Impact of non-thermal plasma on cell signaling in keratinocytes

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Contents

Abbreviations

ANOVA	analysis of variance
ATM	ataxia telangiectasia mutated
AVBB	Annexin V Binding Buffer
CDH1	cadherin 1
cDNA	complementary DNA
Chk1	checkpoint kinase 1
CM	conditioned medium
CSF2	colony stimulating factor 2
COL14A1	collagen, type XIV, alpha 1
COL5A3	collagen, type V, alpha 3
CXCL2	chemokine (C-X-C motif) ligand 2
CXCL5	chemokine (C-X-C motif) ligand 5
DBD	dielectric barrier discharge
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EPR	electron paramagnetic resonance
F13A1	coagulation factor XIIIA1 polypeptide
FCS	fetal calf serum
FGF	fibroblast growth factor
FITC	fluorescein isothiocyanate
FTIR	fourier transformed infrared spectroscopy
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte macrophage colony-stimulating factor
$GS^{\cdot}$	gluthathione thiyl

GSH	gluthathione
H_2O_2	hydrogen peroxide
HaCaT	human adult low calcium temperature keratinocytes
HB-EGF	heparin-binding epidermal growth factor-like growth factor
HMOX1	heme oxygenase-1
HNO_2	nitrous acid
HNO_3	nitric acid
$HO_2^{\cdot}$	hydroperoxyl
HRP	horseradish peroxidase
IFN-γ	interferon-γ
IGF	insulin-like growth factor
IL	interleukin
IL-6R	interleukin-6 receptor
iNOS	inducible nitric oxide synthase
JAK1	janus kinase 1
LPS	lipopolysaccharide
MMP	matrix metallopeptidase
NO	nitric oxide
NO_2	nitrogen dioxide
NO_2^-	nitrite
O and O(1D)	atomic oxygen
$O_2(^1\Delta_g)$	oxygen metastable
$O_2^{\cdot -}$	superoxide anion
O_3	ozone
$^{\cdot}OH$	hydroxyl
PA	plasminogen activator
PBS	phosphate buffered saline
PDGF	platelet-derived growth factor
PTGS2	prostaglandin-endoperoxide synthase 2
RMA	Robust Multichip Average

RNA	ribonucleic acid
RNS	reactive nitrogen species
RONS	reactive oxygen and nitrogen species
ROS	reactive oxygen species
RPL13A	ribosomal protein L13a
RPMI	Roswell Park Memorial Institute
SD	standard derivation
sLm	standard liter per minute
STAT3	signal transducer and activator of transcription 3
TFRC	transferrin receptor
TGF	transforming growth factor
TNFα	tumor necrosis factor α
UV	ultraviolet
VEGF	vascular endothelial growth factor
VTN	vitronectin
VUV	vacuum ultraviolet
WISP1	WNT1 inducible signaling pathway protein 1
WNT5A	Wingless-type MMTV integration site family, member 5A

1 Introduction

1.1 Plasma

Plasma, the fourth state of matter besides solid, liquid and gas (fig. 1) was first described by the American physicist and chemist Irving Langmuir in the nineteen-twenties.[29] Plasma is a partially or completely ionized gas. Gas atoms can be ionized, if they collide with other gas atoms or free electrons. This only happens if the kinetic energy of the collision is equal or larger to the ionization energy.[42] The gas temperature is related to the kinetic energy of the gas particles. With increasing gas temperature, the probability of electron and ion collision ionization rises. Therefore, the transition from the gas to the plasma state is continuous. With increasing temperature more ionizing collisions occur and more free charge carriers are present.[46] Common plasmas in nature are lightnings, stars, flames or northern lights but it is supposed, that 99 % of the matter in the universe is plasma.

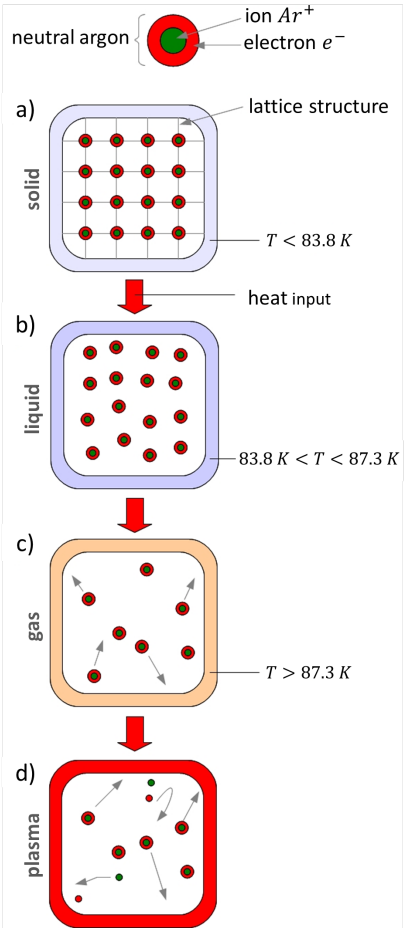


Figure 1: The fourth state of matter explained with argon: With continuous heat input, solid argon (a) can become a liquid (b) or a gas (c). At higher temperatures, some gas atoms are ionized and form a plasma (d).

In the history of plasma technology,

plasma with temperatures larger than 80 °C has very early been used for tissue destruction and ablation, etching, cutting and sterilization of heat resistant medical devices.[35] Recently, non-thermal plasma sources with temperatures near body temperature have been developed. These are suitable for the treatment of heat sensitive materials.[35,86] Especially for the treatment of tissues or cells the application of hot plasma is not an option. Already at temperatures from 40 °C to 50 °C local hypothermia causing modification of the cell membrane, edemas, necrosis and devitalization can occur.[86] A further requirement on the plasma sources for biomedical applications is, that they need to be operable at atmospheric pressure in order to be practicable for clinical application. For this reason, the plasma sources used for biomedical applications are not ignited by heating of the gas, but by acceleration of the electrons by electric fields. The electrons are accelerated to velocities that are sufficient to ionize neutral atoms. The light electrons can easily be accelerated by alternating electric fields. Argon ions however have 80,000 times the weight of electrons and therefore possess to much inertia to be accelerated effectively in high frequency alternating electrical fields. In this way a sufficient amount of fast electrons (corresponding to a high electron temperature) can be achieved, while the temperature of the ions and neutral atoms hardly exceeds room temperature. The transport of heat from the electrons to the ions is also ineffective due to the large mass difference. With a sufficiently high gas flux, a heating of the neutral atoms is prevented almost completely.[46,86]

As plasma is a highly complex system with many variables like the temperature of the gas and of the electrons, gas composition, generated radicals, reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive oxygen and nitrogen species (RONS), ultraviolet (UV) radiation (A, B and C) and electric fields, thorough scientific investigations are absolutely essential.[30,63,86]

1.2 Human Skin

The skin is the largest organ of humans. With a surface of 1.5 - 2 m^2 it weights around 3 to 10 kg and serves as a protection against mechanical, physical or chemical stress.[80] As shown in figure 2, the skin is divided into the epidermis, dermis and subcutis.[73,80] The epidermis is a stratified squamous epithelium with a high regeneration rate and consists of 90 % keratinocytes. The other cells are melanocytes, Langerhans cells and Merkel cells. As keratinocytes make up the majority of the skin, the investigations in the present work focus on a keratinocyte cell line as a model system.

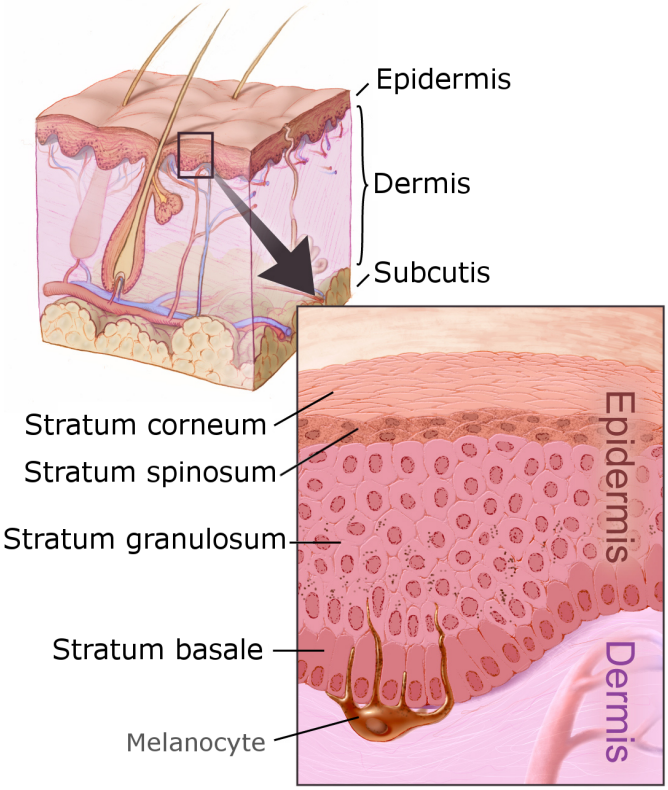


Figure 2: Scheme of the skin.[7]

The epidermis is divided into the layers *stratum basale*, *stratum spinosum*, *stratum granulosum* and *stratum corneum* as depicted in figure 2. The layers correspond to the several differentiation stages of the keratinocytes. These differentiate from the *stratum basale*, the basal layer, to the *stratum corneum*, the horny layer and in the process generate more keratin and actin filaments, which provide the stability of the skin. During this process of differentiation the keratinocytes flatten more and more and at the *stratum corneum* they lose their cell nuclei. The migration of the cells from the basal layer to the horny layer takes around four to six weeks.[73,80]

1.3 Wound Healing

1.3.1 Acute wound

Wound healing is a very well-orchestrated and complex mechanism. Numerous cell types such as keratinocytes, fibroblasts, monocytes, macrophages and neutrophils are involved in this dynamic process. Wound healing is divided into four overlapping phases: Hemostasis, inflammation, proliferation and remodeling.[19,49,54,72] After injury of tissue and blood vessels small molecules like ATP, adenosine, uric acid and arachidonic-acid-derived and other bioactive lipids leak at the wound site.[55] Thereafter, platelets reach the wound space from the damaged blood vessels. They aggregate and clot to plug the defect.[19,54] This fibrin clot additionally consists of cross-linked fibrin, fibronectin, vitronectin, thrombospondin and erythrocytes.[19] It protects against invading microorganisms and provides a matrix for migrating skin and immune cells and a reservoir for released cytokines and growth factors (fig. 3).[19] These secreted signaling molecules play a vital role during wound healing. The different cell types use these molecules for crosstalk and to effect or amplify the behavior of other cells, whereby they can initiate the wound healing phases.[54,89]

Platelet derived growth factor (PDGF), transforming growth factor-β (TGF-

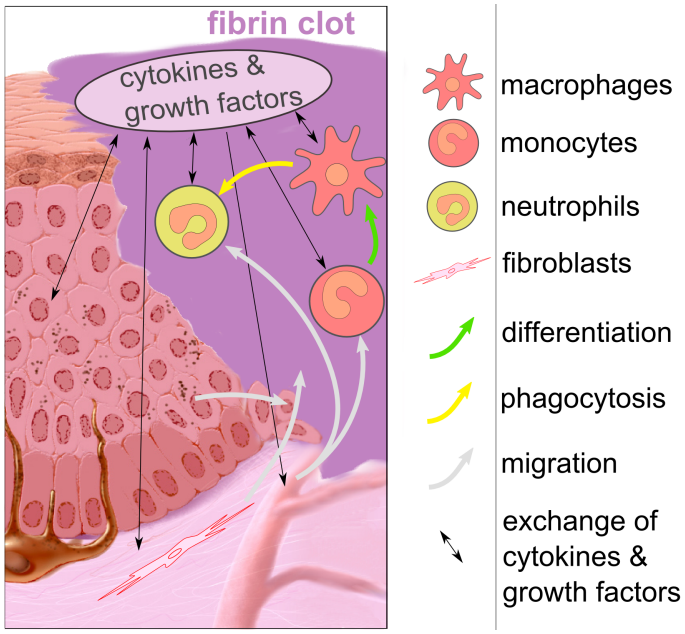


Figure 3: Scheme of wound repair. Modified from [7] .

β) and vascular endothelial growth factor (VEGF) are some of the first molecules, shedded by platelets into the wound site. These chemoattractants recruit circulating inflammatory cells like neutrophils or monocytes.[54] A few minutes after injury, the neutrophils are the first cells that migrate into the wound matrix.[54,89] They infiltrate to remove pathogens, for this purpose neutrophils produce proteases and ROS. They also secrete various growth factors and cytokines to initiate the proliferative phase of wound repair.[89] Monocytes migrate through the blood vessel walls into the wound site and are stimulated by cytokines like interleukin-1α, -1β, -4, -6, -10, -13 (IL-1α, -1β, -4, -6, -10, -13), tumor necrosis factor α (TNFα), interferon-γ (IFN-γ) but also lipopolysaccharide (LPS), a component of gram-negative bacteria membranes. The monocytes degrade the extracellular matrix (ECM) with enzymes, so that they will have space enough to migrate into the wound site.[19] Later, monocytes differentiate into macrophages, as sketched in figure

3.[19] These macrophages produce numerous growth factors (e.g. VEGF) and cytokines to amplify the signals by platelets, neutrophils and monocytes.[54,55] They phagocyte cell and matrix debris but also those neutrophils which did not initiate apoptosis.[19,54]

During proliferation or granulation phase the keratinocytes perform reepithelization while fibroblasts contract the wound. Additionally, angiogenesis (generation of new blood vessels) and the neural response is initiated.[54,89] Angiogenesis is mainly promoted by fibroblast growth factor-2 (FGF-2) and VEGF-A. FGF-2 is secreted by endothelial cells and macrophages, whereas VEGF-A is produced by wound edged keratinocytes, fibroblasts, platelets, neutrophils and macrophages.[3,54,89] Angiopoietin-1 stabilizes the blood vessels, but also chemokines or further growth factors such as IL-8 or granulocyte macrophage colony-stimulating factor (GM-CSF) can effect angiogenesis.[89] After tissue injury the reepithelization is necessary for wound repair. First, the wound edged keratinocytes change their shape, they become flatter and elongate.[19] For migration and proliferation at the wound edge the keratinocytes need to express integrins, collagens, plasminogen activator (PA) and receptors.[54] Important molecules that promote migration and proliferation of the keratinocytes are TGF-α, TGF-β1, epidermal growth factor (EGF), FGF-7, heparin-binding EGF-like growth factor (HB-EGF), matrix metalloproteases-1, -9, -10 (MMP-1, -9, -10), GM-CSF, IL-6 and several chemokines.[19,54,89] Most molecules are mitogenic such as IL-6 which is a crucial kick-starting cytokine for the wound healing process,[89] but some molecules such as MMPs and PA are also important for fragmentation of the ECM to create a path through the fibrin clot for reepithelization.[19] The epidermal migration ceases when the denuded wound space is covered by a monolayer and filled with granulation tissue.[19,54] The latter consists of a very high amount of capillary blood vessels, which transfer nutrients and oxygen into the wound tissue.[55] In order to support the reepithelization the myofibroblasts contract the connective tissue into the wound site.[19] For

this fibroblasts crawl into the fibrin clot, generate large amounts of collagen-rich matrix and can transform into myofibroblasts.[54,89] The last and longest phase during wound repair is the remodeling phase.[19] The collagen is remodeled into larger and more organized fibrils and the cell proliferation and protein synthesis is ceased.[19] After successful wound repair, blood vessels regress and inflammatory cells die and are removed or are extruded with the eschar.[19,55]

1.3.2 Chronic wound

The dynamic wound repair process can fail and cause a non-healing, chronic wound.[72] Different conditions can enhance the risk for chronic wound suffering such as diabetes, aging, venous insufficiency, skin fragility or pressure sores.[21,60,72] A critical colonization by bacteria is associated with non-healing wounds. The microorganisms produce a biofilm which protects themselves from phagocytic cells and is impermeable for antibiotics.[16] Characteristically, chronic wounds have an impaired proliferative phase with a failure of reepithelization and a defective wound ECM. In chronic wounds the wound repair process is additionally arrested in the inflammation phase, which results in a prolonged infiltration of inflammatory cells.[3,60] However, the reasons for chronic wounds are poorly understood,[21,60] but analyses of wound fluids showed imbalances of ROS, RNS and released cytokines and growth factors.[3,50,60,93]

ROS as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$) or hydroxyl ($^{\cdot}OH$) play a very important role during wound repair process.[50,72] The reactive species are normally produced after wounding and are used for the defense of pathogens. Additionally, they act as signaling molecules and can induce proliferation, differentiation or apoptosis. The recruitment of neutrophils into the wound site can also be regulated by ROS.[50,72,84] In chronic wounds an excessive production of ROS is given which can cause oxidative stress.[72] Nucleic acids, proteins and lipids are damaged by ROS which induce a loss

of function and impaired cell migration, proliferation and ECM synthesis.[60] The excessive level of ROS can also cause a dysregulation of cell signaling pathways and finally result in a changed release of growth factors and cytokines.[84] Indeed, the amount of signaling proteins in chronic wound fluids is reduced.[50] To antagonize the oxidative stress antioxidants are crucial, but in chronic wounds an imbalance of oxidants and antioxidants is given.[60,72] Important ROS-detoxifying enzymes are superoxide dismutases, catalase, peroxiredoxins and heme oxygenases.[72] The expression of heme oxygenase-1 (HMOX1) during wound repair is highly up regulated and can therefore be used as a biomarker for oxidative stress in wounds.[84]

Besides ROS, RONS like nitric oxide NO play also a crucial role for wound healing.[72] In an acute wound the inducible nitric oxide synthase (iNOS) catalyzes the NO synthesis from the amino acid L-arginine.[84,93] Its expression, transcription and function is regulated by various growth factors and cytokines and the produced NO can enhance cell proliferation and reepithelization but it can also regulate gene expression and cellular differentiation. NO seems to influence the secretion of cytokines and growth factors. However, in a chronic wound the level of NO is reduced due to the decreased rate of L-arginine, which is necessary for its production.[93]

Another, very important role is played by various growth factors and cytokines during wound healing. They coordinate the dynamic processes in the four different wound repair phases. The cell signaling molecules can act in an autocrine, paracrine, juxtacrine or endocrine way. Thereby they bind to a receptor and activate the downstream cascade of the signaling pathway. If a transcription factor is activated, it binds to a promoter which subsequently regulates the expression of defined target genes. These genes are translated into proteins and can subsequently control cell cycle, motility, differentiation or others.[3] This crucial crosstalk by growth factors and cytokines is imbalanced in chronic wounds. A higher level of many signaling molecules (like GM-CSF, VEGF-A, HB-EGF) is released after wounding, whereas in chronic

wounds the excretion is decreased. And the secretion of other molecules (like IL-8) is drastically increased in chronic wounds.[3,16,19,21]
Until now, no perfect therapy has been developed to heal chronic wounds.[16,21] Many different methods were established or investigated involving maggots or spices. The topical delivery of single or multiple growth factors and cytokines is a very promising therapeutic approach. However, therapies with several mediators were more beneficial because they amplify the stimulation. Some molecules, which enhance wound healing in a combined delivery do not positively influence the healing process in a single application.[1,3,18,50,72]

1.4 Plasma medicine

As plasma medicine is a young and highly interdisciplinary field of research plasma can very efficiently be applied for sterilization and decontamination. It can be used against gram positive and negative bacteria, fungi, viruses and spores.[35,63,86] Plasma is also used for blood coagulation, dental care, cancer treatment and wound healing.[30,35,86] Many research groups have treated eukaryotic cells or tissue with plasma. Cells tend to respond to plasma treatment in a dose dependent manner. At low plasma doses, DNA strand breaks can occur, which are repaired by cellular mechanisms, preventing the cell from inducing apoptosis.[6] In case the applied dose is too large, adherent cells detach and die due to apoptosis or necrosis.[24,35] As different plasma sources have been used in these studies (dielectric barrier discharge, atmospheric pressure plasma jet), the respective lethal treatment times are very different. Besides the duration of treatment, further conditions play crucial roles for cellular response. The cell culture medium can influence the treated cells, but also the humidity in feed gas and environment. The ambience itself can affect cells, too.[8,61,71,91]
As explained in chapter 1.3, in chronic wounds bacteria produce a harming biofilm. Studies showed, that plasma can reduce the amount of bacteria significantly, whereas the eukaryotic cells are still viable. It was repeatedly

shown that for bacteria smaller plasma doses are lethal than for example skin cells, probably as these possess defense mechanisms against components of the plasma.[8,24,35,38] The interactions between plasma, liquids (e.g. wound fluids or cell culture medium) and cells or tissues are largely unknown, also the mechanisms promoting wound healing are controversial.[35] However, it is assumed that the cocktail of generated reactive species and UV radiation is associated with the beneficial effects. Studies revealed the activation of genes and proteins for the defense of oxidative stress[75,87] and the stimulation of cell signaling in skin and immune cells.[2,13]

1.5 Aim of the work

Plasma medicine is a young research field and not much is known about the molecular biological cell response to plasma. A few studies revealed the activation of cell signaling in fibroblasts[2] and immune cells;[13] a first hint, that plasma can influence cell signaling and therefore enhance wound healing.
In the present work, the impact of non-thermal plasma on cell signaling in keratinocytes is to be analyzed. For that reason the HaCaT keratinocyte cell line was treated with the argon-operated non-thermal atmospheric pressure plasma jet kinpen. Due to the well known hormesis response to plasma treatments, doses for the treatment of the keratinocytes have to be found. With regard to wound healing, expression and secretion profiles of signaling molecules have to be conducted. In addition, following questions should be answered: How can the plasma treatment be modulated, so that the cellular responses can be regulated? And which reactive species could be responsible for these responses? Furthermore, for simulation of crosstalk in wounds it is to be analyzed how cells interact with each other post plasma treatment: On the one hand, treated keratinocytes and untreated keratinocytes and on the other hand, keratinocytes and monocytes.

2 Materials and Methods

2.1 Materials

2.1.1 Chemicals and solutions

ammonium acetate	Sigma-Aldrich
Annexin V	Axxora
Argon gas (purity 99.999 %)	Air Liquide
β-mercaptoethanol	Sigma-Aldrich
dH2O, PCR grade	Roche Applied Sciences
DEPC	diethylpyrocarbonate
EDTA	Sigma-Aldrich
ethanol, absolute	Sigma-Aldrich
etoposide	Axxora
FCS	Sigma-Aldrich
glycogen	Ambion
H_2O_2 (30 %)	Sigma-Aldrich
isopropanol	Sigma-Aldrich
L-glutamine (2 mM)	Lonza Group
LPS	Sigma-Aldrich
Nitrogen gas (purity 99.999 %)	Air Liquide
Oxygen gas (purity 99.995 %)	Air Liquide
PBS (10 x, w/o Ca^{2+}/ Mg^{2+})	PAA Laboratories
penicillin (100 U mL^{-1})/ streptomycin (0.1 mg L^{-1})	Lonza Group
RNase ZAP	Sigma-Aldrich
RPMI 1640	Lonza Group
Trypsin/EDTA	Lonza Group

2.1.2 General laboratory equipment

4x72K Array	Roche NimbleGen
beakers	VWR
Buerker counting chamber	VWR
cell culture flasks	TPP
cell scrapers	TPP
FACS tubes	Sarstedt
glass bottles	VWR
HaCaT keratinocytes	German Cancer Research Center DKFZ
LightCycler® 480 Sealing Foil	Roche Diagnostics
microcentrifugation vials	VWR
PCR tubes (0.2 mL, 0.5 mL)	Corning Life Sciences
petri dishes (60 mm)	TPP
pipettes	Eppendorf
pipette filter tips	Eppendorf
pipette tips	Eppendorf
reservoirs	Corning Life Sciences
serological pipettes (5 ml, 20 ml, 25 ml)	Sarstedt
THP-1 monocytes	Cell Lines Services
tubes (15 mL, 50 mL)	Corning Life Sciences
96 well plates	TPP
96 well plates for PCR	Roche Applied Sciences

2.1.3 Kits

CellTox™ Green Cytotoxicity Assay	Promega
Green Caspase-3 Staining Kit	PromoKine
Human IL-6 ELISA MAX™ Deluxe	BioLegend

Human IL-8 ELISA MAX™ Deluxe	BioLegend
Human Inflammatory Cytokines Multi-Analyte ELISArray™ Kit	Qiagen
LEGEND MAX™ Human GM-CSF ELISA Kit with Pre-coated Plates	BioLegend
LEGEND MAX™ Human TNF-α ELISA Kit with Pre-coated Plates	BioLegend
NimbleGen Gene Expression Array	Roche NimbleGen
NimbleGen Hybridization Kit	Roche NimbleGen
NimbleGen One-Color DNA Labeling Kit	Roche NimbleGen
NimbleGen Tracking Control Kit	Roche NimbleGen
NimbleGen Wash Buffer Kit	Roche NimbleGen
Realtime ready Catalog Assay Primer	Roche Applied Sciences
RNA Mini Kit	Bio&SELL
RNase-Free DNase Set	Qiagen
RT^2 First Strand Kit	Qiagen
RT^2 Profiler PCR array	Qiagen
SuperScript Double-Stranded cDNA Synthesis Kit	Invitrogen
Transcriptor First Strand cDNA Synthesis Kit	Roche Applied Sciences
VEGF-A ELISA Kit	Thermo Scientific

2.1.4 Instruments

Centrifuge 5810 R	Eppendorf
clean bench	Thermo Scientific
CNC router Step-Control Zero2	Hylewicz CNC-Technik
CO_2-Incubator 160	Mytron
Gallios™	Beckmann Coulter

kinpen	neoplas
Laboratory hot plate	Labotect
LightCycler® 480 II	Roche Diagnostics
mass flow controller	MKS Instruments
MS 200 Microarray Scanner	Roche NimbleGen
NanoDrop 2000c	Thermo Fisher Scientific
NimbleGen Hybridization System 4	Roche NimbleGen
NimbleGen Microarray Dryer	Roche NimbleGen
Plate reader Infinte 200 PRO	Tecan Group
pipetboy	INTEGRA Biosciences
Tabletop centrifuge Mini Spin	Eppendorf
Thermocycler Professional 96 gradient	Biometra
Thermo block/ Thermomixer	Eppendorf
Vacusafe	INTEGRA Biosciences
Waterbath Memmert WNB14	Memmert
xyz-table	Nanotec Munich

2.1.5 Software

GraphPad Prism 6	Graphpad Software
IPA®	Ingenuity Systems
Kaluza 1.1	Beckmann Coulter
MS Office	Microsoft
Nano Drop operating software	Thermo Scientific
NimbleScan v2.6 software	Roche NimbleGen
PANTHER 8.1	Paul Thomas, University of Southern California
Partek® Genomic Suite™	Partek
Tecan i-Control 1.10	Tecan Group
LightCyler®480 SW 1.5.1	Roche Applied Science

WinPC-NC software	Burkhard Lewetz

2.1.6 Buffers and solutions

Lysis buffer, 25 x	5 % benzalkonium chloride 3 % acetic acid, glacial
Annexin V Binding Buffer	10 mM HEPES 140 mM NaCl 2.5 mM $CaCl_2$ dest. H_2O, ad 1 L
PBS/ EDTA	5 mM EDTA in PBS
Swelling buffer	20 mM tris 1 mM $MgCl_2$ 0.5 mM $CaCl_2$ pH = 6.7
Cell culture medium	RPMI 1640 8 % fetal calf serum 2 mM L-glutamine 0.1 mg L^{-1} streptomycin 100 U mL^{-1} penicillin

2.2 Methods

2.2.1 Cell culture

All experiments were conducted with the human adult low-calcium high-temperature keratinocyte (HaCaT) cell line. For subcultivation cells were washed with 3 mL PBS/ EDTA and subsequently incubated in 5 mL PBS/ EDTA for 10 minutes in the incubator with following conditions: 37 °C, 95 % humidity, 5 % CO_2. In order to detach the keratinocytes they were trypsinized with 5 mL trypsin/EDTA for 4 minutes in the incubator. The

detached cells were gathered and suspended with 5 mL cell culture medium. Subsequently they were centrifuged at 250 g for 3 minutes at room temperature. The supernatant was discarded and the pellet was resuspended in 10 mL medium. In the next step, 20 µL cell suspension were incubated in 460 µL swelling buffer for 7 minutes and after adding 20 µL lysis buffer the cells were counted via Buerker counting chamber. Subsequently, 2 x 10^6 cells in 15 mL medium were transferred into a new cell culture flask with a growth area of 75 cm^2. The subcultivation was repeated twice a week.[5]

2.2.2 Plasma source

The non-thermal atmospheric pressure plasma jet kinpen, depicted in figure 4, was operated with argon at a flow rate of 3 standard liters per minute (sLm). The feed gas flows through a ceramics capillary in which a radio frequency electrode with a radius of 1 mm is mounted. At this inner electrode a voltage of 2 kV_{pp} is applied with a frequency of 1.1 MHz, providing the alternating electric field required for the ignition of the atmospheric pressure plasma. The visible effluent has a length of around 12 mm and the ROS

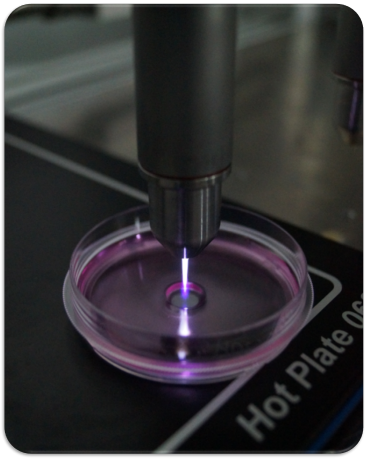


Figure 4: Plasma treatment of 5 mL cell culture medium in a petri dish by the ignited non-thermal atmospheric pressure plasma jet kinpen.

and RNS generated in the effluent are of special interest for their impact on biological systems. Additionally, UV radiation is generated by the kinpen by recombination and excitation processes.[71,86]

Although the jet is operated with pure argon, nitrogen and oxygen from ambient air (main components: 78 % N_2, 21 % O_2) diffuse into the effluent. To study the influence of oxygen and nitrogen, the ambience of the effluent was controlled for some experiments. Therefore, the kinpen was surrounded by a shielding device, depicted in figure 5. The distance from the nozzle of the kinpen to the nozzle of the device was 2.5 mm. As a shielding gas, a mixture of oxygen and nitrogen was used with a total gas flow rate of 5 sLm. The shielding gas mixture was varied in five steps, ranging from pure nitrogen to pure oxygen. The parameters for each shielding gas composition were adjusted 3 minutes before treatment to guarantee constant conditions and homogeneous gas mixtures.

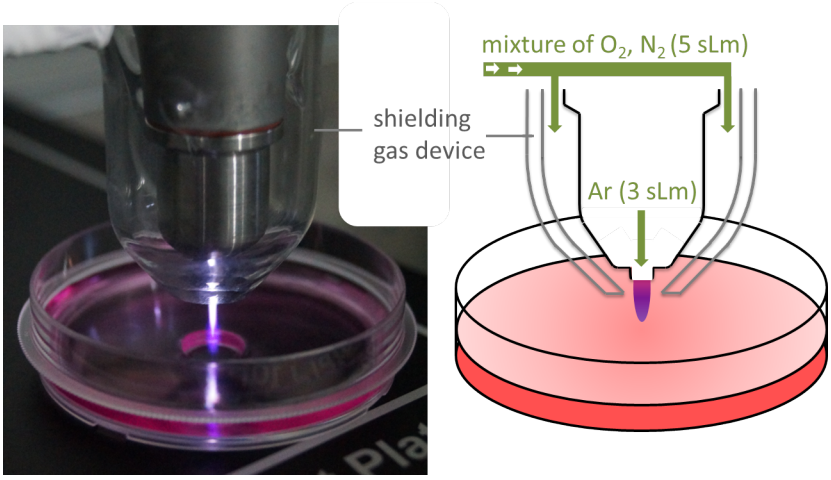


Figure 5: Picture and scheme of the plasma treatment with the kinpen in combination of the shielding gas device.

2.2.3 Plasma treatment

Both, treatment with and without a shielding device were conducted in the same way: The cells were prepared one day prior to indirect plasma treatment. Therefore 1 x 10^6 cells in 5 mL medium were seeded in a 60 mm petri dish. For attachment they remained in the incubator for 24 hours. To ensure same conditions for every treatment, cell culture medium was incubated in a cell culture flask for 24 hours, too. Hence, CO_2 amount, temperature and pH of the medium were constant for every treatment. The plasma was ignited 1 hour prior to the treatment to remove humidity from the tubing, which can enter through tube openings or diffuse through the tube walls from the ambience and influence the cellular response.[91]
For treatment, a 60 mm petri dish containing 5 mL cell culture medium was positioned on a warm plate (37 °C) and subsequently treated for desired time. The kinpen, which was fixed in a xyz-table, was moved automatically by a computer system (WinPC-NC software) along the treatment path sketched in figure 6. Due to the evaporation during plasma treatment the respective amount of distilled sterile water was added to the treated medium (300 µL to 180 s treated medium). Immediately after treatment the medium on the cells was aspirated and replaced by the treated one. For the untreated control cells, a medium change with untreated medium was done. For the treatments with H_2O_2 , insulin or etoposide the desired concentrations were produced in 5 mL medium and subsequently added to the cells. After treatment, the cells were incubated in the incubator for the desired time.

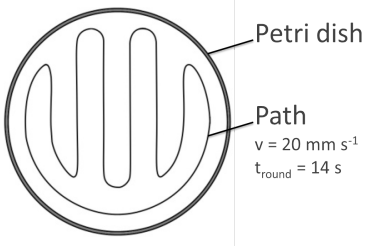


Figure 6: Path of the computer controlled plasma treatment.

2.2.4 Preparation of conditioned medium

Due to plasma treatments keratinocytes can change their gene and protein expression profiles and it is unclear if these changes can impact untreated cells. Therefore, the influence of cell culture medium conditioned by plasma treated and starved keratinocytes on other starved keratinocytes was studied. The cells were treated as follows. At first, 1 x 10^6 HaCaT keratinocytes were seeded in 5 mL standard cell culture medium (containing 8 % FCS) in a 60 mm petri dish. After an incubation time of 24 hours the cells were starved

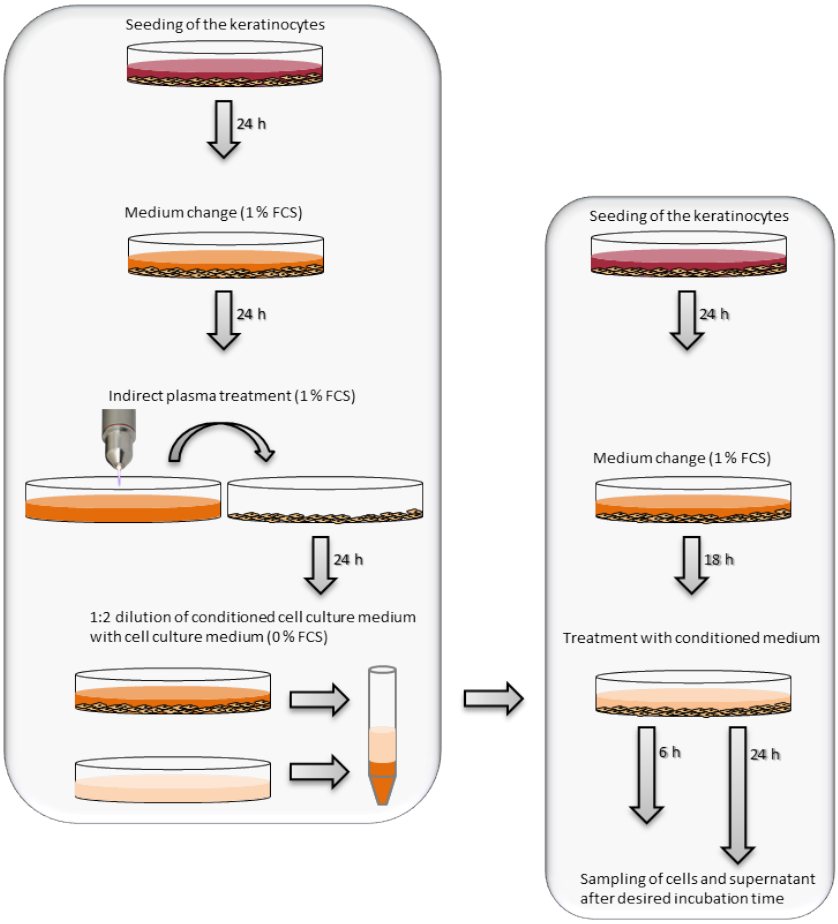


Figure 7: Treatment of starved keratinocytes with conditioned, plasma treated medium.

by a medium change with a medium containing only 1 % FCS. One day later, these cells were treated with plasma in an indirect way, as described in chapter 2.2.3 on page 22. The keratinocytes were allowed to modify the medium by secretion of molecules like cytokines and simultaneously they consumed the medium within 24 hours. The conditioned medium was diluted 1:2 with cell culture medium without FCS, thereby 3 mL conditioned medium was mixed with 3 mL medium without FCS. This diluted medium was given onto other cells as shown in figure 7. These cells were starved 24 hours post seeding, too. However, the starved keratinocytes were treated with the treated and conditioned medium by the other cells 18 hours after starving. They were incubated for 6 or 24 hours and cells and supernatants were used for further gene expression and protein secretion analysis explained in chapters 2.2.7 and 2.2.8.

2.2.5 Co-culture with keratinocytes and monocytes

Besides the interactions between plasma treated and untreated keratinocytes the crosstalk between different cell types is also very interesting for wound healing. Therefore the impact of plasma on a co-culture with skin and immune cells was studied. Keratinocytes (HaCaT cell line) and monocytes (THP-1 cell line) were used for this study. In order to compare the results of the co-culture, the same treatments were conducted with skin or immune cells alone. The treatment of the co-culture was performed as follows: The keratinocytes (1 x 10^6 in 5 mL medium) were seeded in a 60 mm dish and incubated for 24 hours for attachment. Subsequently, 1 x 10^6 monocytes were centrifuged and resuspended with either untreated medium, 180 s plasma treated medium or 180 s plasma treated medium with LPS (1 µg µL^{-1}). The monocyte suspensions replaced the keratinocyte cell culture medium.
The treatment of the keratinocytes alone was performed in a similar way, but the medium was directly added to the HaCaT cells and not used to resuspend the monocytes. The treatment of the monocytes alone was also

similar. However, the THP-1 cells were resuspended with the treated or non treated medium and were not aliquoted to the keratinocytes, but just seeded in a 60 mm dish. Co-culture and mono-cultures were incubated for 24 hours and the supernatants were collected for analyses of secreted proteins. All experiments were done in collaboration with Lena Bundscherer, who handled the monocytes (explained in detail by [13]).

2.2.6 Cell survival

Cytotoxicity assay

The analysis of cell viability was performed with the CellTox™ Green Cytotoxicity Assay (Promega). The CellTox™ Green Dye is not cell permeable and stains intracellular DNA after cell membrane becomes leaky due to cell death. For the measurements 15,000 keratinocytes in 75 µL cell culture medium were seeded per well in a 96 well-plate. Besides plasma and H_2O_2 treatments the cells were also treated with a lysis buffer as a positive control. After treatment, the cells were incubated for 24 hours. The CellTox™ Green Dye was added and the plate was mixed for 1 minute by the microplate reader (Infinte 200 PRO, Tecan Group). After an incubation time of 15 minutes, the fluorescence was detected at 490 nm (excitation wavelength) and 525 nm (emission wavelength). At least six replicates were measured for each treatment.[4]

Early apoptosis

The amount of early apoptotic cells after plasma treatment was detected with a measurement of Annexin V positive cells. Therefore, 1 x 10^6 cells were seeded in a 60 mm petri dish for plasma treatment. After treatment they were incubated for 12 hours. The cell culture medium was gathered and the cells were trypsinized and added to the cell culture medium. Cell culture medium and cell suspension were centrifuged at 160 g for 5 minutes at room

temperature. The supernatant was removed and the pellet was suspended with 1 mL Annexin V Binding Buffer (AVBB). The cells were counted via Buerker counting chamber and 0.3 x 10^6 cells were centrifuged. The cells were resuspended in 400 µL AVBB with 1 µL Annexin V-fluorescein isothiocyanate (FITC) and incubated for 10 minutes in the dark. Subsequently they were centrifuged again and washed with 400 µL AVBB. The cells were resuspended with 300 µL AVBB and measured with the flow cytometer Gallios^{TM} (Beckmann Coulter) and analyzed with the Kaluza® software (Beckmann Coulter). This assay was repeated 3 times in independent experiments.

Late apoptosis

Late apoptosis was measured with the Green Caspase-3 Staining Kit (PromoKine). One million cells in a 60 mm petri dish were treated with plasma and incubated for 18 hours. The cells were detached with trypsin and 0.3 x 10^6 cells in 300 µL medium were incubated with 1 µL labeled and cell permeable FITC-DEVD-FMK for 1 hour in the incubator. Afterwards, they were washed twice. Therefore they were centrifuged at 3,000 rpm for 5 minutes, the supernatant was removed and the cells were resuspended in 500 µL wash buffer. After washing they were resuspended in 300 µL wash buffer and measured with the Gallios™ flow cytometer and analyzed with the Kaluza® software.

2.2.7 Gene expression analysis

RNA isolation

After plasma treatment and incubation time the medium was removed and the keratinocytes were washed with 1 mL of ice-cold PBS. Subsequently, 1 mL ice-cold PBS was added to the cells and the cell layer was detached via cell scraper and transferred into a micro vial. The cells were centrifuged at 250 g at 4 °C for 5 minutes. The supernatant was removed and the cell

pellet deep-frozen at -80 °C or the RNA was isolated. For RNA isolation the RNA Mini Kit by Bio&SELL was used.[5] Therefore, 400 µL lysis buffer SM was added to the cell pellet which was incubated for 2 minutes. The pellet was resuspended with the lysis buffer and incubated for 3 minutes. The cell suspension was aliquoted into the blue shredder column and centrifuged (10,000 g, 2 minutes). The filtrate was mixed with ethanol (70 %) and aliquoted into the purple centrifugation column for centrifugation (10,000 g, 2 minutes). DNA was digested due to the addition of 5 µL DNase I (6.8 Units) and 35 µL RDD buffer (RNase-Free DNase Set by Qiagen) which was than incubated for 15 minutes. Two wash steps followed: First 500 µL wash buffer IT were aliquoted to the column and centrifuged at 10,000 g for 1 minute and subsequently the column was washed with 700 µL wash buffer MT at 10,000 g for 3 minutes. For elution the column was incubated for 1 minute with 40 µL RNase-free water and eluted due to centrifugation at 6,000 g for 1 minute. Either the isolated RNA was stored at -80 °C or the cDNA synthesis was performed. Therefore, the concentration of isolated RNA was measured with a spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific) and 1 µg μL^{-1} isolated RNA was used for cDNA synthesis.

cDNA synthesis

For studies with a RT^2 Profiler PCR array (Qiagen) the cDNA was synthesized by RT^2 First Strand Kit (Qiagen), otherwise the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science) was used.
For cDNA synthesis with the RT^2 First Strand Kit a gDNA elimination mix was prepared. Therefore, 2 µL GE buffer and 1 µg isolated RNA were necessary. In order to reach a total volume of 10 µL, RNase-free water was used. The elimination mix was incubated for 5 minutes at 42 °C in the thermocycler. Subsequently, 10 µL reverse transcriptase mix, which consisted of 4 µL BC3 buffer, 1 µL Control P2, 2 µL RE3 RT Mix and 3 µL RNase-free water, was aliquoted to the elimination mix, which was then incubated for 15

minutes at 42 °C and 5 minutes at 95 °C in the thermocycler. Subsequently, 91 µL RNase-free water was added and either stored at -20 °C or used for the analysis with the RT^2 Profiler PCR array.
The cDNA synthesis with the Transcriptor First Strand cDNA Synthesis was performed as described in the manual: First, 1 µg RNA was mixed with 1 µL anchored-oligo(dT) primer, 1 µL random hexamer primer and RNase-free water was used to achieve a total volume of 13 µL. The solution was heated for 10 minutes at 65 °C. Subsequently, 4 µL Transcriptor RT reaction buffer, 2 µL dNTPs, 0.5 µL RNase inhibitor and 0.5 µL reverse transcriptase were added. The solution was then heated for 45 minutes at 55 °C and 5 minutes at 85 °C by the thermocycler and afterwards either stored at -20 °C or used for DNA microarray analysis or quantitative polymerase chain reaction (qPCR).
The studies of the transcriptome after plasma treatment were done with i.) DNA microarray, ii.) qPCR using the RT^2 Profiler PCR array or iii.) qPCR using Realtime ready Catalog Assay which will be explained in the subsequent paragraphs.

DNA microarray

For the analysis of the transcriptome by DNA microarray, the cells were treated with plasma and incubated for 3 hours. Four independent biological experiments were performed. At a time the samples of two experiments were pooled during RNA isolation as sketched in figure 8. And subsequently the two, pooled RNAs were again pooled yielding one sample for single strand cDNA synthesis (Transcriptor First Strand cDNA Synthesis Kit). The second strand cDNA synthesis was performed with the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen): 20 µL single strand cDNA was mixed with 17.05 µL diethylpyrocarbonate (DEPC) water, 10 µL 5 x Second Strand Buffer, 1 µL dNTP Mix (10 mM), 0.35 µL DNA ligase (10 U µL^{-1}), 15 µL DNA polymerase I (10 U µL^{-1}) and 0.35 µL RNase H (2 U µL^{-1}). This was

incubated in the thermocycler for 2 hours at 16 °C. Later, 0.6 µL of T4 DNA polymerase (5 U μL^{-1}) were added to the solution and incubated for 5 minutes and 16 °C, again. The solution was placed on ice and 3.3 µL EDTA (0.5 M) were added. Afterwards the cDNA was precipitated. Therefore, 5.5 µL 7.5 M ammonium acetate and 5 µL glycogen (5 mg ml^{-1}) were added. In a micro-centrifugation tube 120 µL ice-cold ethanol were aliquoted and the solution was added. It was then centrifuged at 12,000 g for 20 minutes at 4 °C. The supernatant was decanted and 200 µL ice-cold ethanol (80 %) was aliquoted to the pellet. A centrifugation step followed (12,000 g, 20 minutes, 4 °C) twice. Later, the pellet was dried at 37 °C in a thermoblock for 10 minutes, while the tube was open. The pellet was rehydrated with 20 µL PCR-clean water and the quality of the cDNA was controlled with the spectrophotometer NanoDrop 2000c.

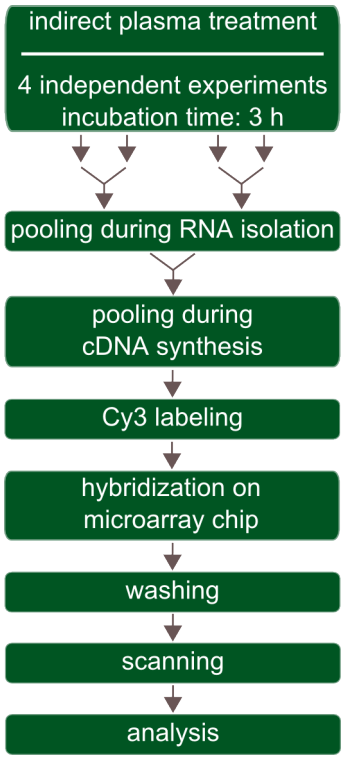


Figure 8: Scheme of the DNA microarray procedure.

The Cy3 labeling was performed with the NimbleGen One-Color DNA Labeling Kit (Roche NimbleGen). Therefore, 1 µg cDNA was aliquoted to 40 µL diluted Cy3 random nonamers and PCR-clean water to a total volume of 80 µL in a 0.2 mL microcentrifugation tube. A heat denaturation followed at 98 °C for 10 minutes in the thermocycler, subsequently the reaction was immediately placed on ice for 2 minutes. The dNTP/ klenow mastermix, consisting of 10 µL dNTPs

(10 mM), PCR-clean water and klenow-fragment (50 U μL^{-1}) was aliquoted to the denatured sample and heated for 2 hours at 37 °C, protected from light. The reaction was stopped by the addition of 21.5 µL stop solution and then transferred into a microcentrifugation tube containing 110 µL isopropanol. The solution was incubated for 10 minutes at room temperature protected from light and subsequently centrifuged at 12,000 g for 10 minutes. The supernatant was removed and the pellet was visible in a pink color. The pellet was resuspended with 500 µL ice-cold ethanol (80 %) and centrifuged at 12,000 g for 2 minutes. The supernatant was carefully removed with an pipette and the pellet was dried in an thermoblock at 37 °C for 10 minutes. The sample could either be stored at -20 °C for 1 month or used for next steps. Afterwards, the pellet was rehydrated with 25 µL PCR-clean water by vortexing it for 30 seconds. It was incubated for 5 minutes in the dark and the double stranded DNA concentration was measured with the spectrophotometer. 4 µg of Cy3-labeled cDNA was dried in a vacuum concentrator and could be stored for 1 month at -20 °C.

Hybridization and washing of the DNA was performed with the NimbleGen Hybridization Kit and NimbleGen Tracking Control Kit by Roche NimbleGen. The pellet was resuspended with 3.3 µL sample tracking control and mixed for 15 seconds using a vortexer with 8.7 µL of hybridization mastermix. The hybridization mastermix consisted of 29.5 µL 2x hybridization buffer, 11.8 µL hybridization component A and 1.2 µL alignment oligo. The reaction was heated to 95 °C for 5 minutes and subsequently kept at 42 °C for at least 5 minutes. The sample was positioned in the hybridization system (NimbleGen Hybridization System 4), which had a temperature of 42 °C. The mixer was prepared and the sample was loaded into the microarray chip (4x72K Array). The hybridization was performed for 20 hours at 42 °C in the hybridization system. Afterwards, the chip was removed and washed with the NimbleGen Wash Buffer Kit. At first, the hybridized array was washed for 2 minutes in Wash I, subsequently for 1 min in Wash II and then 15 s in

Wash III. The array was spin dried in the NimbleGen Microarray Dryer for 2 min and then scanned with a resolution of 2 µm by the MS 200 Microarray Scanner. With the NimbleScan v2.6 software a background correction of the signals was performed via Robust Multichip Average (RMA) algorithm and the signal intensities of the raw data were converted into gene expression levels which were then used for the analysis with the Partek® Genomic Suite™ software. The statistical analysis was performed and genes were regarded as significantly up or down regulated, if they were at least twofold changed. The *P*rotein *An*alysis *Th*rough *E*volutionary *R*elationships (PANTHER) classification system is an open-source software (PANTHER 8.1) and was used to classify the changed genes and assort them into biological function groups. Molecular interaction pathways and networks were generated with the software IPA®.

qPCR

The gene expression profile of the HaCaT cells was analyzed after plasma treatment. The qPCR was either conducted with a human wound healing RT^2 Profiler PCR array (Qiagen) or in separate reactions with the Realtime ready Catalog Assay (Roche Applied Science).
For the qPCRs with the RT^2 Profiler PCR array one 96 well-plate was used for each cDNA be analyzed. Into each well a forward and reverse primer of one gene was located. Besides housekeeping genes, 84 different genes were analyzed. The cDNA was synthesized with the RT^2 First Strand Kit. A mastermix composed of 1350 µL RT^2 SYBR Green Mastermix, 102 µL synthesized cDNA and 1248 µL RNase-free water, was prepared. Into each well 25 µL mastermix was aliquoted and the well-plate was sealed with a foil. After centrifugation (1,000 g, 1 minute) the qPCR was performed with the LightCycler® 480 (Roche Diagnostics). For further experimental controls a melting curve was measured. The data analysis was performed at the free PCR Array Data Analysis Web portal of Qiagen. The quantification was

performed automatically by the software using the Livak or $\Delta\Delta C_T$ method.[48] The gene expression was considered significantly changed if a twofold up or down regulation compared to the untreated control was detected. The genes which were analyzed are listed in the appendix.
Besides this, qPCR was also performed with the Realtime ready Catalog Assay by Roche. Here, 1.3 µg μL^{-1} cDNA was twenty-fold diluted with PCR-clean water. Each reaction was conducted with 4 µL PCR-clean water, 10 µL LightCycler Probes Master (Roche Diagnostics), 1 µL RealTime ready Assay Primer (VEGFA, IL6, HBEGF, CSF2, PTGS2, RPL13A or TFRC; Roche Diagnostics) and 5 µL twenty-fold diluted cDNA. VEGFA, IL6, HBEGF, CSF2 and PTGS2 were the investigated target genes, whereas RPL13A or TFRC were used as housekeeping gene controls. Housekeeping genes were used as internal controls and should not be regulated by plasma treatments. In previous experiments the expressions of numerous housekeeping genes were analyzed after plasma treatment and the expressions of RPL13A and TFRC were not regulated. Each sample was measured as a technical triplicate, the two house-keeping genes (RPL13A, TFRC) as duplicates and one no template control without DNA were part of every run. The qPCR was conducted with the LightCycler® 480. The Livak method or so called $\Delta\Delta C_t$ method was used for fold change calculation.[48]

2.2.8 Protein expression analysis

Analyses of secreted proteins were conducted with the Enzyme-linked immunosorbent assay (ELISA). Different ELISAs from various providers were used and conducted as prescribed in the respective manuals. Here, only a general explanation is given. The interleukins IL-6 and -8 were detected with the Human IL-6 ELISA MAX™ Deluxe and Human IL-8 ELISA MAX™ Deluxe from BioLegend. For the detection of GM-CSF and TNF-α the LEGEND MAX™ Human GM-CSF ELISA Kit and LEGEND MAX™ Human TNF-α ELISA Kit (BioLegend) were used. VEGF-A was measured with the

Human VEGF-A ELISA Kit from Thermo Scientific and the multi analyte ELISAs were performed with the Human Inflammatory Cytokines Multi-Analyte ELISArray^{TM} Kit from Qiagen. One day prior to the measurement, each well of the 96 well-plate had to be coated with a capture antibody. The antibody was diluted with a coating buffer and aliquoted into each well of the well-plate. The plate was incubated overnight at 4 °C. This step could be excluded, if the plates were pre-coated (GM-CSF, TNF-α, VEGF-A, multi analyte). The plate was washed 3 to 4 times by adding wash buffer into each well and subsequently tapping the plate on absorbent paper. Non-specific binding was minimized by the addition of blocking buffer which was incubated for 1 hour at a plate shaker. The plate was washed again and standard or sample was aliquoted as triplicates in the appropriated wells. Before the standard was added into the wells, a standard curve was prepared. The lyophilized standard was reconstituted with assay diluent and this standard stock solution was diluted with assay diluent to prepare the standard curve. Standard and samples were incubated for 2 hours while shaking. The plate was washed again and the detection antibody was added to the wells. If the antibody was horseradish peroxidase (HRP)-conjugated the antibody was incubated for 30 minutes until substrate was added. If the secondary antibody was not HRP-conjugated, they were incubated for 1 hour while shaking and subsequently washed. Diluted avidin-HRP solution was added and an incubation time of 30 minutes followed. The plate was washed again and substrate solution was given into each well and incubated for 15 minutes in the dark. Thereby positive samples were colored in blue. Immediately stop solution was added to the substrate solution and the color changed from blue to yellow. The absorbance was measured with the plate reader at 450 nm and a reference wavelength of 570 nm.

3 Results

3.1 Impact of plasma on HaCaT cells

The investigated human HaCaT keratinocytes were treated with the non-thermal atmospheric pressure plasma jet kinpen. The impact of plasma on cell survival was analyzed. Furthermore, gene expression and protein secretion of wound healing related mediators after plasma treatment was investigated

3.1.1 Cell survival

To study the impact of plasma on cell survival, especially apoptosis, three different experiments were performed. The cytotoxicity of plasma on the human keratinocyte cell line HaCaT was studied and is shown in figure 9.

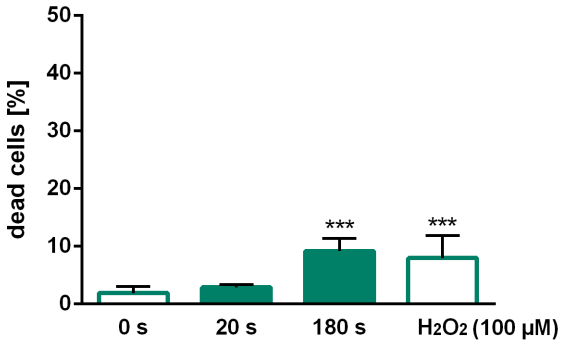
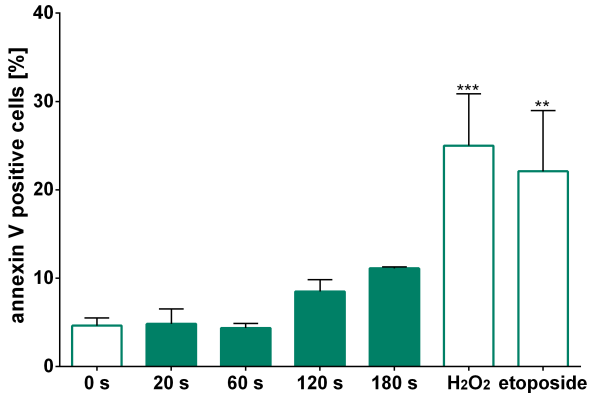
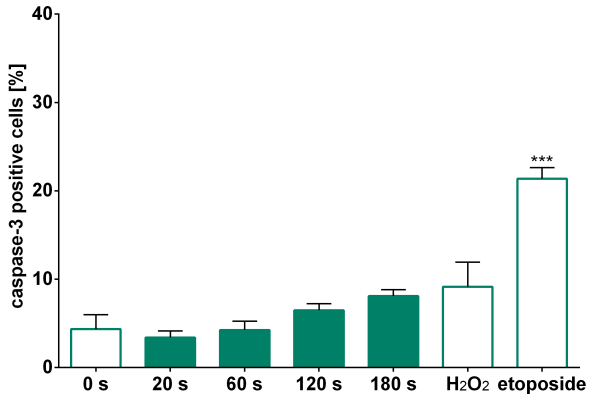


Figure 9: Cytotoxicity of HaCaT cells after plasma treatment. The keratinocytes were either non treated , exposed to plasma for a short (20 s) or long (180 s) time, or they were treated with 100 µM H_2O_2. The bars and error bars are presented in mean and standard derivation (SD). Analysis was performed by Dunnett's test as a follow up for one-way analysis of variance (ANOVA). Three independent experimental repetitions with six technical replicates were performed. The long term plasma treatment (180 s) and H_2O_2 exposure was significantly changed to the untreated control (significance level: $\alpha = 0.001$ (***)) .



(a) Early apoptotic cells 12 hours post treatment.



(b) Late apoptotic cells 18 hours post treatment.

Figure 10: Detection of early (10a) and late (10b) apoptosis after plasma, hydrogen peroxide (100 µM) or etoposide (10 µM) treatment. Each experiment was repeated three times (n = 3). Mean values and SD are given and the statistical analysis was performed via one-way ANOVA following Dunnett's test (significance levels: $\alpha = 0.01$ (**), $\alpha = 0.001$ (***)).

The number of dead cells increased with the plasma treatment time. An exposure of 180 s induced cell death of 10 %, which was similar to the treatment with 100 µM H_2O_2. The short term plasma treatment (20 s) was not cytotoxic for the HaCaT cells and the amount of dead cells was similar to the untreated control cells. The cellular cytotoxicity was studied in greater detail

by apoptosis investigations, presented in figure 10 on the previous page. Both, early and late apoptosis was detected after plasma exposure. Early apoptosis was measured with the FITC labeled annexin V 12 hours post plasma treatment, whereas late apoptosis was detected via caspase-3 activity 18 hours after exposure. The incubation times were determined in previous studies (data not shown) where these time points yielded the highest signal. The cells showed a similar behavior as observed for the cytotoxicity experiment - the longer the plasma treatment, the higher the amount of early and late apoptotic cells. The longest plasma treatment (180 s) induced 10 % annexin V positive cells after 12 hours, whereas after 18 hours less cells were caspase-3 positive (7.5 %). A treatment time until 60 s did not even increase apoptosis neither early nor late apoptosis compared to untreated cells (4.5 %). H_2O_2 and etoposide were used as positive controls. The treatment with H_2O_2 induced in 25 % of the cells early apoptosis and only in 9 % late apoptosis, whereas 22 % of the cells were both annexin V and caspase-3 positive after etoposide (10 µM) treatment.

3.1.2 Transcription profile

The human keratinocytes were treated with plasma in order to analyze the impact on the expression profile of defined genes. After plasma treatment they were incubated for 6 or 12 hours and the qPCRs were performed with the human wound healing RT^2 Profiler PCR array by Qiagen. As described by Barton et al. the 84 studied genes belong to different subgroups like extracellular matrix, cell adhesion, growth factors, inflammatory cytokines and chemokines and signal transduction. Genes for the following proteins were investigated: Collagens, E-cadherin, integrins, angiopoietin 1, GM-CSF, G-CSF, EGF, FGFs, HB-EGF, IGF-1, TGF-α, TGF-β1, TNF, VEGF-A, chemokine ligands, IFN-γ, interleukins, WNT-pathway related proteins and EGFR. The human keratinocyte cell line HaCaT reacts with a change of gene expression due to plasma treatment. Table 1 displays the 21 genes, which

were significantly up or down regulated after plasma exposure.[5]

Table 1: Genes which showed a significantly changed gene expression after plasma treatment compared to untreated control cells. A positive fold regulation describes an up regulation and a negative fold regulation a down regulation.[5]

short gene name	gene name	group	treatment and incubation time	fold regulation
ACTA2	Actin, alpha 2	ECM & Cell Adhesion	120 s, 6 h	4
			180 s, 6 h	8
ANGPT1	Angiopoietin 1	Growth Factors	120 s, 6 h	-52
			180 s, 6 h	-45
CCL2	Chemokine (C-C motif) ligand 2	Cytokines & Chemokines	120 s, 6 h	-5
			180 s, 12 h	-5
CDH1	Cadherin 1, type 1, E-cadherin	ECM & Cell Adhesion	180 s, 6 h	-13
			120 s, 12 h	5
COL5A2	Collagen, type V, alpha 2	ECM & Cell Adhesion	180 s, 6 h	20
CSF2	Colony stimulating factor 2	Growth Factors	180 s, 6 h	13
			120 s, 12 h	7
			180 s, 12 h	6
CSF3	Colony stimulating factor 3	Growth Factors	180 s, 6 h	6
CXCL1	Chemokine (C-X-C motif) ligand 1	Cytokines & Chemokines	180 s, 12 h	-4
CXCL2	Chemokine (C-X-C motif) ligand 2	Cytokines & Chemokines	180 s, 6 h	12
FGF10	Fibroblast growth factor 10	Growth Factors	180 s, 12 h	9

short gene name	gene name	group	treatment and incubation time	fold regulation
HBEGF	Heparin-binding EGF-like growth factor	Growth Factors	120 s, 6 h	6
			180 s, 6 h	4
			180 s, 12 h	5
IL1B	Interleukin 1, beta	Cytokines & Chemokines	180 s, 12 h	5
IL6	Interleukin 6	Cytokines & Chemokines	120 s, 6 h	7
			180 s, 6 h	29
			120 s, 12 h	9
			180 s, 12 h	21
ITGA5	Integrin, alpha 5	ECM & Cell Adhesion	180 s, 6 h	6
ITGB6	Integrin, beta 6	Extracellular Matrix & Cell Adhesion	180 s, 6 h	5
MMP9	Matrix metallopeptidase 9	ECM & Cell Adhesion	180 s, 6 h	5
PLAUR	Plasminogen activator, urokinase receptor	ECM & Cell Adhesion	180 s, 6 h	5
PTGS2	Prostaglandin-endoperoxide synthase 2	Signal Transduction	180 s, 6 h	7
TAGLN	Transgelin	ECM & Cell Adhesion	180 s, 6 h	4
VEGFA	Vascular endothelial growth factor A	Growth Factors	120 s, 6 h	4
			180 s, 6 h	4
B2M	Beta-2-microglobulin	House Keeping	180 s, 6 h	-10

The distribution of these genes in different subgroups is depicted in figure 11. Most genes, which were changed due to plasma treatment, are signaling molecules and encode for growth factors or inflammatory cytokines and chemokines. But also genes, responsible for ECM and cell adhesion, were changed by plasma treatment. One signal transducing gene and one house keeping gene was also changed.

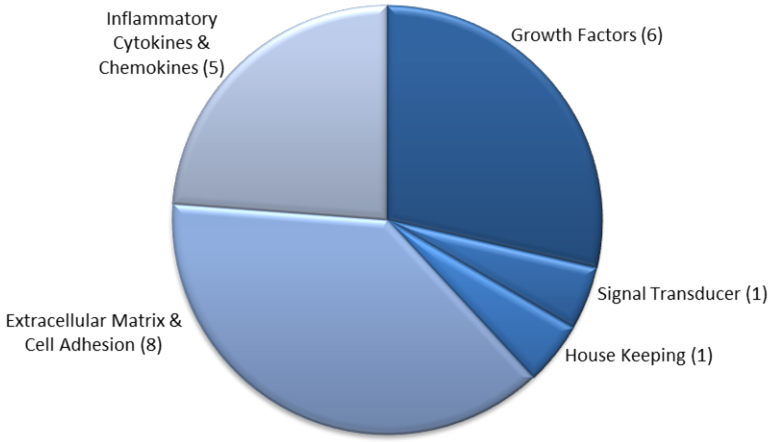


Figure 11: Distribution of the significantly changed genes from table 1. Numbers represent the total number of genes for each subgroup.

As shown in table 1 Cadherin 1 is a protein belonging to the extracellular matrix and after plasma treatment it was both down and up regulated. The integrins ITGA5 and ITGB6 also became up regulated due to plasma treatment. Furthermore, the transcription of various growth factors like VEGF-A, GM-CSF or HB-EGF was activated post plasma treatment. The gene VEGFA, which encodes the growth factor VEGF-A was changed within the first six hours after treatment. Both 120 s and 180 s plasma treatment induced a fourfold up regulation of the gene expression. The HaCaT cells also

increased the expression of CSF2, the gene which encodes GM-CSF, after plasma treatment. Twelve hours post treatment a sixfold up regulation after 120 s treatment and a sevenfold up regulation after 180 s was observed. For the short incubation time of 6 hours and a plasma treatment duration of 180 s the gene expression was 13-fold increased, whereas 120 s plasma treatment did not change the expression rate of CSF2. Another growth factor, which was regulated by plasma is HB-EGF. After the first six hours this gene showed a sixfold and twelvefold up regulation for 120 s and 180 s of treatment. After 12 h incubation, this up regulation vanished and 180 s of treatment even lead to a minor decrease of gene expression. Another growth factor is angiopoietin-1, which expression rate was rapidly declined within the first six hours after plasma treatment. Besides the growth factors also the gene expressions of cytokines were influenced by plasma. Three of them were chemokines (CCL2, CXCL1, CXCL2) and two of them interleukins (IL-1β, IL-6). The gene which encodes IL-6 was up regulated after both treatments of each incubation time (6 and 12 hours). And for 180 s the gene expression was exceedingly increased (29- and 21-fold) at both times.[5] Prostaglandin-endoperoxide synthase 2 was sevenfold up regulated after 180 s treatment and 6 hours incubation, whereas for 120 s no significant changes were detected. After the longer incubation time (12 hours) both samples (120 and 180 s) did not show any changes in regulation.

The genes (VEGFA, CSF2, IL6, HBEGF and PTGS2) which encode the proteins VEGF-A, GM-CSF, IL-6, HB-EGF and PTGS2 were separately detected by qPCR. Besides the short and long plasma treatments, the cells were also treated with 100 µM H_2O_2. As depicted in figure 12, VEGFA was only up regulated by 180 s plasma treatment (2.4-fold) and H_2O_2 exposure (4.7-fold) after 6 hours incubation. CSF2, which encodes the protein GM-CSF, was significantly up regulated (2.7-fold) due to a plasma treatment of 180 s and an incubation time of 12 hours (fig. 13). A 2.1-fold up regulation of the gene PTGS2 could be observed at an incubation time of 6 hours after

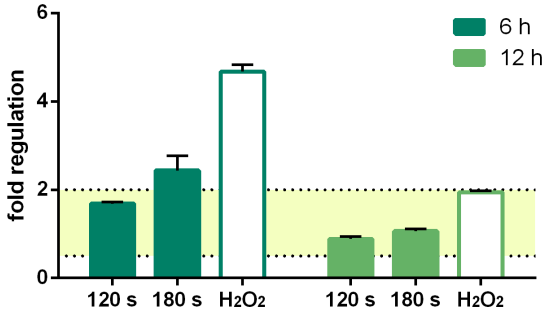


Figure 12: Changed gene expression of VEGFA after plasma or H_2O_2 (100 µM) treatment. The HaCaT cells were incubated for 6 or 12 hours. The data analysis was performed according to the $\Delta\Delta C_T$ method. Was a gene not twofold regulated (between 0.5 and 2; colored area) the gene was not significantly changed. A fold-regulation above 2 displays an up-regulation.

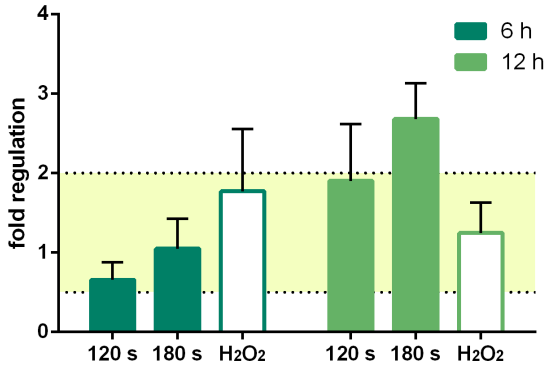


Figure 13: Changed gene expression of CSF2 (which encodes GM-CSF) after plasma or H_2O_2 (100 µM) treatment. The HaCaT cells were incubated for 6 or 12 hours. The data analysis was performed according to the $\Delta\Delta C_T$ method. Was a gene not twofold regulated (between 0.5 and 2; colored area) the gene was not significantly changed. A fold-regulation above 2 displays an up-regulation.

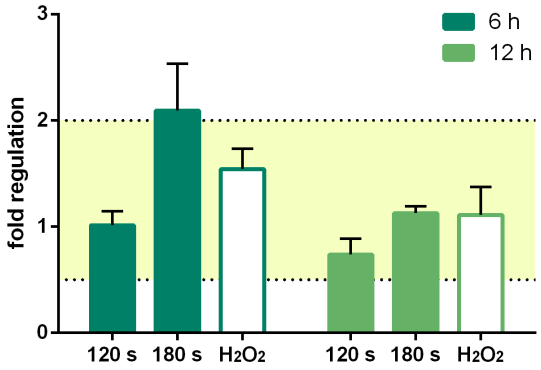


Figure 14: Changed gene expression of PTGS2 after plasma or H_2O_2 (100 µM) treatment. The HaCaT cells were incubated for 6 or 12 hours. The data analysis was performed according to the $\Delta\Delta C_T$ method. Was a gene not twofold regulated (between 0.5 and 2; colored area) the gene was not significantly changed. A fold-regulation above 2 displays an up-regulation.

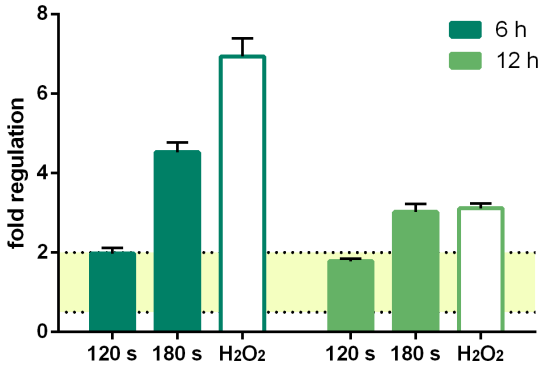


Figure 15: Changed gene expression of HBEGF after plasma or H_2O_2 (100 µM) treatment. The HaCaT cells were incubated for 6 or 12 hours. The data analysis was performed according to the $\Delta\Delta C_T$ method. Was a gene not twofold regulated (between 0.5 and 2; colored area) the gene was not significantly changed. A fold-regulation above 2 displays an up-regulation.

180 s plasma treatment (fig. 14). The gene encoding HB-EGF was significantly up regulated after 180 s plasma exposure and H_2O_2 treatment after 6 as well as 12 hours incubation time.
As shown in figure 15 the changed gene expression after 6 hours was more increased by the treatment with 100 µM H_2O_2, whereas after 12 hours both 180 s plasma and H_2O_2 induced a similar changed gene expression. The gene expression of IL6 was 2.3-fold increased after both 180 s plasma and H_2O_2 treatment at an incubation time of 6 hours, as depicted in figure 16. Twelve hours post treatment, the up regulation was significantly increased for 120 and 180 s plasma and H_2O_2 treatment, whereas H_2O_2 induced the highest increase.

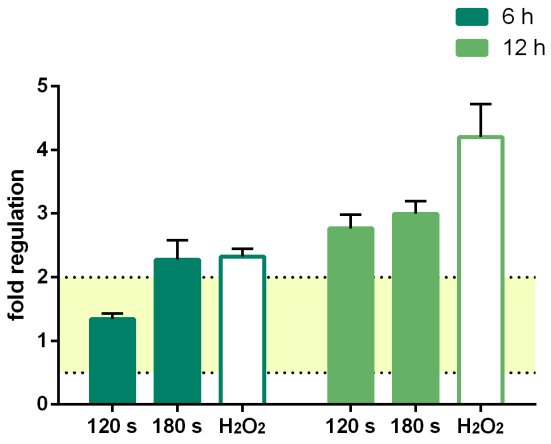


Figure 16: Changed gene expression of IL6 after plasma or H_2O_2 (100 µM) treatment. The HaCaT cells were incubated for 6 or 12 hours. The data analysis was performed according to the $\Delta\Delta C_T$ method. Was a gene not twofold regulated (between 0.5 and 2; colored area) the gene was not significantly changed. A fold-regulation above 2 displays an up-regulation.

3.1.3 Secretion profile

Due to the changed gene expression of various growth factors and cytokines the secretion profile of plasma treated HaCaT keratinocytes was investigated by ELISA. The release of VEGF-A, an angiogenesis promoting growth factor, was detected 6, 12 and 24 hours post treatment (fig. 17). A treatment with 20

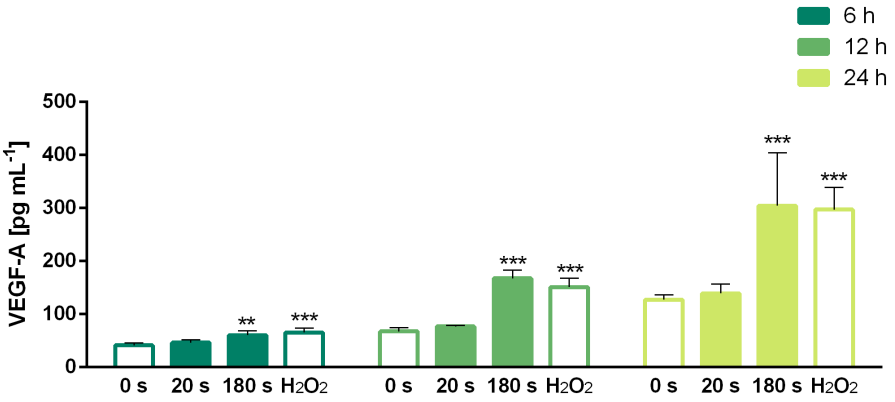


Figure 17: Secretion of VEGF-A by keratinocytes 6, 12 or 24 hours post treatment. The treatments were either, 20 s or 180 s plasma, 100 µM H_2O_2 or non treatment. It was repeated three times in independent experiments and measured with each technical duplicates.Bars and error bars represent mean and SD. Statistical analysis was performed with Dunnett's test as follow up by one-way ANOVA. Significance levels: $\alpha = 0.001$ (***) and $\alpha = 0.01$ (**).

s plasma did not induce a significantly changed protein secretion compared to the untreated cells for each incubation time (6, 12 and 24 h). Upon plasma treatment for 180 s, the release of VEGF-A significantly rose for all incubation times. The amount of secreted VEGF-A doubled between 6 and 12 hours, after 24 hours the amount doubled again. Interestingly, the level of secreted VEGF-A also increased by untreated cells within time. The cells, treated with 100 µM H_2O_2 behaved similar as the cells treated with 180 s plasma.

A significantly increased secretion of the granulocyte macrophage colony-stimulating factor was detected 6 hours post 180 s plasma treatment (fig. 18). The treatment with H_2O_2 also induced a slight increase, yet not a significant one. A exposure of 20 s treated medium did not influence the release of GM-CSF 6 hours later and after 12 or 24 hours the secretion was even decreased in comparison to the untreated control cells. The level of secreted GM-CSF rose with the incubation time of 6, 12 or 24 hours in untreated cells (fig. 18). The cells, treated for 20 s with plasma also increased the level of secreted GM-CSF with time, but in comparison to untreated cells they were even decreased after 12 and 24 hours. In contrast 180 s treated cells significantly increased the secretion of GM-CSF 6 and 12 hours after exposure, whereas after 24 hours the secretion returned to the baseline level. Hydrogen peroxide did not induce a significantly changed protein secretion, but after six hours a slight increase was detectable.

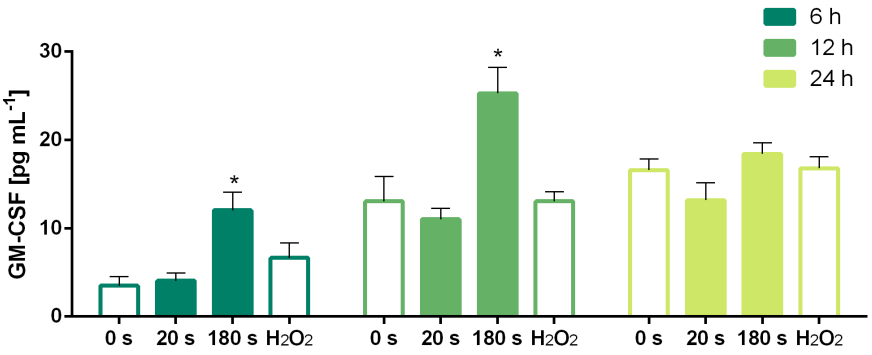


Figure 18: Secretion of GM-CSF by keratinocytes 6, 12 or 24 hours post treatment. The treatments were either, 20 s or 180 s plasma, 100 µM H_2O_2 or non treatment. It was repeated three times in three independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analysis was performed with Dunnett's test as follow up by one-way ANOVA. Significance levels: $\alpha = 0.05$ (*), $\alpha = 0.001$ (***).

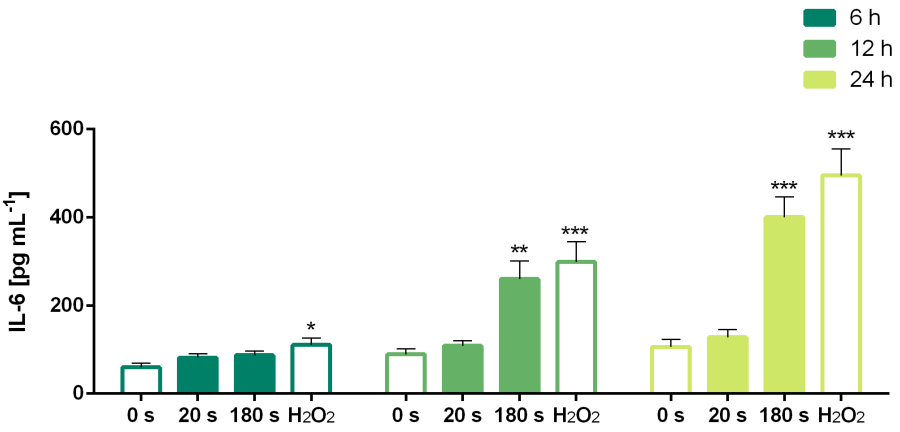


Figure 19: Secretion of IL-6 by keratinocytes 6, 12 or 24 hours post treatment. The treatments were either, 20 s or 180 s plasma, 100 µM H_2O_2 or non treatment. It was repeated three times in three independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analysis was performed with Dunnett's test as follow up by one-way ANOVA. Significance level: $\alpha = 0.05$ (*).

Besides that, an increase of secreted IL-6 after plasma or H_2O_2 treatment was observed 6, 12 and 24 hours later, illustrated in figure 19. However, a treatment of 20 s induced only a slight increase which was not significant at any incubation time. The long term plasma treatment induced a significant rise after 12 and 24 hours and the treatment with 100 µM H_2O_2, which was higher than 180 s response in all cases, even showed an increase after 6 hours. The release of the cytokine IL-8 by HaCaTs after plasma treatment is shown in figure 20. A secretion was detected 6, 12 and 24 hours after treatment, but only after 6 hours a significant change could be measured. Here, the longer the plasma treatment, the more secreted IL-8, while 100 µM H_2O_2 induced the highest amount of released cytokine.
Several other cell signaling molecules were analyzed for secretion after plasma treatment. Most of them were not released by the investigated HaCaT keratinocytes, those are listed in table 2 and can be classified into growth factors

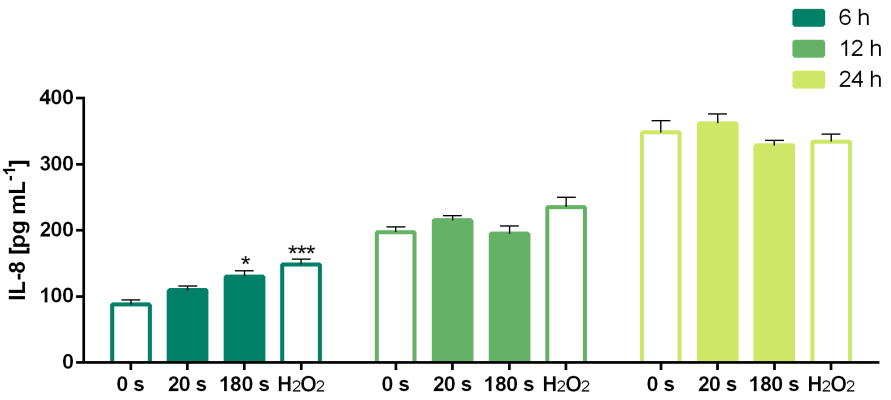


Figure 20: Secretion of the cytokine IL-8 by keratinocytes 6, 12 or 24 hours post treatment. The treatments were either, 20 s or 180 s plasma, 100 µM H_2O_2 or non treatment. It was repeated three times in three independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analysis was performed with Dunnett's test as follow up by one-way ANOVA. Significance levels: $\alpha = 0.05$ (*), $\alpha = 0.01$ (**), $\alpha = 0.001$ (***).

(5) and cytokines (10). These mediators can be either pro-inflammatory or anti-inflammatory, but some could also be both (e.g. IL-10).

Table 2: Cell signaling molecules, which were neither detectable nor regulated by plasma treatment. If these molecules are pro- or anti-inflammatory is labeled with "+" or "-". "n.c" and "n.d." stands for "not changed" or "not detectable" mediators.

short protein name	protein name	group	+/ -	n.c./ n.d.
IL-1α	interleukin-1 alpha	cytokine	+	n.d.
IL-1β	interleukin-1 beta	cytokine	+ -	n.d.
IL-2	interleukin-2	cytokine	+	n.d.
IL-4	interleukin-4	cytokine	+ -	n.d.

short protein name	protein name	group	+/ -	n.c./ n.d.
IL-10	interleukin-10	cytokine	+ -	n.d.
IL-12	interleukin-12	cytokine	+	n.d.
IL-17α	interleukin-17 alpha	cytokine	+	n.d.
IFN-γ	interferon gamma	cytokine	+ -	n.d.
TGF-β1	transforming growth factor beta 1	cytokine	+ -	n.c.
TNF-α	tumor necrosis factor alpha	cytokine	+ -	n.c.
EGF	epidermal growth factor	growth factor	-	n.c.
HB-EGF	heparin-binding EGF-like growth factor	growth factor	-	n.d.
FGF-2	basic fibroblast growth factor	growth factor	-	n.c.
PDGF-BB	platelet-derived growth factor-BB	growth factor	-	n.d.
NGF-b	nerve growth factor	growth factor	-	n.d.

3.2 Impact of the shielding gas

During plasma treatment oxygen and nitrogen from the surrounding ambience can diffuse into the plasma effluent which leads to a generation of ROS and RNS. The shielding gas device was used to control this generation. The shielding gas was a mixture of oxygen and nitrogen which was ranged from pure oxygen to pure nitrogen while the core plasma remains unchanged. With these treatments the impact of reactive species classes, such as ROS, RNS or RONS, generated by plasma could be correlated with the cell responses. Therefore, the impact on cell survival was studied first. Furthermore, the transcriptome as studied and the secretion of defined proteins was investigated.

3.2.1 Cell survival

The influence of five different shielding gas mixtures on the HaCaT cells was investigated with the cytotoxicity assay. As shown in figure 21, the amount of dead cells did not significantly increase within 20 s plasma treatment for each shielding gas composition. With increasing amount of oxygen in the

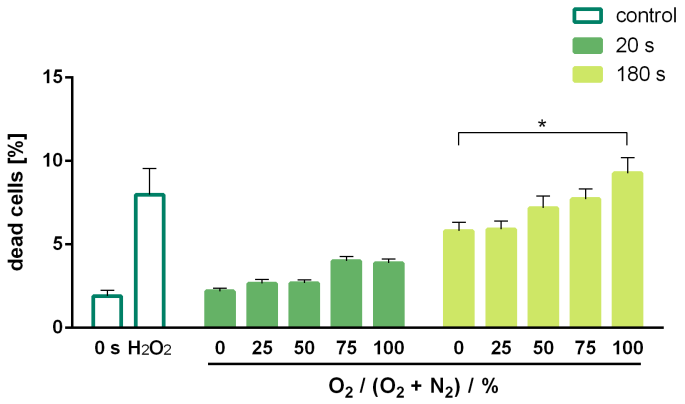


Figure 21: Cytotoxicity of plasma in combination with a shielding gas. The cells were either treated with plasma or 100 µM H_2O_2. The x-axis indicates the O_2 amount of the O_2 to N_2 shielding gas mixture in percentage. Bars and error bars are presented in mean and SD. Statistically analysis: Tukey's multiple comparisons test as follow up for one-way ANOVA. Three independent experimental repetitions with technical triplicates were performed (significance level: $\alpha = 0.05$ (*)) .

shielding gas the cytotoxicity of plasma rose (2.2 to 3.9 %), but also 1.9 % of the untreated control cells were dead. In case of 180 s plasma treatment, the correlation of the cytotoxicity to oxygen was more pronounced. The plasma surrounded by pure oxygen was significantly more cytotoxic (9.3 % dead cells) than a plasma surrounded by pure nitrogen (5.8 % dead cells). The treatment with 100 µM H_2O_2 induced a similar cytotoxicity as 180 s plasma treatment with a high rate of oxygen in the shielding gas. In addition, the caspase-3 activity after plasma treatment in combination with a shielding device was analyzed and is illustrated in figure 22. The cells, treated for

20 s with plasma, showed for each shielding gas a caspase-3 activity of 6.5 %, similar to the untreated cells (6.1 %). However, a treatment of 180 s induced a caspase-3 activity increase of 20.4 % with each shielding gas with the exception of a pure nitrogen shielding gas (12.5 %). The treatment of 100 µM H_2O_2 let the cells activate caspase-3 for 18 %.

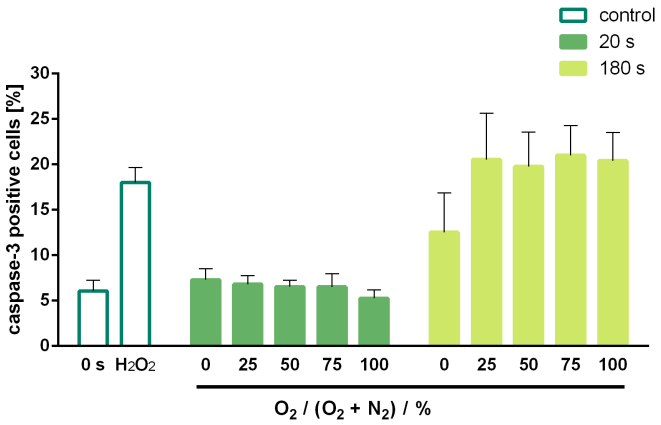


Figure 22: Caspase-3 activity after plasma treatment with a shielding gas. The cells were either treated with plasma in combination of a shielding gas or with 100 µM H_2O_2. The x-axis indicates the O_2 amount of the O_2 to N_2 shielding gas mixture in percentage. Bars and error bars are presented in mean and SD. Three independent experimental repetitions with technical triplicates were performed.

3.2.2 Transcription profile

A cytokine and growth factor profile at transcription and secretion level was also made after treating the HaCaT keratinocytes with plasma in combination with the shielding device. First, a DNA microarray was conducted. The heat map (fig. 23) represents all genes which were significantly (at least twofold) up (blue) or down (red) regulated in at least one treatment. The genes are plotted horizontally and genes, which were unchanged are white. As shown in figure 23, the shielding gas surrounding the plasma effluent had an

influence on the transcriptome of the investigated keratinocytes. The number of regulated genes varied by treatments with different gas mixtures. A pure oxygen gas induced the highest change in the transcriptome, 539 genes were changed after 180 s plasma treatment, whereas a gas mixture of 25 % O_2 and 75 % N_2 changed only 42 genes after the same treatment duration. Mainly, the longer the treatment duration, the higher the amount of changed genes. But for the treatment at which the ratio of oxygen and nitrogen was equal, most changed genes were detected for 20 s (202 genes) and not 180 s (171 genes). The heat map (fig. 23) visualizes that genes were usually either up or down regulated for all conditions and while there were only two genes which were both up and down regulated for different conditions. Furthermore, there is no gene for which the gene expression was changed for all investigated treatment conditions. Some genes were only changed due to a long treatment time independent of the shielding gas composition. Shielding gas with 25 % oxygen or 25 % nitrogen induced a reduction of changed gene expression: Less than 100 changed genes were found for both a short and long treatment time. But also a pure oxygen shielding gas changed only 22 genes for a short treatment time, whereas the long treatment duration regulated 539 genes. This strong difference between the short and long treatment time could not be observed for all shielding gas compositions. For example, 20 and 180 s at both 0 % and 50 % O_2 in the gas mixture induced a changed gene expression of a similar amount of genes, whereas these genes were not in both treatment times identical.

PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System, a open-source online analysis software, was used to classify the genes into subgroups of biological processes. These genes can also belong to more than one subgroup. Figure 24 displays these pie charts for both treatment times (20 and 180 s) with pure nitrogen as shielding gas. For both 20 and 180 s treatment the biggest subgroups were cell communication, cellular process and metabolic process. After a treatment of 20 s 39 genes

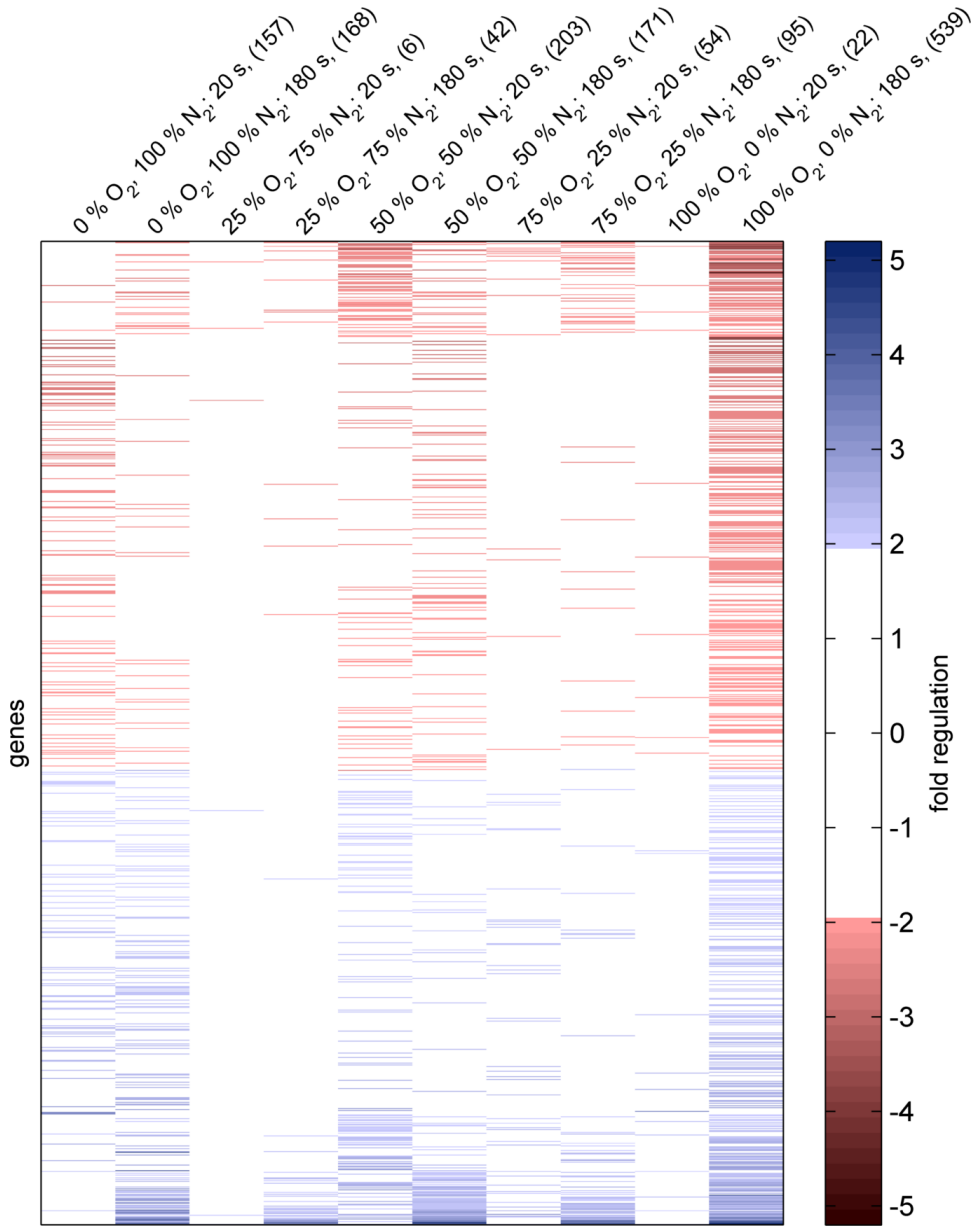


Figure 23: Heat map illustrates the fold regulation of all genes, which were significantly up (above 2; blue), down (below -2; red) or not (white) regulated by plasma treatment. The cells were treated either for 20 or 180 s. The amount of O_2 and N_2 were in percent and the numbers in brackets represent the number of significantly changed genes.

belong to cell communication and after 180 s 48 genes were part of this subgroup. The plasma treatment in combination with shielding gas mixture containing 25 % oxygen and 75 % nitrogen changed 6 genes after a short and 42 genes after a long treatment duration. After 180 s treatment most regulated genes belong to the subgroups metabolic process, cellular process and cell communication. This distribution was also found for both treatment times by using the shielding gas with 50 % oxygen and 50 % nitrogen (fig. 26). Keratinocytes treated with plasma and an oxygen dominated shielding gas mixture (75 % O_2 and 25 % N_2) showed a similar profile (fig. 27). But after 180 s more genes were responsible for metabolic process than cell communication, whereas after 20 s it was the other way around. A pure oxygen shielding induced the highest rate of changed genes for the long treatment time. Most of the 539 genes were a part of the subgroup metabolic process (fig. 28).

Cell signaling molecules like growth factors and cytokines belong to the groups cellular process and cell communication and selected molecules were subsequently analyzed by qPCR and ELISA for validation. Therefore the cells were treated with plasma in combination with the 5 different shielding gas compositions and after 6 hours incubation time the gene expression profile was analyzed. One gene was VEGFA and the changed gene expression is illustrated in figure 29. A short treatment time did not induce an up or down regulation of VEGFA, but a 180 s treatment did. In addition the shielding gas composition had a significant influence on the gene expression. A pure nitrogen or pure oxygen shielding gas resulted in a 2.7-fold up regulation of VEGFA, whereas a mixture of 25 % oxygen and 75 % nitrogen decreased the gene expression (2.1-fold). The highest increase was reached with the oxygen dominated shielding gas mixture (75 % O_2, 25 % N_2), VEGFA was 3.6-fold up regulated. A 3.1-fold changed gene expression was observed for the plasma treatment with a shielding gas consisting of same ratio of oxygen and nitrogen. The gene CSF2 behaved similar as VEGFA, as shown in figure

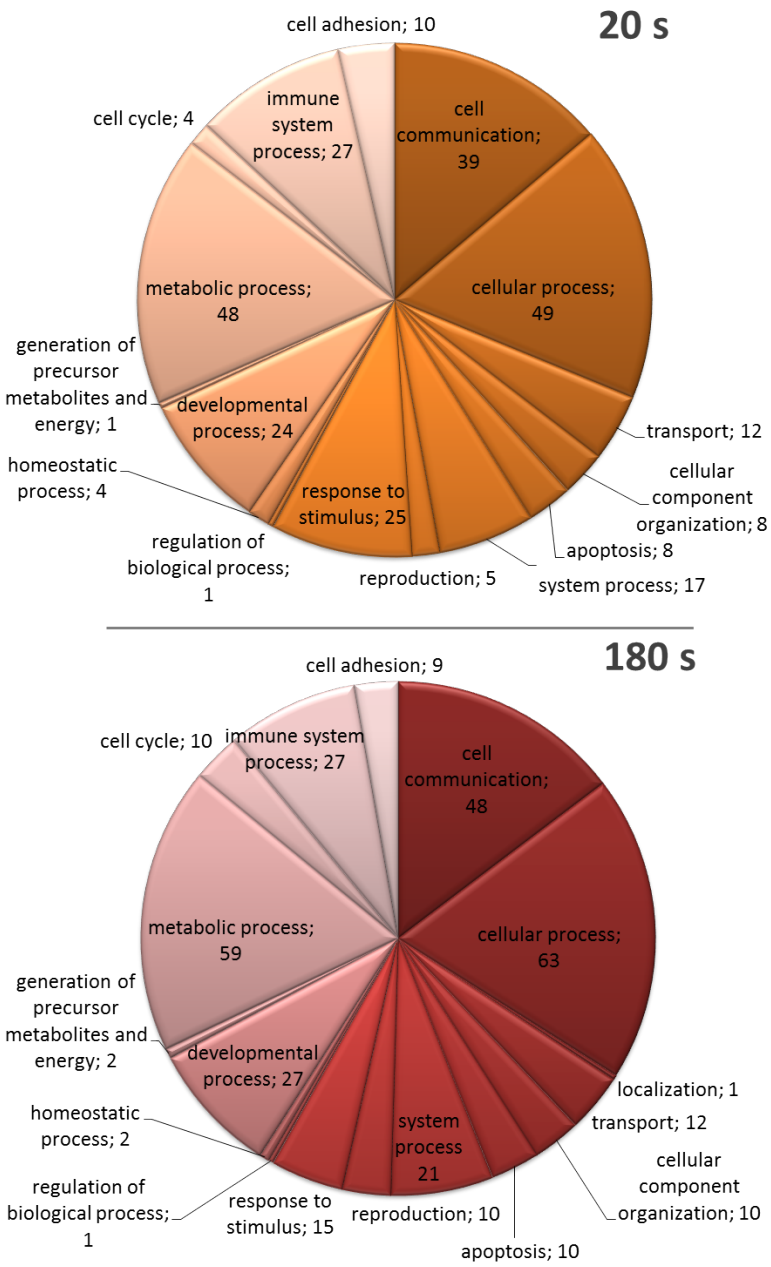


Figure 24: Biological process subgroups, classified by PANTHER Classification System, of the genes which where significantly changed after 20 s or 180 s treatment with a shielding gas mixture of 0 % O_2 and 100 % N_2. In brackets are the numbers of genes.

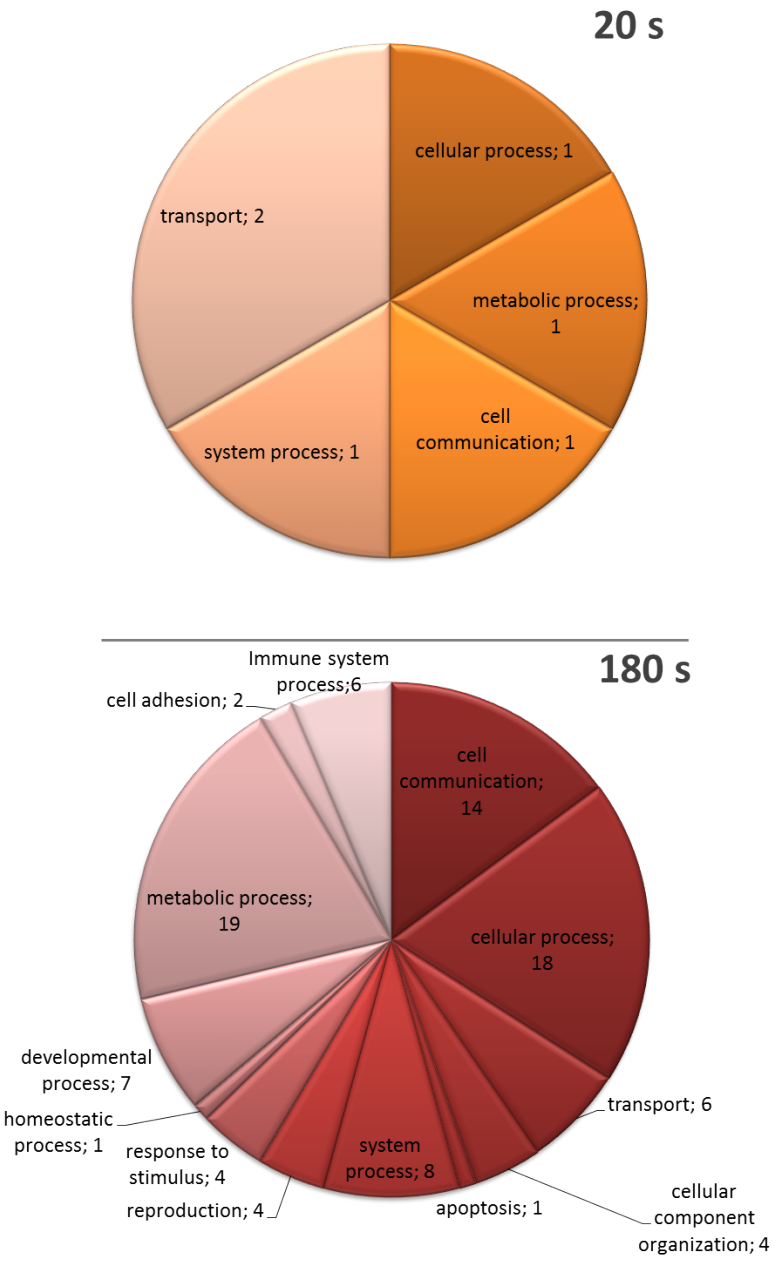


Figure 25: Biological process subgroups, classified by PANTHER Classification System, of the genes which where significantly changed after 20 s or 180 s treatment with a shielding gas mixture of 25 % O_2 and 75 % N_2. In brackets are the numbers of genes.

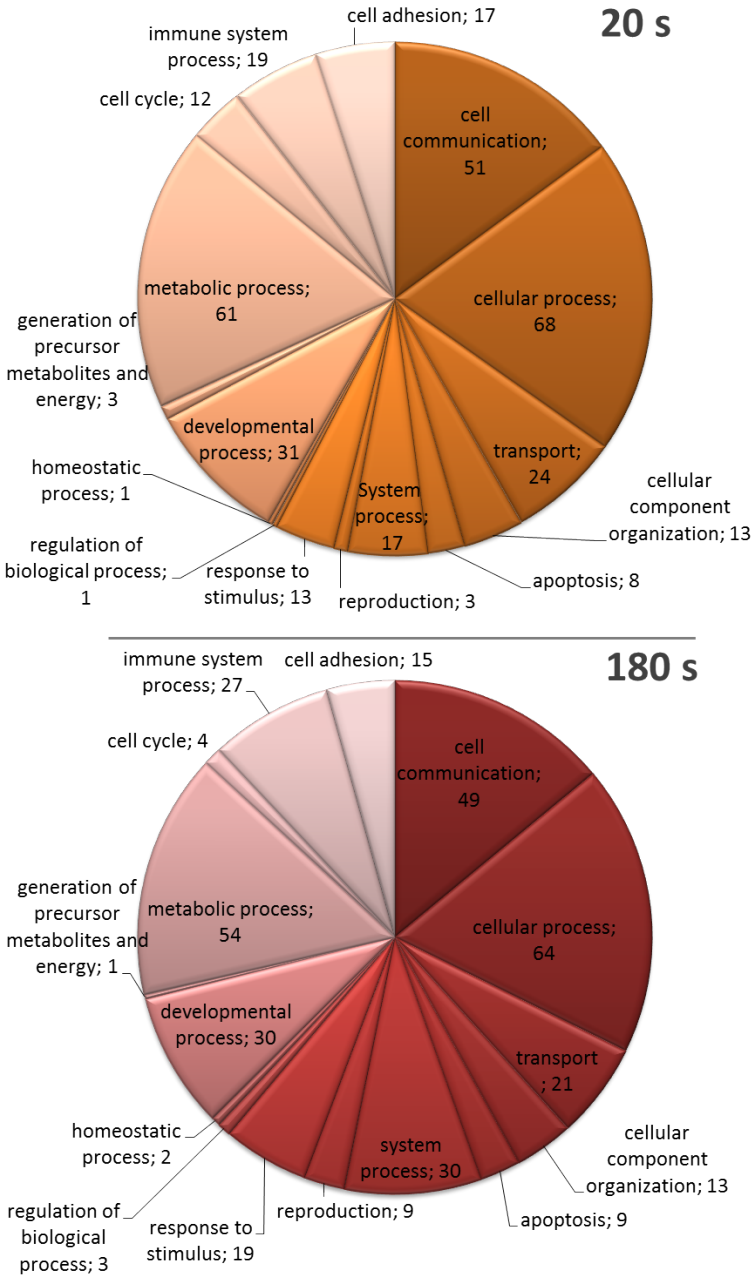


Figure 26: Biological process subgroups, classified by PANTHER Classification System, of the genes which where significantly changed after 20 s or 180 s treatment with a shielding gas mixture of 50 % O_2 and 50 % N_2. In brackets are the numbers of genes.

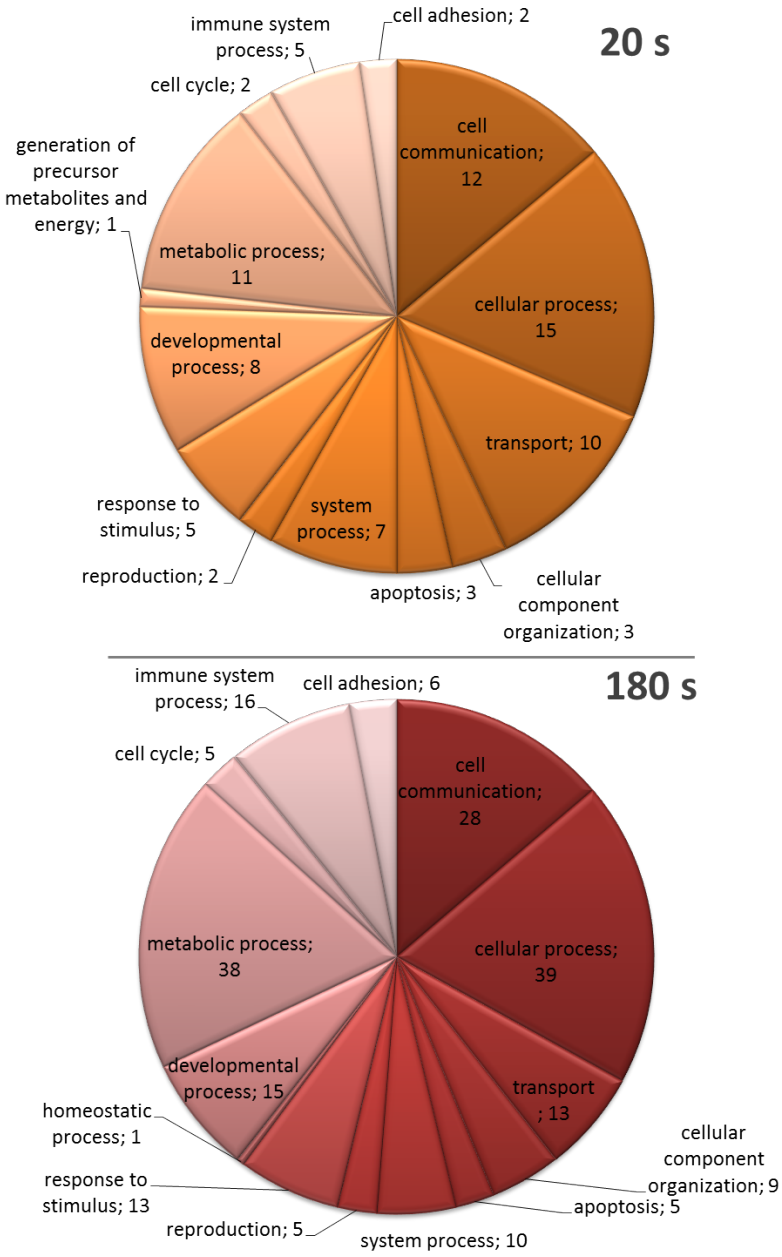


Figure 27: Biological process subgroups, classified by PANTHER Classification System, of the genes which where significantly changed after 20 s or 180 s treatment with a shielding gas mixture of 75 % O_2 and 25 % N_2. In brackets are the numbers of genes.

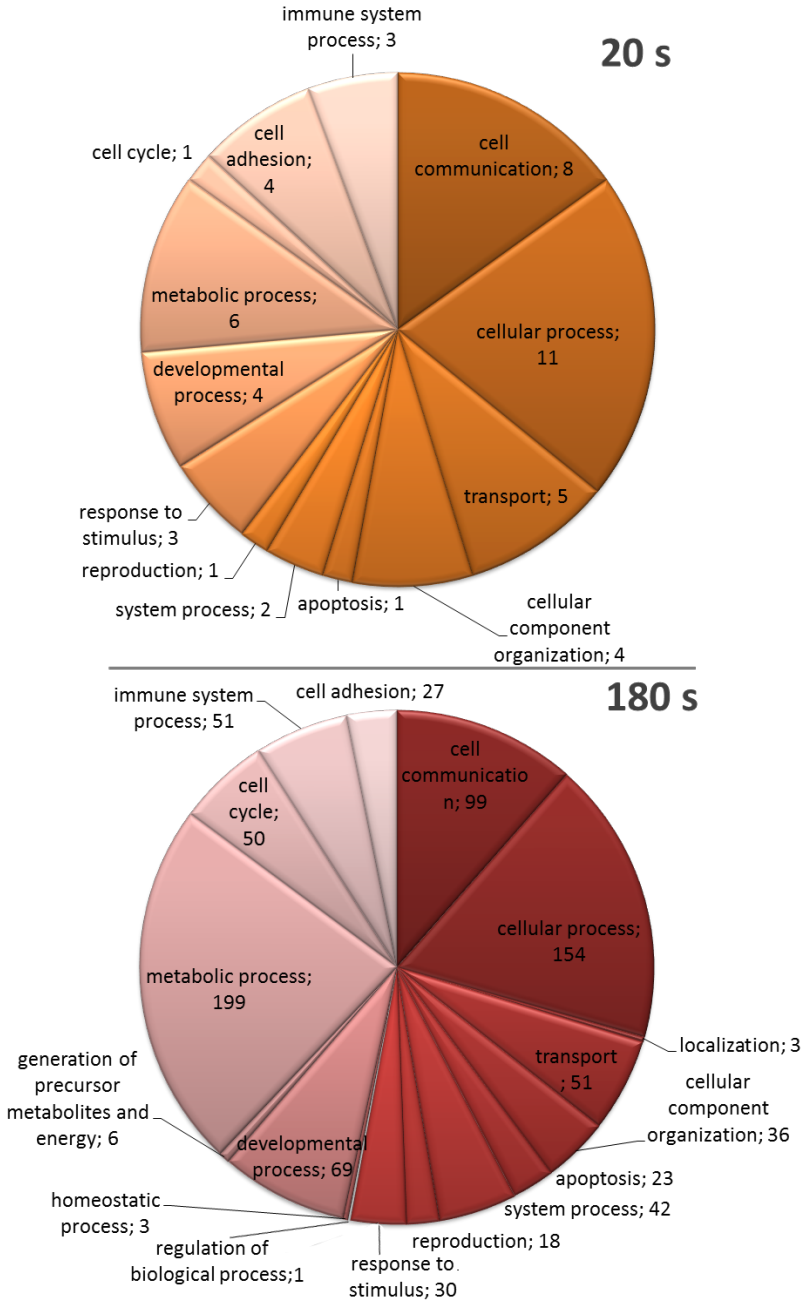


Figure 28: Biological process subgroups, classified by PANTHER Classification System, of the genes which where significantly changed after 20 s or 180 s treatment with a shielding gas mixture of 100 % O_2 and 0 % N_2. In brackets are the numbers of genes.

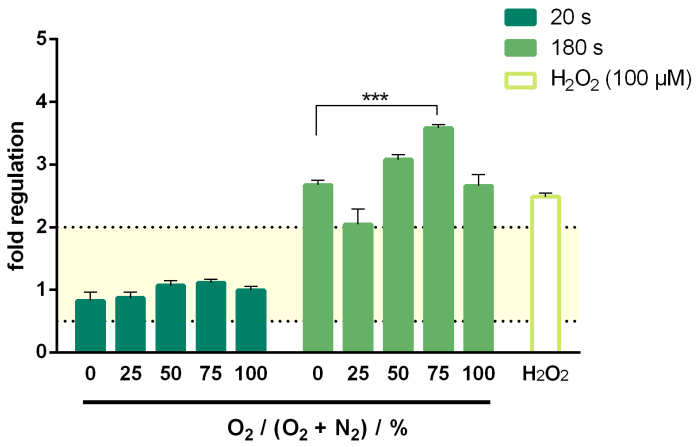


Figure 29: Fold regulation of the gene VEGFA, 6 h after plasma treatment. Detected via qPCR. Values in the colored area were not significantly changed (above 2: up regulated; below 0.5: down regulated). Further analysis: Tukey's multiple comparisons test as a follow up for one-way ANOVA. (significance level: $\alpha = 0.001$ (***)). Bars and error bars are mean and SD.

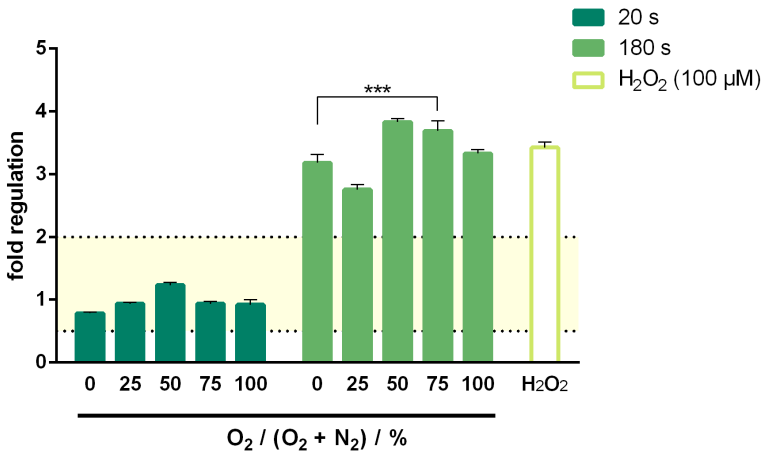


Figure 30: Fold regulation of the gene CSF2, 6 h after plasma treatment was detected via qPCR. Values in the colored area were not significantly changed (above 2: up regulated; below 0.5: down regulated). Further analysis:Tukey's multiple comparisons test as a follow up for one-way ANOVA. (significance level: $\alpha = 0.001$ (***)) . Bars and error bars were mean and SD.

30. The gene expression was not changed after a short but after long treatment time. Again, a pure nitrogen or oxygen shielding gas induced a similar

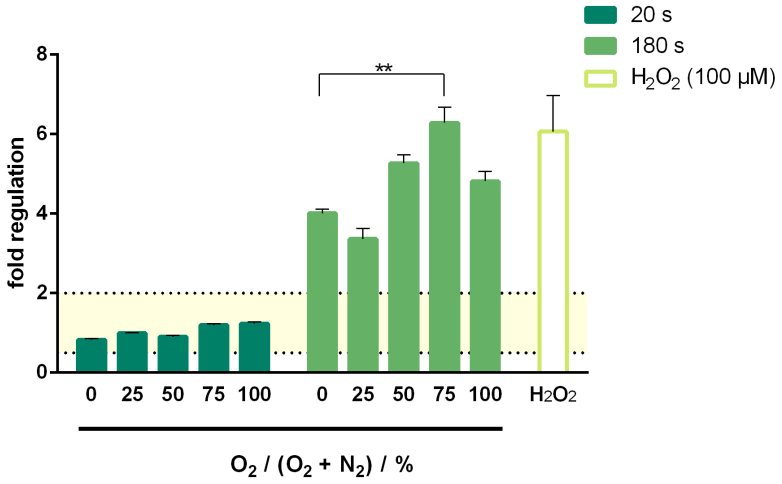


Figure 31: Fold regulation of the gene HBEGF, 6 h after plasma treatment. Detected via qPCR. Values in the colored area were not significantly changed (above 2: up regulated; below 0.5: down regulated). Further analysis: Tukey's multiple comparisons test as a follow up for one-way ANOVA. (significance level: $\alpha = 0.01$ (**)). Bars and error bars are mean and SD.

up regulation (3.2- or 3.3-fold) and the minimum was at 25 % O_2 and 75 % N_2 (2.8-fold). A considerable increase of the gene expression was observed for a gas mixture with 50 and 75 % oxygen (3.8- and 3.7-fold). Another gene, which was analyzed by qPCR was HBEGF. Its expression was significantly changed due to a long but not a short treatment duration. The slight increase (3.4-fold) was detected at a treatment with a shielding gas containing 25 % O_2 and 75 % N_2. The maximum was at 75 % O_2 and 25 % N_2 (6.3-fold), which was similar to the hydrogen peroxide treated cells (6.1-fold). A pure nitrogen, pure oxygen and equal amounts of oxygen and nitrogen in the gas mixtures induced a 4-, 4.8- and 5.3-fold up regulated HBEGF. Besides that, the gene expression of IL6 was studied (fig. 32). The shielding gases with 0 %, 25 % or 50 % oxygen induced a slight up regulation of IL6, whereas the

oxygen dominated gas mixtures (75 % and 100 % O_2) rapidly increased the IL6 expression.

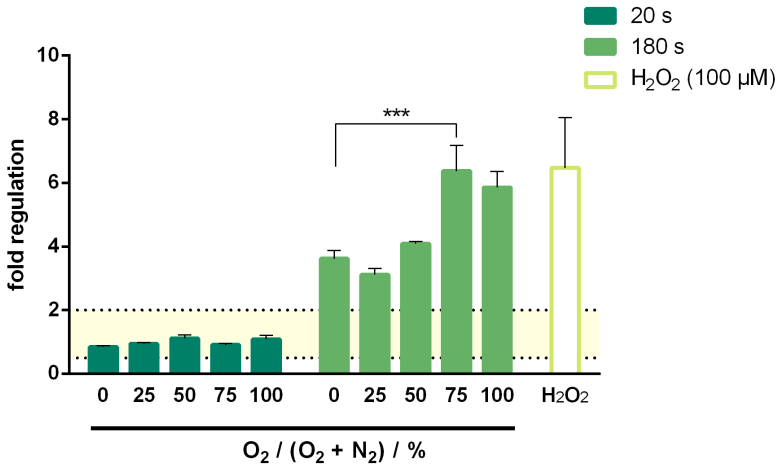
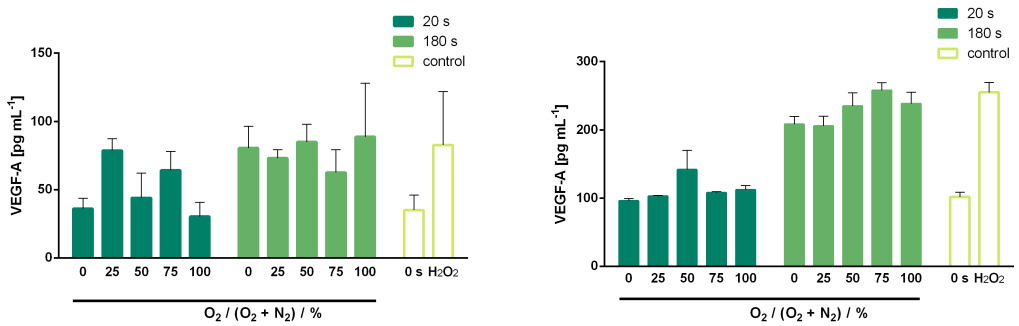


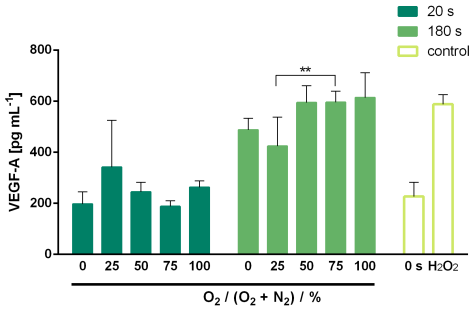
Figure 32: Fold regulation of the gene IL6, 6 h after plasma treatment. Detected via qPCR. Values in the colored area were not significantly changed (above 2: up regulated; below 0.5: down regulated). Further analysis: Tukey's multiple comparisons test as a follow up for one-way ANOVA. (significance level: $\alpha = 0.001$ (***)). Bars and error bars are mean and SD.

3.2.3 Secretion profile

The gene expressions of VEGFA, CSF2, HBEGF and IL6 were significantly changed due to plasma treatment in combination with a shielding gas. Following the secretion of the proteins encoded by these genes was investigated with ELISA. The secretion of VEGF-A was detected 6, 12 and 24 hours post plasma treatment. As shown in figure 33a the release of VEGF-A was enhanced only after a 20 s treatment in combination of a shielding gas containing 25 or 75 % oxygen 6 hours later. The amount of VEGF-A in the medium was increased by treatments for 180 s and an additional influence of the shielding gases was not observed. The cells treated with plasma and a shielding gas containing 75 % O_2 showed a lower increase of VEGF-A. The



(a) VEGF-A release 6 h post plasma treatment.(b) VEGF-A release 12 h post plasma treatment.

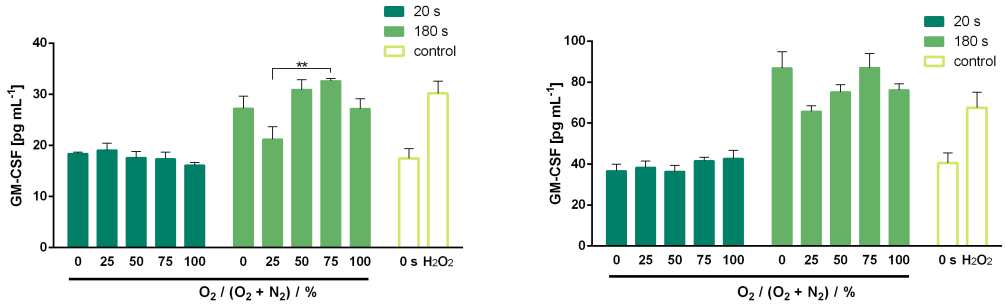


(c) VEGF-A release 24 h post plasma treatment.

Figure 33: Secretion of VEGF-A 6, 12 or 24 h after plasma treatment, measured by ELISA. The keratinocytes were treated with H_2O_2 (100 µM) or plasma in combination of a shielding gas. Bars and error bars are mean and SD. Statistical analysis: Tukey's multiple comparisons test as a follow up for one-way ANOVA. Four experimental repetitions and technical triplicates were measured (significance level: $\alpha = 0.01$ (**)).

secretion of VEGF-A was also measured 12 hours post plasma treatment (fig. 33b). Due to a short treatment time the keratinocytes did not increase the secretion, but for a long time they did. The more oxygen in the gas mixture, the more release of VEGF-A after 180 s treatments. However, these changes were not significant. The treatment with hydrogen peroxide enhanced the VEGF-A release similar to the maximum which was reached by plasma treat-

ment. The secretion of VEGF-A 24 hours after treatment is illustrated in figure 33c. A 20 s treatment enhanced the release only for a shielding gas mixture of 25 % O_2 and 75 % N_2, but due to the error bars this increase was negligible. A long plasma treatment induced increases of the secretion for all shielding gases, whereas 50, 75 or 100 % oxygen in the shielding gas caused the highest ratio of VEGF-A release. The slightest increase was observed at 25 % oxygen, which was significantly higher than the oxygen dominated gas mixtures.

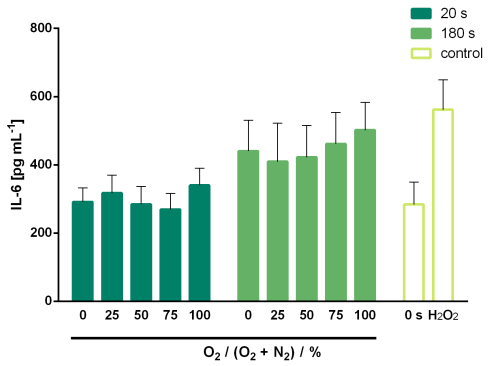


(a) GM-CSF release 6 h post plasma treatment. (b) GM-CSF release 24 h post plasma treatment.

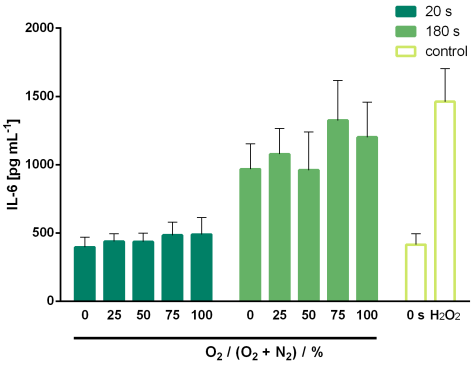
Figure 34: Secretion of GM-CSF 6 and 24 h after plasma treatment, measured by ELISA. The keratinocytes were treated with H_2O_2 (100 µM) or plasma in combination of a shielding gas. Bars and error bars are mean and SD. Statistical analysis: Tukey's multiple comparisons test as a follow up for one-way ANOVA. Four experimental repetitions and technical triplicates were measured (significance level: $\alpha = 0.01$ (**)).

Another stimulating factor which was analyzed, is GM-CSF. The secretion was detected 6 and 24 hours after plasma treatment and is illustrated in figure 34. At both incubation times the short treatment duration did not influence the release of GM-CSF compared to the untreated cells, independent of the shielding gas used. At a plasma treatment time of 180 s the secretion was increased. After an incubation time of 6 hours (fig. 34a), GM-CSF was

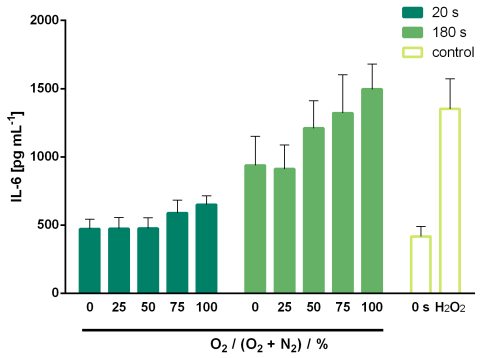
secreted the most due to a shielding gas mixture containing 75 % O_2 and 25 % N_2 (32.6 pg mL^{-1}) , whereas lowest release was detected after the treatment with 25 % O_2 and 75 % N_2 (21.2 pg mL^{-1}). Pure nitrogen or oxygen shielding gas influenced the GM-CSF secretion in the same manner (27.3 and 27.2 pg mL^{-1}) and a gas mixture with equal amounts of O_2 and N_2 induced a release of 30.6 pg mL^{-1}.



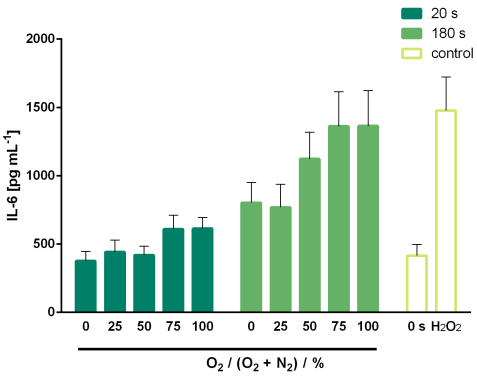
(a) IL-6 release 6 h post plasma treatment.



(b) IL-6 release 12 h post plasma treatment.



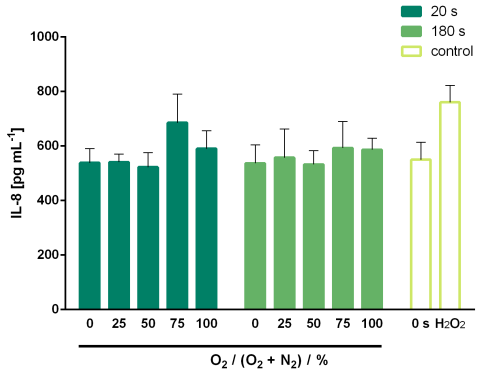
(c) IL-6 release 18 h post plasma treatment.



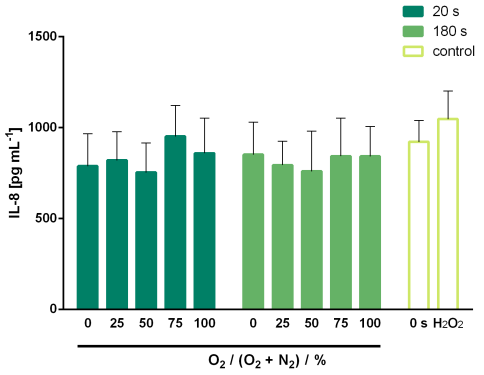
(d) IL-6 release 24 h post plasma treatment.

Figure 35: Secretion of IL-6 6, 12, 18 or 24 h after plasma treatment, measured by ELISA. The keratinocytes were treated with H_2O_2 (100 µM) or plasma in combination of a shielding gas. Bars and error bars are mean and SD. Four experimental repetitions and technical triplicates were measured.

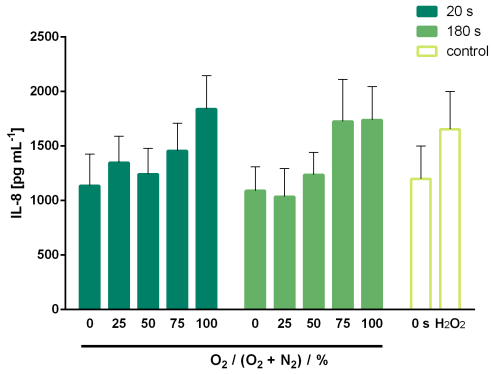
After 24 hours the behavior was similar. No changes compared to the untreated keratinocytes after a short but after a long plasma treatment were observed. Most GM-CSF was released by the keratinocytes due to plasma treatments with shielding gas mixtures consisting of pure nitrogen or 75 % O_2 and 25 % N_2. The GM-CSF concentrations in the cell culture medium were 86.8 and 87 pg mL^{-1} under that conditions.



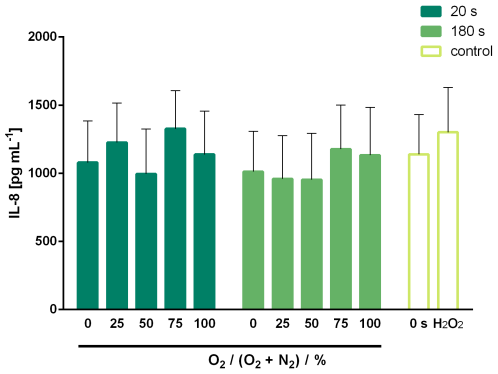
(a) IL-8 release 6 h post plasma treatment.



(b) IL-8 release 12 h post plasma treatment.



(c) IL-8 release 18 h post plasma treatment.



(d) IL-8 release 24 h post plasma treatment.

Figure 36: Secretion of IL-8 6, 12, 18 or 24 h after plasma treatment, measured by ELISA. The keratinocytes were treated with H_2O_2 (100 µM) or plasma in combination of a shielding gas. Bars and error bars are mean and SD. Four experimental repetitions and technical triplicates were measured.

A reduced release was detected with 50 or 100 % oxygen in the shielding gas mixture (75.2 and 76.2 pg mL^{-1}). Only 65.7 pg mL^{-1} secreted GM-CSF was measured due to a treatment with nitrogen dominated gas mixture (25 % O_2; 75 % N_2), which was similar as the hydrogen peroxide treated cells (67.5 pg mL^{-1}).

The effect of shielding gases during plasma treatment on the IL-6 was studied, too. Therefore, the keratinocytes were plasma treated and incubated for 6, 12, 18 or 24 hours (fig. 35). Within the first 12 hours the level of secreted IL-6 rose even for the untreated cells from 300 to 450 pg mL^{-1} subsequently it remained constant. The cells which were treated for 20 s with plasma showed no differences in secretion compared to control cells but after 18 and 24 hours it seemed that the oxygen dominated shielding gases (75 and 100 % O_2) induced an slight increase. The cells which were treated for 180 s released a higher amount of IL-6. After 6, 18 and 24 hours the more oxygen in the shielding gas, the more secreted IL-6. A treatment with 100 µM hydrogen peroxide let the keratinocytes release the most IL-6 exception to 18 h incubation time. After 12 hours, the maximum was reached by treatment with a shielding gas mixture of 75 % oxygen (1324 pg mL^{-1}) followed by a pure oxygen gas shielding (1202 pg mL^{-1}). Pure nitrogen and 50 % nitrogen made the cells release 968 and 960 pg mL^{-1} IL-6. An increased amount was found at 25 % oxygen (1077 pg mL^{-1}). Another cytokine which was analyzed 6, 12, 18 and 24 hours after plasma treatment was IL-8, illustrated in figure 36. Interestingly, the secretion of IL-8 was not changed due to a short or long plasma treatment. But the keratinocytes treated with 100 µM H_2O_2 slightly increased the release of IL-8 at all incubation times. Were the cells treated for 180 s and subsequently incubated for 18 hours an increase of secretion was observed for the shielding gas mixtures consisting of 75 or 100 % oxygen. But this behavior was additionally observed in the cells which were treated for 20 seconds only.

3.3 Impact of conditioned medium on HaCaT cells

For the treatments of wounds with plasma it is necessary to investigate the impact of secreted cell signaling proteins by plasma treated cells on untreated neighboring cells. For that reason a simplified *in vitro* experiment was developed. Medium was conditioned and modified by plasma treated cells which was then added on other cells.

As explained in paragraph 2.2.4 (fig. 7 on page 23) the keratinocytes were starved by FCS reduction of the cell culture medium for 24 hours and were then treated with medium which was taken from other keratinocytes. These cells were starved as well for 24 hours and additionally treated with plasma before their medium was transferred 24 hours later. The plasma treated and conditioned medium stayed for 6 hours on the cells and after incubation time the expression levels of defined genes were analyzed by qPCR (RT^2 Profiler PCR array). The keratinocytes changed the expression of 15 genes after treatment. A heat map (fig. 37) illustrates the fold regulation of the changed genes during the different treatments, which were either performed with plasma (20, 60, 180 s) or H_2O_2 (30, 100 µM). Interestingly, most genes were changed due to treatment with 100 µM H_2O_2 (11 genes) but also a 30 µM H_2O_2 treatment altered the expression of 7 genes in the investigated keratinocytes. The longest plasma treatment (180 s) influenced only one gene, whereas the shorter plasma treatments (20 and 60 s) changed 3 genes. The genes vitronectin (VTN) and colony stimulating factor 2 (CSF2), which encode the proteins vitronectin and GM-CSF, were the only genes which were changed at three different treatments. CSF2 was up regulated due to a 20 and 60 s plasma and 100 µM H_2O_2 treatment. However, VTN was up regulated after 30 µM H_2O_2 and 60 s plasma treatment, but 20 s induced a down regulation. Coagulation factor XIIIA1 polypeptide(F13A1) and WNT1 inducible signaling pathway protein 1(WISP1) were up regulated after both H_2O_2 treatments, whereas collagen, type V, alpha 3 (COL5A3) and chemokine (C-X-C motif) ligand 5 (CXCL5) were down regulated by

30 µM and up regulated by 100 µM H_2O_2. Collagen, type XIV, alpha 1 (COL14A1) was rapidly down regulated after 180 s plasma treatment and up regulated due to 100 µM H_2O_2. Wingless-type MMTV integration site family, member 5A (WNT5A) was also up regulated by the higher H_2O_2 concentration, and down regulated by 20 s plasma treatment. Table 3 contains the exact fold regulations, short gene names, gene names and functional gene grouping. From this follows that the most changed genes belong to the group extracellular matrix and cell adhesion (7 genes). Additionally, 4 growth factors were influenced, 2 inflammatory cytokines and chemokines and 2 signal transducers.

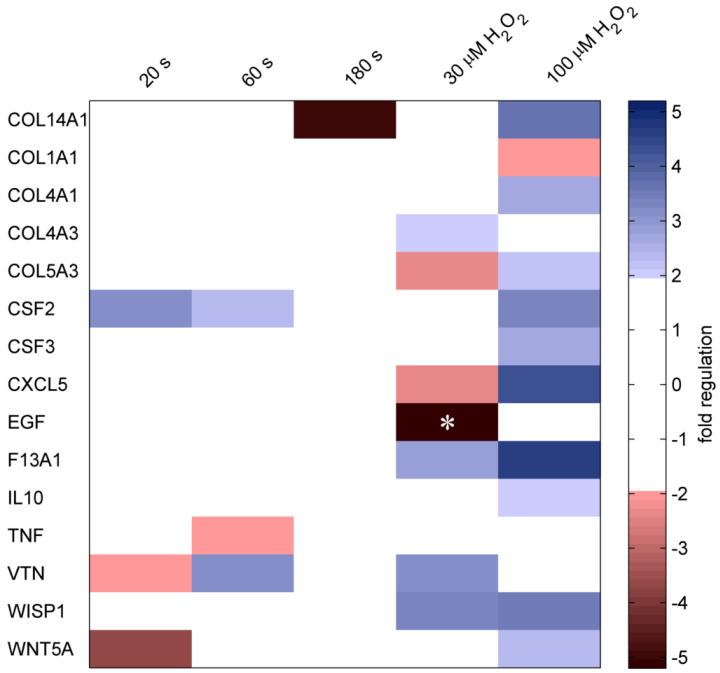


Figure 37: Heat map of the genes which were significantly up (above 2; blue) or down (below -2; red) regulated due to a treatment of starved HaCaT cells with plasma treated and conditioned medium. Every gene, which was not significantly changed is displayed in white. EGF was labeled with a star (*) because the regulation was less than -5 (-55.7).

Table 3: Genes which were significantly up (positive) or down (negative) regulated due to starving and plasma treated conditioned medium. Following abbreviations are used for the groups: ECM & Cell Adhesion (ECM & CA); Growth Factors (GF); Cytokine & Chemokine (C & C); Signal Transduction (ST)

short gene name	gene name	group	treatment	fold reg.
COL14A1	Collagen, type XIV, alpha 1	ECM & CA	180 s	-4.9
			100 µM H_2O_2	3.6
COL1A1	Collagen, type I, alpha 1	ECM & CA	100 µM H_2O_2	-2
COL4A1	Collagen, type IV, alpha 1	ECM & CA	100 µM H_2O_2	2.7
COL4A3	Collagen, type IV, alpha 3	ECM & CA	30 µM H_2O_2	2.1
COL5A3	Collagen, type V, alpha 3	ECM & CA	30 µM H_2O_2	-2.4
			100 µM H_2O_2	2.2
CSF2	Colony stimulating factor 2	GF	20 s	3.1
			60 s	2.3
			100 µM H_2O_2	3.4
CSF3	Colony stimulating factor 3	GF	100 µM H_2O_2	2.7
CXCL5	Chemokine (C-X-C motif) ligand 5	C & C	30 µM H_2O_2	-2.3
			100 µM H_2O_2	4.3
EGF	Epidermal growth factor	GF	30 µM H_2O_2	-55.7
F13A1	Coagulation factor XIII, A1 polypeptide	ECM & CA	30 µM H_2O_2	2.8
			100 µM H_2O_2	4.7
IL10	Interleukin 10	C & C	100 µM H_2O_2	2
TNF	Tumor necrosis factor	GF	60 s	-2.1
VTN	Vitronectin	ECM & CA	20 s	-2.1
			60 s	3.1
			30 µM H_2O_2	3.1

short gene name	gene name	group	treatment	fold reg.
WISP1	WNT1 inducible signaling pathway protein 1	ST	30 µM H_2O_2	3.3
			100 µM H_2O_2	3.5
WNT5A	Wingless-type MMTV integr. site family, member 5A	ST	20 s	-3.6
			100 µM H_2O_2	2.3

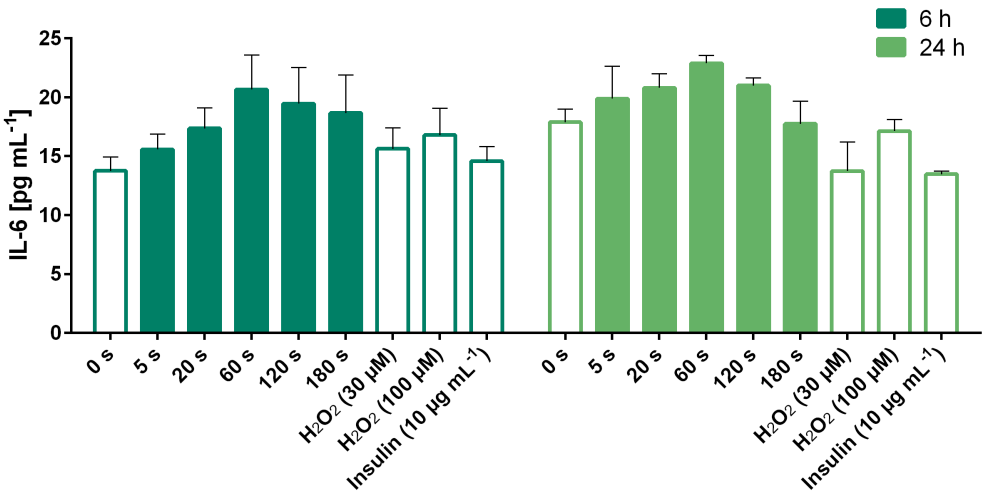


Figure 38: IL-6 secretion measured by ELISA. The cells were treated with conditioned medium from keratinocytes which were starved and than treated with plasma, H_2O_2 or insulin. The conditioned medium was let on the cells for 6 or 24 hours. Bars and error bars represent mean and SD.

Besides studies of the expression of defined genes, an analysis of the secretion was also performed. From further experiments IL-6 was known to be highly secreted by the investigated HaCaT keratinocytes, therefore its release due to conditioned medium was studied and is illustrated in figure 38. The secretion of IL-6 was not significantly changed by the starved keratinocytes after 6 or 24 hours. The treatments with plasma showed a maximum at 60 s and

after 24 h the 180 s treatment induced a similar release as the untreated cells. After 6 and 24 h the higher H_2O_2 concentration (100 µM) led to an increased secretion than lower concentration (30 µM). But it was not significantly changed compared to the control cells. Also the treatment with insulin (10 µg mL^{-1}) did not modify the IL-6 secretion. After 6 hours it even seemed to be slightly decreased.

3.4 Impact of plasma on a co-culture

An *in vitro* co-culture model system involving the keratinocyte cell line HaCaT and the monocyte cell line THP-1 was used to study the crosstalk between these skin and immune cells after plasma treatment. Therefore, the co-cultured skin and immune cells were either untreated or treated with plasma (180 s). In order to simulate the presence of bacteria 1 µg mL^{-1}

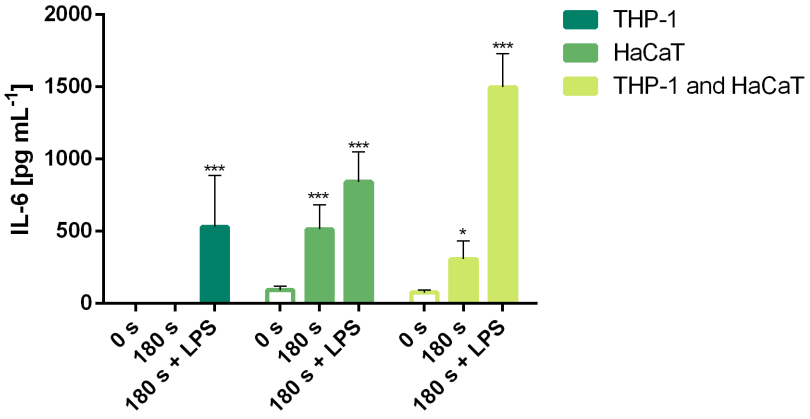


Figure 39: Secretion of IL-6 of the THP-1 or HaCaT mono-culture and the co-culture. The cells were either untreated, treated for 180 s with plasma or with plasma and LPS (10 µg mL^{-1}). The experiment was repeated in four independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analysis was performed with Dunnett's test as follow up by one-way ANOVA for each culture. Significance levels: $\alpha = 0.05$ (*), $\alpha = 0.001$ (***).

lipopolysaccharide (LPS) was added to the plasma treated medium in an additional treatment condition. For comparison mono-cultures with HaCaT cells or THP-1 cells under equivalent conditions were performed, too. After treatment the cells were incubated for 24 hours and the cell culture medium was used to analyze the secretion of 12 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17A, IFN-γ, TNFα, GM-CSF) with

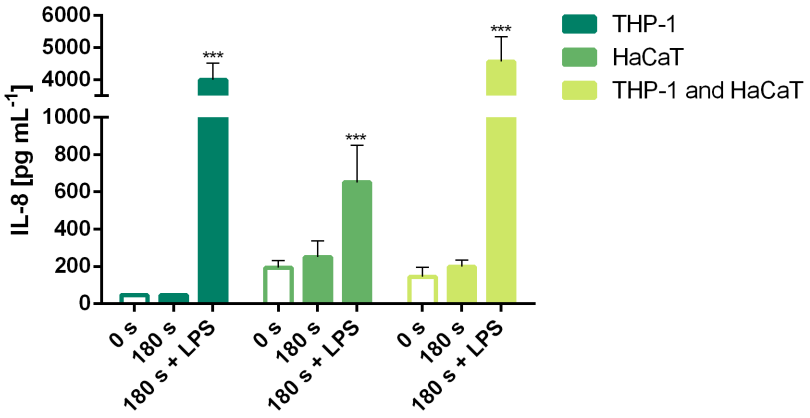


Figure 40: Secretion of IL-8 by THP-1 or HaCaT mono-culture and co-culture. The cells were either untreated, treated with plasma for 180 s or with plasma (180 s) and LPS (10 µg mL^{-1}). The experiment was repeated in four independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analysis was performed with Dunnett's test as follow up by one-way ANOVA for each culture. Significance level: $\alpha = 0.001$ (***).

the human inflammatory cytokines multi-analyte ELISArray Kit by Qiagen. The cytokines IL-6, IL-8, TNFα and GM-CSF could be detected and were subsequently analyzed with more specific ELISAs. As shown in figure 39, the monocytes (THP-1) secreted IL-6 only due to a treatment with plasma and LPS, whereas the keratinocytes (HaCaT) released it under all treatment conditions. A significant increase was observed for a treatment with plasma alone, which was similar to the secretion by the monocytes (180 s + LPS).

The combination of plasma and LPS induced a larger increase of IL-6 by the keratinocytes. The secretion of the untreated co-culture was similar to untreated keratinocytes, whereas a 180 s plasma treatment showed a smaller increase as the keratinocyte mono-culture. The treatment with plasma in combination with LPS induced a doubled release compared to the HaCaT mono-culture.

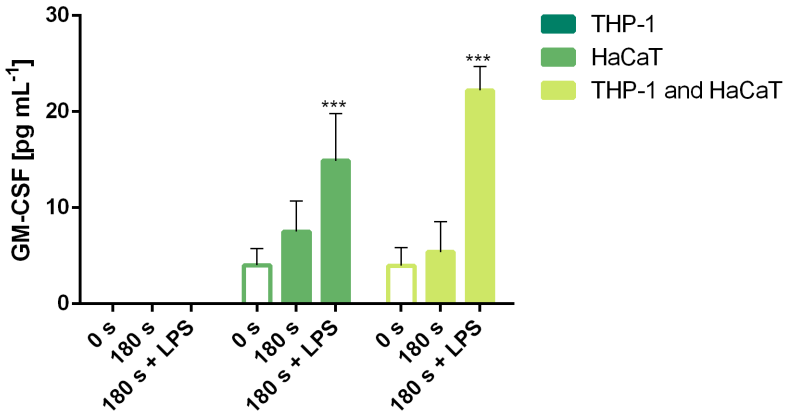


Figure 41: Secretion of GM-CSF by THP-1 or HaCaT mono-culture and co-culture. The cells were either untreated, treated with plasma for 180 s or with plasma (180 s) and LPS (10 µg mL^{-1}). The experiment was repeated in four independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analyses were performed with Dunnett's test as follow up by one-way ANOVA for each culture. Significance level: $\alpha = 0.001$ (***).

Another cytokine which was analyzed is IL-8, illustrated in figure 40. Differences in the baseline of secreted IL-8 were observed. The THP-1 mono-culture secreted less IL-8 than co-culture or HaCaT mono-culture. A treatment with plasma alone did not significantly change the secretion for all cultures. In case LPS was added to the treated medium, the IL-8 release increased rapidly in all cultures. The secretion of the co-culture and THP-1 mono-culture even reached a concentration between 4,000 and 5,000 pg mL^{-1},

whereas the keratinocytes just reached around 700 pg mL^{-1}.
GM-CSF, which is shown in figure 41, was not secreted at any treatment by the immune cells alone. Both untreated keratinocytes and untreated co-cultured cells released the same amount of GM-CSF, whereas after plasma treatment the keratinocytes secreted more. A significant increase of re-

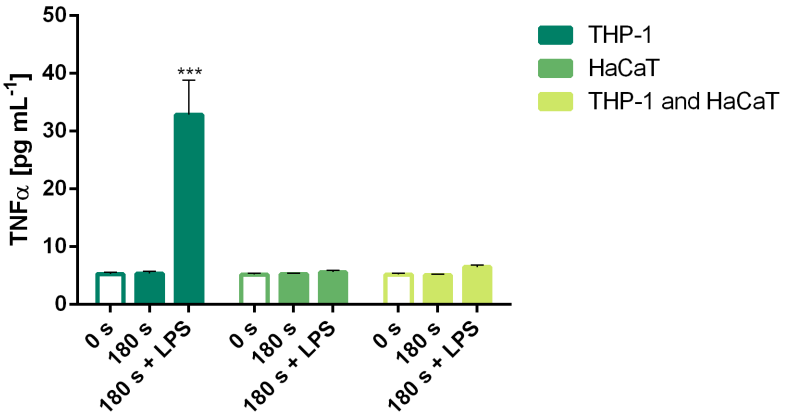


Figure 42: Secretion of TNFα by THP-1 or HaCaT mono-culture and co-culture. The cells were either untreated, treated with plasma for 180 s or with plasma (180 s) and LPS (10 µg mL^{-1}). The experiment was repeated in four independent experiments and measured with technical duplicates. Bars and error bars represent mean and SD. Statistical analyses were performed with Dunnett's test as follow up by one-way ANOVA for each culture. Significance level: $\alpha = 0.001$ (***).

leased GM-CSF was detected in HaCaT mono-culture and THP-1-HaCaT co-culture after plasma treatment in combination with LPS. However, the release by the co-cultured cells was increased in comparison to the skin cells alone.
The detected TNFα in immune or skin cell mono-culture or co-culture is shown in figure 42. All cultures did not raise the TNFα secretion after plasma treatment, but the immune cells alone significantly increased the TNFα secretion due to additional LPS. However, this increase was not observed in

co-cultured cells.

4 Discussion

Plasma medicine is a young research field and further research is needed to understand the interactions and mechanisms in plasma, liquids and cells during and after plasma treatments. First studies investigated the molecular biological impact on skin and immune cells. However, there is a need for extensive research on genes and proteins linked to wound healing. Especially the effect of plasma on cell signaling mediators such as cytokines and growth factors in keratinocytes has not been investigated, yet. Also the crosstalk with untreated keratinocytes or immune cells was not studied until now. These aspects of how plasma impacts cell signaling in HaCaT keratinocytes and which generated species could be responsible for the cellular response was investigated in the present work and is discussed in the following paragraphs.

4.1 Impact of plasma on HaCaT cells

In the present work the impact of plasma on cell signaling in human keratinocyte cell line HaCaT was studied. Therefore the determination of applicable treatment durations was necessary first. Cell survival after plasma treatment was analyzed with three different experiments. First, the cytotoxicity was detected and then early and late apoptosis was analyzed by annexin V and caspase-3 staining. Furthermore, to investigate how plasma impacts wound healing related mediators as cytokines and growth factors in keratinocytes gene expression and protein secretion profiles were produced. This could give first hints how signaling is influenced in cells by plasma.
Cytotoxicity was measured 24 hours after treatment (fig. 9). After a short treatment duration plasma was not cytotoxic to the investigated HaCaT cells, because both untreated and 20 s plasma showed nearly the same amount of dead cells (2 and 3 %). However, the long-termed plasma treatment was cytotoxic for only 10 % of the cells. Because 90 % of the cells were still viable this treatment duration was also used for further experiments. In almost all

experiments the cells were additionally treated with 100 µM H_2O_2 in order to investigate its sole influence. Hydrogen peroxide is very often correlated to cellular responses after plasma treatment[88,91] and during a 180 s plasma treatment with the kinpen around 90 to 100 µM H_2O_2 are generated (personal communications: H. Tresp, J. Winter).

Cell survival was analyzed in greater detail by detecting the amount of early or late apoptotic cells after plasma treatment (fig. 10a and 10b). After 12 hours the early apoptotic cells were measured via annexin V detection and after 18 hours the late apoptotic cells were detected via caspase-3 activity. The plasma dose dependency was also identified in early and late apoptotic cells. The longer the treatment time the higher the amount of apoptotic cells, however even a treatment duration of 180 s did not induce a significant increase in early and late apoptosis (around 10 % compared to 4.5 % in untreated cells) and the HaCaT cells tolerated the plasma treatment. After a treatment with 100 µM H_2O_2 25 % of the cells were early apoptotic but only 9 % were late apoptotic. Thus, the HaCaT cells can prevent the H_2O_2 induced apoptosis process. The treatments with etoposide also induced a significant increase of apoptotic cells, but it was not reduced with increasing incubation time. It is known, that etoposide can induce single- and double-strand breaks which could cause apoptosis and cell death. Is the concentration of etoposide to high, the cells can not repair their DNA to prevent cell death.[33] Plasma can also induce cell damage with increasing DNA strand breaks in HaCaT cells which could induce apoptosis. But it was also shown, that the DNA damage can be decreased to baseline level within 24 hours.[6,88] The reduction of DNA damage is caused by repair mechanisms of the HaCaT keratinocytes. The enzymes ataxia telangiectasia mutated (ATM) and checkpoint kinase 1 (Chk1), which are sensors for DNA double- or single-strand breaks, were phosphorylated and therefore activated after plasma treatment in HaCaT cells and could induce the repair mechanism.[65] Furthermore, it was shown, that an indirect plasma treatment for 180 s with the kinpen was not muta-

genic.[40]

There are first hints, that plasma could stimulate the wound healing process[58,63,82] but until now it is unclear how plasma influences cells. Wound healing is a very complex process and is orchestrated via the regulation of defined genes and their subsequent translation into proteins. They can belong to the extracellular matrix, cell adhesion, signal transduction, growth factors or cytokines. It was shown in previous studies, that plasma influences the gene expression and protein activation in cells. Besides ECM and cell adhesion molecules also signaling mediators can be stimulated by plasma.[2,13,32] To study how treatments with the atmospheric pressure plasma jet kinpen regulates wound healing related genes and proteins a gene expression and protein secretion profile of the HaCaT cells was conducted. The gene expression profile was generated with the analysis of 84 wound healing related genes by qPCR. The analyzed genes encode for ECM, cell adhesion, signal transduction, growth factors or cytokines. Due to the fact that some of their proteins were activated by plasma in previous studies it was assumed that the investigated keratinocytes could also activate these genes. A treatment with plasma induced a significantly changed gene expression in 21 genes, of which 11 genes encode growth factors, cytokines or chemokines. Eight genes were related to ECM and cell adhesion and one gene (PTGS2) was a signal transducer. The gene B2M, whose expression was changed by plasma treatment, is a house keeping gene. Due to the fact that this house keeping gene was regulated by plasma it is necessary for genetic analyses like qPCR to investigate different house keeping genes before they can be used as internal standards. In case the expression of the house keeping gene is regulated by plasma it can not be used.

In addition to the gene expression profile a secretion profile was generated to find out how plasma impacts the release of important cell signaling molecules: 19 wound healing related cytokines and growth factors were analyzed by ELISA. The release of 12 cytokines and 7 growth factors was studied, whereas

the secretion of only 2 cytokines (IL-6, IL-8) and 2 growth factors (VEGF-A, GM-CSF) was changed by plasma treatment in the investigated keratinocyte cell line. Both gene expression profile and protein secretion profile are discussed on the next pages.

The following discussion of the cellular response is adapted from the work of Barton et al.([5]). Some of the most prominently changed genes encode for proteins involved in angiogenesis, the generation of new blood vessels, which is an important process during wound healing. The plasma and H_2O_2 treatments of the HaCaT cells enhanced the transcription rate of the angiogenesis factor VEGF-A within the first six hours after treatment. The secretion of this growth factor was also increased after long-termed plasma and H_2O_2 treatments. A steady rise of VEGF-A release with increasing incubation time was observed. The increase of VEGFA gene expression was decreased to baseline level within time and therefore it is expectable that also the secretion of VEGF-A will decrease. Furthermore, after 24 hours an increase was still detectable and longer incubation times were not applied. A transient up regulation of VEGF-A could induce or speed up the angiogenesis in damaged tissues.[64,79] However, a permanent increase of VEGF-A, which was not shown in the gene expression results, could also have negative effects and is associated with diseases like psoriasis.[57] It can be hypothesized that plasma triggers short term signals finally leading to the secretion of VEGF-A. It is known that RNS like peroxynitrite, which is formed by superoxide anion and nitric oxide, augments a VEGF-A release in fibroblasts.[81] As RNS are generated by the plasma jet, the same effect could lead to the observed increase in HaCaT cells after plasma treatment. However, VEGF-A transcription and translation could also be enhanced by ROS such as H_2O_2,[15] which is also shown in the H_2O_2 treated HaCaT cells. An up regulation of heme oxygenase 1 (HMOX1) is involved in the H_2O_2-induced VEGF-A up regulation, but also p38 MAP-kinase, PI 3-kinase and other transcription factors are involved.[15] It was shown, that HMOX1 and p38 MAPK were in-

creased at gene and protein level by plasma and H_2O_2 treatments in HaCaT keratinocytes.[65,74] As sketched in figure 43 VEGF-A could also be activated by further signaling molecules (e.g. IL-6, GM-CSF) which were activated by plasma, too. Due to that complexity VEGF-A was probably stimulated by both plasma generated reactive species and further signaling molecules.[5]

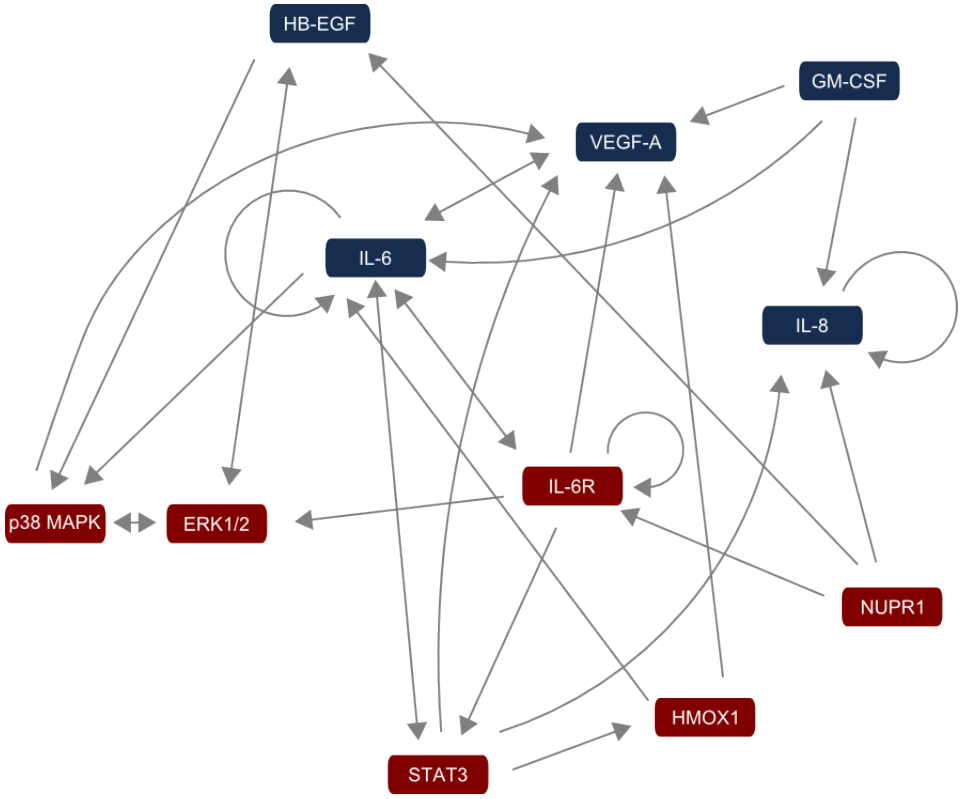


Figure 43: Network of secretable cytokines and growth factors (blue) and intracellular proteins (red).

Another angiogenesis factor is angiopoietin 1, which has rapidly declined within the first six hours after plasma treatment (table 1). It has been reported to be up regulated during wound healing[39,51] and other studies indicate that interleukin-6 can cause a down regulation of angiopoietin 1 and at the same time a VEGF up regulation[43] , which is in good agreement with the

detected changes after plasma treatment in the experiments of the present work.[5]

Angiogenesis can also be influenced by GM-CSF. An overexpression of CSF2, the gene which encodes GM-CSF, improves wound healing by recruitment of leukocytes, enhances keratinocyte proliferation and increases angiogenesis by VEGF up regulation (fig. 43).[9,10,23] Keratinocytes are able to produce this stimulating factor immediately post skin injury.[23,52] Plasma treatments induced both an up regulated gene expression of CSF2 and an increased secretion of its synthesized protein GM-CSF. The secretion profile of GM-CSF showed a maximum after 12 hours post plasma treatment and within 24 hours it was observed at baseline level. Studies have shown a correlation between UV radiation and increase of GM-CSF and IL-6 production.[22,37] ROS, which were generated during UV radiation, could be made responsible for this cellular reaction. However, a H_2O_2 concentration of 100 µM did not induce changes in neither gene nor protein level. Therefore, the increase after plasma treatment seemed not to be induced by H_2O_2, but further reactive oxygen species could have triggered the CSF2 gene expression and GM-CSF protein secretion.[5] Plasma generated ROS, such as superoxide anion ($O_2^{\cdot-}$), hydroperoxyl ($HO_2^{\cdot}$) and hydroxyl ($^{\cdot}OH$) radicals can be scavenged by the cells with glutathione (GSH) and the enzymes glutathione peroxidases, glutathione reductase and glutathione S-transferases.[26,27,30] The vacuum ultraviolet (VUV) radiation of the kinpen induced during a 180 s treatment generation of glutathione thiyl radicals ($GS^{\cdot}$) in THP-1 monocytes (personal communication: H. Tresp). Furthermore, $O_2^{\cdot-}$,$^{\cdot}OH$ and up to now, two unidentified radicals could be detected by electron paramagnetic resonance (EPR) spectroscopy (personal communication: H. Tresp). In addition the gene expression of several glutathione peroxidases, glutathione reductase and gluathione S-transferases were activated in the transcriptome of HaCaT cells after plasma treatment.[75] This could be a hint, that the stimulation of GM-CSF after a long termed plasma treatment was induced by other ROS

such as $O_2^{\cdot-}$ or $HO_2^{\cdot}$. However, the specific activation by $^{\cdot}OH$ can be excluded, because $^{\cdot}OH$ is highly reactive and does not interact in a specific way.[30]

Angiogenesis could also be regulated via the protein prostaglandin-endoperoxide synthase 2, which is encoded by the gene PTGS2. Table 1 and figure 14 illustrate a PTGS2 up regulation only 6 hours after a long termed plasma treatment. An activation of the protein PTGS2 after plasma treatment was not detected (data not shown). It is known that the expression rate of PTGS2 can be increased via UV radiation, followed by ROS production. The reason for activation only in the first 6 hours could be that PTGS2 is an intermediate early gene in wound healing.[97] After 12 hours the activation of PTGS2 was decreased and the expression was not significantly changed compared to the untreated control cells. This could be a hint that PTGS2 is only up regulated by higher amounts of generated ROS. It is also known that PTGS2 could be stimulated by cytokines and growth factors.[77] The changes of PTGS2 at gene level did not influence its translation rate which could mean that the stimulus by ROS and other mediators was not intensive enough. In addition to angiogenesis, PTGS2 can also promote cell proliferation[14] and therefore it is a very important actor during wound healing.[5]

HBEGF, a gene encoding the growth factor HB-EGF is another growth factor, which was regulated by plasma. In human wounds HBEGF is an early gene, which is up regulated within the first hours.[59] Interestingly this behavior was shown in HaCaT cells, too. HB-EGF is well known to induce keratinocyte proliferation and regeneration during injury. It also has mitogenic and cell survival promoting properties on fibroblasts and further multiple cell types.[25,41,44,59] The expression of HBEGF was up regulated 6 hours after plasma treatment and after 12 hours it was minor decreased. Its expression is described to be rapidly increased by oxidative stress, tissue damage, during wound healing and regeneration.[25] After oxidative stress using H_2O_2 an increased mRNA level was shown.[5,44,56] Therefore, an increased gene ex-

pression was expected post plasma treatment.[5] However, a secretion of the protein HB-EGF was not detected neither after plasma nor H_2O_2 treatment. HB-EGF is synthesized as a transmembrane form and can be proteolytically processed to soluble form.[3,89] The transmembrane form can stimulate adjacent cells in a juxtacrine manner.[89] It could be, that the investigated HaCaT cells activated the HB-EGF translation after plasma and H_2O_2 treatment, but it was synthesized in the membrane-anchored form and subsequently not transformed into soluble form. This would explain why HB-EGF was not detectable in the cell culture medium. Fluorescence microscopy or flow cytometry could be used for detection of membrane-anchored HB-EGF in further investigations.

Besides the release of growth factors and angiogenesis promoting mediators, another important mechanism during wound healing is the migration of keratinocytes. One cytokine that stimulates migration is interleukin-6 whose regulation by plasma is demonstrated in table 1 and figures 16 and 19. The gene was significantly up regulated by plasma and H_2O_2 6 and 12 hours after treatment.[5] However, the plasma treated cells did only secrete IL-6 after 12 and 24 hours, whereas a H_2O_2 treatment induced the release even after 6 hours. Similar to the secretion of VEGF-A or GM-CSF a short plasma treatment (20 s) did not lead to a changed secretion profile, only the long treatment duration (180 s) stimulated the release. With increasing incubation time the secretion of IL-6 in plasma treated cells rose and 100 µM H_2O_2 always induced a higher secretion rate. It is known that both ROS[67,95,98] and RNS[53] can increase the level of IL-6 in a dose dependent manner, which could explain these results.[5] Moreover, the up regulation of IL-6 is also correlated with an increase of further stimulating factors like GM-CSF or HMOX1 (fig. 43), which were found to be up regulated by plasma, too.[22,37] Interleukin-6 is a pleiotropic cytokine and can be both pro- and anti-inflammatory, dependent on the amount of secretion over a certain period of time. It can enhance wound healing[52] and be stimulated in an autocrine or paracrine

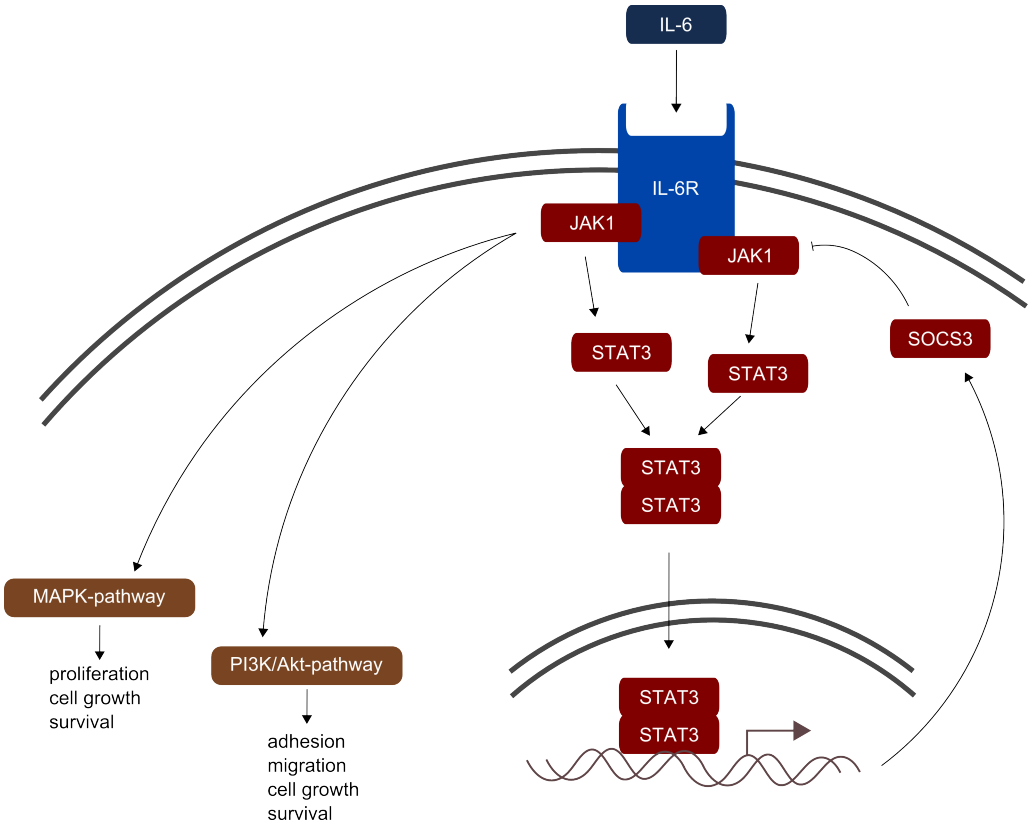


Figure 44: IL-6 signaling pathway.

manner by binding to the IL-6 receptor (IL-6R) as sketched in figure 44. Then, the janus kinase 1 (JAK1) binds to IL-6R and become autophosphorylated. Subsequently, the signal transducer and activator of transcription 3 (STAT3) protein is recruited, phosphorylated and dimerized. The STAT3 homodimer translocates into the nucleus where it binds to the DNA and activates the transcription of the target genes, such as SOCS3, IL6, FOS and JUNB.[34,62,96] SOCS3 acts as a feedback inhibitor, which can inhibit the canonical IL-6 JAK/STAT pathway.[62] Is the canonical pathway inhibited or some how not available, the MAPK- and PI3K/Akt-pathways will be activated and the IL6 gene expression and secretion even so can be activated.[11,17,34,94,99] It was shown by cell-based ELISA, that the kinase JAK1

was not phosphorylated and therefore not activated 5 min, 30 min, 3 h or 24 h after plasma, H_2O_2 (100 µM) or IL-6 (100 ng mL^{-1}) treatment (data not shown). Furthermore, by the automated western analysis Simple Western™ (Simon from ProteinSimple) no changes of the proteins STAT3 and SOCS3 were detected due to plasma, H_2O_2 or IL-6 treatment (incubation times: 30 min, 3 h; data not shown). And the target genes SOCS3, FOS and JUNB were also not significantly changed 6 hours post treatment (measured by qPCR; data not shown). These data support the notion that the canonical JAK/STAT pathway was not active during plasma or H_2O_2 treatment in HaCaT cells. An inhibition of the pathway due to SOCS3 can be excluded because it was neither expressed at gene nor protein level. However, the IL-6 transcription and translation were highly activated by plasma and H_2O_2 treatments. For that reason it is hypothesized that the IL6 gene expression and protein secretion were activated by other signaling pathways such as MAPK- or PI3K/Akt-pathways. Both pathways can regulate the IL-6 transcription and secretion[17,94] and it was shown in other studies that p38 MAPK signaling pathway is highly activated in HaCaT cells after plasma treatment with the kinpen.[65] These data support the assumption, that IL-6 is regulated via MAPK-pathway alone or in combination with PI3K/Akt-pathway, but not via JAK/STAT-pathway.

A further pleiotropic cytokine, which is important for keratinocyte reepithelization during wound healing is IL-8. It is mitogenic and can stimulate proliferation and migration in keratinocytes and is also used for recruitment of immune cells into the wound site.[69] As depicted in figure 20 the secretion of IL-8 by the HaCaT keratinocytes was only changed after the first six hours post plasma (180 s) and H_2O_2 treatment. A longer incubation time let the release decline to baseline level. Interestingly, the IL-8 secretion of untreated cells increased with the incubation time, too. This baseline level increase was also observed for other molecules (VEGF-A, GM-CSF, IL-6) and could attribute to the normal proliferative phase during cell culture. The cells were

seeded and after 24 hours the medium was changed. Due to the nutrients in the fresh medium the cells proliferate and secrete cytokines and growth factors into the cell culture medium. Therefore the total amount of molecules in the medium increased with time. The secretion of IL-8 can be induced by ROS, RNS or other cytokines.[68,90] One hypothesis for the short time of IL-8 release can be, that the stimulus by plasma generated ROS or RNS was not intensive enough. The time of secretion could not be elongated and IL-8 was not detectable. It may be that IL-8 was primary activated by reactive species with a very short lifetime and due to the indirect plasma treatment of the HaCaT cells the stimulative reactive species did not reach the cells. Another possibility is, that the cytokines which can activate IL-8 were not stimulated by plasma and therefore they could not influence the IL-8 secretion. Furthermore, the increased secretion was only observed 6 hours post plasma treatment but an earlier incubation time was not investigated. For that reason it could be that the early cell response via IL-8 secretion was not detected.

Other important cytokines and growth factors inducing keratinocyte migration are epidermal growth factor (EGF), interleukin-1α (IL-1α) and transforming growth factor-β1 (TGFβ1).[85] However, EGF and TGFβ1 were not found to be significantly regulated in transcription and secretion by plasma in the investigated keratinocyte cell line. IL-1α was even not secreted by the untreated control cells. During migration cellular adhesion plays a very important role and it is known, that after ROS or plasma exposure the adhesion molecule E-cadherin was less detectable.[28,31] Therefore it was to be expected that the gene CDH1, which encodes E-cadherin, was down regulated post plasma exposure. However, the results showed both a down and an up regulation (table 1) of CDH1. One hypothesis is that the cells decreased CDH1 to induce proliferation and subsequently it was up regulated to affect adhesion again. Furthermore, it can be hypothesized that the decrease of the protein E-cadherin in the other studies was due to its degradation by the

plasma generated reactive species. For the readjustment of E-cadherin the keratinocytes increased the expression rate of the gene CDH1.
However, Haertel et al. showed that the abundance of the epidermal growth factor receptor (EGFR) was decreased post plasma treatment,[31] but in the present experiments the amount of EGFR gene expression was not significantly changed compared to untreated cells. This may be caused by the different experimental setups, e.g. the incubation time (6 and 12 h vs. 24 h). Additionally, in the present study the mRNA level and not the proteins were analyzed. One hypothesis is that the receptor at the cell membrane was modified by plasma generated ROS and due to this morphological changes the used antibody was not able to bind. It also could be that the receptor was internalized and was therefore not detected. Two further studies investigated the influence of plasma on the adhesion molecules α2-integrin and β1-integrin of the human HaCaT keratinocytes. Interestingly, two different plasma sources were used: on the one hand a dielectric barrier discharge (DBD), and on the other hand the kinpen plasma jet, which was used for these experiments, too. It was shown that the effects on integrins could vary by different plasma sources and experimental designs.[31,32] Additionally, in the work of Haertel et al. the incubation time was extended to 24 hours. These changes in the experimental design could be the reason why α2-integrin and β1-integrin were changed in their but not in the present experiments after plasma exposure.[5]
Further cytokines and growth factors were studied in the present work and most of them were not changed or detected after plasma or H_2O_2 treatment in the HaCaT keratinocytes (table 2). The mediators IL-2, -4, -10 and IFN-γ were not secreted by the investigated cells and the gene expression of these mediators was also not changed. FGF-2 and TNF-α were neither changed at gene nor protein level after plasma treatment and a secretion of IL-12, -17α, PDGF-BB and NGF-b could not be detected.
It has been shown that plasma stimulates the keratinocytes in a dose de-

pendent manner but a treatment duration of 180 s was still tolerable and not cytotoxic. It was also shown in the present work, that plasma can activate the expression of important genes, which are linked to ECM, cell adhesion and cell signaling and are therefore very important for wound healing. Plasma also impacts gene expression and protein secretions of the cytokines and growth factors VEGF-A, HB-EGF, IL-6, IL-8 and GM-CSF in a dose dependent manner.

4.2 Impact of the shielding gas

It was shown in previous studies, that reactive oxygen and nitrogen species can influence cellular responses during plasma treatment.[45,47,88] During treatments with the argon-operated atmospheric pressure plasma jet kinpen, ROS and RNS are generated from oxygen and nitrogen diffusing into the effluent of the jet.[70,71] The application of the shielding gas device during plasma treatment gives some control over the generated ROS and RNS. The shielding gas was a mixture of O_2 and N_2 which was varied from pure O_2 to pure N_2. Besides oxygen and nitrogen humidity can also influence the plasma and the amount of water-related species such as $^{\cdot}OH$, H_2O_2, $HO_2^{\cdot}$, nitrous acid (HNO_2) and nitric acid (HNO_3).[76,92] Another aspect of continuous variations of the shielding gas is, that cellular responses can be correlated to reactive oxygen or reactive nitrogen species, while the core plasma remains unchanged. This experiment gives first insights into the dependence of cellular response of the human keratinocyte cell line HaCaT on ROS or RNS dominated plasma. It was shown by fourier transformed infrared spectroscopy (FTIR) measurements that the shielding gas compositions can vary the amount of ozone (O_3) and nitrogen dioxide (NO_2) in the far field of the kinpen.[76] To understand the mechanisms of plasma treatments *in vitro* it is necessary to also study the generation and transfer of species into the liquids (in this case cell culture medium) which then could interact with the cells. H_2O_2, nitrite (NO_2^-) and pH were measured in medium after a plasma

treatment with the kinpen for 180 s. It was shown, that the pH was almost unchanged when varying the shielding gas from pure oxygen to pure nitrogen. NO_2^- has its maximum at 25 % oxygen and 75 % nitrogen. The amount of H_2O_2 in the cell culture medium was not changed by the shielding gas, however a significant increase was detected for a pure nitrogen shielding gas.[83] O_3 and NO_2 measured in the far field of the plasma jet and H_2O_2 in cell culture medium upon variation of the shielding gas are depicted in figure 45.

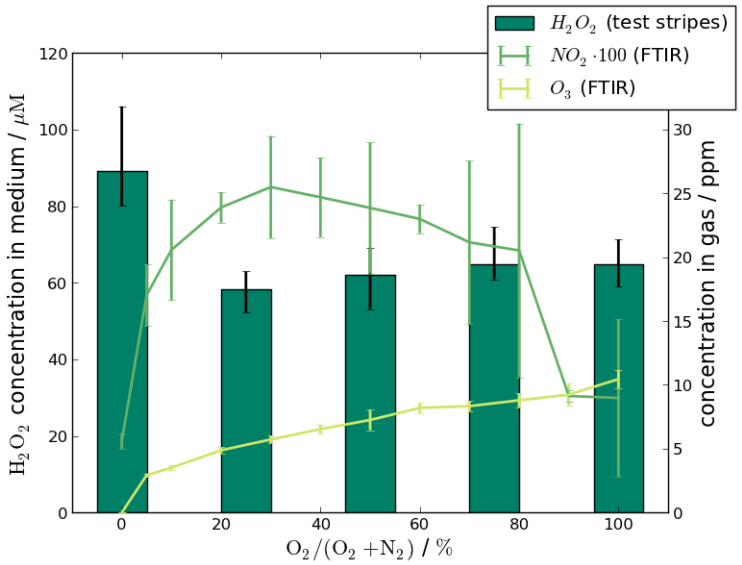


Figure 45: Concentrations of O_3, NO_2 (detected in the far field, FTIR)[76] and H_2O_2 (detected in medium, test stripes).[83]

It is known, that the cell response of the HaCaT keratinocytes depends on the duration of the plasma treatment. To find out which plasma generated species correlate to its cytotoxicity the cells were treated with the kinpen in combination of the shielding gas device. The cytotoxicity and caspase-3 activity experiments reflected the dose dependence: The longer the treatment the higher the amount of dead cells. The cytotoxicity experiment (fig. 21) showed a significant influence of the shielding gas composition for the long

termed plasma treatment: The higher the amount of oxygen in the shielding gas, the higher the amount of dead cells. H_2O_2 was probably the main actor for cytotoxicity as the positive control using H_2O_2 alone was responsible for a similar amount of dead cells. However, as depicted in figure 45 the concentration of plasma generated H_2O_2 was not changed with increasing oxygen in the shielding device. This indicates that also other reactive oxygen species are cytotoxic for the keratinocytes. It is expected, that with increasing oxygen amount in the shielding gas, the concentrations of O_3, atomic oxygen (O and O(1D)) and the oxygen metastable $O_2(^1\Delta_g)$ rise in the plasma effluent.[76] It is also assumed, that the amount of $O_2^{\cdot -}$ in the plasma treated liquid increases with oxygen in the shielding gas. $O_2^{\cdot -}$ in the liquid phase can be generated from $HO_2^{\cdot}$ which is present in the plasma as investigated by plasma chemistry simulations.[76]

A DNA microarray was performed to study the influence of the shielding gas composition on the transcriptome of the investigated HaCaT keratinocytes. The heat map of the significantly changed genes showed (fig. 23) that the shielding gas composition seemed to influence the expression profile. However, a clear dependency did not crystallize. The analyses with the PANTHER Classification System showed that for the long termed plasma treatment most genes which were changed belong to the biological process subgroups cell communication, cellular process and metabolic process (fig. 24-28). The shielding gas composition does not seem to influence the distribution of the subgroups. And also during plasma treatment of the HaCaT cells without shielding device the most changed genes belonged to these three subgroups.[75] Although many genes belong to cell communication subgroup, not all growth factors and cytokines which were known to be changed by plasma were found in the microarray data. The genes IL6, VEGFA and CSF2 were not found, but HBEGF, IL-8 and PTGS2 could be identified. The qPCRs without the shielding device were performed 6 hours post treatment and the DNA microarray 3 hours post treatment, this could be a cause for these vari-

ances. The DNA microarray served as a screening method and the molecules which were known to be stimulated by plasma were subsequently validated in qPCR and ELISA measurements.

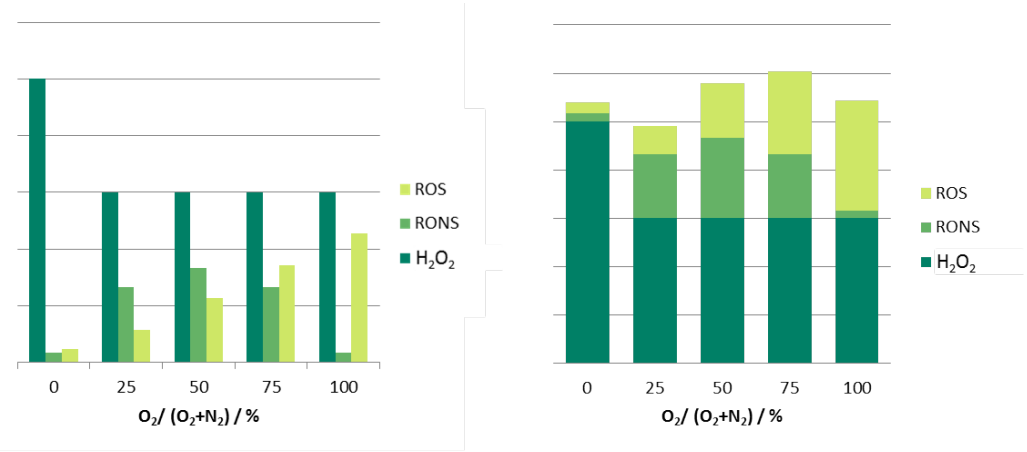
The gene expression of the angiogenesis growth factor VEGFA was significantly up regulated after a long termed plasma treatment and the shielding gas composition had a significant influence on its expression. The minimum was detected at 25 % O_2 and the maximum at 75 % O_2 in the shielding gas. The secretion of VEGF-A was measured 6, 12 and 24 hours post treatment, whereas after 6 hours no significant changes were detected (fig. 33a). After 12 and 24 hours the cells treated for 180 s increased the release of VEGF-A. The same shape (minimum at 25 % O_2, maximum at 75 % O_2), resembling a rotated S-curve, as for gene expression could be detected, whereas after 24 hours the maximum formed a plateau (50, 75 and 100 % O_2).

Both gene expression and protein release of GM-CSF, which can enhance angiogenesis and keratinocyte proliferation, were studied after plasma treatment in combination of the shielding gas device. The shielding gas composition significantly changed GM-CSF after long termed plasma treatment. Similar to VEGF-A the S-shape was detectable with its minimum at 25 % O_2.

HB-EGF, which can also enhance the proliferation of keratinocytes, was also changed by plasma. The shielding gas composition significantly changed the gene expression in a similar way as for VEGF-A and GM-CSF (S-shape). Identically to the treatments without shielding device a release of HB-EGF was not detectable. A feasible reason can be that the membrane-anchored form was synthesized but not transformed into the soluble form.

The growth factors VEGF-A, GM-CSF and HB-EGF were all stimulated in a similar way by the plasma treatments upon shielding gas variation: The cellular response in dependence on the oxygen content in the shielding gas results in a S-shape pattern. The minimum always occurred at a treatment with 25 % O_2 in the gas mixture, whereas the maximum varied, but it was

mostly observed at a O_2 dominated shielding gas mixture. In figure (46a) the



(a) separated (b) added up

Figure 46: Qualitative patterns of ROS, RONS and H_2O_2 upon shielding gas variation separated (46a) and added up (46b).

qualitative patterns of H_2O_2, ROS and reactive oxygen and nitrogen species (RONS) are depicted based on the measurements in figure 45: The shape of H_2O_2 was directly measured in the liquid phase. RONS are expected to have a pronounced minimum at 0% oxygen and 0% nitrogen in the shielding gas as both components are required in order to form species like NO, HNO_3 etc. in the plasma and thus result in a convex shape like measured for NO_2. ROS like O and $O_2(^1\Delta_g)$ are expected to rise continuously in the plasma with the oxygen content, as measured for O_3. In figure (46b) the effects of H_2O_2, ROS and RONS are weighted and added up to form the typical S-shape. H_2O_2 was weighted to have the biggest influence on the cellular response as the positive controls using H_2O_2 yield strong cellular responses. The combination of these species was probably responsible for the changes which were observed at gene and protein level. While H_2O_2 was probably the main actor, the influences of other ROS and RONS were not negligible. This clearly shows, that plasma components different from H_2O_2 play a crucial role in cellular

response.
Cytokines which were studied are IL-6 and IL-8, whereas IL-8 was not changed by treatments. However, gene expression and protein secretion of IL-6 were significantly influenced by the shielding gas composition. The higher the amount of O_2 in the shielding gas, the higher the expression or secretion of IL-6. The main actor of IL-6 stimulation was H_2O_2 but the changes upon the shielding gas variation were due to other ROS. In addition, the expression of the ROS-induced HMOX1 was also increased by shielding gas mixtures containing O_2 (DNA microarray data; appendix).
For the first time it was shown, that the compound of plasma generated RONS significantly influences cytokines and growth factors at transcriptome and protein level. While H_2O_2 was identified as a main actor for gene expression and protein secretion activation after plasma treatment, it could be excluded as the sole component responsible for the observed cellular response.

4.3 Impact of conditioned medium on HaCaT cells

In paragraphs 4.1 and 4.2 it was shown that plasma induces the activation of various cell signaling molecules by its generated species. If plasma would be applied for wound healing it is essential to know how plasma activated cells can influence other cells. Until now, the effect of conditioned medium (CM) of plasma treated cells on untreated cells was not investigated, although it could give deeper insights into the mechanisms during plasma treatment.
To simulate *in vitro* how plasma treated keratinocytes at the wound site can influence the neighboring non-treated keratinocytes by secretion of mediators a method using conditioned medium (CM) was developed: The HaCaT keratinocytes were seeded into a petri dish and incubated for 24 hours. Subsequently they were starved with medium containing only 1 % FCS. After 24 hours they were indirectly treated with plasma, hydrogen peroxide or insulin. The cells consequently could alter the cell culture medium by secretion of cytokines and growth factors and they could alter the composition of the

medium due to their metabolism. After 24 hours, this conditioned medium was collected and diluted (1:2) with fresh medium without FCS, which was than added to HaCaT cells. These cells were also starved (for 18 hours) before. The cells and supernatant were collected after desired time for analyses of the gene expression profile and IL-6 secretion.
To ensure that the effect of the abundance of FCS, which consists of numerous aminoacids, peptides, proteins, sugars and further important nutrients was excluded, the cells were starved prior to plasma treatment. Morphological changes of the HaCaT keratinocytes after starving were not observed.
The heat map (fig. 37 on page 68) displays all genes which were significantly up or down regulated by the keratinocytes after CM treatment. The 30 and 100 µM H_2O_2-CM treated cells changed the expression of the most genes (7 and 11 of 84 genes), whereas the 20 and 60 s plasma-CM treatments changed only 3 genes and 180 s plasma-CM treatment decreased only 1 gene. The genes which were changed by treatments with plasma-CM are probably due to the cytokines and growth factors which were secreted after plasma treatment and which modified the conditioned medium. The direct influence of plasma generated H_2O_2 can be excluded. On the one hand is the lifetime of plasma generated H_2O_2 in cell culture medium relatively short (personal communications: H. Tresp, J. Winter) and on the other hand it is degraded by the catalase of the HaCaT cells within 24 hours (personal communication: K. Wende). However, the commercial H_2O_2 is stabilized[78] and therefore it is assumed that HaCaT cells did not catalyze H_2O_2 completely and significant quantities were added to the investigated cells with the conditioned medium.
Besides the gene expression profile the secretion of IL-6 was detected 6 and 24 hours post conditioned medium treatment (fig. 38). No significant changes of IL-6 could be observed, this raises the assumption and is in good agreement with the previous results, that IL-6 was directly induced by plasma generated ROS. The reactive oxygen species which would induce the IL-6 secretion were not present and therefore could not stimulate IL-6.

These data reveal the effect of CM by plasma treated keratinocytes on untreated keratinocytes and simulate in a very simple *in vitro* experiment neighboring cells of the wound site. In comparison to normal plasma treatment (see paragraph 4.1) the effect of conditioned medium was not that intensive on the genes investigated in both experiments. It was also shown that hydrogen peroxide had larger effects on the expression of the investigated genes than proteins which were secreted by other plasma treated cells. This could mean that plasma acts locally and the effects on neighboring cells are marginal.

4.4 Impact of plasma on a co-culture

The investigated HaCaT keratinocytes secrete cytokines and growth factors post plasma treatment with the kinpen and it is also known that immune cells as monocytes activate signaling pathways due to plasma treatment.[12] For that reason it is of high interest to find out how plasma influences the crosstalk between skin and immune cells. A co-culture of the human keratinocyte cell line HaCaT and the human monocyte cell line THP-1 was conducted, which is a very common method to study their crosstalk.[36,66] For the first time the crosstalk between human keratinocytes and monocytes after plasma treatment was investigated. To simulate a pathogens invasion in this co-culture LPS was added, which could therefore simulate a plasma treatment of a chronic wound in a very simple manner. Cytokine profiling was conducted after plasma treatment alone or plasma and LPS treatment. Besides the co-culture HaCaT and THP-1 mono-cultures were investigated, too. After treatment, the cells were incubated for 24 hours.
The secretion of 12 cytokines was analyzed, whereas only four (IL-6, IL-8, TNFα and GM-CSF) were changed due to treatment. The THP-1 mono-culture released none of the investigated cytokines due to a plasma treatment alone (which was also shown by[12]), whereas plasma and LPS together induced a release of IL-6, IL-8 and TNFα. The mono-cultured keratinocytes

showed the identical secretion profile as discussed in paragraph 4.1 without co-culture. A 180 s plasma treatment induced a significantly increased release of IL-6 in the HaCaT mono-culture while the three other molecules (IL-8, TNFα and GM-CSF) were not regulated anymore after 24 hours. However, the treatment with LPS in combination of plasma induced highly augmented secretions of IL-6, IL-8 and GM-CSF. These enormous secretions caused by LPS, were due to its property of activating the production of various cytokines.[68] During co-culturing the cells secreted similar amounts as they did in mono-culture. However, plasma treatment alone did only significantly change the IL-6 release in co-culture, which seems to be induced by the keratinocytes. A treatment with plasma in combination of LPS induced an amplified secretion of IL-6, -8 and GM-CSF in co-cultured cells. The concentrations of these mediators were higher in co-culture than mono-culture, because both cell types secreted them. For these three mediators (IL-6, -8 and GM-CSF) it was shown that the cells did not change their secretion profile during co-culture after plasma treatment.

Interestingly, TNFα was only secreted by THP-1 mono-cultured cells after LPS stimulation and plasma treatment. It is known that TNFα can be induced by LPS but it is also known that it can be blocked or suppressed by anti-inflammatory cytokines.[20] In the co-culture the secretion of TNFα was probably inhibited by the presence of the keratinocytes. While a low level of TNFα can promote wound healing high levels are known to impair reepithelization.[3] This suppression of TNFα could therefore stimulate the wound healing process.

It was shown that co-cultured cells mainly behaved as mono-cultured cells and the crosstalk was not inhibited. However, it could also be shown that the expression of TNFα was suppressed which could lead to a positive instead of a negative stimulus for wounds.

5 Outlook

The interest for the application of plasma for wound healing increased in the last years. However, not much is known about the intracellular mechanisms during plasma treatments. First studies showed, that plasma can affect cell signaling pathways in fibroblasts or immune cells. Although keratinocytes are damaged during injuries and are involved in wound healing no studies about cell signaling by plasma have been conducted, yet. The aim of the present work was to investigate the impact of plasma on cell signaling in the human keratinocyte cell line HaCaT. It reveals the activation of various cell signaling molecules by plasma. And for the first time it was shown which compounds of plasma could cause the cellular responses. It was also shown that the crosstalk between (i) treated and untreated keratinocytes and (ii) monocytes and keratinocytes was hardly changed by plasma.

Due to the fact that plasma medicine is a very young research field, a lot of further investigations have to be done. Screenings of the transcriptome, proteome and secretome are necessary to understand the cellular responses after plasma treatment. Furthermore, the impact of plasma on other cell types, at least fibroblasts, melanocytes, monocytes, macrophages and neutrophils should be sufficiently studied *in vitro* (cell lines, primary cells) and *ex vivo* (suction blisters, punch biopsies, blood). In the present work it was shown, that many genes and proteins which were activated by plasma induce migration, angiogenesis, proliferation and the recruitment of immune cells. Therefore, the application of plasma *in vivo* is necessary to study these effects after plasma treatment. Animal models could be used for these experiments but due to the fact that plasma should be used to heal wounds in humans more clinical trials are required, too.

It is also known that different plasma sources induce different cell responses. Therefore, it is crucial to research which plasma generated species are beneficial for cells and how plasma sources can be modified to produce these stimulating species.

6 Summary

There is a growing interest in the application of non-thermal atmospheric pressure plasma for the treatment of wounds. Due to the generation of various ROS and RNS, UV radiation and electric fields plasma is a very promising tool which can stimulate skin and immune cells. However, not much is known about the mammalian cell responses after plasma treatments on a molecular level. The present work focusses on the impact of plasma on cell signaling in the human keratinocyte cell line HaCaT by using the methods DNA microarray, qPCR, ELISA and flow cytometry. Here, cell signaling mediators such as cytokines and growth factors which could promote wound healing by enhancing angiogenesis, reepithelization, migration and proliferation were of major interest. Additionally, the crosstalk between keratinocytes and monocytes was studied using a co-culture.

For the first time extensive investigations on the impact of plasma on cell signaling in human keratinocytes were conducted. The most prominent cytokines and growth factors which were regulated by plasma at gene and protein level were VEGF-A, GM-CSF, HB-EGF, IL-8, and IL-6. The latter was not activated due to the JAK/STAT-pathway but probably by a combined activation of MAPK- and PI3K/Akt-pathways. By the use of conditioned medium it was found out that ROS and RNS generated directly after plasma treatment induced larger effects on cell signaling in keratinocytes than the subsequently secreted growth factors and cytokines. Furthermore, monocytes and keratinocytes hardly altered their secretion profiles in co-culture. From these results it is deduced that the plasma generated reactive species are the main actors during cell signaling. In order to differentiate the impact of ROS and RNS on the cellular response the ambience of the plasma effluent was controlled, varying the ambient gas composition from pure nitrogen to pure oxygen. Thereby a first step towards the attribution of the cellular response to specific plasma generated reactive species was achieved. While

IL-6 expression correlated with ROS generated by the plasma source, the cell signaling mediators VEGF-A, GM-CSF and HB-EGF were significantly changed by RONS. Above all hydrogen peroxide was found to play a dominant role for observed cell responses.

In summary, plasma activates wound healing related cell signaling mediators as cytokines and growth factors in keratinocytes. It was also shown that the generated reactive species mainly induced cell signaling. For the first time cell responses can be correlated to ROS and RONS in plasma treated cells. These results underline the potential of non-thermal atmospheric pressure plasma sources for their applications in wound treatment.

7 Zusammenfassung

Das Interesse an einer Anwendung von nichtthermalem Atmosphärendruckplasma zur Behandlung von Wunden wächst stetig. Durch die Erzeugung von reaktiven Sauerstoffspezies (ROS), reaktiven Stickstoffspezies (RNS), UV-Strahlung und elektrischen Feldern ist Plasma für eine medizinische Anwendung äußerst vielversprechend. Durch Plasmabehandlungen können sowohl Haut- als auch Immunzellen stimuliert werden. Dennoch sind Zellantworten auf molekularer Ebene weitestgehend unerforscht. In der vorliegenden Arbeit wurde der Einfluss von Plasma auf die Signaltransduktion in der humanen Keratinozytenzelllinie HaCaT mittels DNA microarray, qPCR, ELISA und Durchflußzytometer untersucht. Der Fokus lag hierbei auf Signalmolekülen wie Zytokinen und Wachstumsfaktoren, welche den Wundheilungsprozess durch Förderung von Angiogenese, Reepithelisierung, Migration und Proliferation stimulieren können. Zur Untersuchung der Kommunikation zwischen Haut- und Immunzellen wurde zusätzlich eine Ko-Kultur mit Keratinozyten (HaCaT Zelllinie) und Monozyten (THP-1 Zelllinie) durchgeführt.

Erstmalig wurden umfangreiche Untersuchungen zur Signaltransduktion in einer humanen Keratinozytenzelllinie nach Plasmabehandlung durchgeführt. Die wichtigsten Zytokine und Wachstumsfaktoren, welche sowohl auf Gen- als auch auf Proteinebene durch Plasma aktiviert wurden, sind VEGF-A, HB-EGF, GM-CSF, IL-8 und IL-6. Es konnte zudem gezeigt werden, dass IL-6 nicht über den JAK/STAT-Signalweg, sondern vermutlich durch eine kombinierte Aktivierung vom MAPK- und PI3K/Akt-Signalwegen stimuliert wurde. Des Weiteren konnte mittels konditioniertem Medium gezeigt werden, dass die plasmagenerierten ROS und RNS einen höheren Einfluss auf die Signaltransduktion hatten, als die darauffolgende Ausschüttung von Zytokinen und Wachstumsfaktoren. Die Sezernierung von Signalmolekülen in den ko-kultivierten Keratinozyten und Monozyten war im Vergleich zu den jeweiligen Mono-Kulturen kaum verändert. Dies ist ein weiteres Indiz dafür,

dass die durch Plasma gebildeten reaktiven Spezies die Hauptrolle bei der Signaltransduktion während der Plasmabehandlung spielen. Um den Einfluss von ROS und RNS in der Zellantwort zu differenzieren wurde die Umgebung vom Plasmaeffluenten kontrolliert. Die Zusammensetzung des Umgebungsgases wurde von reinem Sauerstoff zu reinem Stickstoff variiert. Hierdurch wurde ein erster Schritt hin zur Zuordnung von Zellantworten zu plasmagenerierten reaktiven Spezies gemacht. Während die IL-6 Expression mit ROS korreliert, werden VEGF-A, GM-CSF und HB-EGF durch RONS stimuliert. Darüber hinaus wurde Wasserstoffperoxid, welches durch Plasma gebildet wird, als besonders bedeutend für die Zellantwort identifiziert.

Zusammenfassend konnte gezeigt werden, dass Plasma wichtige Signalmoleküle für die Wundheilung in Keratinozyten aktiviert. Die Zytokine und Wachstumsfaktoren wurden größtenteils durch die reaktiven Spezies stimuliert. Zudem konnte erstmalig gezeigt werden, dass die plasmainduzierten Zellantworten mit ROS und RONS korrelieren. Die erzielten Ergebnisse unterstreichen das Potential von Atmosphärendruck-Plasmaquellen für die Anwendung in der Wundbehandlung.

References

[1] D. M. Ansell, K. A. Holden, and M. J. Hardman. Animal models of wound repair: Are they cutting it? *Experimental Dermatology*, 21(8):581–585, 2012.

[2] S. Arndt, P. Unger, E. Wacker, T. Shimizu, J. Heinlin, Y.-F. Li, H. M. Thomas, G. E. Morfill, J. L. Zimmermann, A.-K. Bosserhoff, et al. Cold Atmospheric Plasma (CAP) Changes Gene Expression of Key Molecules of the Wound Healing Machinery and Improves Wound Healing In Vitro and In Vivo. *PloS one*, 8(11): doi 10.1371/journal.pone.0079325, 2013.

[3] S. Barrientos, O. Stojadinovic, M. S. Golinko, H. Brem, and M. Tomic-Canic. Growth factors and cytokines in wound healing. *Wound Repair and Regeneration*, 16(5):585–601, 2008.

[4] A. Barton, K. Wende, L. Bundscherer, K. Weltmann, U. Lindequist, and K. Masur. Non-Thermal Atmospheric Pressure Plasma Treatment of Human Cells: The Effect of Ambient Conditions. In *ISPC 21 Conference Proceedings*, 2013.

[5] A. Barton, K. Wende, L. Bundscherer, S. Hasse, A. Schmidt, S. Bekeschus, K.-D. Weltmann, U. Lindequist, and K. Masur. Non-thermal plasma increases expression of wound healing related genes in a keratinocyte cell line. *Plasma Medicine*, 2013, submitted.

[6] S. Blackert, B. Haertel, K. Wende, T. von Woedtke, and U. Lindequist. Influence of non-thermal atmospheric pressure plasma on cellular structures and processes in human keratinocytes (HaCaT). *Journal of Dermatological Science*, 70(3):173–181, 2013.

[7] D. Bliss. National Cancer Institute. *http://visualsonline.cancer.gov/ details.cfm?imageid=4366*, 2013.11.06.

[8] B. Boekema, S. Hofmann, B. van Ham, P. Bruggeman, and E. Middelkoop. Antibacterial plasma at safe levels for skin cells. *Journal of Physics D: Applied Physics*, 46(42): doi 10.1088/0022–3727/46/42/422001, 2013.

[9] S. Braunstein, G. Kaplan, A. B. Gottlieb, M. Schwartz, G. Walsh, R. M. Abalos, T. T. Fajardo, L. S. Guido, and J. G. Krueger. GM-CSF activates regenerative epidermal growth and stimulates keratinocyte proliferation in human skin in vivo. *Journal of Investigative Dermatology*, 103(4):601–604, 1994.

[10] K. Breuhahn, A. Mann, G. Müller, A. Wilhelmi, P. Schirmacher, A. Enk, and M. Blessing. Epidermal overexpression of granulocyte-macrophage colony-stimulating factor induces both keratinocyte proliferation and apoptosis. *Cell Growth and Differentiation*, 11(2):111–121, 2000.

[11] B. Bruno, L. Giaccone, M. Rotta, K. Anderson, and M. Boccadoro. Novel targeted drugs for the treatment of multiple myeloma: from bench to bedside. *Leukemia*, 19(10):1729–1738, 2005.

[12] L. Bundscherer. *Immune-modulatory effects of non-thermal plasma*. PhD thesis, Ernst-Moritz-Arndt-University Greifswald, 2013.

[13] L. Bundscherer, K. Wende, K. Ottmüller, A. Barton, A. Schmidt, S. Bekeschus, S. Hasse, K.-D. Weltmann, K. Masur, and U. Lindequist. Impact of non-thermal plasma treatment on MAPK signaling pathways of human immune cell lines. *Immunobiology*, 218(10):1248–1255, 2013.

[14] W. Chen, Q. Tang, M. S. Gonzales, G. T. Bowden, et al. Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. *Oncogene*, 20(29):3921–3926, 2001.

[15] J. Cisowski, A. Łoboda, A. Józkowicz, S. Chen, A. Agarwal, and J. Dulak. Role of heme oxygenase-1 in hydrogen peroxide-induced VEGF synthesis:

effect of HO-1 knockout. *Biochemical and biophysical research communications*, 326(3):670–676, 2005.

[16] R. A. Clark, K. Ghosh, and M. G. Tonnesen. Tissue engineering for cutaneous wounds. *Journal of Investigative Dermatology*, 127(5):1018–1029, 2007.

[17] R. Craig, A. Larkin, A. M. Mingo, D. J. Thuerauf, C. Andrews, P. M. McDonough, and C. C. Glembotski. p38 MAPK and NF-κB Collaborate to Induce Interleukin-6 Gene Expression and Release Evidence For A Cytoprotective Autocrine Signaling Pathway In A Cardiac Myocyte Model System. *Journal of Biological Chemistry*, 275(31):23814–23824, 2000.

[18] J. D. Crawford. Vasculogenic Cytokines in Wound Healing. *BioMed research international*, 2013:190486, 2013.

[19] B. M. Delavary, W. M. van der Veer, M. van Egmond, F. B. Niessen, and R. H. Beelen. Macrophages in skin injury and repair. *Immunobiology*, 216(7):753–762, 2011.

[20] C. A. Dinarello. Proinflammatory cytokines. *CHEST Journal*, 118(2):503–508, 2000.

[21] S. A. Eming, M. Koch, A. Krieger, B. Brachvogel, S. Kreft, L. Bruckner-Tuderman, T. Krieg, J. D. Shannon, and J. W. Fox. Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. *Journal of Proteome Research*, 9(9):4758–4766, 2010.

[22] Y. Fang, S.-J. Gong, Y.-H. Xu, B. Hambly, and S. Bao. Impaired cutaneous wound healing in granulocyte/macrophage colony-stimulating factor knockout mice. *British Journal of Dermatology*, 157(3):458–465, 2007.

[23] Y. Fang, J. Shen, M. Yao, K. Beagley, B. Hambly, and S. Bao. Granulocyte-macrophage colony-stimulating factor enhances wound healing in diabetes

via upregulation of proinflammatory cytokines. *British Journal of Dermatology*, 162(3):478–486, 2010.

[24] H. Feng, R. Wang, P. Sun, H. Wu, Q. Liu, J. Fang, W. Zhu, F. Li, and J. Zhang. A study of eukaryotic response mechanisms to atmospheric pressure cold plasma by using Saccharomyces cerevisiae single gene mutants. *Applied Physics Letters*, 97(13):131501 – 131501–3, 2010.

[25] J. Feng, O. N. El-Assal, and G. E. Besner. Heparin-binding EGF-like growth factor (HB-EGF) and necrotizing enterocolitis. *Seminars in Pediatric Surgery*, 14(3):167–174, 2005.

[26] B. Fiser, B. Jójárt, I. G. Csizmadia, and B. Viskolcz. Glutathione–Hydroxyl Radical Interaction: A Theoretical Study on Radical Recognition Process. *PloS one*, 8(9): doi 10.1371/journal.pone.0073652, 2013.

[27] H. J. Forman, H. Zhang, and A. Rinna. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular aspects of medicine*, 30(1):1–12, 2009.

[28] T. Fukawa, H. Kajiya, S. Ozeki, T. Ikebe, and K. Okabe. Reactive oxygen species stimulates epithelial mesenchymal transition in normal human epidermal keratinocytes via TGF-beta secretion. *Experimental Cell Research*, 318(15):1926–1932, 2012.

[29] R. J. Goldston and P. H. Rutherford. *Plasmaphysik: eine Einführung*. Vieweg, 1998.

[30] D. B. Graves. The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. *Journal of Physics D: Applied Physics*, 45(26):263001, 2012.

[31] B. Haertel, M. Hähnel, S. Blackert, K. Wende, T. Woedtke, and U. Lindequist. Surface molecules on HaCaT keratinocytes after interaction with

non-thermal atmospheric pressure plasma. *Cell Biology International*, 36(12):1217–1222, 2012.

[32] B. Haertel, K. Wende, T. von Woedtke, K. D. Weltmann, and U. Lindequist. Non-thermal atmospheric-pressure plasma can influence cell adhesion molecules on HaCaT-keratinocytes. *Experimental Dermatology*, 20(3):282–284, 2011.

[33] K. Hande. Etoposide: four decades of development of a topoisomerase II inhibitor. *European Journal of Cancer*, 34(10):1514–1521, 1998.

[34] D. A. Harrison. The Jak/STAT pathway. *Cold Spring Harbor Perspectives in Biology*, 4(3): doi 10.1101/cshperspect.a011205, 2012.

[35] J. Heinlin, G. Isbary, W. Stolz, G. Morfill, M. Landthaler, T. Shimizu, B. Steffes, T. Nosenko, J. Zimmermann, and S. Karrer. Plasma applications in medicine with a special focus on dermatology. *Journal of the European Academy of Dermatology and Venereology*, 25(1):1–11, 2011.

[36] J. Hennen, P. Aeby, C. Goebel, T. Schettgen, A. Oberli, M. Kalmes, and B. Blömeke. Cross talk between keratinocytes and dendritic cells: impact on the prediction of sensitization. *Toxicological Sciences*, 123(2):501–510, 2011.

[37] G. Imokawa, Y. Yada, M. Kimura, and N. Morisaki. Granulocyte/macrophage colony-stimulating factor is an intrinsic keratinocyte-derived growth factor for human melanocytes in UVA-induced melanosis. *Biochemical Journal*, 313:625–631, 1996.

[38] G. Isbary, G. Morfill, H. Schmidt, M. Georgi, K. Ramrath, J. Heinlin, S. Karrer, M. Landthaler, T. Shimizu, B. Steffes, et al. A first prospective randomized controlled trial to decrease bacterial load using cold atmospheric argon plasma on chronic wounds in patients. *British Journal of Dermatology*, 163(1):78–82, 2010.

[39] N. S. Ismail, E. A. Pravda, D. Li, S.-C. Shih, and S. M. Dallabrida. Angiopoietin-1 reduces H2O2-induced increases in reactive oxygen species and oxidative damage to skin cells. *Journal of Investigative Dermatology*, 130(5):1307–1317, 2010.

[40] L. Jatsch. Etablierung eines HPRT-Genmutationstests in V79-Zellen für die Untersuchung von Atmosphärendruck-Niedertemperaturplasmen. Diploma Thesis, Ernst-Moritz-Arndt-University Greifswald, 2013.

[41] N. R. Johnson and Y. Wang. Controlled delivery of heparin-binding EGF-like growth factor yields fast and comprehensive wound healing. *Journal of Controlled Release*, 166(2):124–129, 2012.

[42] M. Kaufmann. *Plasmaphysik und Fusionsforschung*. Teubner, Stuttgart Leipzig Wiesbaden, 2003.

[43] K. Kayakabe, T. Kuroiwa, N. Sakurai, H. Ikeuchi, A. T. Kadiombo, T. Sakairi, T. Matsumoto, A. Maeshima, K. Hiromura, and Y. Nojima. Interleukin-6 promotes destabilized angiogenesis by modulating angiopoietin expression in rheumatoid arthritis. *Rheumatology*, 51(9):1571–1579, 2012.

[44] J. Kim, R. M. Adam, and M. R. Freeman. Trafficking of nuclear heparin-binding epidermal growth factor–like growth factor into an epidermal growth factor receptor–dependent autocrine loop in response to oxidative stress. *Cancer Research*, 65(18):8242–8249, 2005.

[45] M. G. Kong, G. Kroesen, G. Morfill, T. Nosenko, T. Shimizu, J. Van Dijk, and J. Zimmermann. Plasma medicine: an introductory review. *New Journal of Physics*, 11(11):115012, 2009.

[46] M. A. Lieberman and A. J. Lichtenberg. *Principles of plasma discharges and materials processing*. Cambridge Univ Press, 1994.

[47] J. Liebmann, J. Scherer, N. Bibinov, P. Rajasekaran, R. Kovacs, R. Gesche, P. Awakowicz, and V. Kolb-Bachofen. Biological effects of nitric oxide generated by an atmospheric pressure gas-plasma on human skin cells. *Nitric Oxide*, 24(1):8–16, 2011.

[48] K. J. Livak and T. D. Schmittgen. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-delta delta CT Method. *Nature Protocols*, 25(4):402–408, 2001.

[49] A. E. K. Loo, R. Ho, and B. Halliwell. Mechanism of hydrogen peroxide-induced keratinocyte migration in a scratch-wound model. *Free Radical Biology and Medicine*, 51(4):884–892, 2011.

[50] A. E. K. Loo, Y. T. Wong, R. Ho, M. Wasser, T. Du, W. T. Ng, and B. Halliwell. Effects of Hydrogen Peroxide on Wound Healing in Mice in Relation to Oxidative Damage. *PloS one*, 7(11):e49215, 2012.

[51] C. M. Loyd, D. Diaconu, W. Fu, G. N. Adams, E. Brandt, D. A. Knutsen, J. A. Wolfram, T. S. McCormick, and N. L. Ward. Transgenic overexpression of keratinocyte-specific VEGF and Ang1 in combination promotes wound healing under nondiabetic but not diabetic conditions. *International Journal of Clinical and Experimental Pathology*, 5(1):1–11, 2012.

[52] A. Mann, K. Breuhahn, P. Schirmacher, and M. Blessing. Keratinocyte-Derived Granulocyte- Macrophage Colony Stimulating Factor Accelerates Wound Healing: Stimulation of Keratinocyte Proliferation, Granulation Tissue Formation, and Vascularization. *Journal of Investigative Dermatology*, 117(6):1382–1390, 2001.

[53] L. D. Martin, T. M. Krunkosky, J. A. Voynow, and K. B. Adler. The role of reactive oxygen and nitrogen species in airway epithelial gene expression. *Environmental health perspectives*, 106(5):1197, 1998.

[54] P. Martin. Wound healing–aiming for perfect skin regeneration. *Science*, 276(5309):75–81, 1997.

[55] P. Martin and S. J. Leibovich. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends in cell biology*, 15(11):599–607, 2005.

[56] C. Mathay, S. Giltaire, F. Minner, E. Bera, M. Hérin, and Y. Poumay. Heparin-binding EGF-like growth factor is induced by disruption of lipid rafts and oxidative stress in keratinocytes and participates in the epidermal response to cutaneous wounds. *Journal of Investigative Dermatology*, 128(3):717–727, 2007.

[57] S. Merighi, S. Gessi, K. Varani, D. Fazzi, and P. A. Borea. Hydrogen sulfide modulates the release of nitric oxide and VEGF in human keratinocytes. *Pharmacological Research*, 66(5):428–436, 2012.

[58] H.-R. Metelmann, T. von Woedtke, R. Bussiahn, K.-D. Weltmann, M. Rieck, R. Khalili, F. Podmelle, and P. D. Waite. Experimental Recovery of CO2-Laser Skin Lesions by Plasma Stimulation. *American Journal of Cosmetic Surgery*, 29(1):52–56, 2012.

[59] Y. Miyazaki, Y. Shinomura, S. Tsutsui, Y. Yasunaga, S. Zushi, S. Higashiyama, N. Taniguchi, and Y. Matsuzawa. Oxidative stress increases gene expression of heparin-binding EGF-like growth factor and amphiregulin in cultured rat gastric epithelial cells. *Biochemical and biophysical research communications*, 226(2):542–546, 1996.

[60] R. Moseley, J. R. Hilton, R. J. Waddington, K. G. Harding, P. Stephens, and D. W. Thomas. Comparison of oxidative stress biomarker profiles between acute and chronic wound environments. *Wound repair and regeneration*, 12(4):419–429, 2004.

[61] P. Muranyi, J. Wunderlich, and M. Heise. Influence of relative gas humidity on the inactivation efficiency of a low temperature gas plasma. *Journal of Applied Microbiology*, 104(6):1659–1666, 2008.

[62] P. J. Murray. The JAK-STAT signaling pathway: input and output integration. *The Journal of Immunology*, 178(5):2623–2629, 2007.

[63] A. V. Nastuta, I. Topala, C. Grigoras, V. Pohoata, and G. Popa. Stimulation of wound healing by helium atmospheric pressure plasma treatment. *Journal of Physics D: Applied Physics*, 44(10):105204, 2011.

[64] R. Negrao, R. Costa, D. Duarte, T. T. Gomes, I. Azevedo, and R. Soares. Different effects of catechin on angiogenesis and inflammation depending on VEGF levels. *The Journal of Nutritional Biochemistry*, 24(2):435–444, 2013.

[65] K. Ottmüller. Non-thermal plasma-induced MAPK and p53 signaling in keratinocytes and immune cells. Diploma Thesis, Ernst-Moritz-Arndt-University Greifswald, 2012.

[66] L. J. Park, S. M. Ju, H. Y. Song, J. A. Lee, M. Y. Yang, Y. H. Kang, H. J. Kwon, T. Kim, S. Y. Choi, and J. Park. The enhanced monocyte adhesiveness after UVB exposure requires ROS and NF-kappaB signaling in human keratinocyte. *Journal of Biochemistry and Molecular Biology*, 39(5):618, 2006.

[67] J. S. Reis, C. A. V. Amaral, C. M. O. Volpe, J. S. Fernandes, E. A. Borges, C. A. Isoni, P. M. F. d. Anjos, and J. A. N. Machado. Oxidative stress and interleukin-6 secretion during the progression of type 1 diabetes. *Arquivos Brasileiros de Endocrinologia & Metabologia*, 56(7):441–448, 2012.

[68] D. G. Remick and L. Villarete. Regulation of cytokine gene expression by reactive oxygen and reactive nitrogen intermediates. *Journal of Leukocyte Biology*, 59(4):471–475, 1996.

[69] H.-O. Rennekampff, J. F. Hansbrough, V. Kiessig, C. Doré, M. Sticherling, and J.-M. Schröder. Bioactive interleukin-8 is expressed in wounds and enhances wound healing. *Journal of Surgical Research*, 93(1):41–54, 2000.

[70] S. Reuter, H. Tresp, K. Wende, M. U. Hammer, J. Winter, K. Masur, A. Schmidt-Bleker, and K.-D. Weltmann. From RONS to ROS: Tailoring plasma jet treatment of skin cells. *Plasma Science & Technology*, 40(11):2986–2993, 2012.

[71] S. Reuter, J. Winter, A. Schmidt-Bleker, H. Tresp, M. U. Hammer, and K.-D. Weltmann. Controlling the ambient air affected reactive species composition in the effluent of an argon plasma jet. *IEEE Transactions on Plasma Science*, 40(11):2788–2794, 2012.

[72] M. Schäfer and S. Werner. Oxidative stress in normal and impaired wound repair. *Pharmacological research*, 58(2):165–171, 2008.

[73] T. H. Schiebler. *Anatomie*. Springer DE, 2005.

[74] A. Schmidt. 2013, in preparation.

[75] A. Schmidt, K. Wende, S. Bekeschus, L. Bundscherer, A. Barton, K. Ottmüller, K.-D. Weltmann, and K. Masur. Non-thermal plasma treatment is associated with changes in transcriptome of human epithelial skin cells. *Free Radical Research*, 47(8):1–32, 2013.

[76] A. Schmidt-Bleker, J. Winter, S. Iseni, M. Dünnbier, K.-D. Weltmann, and S. Reuter. Reactive Species Output of a Plasma Jet with Shielding Gas Device - Combination of FTIR Absorption Spectroscopy and Gas Phase Modelling. 2013, in preparation.

[77] T. Shirahama and C. Sakakura. Overexpression of cyclooxygenase-2 in squamous cell carcinoma of the urinary bladder. *Clinical Cancer Research*, 7(3):558–561, 2001.

[78] Sigma-Aldrich. *Material safety data sheet; Hydrogen peroxide solution.*

[79] R. Slusarz, G. Gadomska, M. Biercewicz, L. Grzelak, M. T. Szewczyk, D. Rość, and W. Beuth. The influence of selected demographic factors and wound location on the concentration of vascular endothelial growth factor (VEGF-A) in the wound healing process after neurosurgery: Brief report. *Wound Repair and Regeneration*, 20(5):667–675, 2012.

[80] W. Sterry. *Kurzlehrbuch Dermatologie*. Georg Thieme Verlag, 2011.

[81] H. Sugiura, X. Liu, T. Kobayashi, S. Togo, R. F. Ertl, S. Kawasaki, K. Kamio, X. Q. Wang, L. Mao, L. Shen, et al. Reactive nitrogen species augment fibroblast-mediated collagen gel contraction, mediator production, and chemotaxis. *American journal of respiratory cell and molecular biology*, 34(5):592–599, 2006.

[82] R. S. Tipa and G. M. Kroesen. Plasma-stimulated wound healing. *Plasma Science, IEEE Transactions on*, 39(11):2978–2979, 2011.

[83] H. Tresp, M. U. Hammer, K.-D. Weltmann, and S. Reuter. Effects of atmosphere composition and liquid type on plasma generated reactive species in biologically relevant solutions. *Plasma Medicine*, 2013, submitted.

[84] F. A. Wagener, C. E. Carels, and D. Lundvig. Targeting the Redox Balance in Inflammatory Skin Conditions. *International Journal of Molecular Sciences*, 14(5):9126–9167, 2013.

[85] Z. Wang, Y. Wang, F. Farhangfar, M. Zimmer, and Y. Zhang. Enhanced Keratinocyte Proliferation and Migration in Co-culture with Fibroblasts. *PloS one*, 7(7):e40951, 2012.

[86] K.-D. Weltmann, E. Kindel, R. Brandenburg, C. Meyer, R. Bussiahn, C. Wilke, and T. von Woedtke. Atmospheric Pressure Plasma Jet for Medical Therapy: Plasma Parameters and Risk Estimation. *Contributions to Plasma Physics*, 49(9):631–640, 2009.

[87] K. Wende, A. Barton, S. Bekeschus, L. Bundscherer, A. Schmidt, K.-D. Weltmann, and K. Masur. Proteomic tools to characterize non-thermal plasma effects in eukaryotic cells. *Plasma Medicine*, 2013, submitted.

[88] K. Wende, S. Straßenburg, B. Haertel, M. Harms, S. Holtz, A. Barton, K. Masur, T. von Woedtke, and U. Lindequist. Atmospheric pressure plasma jet treatment evokes transient oxidative stress in HaCaT keratinocytes and influences cell physiology. *Cell Biology International*, page doi 10.1002/CBIN.10200, 2013.

[89] S. Werner and R. Grose. Regulation of Wound Healing by Growth Factors and Cytokines. *Physiological Reviews*, 83(3):835–870, 2003.

[90] C. Wetzler, H. Kämpfer, J. Pfeilschifter, and S. Frank. Keratinocyte-Derived Chemotactic Cytokines: Expressional Modulation by Nitric Oxide in Vitro and during Cutaneous Wound Repair in Vivo. *Biochemical and biophysical research communications*, 274(3):689–696, 2000.

[91] J. Winter, K. Wende, K. Masur, S. Iseni, M. Dünnbier, M. Hammer, H. Tresp, K. Weltmann, and S. Reuter. Feed gas humidity: a vital parameter affecting a cold atmospheric-pressure plasma jet and plasma-treated human skin cells. *Journal of Physics D: Applied Physics*, 46(29):295401, 2013.

[92] J. Winter. 2013, in preparation.

[93] M. B. Witte and A. Barbul. Role of nitric oxide in wound repair. *The American Journal of Surgery*, 183(4):406–412, 2002.

[94] L. Yang, L. Wang, H.-K. Lin, P.-Y. Kan, S. Xie, M.-Y. Tsai, P.-H. Wang, Y.-T. Chen, and C. Chang. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. *Biochemical and biophysical research communications*, 305(3):462–469, 2003.

[95] L. Yao, D.-N. Hu, M. Chen, and S.-S. Li. Subtoxic levels hydrogen peroxide-induced expression of interleukin-6 by epidermal melanocytes. *Archives of dermatological research*, 304(10):831–838, 2012.

[96] A. Yoshimura. Signal transduction of inflammatory cytokines and tumor development. *Cancer Science*, 97(6):439–447, 2006.

[97] P. Zhan, Q. Qian, and L.-K. Yu. Prognostic value of COX-2 expression in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Journal of Thoracic Disease*, 5(1):40–47, 2013.

[98] J. Zhang, G. Johnston, B. Stebler, and E. T. Keller. Hydrogen Peroxide Activates NF κ B and the Interleukin-6 Promoter Through NF κ B-Inducing Kinase. *Antioxidants and Redox Signaling*, 3(3):493–504, 2001.

[99] S.-Q. Zhang, X. Luo, S. Yang, J.-L. Liu, C.-J. Yang, X.-Y. Yin, H.-L. Huang, and X.-J. Zhang. Autoinhibition of IL-15 Expression in KC Cells is ERK1/2 and PI3K Dependent. *Scandinavian journal of immunology*, 68(4):397–404, 2008.

List of Figures

List of Tables

8 Appendix

PCR program Qiagen:

name	cycle	analysis mode	target (°C)	acquisition mode	hold (mm:ss)	ramp rate (°C s^{-1})
heat activation	1	none	95	none	10:00	4.4
PCR cycle	45	quantification	95	none	00:15	1
			60	single	01:00	1
melt curve	1	melting curve	60	none	00:15	4.4
			95	continuous		0.03

PCR program Roche:

name	cycle	analysis mode	target (°C)	acquisition mode	hold (mm:ss)	ramp rate (°C s^{-1})
pre-incubation	1	none	95	none	10:00	4.4
amplification	45	quantification	95	none	00:10	4.4
			60	none	00:30	2.2
			72	single	00:01	4.4
cooling	1	none	40	none	00:30	2.2

Analyzed genes qPCR array by Qiagen:

Pos.	GeneBank	Symbol	Description
A01	NM_001613	ACTA2	Actin, alpha 2, smooth muscle, aorta
A02	NM_005159	ACTC1	Actin, alpha, cardiac muscle 1
A03	NM_001146	ANGPT1	Angiopoietin 1
A04	NM_002982	CCL2	Chemokine (C-C motif) ligand 2
A05	NM_006273	CCL7	Chemokine (C-C motif) ligand 7
A06	NM_000074	CD40LG	CD40 ligand
A07	NM_004360	CDH1	Cadherin 1, type 1, E-cadherin (epithelial)
A08	NM_021110	COL14A1	Collagen, type XIV, alpha 1
A09	NM_000088	COL1A1	Collagen, type I, alpha 1
A10	NM_000089	COL1A2	Collagen, type I, alpha 2
A11	NM_000090	COL3A1	Collagen, type III, alpha 1
A12	NM_001845	COL4A1	Collagen, type IV, alpha 1
B01	NM_000091	COL4A3	Collagen, type IV, alpha 3 (Goodpasture antigen)
B02	NM_000093	COL5A1	Collagen, type V, alpha 1
B03	NM_000393	COL5A2	Collagen, type V, alpha 2
B04	NM_015719	COL5A3	Collagen, type V, alpha 3
B05	NM_000758	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
B06	NM_000759	CSF3	Colony stimulating factor 3 (granulocyte)
B07	NM_001901	CTGF	Connective tissue growth factor
B08	NM_001904	CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa
B09	NM_001911	CTSG	Cathepsin G
B10	NM_000396	CTSK	Cathepsin K
B11	NM_001333	CTSL2	Cathepsin L2
B12	NM_001511	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
C01	NM_005409	CXCL11	Chemokine (C-X-C motif) ligand 11

C02	NM_002089	CXCL2	Chemokine (C-X-C motif) ligand 2
C03	NM_002994	CXCL5	Chemokine (C-X-C motif) ligand 5
C04	NM_001963	EGF	Epidermal growth factor
C05	NM_005228	EGFR	Epidermal growth factor receptor
C06	NM_000129	F13A1	Coagulation factor XIII, A1 polypeptide
C07	NM_001993	F3	Coagulation factor III (thromboplastin, tissue factor)
C08	NM_000508	FGA	Fibrinogen alpha chain
C09	NM_004465	FGF10	Fibroblast growth factor 10
C10	NM_002006	FGF2	Fibroblast growth factor 2 (basic)
C11	NM_002009	FGF7	Fibroblast growth factor 7
C12	NM_001945	HBEGF	Heparin-binding EGF-like growth factor
D01	NM_000601	HGF	Hepatocyte growth factor (hepapoietin A; scatter factor)
D02	NM_000619	IFNG	Interferon, gamma
D03	NM_000618	IGF1	Insulin-like growth factor 1 (somatomedin C)
D04	NM_000572	IL10	Interleukin 10
D05	NM_000576	IL1B	Interleukin 1, beta
D06	NM_000586	IL2	Interleukin 2
D07	NM_000589	IL4	Interleukin 4
D08	NM_000600	IL6	Interleukin 6 (interferon, beta 2)
D09	NM_002184	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)
D10	NM_181501	ITGA1	Integrin, alpha 1
D11	NM_002203	ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
D12	NM_002204	ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
E01	NM_000885	ITGA4	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
E02	NM_002205	ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
E03	NM_000210	ITGA6	Integrin, alpha 6
E04	NM_002210	ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
E05	NM_002211	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
E06	NM_000212	ITGB3	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
E07	NM_002213	ITGB5	Integrin, beta 5
E08	NM_000888	ITGB6	Integrin, beta 6
E09	NM_002745	MAPK1	Mitogen-activated protein kinase 1
E10	NM_002746	MAPK3	Mitogen-activated protein kinase 3
E11	NM_002415	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
E12	NM_002421	MMP1	Matrix metallopeptidase 1 (interstitial collagenase)
F01	NM_004530	MMP2	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
F02	NM_002423	MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)
F03	NM_004994	MMP9	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
F04	NM_002607	PDGFA	Platelet-derived growth factor alpha polypeptide
F05	NM_000930	PLAT	Plasminogen activator, tissue
F06	NM_002658	PLAU	Plasminogen activator, urokinase
F07	NM_002659	PLAUR	Plasminogen activator, urokinase receptor
F08	NM_000301	PLG	Plasminogen
F09	NM_000314	PTEN	Phosphatase and tensin homolog
F10	NM_000963	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
F11	NM_006908	RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
F12	NM_001664	RHOA	Ras homolog gene family, member A
G01	NM_000602	SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
G02	NM_003150	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
G03	NM_003186	TAGLN	Transgelin
G04	NM_003236	TGFA	Transforming growth factor, alpha
G05	NM_000660	TGFB1	Transforming growth factor, beta 1
G06	NM_003243	TGFBR3	Transforming growth factor, beta receptor III
G07	NM_003254	TIMP1	TIMP metallopeptidase inhibitor 1
G08	NM_000594	TNF	Tumor necrosis factor
G09	NM_003376	VEGFA	Vascular endothelial growth factor A
G10	NM_000638	VTN	Vitronectin
G11	NM_003882	WISP1	WNT1 inducible signaling pathway protein 1
G12	NM_003392	WNT5A	Wingless-type MMTV integration site family, member 5A
H01	NM_001101	ACTB	Actin, beta
H02	NM_004048	B2M	Beta-2-microglobulin
H03	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	NM_001002	RPLP0	Ribosomal protein, large, P0
H06	SA_00105	HGDC	Human Genomic DNA Contamination

H07 SA_00104	RTC	Reverse Transcription Control
H08 SA_00104	RTC	Reverse Transcription Control
H09 SA_00104	RTC	Reverse Transcription Control
H10 SA_00103	PPC	Positive PCR Control
H11 SA_00103	PPC	Positive PCR Control
H12 SA_00103	PPC	Positive PCR Control

Results of the DNA microarray (20 s; 0 % oxygen and 100 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)
3,327	NM_012369	OR2F1	olfactory receptor, family 2, subfamily F, member 1	Plasma Membrane	G-protein coupled receptor
3,232	NM_198951	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	Cytoplasm	enzyme
3,145	NM_007072	HHLA2	HERV-H LTR-associating 2	Other	other
2,779	NM_001005515	OR5H15	olfactory receptor, family 5, subfamily H, member 15	Plasma Membrane	G-protein coupled receptor
2,515	NM_022049	GPR88	G protein-coupled receptor 88	Plasma Membrane	G-protein coupled receptor
2,446	NM_016610	TLR8	toll-like receptor 8	Plasma Membrane	transmembrane receptor
2,438	NM_012113	CA14	carbonic anhydrase XIV	Plasma Membrane	enzyme
2,403	NM_012344	NTSR2	neurotensin receptor 2	Plasma Membrane	G-protein coupled receptor
2,378	NM_003140	SRY	sex determining region Y	Nucleus	transcription regulator
2,378	NM_001018036	TSHR	thyroid stimulating hormone receptor	Plasma Membrane	G-protein coupled receptor
2,344	NM_006121	KRT1	keratin 1	Cytoplasm	other
2,340	NM_000348	SRD5A2	steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2)	Cytoplasm	enzyme
2,335	NM_001003745	OR10A3	olfactory receptor, family 10, subfamily A, member 3	Plasma Membrane	G-protein coupled receptor
2,303	NM_207498	RAB44	RAB44, member RAS oncogene family	Other	other
2,299	NM_153448	ESX1	ESX homeobox 1	Nucleus	transcription regulator
2,293	NM_133637	DQX1	DEAQ box RNA-dependent ATPase 1	Other	enzyme
2,281	NM_001005179	OR56A4	olfactory receptor, family 56, subfamily A, member 4	Plasma Membrane	G-protein coupled receptor
2,277	XM_172995	C19orf38	chromosome 19 open reading frame 38	Other	other
2,268	NM_152507	C21orf128	chromosome 21 open reading frame 128	Other	other
2,257	NM_014033	METTL7A	methyltransferase like 7A	Other	other
2,231	NM_133272	FCAR	Fc fragment of IgA, receptor for	Plasma Membrane	other

2,230	NM_015879	ST8SIA3	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3	Cytoplasm	enzyme
2,228	NM_005300	GPR34	G protein-coupled receptor 34	Plasma Membrane	G-protein coupled receptor
2,221	NM_000684	ADRB1	adrenoceptor beta 1	Plasma Membrane	G-protein coupled receptor
2,220	NM_001495	GFRA2	GDNF family receptor alpha 2	Plasma Membrane	transmembrane receptor
2,216	NM_000869	HTR3A	5-hydroxytryptamine (serotonin) receptor 3A, ionotropic	Plasma Membrane	ion channel
2,215	NM_182516	KNCN	kinocilin	Cytoplasm	other
2,202	NM_004345	CAMP	cathelicidin antimicrobial peptide	Cytoplasm	other
2,197	XM_497642	TARM1	T cell-interacting, activating receptor on myeloid cells 1	Other	other
2,191	NM_016200	NAA38	N(alpha)-acetyltransferase 38, NatC auxiliary subunit	Nucleus	other
2,171	NM_178545	TMEM52	transmembrane protein 52	Other	other
2,170	NM_006229	PNLIPRP1	pancreatic lipase-related protein 1	Extracellular Space	enzyme
2,169	NM_006160	NEUROD2	neuronal differentiation 2	Nucleus	transcription regulator
2,169	NM_178168	OR10A5	olfactory receptor, family 10, subfamily A, member 5	Plasma Membrane	G-protein coupled receptor
2,140	NM_080612	GAB3	GRB2-associated binding protein 3	Other	other
2,131	NM_001522	GUCY2F	guanylate cyclase 2F, retinal	Plasma Membrane	kinase
2,129	NM_021572	ENPP5	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)	Extracellular Space	enzyme
2,124	NM_198474	OLFML1	olfactomedin-like 1	Extracellular Space	other
2,116	NM_000324	RHAG	Rh-associated glycoprotein	Plasma Membrane	peptidase
2,113	NM_031497	PCDHA3	protocadherin alpha 3	Plasma Membrane	other
2,104	NM_000369	TSHR	thyroid stimulating hormone receptor	Plasma Membrane	G-protein coupled receptor
2,096	NM_032136	TKTL2	transketolase-like 2	Cytoplasm	enzyme
2,094	NM_000419	ITGA2B	integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41)	Plasma Membrane	transmembrane receptor
2,088	NM_052964	CLNK	cytokine-dependent hematopoietic cell linker	Cytoplasm	other
2,078	NM_032571	EMR3	egf-like module containing, mucin-like, hormone receptor-like 3	Plasma Membrane	G-protein coupled receptor
2,071	NM_024869	FAM110D	family with sequence similarity 110, member D	Other	other

2,068	XM_292820	PALM3	paralemmin 3	Other	other
2,062	NM_003841	TNFRSF10C	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain	Plasma Membrane	transmembrane receptor
2,045	NM_144622	DCST2	DC-STAMP domain containing 2	Other	other
2,045	NM_001972	ELANE	elastase, neutrophil expressed	Extracellular Space	peptidase
2,033	NM_020212	WDR93	WD repeat domain 93	Other	other
2,030	NM_032126	TEX35	testis expressed 35	Nucleus	other
2,028	NM_144702	LRRC71	leucine rich repeat containing 71	Other	other
2,025	NM_000032	ALAS2	aminolevulinate, delta-, synthase 2	Cytoplasm	enzyme
2,025	NM_152394	FAM194A	family with sequence similarity 194, member A	Other	other
2,017	NM_001001965	OR4D5	olfactory receptor, family 4, subfamily D, member 5	Plasma Membrane	G-protein coupled receptor
2,015	NM_004042	ARSF	arylsulfatase F	Extracellular Space	enzyme
2,008	NM_173489	MROH2B	maestro heat-like repeat family member 2B	Other	other
2,007	NM_080389	DEFB104A/ DEFB104B	defensin, beta 104A	Extracellular Space	other
2,007	NM_001012959	DISC1	disrupted in schizophrenia 1	Cytoplasm	other
2,006	NM_152439	BEST3	bestrophin 3	Nucleus	ion channel
2,005	NM_006042	HS3ST3A1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1	Cytoplasm	enzyme
2,000	NM_207403	RFX8	RFX family member 8, lacking RFX DNA binding domain	Other	other
2,000	NM_003305	TRPC3	transient receptor potential cation channel, subfamily C, member 3	Plasma Membrane	ion channel
-2,002	NM_194251	GPR151	G protein-coupled receptor 151	Plasma Membrane	G-protein coupled receptor
-2,005	NM_182535	LINC00656	long intergenic non-protein coding RNA 656	Other	other
-2,006	NM_000928	PLA2G1B	phospholipase A2, group IB (pancreas)	Extracellular Space	enzyme
-2,007	NM_172084	CAMK2B	calcium/calmodulin-dependent protein kinase II beta	Cytoplasm	kinase
-2,008	NM_175619	ZAR1	zygote arrest 1	Cytoplasm	other
-2,010	NM_181604	KRTAP6-2	keratin associated protein 6-2	Other	other
-2,013	NM_030957	ADAMTS10	ADAM metallopeptidase with thrombospondin type 1 motif, 10	Extracellular Space	peptidase
-2,016	NM_004019	DMD	dystrophin	Plasma Membrane	other
-2,019	NM_201550	LRRC10	leucine rich repeat containing 10	Nucleus	other
-2,026	NM_001009994	RIPPLY2	ripply2 homolog (zebrafish)	Nucleus	other
-2,027	NM_001012452	GOLGA8F	golgin A8 family, member F	Other	other

-2,030	NM_173080	SPRR4	small proline-rich protein 4	Cytoplasm	other
-2,032	NM_178456	C20orf85	chromosome 20 open reading frame 85	Other	other
-2,034	NM_015831	ACHE	acetylcholinesterase	Plasma Membrane	enzyme
-2,044	NM_173092	KCNH6	potassium voltage-gated channel, subfamily H (eag-related), member 6	Plasma Membrane	ion channel
-2,047	NM_001005165	OR52E4	olfactory receptor, family 52, subfamily E, member 4	Plasma Membrane	G-protein coupled receptor
-2,048	NM_032512	PDZD4	PDZ domain containing 4	Cytoplasm	other
-2,049	NM_001012708	KRTAP5-3/KRTAP5-5	keratin associated protein 5-5	Other	other
-2,051	NM_001005276	OR2AE1	olfactory receptor, family 2, subfamily AE, member 1	Plasma Membrane	other
-2,054	NM_032425	HHIPL1	HHIP-like 1	Other	other
-2,057	NM_198464	PRSS55	protease, serine, 55	Other	other
-2,062	XM_291816	OTOG	otogelin	Extracellular Space	enzyme
-2,063	XM_496111	NPW	neuropeptide W	Extracellular Space	other
-2,071	NM_001001875	TPD52L3	tumor protein D52-like 3	Other	other
-2,073	NM_005624	CCL25	chemokine (C-C motif) ligand 25	Extracellular Space	cytokine
-2,075	NM_000631	NCF4	neutrophil cytosolic factor 4, 40kDa	Cytoplasm	enzyme
-2,081	NM_000669	ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide	Cytoplasm	enzyme
-2,083	NM_003571	BFSP2	beaded filament structural protein 2, phakinin	Cytoplasm	other
-2,101	NM_152860	SP7	Sp7 transcription factor	Nucleus	transcription regulator
-2,106	NM_172369	C1QC	complement component 1, q subcomponent, C chain	Extracellular Space	other
-2,109	NM_005099	ADAMTS4	ADAM metallopeptidase with thrombospondin type 1 motif, 4	Extracellular Space	peptidase
-2,120	NM_000717	CA4	carbonic anhydrase IV	Plasma Membrane	enzyme
-2,121	NM_013371	IL19	interleukin 19	Extracellular Space	cytokine
-2,122	NM_031496	PCDHA2	protocadherin alpha 2	Plasma Membrane	other
-2,124	NM_175769	TCF23	transcription factor 23	Nucleus	other
-2,132	NM_005187	CBFA2T3	core-binding factor, runt domain, alpha subunit 2; translocated to, 3	Nucleus	transcription regulator
-2,133	NM_022006	FXYD7	FXYD domain containing ion transport regulator 7	Plasma Membrane	ion channel
-2,138	XM_372741	LOC390956	peptidyl-prolyl cis-trans isomerase A-like	Other	other

-2,140	NM_030779	KCNH6	potassium voltage-gated channel, subfamily H (eag-related), member 6	Plasma Membrane	ion channel
-2,147	NM_177477	LYNX1	Ly6/neurotoxin 1	Plasma Membrane	transporter
-2,169	NM_001700	AZU1	azurocidin 1	Cytoplasm	peptidase
-2,169	NM_005535	IL12RB1	interleukin 12 receptor, beta 1	Plasma Membrane	transmembrane receptor
-2,175	NM_001017440	CALN1	calneuron 1	Cytoplasm	other
-2,188	NM_001001912	OR4E2	olfactory receptor, family 4, subfamily E, member 2	Plasma Membrane	G-protein coupled receptor
-2,204	NM_013251	TAC3	tachykinin 3	Extracellular Space	other
-2,218	NM_001002916	H2BFWT	H2B histone family, member W, testis-specific	Other	other
-2,223	NM_001025466	LOC338797	uncharacterized LOC338797	Other	other
-2,230	NM_001012728	DPRX	divergent-paired related homeobox	Other	other
-2,231	NM_032963	CCL14	chemokine (C-C motif) ligand 14	Extracellular Space	cytokine
-2,235	NM_002921	RGR	retinal G protein coupled receptor	Plasma Membrane	G-protein coupled receptor
-2,243	NM_001004434	SLC30A2	solute carrier family 30 (zinc transporter), member 2	Plasma Membrane	transporter
-2,245	NM_033554	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	Plasma Membrane	transmembrane receptor
-2,247	NM_001007249	OR8G2	olfactory receptor, family 8, subfamily G, member 2	Plasma Membrane	G-protein coupled receptor
-2,266	NM_000589	IL4	interleukin 4	Extracellular Space	cytokine
-2,283	NM_001013735	FOXB2	forkhead box B2	Nucleus	transcription regulator
-2,284	NM_144605	SEPT12	septin 12	Cytoplasm	other
-2,288	NM_006984	CLDN10	claudin 10	Plasma Membrane	other
-2,293	NM_001911	CTSG	cathepsin G	Cytoplasm	peptidase
-2,296	NM_007084	SOX21	SRY (sex determining region Y)-box 21	Nucleus	transcription regulator
-2,301	NM_005173	ATP2A3	ATPase, Ca++ transporting, ubiquitous	Cytoplasm	transporter
-2,305	NM_182833	GDPD4	glycerophosphodiester phosphodiesterase domain containing 4	Other	kinase
-2,307	NM_080390	TCEAL2	transcription elongation factor A (SII)-like 2	Other	other
-2,326	NM_012146	DUX1	double homeobox 1	Nucleus	transcription regulator
-2,332	NM_181611	KRTAP19-5	keratin associated protein 19-5	Other	other
-2,335	NM_017712	PGPEP1	pyroglutamyl-peptidase I	Cytoplasm	peptidase

-2,359	NM_022479	WBSCR17	Williams-Beuren syndrome chromosome region 17	Cytoplasm	enzyme
-2,375	NM_001004476	OR10K2	olfactory receptor, family 10, subfamily K, member 2	Plasma Membrane	G-protein coupled receptor
-2,383	NM_005368	MB	myoglobin	Cytoplasm	transporter
-2,396	XM_927671	ANKRD31	ankyrin repeat domain 31	Extracellular Space	other
-2,418	NM_003235	TG	thyroglobulin	Extracellular Space	other
-2,436	NM_173502	PRSS36	protease, serine, 36	Extracellular Space	peptidase
-2,444	NM_001004735	OR5D14	olfactory receptor, family 5, subfamily D, member 14	Plasma Membrane	G-protein coupled receptor
-2,446	NM_001031680	RUNX3	runt-related transcription factor 3	Nucleus	transcription regulator
-2,459	XM_374386	ELFN1	extracellular leucine-rich repeat and fibronectin type III domain containing 1	Plasma Membrane	other
-2,491	NM_001013628	DCAF12L2	DDB1 and CUL4 associated factor 12-like 2	Other	other
-2,491	NM_152762	TSGA10IP	testis specific, 10 interacting protein	Other	other
-2,492	NM_001029871	RSPO4	R-spondin 4	Plasma Membrane	other
-2,520	XM_293354	DCAF8L2	DDB1 and CUL4 associated factor 8-like 2	Other	other
-2,525	NM_178428	LCE2A	late cornified envelope 2A	Other	other
-2,526	NM_017716	MS4A12	membrane-spanning 4-domains, subfamily A, member 12	Other	other
-2,536	NM_000578	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	Plasma Membrane	transporter
-2,546	NM_018687	C19orf80	chromosome 19 open reading frame 80	Extracellular Space	other
-2,547	NM_002619	PF4	platelet factor 4	Extracellular Space	cytokine
-2,605	NM_173857	VN1R4	vomeronasal 1 receptor 4	Plasma Membrane	G-protein coupled receptor
-2,658	NM_024686	TTLL7	tubulin tyrosine ligase-like family, member 7	Plasma Membrane	other
-2,670	NM_024924	ZNF702P	zinc finger protein 702, pseudogene	Nucleus	other
-2,680	NM_020974	SCUBE2	signal peptide, CUB domain, EGF-like 2	Other	other
-2,712	NM_198687	KRTAP10-4	keratin associated protein 10-4	Other	other
-2,769	NM_006119	FGF8	fibroblast growth factor 8 (androgen-induced)	Extracellular Space	growth factor
-2,895	NM_000498	CYP11B2	cytochrome P450, family 11, subfamily B, polypeptide 2	Cytoplasm	enzyme
-2,938	XM_371491	TMEM247	transmembrane protein 247	Cytoplasm	other
-3,027	NM_173509	FAM163A	family with sequence similarity 163, member A	Other	other
-3,345	NM_198689	KRTAP10-6/KRTAP10-7	keratin associated protein 10-7	Other	other

Results of the DNA microarray (20 s; 25 % oxygen and 75 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)
2,631	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
2,100	NM_001014842	TM9SF1	transmembrane 9 superfamily member 1	Plasma Membrane	transporter
2,024	NM_032849	MEDAG	mesenteric estrogen-dependent adipogenesis	Cytoplasm	other
-2,028	XM_209163	LOC284379	solute carrier family 7 (cationic amino acid transporter, y+ system), member 3 pseudogene	Other	other
-2,034	NM_153031	SATB2-AS1	SATB2 antisense RNA 1	Other	other
-2,458	NM_001037730	DEFB115	defensin, beta 115	Extracellular Space	other

Results of the DNA microarray (20 s; 50 % oxygen and 50 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)
3,312	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
3,025	NM_006132	BMP1	bone morphogenetic protein 1	Extracellular Space	peptidase
2,943	NM_001039517	RUSC1-AS1	RUSC1 antisense RNA 1	Other	other
2,910	NM_175739	SERPINA9	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	Extracellular Space	other
2,829	NM_003179	SYP	synaptophysin	Cytoplasm	transporter
2,812	NM_006039	MRC2	mannose receptor, C type 2	Plasma Membrane	transmembrane receptor
2,702	NM_173593	B4GALNT3	beta-1,4-N-acetyl-galactosaminyl transferase 3	Other	enzyme
2,675	NM_002779	PSD	pleckstrin and Sec7 domain containing	Cytoplasm	other
2,650	XM_496502	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	Other	other
2,645	NM_139021	MAPK15	mitogen-activated protein kinase 15	Cytoplasm	kinase
2,612	NM_145042	TUBA3FP	tubulin, alpha 3f, pseudogene	Other	other
2,577	NM_001004134	OR10AD1	olfactory receptor, family 10, subfamily AD, member 1	Plasma Membrane	G-protein coupled receptor
2,569	NM_173158	NR4A1	nuclear receptor subfamily 4, group A, member 1	Nucleus	ligand-dependent nuclear receptor

2,567	NM_181484	ZGPAT	zinc finger, CCCH-type with G patch domain	Nucleus	transcription regulator
2,552	NM_181657	LTB4R	leukotriene B4 receptor	Plasma Membrane	G-protein coupled receptor
2,537	NM_198427	BCAN	brevican	Extracellular Space	other
2,535	NM_153487	MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	Plasma Membrane	other
2,504	NM_001670	ARVCF	armadillo repeat gene deleted in velocardiofacial syndrome	Plasma Membrane	other
2,474	NM_203299	C9orf131	chromosome 9 open reading frame 131	Other	other
2,473	NM_000804	FOLR3	folate receptor 3 (gamma)	Extracellular Space	other
2,461	XM_497334	TSPAN11	tetraspanin 11	Other	other
2,431	NM_005453	ZBTB22	zinc finger and BTB domain containing 22	Nucleus	other
2,427	NM_001014842	TM9SF1	transmembrane 9 superfamily member 1	Plasma Membrane	transporter
2,387	NM_003632	CNTNAP1	contactin associated protein 1	Plasma Membrane	other
2,384	NM_001033953	CALCA	calcitonin-related polypeptide alpha	Plasma Membrane	other
2,384	NM_138690	GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	Plasma Membrane	ion channel
2,380	NM_005710	PQBP1	polyglutamine binding protein 1	Nucleus	transcription regulator
2,377	NM_015503	SH2B1	SH2B adaptor protein 1	Cytoplasm	other
2,364	NM_024891	KMT2A	lysine (K)-specific methyltransferase 2A	Nucleus	transcription regulator
2,352	XM_030729	NUTM2F/ NUTM2G	NUT family member 2G	Other	other
2,347	NM_024313	NOL12	nucleolar protein 12	Nucleus	other
2,328	NM_020795	NLGN2	neuroligin 2	Plasma Membrane	enzyme
2,319	NM_002420	TRPM1	transient receptor potential cation channel, subfamily M, member 1	Plasma Membrane	ion channel
2,309	NM_000691	ALDH3A1	aldehyde dehydrogenase 3 family, member A1	Cytoplasm	enzyme
2,292	NM_201574	SLC4A3	solute carrier family 4, anion exchanger, member 3	Plasma Membrane	transporter
2,237	NM_207385	C16orf47	chromosome 16 open reading frame 47	Other	other
2,232	NM_001585	MPPED1	metallophosphoesterase domain containing 1	Other	other
2,216	NM_033413	LRRC46	leucine rich repeat containing 46	Other	other
2,199	NM_000638	VTN	vitronectin	Extracellular Space	other
2,193	NM_022803	UCP3	uncoupling protein 3 (mitochondrial, proton carrier)	Cytoplasm	transporter

2,191	NM_203411	TMEM88	transmembrane protein 88	Other	other
2,189	NM_138429	CLDN15	claudin 15	Plasma Membrane	other
2,185	NM_004962	GDF10	growth differentiation factor 10	Extracellular Space	growth factor
2,183	NM_014700	RAB11FIP3	RAB11 family interacting protein 3 (class II)	Cytoplasm	other
2,180	NM_032647	CBX2	chromobox homolog 2	Nucleus	transcription regulator
2,164	NM_004443	EPHB3	EPH receptor B3	Plasma Membrane	kinase
2,160	NM_001004426	PLA2G6	phospholipase A2, group VI (cytosolic, calcium-independent)	Cytoplasm	enzyme
2,158	NM_016564	CEND1	cell cycle exit and neuronal differentiation 1	Other	other
2,156	NM_001014447	CPZ	carboxypeptidase Z	Extracellular Space	peptidase
2,154	NM_001672	ASIP	agouti signaling protein	Extracellular Space	other
2,154	NM_001002034	FAM109B	family with sequence similarity 109, member B	Other	other
2,152	NM_012458	TIMM13	translocase of inner mitochondrial membrane 13 homolog (yeast)	Cytoplasm	transporter
2,150	NM_001864	COX7A1	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)	Cytoplasm	enzyme
2,146	NM_001136	AGER	advanced glycosylation end product-specific receptor	Plasma Membrane	transmembrane receptor
2,126	NM_001005367	TTYH1	tweety homolog 1 (Drosophila)	Plasma Membrane	ion channel
2,121	NM_144610	SGK494	uncharacterized serine/threonine-protein kinase SgK494	Other	kinase
2,116	NM_000152	GAA	glucosidase, alpha; acid	Cytoplasm	enzyme
2,108	NM_000757	CSF1	colony stimulating factor 1 (macrophage)	Extracellular Space	cytokine
2,101	NM_022377	ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	Plasma Membrane	other
2,091	NM_001018103	POLR2M	polymerase (RNA) II (DNA directed) polypeptide M	Nucleus	other
2,090	NM_003717	NPFF	neuropeptide FF-amide peptide precursor	Extracellular Space	other
2,089	NM_031289	GSG1	germ cell associated 1	Cytoplasm	other
2,080	NM_174936	PCSK9	proprotein convertase subtilisin/kexin type 9	Extracellular Space	peptidase
2,073	NM_144968	RIBC1	RIB43A domain with coiled-coils 1	Other	other
2,071	NM_213655	WNK1	WNK lysine deficient protein kinase 1	Cytoplasm	kinase
2,069	NM_152237	GAS2L1	growth arrest-specific 2 like 1	Cytoplasm	other
2,065	XM_039733	EFR3B	EFR3 homolog B (S. cerevisiae)	Other	other
2,065	NM_001039766	LINC00893	long intergenic non-protein coding RNA 893	Other	other
2,057	NM_006687	ACTL7A	actin-like 7A	Nucleus	other
2,057	NM_016453	NCKIPSD	NCK interacting protein with SH3 domain	Other	other

2,054	NM_014272	ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif, 7	Extracellular Space	peptidase
2,053	NM_013959	NRG1	neuregulin 1	Other	growth factor
2,047	NM_018996	TNRC6C	trinucleotide repeat containing 6C	Cytoplasm	other
2,046	NM_030907	RSG1	REM2 and RAB-like small GTPase 1	Cytoplasm	other
2,043	NM_153213	ARHGEF19	Rho guanine nucleotide exchange factor (GEF) 19	Other	other
2,043	NM_182981	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
2,041	NM_015950	MRPL2	mitochondrial ribosomal protein L2	Extracellular Space	other
2,041	NM_032794	SLC44A4	solute carrier family 44, member 4	Plasma Membrane	transporter
2,040	NM_020998	MST1	macrophage stimulating 1 (hepatocyte growth factor-like)	Extracellular Space	growth factor
2,038	NM_001004490	OR2AG2	olfactory receptor, family 2, subfamily AG, member 2	Plasma Membrane	G-protein coupled receptor
2,033	NM_147169	C9orf24	chromosome 9 open reading frame 24	Cytoplasm	other
2,033	NM_001031682	GPER	G protein-coupled estrogen receptor 1	Plasma Membrane	G-protein coupled receptor
2,033	NM_198406	PAQR6	progestin and adipoQ receptor family member VI	Other	other
2,032	NM_177977	HAP1	huntingtin-associated protein 1	Cytoplasm	other
2,031	NM_014718	CLSTN3	calsyntenin 3	Plasma Membrane	other
2,026	NM_152335	C15orf27	chromosome 15 open reading frame 27	Other	other
2,026	NM_004260	RECQL4	RecQ protein-like 4	Nucleus	enzyme
2,026	NM_001035235	SRA1	steroid receptor RNA activator 1	Nucleus	transcription regulator
2,024	NM_021948	BCAN	brevican	Extracellular Space	other
2,023	NM_153215	LSMEM2	leucine-rich single-pass membrane protein 2	Other	other
2,022	NM_138769	RHOT2	ras homolog family member T2	Cytoplasm	enzyme
2,020	NM_001164	APBB1	amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	Cytoplasm	transcription regulator
2,020	NM_001004473	OR10K1	olfactory receptor, family 10, subfamily K, member 1	Plasma Membrane	other
2,019	NM_152498	WDR65	WD repeat domain 65	Extracellular Space	other
2,018	NM_022049	GPR88	G protein-coupled receptor 88	Plasma Membrane	G-protein coupled receptor
2,017	NM_001014987	LAT	linker for activation of T cells	Plasma Membrane	kinase
2,017	NM_022369	STRA6	stimulated by retinoic acid 6	Plasma Membrane	other
2,012	NM_000364	TNNT2	troponin T type 2 (cardiac)	Cytoplasm	other
2,012	XM_038604	UNC13A	unc-13 homolog A (C. elegans)	Plasma Membrane	other
2,012	NM_015872	ZBTB7B	zinc finger and BTB domain containing 7B	Nucleus	other

2,010	NM_013353	TMOD4	tropomodulin 4 (muscle)	Other	other
2,004	NM_003790	TNFRSF25	tumor necrosis factor receptor superfamily, member 25	Plasma Membrane	transmembrane receptor
2,002	NM_181720	ARHGAP30	Rho GTPase activating protein 30	Cytoplasm	other
2,002	NM_004584	RAD9A	RAD9 homolog A (S. pombe)	Nucleus	enzyme
2,001	NM_031264	CDHR5	cadherin-related family member 5	Plasma Membrane	other
2,001	NM_004532	MUC4	mucin 4, cell surface associated	Extracellular Space	growth factor
-2,002	NM_016105	FKBP7	FK506 binding protein 7	Cytoplasm	enzyme
-2,004	NM_003750	EIF3A	eukaryotic translation initiation factor 3, subunit A	Cytoplasm	translation regulator
-2,005	NM_032047	B3GNT5	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5	Cytoplasm	enzyme
-2,008	NM_000998	RPL37A	ribosomal protein L37a	Cytoplasm	other
-2,012	NM_004385	VCAN	versican	Extracellular Space	other
-2,016	NM_018178	GOLPH3L	golgi phosphoprotein 3-like	Cytoplasm	other
-2,018	NM_016101	NIP7	NIP7, nucleolar pre-rRNA processing protein	Nucleus	other
-2,024	NM_001964	EGR1	early growth response 1	Nucleus	transcription regulator
-2,026	NM_173657	C3orf33	chromosome 3 open reading frame 33	Other	other
-2,027	NM_016583	BPIFA1	BPI fold containing family A, member 1	Extracellular Space	other
-2,028	NM_138444	KCTD12	potassium channel tetramerization domain containing 12	Plasma Membrane	ion channel
-2,031	NM_178839	LRRTM1	leucine rich repeat transmembrane neuronal 1	Plasma Membrane	other
-2,033	NM_203298	CHCHD1	coiled-coil-helix-coiled-coil-helix domain containing 1	Nucleus	other
-2,034	NM_001391	DTNA	dystrobrevin, alpha	Plasma Membrane	other
-2,035	NM_181581	DUS4L	dihydrouridine synthase 4-like (S. cerevisiae)	Other	other
-2,036	NM_014736	KIAA0101	KIAA0101	Nucleus	other
-2,043	NM_012353	OR1C1	olfactory receptor, family 1, subfamily C, member 1	Plasma Membrane	G-protein coupled receptor
-2,046	NM_020774	MIB1	mindbomb E3 ubiquitin protein ligase 1	Cytoplasm	other
-2,051	NM_003338	UBE2D1	ubiquitin-conjugating enzyme E2D 1	Cytoplasm	enzyme
-2,055	NM_031296	RAB33B	RAB33B, member RAS oncogene family	Cytoplasm	enzyme
-2,062	NM_152903	KBTBD6	kelch repeat and BTB (POZ) domain containing 6	Other	other
-2,063	NM_014011	SOCS5	suppressor of cytokine signaling 5	Extracellular Space	cytokine
-2,063	NM_153367	ZCCHC24	zinc finger, CCHC domain containing 24	Other	other
-2,065	NM_004708	PDCD5	programmed cell death 5	Nucleus	other
-2,065	NM_017666	ZNF280C	zinc finger protein 280C	Nucleus	other

-2,076	NM_001005205	OR8J1	olfactory receptor, family 8, subfamily J, member 1	Plasma Membrane	G-protein coupled receptor
-2,078	NM_015000	STK38L	serine/threonine kinase 38 like	Cytoplasm	kinase
-2,081	NM_052850	GADD45GIP1	growth arrest and DNA-damage-inducible, gamma interacting protein 1	Nucleus	other
-2,086	NM_001917	DAO	D-amino-acid oxidase	Cytoplasm	enzyme
-2,091	NM_002906	RDX	radixin	Cytoplasm	other
-2,091	NM_001006947	UHRF1BP1L	UHRF1 binding protein 1-like	Other	other
-2,095	NM_138782	FCHO2	FCH domain only 2	Cytoplasm	other
-2,096	NM_001033925	TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1	Nucleus	transcription regulator
-2,101	XM_927671	ANKRD31	ankyrin repeat domain 31	Extracellular Space	other
-2,101	XM_495961	MZT1	mitotic spindle organizing protein 1	Cytoplasm	other
-2,101	NM_003825	SNAP23	synaptosomal-associated protein, 23kDa	Plasma Membrane	transporter
-2,103	NM_020310	MNT	MNT, MAX dimerization protein	Nucleus	transcription regulator
-2,103	NM_000321	RB1	retinoblastoma 1	Nucleus	transcription regulator
-2,111	NM_005455	ZRANB2	zinc finger, RAN-binding domain containing 2	Nucleus	transcription regulator
-2,113	NM_032024	C10orf11	chromosome 10 open reading frame 11	Other	other
-2,116	NM_014372	RNF11	ring finger protein 11	Nucleus	other
-2,119	NM_134470	IL1RAP	interleukin 1 receptor accessory protein	Plasma Membrane	transmembrane receptor
-2,124	NM_024725	CCDC82	coiled-coil domain containing 82	Other	other
-2,129	NM_000943	PPIC	peptidylprolyl isomerase C (cyclophilin C)	Cytoplasm	enzyme
-2,132	NM_004380	CREBBP	CREB binding protein	Nucleus	transcription regulator
-2,132	NM_003359	UGDH	UDP-glucose 6-dehydrogenase	Nucleus	enzyme
-2,142	NM_022459	XPO4	exportin 4	Nucleus	transporter
-2,151	NM_002157	HSPE1	heat shock 10kDa protein 1 (chaperonin 10)	Cytoplasm	enzyme
-2,163	NM_000143	FH	fumarate hydratase	Cytoplasm	enzyme
-2,172	NM_144701	IL23R	interleukin 23 receptor	Plasma Membrane	transmembrane receptor
-2,176	NM_152773	TCTEX1D2	Tctex1 domain containing 2	Other	other
-2,183	NM_015571	SENP6	SUMO1/sentrin specific peptidase 6	Cytoplasm	peptidase
-2,186	NM_002495	NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	Cytoplasm	enzyme

-2,195	NM_016271	RNF138	ring finger protein 138, E3 ubiquitin protein ligase	Other	other
-2,196	NM_006626	ZBTB6	zinc finger and BTB domain containing 6	Nucleus	other
-2,200	NM_018112	TMEM38B	transmembrane protein 38B	Nucleus	ion channel
-2,202	NM_000028	AGL	amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase	Cytoplasm	enzyme
-2,205	NM_004052	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	Cytoplasm	other
-2,206	NM_001031812	CSNK1G3	casein kinase 1, gamma 3	Cytoplasm	kinase
-2,214	NM_173177	C1D	C1D nuclear receptor corepressor	Nucleus	transcription regulator
-2,236	NM_000791	DHFR	dihydrofolate reductase	Nucleus	enzyme
-2,237	NM_018482	ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1	Plasma Membrane	other
-2,252	NM_022756	MEAF6	MYST/Esa1-associated factor 6	Nucleus	other
-2,297	NM_018137	PRMT6	protein arginine methyltransferase 6	Nucleus	enzyme
-2,300	NM_006085	BPNT1	3'(2'), 5'-bisphosphate nucleotidase 1	Nucleus	phosphatase
-2,313	NM_002493	NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa	Cytoplasm	enzyme
-2,315	NM_012089	ABCB10	ATP-binding cassette, sub-family B (MDR/TAP), member 10	Cytoplasm	transporter
-2,323	NM_018078	LARP1B	La ribonucleoprotein domain family, member 1B	Extracellular Space	other
-2,340	NM_017655	GIPC2	GIPC PDZ domain containing family, member 2	Cytoplasm	other
-2,351	NM_001004749	OR51A7	olfactory receptor, family 51, subfamily A, member 7	Plasma Membrane	G-protein coupled receptor
-2,361	NM_001004693	OR2T10	olfactory receptor, family 2, subfamily T, member 10	Plasma Membrane	other
-2,371	NM_001167	XIAP	X-linked inhibitor of apoptosis	Cytoplasm	enzyme
-2,372	NM_012300	FBXW11	F-box and WD repeat domain containing 11	Cytoplasm	enzyme
-2,375	NM_001949	E2F3	E2F transcription factor 3	Nucleus	transcription regulator
-2,375	NM_017665	ZCCHC10	zinc finger, CCHC domain containing 10	Other	other
-2,383	NM_152626	ZNF92	zinc finger protein 92	Nucleus	transcription regulator
-2,386	NM_024920	DNAJB14	DnaJ (Hsp40) homolog, subfamily B, member 14	Other	enzyme
-2,393	NM_020640	DCUN1D1	DCN1, defective in cullin neddylation 1, domain containing 1	Nucleus	other
-2,421	NM_005999	TSNAX	translin-associated factor X	Nucleus	transporter
-2,430	NM_001008211	OPTN	optineurin	Cytoplasm	other

-2,433	NM_033342	TRIM7	tripartite motif containing 7	Cytoplasm	other
-2,435	NM_004775	B4GALT6	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6	Cytoplasm	enzyme
-2,448	NM_002546	TNFRSF11B	tumor necrosis factor receptor superfamily, member 11b	Plasma Membrane	transmembrane receptor
-2,455	NM_153689	C2orf69	chromosome 2 open reading frame 69	Other	other
-2,460	NM_018227	UBA6	ubiquitin-like modifier activating enzyme 6	Cytoplasm	enzyme
-2,493	NM_031453	FAM107B	family with sequence similarity 107, member B	Other	other
-2,528	NM_015235	CSTF2T	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant	Nucleus	other
-2,529	NM_003655	CBX4	chromobox homolog 4	Nucleus	transcription regulator
-2,598	NM_023927	GRAMD3	GRAM domain containing 3	Extracellular Space	other
-2,620	NM_001012985	COA6	cytochrome c oxidase assembly factor 6 homolog (S. cerevisiae)	Cytoplasm	other
-2,682	NM_018841	GNG12	guanine nucleotide binding protein (G protein), gamma 12	Plasma Membrane	enzyme
-2,689	NM_001017992	ACTBL2	actin, beta-like 2	Other	other
-2,740	NM_015199	ANKRD28	ankyrin repeat domain 28	Cytoplasm	other
-2,748	NM_001029885	GLTPD1	glycolipid transfer protein domain containing 1	Other	other
-2,814	NM_207181	NPHP1	nephronophthisis 1 (juvenile)	Nucleus	other
-3,028	NM_001621	AHR	aryl hydrocarbon receptor	Nucleus	ligand-dependent nuclear receptor
-3,849	NM_001012968	SPIN4	spindlin family, member 4	Other	other

Results of the DNA microarray (20 s; 75 % oxygen and 25 % nitrogen):

Fold Change	**ID**	**Symbol**	**Entrez Gene Name**	**Location**	**Type(s)**
3,059	NM_001004134	OR10AD1	olfactory receptor, family 10, subfamily AD, member 1	Plasma Membrane	G-protein coupled receptor
2,552	NM_001031855	LONRF3	LON peptidase N-terminal domain and ring finger 3	Other	other
2,450	NM_181530	WFDC3	WAP four-disulfide core domain 3	Extracellular Space	other
2,445	NM_025237	SOST	sclerostin	Extracellular Space	other
2,444	NM_005300	GPR34	G protein-coupled receptor 34	Plasma Membrane	G-protein coupled receptor

2,442	NM_182978	GNAL	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	Cytoplasm	enzyme
2,406	NM_177402	SYT2	synaptotagmin II	Cytoplasm	transporter
2,389	NM_002071	GNAL	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	Cytoplasm	enzyme
2,362	NM_005609	PYGM	phosphorylase, glycogen, muscle	Cytoplasm	enzyme
2,348	NM_203411	TMEM88	transmembrane protein 88	Other	other
2,317	NM_013353	TMOD4	tropomodulin 4 (muscle)	Other	other
2,258	NM_139158	CDK15	cyclin-dependent kinase 15	Plasma Membrane	kinase
2,256	NM_178314	RILPL1	Rab interacting lysosomal protein-like 1	Cytoplasm	other
2,228	NM_144968	RIBC1	RIB43A domain with coiled-coils 1	Other	other
2,198	NM_174934	SCN4B	sodium channel, voltage-gated, type IV, beta subunit	Plasma Membrane	ion channel
2,187	NM_198427	BCAN	brevican	Extracellular Space	other
2,173	XM_096472	C11orf94	chromosome 11 open reading frame 94	Other	other
2,171	NM_001008226	FAM154B	family with sequence similarity 154, member B	Other	other
2,168	NM_178168	OR10A5	olfactory receptor, family 10, subfamily A, member 5	Plasma Membrane	G-protein coupled receptor
2,168	NM_181525	WFDC3	WAP four-disulfide core domain 3	Extracellular Space	other
2,158	NM_016348	FAXDC2	fatty acid hydroxylase domain containing 2	Other	other
2,148	NM_001242	CD27	CD27 molecule	Plasma Membrane	transmembrane receptor
2,148	NM_001499	GLE1	GLE1 RNA export mediator	Nucleus	other
2,122	NM_006248	PRB1/PRB2	proline-rich protein BstNI subfamily 2	Other	other
2,120	NM_181522	WFDC3	WAP four-disulfide core domain 3	Extracellular Space	other
2,116	NM_001004460	OR10A2	olfactory receptor, family 10, subfamily A, member 2	Plasma Membrane	G-protein coupled receptor
2,115	NM_173652	CYP1B1-AS1	CYP1B1 antisense RNA 1	Other	other
2,111	NM_173565	RSPH10B/ RSPH10B2	radial spoke head 10 homolog B (Chlamydomonas)	Extracellular Space	other
2,084	NM_005523	HOXA11	homeobox A11	Nucleus	transcription regulator
2,069	NM_203299	C9orf131	chromosome 9 open reading frame 131	Other	other
2,035	NM_001005567	OR51B5	olfactory receptor, family 51, subfamily B, member 5	Plasma Membrane	G-protein coupled receptor
2,034	NM_177550	SLC13A5	solute carrier family 13 (sodium-dependent citrate transporter), member 5	Plasma Membrane	transporter
2,027	NM_153347	TMEM86A	transmembrane protein 86A	Other	other

2,021	NM_004541	NDUFA1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa	Cytoplasm	enzyme
2,020	NM_000392	ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Plasma Membrane	transporter
2,020	NM_002120	HLA-DOB	major histocompatibility complex, class II, DO beta	Plasma Membrane	transmembrane receptor
2,017	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
2,015	NM_018943	TUBA8	tubulin, alpha 8	Cytoplasm	other
2,014	NM_001025598	ARHGAP30	Rho GTPase activating protein 30	Cytoplasm	other
2,011	NM_138690	GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	Plasma Membrane	ion channel
-2,008	NM_005509	DMXL1	Dmx-like 1	Extracellular Space	other
-2,016	NM_004052	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	Cytoplasm	other
-2,017	NM_014344	FJX1	four jointed box 1 (Drosophila)	Extracellular Space	other
-2,025	NM_033342	TRIM7	tripartite motif containing 7	Cytoplasm	other
-2,035	NM_023927	GRAMD3	GRAM domain containing 3	Extracellular Space	other
-2,045	NM_001004749	OR51A7	olfactory receptor, family 51, subfamily A, member 7	Plasma Membrane	G-protein coupled receptor
-2,069	NM_004380	CREBBP	CREB binding protein	Nucleus	transcription regulator
-2,088	NM_018394	ABHD10	abhydrolase domain containing 10	Cytoplasm	enzyme
-2,150	NM_001029885	GLTPD1	glycolipid transfer protein domain containing 1	Other	other
-2,160	NM_138799	MBOAT2	membrane bound O-acyltransferase domain containing 2	Cytoplasm	enzyme
-2,178	NM_020943	CWC22	CWC22 spliceosome-associated protein homolog (S. cerevisiae)	Nucleus	other
-2,229	NM_015235	CSTF2T	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant	Nucleus	other
-2,311	NM_015571	SENP6	SUMO1/sentrin specific peptidase 6	Cytoplasm	peptidase
-2,328	NM_001030050	KLK3	kallikrein-related peptidase 3	Extracellular Space	peptidase

Results of the DNA microarray (20 s; 100 % oxygen and 0 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)

3,121	NM_024101	MLPH	melanophilin	Cytoplasm	other
2,518	NM_012069	ATP1B4	ATPase, Na+/K+ transporting, beta 4 polypeptide	Plasma Membrane	transporter
2,417	NM_001001871	HSFY1/HSFY2	heat shock transcription factor, Y-linked 1	Nucleus	transcription regulator
2,241	XM_372760	PRAMEF12	PRAME family member 12	Other	other
2,239	NM_015478	L3MBTL1	l(3)mbt-like 1 (Drosophila)	Nucleus	other
2,153	NM_176887	TAS2R46	taste receptor, type 2, member 46	Plasma Membrane	G-protein coupled receptor
2,126	NM_013959	NRG1	neuregulin 1	Other	growth factor
2,062	NM_178168	OR10A5	olfactory receptor, family 10, subfamily A, member 5	Plasma Membrane	G-protein coupled receptor
2,053	NM_001011544	MAGEA11	melanoma antigen family A, 11	Other	other
2,051	NM_001012414	TRIM61	tripartite motif containing 61	Other	other
2,037	NM_005300	GPR34	G protein-coupled receptor 34	Plasma Membrane	G-protein coupled receptor
2,015	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
-2,007	NM_198462	DCDC5	doublecortin domain containing 5	Other	other
-2,017	NM_032955	AIF1	allograft inflammatory factor 1	Nucleus	other
-2,042	NM_182758	WDR72	WD repeat domain 72	Other	other
-2,061	NM_172369	C1QC	complement component 1, q subcomponent, C chain	Extracellular Space	other
-2,063	NM_000905	NPY	neuropeptide Y	Extracellular Space	other
-2,088	NM_014257	CLEC4M	C-type lectin domain family 4, member M	Plasma Membrane	other
-2,116	NM_024686	TTLL7	tubulin tyrosine ligase-like family, member 7	Plasma Membrane	other
-2,158	NM_014241	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	Other	phosphatase
-2,165	NM_001004310	FCRL6	Fc receptor-like 6	Plasma Membrane	other
-2,258	NM_182904	P4HA3	prolyl 4-hydroxylase, alpha polypeptide III	Other	enzyme

Results of the DNA microarray (180 s; 0 % oxygen and 100 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)
3,121	NM_024101	MLPH	melanophilin	Cytoplasm	other

2,518	NM_012069	ATP1B4	ATPase, Na+/K+ transporting, beta 4 polypeptide	Plasma Membrane	transporter
2,417	NM_001001871	HSFY1/HSFY2	heat shock transcription factor, Y-linked 1	Nucleus	transcription regulator
2,241	XM_372760	PRAMEF12	PRAME family member 12	Other	other
2,239	NM_015478	L3MBTL1	l(3)mbt-like 1 (Drosophila)	Nucleus	other
2,153	NM_176887	TAS2R46	taste receptor, type 2, member 46	Plasma Membrane	G-protein coupled receptor
2,126	NM_013959	NRG1	neuregulin 1	Other	growth factor
2,062	NM_178168	OR10A5	olfactory receptor, family 10, subfamily A, member 5	Plasma Membrane	G-protein coupled receptor
2,053	NM_001011544	MAGEA11	melanoma antigen family A, 11	Other	other
2,051	NM_001012414	TRIM61	tripartite motif containing 61	Other	other
2,037	NM_005300	GPR34	G protein-coupled receptor 34	Plasma Membrane	G-protein coupled receptor
2,015	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
-2,007	NM_198462	DCDC5	doublecortin domain containing 5	Other	other
-2,017	NM_032955	AIF1	allograft inflammatory factor 1	Nucleus	other
-2,042	NM_182758	WDR72	WD repeat domain 72	Other	other
-2,061	NM_172369	C1QC	complement component 1, q subcomponent, C chain	Extracellular Space	other
-2,063	NM_000905	NPY	neuropeptide Y	Extracellular Space	other
-2,088	NM_014257	CLEC4M	C-type lectin domain family 4, member M	Plasma Membrane	other
-2,116	NM_024686	TTLL7	tubulin tyrosine ligase-like family, member 7	Plasma Membrane	other
-2,158	NM_014241	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	Other	phosphatase
-2,165	NM_001004310	FCRL6	Fc receptor-like 6	Plasma Membrane	other
-2,258	NM_182904	P4HA3	prolyl 4-hydroxylase, alpha polypeptide III	Other	enzyme

Results of the DNA microarray (180 s; 25 % oxygen and 75 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)
3,717	NM_002133	HMOX1	heme oxygenase (decycling) 1	Cytoplasm	enzyme
3,470	NM_024111	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)	Cytoplasm	other

2,903	NM_013370	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
2,900	NM_182981	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
2,876	NM_004744	LRAT	lecithin retinol acyltransferase (phosphatidylcholine–retinol O-acyltransferase)	Cytoplasm	enzyme
2,571	NM_000565	IL6R	interleukin 6 receptor	Plasma Membrane	transmembrane receptor
2,446	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
2,388	NM_014331	SLC7A11	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	Plasma Membrane	transporter
2,366	NM_020299	AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	Cytoplasm	enzyme
2,330	NM_000574	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	Plasma Membrane	other
2,263	NM_002943	RORA	RAR-related orphan receptor A	Nucleus	ligand-dependent nuclear receptor
2,255	NM_025218	ULBP1	UL16 binding protein 1	Plasma Membrane	transmembrane receptor
2,251	NM_025237	SOST	sclerostin	Extracellular Space	other
2,243	NM_003714	STC2	stanniocalcin 2	Extracellular Space	other
2,237	NM_001018073	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	Cytoplasm	kinase
2,197	NM_001039517	RUSC1-AS1	RUSC1 antisense RNA 1	Other	other
2,188	NM_057091	ARTN	artemin	Extracellular Space	growth factor
2,174	NM_005098	MSC	musculin	Cytoplasm	transcription regulator
2,166	NM_000691	ALDH3A1	aldehyde dehydrogenase 3 family, member A1	Cytoplasm	enzyme
2,149	NM_003739	AKR1C3	aldo-keto reductase family 1, member C3	Cytoplasm	enzyme
2,073	NM_020689	SLC24A3	solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	Plasma Membrane	transporter
2,059	NM_015404	DFNB31	deafness, autosomal recessive 31	Plasma Membrane	other
2,054	NM_177977	HAP1	huntingtin-associated protein 1	Cytoplasm	other
2,049	NM_139248	LIPH	lipase, member H	Extracellular Space	enzyme
2,044	NM_005771	DHRS9	dehydrogenase/reductase (SDR family) member 9	Cytoplasm	enzyme
2,040	NM_001014842	TM9SF1	transmembrane 9 superfamily member 1	Plasma Membrane	transporter
2,009	NM_153487	MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	Plasma Membrane	other

2,009	NM_004756	NUMBL	numb homolog (Drosophila)-like	Cytoplasm	other
2,008	NM_031476	CRISPLD2	cysteine-rich secretory protein LCCL domain containing 2	Cytoplasm	other
-2,002	NM_014241	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	Other	phosphatase
-2,009	NM_021957	GYS2	glycogen synthase 2 (liver)	Cytoplasm	enzyme
-2,085	NM_001809	CENPA	centromere protein A	Nucleus	other
-2,099	NM_005320	HIST1H1D	histone cluster 1, H1d	Nucleus	other
-2,102	NM_012467	TPSG1	tryptase gamma 1	Extracellular Space	peptidase
-2,105	NM_018410	HJURP	Holliday junction recognition protein	Nucleus	other
-2,164	NM_003535	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,181	NM_013381	TRHDE	thyrotropin-releasing hormone degrading enzyme	Plasma Membrane	peptidase
-2,183	NM_007226	NXPH2	neurexophilin 2	Extracellular Space	other
-2,219	NM_001004485	OR13F1	olfactory receptor, family 13, subfamily F, member 1	Plasma Membrane	G-protein coupled receptor
-2,257	NM_032602	GJA10	gap junction protein, alpha 10, 62kDa	Plasma Membrane	transporter
-2,328	NM_001005205	OR8J1	olfactory receptor, family 8, subfamily J, member 1	Plasma Membrane	G-protein coupled receptor
-2,331	NM_000905	NPY	neuropeptide Y	Extracellular Space	other

Results of the DNA microarray (180 s; 50 % oxygen and 50 % nitrogen):

Fold Change	**ID**	**Symbol**	**Entrez Gene Name**	**Location**	**Type(s)**
4,633	NM_002133	HMOX1	heme oxygenase (decycling) 1	Cytoplasm	enzyme
4,253	NM_024111	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)	Cytoplasm	other
3,484	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
3,335	NM_182981	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
3,269	NM_013370	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
3,045	NM_004744	LRAT	lecithin retinol acyltransferase (phosphatidylcholine–retinol O-acyltransferase)	Cytoplasm	enzyme
3,006	NM_001018073	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	Cytoplasm	kinase

2,965	NM_031476	CRISPLD2	cysteine-rich secretory protein LCCL domain containing 2	Cytoplasm	other
2,819	NM_001039517	RUSC1-AS1	RUSC1 antisense RNA 1	Other	other
2,819	NM_025237	SOST	sclerostin	Extracellular Space	other
2,809	NM_003714	STC2	stanniocalcin 2	Extracellular Space	other
2,678	NM_001004134	OR10AD1	olfactory receptor, family 10, subfamily AD, member 1	Plasma Membrane	G-protein coupled receptor
2,660	NM_203299	C9orf131	chromosome 9 open reading frame 131	Other	other
2,648	NM_001008272	TAGLN3	transgelin 3	Extracellular Space	other
2,578	NM_000565	IL6R	interleukin 6 receptor	Plasma Membrane	transmembrane receptor
2,563	NM_005098	MSC	musculin	Cytoplasm	transcription regulator
2,521	NM_198485	TPRG1	tumor protein p63 regulated 1	Cytoplasm	other
2,481	NM_014331	SLC7A11	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	Plasma Membrane	transporter
2,477	NM_005300	GPR34	G protein-coupled receptor 34	Plasma Membrane	G-protein coupled receptor
2,456	NM_001013624	ZNF385C	zinc finger protein 385C	Other	other
2,450	NM_005525	HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	Cytoplasm	enzyme
2,446	NM_139248	LIPH	lipase, member H	Extracellular Space	enzyme
2,397	NM_173199	NR4A3	nuclear receptor subfamily 4, group A, member 3	Nucleus	ligand-dependent nuclear receptor
2,374	NM_002779	PSD	pleckstrin and Sec7 domain containing	Cytoplasm	other
2,355	NM_181847	AMIGO2	adhesion molecule with Ig-like domain 2	Plasma Membrane	other
2,340	NM_052957	ACRC	acidic repeat containing	Other	other
2,339	NM_020778	ALPK3	alpha-kinase 3	Nucleus	kinase
2,339	NM_006981	NR4A3	nuclear receptor subfamily 4, group A, member 3	Nucleus	ligand-dependent nuclear receptor
2,333	NM_006132	BMP1	bone morphogenetic protein 1	Extracellular Space	peptidase
2,310	NM_001018103	POLR2M	polymerase (RNA) II (DNA directed) polypeptide M	Nucleus	other
2,297	NM_025218	ULBP1	UL16 binding protein 1	Plasma Membrane	transmembrane receptor
2,293	NM_153487	MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	Plasma Membrane	other
2,281	NM_153347	TMEM86A	transmembrane protein 86A	Other	other
2,277	NM_015404	DFNB31	deafness, autosomal recessive 31	Plasma Membrane	other

2,252	NM_139021	MAPK15	mitogen-activated protein kinase 15	Cytoplasm	kinase
2,247	NM_057091	ARTN	artemin	Extracellular Space	growth factor
2,247	NM_000364	TNNT2	troponin T type 2 (cardiac)	Cytoplasm	other
2,244	NM_031459	SESN2	sestrin 2	Cytoplasm	other
2,242	NM_005609	PYGM	phosphorylase, glycogen, muscle	Cytoplasm	enzyme
2,225	NM_004312	ARR3	arrestin 3, retinal (X-arrestin)	Cytoplasm	other
2,216	NM_001010893	SLC10A5	solute carrier family 10 (sodium/bile acid cotransporter family), member 5	Other	other
2,210	NM_001945	HBEGF	heparin-binding EGF-like growth factor	Extracellular Space	growth factor
2,208	NM_002943	RORA	RAR-related orphan receptor A	Nucleus	ligand-dependent nuclear receptor
2,198	NM_177977	HAP1	huntingtin-associated protein 1	Cytoplasm	other
2,188	NM_052890	PGLYRP2	peptidoglycan recognition protein 2	Plasma Membrane	transmembrane receptor
2,179	NM_012385	NUPR1	nuclear protein, transcriptional regulator, 1	Nucleus	transcription regulator
2,165	NM_000171	GLRA1	glycine receptor, alpha 1	Plasma Membrane	ion channel
2,164	NM_020642	AKIP1	A kinase (PRKA) interacting protein 1	Nucleus	other
2,162	NM_000574	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	Plasma Membrane	other
2,155	NM_007180	TREH	trehalase (brush-border membrane glycoprotein)	Plasma Membrane	enzyme
2,154	NM_019074	DLL4	delta-like 4 (Drosophila)	Extracellular Space	other
2,140	NM_033514	LIMS3/LIMS3L	LIM and senescent cell antigen-like domains 3	Other	other
2,129	NM_002514	NOV	nephroblastoma overexpressed	Extracellular Space	growth factor
2,117	NM_173565	RSPH10B/ RSPH10B2	radial spoke head 10 homolog B (Chlamydomonas)	Extracellular Space	other
2,110	NM_002773	PRSS8	protease, serine, 8	Extracellular Space	peptidase
2,108	NM_170600	SH2D3C	SH2 domain containing 3C	Cytoplasm	other
2,108	NM_001014842	TM9SF1	transmembrane 9 superfamily member 1	Plasma Membrane	transporter
2,107	NM_178450	MARCH3	membrane-associated ring finger (C3HC4) 3, E3 ubiquitin protein ligase	Cytoplasm	other
2,103	NM_005453	ZBTB22	zinc finger and BTB domain containing 22	Nucleus	other
2,096	NM_021810	CDH26	cadherin 26	Plasma Membrane	other
2,092	NM_018436	ALLC	allantoicase	Other	enzyme
2,092	NM_005771	DHRS9	dehydrogenase/reductase (SDR family) member 9	Cytoplasm	enzyme
2,080	NM_032647	CBX2	chromobox homolog 2	Nucleus	transcription regulator
2,080	NM_144593	RHEBL1	Ras homolog enriched in brain like 1	Cytoplasm	enzyme

2,074	NM_001039664	TNFRSF25	tumor necrosis factor receptor superfamily, member 25	Plasma Membrane	transmembrane receptor
2,063	NM_003749	IRS2	insulin receptor substrate 2	Cytoplasm	enzyme
2,054	NM_016348	FAXDC2	fatty acid hydroxylase domain containing 2	Other	other
2,046	NM_020299	AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	Cytoplasm	enzyme
2,044	NM_176891	IFNE	interferon, epsilon	Extracellular Space	cytokine
2,043	NM_001012454	FAM71F2	family with sequence similarity 71, member F2	Other	other
2,039	XM_373925	LOC388813	uncharacterized protein ENSP00000383407-like	Other	other
2,035	NM_000638	VTN	vitronectin	Extracellular Space	other
2,031	NM_017415	KLHL3	kelch-like family member 3	Cytoplasm	other
2,029	NM_206827	RASL11A	RAS-like, family 11, member A	Nucleus	other
2,025	NM_004443	EPHB3	EPH receptor B3	Plasma Membrane	kinase
2,022	NM_004816	FAM189A2	family with sequence similarity 189, member A2	Other	other
2,019	NM_138690	GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	Plasma Membrane	ion channel
2,008	NM_181657	LTB4R	leukotriene B4 receptor	Plasma Membrane	G-protein coupled receptor
2,004	NM_024013	IFNA1/IFNA13	interferon, alpha 1	Extracellular Space	cytokine
2,002	NM_153268	PLCXD2	phosphatidylinositol-specific phospholipase C, X domain containing 2	Other	enzyme
-2,001	NM_019888	MC3R	melanocortin 3 receptor	Plasma Membrane	G-protein coupled receptor
-2,002	NM_001004462	OR10G4	olfactory receptor, family 10, subfamily G, member 4	Plasma Membrane	G-protein coupled receptor
-2,003	NM_003835	RGS9	regulator of G-protein signaling 9	Cytoplasm	enzyme
-2,005	XM_370707	TMEM233	transmembrane protein 233	Other	other
-2,006	NM_018485	C5AR2	complement component 5a receptor 2	Plasma Membrane	other
-2,006	NM_004321	KIF1A	kinesin family member 1A	Cytoplasm	other
-2,010	NM_005161	APLNR	apelin receptor	Plasma Membrane	G-protein coupled receptor
-2,020	NM_032803	SLC7A3	solute carrier family 7 (cationic amino acid transporter, y+ system), member 3	Plasma Membrane	transporter
-2,027	NM_000851	GSTM5	glutathione S-transferase mu 5	Cytoplasm	enzyme
-2,027	NM_170610	HIST1H2BA	histone cluster 1, H2ba	Nucleus	other
-2,030	NM_032024	C10orf11	chromosome 10 open reading frame 11	Other	other
-2,035	NM_207335	KBTBD12	kelch repeat and BTB (POZ) domain containing 12	Other	other

-2,046	NM_006982	ALX1	ALX homeobox 1	Nucleus	transcription regulator
-2,056	NM_145273	CD300LG	CD300 molecule-like family member g	Other	other
-2,060	NM_003535	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,067	NM_001001821	OR2T3/OR2T34	olfactory receptor, family 2, subfamily T, member 34	Plasma Membrane	other
-2,068	NM_015855	WT1-AS	WT1 antisense RNA	Other	other
-2,074	NM_178176	MOGAT3	monoacylglycerol O-acyltransferase 3	Other	enzyme
-2,074	NM_144713	RMDN2	regulator of microtubule dynamics 2	Cytoplasm	other
-2,078	NM_001809	CENPA	centromere protein A	Nucleus	other
-2,086	NM_032298	SYT3	synaptotagmin III	Cytoplasm	transporter
-2,087	NM_203400	RPRML	reprimo-like	Other	other
-2,089	NM_032147	USP44	ubiquitin specific peptidase 44	Nucleus	peptidase
-2,100	NM_000486	AQP2	aquaporin 2 (collecting duct)	Plasma Membrane	transporter
-2,100	NM_001004705	OR4D10	olfactory receptor, family 4, subfamily D, member 10	Plasma Membrane	G-protein coupled receptor
-2,102	NM_001037668	DEFB107A/ DEFB107B	defensin, beta 107A	Extracellular Space	other
-2,102	NM_153031	SATB2-AS1	SATB2 antisense RNA 1	Other	other
-2,107	NM_021193	HOXD12	homeobox D12	Nucleus	transcription regulator
-2,108	NM_207414	MROH5	maestro heat-like repeat family member 5	Other	other
-2,112	NM_182617	ACSM2B	acyl-CoA synthetase medium-chain family member 2B	Cytoplasm	enzyme
-2,112	NM_003055	SLC18A3	solute carrier family 18 (vesicular acetylcholine), member 3	Plasma Membrane	transporter
-2,114	XM_295195	COL28A1	collagen, type XXVIII, alpha 1	Extracellular Space	other
-2,117	NM_001012416	KRTAP5-6	keratin associated protein 5-6	Other	other
-2,118	NM_001031853	INSC	inscuteable homolog (Drosophila)	Other	other
-2,119	NM_001001666	ANO7	anoctamin 7	Plasma Membrane	ion channel
-2,119	NM_000420	KEL	Kell blood group, metallo-endopeptidase	Plasma Membrane	peptidase
-2,129	XM_927351	PIRT	phosphoinositide-interacting regulator of transient receptor potential channels	Other	other
-2,134	NM_001005324	OR10V1	olfactory receptor, family 10, subfamily V, member 1	Plasma Membrane	G-protein coupled receptor
-2,141	NM_012353	OR1C1	olfactory receptor, family 1, subfamily C, member 1	Plasma Membrane	G-protein coupled receptor

-2,142	NM_002594	PCSK2	proprotein convertase subtilisin/kexin type 2	Extracellular Space	peptidase
-2,150	NM_000667	ADH1A	alcohol dehydrogenase 1A (class I), alpha polypeptide	Cytoplasm	enzyme
-2,156	NM_198483	RUFY4	RUN and FYVE domain containing 4	Other	other
-2,170	NM_001007563	IGFBPL1	insulin-like growth factor binding protein-like 1	Other	other
-2,178	NM_173544	FAM129C	family with sequence similarity 129, member C	Other	other
-2,180	NM_005889	APOBEC1	apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	Cytoplasm	enzyme
-2,181	NM_207181	NPHP1	nephronophthisis 1 (juvenile)	Nucleus	other
-2,194	XM_927661	SMIM10	small integral membrane protein 10	Other	other
-2,207	NM_000278	PAX2	paired box 2	Nucleus	transcription regulator
-2,215	NM_015687	FILIP1	filamin A interacting protein 1	Cytoplasm	other
-2,223	NM_018980	TAS2R5	taste receptor, type 2, member 5	Plasma Membrane	G-protein coupled receptor
-2,225	NM_002001	FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	Plasma Membrane	transmembrane receptor
-2,226	NM_012378	OR8B8	olfactory receptor, family 8, subfamily B, member 8	Plasma Membrane	G-protein coupled receptor
-2,227	NM_145028	ARMC12	armadillo repeat containing 12	Other	other
-2,235	NM_007256	SLCO2B1	solute carrier organic anion transporter family, member 2B1	Plasma Membrane	transporter
-2,237	NM_003240	LEFTY2	left-right determination factor 2	Extracellular Space	growth factor
-2,244	NM_001832	CLPS	colipase, pancreatic	Extracellular Space	other
-2,252	NM_021250	LILRA5	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	Plasma Membrane	other
-2,253	XM_211174	LOC283710	uncharacterized LOC283710	Other	other
-2,254	NM_001030050	KLK3	kallikrein-related peptidase 3	Extracellular Space	peptidase
-2,255	NM_203471	LGALS14	lectin, galactoside-binding, soluble, 14	Nucleus	other
-2,259	NM_000794	DRD1	dopamine receptor D1	Plasma Membrane	G-protein coupled receptor
-2,261	NM_004391	CYP8B1	cytochrome P450, family 8, subfamily B, polypeptide 1	Cytoplasm	enzyme
-2,274	NM_016347	NAT8B	N-acetyltransferase 8B (GCN5-related, putative, gene/pseudogene)	Other	other
-2,293	NM_152460	C17orf77	chromosome 17 open reading frame 77	Other	other
-2,293	NM_174892	CD300LB	CD300 molecule-like family member b	Plasma Membrane	other

-2,298	NM_022454	SOX17	SRY (sex determining region Y)-box 17	Nucleus	transcription regulator
-2,318	NM_033440	CELA2A	chymotrypsin-like elastase family, member 2A	Extracellular Space	peptidase
-2,327	NM_181866	ACOT7	acyl-CoA thioesterase 7	Cytoplasm	enzyme
-2,345	XM_293529	LRRIQ4	leucine-rich repeats and IQ motif containing 4	Other	other
-2,346	NM_182828	GDF7	growth differentiation factor 7	Extracellular Space	growth factor
-2,346	NM_178356	LCE4A	late cornified envelope 4A	Other	other
-2,350	NM_006685	SMR3B	submaxillary gland androgen regulated protein 3B	Nucleus	other
-2,363	NM_001005325	OR6M1	olfactory receptor, family 6, subfamily M, member 1	Plasma Membrane	other
-2,394	NM_199131	VAX1	ventral anterior homeobox 1	Nucleus	transcription regulator
-2,410	NM_005320	HIST1H1D	histone cluster 1, H1d	Nucleus	other
-2,421	NM_001004728	OR5A1	olfactory receptor, family 5, subfamily A, member 1	Plasma Membrane	G-protein coupled receptor
-2,426	NM_181600	KRTAP13-4	keratin associated protein 13-4	Other	other
-2,504	NM_152629	GLIS3	GLIS family zinc finger 3	Nucleus	transcription regulator
-2,533	NM_144701	IL23R	interleukin 23 receptor	Plasma Membrane	transmembrane receptor
-2,533	NM_206880	OR2V2	olfactory receptor, family 2, subfamily V, member 2	Plasma Membrane	G-protein coupled receptor
-2,581	NM_012352	OR1A2	olfactory receptor, family 1, subfamily A, member 2	Plasma Membrane	G-protein coupled receptor
-2,584	XM_926213	NBPF4/NBPF6	neuroblastoma breakpoint family, member 4	Other	other
-2,629	NM_181623	KRTAP15-1	keratin associated protein 15-1	Other	other
-2,701	NM_001004711	OR4D9	olfactory receptor, family 4, subfamily D, member 9	Plasma Membrane	G-protein coupled receptor
-2,742	NM_003037	SLAMF1	signaling lymphocytic activation molecule family member 1	Plasma Membrane	transmembrane receptor
-2,794	NM_007374	SIX6	SIX homeobox 6	Nucleus	transcription regulator
-2,859	NM_001001690	LOC100287792	uncharacterized LOC100287792	Other	other
-2,941	NM_021225	PROL1	proline rich, lacrimal 1	Extracellular Space	other
-2,971	NM_001004749	OR51A7	olfactory receptor, family 51, subfamily A, member 7	Plasma Membrane	G-protein coupled receptor
-2,998	NM_014442	SIGLEC8	sialic acid binding Ig-like lectin 8	Plasma Membrane	transmembrane receptor

-3,230	NM_139250	CTAG1A/CTAG1B	Cancer/testis antigen 1B	Plasma Membrane	other

Results of the DNA microarray (180 s; 75 % oxygen and 25 % nitrogen):

Fold Change	ID	Symbol	Entrez Gene Name	Location	Type(s)
4,225	NM_024111	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)	Cytoplasm	other
3,609	NM_182981	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
3,598	NM_013370	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
3,395	NM_002133	HMOX1	heme oxygenase (decycling) 1	Cytoplasm	enzyme
2,966	NM_001018073	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	Cytoplasm	kinase
2,916	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
2,795	NM_004744	LRAT	lecithin retinol acyltransferase (phosphatidylcholine–retinol O-acyltransferase)	Cytoplasm	enzyme
2,584	NM_177977	HAP1	huntingtin-associated protein 1	Cytoplasm	other
2,563	NM_003714	STC2	stanniocalcin 2	Extracellular Space	other
2,552	NM_001945	HBEGF	heparin-binding EGF-like growth factor	Extracellular Space	growth factor
2,488	NM_001008272	TAGLN3	transgelin 3	Extracellular Space	other
2,469	NM_025194	ITPKC	inositol-trisphosphate 3-kinase C	Cytoplasm	kinase
2,450	NM_012113	CA14	carbonic anhydrase XIV	Plasma Membrane	enzyme
2,419	NM_001001965	OR4D5	olfactory receptor, family 4, subfamily D, member 5	Plasma Membrane	G-protein coupled receptor
2,379	NM_002943	RORA	RAR-related orphan receptor A	Nucleus	ligand-dependent nuclear receptor
2,372	NM_000574	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	Plasma Membrane	other
2,371	NM_139021	MAPK15	mitogen-activated protein kinase 15	Cytoplasm	kinase
2,359	NM_174934	SCN4B	sodium channel, voltage-gated, type IV, beta subunit	Plasma Membrane	ion channel
2,331	NM_031459	SESN2	sestrin 2	Cytoplasm	other
2,327	NM_014331	SLC7A11	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	Plasma Membrane	transporter
2,302	NM_021642	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	Plasma Membrane	transmembrane receptor

2,297	NM_203299	C9orf131	chromosome 9 open reading frame 131	Other	other
2,297	NM_031476	CRISPLD2	cysteine-rich secretory protein LCCL domain containing 2	Cytoplasm	other
2,292	NM_001014842	TM9SF1	transmembrane 9 superfamily member 1	Plasma Membrane	transporter
2,284	NM_020184	CNNM4	cyclin M4	Plasma Membrane	other
2,270	NM_005771	DHRS9	dehydrogenase/reductase (SDR family) member 9	Cytoplasm	enzyme
2,253	NM_005609	PYGM	phosphorylase, glycogen, muscle	Cytoplasm	enzyme
2,229	NM_000565	IL6R	interleukin 6 receptor	Plasma Membrane	transmembrane receptor
2,164	NM_000364	TNNT2	troponin T type 2 (cardiac)	Cytoplasm	other
2,157	NM_013959	NRG1	neuregulin 1	Other	growth factor
2,148	NM_018948	ERRFI1	ERBB receptor feedback inhibitor 1	Cytoplasm	other
2,141	NM_005217	DEFA1 (includes others)	defensin, alpha 1	Cytoplasm	other
2,138	NM_145244	DDIT4L	DNA-damage-inducible transcript 4-like	Other	other
2,138	NM_003949	HAP1	huntingtin-associated protein 1	Cytoplasm	other
2,131	NM_032974	CASP10	caspase 10, apoptosis-related cysteine peptidase	Cytoplasm	peptidase
2,128	NM_001034954	SORBS1	sorbin and SH3 domain containing 1	Plasma Membrane	other
2,120	NM_001008394	EID3	EP300 interacting inhibitor of differentiation 3	Cytoplasm	other
2,119	NM_001037668	DEFB107A/ DEFB107B	defensin, beta 107A	Extracellular Space	other
2,112	NM_052957	ACRC	acidic repeat containing	Other	other
2,109	NM_001039664	TNFRSF25	tumor necrosis factor receptor superfamily, member 25	Plasma Membrane	transmembrane receptor
2,106	NM_025218	ULBP1	UL16 binding protein 1	Plasma Membrane	transmembrane receptor
2,100	NM_001039517	RUSC1-AS1	RUSC1 antisense RNA 1	Other	other
2,100	NM_025237	SOST	sclerostin	Extracellular Space	other
2,091	NM_000452	SLC10A2	solute carrier family 10 (sodium/bile acid cotransporter family), member 2	Plasma Membrane	transporter
2,074	NM_022119	PRSS22	protease, serine, 22	Extracellular Space	peptidase
2,059	NM_198853	TRIM73/ TRIM74	tripartite motif containing 74	Other	other
2,058	NM_001030287	ATF3	activating transcription factor 3	Nucleus	transcription regulator
2,047	NM_181711	GRASP	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein	Plasma Membrane	other

2,046	NM_001010893	SLC10A5	solute carrier family 10 (sodium/bile acid cotransporter family), member 5	Plasma Membrane	other
2,032	NM_001365	DLG4	discs, large homolog 4 (Drosophila)	Plasma Membrane	kinase
2,022	NM_000584	IL8	interleukin 8	Extracellular Space	cytokine
2,014	NM_176891	IFNE	interferon, epsilon	Extracellular Space	cytokine
2,013	NM_139248	LIPH	lipase, member H	Extracellular Space	enzyme
2,012	NM_001008496	PIWIL3	piwi-like RNA-mediated gene silencing 3	Other	other
2,000	NM_144593	RHEBL1	Ras homolog enriched in brain like 1	Cytoplasm	enzyme
-2,007	NM_015076	CDK19	cyclin-dependent kinase 19	Nucleus	kinase
-2,010	NM_001001325	SPINK14	serine peptidase inhibitor, Kazal type 14 (putative)	Other	other
-2,014	NM_001809	CENPA	centromere protein A	Nucleus	other
-2,017	NM_024772	ZMYM1	zinc finger, MYM-type 1	Other	other
-2,032	NM_207418	FAM72D	family with sequence similarity 72, member D	Nucleus	other
-2,032	NM_023927	GRAMD3	GRAM domain containing 3	Extracellular Space	other
-2,032	NM_001555	IGSF1	immunoglobulin superfamily, member 1	Plasma Membrane	other
-2,033	NM_003521	HIST1H2BM	histone cluster 1, H2bm	Nucleus	other
-2,047	NM_018482	ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1	Plasma Membrane	other
-2,054	NM_016426	GTSE1	G-2 and S-phase expressed 1	Cytoplasm	other
-2,063	NM_017655	GIPC2	GIPC PDZ domain containing family, member 2	Cytoplasm	other
-2,072	NM_005156	PTBP3	polypyrimidine tract binding protein 3	Nucleus	other
-2,073	NM_148170	CTSC	cathepsin C	Cytoplasm	peptidase
-2,081	NM_013381	TRHDE	thyrotropin-releasing hormone degrading enzyme	Plasma Membrane	peptidase
-2,107	NM_000065	C6	complement component 6	Extracellular Space	other
-2,116	NM_012484	HMMR	hyaluronan-mediated motility receptor (RHAMM)	Plasma Membrane	transmembrane receptor
-2,121	NM_021058	HIST1H2BJ/HIST1H2BK	histone cluster 1, H2bk	Nucleus	other
-2,126	NM_018082	POLR3B	polymerase (RNA) III (DNA directed) polypeptide B	Nucleus	enzyme
-2,149	NM_001039884	ZNF826P	zinc finger protein 826, pseudogene	Other	other
-2,154	NM_018238	AGK	acylglycerol kinase	Cytoplasm	kinase
-2,174	NM_001813	CENPE	centromere protein E, 312kDa	Nucleus	other
-2,194	NM_003513	HIST1H2AB/HIST1H2AE	histone cluster 1, H2ae	Other	other
-2,199	NM_030760	S1PR5	sphingosine-1-phosphate receptor 5	Plasma Membrane	G-protein coupled receptor
-2,203	NM_022459	XPO4	exportin 4	Nucleus	transporter

-2,212	NM_153453	VGLL2	vestigial like 2 (Drosophila)	Nucleus	transcription regulator
-2,215	NM_022756	MEAF6	MYST/Esa1-associated factor 6	Nucleus	other
-2,219	NM_133271	FCAR	Fc fragment of IgA, receptor for	Plasma Membrane	other
-2,223	NM_003533	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,228	NM_018330	KIAA1598	KIAA1598	Other	other
-2,265	NM_021957	GYS2	glycogen synthase 2 (liver)	Cytoplasm	enzyme
-2,273	NM_015199	ANKRD28	ankyrin repeat domain 28	Cytoplasm	other
-2,290	NM_134470	IL1RAP	interleukin 1 receptor accessory protein	Plasma Membrane	transmembrane receptor
-2,291	NM_003531	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,295	NM_017779	DEPDC1	DEP domain containing 1	Nucleus	transcription regulator
-2,307	NM_001039547	GK5	glycerol kinase 5 (putative)	Other	kinase
-2,315	NM_003199	TCF4	transcription factor 4	Nucleus	transcription regulator
-2,325	NM_019857	CTPS2	CTP synthase 2	Cytoplasm	enzyme
-2,359	NM_020926	BCOR	BCL6 corepressor	Nucleus	transcription regulator
-2,362	NM_017769	G2E3	G2/M-phase specific E3 ubiquitin protein ligase	Cytoplasm	enzyme
-2,490	NM_005320	HIST1H1D	histone cluster 1, H1d	Nucleus	other

Results of the DNA microarray (180 s; 100 % oxygen and 0 % nitrogen):

Fold Change	**ID**	**Symbol**	**Entrez Gene Name**	**Location**	**Type(s)**
4,956	NM_024111	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)	Cytoplasm	other
4,849	NM_004256	SLC22A13	solute carrier family 22 (organic anion transporter), member 13	Plasma Membrane	transporter
4,209	NM_182981	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor
4,184	NM_005525	HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1	Cytoplasm	enzyme
4,171	NM_002133	HMOX1	heme oxygenase (decycling) 1	Cytoplasm	enzyme
3,865	NM_013370	OSGIN1	oxidative stress induced growth inhibitor 1	Other	growth factor

3,487	NM_001018073	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	Cytoplasm	kinase
3,132	NM_203299	C9orf131	chromosome 9 open reading frame 131	Other	other
3,123	NM_177977	HAP1	huntingtin-associated protein 1	Cytoplasm	other
3,021	NM_153487	MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	Plasma Membrane	other
3,002	NM_139021	MAPK15	mitogen-activated protein kinase 15	Cytoplasm	kinase
2,981	NM_001004134	OR10AD1	olfactory receptor, family 10, subfamily AD, member 1	Plasma Membrane	G-protein coupled receptor
2,949	NM_012385	NUPR1	nuclear protein, transcriptional regulator, 1	Nucleus	transcription regulator
2,937	NM_001039517	RUSC1-AS1	RUSC1 antisense RNA 1	Other	other
2,923	NM_013959	NRG1	neuregulin 1	Other	growth factor
2,895	NM_005453	ZBTB22	zinc finger and BTB domain containing 22	Nucleus	other
2,871	NM_001008394	EID3	EP300 interacting inhibitor of differentiation 3	Cytoplasm	other
2,853	NM_001864	COX7A1	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)	Cytoplasm	enzyme
2,803	NM_003395	WNT9A	wingless-type MMTV integration site family, member 9A	Extracellular Space	other
2,799	NM_003949	HAP1	huntingtin-associated protein 1	Cytoplasm	other
2,789	NM_176891	IFNE	interferon, epsilon	Extracellular Space	cytokine
2,768	NM_003714	STC2	stanniocalcin 2	Extracellular Space	other
2,763	NM_172006	WFDC10B	WAP four-disulfide core domain 10B	Extracellular Space	other
2,759	NM_181657	LTB4R	leukotriene B4 receptor	Plasma Membrane	G-protein coupled receptor
2,759	NM_001014842	TM9SF1	transmembrane 9 superfamily member 1	Plasma Membrane	transporter
2,737	NM_000565	IL6R	interleukin 6 receptor	Plasma Membrane	transmembrane receptor
2,717	NM_153347	TMEM86A	transmembrane protein 86A	Other	other
2,704	NM_004443	EPHB3	EPH receptor B3	Plasma Membrane	kinase
2,701	NM_001010893	SLC10A5	solute carrier family 10 (sodium/bile acid cotransporter family), member 5	Other	other
2,693	NM_198427	BCAN	brevican	Extracellular Space	other
2,685	NM_006132	BMP1	bone morphogenetic protein 1	Extracellular Space	peptidase
2,681	NM_006039	MRC2	mannose receptor, C type 2	Plasma Membrane	transmembrane receptor
2,680	NM_022377	ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	Plasma Membrane	other

2,669	XM_030729	NUTM2F/ NUTM2G	NUT family member 2G	Other	other
2,662	NM_022119	PRSS22	protease, serine, 22	Extracellular Space	peptidase
2,612	NM_006271	S100A1	S100 calcium binding protein A1	Cytoplasm	other
2,598	NM_002779	PSD	pleckstrin and Sec7 domain containing	Cytoplasm	other
2,590	NM_005609	PYGM	phosphorylase, glycogen, muscle	Cytoplasm	enzyme
2,576	NM_020439	CAMK1G	calcium/calmodulin-dependent protein kinase IG	Cytoplasm	kinase
2,575	NM_020184	CNNM4	cyclin M4	Plasma Membrane	other
2,574	NM_020795	NLGN2	neuroligin 2	Plasma Membrane	enzyme
2,573	NM_198853	TRIM73/ TRIM74	tripartite motif containing 74	Other	other
2,571	NM_004744	LRAT	lecithin retinol acyltransferase (phosphatidylcholine–retinol O-acyltransferase)	Cytoplasm	enzyme
2,570	NM_172131	WFDC10B	WAP four-disulfide core domain 10B	Extracellular Space	other
2,561	NM_001024211	S100A13	S100 calcium binding protein A13	Cytoplasm	other
2,559	XM_496502	POM121L9P	POM121 transmembrane nucleoporin-like 9, pseudogene	Other	other
2,552	NM_032855	HSH2D	hematopoietic SH2 domain containing	Cytoplasm	other
2,548	NM_153268	PLCXD2	phosphatidylinositol-specific phospholipase C, X domain containing 2	Other	enzyme
2,540	NM_003976	ARTN	artemin	Extracellular Space	growth factor
2,535	NM_001013714	LINC00969	long intergenic non-protein coding RNA 969	Other	other
2,528	NM_016943	TAS2R3	taste receptor, type 2, member 3	Plasma Membrane	G-protein coupled receptor
2,522	NM_138690	GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	Plasma Membrane	ion channel
2,521	NM_020884	MYH7B	myosin, heavy chain 7B, cardiac muscle, beta	Other	other
2,497	NM_032166	ATRIP	ATR interacting protein	Nucleus	kinase
2,495	NM_001365	DLG4	discs, large homolog 4 (Drosophila)	Plasma Membrane	kinase
2,493	NM_001039773	LOC644083	uncharacterized LOC644083	Other	other
2,491	NM_001025598	ARHGAP30	Rho GTPase activating protein 30	Cytoplasm	other
2,491	NM_198406	PAQR6	progestin and adipoQ receptor family member VI	Other	other
2,491	NM_002773	PRSS8	protease, serine, 8	Extracellular Space	peptidase
2,487	NM_001039664	TNFRSF25	tumor necrosis factor receptor superfamily, member 25	Plasma Membrane	transmembrane receptor
2,485	NM_001024858	SPTB	spectrin, beta, erythrocytic	Plasma Membrane	other
2,481	NM_145042	TUBA3FP	tubulin, alpha 3f, pseudogene	Other	other
2,478	NM_021046	KRTAP5-8	keratin associated protein 5-8	Other	other

2,471	NM_002648	PIM1	pim-1 oncogene	Cytoplasm	kinase
2,449	NM_000691	ALDH3A1	aldehyde dehydrogenase 3 family, member A1	Cytoplasm	enzyme
2,441	NM_153710	C9orf96	chromosome 9 open reading frame 96	Other	kinase
2,431	NM_138769	RHOT2	ras homolog family member T2	Cytoplasm	enzyme
2,420	NM_173158	NR4A1	nuclear receptor subfamily 4, group A, member 1	Nucleus	ligand-dependent nuclear receptor
2,415	NM_025237	SOST	sclerostin	Extracellular Space	other
2,413	NM_015404	DFNB31	deafness, autosomal recessive 31	Plasma Membrane	other
2,412	NM_002308	LGALS9	lectin, galactoside-binding, soluble, 9	Extracellular Space	other
2,399	XM_370681	RPL13AP20	ribosomal protein L13a pseudogene 20	Other	other
2,391	NM_001024656	ASPDH	aspartate dehydrogenase domain containing	Other	other
2,389	NM_024963	FBXL18	F-box and leucine-rich repeat protein 18	Other	enzyme
2,387	NM_016348	FAXDC2	fatty acid hydroxylase domain containing 2	Other	other
2,386	NM_015644	TTLL3	tubulin tyrosine ligase-like family, member 3	Extracellular Space	enzyme
2,380	NM_007254	PNKP	polynucleotide kinase 3'-phosphatase	Nucleus	kinase
2,379	NM_005677	COLQ	collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase	Extracellular Space	other
2,372	NM_032213	ELMOD3	ELMO/CED-12 domain containing 3	Other	other
2,369	NM_033514	LIMS3/LIMS3L	LIM and senescent cell antigen-like domains 3	Other	other
2,365	NM_001015053	HDAC5	histone deacetylase 5	Nucleus	transcription regulator
2,363	NM_144990	SLFNL1	schlafen-like 1	Other	other
2,360	NM_001945	HBEGF	heparin-binding EGF-like growth factor	Extracellular Space	growth factor
2,352	NM_173201	ATP2A1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	Cytoplasm	transporter
2,351	NM_016938	EFEMP2	EGF containing fibulin-like extracellular matrix protein 2	Extracellular Space	other
2,348	NM_000804	FOLR3	folate receptor 3 (gamma)	Extracellular Space	other
2,347	NM_018654	GPRC5D	G protein-coupled receptor, family C, group 5, member D	Plasma Membrane	G-protein coupled receptor
2,338	NM_001002034	FAM109B	family with sequence similarity 109, member B	Other	other
2,334	NM_031459	SESN2	sestrin 2	Cytoplasm	other
2,330	NM_006676	USP20	ubiquitin specific peptidase 20	Cytoplasm	peptidase
2,327	NM_001672	ASIP	agouti signaling protein	Extracellular Space	other
2,326	NM_023087	CAPN10	calpain 10	Cytoplasm	peptidase
2,324	NM_007056	CLASRP	CLK4-associating serine/arginine rich protein	Nucleus	other
2,319	NM_138356	SHF	Src homology 2 domain containing F	Other	other
2,317	NM_016564	CEND1	cell cycle exit and neuronal differentiation 1	Other	other

2,315	NM_004584	RAD9A	RAD9 homolog A (S. pombe)	Nucleus	enzyme
2,313	NM_000574	CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	Plasma Membrane	other
2,313	NM_003961	RHBDL1	rhomboid, veinlet-like 1 (Drosophila)	Plasma Membrane	peptidase
2,313	NM_016642	SPTBN5	spectrin, beta, non-erythrocytic 5	Plasma Membrane	other
2,311	NM_004320	ATP2A1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	Cytoplasm	transporter
2,302	NM_001031682	GPER	G protein-coupled estrogen receptor 1	Plasma Membrane	G-protein coupled receptor
2,302	NM_012108	STAP1	signal transducing adaptor family member 1	Cytoplasm	other
2,298	NM_181686	KRTAP12-1	keratin associated protein 12-1	Other	other
2,295	NM_000364	TNNT2	troponin T type 2 (cardiac)	Cytoplasm	other
2,262	NM_025194	ITPKC	inositol-trisphosphate 3-kinase C	Cytoplasm	kinase
2,262	NM_181484	ZGPAT	zinc finger, CCCH-type with G patch domain	Nucleus	transcription regulator
2,260	NM_053006	TSSK2	testis-specific serine kinase 2	Cytoplasm	kinase
2,259	NM_130900	RAET1L	retinoic acid early transcript 1L	Other	other
2,259	NM_020659	TTYH1	tweety homolog 1 (Drosophila)	Plasma Membrane	ion channel
2,258	NM_001004474	OR10S1	olfactory receptor, family 10, subfamily S, member 1	Plasma Membrane	G-protein coupled receptor
2,255	NM_031476	CRISPLD2	cysteine-rich secretory protein LCCL domain containing 2	Cytoplasm	other
2,253	NM_023083	CAPN10	calpain 10	Cytoplasm	peptidase
2,253	NM_005098	MSC	musculin	Cytoplasm	transcription regulator
2,253	NM_000638	VTN	vitronectin	Extracellular Space	other
2,249	NM_057091	ARTN	artemin	Extracellular Space	growth factor
2,243	NM_018948	ERRFI1	ERBB receptor feedback inhibitor 1	Cytoplasm	other
2,242	NM_173515	CNKSR3	CNKSR family member 3	Plasma Membrane	kinase
2,242	NM_018653	GPRC5C	G protein-coupled receptor, family C, group 5, member C	Plasma Membrane	G-protein coupled receptor
2,241	NM_181885	RXFP4	relaxin/insulin-like family peptide receptor 4	Plasma Membrane	G-protein coupled receptor
2,235	NM_031264	CDHR5	cadherin-related family member 5	Plasma Membrane	other
2,232	NM_015997	RRNAD1	ribosomal RNA adenine dimethylase domain containing 1	Other	other
2,229	NM_001012454	FAM71F2	family with sequence similarity 71, member F2	Other	other
2,228	NM_001258	CDK3	cyclin-dependent kinase 3	Other	kinase

2,226	NM_001013632	TCTEX1D4	Tctex1 domain containing 4	Cytoplasm	other
2,219	NM_001437	ESR2	estrogen receptor 2 (ER beta)	Nucleus	ligand-dependent nuclear receptor
2,218	NM_001158	AOC2	amine oxidase, copper containing 2 (retina-specific)	Other	enzyme
2,218	NM_006484	DYRK1B	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B	Nucleus	kinase
2,210	NM_001029881	CIB4	calcium and integrin binding family member 4	Other	other
2,209	NM_133644	GTPBP3	GTP binding protein 3 (mitochondrial)	Cytoplasm	enzyme
2,205	NM_052957	ACRC	acidic repeat containing	Other	other
2,198	NM_013358	PADI1	peptidyl arginine deiminase, type I	Cytoplasm	enzyme
2,197	NM_181847	AMIGO2	adhesion molecule with Ig-like domain 2	Plasma Membrane	other
2,195	NM_015503	SH2B1	SH2B adaptor protein 1	Cytoplasm	other
2,192	NM_182520	C22orf15	chromosome 22 open reading frame 15	Other	other
2,182	NM_012458	TIMM13	translocase of inner mitochondrial membrane 13 homolog (yeast)	Cytoplasm	transporter
2,174	NM_002616	PER1	period circadian clock 1	Nucleus	other
2,173	NM_032794	SLC44A4	solute carrier family 44, member 4	Plasma Membrane	transporter
2,171	NM_024318	LILRA6	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 6	Other	other
2,169	NM_004409	DMPK	dystrophia myotonica-protein kinase	Cytoplasm	kinase
2,166	NM_173525	C11orf42	chromosome 11 open reading frame 42	Other	other
2,164	NM_024866	ADM2	adrenomedullin 2	Extracellular Space	other
2,164	NM_025092	ATHL1	ATH1, acid trehalase-like 1 (yeast)	Other	other
2,161	NM_018055	NODAL	nodal growth differentiation factor	Extracellular Space	growth factor
2,159	NM_005886	KATNB1	katanin p80 (WD repeat containing) subunit B 1	Cytoplasm	enzyme
2,158	NM_001035235	SRA1	steroid receptor RNA activator 1	Nucleus	transcription regulator
2,157	NM_000171	GLRA1	glycine receptor, alpha 1	Plasma Membrane	ion channel
2,157	NM_001040078	LGALS9C	lectin, galactoside-binding, soluble, 9C	Other	other
2,154	NM_145008	YPEL4	yippee-like 4 (Drosophila)	Nucleus	other
2,153	NM_145804	ABTB2	ankyrin repeat and BTB (POZ) domain containing 2	Other	other
2,153	NM_016363	GP6	glycoprotein VI (platelet)	Plasma Membrane	transmembrane receptor
2,151	NM_173593	B4GALNT3	beta-1,4-N-acetyl-galactosaminyl transferase 3	Other	enzyme
2,149	NM_145296	CADM4	cell adhesion molecule 4	Plasma Membrane	other

2,149	NM_005090	JMJD7-PLA2G4B	JMJD7-PLA2G4B readthrough	Cytoplasm	other
2,143	NM_133175	APBB3	amyloid beta (A4) precursor protein-binding, family B, member 3	Cytoplasm	other
2,143	NM_178502	DTX3	deltex homolog 3 (Drosophila)	Cytoplasm	other
2,140	NM_031918	KLF16	Kruppel-like factor 16	Nucleus	transcription regulator
2,137	NM_001013624	ZNF385C	zinc finger protein 385C	Other	other
2,126	NM_001335	CTSW	cathepsin W	Cytoplasm	peptidase
2,126	NM_014331	SLC7A11	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	Plasma Membrane	transporter
2,121	NM_001005914	SEMA3B	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	Extracellular Space	other
2,120	NM_001025161	CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6	Cytoplasm	enzyme
2,118	NM_198490	RAB43	RAB43, member RAS oncogene family	Other	enzyme
2,115	NM_012323	MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	Nucleus	transcription regulator
2,113	NM_153342	TMEM150A	transmembrane protein 150A	Other	other
2,111	NM_006301	MAP3K12	mitogen-activated protein kinase kinase kinase 12	Cytoplasm	kinase
2,110	NM_152468	TMC8	transmembrane channel-like 8	Other	other
2,108	NM_016391	NOP16	NOP16 nucleolar protein	Nucleus	other
2,104	NM_001031738	TMEM150A	transmembrane protein 150A	Other	other
2,103	NM_207387	FAM211A	family with sequence similarity 211, member A	Other	other
2,100	NM_001013838	RLTPR	RGD motif, leucine rich repeats, tropomodulin domain and proline-rich containing	Other	other
2,099	NM_144505	KLK8	kallikrein-related peptidase 8	Extracellular Space	peptidase
2,099	NM_002943	RORA	RAR-related orphan receptor A	Nucleus	ligand-dependent nuclear receptor
2,098	NM_182683	UPK3B	uroplakin 3B	Plasma Membrane	other
2,096	NM_006483	DYRK1B	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B	Nucleus	kinase
2,095	NM_004807	HS6ST1	heparan sulfate 6-O-sulfotransferase 1	Plasma Membrane	enzyme
2,094	NM_139239	NFKBID	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta	Nucleus	transcription regulator
2,092	NM_020325	ABCD4	ATP-binding cassette, sub-family D (ALD), member 4	Cytoplasm	transporter
2,091	NM_014700	RAB11FIP3	RAB11 family interacting protein 3 (class II)	Cytoplasm	other

2,085	NM_001005862	ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Plasma Membrane	kinase
2,082	NM_002373	MAP1A	microtubule-associated protein 1A	Cytoplasm	other
2,079	NM_000359	TGM1	transglutaminase 1	Plasma Membrane	enzyme
2,078	NM_003632	CNTNAP1	contactin associated protein 1	Plasma Membrane	other
2,077	NM_031295	ABHD11	abhydrolase domain containing 11	Cytoplasm	enzyme
2,075	NM_003466	PAX8	paired box 8	Nucleus	transcription regulator
2,075	NM_174934	SCN4B	sodium channel, voltage-gated, type IV, beta subunit	Plasma Membrane	ion channel
2,074	NM_001018060	AIFM3	apoptosis-inducing factor, mitochondrion-associated, 3	Cytoplasm	enzyme
2,073	NM_182556	SLC25A45	solute carrier family 25, member 45	Cytoplasm	transporter
2,069	NM_000152	GAA	glucosidase, alpha; acid	Cytoplasm	enzyme
2,068	NM_005091	PGLYRP1	peptidoglycan recognition protein 1	Plasma Membrane	transmembrane receptor
2,067	NM_004819	SYMPK	symplekin	Cytoplasm	other
2,066	NM_198540	B3GNT8	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8	Cytoplasm	enzyme
2,064	NM_181720	ARHGAP30	Rho GTPase activating protein 30	Cytoplasm	other
2,064	NM_013279	MYRF	myelin regulatory factor	Nucleus	transcription regulator
2,058	NM_003388	CLIP2	CAP-GLY domain containing linker protein 2	Cytoplasm	transcription regulator
2,057	NM_017705	PAQR5	progestin and adipoQ receptor family member V	Other	other
2,056	NM_207581	DUOXA2	dual oxidase maturation factor 2	Cytoplasm	other
2,056	NM_006900	IFNA1/IFNA13	interferon, alpha 1	Extracellular Space	cytokine
2,056	NM_032107	L3MBTL1	l(3)mbt-like 1 (Drosophila)	Nucleus	other
2,056	NM_025228	TRAF3IP3	TRAF3 interacting protein 3	Other	other
2,055	NM_033557	YIF1B	Yip1 interacting factor homolog B (S. cerevisiae)	Other	other
2,053	NM_001012509	SLC45A2	solute carrier family 45, member 2	Plasma Membrane	other
2,052	NM_138414	CCDC101	coiled-coil domain containing 101	Nucleus	other
2,049	NM_001002035	DEFB108B	defensin, beta 108B	Extracellular Space	other
2,048	NM_006487	FBLN1	fibulin 1	Extracellular Space	other
2,046	NM_006244	PPP2R5B	protein phosphatase 2, regulatory subunit B', beta	Cytoplasm	phosphatase
2,044	NM_023084	CAPN10	calpain 10	Cytoplasm	peptidase
2,043	NM_052877	MED8	mediator complex subunit 8	Nucleus	other

2,037	NM_001032364	GGT1	gamma-glutamyltransferase 1	Plasma Membrane	enzyme
2,036	NM_017711	GDPD2	glycerophosphodiester phosphodiesterase domain containing 2	Plasma Membrane	enzyme
2,035	NM_015259	ICOSLG	inducible T-cell co-stimulator ligand	Plasma Membrane	other
2,034	NM_017765	PQLC2	PQ loop repeat containing 2	Cytoplasm	transporter
2,032	NM_021948	BCAN	brevican	Extracellular Space	other
2,030	NM_144671	FAM109A	family with sequence similarity 109, member A	Other	other
2,030	NM_003593	FOXN1	forkhead box N1	Nucleus	transcription regulator
2,028	NM_001005367	TTYH1	tweety homolog 1 (Drosophila)	Plasma Membrane	ion channel
2,027	NM_033510	DISP2	dispatched homolog 2 (Drosophila)	Other	other
2,027	NM_005919	MEF2BNB-MEF2B	MEF2BNB-MEF2B readthrough	Nucleus	transcription regulator
2,025	NM_002475	MYL6B	myosin, light chain 6B, alkali, smooth muscle and non-muscle	Cytoplasm	other
2,024	NM_006096	NDRG1	N-myc downstream regulated 1	Nucleus	kinase
2,020	NM_000290	PGAM2	phosphoglycerate mutase 2 (muscle)	Cytoplasm	phosphatase
2,020	NM_013373	ZDHHC8	zinc finger, DHHC-type containing 8	Cytoplasm	enzyme
2,019	NM_021625	TRPV4	transient receptor potential cation channel, subfamily V, member 4	Plasma Membrane	ion channel
2,017	NM_003116	SPAG4	sperm associated antigen 4	Cytoplasm	other
2,012	NM_001024401	SBK1	SH3-binding domain kinase 1	Other	kinase
2,011	NM_001520	GTF3C1	general transcription factor IIIC, polypeptide 1, alpha 220kDa	Nucleus	transcription regulator
2,009	NM_002701	POU5F1	POU class 5 homeobox 1	Nucleus	transcription regulator
2,008	NM_006034	TP53I11	tumor protein p53 inducible protein 11	Other	other
2,003	NM_004860	FXR2	fragile X mental retardation, autosomal homolog 2	Cytoplasm	other
2,003	NM_178275	IGFN1	immunoglobulin-like and fibronectin type III domain containing 1	Nucleus	other
2,001	NM_015596	KLK13	kallikrein-related peptidase 13	Extracellular Space	peptidase
2,000	NM_000106	CYP2D6	cytochrome P450, family 2, subfamily D, polypeptide 6	Cytoplasm	enzyme
-2,001	NM_032307	C9orf64	chromosome 9 open reading frame 64	Other	other
-2,001	NM_021230	KMT2C	lysine (K)-specific methyltransferase 2C	Nucleus	transcription regulator
-2,001	NM_004708	PDCD5	programmed cell death 5	Nucleus	other

-2,002	NM_003539	HIST1H4A (includes others)	histone cluster 1, H4a	Nucleus	other
-2,003	NM_001002292	WLS	wntless homolog (Drosophila)	Cytoplasm	other
-2,006	NM_015355	SUZ12	SUZ12 polycomb repressive complex 2 subunit	Nucleus	enzyme
-2,010	NM_031302	GLT8D2	glycosyltransferase 8 domain containing 2	Other	enzyme
-2,013	NM_003338	UBE2D1	ubiquitin-conjugating enzyme E2D 1	Cytoplasm	enzyme
-2,015	NM_022145	CENPK	centromere protein K	Nucleus	other
-2,015	NM_033642	FGF13	fibroblast growth factor 13	Extracellular Space	growth factor
-2,015	NM_001006640	TCEAL1	transcription elongation factor A (SII)-like 1	Nucleus	transcription regulator
-2,020	NM_006085	BPNT1	3'(2'), 5'-bisphosphate nucleotidase 1	Nucleus	phosphatase
-2,020	NM_001018037	VPS13A	vacuolar protein sorting 13 homolog A (S. cerevisiae)	Cytoplasm	transporter
-2,021	NM_002296	LBR	lamin B receptor	Nucleus	enzyme
-2,021	NM_000297	PKD2	polycystic kidney disease 2 (autosomal dominant)	Plasma Membrane	ion channel
-2,021	NM_003318	TTK	TTK protein kinase	Nucleus	kinase
-2,022	NM_004483	GCSH	glycine cleavage system protein H (aminomethyl carrier)	Cytoplasm	enzyme
-2,024	NM_012484	HMMR	hyaluronan-mediated motility receptor (RHAMM)	Plasma Membrane	transmembrane receptor
-2,025	NM_015235	CSTF2T	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant	Nucleus	other
-2,026	NM_014962	BTBD3	BTB (POZ) domain containing 3	Other	other
-2,026	NM_003082	SNAPC1	small nuclear RNA activating complex, polypeptide 1, 43kDa	Nucleus	other
-2,027	NM_015496	KIAA1429	KIAA1429	Nucleus	other
-2,028	NM_000051	ATM	ataxia telangiectasia mutated	Nucleus	kinase
-2,028	NM_003543	HIST1H4A (includes others)	histone cluster 1, H4a	Nucleus	other
-2,029	NM_003545	HIST1H4A (includes others)	histone cluster 1, H4a	Nucleus	other
-2,030	NM_001005409	SF3A1	splicing factor 3a, subunit 1, 120kDa	Nucleus	other
-2,031	NM_004052	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3	Cytoplasm	other
-2,035	NM_001034915	ESRP1	epithelial splicing regulatory protein 1	Nucleus	other
-2,035	NM_020715	PLEKHH1	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1	Cytoplasm	other

-2,036	NM_016441	CRIM1	cysteine rich transmembrane BMP regulator 1 (chordin-like)	Extracellular Space	kinase
-2,037	NM_006317	BASP1	brain abundant, membrane attached signal protein 1	Nucleus	transcription regulator
-2,039	NM_018086	FIGN	fidgetin	Nucleus	other
-2,039	NM_001584	MPPED2	metallophosphoesterase domain containing 2	Other	other
-2,041	NM_004492	GTF2A2	general transcription factor IIA, 2, 12kDa	Nucleus	transcription regulator
-2,042	NM_013296	GPSM2	G-protein signaling modulator 2	Nucleus	other
-2,044	NM_020666	CLK4	CDC-like kinase 4	Nucleus	kinase
-2,045	NM_003513	HIST1H2AB/ HIST1H2AE	histone cluster 1, H2ae	Other	other
-2,048	NM_013233	STK39	serine threonine kinase 39	Nucleus	kinase
-2,049	NM_018364	RSBN1	round spermatid basic protein 1	Nucleus	other
-2,050	NM_022763	FNDC3B	fibronectin type III domain containing 3B	Cytoplasm	other
-2,050	NM_017819	TRMT10C	tRNA methyltransferase 10 homolog C (S. cerevisiae)	Cytoplasm	other
-2,051	NM_172071	RC3H1	ring finger and CCCH-type domains 1	Cytoplasm	enzyme
-2,052	NM_022776	OSBPL11	oxysterol binding protein-like 11	Cytoplasm	other
-2,053	NM_019605	SERTAD4	SERTA domain containing 4	Other	other
-2,054	NM_003071	HLTF	helicase-like transcription factor	Nucleus	transcription regulator
-2,054	NM_000899	KITLG	KIT ligand	Extracellular Space	growth factor
-2,054	NM_003343	UBE2G2	ubiquitin-conjugating enzyme E2G 2	Cytoplasm	enzyme
-2,055	NM_015335	MED13L	mediator complex subunit 13-like	Nucleus	other
-2,056	NM_018120	ARMC1	armadillo repeat containing 1	Cytoplasm	other
-2,056	NM_007139	ZNF92	zinc finger protein 92	Nucleus	transcription regulator
-2,057	NM_203495	COMMD6	COMM domain containing 6	Other	other
-2,057	NM_013438	UBQLN1	ubiquilin 1	Cytoplasm	other
-2,059	NM_153226	SLC35G1	solute carrier family 35, member G1	Plasma Membrane	other
-2,060	NM_000109	DMD	dystrophin	Plasma Membrane	other
-2,061	NM_080664	DTD2	D-tyrosyl-tRNA deacylase 2 (putative)	Other	other
-2,062	NM_152902	TIPRL	TIP41, TOR signaling pathway regulator-like (S. cerevisiae)	Other	other
-2,067	NM_002158	FOXN2	forkhead box N2	Nucleus	transcription regulator
-2,067	NM_013381	TRHDE	thyrotropin-releasing hormone degrading enzyme	Plasma Membrane	peptidase

-2,067	NM_007013	WWP1	WW domain containing E3 ubiquitin protein ligase 1	Cytoplasm	enzyme
-2,067	NM_021148	ZNF273	zinc finger protein 273	Nucleus	other
-2,074	NM_005192	CDKN3	cyclin-dependent kinase inhibitor 3	Other	phosphatase
-2,075	NM_014344	FJX1	four jointed box 1 (Drosophila)	Extracellular Space	other
-2,075	NM_001004301	ZNF813	zinc finger protein 813	Other	other
-2,077	NM_001002909	GPATCH8	G patch domain containing 8	Other	other
-2,078	NM_018290	PGM2	phosphoglucomutase 2	Cytoplasm	enzyme
-2,081	NM_181783	TMTC3	transmembrane and tetratricopeptide repeat containing 3	Other	other
-2,082	NM_016020	TFB1M	transcription factor B1, mitochondrial	Cytoplasm	transcription regulator
-2,083	NM_018353	MIS18BP1	MIS18 binding protein 1	Nucleus	other
-2,084	NM_003112	SP4	Sp4 transcription factor	Nucleus	transcription regulator
-2,088	NM_017693	BIVM	basic, immunoglobulin-like variable motif containing	Extracellular Space	other
-2,088	NM_001981	EPS15	epidermal growth factor receptor pathway substrate 15	Cytoplasm	other
-2,088	NM_018841	GNG12	guanine nucleotide binding protein (G protein), gamma 12	Plasma Membrane	enzyme
-2,089	NM_012310	KIF4A	kinesin family member 4A	Nucleus	other
-2,089	NM_006055	LANCL1	LanC lantibiotic synthetase component C-like 1 (bacterial)	Plasma Membrane	other
-2,089	NM_004589	SCO1	SCO1 cytochrome c oxidase assembly protein	Cytoplasm	other
-2,092	NM_001002264	EPSTI1	epithelial stromal interaction 1 (breast)	Other	other
-2,092	NM_005322	HIST1H1B	histone cluster 1, H1b	Nucleus	other
-2,093	NM_016603	FAM13B	family with sequence similarity 13, member B	Cytoplasm	other
-2,093	NM_001005500	OR4M1	olfactory receptor, family 4, subfamily M, member 1	Plasma Membrane	G-protein coupled receptor
-2,094	NM_018269	ADI1	acireductone dioxygenase 1	Nucleus	enzyme
-2,094	NM_001013746	ZNF107	zinc finger protein 107	Nucleus	other
-2,094	NM_003414	ZNF267	zinc finger protein 267	Nucleus	other
-2,097	NM_175063	EMC10	ER membrane protein complex subunit 10	Other	other
-2,098	NM_181581	DUS4L	dihydrouridine synthase 4-like (S. cerevisiae)	Other	other
-2,099	NM_015275	KIAA1033	KIAA1033	Cytoplasm	other
-2,100	NM_022900	CASD1	CAS1 domain containing 1	Cytoplasm	enzyme
-2,102	NM_016343	CENPF	centromere protein F, 350/400kDa	Nucleus	other

-2,104	XM_055636	CCDC85A	coiled-coil domain containing 85A	Other	other
-2,104	NM_022756	MEAF6	MYST/Esa1-associated factor 6	Nucleus	other
-2,105	NM_017665	ZCCHC10	zinc finger, CCHC domain containing 10	Other	other
-2,106	NM_001017979	RAB28	RAB28, member RAS oncogene family	Plasma Membrane	enzyme
-2,107	NM_020242	KIF15	kinesin family member 15	Nucleus	other
-2,111	NM_002956	CLIP1	CAP-GLY domain containing linker protein 1	Cytoplasm	other
-2,111	NM_002501	NFIX	nuclear factor I/X (CCAAT-binding transcription factor)	Nucleus	transcription regulator
-2,114	NM_022725	FANCF	Fanconi anemia, complementation group F	Nucleus	other
-2,115	NM_001033578	C8orf44-SGK3/SGK3	serum/glucocorticoid regulated kinase family, member 3	Cytoplasm	kinase
-2,120	NM_003514	HIST1H2AM (includes others)	histone cluster 1, H2ag	Nucleus	other
-2,122	NM_006626	ZBTB6	zinc finger and BTB domain containing 6	Nucleus	other
-2,131	NM_003161	RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	Cytoplasm	kinase
-2,134	NM_001001556	GALK2	galactokinase 2	Cytoplasm	kinase
-2,135	NM_032117	MND1	meiotic nuclear divisions 1 homolog (S. cerevisiae)	Nucleus	other
-2,136	NM_003521	HIST1H2BM	histone cluster 1, H2bm	Nucleus	other
-2,137	NM_017769	G2E3	G2/M-phase specific E3 ubiquitin protein ligase	Cytoplasm	enzyme
-2,137	NM_005645	TAF13	TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 18kDa	Nucleus	transcription regulator
-2,140	NM_181781	ZNF326	zinc finger protein 326	Nucleus	transcription regulator
-2,143	NM_176866	PPA2	pyrophosphatase (inorganic) 2	Cytoplasm	enzyme
-2,147	NM_015224	FAM208A	family with sequence similarity 208, member A	Other	other
-2,147	NM_018365	MNS1	meiosis-specific nuclear structural 1	Other	other
-2,147	NM_003417	ZNF264	zinc finger protein 264	Nucleus	other
-2,151	NM_015578	LSM14A	LSM14A, SCD6 homolog A (S. cerevisiae)	Cytoplasm	other
-2,151	NM_019012	PLEKHA5	pleckstrin homology domain containing, family A member 5	Other	other
-2,152	NM_003884	KAT2B	K(lysine) acetyltransferase 2B	Nucleus	transcription regulator
-2,152	NM_021033	RAP2A	RAP2A, member of RAS oncogene family	Plasma Membrane	enzyme
-2,152	NM_006606	RBBP9	retinoblastoma binding protein 9	Nucleus	other
-2,152	NM_030934	TRMT1L	tRNA methyltransferase 1 homolog (S. cerevisiae)-like	Other	enzyme

-2,153	NM_018159	NUDT11	nudix (nucleoside diphosphate linked moiety X)-type motif 11	Cytoplasm	phosphatase
-2,154	XM_291016	C2orf74	chromosome 2 open reading frame 74	Other	other
-2,155	NM_022824	FBXL17	F-box and leucine-rich repeat protein 17	Other	other
-2,155	NM_032993	GAR1	GAR1 ribonucleoprotein homolog (yeast)	Nucleus	ion channel
-2,156	NM_176867	PPA2	pyrophosphatase (inorganic) 2	Cytoplasm	enzyme
-2,160	NM_020675	SPC25	SPC25, NDC80 kinetochore complex component	Cytoplasm	other
-2,164	NM_138286	ZNF681	zinc finger protein 681	Other	other
-2,169	NM_153453	VGLL2	vestigial like 2 (Drosophila)	Nucleus	transcription regulator
-2,171	NM_021645	UTP14C	UTP14, U3 small nucleolar ribonucleoprotein, homolog C (yeast)	Nucleus	other
-2,177	NM_003616	GEMIN2	gem (nuclear organelle) associated protein 2	Nucleus	other
-2,182	NM_020119	ZC3HAV1	zinc finger CCCH-type, antiviral 1	Plasma Membrane	other
-2,182	NM_016107	ZFR	zinc finger RNA binding protein	Nucleus	other
-2,184	NM_018983	GAR1	GAR1 ribonucleoprotein homolog (yeast)	Nucleus	ion channel
-2,186	NM_001002843	ZNF280D	zinc finger protein 280D	Other	other
-2,188	NM_018934	PCDHB14	protocadherin beta 14	Plasma Membrane	other
-2,188	NM_022828	YTHDC2	YTH domain containing 2	Other	other
-2,190	NM_018248	NEIL3	nei endonuclease VIII-like 3 (E. coli)	Nucleus	enzyme
-2,190	NM_007106	UBL3	ubiquitin-like 3	Cytoplasm	other
-2,195	NM_004546	NDUFB2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	Cytoplasm	enzyme
-2,199	NM_022757	CCDC14	coiled-coil domain containing 14	Cytoplasm	other
-2,199	NM_021227	OSTC	oligosaccharyltransferase complex subunit (non-catalytic)	Cytoplasm	enzyme
-2,200	NM_001160	APAF1	apoptotic peptidase activating factor 1	Cytoplasm	other
-2,201	NM_001009909	LUZP2	leucine zipper protein 2	Other	other
-2,202	NM_032151	PCBD2	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2	Other	enzyme
-2,203	NM_138444	KCTD12	potassium channel tetramerization domain containing 12	Plasma Membrane	ion channel
-2,205	NM_025103	IFT74	intraflagellar transport 74 homolog (Chlamydomonas)	Cytoplasm	other
-2,210	NM_004114	FGF13	fibroblast growth factor 13	Extracellular Space	growth factor
-2,213	NM_080596	HIST1H2AH	histone cluster 1, H2ah	Nucleus	other
-2,213	NM_031217	KIF18A	kinesin family member 18A	Cytoplasm	enzyme

-2,213	NM_001029884	PLEKHG1	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	Extracellular Space	other
-2,214	NM_001033559	DYX1C1	dyslexia susceptibility 1 candidate 1	Nucleus	other
-2,214	NM_153240	NPHP3	nephronophthisis 3 (adolescent)	Extracellular Space	other
-2,216	NM_015285	WDR7	WD repeat domain 7	Other	other
-2,225	NM_003425	ZNF45	zinc finger protein 45	Nucleus	transcription regulator
-2,226	NM_014900	COBLL1	cordon-bleu WH2 repeat protein-like 1	Extracellular Space	other
-2,226	NM_000153	GALC	galactosylceramidase	Cytoplasm	enzyme
-2,226	NM_014021	SSX2IP	synovial sarcoma, X breakpoint 2 interacting protein	Plasma Membrane	other
-2,226	NM_001018038	VPS13A	vacuolar protein sorting 13 homolog A (S. cerevisiae)	Cytoplasm	transporter
-2,231	NM_052850	GADD45GIP1	growth arrest and DNA-damage-inducible, gamma interacting protein 1	Nucleus	other
-2,231	NM_005321	HIST1H1E	histone cluster 1, H1e	Nucleus	other
-2,232	NM_144724	MARVELD2	MARVEL domain containing 2	Plasma Membrane	other
-2,235	NM_020232	PSMG2	proteasome (prosome, macropain) assembly chaperone 2	Nucleus	other
-2,236	NM_015272	RPGRIP1L	RPGRIP1-like	Cytoplasm	other
-2,237	NM_000143	FH	fumarate hydratase	Cytoplasm	enzyme
-2,237	NM_016195	KIF20B	kinesin family member 20B	Nucleus	enzyme
-2,237	NM_007214	SEC63	SEC63 homolog (S. cerevisiae)	Cytoplasm	transporter
-2,238	NM_152556	C7orf60	chromosome 7 open reading frame 60	Other	other
-2,238	NM_182739	NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa	Cytoplasm	enzyme
-2,241	NM_175876	EXOC8	exocyst complex component 8	Plasma Membrane	other
-2,242	NM_018369	DEPDC1B	DEP domain containing 1B	Cytoplasm	other
-2,248	NM_021204	ENOPH1	enolase-phosphatase 1	Cytoplasm	enzyme
-2,248	NM_133474	ZNF721	zinc finger protein 721	Other	other
-2,253	NM_017779	DEPDC1	DEP domain containing 1	Nucleus	transcription regulator
-2,254	NM_153328	RBBP9	retinoblastoma binding protein 9	Nucleus	other
-2,255	NM_001033925	TIAL1	TIA1 cytotoxic granule-associated RNA binding protein-like 1	Nucleus	transcription regulator
-2,258	NM_207418	FAM72D	family with sequence similarity 72, member D	Nucleus	other
-2,258	NM_012329	MMD	monocyte to macrophage differentiation-associated	Plasma Membrane	other

-2,259	NM_022909	CENPH	centromere protein H	Nucleus	other
-2,268	NM_018112	TMEM38B	transmembrane protein 38B	Nucleus	ion channel
-2,268	NM_006352	ZBTB18	zinc finger and BTB domain containing 18	Nucleus	transcription regulator
-2,270	NM_003838	FPGT	fucose-1-phosphate guanylyltransferase	Cytoplasm	enzyme
-2,271	NM_014612	FAM120A	family with sequence similarity 120A	Cytoplasm	other
-2,274	NM_015186	VPS13A	vacuolar protein sorting 13 homolog A (S. cerevisiae)	Cytoplasm	transporter
-2,277	NM_197962	GLRX2	glutaredoxin 2	Cytoplasm	enzyme
-2,278	NM_144508	CASC5	cancer susceptibility candidate 5	Nucleus	other
-2,278	NM_005334	HCFC1	host cell factor C1 (VP16-accessory protein)	Nucleus	transcription regulator
-2,281	NM_018312	PPP6R3	protein phosphatase 6, regulatory subunit 3	Cytoplasm	other
-2,282	NM_024054	C7orf25	chromosome 7 open reading frame 25	Other	other
-2,282	NM_198507	FAM174A	family with sequence similarity 174, member A	Extracellular Space	other
-2,283	NM_138446	MALSU1	mitochondrial assembly of ribosomal large subunit 1	Extracellular Space	other
-2,288	NM_182639	HPS1	Hermansky-Pudlak syndrome 1	Cytoplasm	other
-2,291	NM_001012339	DNAJC21	DnaJ (Hsp40) homolog, subfamily C, member 21	Other	other
-2,296	NM_194292	SASS6	spindle assembly 6 homolog (C. elegans)	Cytoplasm	other
-2,297	NM_002493	NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa	Cytoplasm	enzyme
-2,305	NM_007280	OIP5	Opa interacting protein 5	Nucleus	other
-2,307	NM_018691	FAM114A2	family with sequence similarity 114, member A2	Other	other
-2,311	NM_017676	GIN1	gypsy retrotransposon integrase 1	Other	other
-2,316	NM_001007254	ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1	Cytoplasm	transporter
-2,316	NM_014897	ZNF652	zinc finger protein 652	Other	other
-2,325	NM_016075	VPS36	vacuolar protein sorting 36 homolog (S. cerevisiae)	Cytoplasm	other
-2,328	NM_005213	CSTA	cystatin A (stefin A)	Cytoplasm	other
-2,328	NM_013328	PYCR2	pyrroline-5-carboxylate reductase family, member 2	Cytoplasm	enzyme
-2,330	NM_015076	CDK19	cyclin-dependent kinase 19	Nucleus	kinase
-2,334	NM_145049	UBLCP1	ubiquitin-like domain containing CTD phosphatase 1	Nucleus	phosphatase
-2,336	NM_003185	TAF4	TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135kDa	Nucleus	transcription regulator
-2,338	NM_017655	GIPC2	GIPC PDZ domain containing family, member 2	Cytoplasm	other

-2,338	NM_022826	MARCH7	membrane-associated ring finger (C3HC4) 7, E3 ubiquitin protein ligase	Extracellular Space	other
-2,341	NM_145261	DNAJC19	DnaJ (Hsp40) homolog, subfamily C, member 19	Cytoplasm	other
-2,346	XM_034274	MYBL1	v-myb myeloblastosis viral oncogene homolog (avian)-like 1	Nucleus	transcription regulator
-2,350	NM_003864	SAP30	Sin3A-associated protein, 30kDa	Nucleus	transcription regulator
-2,351	NM_015458	MTMR9	myotubularin related protein 9	Cytoplasm	phosphatase
-2,352	NM_003359	UGDH	UDP-glucose 6-dehydrogenase	Nucleus	enzyme
-2,357	NM_005455	ZRANB2	zinc finger, RAN-binding domain containing 2	Nucleus	transcription regulator
-2,360	NM_018136	ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)	Nucleus	other
-2,362	NM_001009182	GEMIN2	gem (nuclear organelle) associated protein 2	Nucleus	other
-2,367	NM_001167	XIAP	X-linked inhibitor of apoptosis	Cytoplasm	enzyme
-2,376	NM_005156	PTBP3	polypyrimidine tract binding protein 3	Nucleus	other
-2,376	NM_020654	SENP7	SUMO1/sentrin specific peptidase 7	Nucleus	peptidase
-2,384	NM_002811	PSMD7	proteasome (prosome, macropain) 26S subunit, non-ATPase, 7	Cytoplasm	other
-2,392	NM_002906	RDX	radixin	Cytoplasm	other
-2,398	NM_002157	HSPE1	heat shock 10kDa protein 1 (chaperonin 10)	Cytoplasm	enzyme
-2,401	NM_052966	FAM129A	family with sequence similarity 129, member A	Cytoplasm	other
-2,403	NM_000110	DPYD	dihydropyrimidine dehydrogenase	Cytoplasm	enzyme
-2,404	NM_025009	CEP135	centrosomal protein 135kDa	Cytoplasm	other
-2,404	NM_030760	S1PR5	sphingosine-1-phosphate receptor 5	Plasma Membrane	G-protein coupled receptor
-2,408	NM_152624	DCP2	decapping mRNA 2	Nucleus	enzyme
-2,408	NM_020640	DCUN1D1	DCN1, defective in cullin neddylation 1, domain containing 1	Nucleus	other
-2,409	NM_004242	HMGN3	high mobility group nucleosomal binding domain 3	Nucleus	other
-2,410	NM_022129	PBLD	phenazine biosynthesis-like protein domain containing	Other	enzyme
-2,411	NM_032905	RBM17	RNA binding motif protein 17	Nucleus	other
-2,411	NM_178549	ZNF678	zinc finger protein 678	Nucleus	other
-2,413	NM_003750	EIF3A	eukaryotic translation initiation factor 3, subunit A	Cytoplasm	translation regulator

-2,413	NM_003537	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,417	NM_003655	CBX4	chromobox homolog 4	Nucleus	transcription regulator
-2,431	NM_018518	MCM10	minichromosome maintenance complex component 10	Nucleus	other
-2,439	NM_001007466	TULP4	tubby like protein 4	Cytoplasm	transcription regulator
-2,443	NM_016271	RNF138	ring finger protein 138, E3 ubiquitin protein ligase	Other	other
-2,447	NM_020381	PDSS2	prenyl (decaprenyl) diphosphate synthase, subunit 2	Cytoplasm	enzyme
-2,457	NM_001011708	OLA1	Obg-like ATPase 1	Cytoplasm	other
-2,459	NM_172070	UBR3	ubiquitin protein ligase E3 component n-recognin 3 (putative)	Other	enzyme
-2,467	NM_001012985	COA6	cytochrome c oxidase assembly factor 6 homolog (S. cerevisiae)	Cytoplasm	other
-2,477	NM_006565	CTCF	CCCTC-binding factor (zinc finger protein)	Nucleus	transcription regulator
-2,477	NM_020774	MIB1	mindbomb E3 ubiquitin protein ligase 1	Cytoplasm	other
-2,483	NM_006390	IPO8	importin 8	Nucleus	transporter
-2,485	NM_004701	CCNB2	cyclin B2	Cytoplasm	other
-2,502	NM_003533	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,506	NM_015288	PHF15	PHD finger protein 15	Nucleus	other
-2,533	NM_003205	TCF12	transcription factor 12	Nucleus	transcription regulator
-2,534	NM_004586	RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	Cytoplasm	kinase
-2,545	NM_004667	HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2	Cytoplasm	enzyme
-2,548	NM_003825	SNAP23	synaptosomal-associated protein, 23kDa	Plasma Membrane	transporter
-2,554	NM_006379	SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	Extracellular Space	other
-2,558	NM_173177	C1D	C1D nuclear receptor corepressor	Nucleus	transcription regulator
-2,564	NM_015571	SENP6	SUMO1/sentrin specific peptidase 6	Cytoplasm	peptidase
-2,580	NM_014487	ZNF330	zinc finger protein 330	Nucleus	other
-2,591	NM_001809	CENPA	centromere protein A	Nucleus	other

-2,593	NM_003511	HIST1H2AM (includes others)	histone cluster 1, H2ag	Nucleus	other
-2,593	NM_134470	IL1RAP	interleukin 1 receptor accessory protein	Plasma Membrane	transmembrane receptor
-2,597	NM_014802	C2CD5	C2 calcium-dependent domain containing 5	Cytoplasm	other
-2,600	NM_001813	CENPE	centromere protein E, 312kDa	Nucleus	other
-2,614	NM_000028	AGL	amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase	Cytoplasm	enzyme
-2,637	NM_024949	WWC2	WW and C2 domain containing 2	Other	other
-2,639	NM_017927	MFN1	mitofusin 1	Cytoplasm	enzyme
-2,642	NM_153689	C2orf69	chromosome 2 open reading frame 69	Other	other
-2,643	NM_019592	RNF20	ring finger protein 20, E3 ubiquitin protein ligase	Nucleus	enzyme
-2,648	NM_005327	HADH	hydroxyacyl-CoA dehydrogenase	Cytoplasm	enzyme
-2,649	NM_212552	BOLA3	bolA homolog 3 (E. coli)	Other	other
-2,650	NM_182523	CMC1	COX assembly mitochondrial protein 1 homolog (S. cerevisiae)	Cytoplasm	other
-2,652	NM_152773	TCTEX1D2	Tctex1 domain containing 2	Other	other
-2,660	NM_016126	HSPB11	heat shock protein family B (small), member 11	Extracellular Space	other
-2,672	NM_152641	ARID2	AT rich interactive domain 2 (ARID, RFX-like)	Nucleus	transcription regulator
-2,676	NM_004380	CREBBP	CREB binding protein	Nucleus	transcription regulator
-2,687	NM_148170	CTSC	cathepsin C	Cytoplasm	peptidase
-2,697	NM_019087	ARL15	ADP-ribosylation factor-like 15	Other	other
-2,703	NM_016559	PEX5L	peroxisomal biogenesis factor 5-like	Cytoplasm	ion channel
-2,735	NM_018330	KIAA1598	KIAA1598	Other	other
-2,742	NM_018204	CKAP2	cytoskeleton associated protein 2	Cytoplasm	other
-2,746	NM_018410	HJURP	Holliday junction recognition protein	Nucleus	other
-2,763	NM_018237	CCAR1	cell division cycle and apoptosis regulator 1	Nucleus	transcription regulator
-2,779	NM_001031805	AFG3L1P	AFG3 ATPase family member 3-like 1 (S. cerevisiae), pseudogene	Cytoplasm	other
-2,810	NM_006333	C1D	C1D nuclear receptor corepressor	Nucleus	transcription regulator
-2,841	NM_031407	HUWE1	HECT, UBA and WWE domain containing 1, E3 ubiquitin protein ligase	Nucleus	transcription regulator
-2,854	NM_002623	PFDN4	prefoldin subunit 4	Cytoplasm	other

-2,856	NM_003535	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-2,859	XM_290597	GXYLT1	glucoside xylosyltransferase 1	Cytoplasm	other
-2,875	NM_004796	NRXN3	neurexin 3	Other	transporter
-2,890	NM_014736	KIAA0101	KIAA0101	Nucleus	other
-2,942	NM_003157	NEK4	NIMA-related kinase 4	Nucleus	kinase
-2,952	NM_018137	PRMT6	protein arginine methyltransferase 6	Nucleus	enzyme
-2,956	NM_018227	UBA6	ubiquitin-like modifier activating enzyme 6	Cytoplasm	enzyme
-2,967	NM_178496	MB21D2	Mab-21 domain containing 2	Other	other
-2,984	NM_020926	BCOR	BCL6 corepressor	Nucleus	transcription regulator
-3,019	NM_023927	GRAMD3	GRAM domain containing 3	Extracellular Space	other
-3,019	NM_005320	HIST1H1D	histone cluster 1, H1d	Nucleus	other
-3,062	NM_021058	HIST1H2BJ/ HIST1H2BK	histone cluster 1, H2bk	Nucleus	other
-3,081	NM_012300	FBXW11	F-box and WD repeat domain containing 11	Cytoplasm	enzyme
-3,088	NM_001039547	GK5	glycerol kinase 5 (putative)	Other	kinase
-3,115	NM_003529	HIST1H3A (includes others)	histone cluster 1, H3a	Nucleus	other
-3,140	NM_012089	ABCB10	ATP-binding cassette, sub-family B (MDR/TAP), member 10	Cytoplasm	transporter
-3,203	NM_005999	TSNAX	translin-associated factor X	Nucleus	transporter
-3,244	NM_015199	ANKRD28	ankyrin repeat domain 28	Cytoplasm	other
-3,363	NM_003199	TCF4	transcription factor 4	Nucleus	transcription regulator
-3,411	NM_033342	TRIM7	tripartite motif containing 7	Cytoplasm	other
-3,590	NM_018482	ASAP1	ArfGAP with SH3 domain, ankyrin repeat and PH domain 1	Plasma Membrane	other
-3,623	NM_022739	SMURF2	SMAD specific E3 ubiquitin protein ligase 2	Cytoplasm	enzyme
-3,634	NM_016305	SS18L2	synovial sarcoma translocation gene on chromosome 18-like 2	Other	other
-3,696	NM_207181	NPHP1	nephronophthisis 1 (juvenile)	Nucleus	other
-3,827	NM_014241	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member A	Other	phosphatase
-3,903	NM_024680	E2F8	E2F transcription factor 8	Nucleus	transcription regulator
-3,969	NM_001012968	SPIN4	spindlin family, member 4	Other	other
-3,976	NM_018238	AGK	acylglycerol kinase	Cytoplasm	kinase

-4,193	NM_001949	E2F3	E2F transcription factor 3	Nucleus	transcription regulator
-5,111	NM_006364	SEC23A	Sec23 homolog A (S. cerevisiae)	Cytoplasm	transporter

Eidesstattliche Erklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde. Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Annemarie Barton

Greifswald, 20.12.2013

Curriculum Vitae

Education	
2010 - now	PhD student; Center of innovation competence (ZIK) plasmatis at the Leibniz Institute for Plasma Science and Technology Greifswald, Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany
2010 - now	student of the Greifswald Graduate School of Science (GGSS), Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany
2005 - 2010	Studies of Bioingeneering; Aachen University of Applied Science; Campus Jülich, Germany
2009 - 2010	Diploma Thesis; CardioGenetic Lab, Charité Berlin, Berlin, Germany
1996 - 2005	Max-Planck-Gymnasium, Bielefeld, Germany

Practical Experience	
10/2008 - 03/2009	student research assistent in the field Tissue Engineering; Fraunhofer Institute for Production Technology IPT, Aachen, Germany
02/2006 - 03/2006	internship, Degussa AG, Biotechnological Analytic, Halle-Künsebeck, Germany
07/2005 - 08/2005	internship, Federal Research Institute of Nutrition and Food, Detmold, Germany
semester breaks 2006 - 2008	student assistant in the field Engineering; BSG Sondermaschinenbau GmbH, Blomberg, Germany

Annemarie Barton Greifswald, 20.12.2013

Publications

Publications

Barton A., Wende K., Bundscherer L., Hasse S., Schmidt A., Bekeschus S., Weltmann K.-D., Lindequist U., Masur K. (Submitted 2013): Non-thermal plasma increases expression of wound healing related genes in a keratinocyte cell line. *Plasma Medicine*

Barton A., Wende K., Weltmann K.-D., Masur K., Lindequist U. (in preparation 2013): Correlation of cell signaling to plasma generated ROS and RONS.

Wende K., Barton A., Bekeschus S., Bundscherer L., Schmidt A., Weltmann K.-D., Masur K. (Submitted 2013): Proteomic tools to characterize non-thermal plasma effects in eukaryotic cells. *Plasma Medicine*

Bundscherer L., Wende K., Ottmüller K., Barton A., Schmidt A., Bekeschus S., Hasse S., Weltmann K.-D., Masur K., Lindequist U. (2013): Impact of non-thermal plasma treatment on MAPK signaling pathways of human immune cell lines. *Immunobiology* 218(10): 1248-1255

Schmidt A., Wende K., Bekeschus S., Bundscherer L., Barton A., Ottmüller K., Weltmann K.-D., Masur K. (2013): Non-thermal plasma treatment is associated with changes in transcriptome of human epithelial skin cells. *Free Radical Research* 47(8): 577-609

Wende K., Straßenburg S., Haertel B., Harms M., Holtz S., Barton A., Masur K., von Woedtke T., Lindequist U. (Accepted 2013): Atmospheric pressure plasma jet treatment evokes transient oxidative stress in HaCaT keratinocytes and influences cell physiology. *Cell Biology International*

Bekeschus S., Masur K., Kolata J., Wende K., Schmidt A., Bundscherer L., Barton A., Kramer A., Bröker B., Weltmann K.-D. (2013): Human Mononuclear Cell Survival and Proliferation is Modulated by Cold Atmospheric Plasma Jet. *Plasma Processess and Polymers* 2013(10): 706-713

Conference Proceedings

Reuter S., Winter J., Wende K., Hasse S., Schroeder D., Bundscherer L., Barton A., Masur K., Knake N., Schulz-von der Gathen V., Weltmann K.-D. (2011): Reactive Oxygen Species (ROS) in an Argon Plasma Jet Investigated with Respect to ROS Mediated Apoptosis in Human Cells. *30th International Conference on Phenomena in Ionized Gases (ICPIG)*

Barton A., Holtz, S., Bundscherer L., Masur K., Weltmann K.-D. (2012): Influence of atmospheric pressure plasma on keratinocytes. *39th Meeting of the Arbeitsgemeinschaft Dermatologische Forschung (ADF), Experimental Dermatology*, 21(3)

Bundscherer L., Barton A., Masur K., Weltmann K.-D. (2012): Impact of physical plasma on T lymphocytes. *39th Meeting of the Arbeitsgemeinschaft Dermatologische Forschung (ADF), Experimental Dermatology*, 21(3)

Barton A., Wende K., Bundscherer L., Schmidt A., Bekeschus S., Hasse S., Lindequist U., Weltmann K.-D., Masur K. (2013): Non-Thermal Plasma Treatment of Human Cells: The Effect of Ambient Conditions. *21st International Symposium on Plasma Chemistry (ISPC21)*

Oral presentations

Barton A., Wende K., Bundscherer L., Hasse S., Lindequist U., Masur K. (2011): Studies of Plasma-based Activation of Skin Cells. *Workshop ZIK*

plasmatis, Rostock, Germany
Barton A., Bekeschus S., Bundscherer L., Schmidt A., Wende K., Weltmann K.-D., Lindequist U., Masur K. (2012): Cellular reaction of skin cells after atmospheric pressure plasma treatment. *1st Young Professionals Workshop on Plasma Medicine*, Boltenhagen, Germany

Barton A., Wende K., Bundscherer L., Schmidt A., Bekeschus S., Hasse S., Lindequist U., Weltmann K.-D., Masur K. (2013): Non-Thermal Plasma Treatment of Human Cells: The Effect of Ambient Conditions. *21st International Symposium on Plasma Chemistry (ISPC21)*, Cairns, Australia

Barton A., Wende K., Bundscherer L., Hasse S., Bekeschus S., Schmidt A., Weltmann K.-D., Lindequist U., Masur K. (2013): Growth factors and cytokines are regulated by non-thermal atmospheric pressure plasma. *5th Central European Symposium on Plasma Chemistry (CESPC5)*, Balatonalmádi, Hungary

Barton A., Wende K., Weltmann K.-D., Lindequist U., Masur K. (2013): The impact of non-thermal plasma on intracellular pathways of human keratinocytes. *2nd Young Professionals Workshop on Plasma Medicine*, Kölpinsee, Germany

Poster presentations

Barton A., Bundscherer L., Wende K., Hasse S., Bekeschus S., Masur K., Lindequist U., Kramer A., Weltmann K.-D. (2011): Influence of cold atmospheric pressure plasma on keratinocytes. *10. Workshop Plasmamedizin (ak-adp)*, Erfurt, Germany

Barton A., Holtz, S., Bundscherer L., Masur K., Weltmann K.-D. (2012): Influence of atmospheric pressure plasma on keratinocytes. *39th Meeting of*

the Arbeitsgemeinschaft Dermatologische Forschung (ADF), Marburg, Germany

Barton A., Bundscherer L., Masur K., Lindequist U., Kramer A., Weltmann K.-D. (2012): Impact of cold atmospheric pressure plasma on human skin cell-lines. *4*$^{\text{th}}$ *international conference on plasma medicine (ICPM4)*, Orléans, France

Barton A., Ottmüller K., Schmidt A., Wende K., Lindequist U., Weltmann K.-D., Masur K. (2012): Influence of cold atmospheric pressure plasma on human skin cells. *EMBO Conferences - The molecular and cellular basis of regeneration and tissue repair*, Oxford, United Kingdom

Barton A.*, Masur K.*†, Wende K.†, Bundscherer L., Bekeschus S., Schmidt A., Hasse S., Lindequist U., Weltmann K.-D. (2013): Comparison of apoptosis in a skin and skin cancer cell line after non-thermal plasma exposure. *NextMed 2013*, San Diego, United States of America (* = contributed equally; † = presenting authors)

Other scientific achievements

Finalist Best Paper Award:
Barton A., Wende K., Bundscherer L., Schmidt A., Bekeschus S., Hasse S., Lindequist U., Weltmann K.-D., Masur K. (2013): Non-Thermal Plasma Treatment of Human Cells: The Effect of Ambient Conditions. *21st International Symposium on Plasma Chemistry (ISPC21)*, Cairns, Australia

Organization committee member:
1st Young Professionals Workshop on Plasma Medicine

Member of the International Society for Plasma Medicine (ISPM)

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An dieser Stelle möchte ich mich bei allen Personen bedanken, die mich in dieser spannenden Zeit begleitet und unterstützt haben.

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