The reproducibility of a new computerised planimetric method for the measurement and assessment of removable dental prostheses plaque, rotating needles device as example.

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Dedication

This thesis is dedicated with love and gratitude
to my parents Abdul Kariem and Muna
to my wife Alaa
and my children Sahra, Lana, and Mira
without whom this work would have not been completed.
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List of abbreviations

RDP     Removable Dental Prosthesis
CD      Complete Denture
AHLs    Acylated Homoserine Lactones
EPS     Extracellular Polymeric Substances
MIC     Minimum inhibitory concentration of planktonic cells
MBC     Minimum bacteriocidal concentration of planktonic cells
BMS     Burning mouth syndrome
CPM     Computerised planimetric method
POP     The Percent Of Plaque
base\textsubscript{(B-O)} The vestibular flange and the occlusal and vestibular surfaces of the artificial teeth
RPR     Relative plaque removal
ICC     Intraclass correlation coefficients
1. Introduction

The percentage of old people against younger people has grown considerably during the last few decades in the developed countries and is expected to increase further in the next few decades (1). This demographic shift will have significant impact on oral health care services. More older people will face more teeth loss, morbidity and disabilities and consequently will need an increasing proportion of oral health care services like removable dental prosthesis (RDP) (1, 2). Nevertheless, many surveys of adult dental health indicate that the prevalence of RDP in high developed countries is slightly decreasing, but with great differences between countries, geographical regions within countries and between groups with various backgrounds and age (3-5). This decrease came mainly from Complete Denture wearers (CD). More subjects had maintained a residual dentition and were wearing removable partial dentures, but the decrease in CD use was greater than the increase in removable partial denture RPD use (6-11). The percentage of edentulous was estimated to continue to decrease into the next century (12, 13). Nevertheless, the provision of replacement removable dentures to those currently edentulous or the construction of RDP for new patients will present a considerable task to the dental profession (12, 13). Besides, those patients who have RDP are becoming more clinically demanding either because of oral conditions present at the time of tooth loss or the deterioration (12). And since tooth loss may impair oral function, esthetics and phonation, the need for RDP to restore the dentition will keep its momentous in the near future (14, 15).
2. Literature review

2.1 What Is Biofilms?

Biofilms are composed of microorganisms attached to surfaces and encased in a hydrated polymeric matrix containing polysaccharides, proteins, and nucleic acids (16). Biofilm formation is a way used by microorganisms to ensure their survival (17). In nature, most microbes live as communities in biofilms (17). Bacteria and other microbes embedded in a self-produced and secreted matrix of extracellular polymeric substances (17, 18). Life in a biofilm provides protection to outrage from the surrounding environment, and facilitates survival under hard conditions and environmental insults such as ultraviolet radiation, physicochemical stresses, desiccation, and insufficient supply of nutritive resources (16, 17, 19).

2.2 Formation and Development of Biofilms

Biofilms grow in a three-stage process as shown in, figure 1. The first stage includes the attachment of bacteria to the substratum. This attachment is often a response to intercellular messenger molecules such as acylated homoserine lactones (AHLs) which are used to detect a surface or a high density of cells in the local environment (20). The second stage is the microorganisms growth, which leads to the colonization of the surrounding area and the formation of the biofilm. The third stage includes the biofilms disperse, allowing cells to spawn new biofilm communities elsewhere. Through the last stage, the biofilm developmental cycle closes full circle (16).
2.3 Denture plaque

Chalmers defined dental plaque as a natural biofilm composed of various microorganisms that tenaciously attach to the teeth and other oral surfaces (21). Plaque on RDP is a dense layer of microorganisms and their metabolites, that can develop on the surface of complete denture (CD) or removable partial denture (RDP) (22). Briefly, denture plaque is a classic example of a biofilm that builds up on the RDP.

Like soft and hard intra-oral tissues, immediately after being placed into the oral cavity, the RDP surface begins to be coated with a layer of salivary glycoproteins and immunoglobulins called ‘acquired pellicle’ (23-25). Stedman's Medical Dictionary defined acquired pellicle as a thin film (about 1 µm), derived mainly from salivary glycoproteins, that forms over the surface of a cleansed tooth crown when it is exposed to the saliva (26). Salivary molecules such as proline-rich peptides, statherin, and amylase, are attached on the oral surfaces and the RDP surfaces to form an acquired pellicle, which provides receptors to which only certain microbes are able to attach. Subsequently, salivary glycoproteins act as a nutrient source for the growth of the colonizing organisms (27). On the other hand, the bacterial capsule, which is an amorphous, gelatinous layer, mediates...
the adhesion of bacteria to human tissues or prosthesis such as dentures or implants (28). This adhesion is a prerequisite for colonization and infection (29). The Glycocalyx (slime layer) covers the outer surfaces of many bacteria and allows the bacteria to adhere firmly to various structures, e.g. oral mucosa, teeth, RDPs, heart valves and catheters, and contribute to the formation of biofilms (29). Once the microorganisms adhere to the RDP, they usually tend to aggregate and form intelligent communities of cells embedded in a matrix of polymers of host and bacterial origin (30, 31). Microorganisms produce Extracellular Polymeric Substances (EPS), that is a complex mixture of biopolymers primarily consisting of polysaccharides, as well as proteins, nucleic acids, lipids and humic substances (32). EPS create the intercellular space of microbial aggregates and form the architecture and building of the biofilm matrix (32). Plaque maturation (Figure 2) involves the growth of the attached bacteria to produce a structurally- and functionally-organized biofilm (29). The structure of this community is not flat and compressed but comprise a complex architecture with towers and mushroom or dome-shaped structures with water channels that permit transport of metabolites and nutrients (29). The maintenance of open water channels in multispecies biofilms requires interspecies signalling to direct growth and exopolysaccharide production away from the channels (33). The microorganisms in the biofilm communicate via the signal molecule Autoinducer. Miller and Bassler defined Autoinducer as signalling molecules that are produced in response to changes in cell-population density (34). Plaque maturation is characterized by increasing quantity and diversity of micro-organisms on the RDP surface. But the growth rate has been shown to be far slower than planktonic cells. This is thought to be due reduced level of nutrient supply as a result of the dense population of cells within a biofilm structure (35). After 7 days, it was noticed that streptococci
are the main organisms present, but after 14 days there is a shift to anaerobic rods and filaments (36).

**Figure 2** Summary of plaque development on RDP. The figure is designed by the author according to Bagg et al. in his textbook: Essentials of microbiology for dental students (36).

Clean Surface of the RDP.

After 2 seconds: Acquired pellicle (black colour).

After 1 minute: Pioneer bacteria (yellow colour).

2 Hours: Microcolonies and extracellular polysaccharide.

2 Hours onwards: Biofilm development (*different forms and colours represent different types of bacteria*).

48 hours: Mature plaque.
2.4 Biofilm resistance

Because Biofilms possess a high resistance to antimicrobials, immune cell and antifungal treatment, they gained notoriety ability to cause clinically refractory disease (35, 37-39). The structure of the biofilm matrix, reduce the ability of antifungal or antimicrobial agents to penetrate and diffuse through the biofilm (37). Besides, some microorganisms in the biofilm have the ability to alter the membrane protein composition resulting in a reduction in cell permeability to antimicrobial agents (37). Moreover, it was noticed the increasing presence of multidrug efflux pumps in many biofilms, which can exude antimicrobials (37, 38). Therefore, some researchers reported that, bacteria grown in a biofilm can be up to 1,500 times more resistant to antibiotics, biocides, and immune chemicals compared to the same bacteria grown suspended in liquid culture (16).

For example, Streptococcus sanguinis grown in biofilm increase its tolerance to Chlorhexidine 10 – 50× MIC (MIC = minimum inhibitory concentration of planktonic cells) (27). Streptococcus sobrinus grown in biofilm increase its tolerance to Amine Fluoride 75× MBC (MBC = minimum bacteriocidal concentration of planktonic cells), and to Chlorhexidine about 300× MBC. Porphyromonas gingivalis grown in biofilm increase its tolerance to Metronidazole 2 – 8× MBC to Doxycycline 4 – 64× MBC, and to Amoxicillin 2 – 4× MBC (27).

2.5 The microflora of denture plaque

Normal oral flora is taking an important role in keeping the oral mucosa in a health status (39). There are many factors influencing the oral microbial community structure, such as (40, 41):

- General factors: Age, infancy, pregnancy, illness, administered medicaments, immunity system, diet......
- Local factors: Oral hygiene, saliva quality and quantity, teeth (eruption and the loss of teeth), insertion of partial or complete dentures, trauma, smoking.

Differences in the microflora of denture plaque are highly variable between healthy sites and sites with denture stomatitis. Even the microflora of healthy sites is highly variable between the fitting and the exposed surfaces. In general, denture plaque contains complex heterogeneous layer of microorganisms and their metabolites, that includes more than $10^{11}$ organisms/g (wet weight) (22). The first step in understanding the role of bacteria in oral diseases is the comprehensive insight into the *normal microflora* (42). For instance, streptococci (especially mutans streptococci) and sometimes Candida spp are commonly seen in the relatively stagnant area on the denture-fitting surface, because plaque tends to be more acidogenic (43). Mutans streptococci and members of the mitis-group of streptococci are the primary microorganisms on complete dentures (27). Obligate anaerobes including *A. israelii* and low proportions of Gram negative rods are also detected in denture plaque. *Staphylococcus aureus* – which is a species found commonly in the mucosa of patients with denture stomatitis - can also be isolated from denture plaque over healthy sites (27).

On the other hand, many researchers stated that, approximately 90% of cases of denture-related stomatitis are thought to be caused by yeasts, typically *Candida albicans*, although other species, such as *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii* and *C. dublieniensis*, may also contribute to the pathogenesis of the disease (44-46). Yeasts and bacteria have the ability to cause damage to the oral mucosa, and colonize the surfaces of the RPD as a biofilm more than oral mucosa (44, 45, 47). However, the mucosa beneath the dentures is sparsely colonized with bacteria, irrespective of whether or not the subjects had denture-related stomatitis (48).
Moreover, dentures can serve as a reservoir for halitosis-causing oral bacteria. Halitosis-related species like *F. nucleatum*, *T. forsythia*, *V. atypica* and *K. pneumoniae* were detected in denture plaque (49).

**2.6 Oral diseases caused by RPD**

Oral mucosa lesions associated with RDP may represent acute or chronic reactions to bacterial plaque adhering to RDP, a mechanical denture injury, or a reaction to the material of the RPD (41, 50). The direct factor for oral mucosa lesions associated with RDP is the presence of the removable dentures in the oral cavity. It is also important to mention that mucosal lesions related to RDP are induced in many cases by multiple factors (41). The main predisposing factors can be divided into (41, 50-52):


2. Nutritional deficiencies, iron, folate, or vitamin B12 deficiency (53-55).………
3. High-carbohydrate diet (28, 56).
4. Endocrine disorders, diabetes mellitus, hypothyroidism (57-60)………
5. Immune defects, immunosuppression, AIDS, Sjogren's syndrome, thymic aplasia (52, 61-63)………
6. Acute leukemia, agranulocytosis, malignancies (64-66).
7. Irradiation (41).
8. Drug therapy, Broad-spectrum antibiotics, corticosteroids (61-63).………

B- Local factors: 1. Xerostomia (41).

2. Smoking tobacco (67).
3. Fungal infection (68).
4. Changes in environmental conditions, trauma, denture usage (how long), denture plaque, denture cleaning (method / material) (69-71).

Lesions of the oral mucosa associated with wearing RPD include (Figure 3):

1. Denture Stomatitis (also called (72-74) denture-related stomatitis, denture sore mouth, denture stomatitis, chronic atrophic candidiasis, Candida-associated denture induced stomatitis, and denture-associated erythematous stomatitis) is a term that has been applied to an inflammation of the denture-bearing mucosa which may affect as many as two-thirds of an elderly population of denture wearers (75, 76). Laskaris stated that denture stomatitis is a tissue response to microorganisms living beneath the dentures (77). Newton in 1962 classified denture stomatitis according to clinical criteria into three types (78):
   Type I / pin-point hyperaemic: Localized simple inflammation which is associated with trauma.
   Type II / generalized simple inflammation: Diffuse erythema confined to the mucosa contacting the denture.
   Type III / granular surface: Inflammatory papillary hyperplasia only resolved by surgery.

Improvement of RDP fit and occlusion, oral and RDP hygiene, and topical antimycotics are important steps in denture stomatitis treatment (77). Besides, it is always important to consider the role of systemic disease and its impact on oral function and homeostasis.

2. Angular Cheilitis (also called (73, 79-82) rhagades, perlèche, cheilosis, angular cheilosis, commissural cheilitis, or angular stomatitis) is inflammation and atrophy of the skin folds at the angles of the mouth, well known among denture wearers (79). The lips may exhibit cracking, fissures, atrophy,
literature review

erythema, moist maceration, and ulceration that are exaggerated at the corners of the mouth (81). This may come from the excessive lip licking, thumb sucking, sagging of facial skin in edentulous or elderly persons, or RDP with reduced vertical dimension leads to overclosure (81). The prolonged contact with saliva results in maceration, with possible secondary infection by Candida albicans or staphylococci (83). Treatment consists of correcting the occlusal vertical dimension, and applying topical steroids and/or antifungal ointments (77).

3. Burning mouth syndrome: Burning mouth syndrome BMS is characterized by a burning sensation in one or several oral structures of long-term duration without any detectable local or systemic causes (84, 85). In this disease, the patients express a wide range of oral complaints without visible tissue changes (83). Despite considerable studies conducted on this topic, its aetiology and pathogenesis are still unknown and, as a consequence, there is no causal and/or effective treatment (86, 87). It typically affects women over the age of 50 and wear complete dentures (85). The symptoms often appear for the first time in association with the placement of new dentures (84). Appropriate counselling of the patient, to help him understand the benign nature of the problem, with subsequent elimination of fears is the first main step in disease management. A large variety of Medicines, neuroprotective drugs and therapies have been proposed in BMS treatment. Sadly, the treatment management of this syndrome is still not satisfactory (86-88).

4. Traumatic ulcers (sore spots): Traumatic ulcers are common oral lesions, that can be caused by RDPs irritation / high stresses and lateral movements. Traumatic lesions are usually the result of imperfections in or on the surface of the tissue side of the RDP base. Disharmony in occlusion in either the centric or the eccentric jaw positions can also produce sore spots. Errors in
denture design may cause a denture to move excessively over the mucosa and enhance the occurrence of sore spots (89). For example, incorrect fabrication of the metal frameworks or denture base may increase the functional stress on the mucosa or interfere with the freedom of movement of the surrounding muscles and that may initiate a complaint of burning or frank soreness (90).

Chronic irritation is generally regarded as a modifier rather than an initiator of oral cancer (83). Mechanical trauma from ill-fitting dentures, broken fillings and other factional rubs factors is unlikely to cause oral cancer. These factors will probably hasten the process of cancer, if a cancer is started from another cause (83).

Treatment of traumatic ulcers consists of occlusal adjustment and basal surface refining.

5. Denture-induced hyperplasia (Also termed flabby gums, denture fibroma or flabby ridges). Denture-induced hyperplasia takes the form of single or multiple flaps of fibrous tissue related to the border of a denture (90). The fibrous tissue is a reactive lesion arising from excessive and chronic mechanical pressure on the vestibular oral mucosa. Lesions develop slowly as a result of overextension of denture border or gradual resorption of alveolar bone, causing the denture to sink so that its borders dig into the mucosa (50). Denture-induced hyperplasia is found in up to 10% of denture wearers (50, 91). The lesions can reach a considerable size before the patient becomes aware of the offending denture (90).

Treatment consists of eliminating denture trauma and recalling after 2 weeks. If the size of the lesion is still too large to allow adequate extension of the denture, surgical removal is indicated.
6. Papillary hyperplasia: Papillary hyperplasia or palatal papillomatosis is a painless papillomatous lesion of hard palate in denture wearers (83). The lesion is composed of multiple small nodules that are ovoid to spherical and measuring 2 to 3 mm in diameter on an erythematous background as a result of inflammation (92). The precise cause of papillary hyperplasia is not well understood, although it appears almost exclusively on the hard palate and almost always in association with an ill-fitting or loose removable prosthesis, that creates a potential space between the denture base and tissue (92). Other causes like poor denture hygiene or wearing the denture 24 hours a day are also mentioned by some researchers (93). Tissue hyperplasia has also been related to the presence of the fungal organism in the setting of low-grade chronic trauma (92, 94, 95).

Removal of the RDP may allow the erythema and edema to subside, and the tissues may return to its normal appearance. The condition also may show improvement after topical or systemic antifungal therapy (95). For more advanced lesions, hyperplastic tissue should be removed before adjusting the old RDP or fabrication of a new one. This may be achieved with a scalpel, a fluted burr on a rotary instrument, electrocautery, or laser surgery (96).

7. Contact allergic stomatitis: RDP dental materials occasionally induce allergic reactions locally (contact allergic stomatitis) as well systemically (anaphylactic shock and palmoplantar pustulosis) (97-99). Denture-induced allergic stomatitis is a rare acute or chronic allergic reaction, caused by denture materials or denture cleansing agents (77). Clinically, erythema, edema, vesicles, erosions, and occasionally ulcers may be seen. A burning sensation is a common symptom. Removal of suspected allergens, topical or systemic steroids, and antihistamines are the followed measures in treating contact allergic stomatitis (77).
Figure 3  Oral mucosal lesions related to RDP.

- **Acute Reactions**
  - Allergic Reaction
  - Traumatic Ulcers (sore spots)

- **Chronic Reactions**
  - Denture-induced Hyperplasia/Flabby Ridges
  - Hyperplasia
    - Papillary Hyperplasia
  - Chronic irritation may accelerate the process in oral cancer
  - Allergic Reaction
  - Burning Mouth syndrome
  - Angular Cheilitis
  - Denture Stomatitis

Chronic irritation may accelerate the process in oral cancer.
In addition to what were mentioned previously, there are many other direct and indirect sequelae caused by wearing RRP like: caries, root caries, periodontal disease (especially around the abutments), gagging, residual ridge reduction, atrophy of masticatory muscles, nutritional deficiencies, oral galvanic currents and altered taste perception (22, 84, 100). Finally, many recent studies demonstrated relationships/associations/mutualistic interactions between oral plaque/oral health/periodontitis and systemic diseases such as diabetes, tissue repair capacity, immune cell functions........ (101, 102). Oral plaque microorganisms may disseminate through the blood to infect the vascular endothelium and contribute to the occurrence of atherosclerosis and risk of myocardial ischemia and infarction (103). Sumi et al. proved the high correlation between the bacterial species in denture plaque and pharyngeal microflora (104). Besides, denture plaque could serve as a reservoir for disseminated infections such as gastrointestinal infections (105). The new studies enhance our understanding of the importance of oral health and its connection to the patients’ overall health and their intraoral and general diseases. Disease can be treated not only by targeting the putative pathogens, but also prevented by proper oral and RDP care:

2.7 Materials and methods for cleaning RPD

RDPs require proper care to keep them clean, free from stains and looking their best. Two major approaches are generally recommended to patients for the removal of denture plaque and stains (figure 4). RDPs can be cleaned mechanically or chemically (106). Mechanical methods can be further subdivided into: manual (by hand) or machinal (ultrasonic device or rotating needles device). In this thesis, the term ‘mechanical method’ will be used when plaque is mainly removed by a mechanical force. It is important to mention that nearly all
mechanical approaches have chemical solutions or materials (chemical method) are used.

A- Mechanical methods

- **Brushing:** It is the most widely used mechanical method. The method includes rinsing and brushing away plaque and food with water alone, household soap, a toothpaste, denture cream or abrasives. The brush or the whitening toothpaste can scratch the denture. The tiny scratches could in turn increase the surface area for plaque formation (107). The degree of scratching depends to a large extent on the diameter, length and hardness of bristle; the stiffer the bristles the more abrasive the brush will be. Wear is inversely related to the length of the bristles, on the contrary, the wear is directly proportional to the diameter of the bristle (108-111).

- **The ultrasonic denture cleaner** is a small bathtub containing a liquid, which has high-frequency sound waves (between 20 – 120 kilohertz) projected through the tube. Ultrasound has two mechanisms of action, the first is the movement of liquid resulting from sound waves transferred to the liquid (vibration), and the second, the ultrasound within the liquid causes tiny bubbles (microscopic cavities) to appear and collapse on RPD surfaces, very quickly, all through the liquid (112, 113). The violent collapse of bubbles on the dentures creates localized areas of disruption that loosen and remove debris from the denture surface; this process is known as cavitation (111). This is almost similar to ultrasonic scalers when used to remove plaque and calculus from teeth (111). Ultrasonic RPD cleaner removes denture plaque, but does not effectively reduce the number of microorganisms, therefore the mechanical cleansing ability of the device is improved by adding disinfectant solution to the bathtub (109, 114).
- **Rotating needles device (Sympro - Renfert GmbH):** Rotating needles device is a small bathtub containing cleaning solution and cleaning pins (needles). The tub is surrounded by movable magnets. A rotary magnetic field sets the needles in motion, which causes mechanical removal of the denture plaque. Besides, the rotating needles heat up the cleaning solution to approx. 45°C (113°F), which accelerates the chemical reaction according to the manufacturer product details.

**B- Chemical methods**

The term ‘chemical method’ will be employed when cleansing is dependent on a chemical reaction.

Chemical denture cleaners (so-called denture cleanser), are available in the market in different commercial brands: Periogard, Cepacol, Corega Tabs (sodium perborate/ effervescent tablet), Milton, Mildent, Steradent, Polident, Renew Denture Cleaner, Efferdent…………… (115).

In chemical cleaners, RPD should be immersed entirely within a bath containing a particular cleaner. All RPD chemical cleaners rely on a chemical reaction to remove the denture biofilm and plaque and have a different mode of action to achieve this mission (110, 116, 117).

Normally, denture cleaners contain more than one of these ingredients (115, 118-121):

1. **Alkali Hypochlorite:** Like sodium hypochlorite which is the main constituent of several brands of denture cleanser. It is frequently used as a disinfectant or a stain removal /bleaching agent. Sodium hypochlorite can disrupt the cell membrane of microorganisms and dissolve the mucins that collect on the RPD (109, 111, 122).

2. **Alkali Peroxide:** Peroxide cleansers are commonly used and supplied in the form of tablets and powders. Sodium perborate is soluble in water
and releases hydrogen peroxide which has stain removal and bleaching potency. Sodium perborate serves as a source of active oxygen in many detergents, and cleaning products, and act by producing bubbles which help to remove and disrupt microorganisms from the denture surface by the process of cavitation. Alkali Peroxide is effective only when dentures are soaked for several hours or overnight and are not effective when soaked for 15-30 minutes. (109, 111, 116).

3. Acids: Citric acid appears commonly in denture cleaners. The acid acts as a chemotherapeutic agent that can effectively disrupt biofilms through a sequestering mechanism with calcium ions (123). This mechanism allows citric acid to break calcium bridges and subsequently disrupt the biofilm matrix, which may lead to anti-biofilm activity (119, 124, 125).

4. Sodium polyphosphate: Sodium polyphosphate is a pigment dispersant and strong cleaning agent, normally consumed as a component of commercial detergents and soaps.

5. Potassium monopersulfate: Potassium monopersulfate is a powerful oxidizer with cleaning and bleaching properties.

6. EDTA: Ethylenediaminetetraacetic acid can be used to remove inorganic debris (smear layer) and loosen up calcifications on RPD surfaces.

7. Enzymes: Enzymes like Protease, amylase and mutanase serve in removing denture plaque (126, 127).

8. Additional compounds: For example, vinegar, sodium bicarbonate, flavourings and fragrances. Dye markers that provide a color change when the cleansing process has been completed.
Oral rinses are considered one of the chemical methods for cleaning dentures. They include any dental mouthwash products available for patients, such as 0.2% chlorhexidine gluconate or 0.05% salicylate solution. They are used as a soak like the above products and have a good range of antibacterial properties (111, 122). Finally, it is important to mention that the British Dental Health Foundation and International Dental Health Foundation recommend denture-wearers to avoid using products that contain bleach, as it may weaken and discolor the RPD (128, 129). Nonetheless, manufacturers such as Cepacol, Corega and Polident manufacture cleansers with this ingredient. Besides, oral rinses which contain chlorhexidine solutions do tend to stain dentures with prolonged use (109, 111).

**Figure 4** Materials and methods for cleaning RPD.

2.8 RDP plaque assessment methods

Oral hygiene assessment is rarely carried out for RPD hygiene, because there is a need for standardization of both the methods for RPD hygiene assessment and RPD hygiene management. Several different methods for assessing denture hygiene have been suggested in the current literature, but until now none has been widely accepted. Above all, there is currently no standardized method of assessing RPD plaque/hygiene (130-133).
Assessing denture plaque/hygiene is a very important step to:

1- Monitoring denture hygiene.
2- Assessing and measuring patient compliance with regards to RPD hygiene.
3- Evaluating denture plaque scores before and after using mechanical or chemical methods.
4- Compare the efficacy of different methods or devices in cleaning and removing biofilm and denture plaque.

In general, the methods of assessing RPD plaque can be divided into 3 categories, see table 1.

**Table 1** The methods of assessing RPD plaque.

<table>
<thead>
<tr>
<th>Method</th>
<th>Suitability</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual assessment</td>
<td>Patients’ monitoring and motivation, and evaluating patients’ compliance with RDP hygiene instructions.</td>
<td>Schubert &amp; Schubert index (132), Denture Hygiene Index (133), Ausberger &amp; Elahi index (134), Tarbet index (135), Jeganathan et al. Index (136), Budtz-Jørgensen &amp; Knudsen Index (137), Denture Cleanliness Index (111)……..</td>
</tr>
<tr>
<td>Planimetric assessment</td>
<td>Mostly used in the field of scientific research.</td>
<td>Paper-weighing quantification method (138), Digital planimeter method (139), Point-counting method (dot-grid method) (139), Square-counting method (140), Computerized methods (141).</td>
</tr>
<tr>
<td>Laboratory assessment</td>
<td>Useful for diagnostic purposes or scientific research.</td>
<td>Conventional microbiological method (120), Molecular method (142).</td>
</tr>
</tbody>
</table>

Before we start with the methods, it is important to explain the importance of plaque disclosing agents:

**2.8.1 Plaque disclosing agents**

Biofilm and plaque detection methods vary greatly because plaque is generally colourless (143), therefore, it should be stained prior to assessment. In many clinical studies, biofilm and plaque are clinically detected by using biofilm
disclosing agents and/or protein evaluation and/or microbiological quantification (120, 134, 139, 140, 144-147). Since dental and denture plaque is usually transparent, colourless and not easily visible, RDP wearer frequently is not aware of the quantity or the location of plaque present in the mouth and over the RPD (148). Therefore, it is desirable to use plaque-disclosing compositions to identify areas of the mouth and RPD where plaque buildup is a problem. The use of plaque disclosing agents motivates the RDP wearer and help the examiner by showing the presence and quantity of plaque.

A number of agents have been developed, some of which may be used conveniently and economically at home, whereas others may be used more effectively in a dental office.

The most common plaque disclosing agents are: 1% eosin, 1% neutral red, 5% erythrosine (most widely used), 0.05% methylene blue, 0.3% proflavine, 1% sodic fluorescein (special UV light is needed in order to make the fluorescein dye visible), and Replak (141, 149).

According to Silva et al. work, the agent which presents the greatest disclosing ability and removal facility are 1% eosin, 1% neutral red and 5% erythrosine (149).

2.8.2 RDP Plaque visual assessment (indexes)

Before using or determining the most suitable index to be used within a study, five points should be put into consideration: the population size, duration of study, the type and extent of change anticipated, and above all, the objectives of the study (143, 150). By literature review, only one research was founded, that tested the discriminating power of three RDP plaque indices (151). RDP plaque assessment by visual examination has some advantages as it is the easiest. There is no need for special equipment, making it useful for epidemiological studies (151). However, applying index produce problems and difficulties. The problems come from the subjective nature of the indices and the need for examiner
training. Besides, the calibration should be put into consideration if there is more than one examiner. Nevertheless, if all examiners have not been extensively calibrated to ensure intra- and inter-examiner agreements, calibration does not necessarily grant reliability at differing times within the same centre or between centres (143). This process will increase the time and cost of studies. Besides, visual assessment may generate more random and systematic errors than computer based plaque analyses because it is more subjective (151, 152). On the other hand, computer based plaque analyses when applied on natural teeth proved to be more reliable, (153) precise, (154) objective (153-155) and sensitive (154, 156) than classic plaque indices. These critical points may enable the researcher to reduce the sample size and avoid underpowered trials simultaneously. Without doubt, reducing the sample size will reduce the cost and time of the study. The RDP indexes generate ordinal data, but computerized methods generate interval data which give statistical strength to the studies. Above all, most computerized methods are able to detect and measure small changes in plaque, it is more representative of the true plaque area coverage (157), and can be applied on all types and surfaces of RDPs. However, it is more sophisticated and very time consuming.

After the literature review, 12 indexes were retrieved:

2.8.2.1 Denture Cleanliness Index (DCI) (111)
DCI index can be used with upper and lower/complete and partial dentures. Preparing the RPD: RPD is gently washed under cold water to remove loose debris; a liquid plaque disclosing dye is applied onto the entire RPD fit surface, and left for 30 seconds. The RPD then gently washed under cold water to remove excess dye. The fit surface is inspected visually and scored according to the DCI
index criteria (table 2). Based upon the DCI scores, the patient is given specific denture hygiene instructions (table 3).

Table 2  DCI Index criteria (111).

<table>
<thead>
<tr>
<th>DCI score</th>
<th>Index criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clean denture. No plaque is visibly seen, no staining, no plaque detectable</td>
</tr>
<tr>
<td>1</td>
<td>Denture is visibly clean. Little staining (&lt;25% fit surface stained)</td>
</tr>
<tr>
<td>2</td>
<td>Denture has visible plaque and/or debris. Moderate staining on the fit surface (25-50% fit surface stained)</td>
</tr>
<tr>
<td>3</td>
<td>Denture has visible plaque and/or debris. Severe staining on the fit surface (&gt;50% stained)</td>
</tr>
<tr>
<td>4</td>
<td>Denture has visible calculus deposits, on any surface</td>
</tr>
<tr>
<td>*</td>
<td>Visible defects in denture, in addition to any of the above scores</td>
</tr>
</tbody>
</table>

Table 3  Intervention modalities based on DCI score (111).

<table>
<thead>
<tr>
<th>DCI score</th>
<th>Intervention and patient´s instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No intervention required, reinforce current denture hygiene</td>
</tr>
<tr>
<td>1</td>
<td>Denture hygiene reinforcement</td>
</tr>
<tr>
<td>2</td>
<td>Denture hygiene reinforcement, patient information leaflet</td>
</tr>
<tr>
<td>3</td>
<td>Denture hygiene reinforcement, patient information leaflet and denture hygiene kit</td>
</tr>
<tr>
<td>4</td>
<td>Intervention by clinician to clean dentures, denture hygiene reinforcement, Patient information leaflet and denture hygiene kit</td>
</tr>
<tr>
<td>*</td>
<td>Consider denture reline or remake (depending on the severity of the defect)</td>
</tr>
</tbody>
</table>

2.8.2.2 Denture Hygiene Index (DHI) according to Prof. Wefers (133)

DHI index can be used with upper and lower / complete and partial dentures. Retaining elements are included according to their position. The index divides the RPD to vestibular surface, oral surface and fit surface. The assessment starts always with the vestibular surface, which is divided into three sections; two side sections behind the canines and one front section between the canines, see
The section starts from the cutting edge of the front teeth or from the centre of the occlusal fissures on the premolars and molars to the vestibular RPD flange edge. Similarly, the oral (palatinal or lingual) RPD surface is divided into 3 sections. The denture fit surface is divided into 4 sections by two imaginary lines; the first is the midsagittal line and the second is between second premolar (P2) and first molar (M1). In this way, 10 sections have been created per RDP.

- DHI Score: All 10 sections are assessed in accordance with the principle Yes/1 (presence of biofilm or denture plaque) and No/0 (clean section). The assessment is carried out purely quantitative.

Preparing the RDP: After picking RPD from the mouth, it should be only rinsed under running water; without brushing it.

DHI Score can be represented in one of two forms:

A- In three numbers: DHI index score can be represented in three numbers (vestibular-oral-fit surface). It may have a value between 0-0-0 (= all surfaces clean) to 3-3-4 (all surfaces partially or completely covered with biofilm or denture plaque).

B- In one number: DHI index score can be represented in one number:

Total score of the DHI index = vestibular value + oral value + fit surface value.

If plaque disclosing agents is applied, the prefix "r" is added before the score. And if mineralization is detected, the suffix "c" (calcified) is added after the number of the affected section.

**Examples:**

0-2-4 0 no biofilm or plaque detected in the three RPD vestibular sections.

2 two of the three oral sections are contaminated (covered with biofilm or denture plaque).

4 all the four fit surface sections are contaminated (covered with biofilm or denture plaque).

2-3-2 2 two vestibular sections are contaminated.

3 all the tree oral sections are contaminated.
literature review

2  two fit surface sections are contaminated.
0-1c-4  0  no plaque on the vestibular sections of the RPD.
1c  one oral section is contaminated with mineralized plaque (tartar).
4  all the four fit surface sections are contaminated.

ger 2c-1c-3  letter (r) indicate the use of plaque disclosing agent.
2c  two vestibular sections are contaminated with mineralized plaque.
1c  one oral section is contaminated with mineralized plaque.
3  three fit surface sections are contaminated.

Prof. Wefers believe that the flexibility of this index makes it suitable for both epidemiological studies and routinely patents’ follow-up (133).

Figure 5  The 10 sections in every RPD according to the denture Hygiene Index. The figures are taken from: www.dental.unigreifswald.de/fpk/pdf/prothesenhygiene.pdf
2.8.2.3 McCabe et al. scoring system for stain, plaque and calculus (145)

This system can be used to estimate plaque accumulation on all surfaces of complete dentures. According to our knowledge, it is the only index tried to distinguish between stain, plaque and calculus, and score all of them separately at the same time. The author did not mention how he distinguished between stain, soil/calculus and disclosed Plaque.

**Table 4** McCabe et al. scoring system for Stain, Plaque and Calculus.

<table>
<thead>
<tr>
<th>Score</th>
<th>Stain</th>
<th>Soil / Calculus</th>
<th>Disclosed Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No stain</td>
<td>No calcified deposits</td>
<td>No areas of blue</td>
</tr>
<tr>
<td>2</td>
<td>Slight stains either on denture or teeth.</td>
<td>Some deposits can be seen, especially in problem areas behind the teeth.</td>
<td>Small areas of blue only, either on denture or teeth.</td>
</tr>
<tr>
<td>4</td>
<td>Pronounced stained areas, but much of denture not affected.</td>
<td>More general deposits. May be solid in places.</td>
<td>More pronounced blue areas, but much of denture unaffected.</td>
</tr>
<tr>
<td>6</td>
<td>Considerable areas of stain, especially on teeth and on palate.</td>
<td>Calcified deposits on and between teeth and on large areas of the denture, severe in some areas.</td>
<td>Large areas of denture and teeth stained blue, very heavy stains in places.</td>
</tr>
<tr>
<td>8</td>
<td>Greater part of denture is stained - very heavy in many areas.</td>
<td>Large areas of denture show heavy deposits. Teeth also show heavy deposits.</td>
<td>Greater part of denture and most of teeth show medium-heavy blue staining.</td>
</tr>
<tr>
<td>10</td>
<td>Entire denture and all teeth heavily stained.</td>
<td>Heavy white encrustations on virtually all surfaces and on and between teeth.</td>
<td>Denture and teeth covered in heavy blue stains.</td>
</tr>
</tbody>
</table>

2.8.2.4 ASKD Denture Plaque Index (ASKD-DPI) (158):

ASKD-DPI is a quantitative denture plaque index to measure plaque on the fit (intaglio / interior) surface of complete denture.

The fit surface of the maxillary complete denture is divided into 10 areas, table 5. Four areas on the inner crestal part of fit surface (1 - 4), three areas of flanges (5 - 7), and four areas of the rugae and posterior palatal seal (8 - 10). The fit surface
of mandibular complete dentures is also divided into 10 areas: three areas on the crestal part (1 - 3), three areas of facial flange (4 - 6), and four areas on the lingual flange (7 - 10), table 6.

Preparing the complete denture: Maxillary and mandibular complete dentures are soaked in a bowl of water for 1 minute to remove food debris. Fifteen drops of an erythrosine solution are poured in a bowl, having 30 ml of water at room temperature. The dentures are soaked for 2 minutes and then rinsed under running water for 1 minute to remove unattached dye. Pictures of disclosed plaque on the fit surface of maxillary and mandibular complete dentures are taken with a digital camera (158).

The index score ranged from 0 - 100%, and reflected the percentage of the fit surface of maxillary and mandibular complete dentures that contained plaque.


<table>
<thead>
<tr>
<th>Area designation</th>
<th>Plaque retention area description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mesial of central incisor to distal of right 1st premolar</td>
</tr>
<tr>
<td>2</td>
<td>Mesial of central incisor to distal of left 1st premolar</td>
</tr>
<tr>
<td>3</td>
<td>Distal of 1st premolar to the right posterior end</td>
</tr>
<tr>
<td>4</td>
<td>Distal of 1st premolar to the left posterior end</td>
</tr>
<tr>
<td>5</td>
<td>Distal of right canine to distal of left canine</td>
</tr>
<tr>
<td>6</td>
<td>Distal of right canine to the right posterior end of flange</td>
</tr>
<tr>
<td>7</td>
<td>Distal of left canine to the left posterior end of flange</td>
</tr>
<tr>
<td>8</td>
<td>Rugae area</td>
</tr>
<tr>
<td>9</td>
<td>Posterior palatal area bounded by rugae anteriorly, mid-palatal suture medially, rugae anteriorly, and distal flange laterally on right side</td>
</tr>
<tr>
<td>10</td>
<td>Posterior palatal area bounded by rugae anteriorly, mid-palatal suture medially, rugae anteriorly, and distal flange laterally on left side</td>
</tr>
</tbody>
</table>
According to Almas et al, the index can also be used to classified quantitative percentages based on clinical observation (158):

- 0 - 30%  (low DPI), good denture hygiene.
- 31 - 70%  (moderate DPI), represent fair denture hygiene.
- 71 - 100%  (High DPI), represent bad denture hygiene.


<table>
<thead>
<tr>
<th>Area designation</th>
<th>Plaque retention area description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distal of right canine to distal side of left canine</td>
</tr>
<tr>
<td>2</td>
<td>Distal of right canine to the right posterior end of crestal surface</td>
</tr>
<tr>
<td>3</td>
<td>Distal of left canine to the left posterior end of crestal surface</td>
</tr>
<tr>
<td>4</td>
<td>Distal of right canine to distal of left canine on facial flange</td>
</tr>
<tr>
<td>5</td>
<td>Distal of right canine on facial flange</td>
</tr>
<tr>
<td>6</td>
<td>Distal of left canine on facial flange</td>
</tr>
<tr>
<td>7</td>
<td>Lingual area from midline to half distance to distal end on right side</td>
</tr>
<tr>
<td>8</td>
<td>Disto-lingual half of the lingual flange on right side</td>
</tr>
<tr>
<td>9</td>
<td>Lingual area from midline to half distance to distal end on left side</td>
</tr>
<tr>
<td>10</td>
<td>Disto-lingual half of the lingual flange on left side</td>
</tr>
</tbody>
</table>

**2.8.2.5 Prosthesis Hygiene Index PHI according to Schubert & Schubert (132)**

In 1979 Schubert, H and Schubert, U developed the first hygiene index for RPD. The Schubert & Schubert index was originally based on direct assessment of coloured plaque on the whole denture base (fit surface/ tissue side), without using a photographic technique. Prosthesis hygiene index PHI is a quantitative denture plaque and biofilm index to measure plaque on the fit surfaces of complete dentures. It is used for classifying the cleaning level of complete dentures.
The denture base is divided into nine sections, figure 6. The accumulation of plaque is assessed in each section on a scale from 0 to 4 points:

0  Absence of plaque;
1  small spots of plaque;
2  less than half covered with plaque;
3  half or more than half covered with plaque;
4  the entire section covered with plaque.

After the direct assessment of plaque in every section, the prosthesis hygiene index score is calculated by using this formula:

$$PHI = \frac{\sum \text{of individual scores}}{\sum \text{of evaluated areas on dentures}}$$

PHI score could vary from 0 to 4 for a denture. Denture score of less than 1.5 is defined as excellent, from 1.5 to 2.5 as fair, and more than 2.5 as poor (159).

**Figure 6** The 9 sections in upper CD according to Schubert & Schubert index.
### 2.8.2.6 Ausberger and Elahi index (107, 134)

Ausberger and Elahi index can be used to estimate plaque accumulation on complete dentures. Scoring is carried out after dividing the denture into eight sites; 4 on the labial and buccal surfaces, and 4 on the palatal, or fitting surface, figure 7. The plaque present in each area is scored as follows:

- 0 = no plaque;
- 1 = light plaque, 1% to 25% of area covered;
- 2 = moderate plaque, 26% to 50% of area covered;
- 3 = heavy plaque, 51% to 75% of area covered;
- 4 = very heavy plaque, 76% to 100% of area covered.

The mean plaque score from all 8 sites is obtained by averaging the 8 individual scores.

**Figure 7** The 8 sections in upper CD according to Ausberger and Elahi index.
2.8.2.7 Tarbet index (135)

The Tarbet index was originally constructed to assess plaque on the fitting surface of upper complete removable denture (intaglio surface).

The fit surface of each maxillary denture is divided into four sections approximately equal in area by mentally drawing an anteroposterior line at the midline and another line perpendicular to the midline at about the premolar region. Plaque is coloured by plaque disclosing agent, and each of these quadrants is scored as follows:

0 = no plaque;
1 = light plaque (25% or less of the quadrant covered);
2 = moderate plaque (26% to 50% of the quadrant covered);
3 = heavy plaque (51% to 75% of the quadrant covered); and
4 = very heavy plaque (76% to 100% of the quadrant covered).

A total denture plaque score was obtained by summing the quadrant scores, (Maximum score = 16).

2.8.2.8 Jeganathan et al. Index (136)

Jeganathan et al. index is a modification of Tarbet index. The maxillary denture is removed from the mouth and soaked in a bowel of water for 1 minute to remove food debris. Plaque disclosing agent (Erythrosine) is applied on the fitting surface and left for 1 minute. The denture is rinsed under running tap water to remove the unbound dye. The disclosed denture plaque is scored as follows:

0= no visible biofilm;
1= light biofilm (1 to 25% of the fitting surface covered by biofilm);
2= moderate biofilm (26 to 50% covered);
3= heavy biofilm (51 to 75% covered);
4= very heavy biofilm (76 to 100% covered).
2.8.2.9 Budtz-Jørgensen & Knudsen Index (137, 160)

This index is also for the fitting surfaces of the complete denture. The distribution of the plaque is analysed by the following criteria:

- 0 (Excellent): no visible biofilm;
- 1 (Good): 1/3 or less of the fitting surface covered with plaque;
- 2 (Fair): between 1/3 and 2/3 coverage; and
- 3 (Poor): 2/3 or more covered.

2.8.2.10 Modified Quigley-Hein scale according to Keng et al. (161, 162)

The Quigley-Hein scale is a plaque scoring system developed 1962 to assess plaque accumulation on the front normal teeth (161). A modified Quigley-Hein scale according to Keng et al. was developed to estimate plaque accumulation on complete dentures. The denture is divided into four areas: teeth (including the gingival margins), palate, flange, and fitting surface, and each of these areas is scored as follows:

- 0: no visible plaque
- 1: 0% to 25% of denture area covered with plaque;
- 2: 26% to 50% of denture area covered;
- 3: 51% to 75% of denture area covered; and
- 4: 76% to 100% of denture area covered

The scores of all the surfaces are combined to give the total score.

2.8.2.11 Modified Quigley-Hein scale according to Palenik et al. (161, 163)

A modified Quigley-Hein scale according to Palenik et al. can be used to estimate plaque accumulation on complete dentures. This scale divides the complete
dentures for scoring purposes into three large areas: “teeth,” “palate,” and “tissue side”. Each of these large segments is further divided into 40 equal sized squares, which is surveyed individually for visible plaque according to this table:

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no visible plaque</td>
</tr>
<tr>
<td>1</td>
<td>0% to 25% of denture area (square) covered</td>
</tr>
<tr>
<td>2</td>
<td>26% to 50% of denture area covered</td>
</tr>
<tr>
<td>3</td>
<td>51% to 75% of denture area covered</td>
</tr>
<tr>
<td>4</td>
<td>76% to 100% of denture area covered</td>
</tr>
</tbody>
</table>

Scores from the squares for each of the large divisions are pooled and averaged. The average scores from the large divisions are then added together to determine the total plaque score for each denture.

2.8.2.12 Abelson index (164)

Abelson, D stated that: the process by which dentures accumulate plaque, stain, and calculus is apparently similar to that process which takes place on natural teeth. Therefore, he developed a plaque index to measure plaque accumulation around denture teeth.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No stainable plaque.</td>
</tr>
<tr>
<td>1</td>
<td>Discrete, discontinuous plaque.</td>
</tr>
<tr>
<td>2</td>
<td>Thin layer of continuous lightly stained plaque.</td>
</tr>
<tr>
<td>3</td>
<td>Layer of intensely red staining plaque (up to 1 mm).</td>
</tr>
<tr>
<td>4</td>
<td>Same as No. 3, but material covers more than 1 mm of tooth surface and less than one-third of the tooth.</td>
</tr>
<tr>
<td>5</td>
<td>Same as No. 3, but material covers more than one-third of the tooth.</td>
</tr>
</tbody>
</table>
2.8.3 Laboratory assessment

These methods can be divided into two main categories:

2.8.3.1 Molecular method

Monitoring denture hygiene by measuring the rate of oxygen consumption is a method invented by Cardash et al. (142). According to Cardash et al., simple and rapid test for measuring oral hygiene can be done by measuring the rate of oxygen consumption of oral expectorates of milk. The denture is immersed in 10 ml of sterile milk and agitated for 2 minutes. 3 ml of the previous milk was added to test tubes containing methylene blue. The time required for colour change at the bottom of the test tube, which is indicative of the rate of oxygen consumption, is recorded. The recorded time is correlated with the amount of plaque on dentures.

2.8.3.2 Conventional microbiological method

Taking swabs from the surface of the RPD is a well-known laboratory method to isolate, identify and quantify the microorganisms associated with RPD. Gram-positive bacteria, gram negative bacteria, anaerobic bacteria, aerobic bacteria, and yeast can be detected by this method (45, 68, 120, 165-171).

2.8.4 Planimetric assessment

In this method, an image or images are captured of the RPD in standard conditions. The images are processed in one of these methods:

2.8.4.1 Paper-weighing quantification method

The areas of interest (covered with biofilm) are outlined using a lead pencil or other colouring method. Then, the areas of interest are cut using scissors and the resulting shapes are weighed using a precision scale (139).
Silva et al. projected the obtained slides (photos) on paper (10 X amplification) and the total and stained surfaces were outlined with graphite, cut off and weighed (149).

2.8.4.2 Digital Planimeter method

A digital Planimeter is a measuring instrument used to determine (measure the size) the area of an arbitrary two-dimensional shape. In this method, the figures representing the areas (parts of the total image which is covered with plaque) are measured using a digital Planimeter (139, 172-174), figure 8.

**Figure 8** Digital Planimeter (Placom KP 92N, Tokyo, Japan).

2.8.4.3 Point-counting method

A commonly-used method of estimating the area of a region on a map or image is the dot-grid method (175). Transparent sheet with points (+) on it is placed on the image and the number of points on the targeted area are counted (139, 172-174), figure 9.

**Figure 9** Transparent sheet with points (+) on it is placed on an image.
2.8.4.4 Square-counting method / Square-grid system

In this method, the photo of fit surface of complete denture is projected against a screen with over 100 squares. The presence of plaque was recorded per squares of the grid system (140).

2.8.4.5 Computerized methods

A software like, Adobe Photoshop (157), ImageTool 2.02 (Texas, USA) (151), Scion Image (141) or Optimas 6.51 image analysis software (Media Cybernetics, Silver Spring, MD, USA) (176) were used to measure the covered parts with plaque or the total recognized surface of the RPD.

One of the first computerized methods was computer-graphic analysing system developed in Japan by Minagi et al. 1987. The method was applied on CDs. The system consist of a camera and a microcomputer. Minagi et al. did not describe how they used the system, but they stated that this system showed high reproducibility (146).

Paranhos and Silva (139) tried to compare between the previous planimetric methods (except the square-counting method) and founded that all the four quantitative methods were efficient for measuring quantity of plaque in complete dentures, and may be useful in experimental studies. They also found that, the computerized method with ImageTool software is fast and easy to perform in complete dentures.

Shimura et al. used the computerized method with Adobe Photoshop to assess the relation between the shape of the retainers and the plaque formation on abutment teeth. In their study, they tested the reliability of the of standardized photography, but they did not test the reliability of plaque assessment. They mentioned, that they had repeated pixels counting three different times with three different pictures and the average pixels were calculated (177).
Coulthwaite et al. used two softwares to assess plaque on CD; Adobe Photoshop and Scion Image. They did not describe how they used the softwares and they did not mention that they have tested the reproducibility of their method to assess plaque on CD (141).

Salles et al. used Adobe Photoshop 5.5 and Image Tool 2.0 software (UTHSCSA—University of Texas Health Science Center, San Antonio, TX, USA). They also did not test the reproducibility of their method to assess plaque on CD (178).

Fernandes et al. used Adobe Photoshop 5.5 software and new version (3.0) of Image Tool software to compare the efficacy of three denture brushes on biofilm removal. They also did not test their denture plaque assessment method.

Coulthwaite and Verran founded that computerized method is more representative, sensitive and less subjective than the visual plaque assessment (157).

2.9 Adobe Photoshop

Adobe Photoshop is a worldwide used tool for editing, annotating, enhancing and analysing digital images (179). Digital images play a key role in the work of medical professionals, whether in a diagnostic workflow, communication of findings, or patient education.

Physicians, dentists, and other medical professionals can strengthen communications with patients by presenting procedures alongside before-and-after images, and share findings with peers. Scientists can optimize images for both visual appreciation and further automated analysis. For example, dermatologists could use the Count tool to show skin changes by quantifying and tracking skin changing (wrinkles/texture, ptosis, vascular disorders, and pigmentation disorders) over time (180, 181).
Previous researchers used Adobe Photoshop to assess biofilm, dental plaque and denture plaque (143, 157, 176, 182-184). When Adobe Photoshop® is used properly with good digital image, it can provide detailed colour data in a relatively short period of time, and create a customized colour profile for each image (185). Above all, Adobe Photoshop allows quantitative and qualitative analysis of the data in digital image (186). Measurement, counting, and other analysis tools in Photoshop facilitate performing qualitative and quantitative analysis. Therefore, it was the chosen software in this study.
Aims and Objectives

3. **Aims and Objectives**

3.1 First objective

The first aim of this study was to evaluate reliability and agreement of a new computerized planimetric method for measurement and assessment of plaque on all types of RPDs.

3.2 Second objective

The second was to test the previous instrument (computerized planimetric method) by evaluating the efficacy of the rotating needles device in cleaning specific parts of RPDs:

- the vestibular flange and the occlusal and vestibular surfaces of the artificial teeth (base (B-O)),
- and the veneer (facing) of the secondary crown in double crown retained removable denture.
4. Materials and Methods

4.1 Participants (study population) and the inclusion/exclusion criteria

Our target population was patients with RDPs who were treated in the dental school of Greifswald University. All of the participants were patients who had received and adapted to their RDPs for at least two months. Additionally, the participants did not have any intraoral or extraoral pathologic findings or mental disorders. The study was approved by the medical ethics committee (RegNo: BB141/10). Participants gave their written consent to all study procedures. Forty-nine participants (29 female) with 65 RDPs were included, aged 32–88 years with a mean age of 62.

4.2 Standard Operating Procedure (SOP)

- **Preparing the RDP for photography:** After an RDP was removed from the mouth, it was rinsed under running water for 5 to 10 seconds (according to the size of the RDP) to remove any loose food particles (without brushing). Next, the RDP was dried with jets of air for a further 5 to 10 seconds. Five percent erythrosine was applied on all RDP surfaces and was left for 1 minute. Finally, the RDP was rinsed under running water to remove the unbound dye for 5 to 10 seconds and dried with jets of air for 5 to 10 seconds.

- **Photography:** For every RDP, a plaster plate with an individual silicon key was fabricated to fix the RDP. The silicon key was used to secure the RDP on the plate in the same position and angle before taking the images. The plate was fixed with a magnet on a movable base, which can be tilted forward, backwards, left and right at ±45. A Canon EOS/450D (Canon Inc. Japan) with an objective MR-14EX Macro Canon ultrasonic 60 mm f/2.8 and Ring flash (MR-14EX TTL Macro Lite Flash) were used. The camera’s manual settings were: ISO: 100, exposure: 200, aperture: 22, and white balance: flash. The camera was held with an adjustable reprostand; Hama Reprostativ RS 20, figure 12.
Materials and Methods

Six images were taken for every RDP under the same light conditions: front, back, left, right, occlusion and base (intaglio surfaces/tissue side). The first 4 images (front, back, right and left) were taken at a 45-degree angle, and the last 2 images were taken at a 90-degree angle.

**Figure 10** The plaster plate and the movable base.

![Figure 10](image)

**Figure 11** The movable base was tilted 45° to the left to make a photo of the lingual surface of the right side and the buccal surface of the left side.

![Figure 11](image)

*Cleaning the RDP:* After taking the photos, every RDP was cleaned with the rotating needles device (Sympro - Renfert GmbH) in Universal Sympro fluid for 20 minutes and 1.600 rotational speed (RPM) according to the company instructions.
Materials and Methods

- Rephotography: After picking the RDP from the device, it was rinsed under running water for 10 seconds to remove the cleaner (Universal Sympro fluid). Subsequently, the RDP was dried with jets of air for 5 to 10 seconds. Plaque disclosing agent (5% Erythrosine) was applied on all RDP surfaces and left for 1 minute. Then, the RDP was rinsed under running water to remove the unbound dye for 5 to 10 seconds and dried with jets of air for 5 to 10 seconds. Finally the RDP was reattached on the silicon key and the 6 photos were retaken in the same positions, angles and light condition.

Figure 12 The camera, the reprostand, and the RD is placed on the movable base.
Materials and Methods

Three hundred and ninety (65x6) images were taken before the rotating needles device, and 390 images were taken after. From this database (780 images), 55 images were randomly selected for image analysis.

- **Image analysis**: Adobe Photoshop Version:CS5 EXTENDED 12.0X32 was used for Photo analysis, according to this SOP, which was given to all examiners:

  **A. Browsing folders and opening photos**

  Clicking the File tab will open the computer's drives and folders, enabling the examiner to navigate the computer's folders to locate the image that he want to view, figure 13.

  ![Opening the targeted photo.](image)

  **Figure 13** Opening the targeted photo.

  **B. Duplicate layer (your photo)**

  In the Layers panel, you could see your photo as a layer.

  Select your layer (photo) in the Layers panel.

  Do one of the following

  - Drag the layer to the *Create a New Layer button*, figure 14.
Materials and Methods

- Choose the layer you want to Duplicate (your photo) → click the right button of the mouse → choose duplicate layer, figure 14.

Figure 14 How to duplicate a layer.

C. Selection

In this step, the examiner isolates one or more parts of the image, by selecting specific areas. In the image, we could see the metal, acrylic veneers on double crowns, acrylic resin bases and artificial teeth. We select only the acrylic veneers and acrylic resin bases with artificial teeth using the Magnetic Lasso tool. The Magnetic Lasso tool is one of three lasso tools available in Photoshop: the simple Lasso tool, the Polygonal Lasso and the Magnetic Lasso. The Magnetic Lasso tool can be found as the third icon from the top in the toolbox, figure 15. When the Magnetic Lasso tool is used, the border snaps to the edges of defined areas in the image. It is especially useful for quickly selecting objects with complex edges set against contrast backgrounds.
Materials and Methods

**Figure 15** The Magnetic Lasso tool.

After choosing the Magnetic Lasso tool, the features in the options bar should be set as demonstrated in figure 16.

**Figure 16** The values in the options bar of the Magnetic Lasso tool.

How do we use the Magnetic Lasso tool? Click the edge of the targeted part to set the first fastening point. Fastening points anchor the selection border in place. Release the mouse button and then move the pointer along the edge you want to trace. As you move the pointer, the active segment snaps to the strongest edge in the image, based on the detection width set in the options bar. Periodically, the Magnetic Lasso tool adds fastening points to the selection border to anchor previous segments. If the border does not snap to the desired edge, click once to add a fastening point manually. Continue to trace the edge, and add fastening
Materials and Methods

points as needed. To erase recently drawn segments and fastening points, press the Delete Key until you’ve erased the fastening points for the desired segment. Close the border manually with a magnetic segment, by dragging over the starting point and clicking. After you close the border copy, paste your selection to create a new layer.

D. Select a color range (plaque selection)

Colour Range selection can be used to create selections that are based on similar colour values. Opening the Colour Range panel can be achieved by clicking Select → Colour Range, figure 17. In the colour range window, the Sampled colours tool from the Select menu should be chosen, and both the Localised Colour Clusters box and the invert option box are unchecked, see figure 8.

**Figure 17** Opening the Colour Range panel.
The Fuzziness value has a major effect on the colour range selection; therefore, **the Fuzziness slider should not be changed**, and should remain the same in all images. In this study, the Fuzziness was 10. The first selection starts with the darkest red part of the image. Then the plus eyedropper selects lighter red to expand the colour range until all needed spots (plaque) in the layer are included, figure 18. If there are spots or areas that should not be included in the selection, the minus eyedropper is used.

Assessing the selection is accomplished by choosing White and Black Matte in the Selection Preview menu, figure 19.

After reaching the optimal selection, OK is clicked, and subsequently a new layer is created by the copy and paste.

In some rare cases, the Localised Colour Clusters (in the colour range box) is used to avoid selecting unwanted areas in the image. This will increase the number of the needed selection, but it will make the selection more satisfactory.
Materials and Methods

Figure 19  White Matte to evaluate the selected areas.

Exceptionally, the eraser tool is used to remove unwanted selected areas. Pencil Mode and Opacity of 100% in the options bar are selected during this step. The examiner makes 2 colour selections to control his work. If he is not satisfied, he should make a third and last selection. The mean of these two or three selections is considered the final result.

E. Saving, closing and then reopening

Saving the work by clicking (File → Save as), this will save all the work as a PSD file. Finally, reopen the PSD file to record the number of pixels. Closing and then reopening the PSD file give the software the ability to determine the correct number of pixels.
Materials and Methods

F. Counting the total number of pixels in the selected layer

This can be accomplished by opening the histogram panel. Choose Window → Histogram, and then click the Histogram menu and check both Expanded View and Show Statistics, figure 20.

**Figure 20** The choices in the Histogram panel.

**Figure 21** The number of pixels in the selected layer.

In the *Histogram panel*, these two choices should be made:
- In channel, select the (colours).
- In source, select the (selected layer).

In the *Histogram panel*, the number of pixels is presented.

The percent of plaque (POP) on the targeted part of the RDP is calculated using the following formula:

\[
POP \text{ on the targeted part of RDP} = \frac{\text{Pixels of Plaque}}{\text{Pixels of the targeted part}} \times 100
\]

If the targeted part of the RDP is divided over more than one image, the below formula is applied:

\[
POP \text{ on the targeted parts of RDP} = \frac{\sum \text{Pixels of Plaque [image 1+2+3+…]}}{\sum \text{Pixels of the targeted part [image 1+2+3+…]}} \times 100
\]
4.3 Examiners

The image analysis for each image was obtained by the main examiner 2 times in different sessions, one week apart and 1 time by 3 other examiners over a period of two weeks each. All examiners were students in Greifswald University and were previously trained in the use of Photoshop software.

4.4 Randomisation and blinding

From the database (780 images), 55 images were randomly selected for image analysis.

To ensure examiner blinding, the images were identified by codes in random order and analysed anonymously. One, two or three layers were created after the third step of photo analysis, which is isolating the targeted parts of the photo before making colour selection (plaque selection). Every layer was considered a single photo. A total of 111 images (layers) underwent plaque selection to evaluate the reliability and agreement of the CPM.

4.5 Statistical analysis

4.5.1 Study’s first objective

The first aim of this study was to evaluate the reliability and agreement of a new computerized planimetric method for measurement and assessment of plaque on RPD.

Descriptive statistics were calculated for all examiners to show the dispersion of the image analysis results. The normality of the data was checked by the Shapiro Wilk test, Q-Q-plots and histograms.

Following the recommendation by Shrout & Fleiss, the ICC(2,1) was calculated for both the intra- and inter-examiner (rater) reliability, which considers both examiners and images as random factors (187). The inter-examiner reliability
Materials and Methods

ICC$_{(2,1)}$ was calculated by comparing the main examiner mean with the results of the other three examiners. The ICC$_{(2,1)}$ was used to assess whether the standardised image analysis can be effectively used by a variety of examiners (187).

Three parameters were used to estimate agreement: the standard error of measurement (SEM$_{agreement}$), the smallest detectable change at the 95% confidence level (SDC$_{95\%}$), and the limits of agreement at the 95% confidence level (LoA$_{95\%}$). The variance components needed for calculating the SEM$_{agreement}$ were estimated using the restricted maximum likelihood method. De Vet et al. recommendations were taken into consideration for calculating both the SEM$_{agreement}$ and SDC$_{95\%}$ (188).

The Bland and Altman method (analysis of differences) is one of the best methods for quantifying agreement between the main examiner and each examiner by constructing limits of agreement on a visual aid (plot). The resulting visual aid is an XY plot, in which the Y axis shows the difference between the main examiner and the other examiner, and the X axis represents the average of their measures. In other words, the difference is plotted against the mean of the two examiners. The Bland and Altman method was used to determine the systematic error between the main examiner and each examiner, to investigate the existence of any systematic difference between the measurements (i.e., fixed bias) and to calculate the LoA$_{95\%}$ (189). This formula was used to calculate LoA$_{95\%}$:

Lower/Upper limits of agreement = mean difference ± (1.96+ standard deviation SD).

4.5.2 Study’s second objective

The second aim of this study was to test the previous instrument (computerized planimetric method) by evaluating the efficacy of the rotating needles device in cleaning specific parts of RPDs.
Materials and Methods

In the database, only 34 subjects were found with double crown retained removable denture and veneer over the secondary crown. Our statistical level of analyses was the subject. In other words, each subject was considered as a statistical unit, and if the subject has two RDPs, only one was randomly selected. Finally, 49x2 (before x after) images for 34 RDPs went under image analyses to calculate POP before and after cleaning the RDPs with the rotating needle device in Universal Sympro fluid for 20 minutes and 1.600 RPM.

Descriptive statistics were calculated to show dispersion of the image analysis results. The normality of the data was checked by the Shapiroe Wilk test, Q-Q-plots and histograms.

The POP data were statically analysed using nonparametric Wilcoxon signed-rank test because the data was not normally distributed. The test was applied to compare between plaque accumulation on base\(_{(B-O)}\) and veneer before and after using the rotating needle device.

Wilcoxon signed-rank test was used to estimate the efficacy of the device on both base\(_{(B-O)}\) and veneer. In this test we have used the pixels of plaque before and after, instead of using the POP. This was accomplished after controlling the Pixels of the targeted part in the photo before and the photo after. The values should be approximately stable in both photos. If not, this means there is no standardization in taking the photos or the selection of the targeted part has a low reliability. The Pixels of the targeted part in both photos was statistically tested by ICC(2,1).

The third and last question in this part of the thesis was comparing the efficacy of the rotating needle device on both parts of the RDP by applying the formula:

\[
Relative \, plaque \, removal \, (RPR) = \frac{\text{Pixels of Plaque before} - \text{Pixels of Plaque after}}{\text{Pixels of Plaque before}} \times 100
\]
Materials and Methods

Figure 22  Basic steps in the image analysis for the second objective:

A. Selecting the targeted part; \( A_1 \) the veneer and \( A_2 \) the base (B-O),

B. Plaque selection; \( B_1 \) selecting the plaque on the veneer and \( B_2 \) selecting the plaque on the base (B-O).

This was also accomplished after controlling the Pixels of the targeted part in the photo before and the photo after. The formula above can be used if we have an ICC value for the Pixels of the targeted part before/after >0.9.

P value below 5% were accepted as statistically significant. The statistical analyses were performed using SPSS 23.0 (SPSS, Inc, Chicago, Illinois, United States).
Results

5. Results

5.1 Results of study’s first objective

The first aim of this study was to evaluate the reliability and agreement of a new computerized planimetric method for measurement and assessment of plaque on RPD. 49 participants (29 f, 20 m) were included, having 65 RDPs, age between 32–88 years with a mean age 62. 6x2 images were taken for every RDP - before and after using the rotating needles device - under the same light conditions: front, back, left, right, occlusion and fit surfaces. Finally a database of 780 images was created. 55 photos were randomly selected for photo analysis. To ensure examiner blinding, the 55 images were identified by codes and analysed anonymously.

Image analyses were obtained by the main examiner 2 times in different sessions, one week apart and 1 time by 3 other examiners over a period of two weeks each. One, two or three layers were created after the third step of photo analysis, which is isolating the targeted parts of the photo before making colour / plaque selection. Every layer was considered a single photo. At the end, 111 photos (layers) went under colour / plaque selection.

Descriptive statistics were calculated for all examiners to show dispersion of the photo analysis results. Normality of the data was checked with Shapiroe Wilk test, Q-Q-plots and histograms, see table 7 and figures 22,23,24 and 25. The significant in Shapiro-Wilk test, are lower than 0.05 (the typical alpha level), so we reject the null hypothesis that the data are not different from normal.
Table 7 Test of Normality.

<table>
<thead>
<tr>
<th></th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
</tr>
<tr>
<td>Main examiner *</td>
<td>0.975</td>
</tr>
<tr>
<td>Second examiner</td>
<td>0.970</td>
</tr>
<tr>
<td>Third examiner</td>
<td>0.951</td>
</tr>
<tr>
<td>Fourth examiner</td>
<td>0.952</td>
</tr>
</tbody>
</table>

* Normality test of the mean of the first and second readings of the main examiner.

Figure 23 Normal Q-Q plot and histogram of the main examiner (mean).
Results

Figure 24 Normal Q-Q plot and histogram of the second examiner results.

Figure 25 Normal Q-Q plot and histogram of the third examiner results.

Figure 26 Normal Q-Q plot and histogram of the fourth examiner results.
**Results**

Q-Q plot, histogram, skewness, and kurtosis indicate the data are not normally distributed and exhibits positive skewness, see tables 8 and 9, and figures 22,23,24 and 25.

The median and range presented first in the descriptive statistics table because the median is more representative than the mean when the data are asymmetrically distributed (190), tables 8 and 9.

**Table 8** Descriptive statistics of image analysis for the main examiner in the first and second readings.

<table>
<thead>
<tr>
<th></th>
<th>1st reading</th>
<th>2nd reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid data</td>
<td>111</td>
<td>111</td>
</tr>
<tr>
<td>Missing data</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median</td>
<td>29.01</td>
<td>29.27</td>
</tr>
<tr>
<td>Range</td>
<td>85.48</td>
<td>69.06</td>
</tr>
<tr>
<td>Minimum</td>
<td>.99</td>
<td>.87</td>
</tr>
<tr>
<td>Maximum</td>
<td>86.47</td>
<td>69.92</td>
</tr>
<tr>
<td>Skewness</td>
<td>.517</td>
<td>.305</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>.08</td>
<td>-.71</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>30.40</strong></td>
<td><strong>29.97</strong></td>
</tr>
<tr>
<td><strong>Std. Deviation</strong></td>
<td><strong>17.21</strong></td>
<td><strong>16.82</strong></td>
</tr>
</tbody>
</table>

The first step of image analysis is the selection; the examiner isolated one or more parts of the image. Both the intra-examiner and inter-examiner reliability had excellent ICC (1.00 to 0.98). In the last step of image analysis (counting pixels and calculating the POP), both the intra-examiner and inter-examiner reliability were also excellent, ICC >0.85 at 95% CI, table 10. According to Fleiss, ICC values above 0.75 may be taken to represent excellent reliability (191).
Results

Table 9 Descriptive statistics of photo analysis for all examiners.

<table>
<thead>
<tr>
<th></th>
<th>Main examiner</th>
<th>2nd examiner</th>
<th>3rd examiner</th>
<th>4th examiner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid data</td>
<td>111</td>
<td>111</td>
<td>111</td>
<td>111</td>
</tr>
<tr>
<td>Missing data</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median</td>
<td>29.38</td>
<td>28.63</td>
<td>27.72</td>
<td>27.09</td>
</tr>
<tr>
<td>Range</td>
<td>77.27</td>
<td>78.13</td>
<td>73.74</td>
<td>76.75</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.93</td>
<td>0.00</td>
<td>0.61</td>
<td>0.29</td>
</tr>
<tr>
<td>Maximum</td>
<td>78.20</td>
<td>78.13</td>
<td>74.35</td>
<td>77.04</td>
</tr>
<tr>
<td>Skewness</td>
<td>0.38</td>
<td>0.34</td>
<td>0.35</td>
<td>0.47</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>-0.39</td>
<td>-0.31</td>
<td>-0.66</td>
<td>-0.51</td>
</tr>
<tr>
<td>Mean</td>
<td>30.19</td>
<td>28.95</td>
<td>29.50</td>
<td>28.83</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>16.84</td>
<td>17.766</td>
<td>17.33</td>
<td>19.38</td>
</tr>
</tbody>
</table>

Table 10 Intra-examiner and inter-examiner reliability in the first and last step of image analysis.

<table>
<thead>
<tr>
<th></th>
<th>ICC*</th>
<th>95% Confidence level</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
</tr>
<tr>
<td>The first step of</td>
<td>Intra-examiner ICC</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>photo analysis**</td>
<td>Inter-examiner ICC</td>
<td>0.98</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>The last step of</td>
<td>Intra-examiner ICC</td>
<td>0.96</td>
<td>0.94</td>
<td>0.97</td>
</tr>
<tr>
<td>photo analysis***</td>
<td>Inter-examiner ICC</td>
<td>0.89</td>
<td>0.86</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* Intraclass correlation coefficients.
** Selection, the examiner isolates one or more parts of the image.
*** Colour / plaque selection, counting pixels and calculating the percent of plaque (POP) on the targeted part of the RDP.
Results

Three parameters were used to estimate the agreement. The first two parameters were Standard Error of Measurement SEMagreement and Smallest Detectable Change at the 95% confidence level SDCagreement95%, table 11.

Table 11 Intra-rater and inter-rater agreement values.

<table>
<thead>
<tr>
<th></th>
<th>SEMagreement*</th>
<th>SDCagreement95%**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-rater</td>
<td>3.52%</td>
<td>9.76%</td>
</tr>
<tr>
<td>Inter-rater</td>
<td>5.96%</td>
<td>16.51%</td>
</tr>
</tbody>
</table>

* SEMagreement Standard Error of Measurement agreement.
** SDCagreement95% Smallest Detectable Change at the 95% confidence level.

The third parameter was the limits of agreement at 95% confidence level according to Bland and Altman analysis. A Bland-Altman plot was used to assess the level of agreement between the main examiner and the other three examiners. The Bland and Altman method started with a one-sample t test of differences. The mean value of the difference is the estimated bias, and the standard deviation of the differences measures the random fluctuations around this mean (the estimated bias), table 12. In the one-sample t test, the p-value was bigger than 0.05. This indicates that the mean value of the difference does not differ significantly from 0. In other words, there is no presence of fixed bias. After calculating the LoA95%, a scatter plot was constructed to reveal the agreement between the main examiner and each examiner, figures 26-28.
Results

Table 12 one-sample t test of differences between main and each examiner.

<table>
<thead>
<tr>
<th></th>
<th>t **</th>
<th>df ***</th>
<th>p****</th>
<th>Estimated Bias*****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diff. main vs. second *</td>
<td>1.807</td>
<td>110</td>
<td>0.073</td>
<td>1.232</td>
</tr>
<tr>
<td>Diff. main vs. third</td>
<td>1.405</td>
<td>110</td>
<td>0.163</td>
<td>0.683</td>
</tr>
<tr>
<td>Diff. main vs. fourth</td>
<td>1.747</td>
<td>110</td>
<td>0.083</td>
<td>1.355</td>
</tr>
</tbody>
</table>

* Differences between main and second examiner.
** Test statistic t
*** Degrees of Freedom.
**** Significance level P (2-tailed).
***** Mean value of the difference

Figure 27 Bland-Altman Plot for main examiner vs. second examiner.

* $d_{\text{mean}}$ (estimated bias), represents the mean difference between main examiner and second examiner.
** LoA(95%), limits of agreement at 95% confidence level.
Results

**Figure 28** Bland-Altman Plot for main examiner vs. third examiner.

**Figure 29** Bland-Altman Plot for main examiner vs. fourth examiner.
5.2 Results of study’s second objective

The second objective of the study was to evaluate the efficacy of the rotating needles device in cleaning specific parts of RPD; the base_{B-O} and the veneer. All images in the database for double crown retained removable denture with veneer over the secondary crown were selected. 49x2 (before x after) images for 34 patents (16 men) wearing 34 RDPs (17 upper jaw) went under image analyses. All RDPs are ≥ 3 years old.

**Table 13** Descriptive statistics of image analysis before and after cleaning the RDPs with rotating needles device.

<table>
<thead>
<tr>
<th>Targeted part of RDP</th>
<th>N</th>
<th>Median</th>
<th>Mean</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>***** SD</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>**** P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base_{B-O} Before</td>
<td>34</td>
<td>4507.25</td>
<td>5371.18</td>
<td>4390.35</td>
<td>6352.01</td>
<td>1.14</td>
<td>0.7</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Veneer Before</td>
<td>34</td>
<td>1291.25</td>
<td>1382.03</td>
<td>1157.15</td>
<td>1606.91</td>
<td>0.29</td>
<td>-1.05</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Base_{B-O} After</td>
<td>34</td>
<td>1845.75</td>
<td>2188.25</td>
<td>1720.06</td>
<td>2656.44</td>
<td>0.68</td>
<td>-0.47</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Veneer After</td>
<td>34</td>
<td>830.25</td>
<td>897.49</td>
<td>746.72</td>
<td>1048.25</td>
<td>0.57</td>
<td>-0.17</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>POP Before</td>
<td>34</td>
<td>34.86</td>
<td>39.47</td>
<td>33.59</td>
<td>45.35</td>
<td>0.59</td>
<td>-0.61</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Base_{B-O} POP</td>
<td>34</td>
<td>13.49</td>
<td>17.75</td>
<td>12.95</td>
<td>22.54</td>
<td>2.14</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veneer POP</td>
<td>34</td>
<td>35.70</td>
<td>40.46</td>
<td>32.96</td>
<td>47.96</td>
<td>21.50</td>
<td>0.65</td>
<td>-0.80</td>
<td></td>
</tr>
</tbody>
</table>

* Pixels (number of pixels) of plaque.
** POP; Percent Of Plaque
*** CI; Confidence Interval
**** SD; Standard Deviation
***** P-value in Shapiro-Wilk test

If the P-value in Shapiro-Wilk test is bigger than 0.05, we will accept the null hypothesis Ho that the data are not different from normal. In other words, if the P-value of the Shapiro-Wilk test is greater than 0.05, the data is normal. Normal Q-Q plot and histogram should not show a distinguish violation of normality, table 13 and figures 30, 32 and 33. On the other hand, if the P-value in Shapiro-Wilk test is lower than 0.05, we reject the null hypothesis Ho that the data is not
Results

different from normal. Normal Q-Q plot and histogram should confirm this finding by indicating departures from normality, table 13 and figures 30, 31, 29, 31, 34, 35 and 36.

**Figure 30** Normal Q-Q plot and histogram of the pixels (number of pixels) of plaque on the Base\_B-O before cleaning the RDPs with the rotating needles device.

**Figure 31** Normal Q-Q plot and histogram of the pixels of plaque on the Veneer before cleaning the RDPs with the rotating needles device.
Results

**Figure 32** Normal Q-Q plot and histogram of the pixels of plaque on the Base\(\text{B-O}\) after cleaning the RDPs with the rotating needles device.

**Figure 33** Normal Q-Q plot and histogram of the pixels of plaque on the Veneer after cleaning the RDPs with the rotating needles device.
Results

**Figure 34** Normal Q-Q plot and histogram of POP on the base \( (B-O) \) before cleaning the RDPs with the rotating needles device.

**Figure 35** Normal Q-Q plot and histogram of POP on the veneer before cleaning the RDPs with the rotating needles device.
Results

**Figure 36** Normal Q-Q plot and histogram of POP on the base \( (B-O) \) after cleaning the RDPs with the rotating needles device.

**Figure 37** Normal Q-Q plot and histogram of POP on the veneer after cleaning the RDPs with the rotating needles device.
Results

Testing normality helped in choosing the most appropriate statistical test for every data set in the study. For example, paired sample t-test will be used if the data is normally distributed. On the other hand, nonparametric tests like Wilcoxon signed-rank test should be chosen if the data set showed a violation of normality.

Before starting data analyses, we have checked the number of pixels of the targeted part in the image before and the image after. Because the targeted part should be approximately stable in both photos. This was done for both base\textsubscript{(B-O)} and veneer.

Table 14 Intraclass correlation coefficients for testing the reliability in the image before and after.

<table>
<thead>
<tr>
<th></th>
<th>ICC*</th>
<th>95% Confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound Upper Bound</td>
</tr>
<tr>
<td>Base\textsubscript{(B-O)}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Measures</td>
<td>0.995</td>
<td>0.991 0.998</td>
</tr>
<tr>
<td>Average Measures</td>
<td>0.998</td>
<td>0.995 0.999</td>
</tr>
<tr>
<td>Veneer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Measures</td>
<td>0.998</td>
<td>0.997 0.999</td>
</tr>
<tr>
<td>Average Measures</td>
<td>0.999</td>
<td>0.998 1.000</td>
</tr>
</tbody>
</table>

ICC > 0.99 represent a very high level of standardization in both taking the images and selecting the targeted part with Photoshop.
Results

Table 15  Results of Wilcoxon signed-rank test for the pairs:
- Base<sub>(B-O)</sub> / Pixels (number of pixels) of plaque on the Base<sub>(B-O)</sub> before / Pixels of plaque on the Base<sub>(B-O)</sub> after,
- Veneer / Pixels of plaque on the Veneer before / Pixels of plaque on the Veneer after.

<table>
<thead>
<tr>
<th>Ranks</th>
<th>Negative Ranks</th>
<th>Positive Ranks</th>
<th>Ties</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Mean Rank</td>
<td>17.50</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Sum of Ranks</td>
<td>595.00</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>N</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Mean Rank</td>
<td>17.50</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Sum of Ranks</td>
<td>595.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wilcoxon test</th>
<th>Z</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-5.086&lt;sup&gt;g&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a  Pixels of plaque - Base<sub>(B-O)</sub> after < Pixels of plaque - Base<sub>(B-O)</sub> before
b  Pixels of plaque - Base<sub>(B-O)</sub> after > Pixels of plaque - Base<sub>(B-O)</sub> before
c  Pixels of plaque - Base<sub>(B-O)</sub> after = Pixels of plaque - Base<sub>(B-O)</sub> before
d  Pixels of plaque - Veneer after < Pixels of plaque - Veneer before
e  Pixels of plaque - Veneer after > Pixels of plaque - Veneer before
f  Pixels of plaque - Veneer after = Pixels of plaque - Veneer before
g  Based on positive ranks.
h  Wilcoxon signed-rank test significance.

The rotating needles device was effective in cleaning the veneers because there was a significant decrease between POP on the veneer before cleaning (Median =1291.25 pixel / Mean=1382.03 pixel) and POP on the veneer after using the rotating needles device (Median=830.25 pixel / Mean=897.49 pixel), Z = -5.09, p < 0.001. The device was also effective in cleaning the base<sub>(B-O)</sub>, Z = -5.09, P <0.001,
Results

Median before=4507.25 pixel, after 1845.75 pixel, and Mean before=5371.18, after=2188.25 pixel, Tables 13 and 15.

Table 16 Results of Wilcoxon signed-rank test for the pairs:
- Before / POP on the Base\(_{(B-O)}\) before / POP on the Veneer before,
- After / POP on the Base\(_{(B-O)}\) after / POP on the Veneer after.

<table>
<thead>
<tr>
<th>Ranks</th>
<th>Before Base(_{(B-O)}) / Veneer</th>
<th>After Base(_{(B-O)}) / Veneer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean Rank</td>
</tr>
<tr>
<td>Negative Ranks</td>
<td>8(_{a})</td>
<td>9.50</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>26(_{b})</td>
<td>19.96</td>
</tr>
<tr>
<td>Ties</td>
<td>0(_{c})</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Wilcoxon test</td>
<td></td>
<td>Z = -3.787(_{g})</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>h</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(a\) POP - Veneer before < POP - Base\(_{(B-O)}\) before
\(b\) POP - Veneer before > POP - Base\(_{(B-O)}\) before
\(c\) POP - Veneer before = POP - Base\(_{(B-O)}\) before
\(d\) POP - Veneer after < POP - Base\(_{(B-O)}\) after
\(e\) POP - Veneer after > POP - Base\(_{(B-O)}\) after
\(f\) POP - Veneer after = POP - Base\(_{(B-O)}\) after
\(g\) Based on negative ranks.
\(h\) Wilcoxon signed-rank test significance.

Wilcoxon signed-rank test showed that POP is significantly more on the veneer (median =50.89) than on the Base\(_{(B-O)}\) (median = 34.86) before cleaning the RDP.
Results

(Z = -2.72, p < 0.001). The POP is also significantly more on the veneer than on the base \( B-O \) after cleaning, \( p < 0.001 \), Table 16. The median and mean values (before/after) of POP support this result, Table 13.

Assessing the difference in the device efficacy on the base \( B-O \) and veneers was conducted by using this formula which put into consideration the amount of plaque before treatment:

\[
\text{Relative plaque removal (RPR)} = \frac{\text{Pixels of Plaque before} - \text{Pixels of Plaque after}}{\text{Pixels of Plaque before}} \times 100
\]

Because there is a statistically significant difference in plaque accumulation between the base \( B-O \) and veneer before treatment we could not use Mann-Whitney U test to compare the efficacy of the device on both parts of the RDP. As alternative we have used the RPR formula after controlling the Pixels of the targeted part in the photo before and the photo after. The formula above can be used because the pixels of the targeted part in both images (before-after) have excellent reliability , ICC > 0.99, table 14. Wilcoxon signed-rank test proved that there is a significant difference, and the RPR for the base \( B-O \) (negative ranks =30) is more than the veneer (Positive Ranks = 4), table 17. In other words, the rotating needles device is more effective in cleaning the base \( B-O \) than the veneer.
Results

Table 17  Results of Wilcoxon signed-rank test for testing device efficacy on both parts of RDP; pair:

- Relative plaque removal (RPR) in the plaque (pixels) on the Base\(_{(B-O)}\) before and after cleaning / RPR in the plaque (pixels) on the Veneer before and after cleaning.

<table>
<thead>
<tr>
<th>Ranks</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Ranks</td>
<td>30 a</td>
<td>18.53</td>
<td>556.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>4 b</td>
<td>9.75</td>
<td>39.00</td>
</tr>
<tr>
<td>Ties</td>
<td>0 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilcoxon test</td>
<td>Z</td>
<td>-4.419 d</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>&lt;0.00 e</td>
<td></td>
</tr>
</tbody>
</table>

a  RPR - Veneer < RPR - Base\(_{(B-O)}\).
b  RPR - Veneer > RPR - Base\(_{(B-O)}\).
c  RPR e - Veneer = RPR - Base\(_{(B-O)}\).
d  Based on positive ranks.
e  Wilcoxon signed-rank test significance.
6. Discussion

6.1 Discussion - study’s first objective

Although RDP plaque assessment and RDP cleansing (especially Denture) has been the aim of a very big number of studies over the years; unexpectedly, a very small number of studies was found to possess a moderate or high evidence-based level (106). Moreover, in a systemic review conducted by Souza et al. 2009, which considered the above mentioned big number, surprisingly, none of those studies specifically aimed removable partial dentures (106).

RPD hygiene assessment is rarely carried out, because there is a need for method standardization in both RDP hygiene assessment and RDP hygiene management (111, 130-133). Several different methods for assessing denture hygiene have been suggested in the current literature, but until now none has been widely accepted, or considered the standard method of assessing RPD hygiene (130-133). In general, the methods of assessing RPD plaque can be divided into 3 categories:

- Visual assessment which is suitable for patient monitoring and motivation, and evaluating patients’ compliance with RDP hygiene instructions.
- Planimetric assessment which can be used in the field of scientific research.
- Laboratory assessment which is useful for diagnostic purposes or scientific research.

Because there is a lack of evidence about the efficacy of the different denture cleaning methods (192), we are aiming in this work to develop and test an instrument which could be considered a standard method for assessing plaque on all types of RDPs.

Many research used computerized methods to assess RDP plaque. Unfortunately, not a one mentioned in detail the Standard Operation Procedures (SOP) for
image analyses or proved that the used method is both valid or reliable. The increasing emphasis on evidence-based health care makes it very important to test the used method or instrument before discussing the data collected by them. Reliability and agreement are very important quality characteristics without both of them we could not trust the instrument.

This study showed that our SOP used in image analysis is a reliable and reproducible instrument. The camera reprostand, standard lighting condition, silicon key (to attach and re-attach the RDP) over a movable basis (which can be tilted and blocked in any angel ± 45 right, left, front, back) provide a high level of image standardization. Although some studies required 12 photos to cover all surfaces of complete denture (141), we found that 6 photos for every RDP is enough. Computerized image analysis -which is a type of planimetric plaque assessment- is applied on natural teeth and complete dentures in different ways and methods. For example, some studies used one software package, others used two software packages; the first software was always Adobe Photoshop. Using two software may lead to reader error or bias, and increase the time of image analyses. In general, all methods used two basic steps, selecting a part of the photo (tooth or part of RDP) then selecting plaque. The first step was done in most studies by pen tool or lasso tool (157, 195). In this study, we have used the magnetic lasso tool because it is a quick and easy way to obtain accurate selections and the recommended tool for the beginners (193). The second step was plaque selection. This step was done by one of three methods:

- by drawing or selection tool which highlight the areas covered with plaque (139, 155, 194). This method is very time consuming, especially if we have a big RDP with many small plaque islands.

- by image thresholding which adjusts the image by converting all colours and shades to either black or white and create a grayscale image with high-contrast.
Discussion

Although we believe that it is not the optimal method, it can be argued that image thresholding can be used with natural teeth, because there are two main colours, white (teeth) and red (disclosed plaque). Unfortunately, we cannot use this method with RDP because we have different degree of red in the image, which ranged between dark red (disclosed plaque) to red or light red (RDP base).

- by colour selection. Smith et al. had used this option in Photoshop to quantify plaque on natural teeth. And he set the fussiness at 100 (50 shades above and below the selected pixel) to automatically highlight the red disclosed plaque areas. This setting is not suitable in case of RDP because it will highlight both plaque and RDP base. In our study, colour-range option was used with a predetermined fuzziness setting of 10. Therefore the examiner selection is more precise as it contains a small range of colour. On the other hand, he should make more than one selection to cover all the targeted areas in the image, therefore it is more time consuming.

The Computerized Planimetric Method (CPM) used in this study demonstrated excellent intra-examiner and inter-examiner reliability. ICC values were $\geq 0.9$, suggesting sufficient reliability for making decisions (195). Similar high reliability was recorded in other studies. Smith et al. who used SOP closed to the one used in this study, reported high intra-examiner and inter-examiner reliability. They reported reliability (ICC) of quantifying plaque on the buccal front natural teeth between 0.82 to 0.99 (196), and on the lingual natural tooth surface 0.89 to 0.98 (183). However, Smith et al. method cannot be applied on RDP because he recommended high fuzziness. Our data showed intra-examiner ICC values higher than inter-examiner ICC values. That may be explained by a learning effect of the examiners. These results are similar to the findings from prior studies (183, 196, 197).
Discussion

To assess the effectiveness of a certain method in plaque removal from RDP or to monitor improvement of RDP hygiene in clinical practice, and epidemiological or clinical trial studies, the magnitude of the measurement error might be of more interest than the reliability parameters values such as ICC. Both SEM and SDC can help the clinician to determine if the patient truly complies with the recommendations and instructions, and aid the researcher in evaluating the cleaning method. Moreover, some researcher believe that SEM is a better measure of the quality of an assessment than is reliability (198). Because reliability alone can produce a paradoxical and distorted picture, particularly in the situation where a small number of subjects participate in the study. On the other hand, SEM is recommended to measure the quality of an assessment because it is not subject to such hitches (198). The SEM intra- and inter-rater values were respectively ≈ 3 – 6%. This means that the instrument has a low to acceptable level of measurement error which reflect a good quality of denture plaque assessment, because we are 95% sure of the value ±2x(3 or 6)%.

The SDC intra- and inter-rater values were respectively ≈ 10 – 16%. This means, we are 95% sure that a change in RDP plaque coverage exceed 10 - 16% of the examined area can be detected by our instrument. The decision, if this level of sensitivity is enough or not, is left to the user of the instrument. Until now, there are now other similar studies to compare with. However, it is important to mention that, Shaloub & Addy revealed that most examiners tended to overestimate plaque areas over natural teeth when they used visual assessment (index) (155). Paranhos et al. proved that Jeganathan et al. and Budtz-Jørgensen indices overestimated the plaque coverage area over denture, when compared to a computerised method (151).

It is most unlikely that different examiners will agree exactly, by giving the identical result for all images. Therefore, we have used Bland and Altman plot to
Discussion

know how every examiner is likely to differ from the main examiner. Bland and Altman proposed a method, which is frequently used to assess the limits of agreement between two examiners or two different methods (188, 189). In order to accurately estimate the limits of agreement between 2 examiners, a big sample size is required. According to Bland's recommendations, 100 subjects (photos) are adequate. In this study, 111 was the sample size to determine reproducibility of colour /plaque selection. The bias doesn’t exceed 1.4%, and the one-sample t test proved that there is no presence of fixed bias. Besides, a good agreement for most values can be easily recognized in the three plots, Fig. 4, 5 and 6. The outlier for the second, third and fourth examiner were 3, 7, and 4 respectively. Although the third examiner has the biggest number of outlier, it has the best level of agreement. Therefore, we can confirm that the results collected from the same images by different examiners have a high level of agreement.

6.2 Limitations

Image analyses was performed by standard examiner and 3 other students. Although they were in the final stage of their study, their experience in RDP plaque assessment and image analyses is obviously less compared to experienced clinician. As a consequence, they may have looked differently at the images, to decide what should or shouldn’t be selected.

The fact that the CPM has a limitation because it is completely relying on two dimensional images, cannot be ignored.
6.3 Discussion - study’s second objective

RDP plaque is considered an important reservoir of organisations which could induce local oral diseases such as denture stomatitis or papillary hyperplasia (77, 93).

Many recent studies demonstrated mutualistic interactions / relationships / associations between oral plaque/oral health/periodontitis and systemic diseases such as diabetes, tissue repair capacity, immune cell functions…….. (101, 102). Oral plaque microorganisms may disseminate through the blood to infect the vascular endothelium and contribute to the occurrence of atherosclerosis and risk of myocardial ischemia and infarction (103). Sumi et al. proved the high correlation between the bacterial species in denture plaque and pharyngeal microflora (104). Besides, denture plaque could serve as a resource for pathogens cause disseminated infections such as gastrointestinal infections (105).

The new studies enhance our understanding of the importance of oral health and its connection to the patients’ overall health and their intraoral and general diseases. Diseases which can be treated not only by targeting the putative pathogens, but also prevented with proper oral and RDP cleaning.

RDPs can be cleaned mechanically or chemically (106). Mechanical methods can be further subdivided into: manual (by hand) or machinal (ultrasonic device or rotating needles device). By literature review, it was noticed that there is no study tried to evaluate the efficacy of the rotating needles device.

We are aiming in the second object to test the computerized planimetric method – which was the first object of the theses - by evaluating the efficacy of the rotating needles device in cleaning specific parts of RPD:

- the vestibular flange and the occlusal and vestibular surfaces of the artificial teeth (base(B-O)).
Discussion

- and the veneer (facing) of the secondary crown in double crown retained removable denture.

It was noticed that POP is significantly more on the veneer than on the base (B-O) before and after cleaning the RDP. This result is supported by many other studies which proved that plaque accumulation is varied between different parts of the RDP. Fermandes et al. reported an intense biofilm accumulation, mainly at the site of union of the teeth with the denture base (182). Augsburger and Elahi study pointed out that convex and smooth surfaces present lower biofilm levels than those artistically sculptured and with sharp finishing (134). Tarbet, et al. in a comparative analysis of denture hygiene products, found significant differences between the internal and external surfaces (right and left buccal flanges). They stated that biofilm on the internal unpolished surface is significantly more than the external polished surface (199). The effect of surface polishing on plaque accumulation was tested by Keenan et al. who proved that plaque deposition rate decreases as surface polish level increases (200).

Resin-based composites are the preferable material for making the veneers (facings) on the secondary crowns of double crown retained dentures. The material possesses higher elastic modulus and proved to have a greater degree of safety in double crown retained dentures than ceramic (201). Unfortunately, resin-based composite display a number of flaws, like plaque retention, discoloration, relatively weak bond between the metal base and veneers, and low resistance to abrasion (202-206). This could explain the significant difference in POP between the veneers and base (B-O).

It was noticed in the literature review that some researcher had used the difference (value before minus value after) to compare between treatments (groups) (207, 208). Although we believe that it is not the optimal method, it can be argued that the previous approach can be used if there is no significant
Discussion

difference in the distribution of the analysed parameter between the groups before treatment. To give an example, why it is not advisable to use the raw difference to compare the efficacy of treatments between groups with different pre-treatment distributions, let’s say that we have these three pairs of group means (before-after); (60-30), (80-40), and (90-45). The raw differences are 30, 40, and 45, but the improvement after the treatment is always the same: 50%. Since the distribution of pixels of plaque before treatment was different on base\(_{(b-o)}\) and veneer, we used the relative plaque removal (RPR) formula. By doing so, we put into consideration the different amount of plaque before treatment. However, for using the RPR formula it must be ensured that the number of pixels on the targeted part of the RDP before and after treatment is approximately stable. This means a high level of standardization and reliability in both taking the images and selecting the targeted part with Photoshop software is needed.

In this part of the theses, the statistic level of analyses was the subject. If the subject has two RDPs only one was randomly selected. The statistic unit was not the Photoshop layer as in the first objective, or the image, or the RDP, because they are not independent variables, which is essential for many single-level statistical methods (209). Due to the clustering of layers within the same image, and images within the same RDP, and two RDPs within the same participant, a correlation may exist between variables in the same cluster. In other words, if the patient hygiene is good, both RDPs will have low values of POP and vice versa. Although, considering both RDPs would increase the sample size; this may distort the distribution in the same cluster and lead to biased estimates. To correctly analyse the data, the correlation in the same cluster was taken into account by randomly selecting one RDP if the subject had two.
The used parameter for evaluating the respective RND efficacy in cleaning the veneers, and in cleaning the base\(_{(B-O)}\), were the pixels and not the POP. But from a statistical point, using the POP would give exactly the same results as using the pixels because in Wilcoxon signed-rank tests (which is the non-parametric alternative to the paired t-test), for each pair of values, the relation of the respective values is considered, which is not affected by transforming both values in the same way (e.g. from pixels to POP if the number of pixels on the RDP before and after treatment is stable). Nevertheless, we believe that using the pixels would be more appropriate if the conditions for parametric tests are met. For example, in the paired t-test, the values themselves are considered, not their ranking. In other words, our study scenario may not be repeated in other studies if they have a normally distributed data. One assumption in many statistical tests is that the data are free to vary widely about the mean - there are no imposed limits. Clearly, this is not the case if we use POP, which cannot be less than 0 nor more than 100. However, in comparing plaque accumulation between base\(_{(B-O)}\) and veneer, the POP had to be used due to the difference in the size (and therefore also in the number of pixels) between the two parts of the RDP. The relatively small size of the veneer will produce a small number of pixels, Table 13. And using the POP in this case can quantify the plaque correctly.

Although we have 6 images for every RDP before and 6 after, we have used in the second objective of the theses only one or two images (right/left) before and the same number after. Selecting the most appropriate image for analyses was performed under two criteria: the most appropriate and clear image of the targeted part; secondly, no overlap of the targeted part between the images.
7. Conclusion

7.1 Conclusion - study’s first objective

The Computerized Planimetric Method (CPM) have been applied with increasing success in the fields of science and medicine because of its objectivity, reliability, and high level of standardisation.

Within the limitations of this study, it can be concluded that our SOP for image analysis has an excellent inter- and intra-examiner reproducibility, good level of examiners agreement, acceptable level of sensitivity, acceptable measurement error, and good quality of denture plaque assessment. Above all, it can be used with all types of RDPs.

The method is time consuming, therefore it is more suitable for clinical researches. The method required examiner trained by clinician to decide what is truly stained plaque, and also required image analysis training before mastering the method.

7.2 Conclusion - study’s second objective

Wherever possible, parametric models are preferable to non-parametric methods because of their higher statistical power. When doing so, answering the study question by using the number of pixels instead of POP is recommended if the number of pixels on the targeted part of the RDP does not differ between images before and after.

The results of this study indicate that plaque accumulation is significantly higher on the double crown veneer than on the base(B-O). Therefore, extra attention should be given to the veneer over the secondary crown as they are a potential part for RDP plaque accumulation.

The RND is significantly effective in cleaning the veneer and the base(B-O), but more effective in cleaning the base(B-O).
8. Summary

Objectives

The aim of the study was to develop and evaluate the reliability and agreement of new computer planimetric method for measurement and assessment of plaque on all types of removable dental prostheses RPDs.

The instrument (new method) was tested by evaluating the efficacy of the rotating needles device in cleaning specific parts of RPD

Materials and Methods

From a database containing 780 images, which were taken in a standardized method for 65 RDPs for 49 participants, 55 images were selected randomly for image analysis. Adobe Photoshop software was used according to a standard operating procedure (SOP) by a main examiner two times in different sessions, and 1 time by 3 other examiners. In order to estimate the intra- and inter-examiner reliability, intraclass correlation coefficients ICC\(_{(2,1)}\) was used. Three parameters were used to estimate agreement: standard error of measurement (SEM), smallest detectable change at 95% confidence level (SDC\(_{95}\%\)), and limits of agreement (LoA) according to Bland–Altman method.

In the database, only 34 subjects were found with double crown retained removable denture and veneer over the secondary crown. 49x2 (before x after) images for 34 RDPs went under image analyses to calculate POP before and after cleaning the RDPs with the rotating needle device. Data were analysed using Wilcoxon signed-rank test.

Results

In all steps of image analysis, both intra-examiner and inter-examiner reliability were excellent with ICC\(_{(2,1)}\) values > 0.85 at 95% confidence level. Intra- and inter-
examiner values for both, SEM and SDC_{95%} were ≤ 6% and ≤17% respectively. The Bland–Altman analysis revealed a satisfactory level of agreement.

POP is significantly more on the veneer than on the base_{(B-O)}, P <0.05. The rotating needles device is effective in cleaning the veneer and base_{(B-O)}, P <0.05 with absolute effect size 0.62. No statistical significance was detected in the effectiveness of the rotating needles device between base_{(B-O)} and veneer.

**Conclusion**

This study showed an excellent inter- and intra-examiner reproducibility, satisfactory level of examiners agreement, and acceptable measurement error of the new computer planimetric method. Furthermore, the method can be used with all types of RDPs.

The rotating needles device can significantly reduce plaque on the double crown retained removable denture.

**Clinical significance**

The Computerized Planimetric Method (CPM) is more suitable for clinical researches because of its objectivity, reliability, high level of standardization, and the ability to detect and quantify small changes in plaque.

Extra attention should be given to the veneer over the secondary crown as they are a potential part for RDP plaque accumulation.
9. References


References


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12 Curriculum Vitae (CV)

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2010 – present: Member in the staff of the Department of Prosthodontics, Greifswald University, Germany.

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2003: Postgraduate Diploma in removable prosthodontics. Dental school, Damascus University, Syria. Awarded with a very good grade.

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Curriculum Vitae

1999-2001  Resident in The Orthodontic Department at Abdulkader Mahloul Hospital.

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1999  License to practice the Profession of Dentistry in Syria as an oral surgeon.

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Acknowledgements

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Greifswald, May 2016
14 Declaration - Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät, keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Ethical Approval: The study was approved by the medical ethics committee of the University Medicine Greifswald (RegNo: BB141/10).

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Conflict of Interest: None declared.

Datum Unterschrift
15.1 Standard Operating Procedure (SOP)

Image analysis to assess plaque on removable dental prostheses

This SOP provides a step-by-step instructions to guide you easily through each task. It will show you how to perform each task correctly in Adobe Photoshop Version:CS5 EXTENDED 12.0X32

A. Browsing folders and opening images: Clicking the File tab will open the computer's drives and folders, enabling the examiner to navigate the computer's folders to locate the image that he want to view.

B. Duplicate layer (your image):
In the Layers panel, you can see your image as a layer.

Select your layer (image) in the Layers panel → click the right button of the mouse → choose duplicate layer.

C. Selection:
In this step, the examiner isolates one or more parts of the image, by selecting specific areas. In the image, we could see the metal, acrylic veneers on double crowns, acrylic resin bases and artificial teeth. We select only the acrylic veneers and acrylic resin bases with artificial teeth using the Magnetic Lasso tool. The Magnetic Lasso tool is one of three lasso tools available in Photoshop: the simple Lasso tool, the Polygonal Lasso and the Magnetic Lasso. The Magnetic Lasso tool can be found as the third icon from the top in the toolbox.

After choosing the Magnetic Lasso tool, the features in the options' bar should be set as demonstrated in Table 1.
How do we use the Magnetic Lasso tool? Click the edge of the targeted part to set the first fastening point. Fastening points anchor the selection border in place. Release the mouse button and then move the pointer along the edge you want to trace. As you move the pointer, the active segment snaps to the strongest edge in the image, based on the detection width set in the options bar. Periodically, the Magnetic Lasso tool adds fastening points to the selection border to anchor previous segments. If the border does not snap to the desired edge, click once to add a fastening point manually. Continue to trace the edge, and add fastening points as needed. To erase recently drawn segments and fastening points, press the Delete Key until you’ve erased the fastening points for the desired segment. Close the border manually with a magnetic segment, by dragging over the starting point and clicking. After you close the border copy, paste your selection to create a new layer.

Table 1 The values in the options bar of the Magnetic Lasso tool.

<table>
<thead>
<tr>
<th>Options</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feather</td>
<td>0 PX</td>
</tr>
<tr>
<td>Anti-alias</td>
<td>Do not click this option</td>
</tr>
<tr>
<td>Width</td>
<td>10 PX</td>
</tr>
<tr>
<td>Contrast</td>
<td>10</td>
</tr>
<tr>
<td>Frequency</td>
<td>50</td>
</tr>
<tr>
<td>Tablet Pressure</td>
<td>Do not click tablet pressure</td>
</tr>
<tr>
<td>Refine Edge</td>
<td>Do not refine edge</td>
</tr>
</tbody>
</table>
Appendix

D. Select a colour range (plaque selection):

Colour Range selection can be used to create selections that are based on similar colour values. Opening the Colour Range panel can be achieved by clicking Select → Colour Range. In the colour range window, the Sampled colours tool from the Select menu should be chosen, and both the Localised Colour Clusters box and the invert option box are unchecked.

The Fuzziness value has a major effect on the colour range selection; therefore, the **Fuzziness slider should not be changed**, and should remain the same in all images. In this study, the Fuzziness was 10. The first selection starts with the darkest red part of the image. Then the plus eyedropper selects lighter red to expand the colour range until all needed spots (plaque) in the layer are included. If there are spots or areas that should not be included in the selection, the minus eyedropper is used.

Assessing the selection is accomplished by choosing White and Black Matte in the Selection Preview menu.

After reaching the optimal selection, OK is clicked, and subsequently a new layer is created by the copy and paste, **Fig. 1.**

In some rare cases, the Localised Colour Clusters (in the colour range box) is used to avoid selecting unwanted areas in the image. This will increase the number of the needed selection, but it will make the selection more satisfactory.

Exceptionally, the eraser tool is used to remove unwanted selected areas. Pencil Mode and Opacity of 100% in the options bar are selected during this step.

The examiner makes 2 colour selections to control his work. If he is not satisfied, he should make a third and last selection. The mean of these two or three selections is considered the final result.
Fig. 1. Basic steps of the image analysis:

A. Combined attachment- and double crown-retained RDP.

B. Selection: The examiner isolates one or more parts of the image.

C. Plaque selection: By using the colour range selection the examiner can make selections based on the colour of the disclosed plaque.
Appendix

5. Saving, closing and then reopening:

Saving the work by clicking (File → Save as), this will save all the work as a **PSD file**. Finally, reopen the PSD file to record the number of pixels. Closing and then reopening the PSD file give the software the ability to determine the correct number of pixels.

6. Counting the total number of pixels in the selected layer:

This can be accomplished by opening the histogram panel. Choose Window → Histogram, and then click the Histogram menu and check both **Expanded View** and **Show Statistics**.

In the *Histogram panel*, these two choices should be made:

- In **channel**, select the (colours).
- In **source**, select the (selected layer).

In the *Histogram panel*, the number of pixels is presented.

The percent of plaque (POP) on the targeted part of the RDP is calculated using the following formula:

\[
\text{POP on the targeted part of RDP} = \frac{\text{Pixels of Plaque}}{\text{Pixels of the targeted part}} \times 100
\]

If the targeted part of the RDP is divided over more than one image, the below formula is applied:

\[
\text{POP on the targeted parts of RDP} = \frac{\sum \text{Pixels of Plaque [image} \quad 1 + 2 + 3 + \cdots \text{]}}{\sum \text{Pixels of the targeted part [image} \quad 1 + 2 + 3 + \cdots \text{]}} \times 100
\]

Do not use the last formula if the targeted part overlap between the images.

August 2016, Greifswald. DDS. Ahmad Al Jaghsi Dipl. M.Sc. M.Sc

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