Entwicklung und Analyse einer Adhäsionsmessmethode für intestinal anzuwendende mukoadhäsive Polyvinylalkohol-Filme

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1 Einleitung und Zielstellung

1.1 Einleitung

Nach der Definition des Arzneimittelgesetzes § 2 Arzneimittelbegriff sind Arzneimittel Stoffe und Zubereitungen, die zur Anwendung am menschlichen Körper bestimmt sind, um Krankheiten zu heilen, zu lindern oder zu verhüten oder die im menschlichen Körper angewendet werden können, um z.B. physiologische Funktionen durch pharmakologische, immunologische oder metabolische Wirkung wiederherzustellen. Um das Ziel einer möglichst hohen therapeutischen Wirksamkeit zu erreichen, kann es beispielsweise bei oralen Darreichungsformen erforderlich sein, dass die Arzneiform zunächst gezielt in bestimmte Abschnitte des Magen-Darm-Traktes transportiert und erst dort freigesetzt wird, um die Resorption und anschließende Verteilung des Wirkstoffs über den Blutkreislauf des Körpers zu ermöglichen. Die sogenannte orale Bioverfügbarkeit von Arzneistoffen, d. h. der Anteil des resorbierten Wirkstoffs an der Gesamtdosis, hängt dabei nicht nur vom Arzneistoff selbst ab, sondern in nicht unerheblichem Maße auch von den Eigenschaften der Arzneiform, mit der der Wirkstoff an den vorgesehenen Ort transportiert werden soll. Niedermolekulare Wirkstoffe waren jahrzehntelang die dominierende Klasse von Therapeutika [1]. Aufgrund ihrer Größe und ihres geringen Molekulargewichts von weniger als 900 Dalton diffundieren diese Arzneistoffe schnell durch biologische Membranen [2]. Das Ausmaß der Resorption dieser Substanzen hängt in erster Linie von ihren physikochemischen Eigenschaften ab. Voraussetzung für eine schnelle Diffusion durch Membranen und eine damit verbundene Resorption ist die Löslichkeit des Wirkstoffs im umgebenden Medium [3,4]. Dementsprechend liegt der Fokus bei der Entwicklung von Vehikeln für niedermolekulare Therapeutika vor allem auf der Beeinflussung der Löslichkeit und der Permeabilität sowie auf der kontrollierten Freisetzung [4]. In den letzten Jahren hat sich eine neue Generation von Wirkstoffen etabliert. Proteinarzneistoffe, Nukleinsäuren sowie Antikörper wurden entwickelt, um eine rationale Therapie zu ermöglichen. Aufgrund ihrer Struktur sind diese Arzneistoffe besonders anfällig für Stabilitätsprobleme, so dass die Entwicklung protektiver Darreichungsformen für den unbeschädigten Transport der Arzneistoffe zu ihrer Zielstruktur und die gezielte Freisetzung dort notwendig wurde und somit die Entwicklung neuer Arzneistoffe innovative Darreichungsformen erfordert [1].

Die Entwicklung geeigneter Darreichungsformen erfordert eine detaillierte Kenntnis des spezifischen Applikationsortes. Dieses Wissen ist relevant für die Entwicklung von Darreichungsformen, aber auch für die Etablierung prädiktiver Methoden wie biorelevante *in vitro*-Tests und *in silico*-Modelle, die, um aussagekräftige Ergebnisse liefern zu können, auf möglichst viele physiologische und möglicherweise pathologisch assoziierte Informationen über den Applikationsort angewiesen sind [5]. Im weiteren Verlauf der Arzneimittelentwicklung werden Tiermodelle in präklinischen Studien zur Dosisfindung eingesetzt. Dabei ist es im Sinne der Übertragbarkeit wichtig, ein geeignetes Tiermodell zu finden, dessen Physiologie der des zu untersuchenden menschlichen Organs ähnelt, weshalb die genaue

Kenntnis des Applikationsortes daher nicht nur beim Menschen, sondern auch beim Versuchstier relevant ist.

Die meisten verordneten Therapeutika in Deutschland werden peroral verabreicht, da dies aufgrund des nicht-invasiven Verabreichungsweges und der einfachen Handhabung die Compliance der Patienten fördert [6]. Das Hauptresorptionsorgan für die peroral applizierten Arzneistoffe ist der Dünndarm. Daher ist die Physiologie dieses Teils des Gastrointestinaltrakts (GIT) von besonderem Interesse für das Design von Arzneiformen, die Entwicklung biorelevanter *in vitro*-Testmethoden, die Auswahl geeigneter Tiermodelle und die *in silico*-Modulation. Die ersten Methoden zur Aufklärung physiologischer Prozesse im GIT waren invasive Methoden. René-Antoine Ferchault de Réaumur und Lazarro Spallanzani waren zwei der ersten Wissenschaftler, die Anfang des 18. Jahrhunderts die Verdauungsvorgänge im Magen von Vögeln invasiv untersuchten. Mit dem technischen Fortschritt wurde es möglich, die Anatomie des Gastrointestinaltrakts mit Endoskopen und Koloskopen zu untersuchen. Der Wunsch, in Speiseröhre und Magen hineinschauen zu können und im wahrsten Sinne des Wortes „Licht ins Dunkel“ zu bringen, wurde bereits 1806 von Philipp Bozzini in die Tat umgesetzt [7]. Er gilt als einer der Wegbereiter der Ösophagoskopie, indem er ein „Winkelrohr“ vorschlug, mit dem er über Spiegel in die Speiseröhre blicken wollte [8]. Zur Ausleuchtung der dunklen Körperhöhlen benutzte Bozzini eine Kerze, deren Licht über die Spiegel geleitet wurde. Die erste echte Ösophago- und Gastroskopie wurde von Adolf Kussmaul und seinem Kollegen Julius Müller an einem Schwertschlucker durchgeführt [9]. In den folgenden Jahren konnten durch die Entwicklung flexibler Endoskope und die Integration einer internen Lichtquelle weitere anatomische und physiologische Erkenntnisse gewonnen werden. Seitdem können mit Hilfe von Endoskopen und Kathetern wertvolle Informationen erhoben werden. Dazu gehören z.B. die Einführung von pH-Metern zur Messung des gastralen und duodenalen pH-Wertes [10], von Manometern zur Bestimmung der Druckverhältnisse sowie die Aspiration luminaler Flüssigkeiten mit speziellen Sonden [10]. Alle diese Methoden haben jedoch neben dem hohen apparativen Aufwand und den damit verbundenen Kosten den Nachteil der Invasivität. Durch den Eingriff in den lebenden Körper können die Messsysteme selbst unerwünschter Weise die Datenerfassung beeinflussen [11]. Untersuchungen der tieferen Abschnitte des GIT, insbesondere des Dünndarms, wurden unter Verwendung von Narkotika durchgeführt, die die Motilität der Verdauungsorgane beeinflussen können. Daraus ergab sich die Notwendigkeit, nicht-invasive Methoden zu entwickeln, um die Physiologie des GIT zu verstehen.

Um dieses Ziel zu erreichen, wurden in der Mitte des letzten Jahrhunderts telemetrische Messsysteme entwickelt, die den physiologischen Zustand des GIT möglichst wenig beeinflussen sollten. Im Jahre 1957 wurden die ersten schluckbaren Messgeräte, die „*radio pill*“ von Vladimir Zworykin und John Farrar bzw. die „*endoradiosonde*“ von Stuart Mackay und Bertil Jacobson erfolgreich getestet [11,12]. Diese telemetrischen Systeme wurden in den folgenden Jahren ständig weiterentwickelt, so dass heute Systeme zur Messung von Temperatur, pH-Wert, Druck und intraluminalen Gasen zur Verfügung stehen [13,14]. Weiterhin sind kapselähnliche Devices erhältlich, die ein Wirkstoffreservoir besitzen, das

gezielt am vorgesehenen Abschnitt des GIT geöffnet werden kann [15,16]. Zur Aufklärung anatomischer und ggf. pathophysiologischer Zustände des GIT stehen verschiedene schluckbare Kamerakapselsysteme zur Verfügung, wobei all diese schluckbaren Messsysteme ähnlich aufgebaut sind. Der zu applizierende Teil besteht aus einem kleinen Sender, der die aufgenommenen Daten an ein externes Empfängermodul sendet [17]. Das schluckbare Messgerät besteht neben einer Stromquelle aus einem Modul zur Messwertaufnahme und einer Antenne zum Senden der Daten. Die aufgenommenen Daten werden dann an den Empfänger gesendet. Die Empfangseinheit besteht aus einer Empfangsantenne, einem Datenverarbeitungsmodul und einer Ausgabeeinheit oder einem Datenspeicher [17]. Die Etablierung telemetrischer Messsysteme in der Forschung hat in gewissem Maße zu einer Veränderung der bis dahin vorherrschenden Annahmen über die Physiologie geführt. Vor der Einführung von schluckbaren pH-Messkapseln ging man lange Zeit davon aus, dass die pH-Werte im GIT weitestgehend statisch sind. Der Magen galt als sauer (pH 1-2), der Dünndarm als neutral (pH 6-7) und das Kolon als schwach sauer (pH 5-6) [5]. Diese Annahmen konnten mit Hilfe von telemetrischen Kapseln widerlegt werden. Entgegen früheren Annahmen sind die pH-Werte im GIT sehr dynamisch und von einer Vielzahl von Faktoren abhängig. Dressman et al. [18] konnten in Untersuchungen zur Bestimmung des pH-Wertes im oberen GIT bei gesunden Probanden zeigen, dass die mit dem telemetrischen Messsystem Heidelberg Capsule gemessenen pH-Profile im Magen und Duodenum stark von der Nahrungsaufnahme beeinflusst werden. Im nüchternen Zustand entsprachen die gemessenen Mediane den bisher angenommenen Werten (pH 1,7 im Magen und pH 6,1 im Duodenum). Nach Einnahme einer hochkalorischen Mahlzeit stiegen die gemessenen pH-Werte im Magen auf 6,7 und sanken im Duodenum auf 5,4 ab. Innerhalb von 2 bzw. 4 Stunden nach Einnahme der Mahlzeit glichen sich die Werte wieder den Nüchternwerten an. Auf der Grundlage dieser Untersuchungen war es nun eher möglich, Schlussfolgerungen für Mindestanforderungen bei der Etablierung von biorelevanten *in vitro*-Testverfahren zur Abbildung des humanen GIT zu formulieren, bei denen mindestens ein geeignetes pH- und Druckprofil gewählt werden sollte, um die physiologischen Verhältnisse *in vivo* abbilden zu können. Dies führte weiter dazu, dass auch die zumeist für den humanen GIT als besonders relevant angesehenen Spezies Schwein oder Hund , kritisch beurteilt wurden [19,20]. Für die Entwicklung neuer Arzneiformen und geeigneter *in vitro*-Tests ist jedoch nicht nur der pH-Wert von Bedeutung, sondern auch die bereits erwähnten im GIT herrschenden Drücke. Ein anschauliches Beispiel hierfür liefern die Untersuchungen von Garbacz et al. [21], die in einem *in vitro*-Stresstester Diclofenac-Matrixtabletten hinsichtlich ihrer Freisetzung unter simulierten physiologischen Druckbedingungen charakterisierten. Dabei konnte gezeigt werden, dass mit zunehmender Quellung der Matrix *dose dumping*-Effekte auftraten, sobald Druckereignisse simuliert wurden.

Mit Hilfe solcher telemetrischer Messsysteme können auf nicht-invasive Weise wertvolle Informationen über die physiologischen Bedingungen, denen eine Darreichungsform ausgesetzt ist, gewonnen werden. Insbesondere die Entwicklung neuer Wirkstoffe erfordert eine genaue Kenntnis des Applikationsortes und eventuell auftretender Bedingungen, die die Bioverfügbarkeit der Arzneistoffe reduzieren können.

Eigenschaften eines Arzneistoffs, die einen wesentlichen Einfluss auf die orale Bioverfügbarkeit haben, sind beispielsweise die Löslichkeit und Permeabilität. Um Arzneistoffe klassifizieren und solche mit möglichen Bioverfügbarkeitsdefiziten identifizieren zu können, wurde das Biopharmazeutische Klassifizierungssystem, kurz BCS (engl. *Biopharmaceutics Classification System*), entwickelt. Es teilt Arzneistoffe in vier Kategorien ein. Als Entscheidungskriterien werden die Löslichkeit und die Permeabilität des Arzneistoffs herangezogen. Ist die gesamte maximale Einzeldosis des Wirkstoffs in maximal 250 mL eines wässrigen Mediums über einen pH-Bereich von 1,2 bis 6,8 bei 37 °C löslich, gilt der Arzneistoff nach der ICH-Guideline als gut löslich [22]. Eine gute Permeation des Wirkstoffs ist gegeben, wenn der Wirkstoff peroral zu ≥ 85 % bioverfügbar ist, bezogen auf die parenterale Gabe. Die Bioverfügbarkeit wird vorzugsweise in humanen pharmakokinetischen Studien generiert, kann aber auch *in vitro* an Caco-2-Zelllinien untersucht werden [22]. Aus diesen Kriterien ergeben sich vier Klassen. Klasse I umfasst Wirkstoffe, die eine gute Löslichkeit und eine hohe Permeabilität aufweisen. Ihre Bioverfügbarkeit ist daher weitgehend unabhängig von der Arzneiform selbst. In der Klasse III finden sich Arzneistoffe, die gut löslich, aber schlecht permeabel sind. Daraus ergibt sich für diese Klasse, dass die Resorption der geschwindigkeitsbestimmende Schritt für den Wirkeintritt ist. Die meisten Arzneistoffe, die sich heute in der Entwicklung befinden, gehören zu den beiden anderen Klassen. Sie zeichnen sich durch eine schlechte Löslichkeit aus, wobei Stoffe der Klasse II noch gut permeabel sind, während Stoffe der Klasse IV zusätzlich schlecht resorbiert werden. Gerade Stoffe mit schlechter Löslichkeit stellen die Formulierungsentwicklung vor große Herausforderungen. Da sich „schlechte Löslichkeit“ auf den gesamten pH-Bereich von 1,2 bis 6,8 bezieht, gibt es einige Arzneistoffe, die aufgrund ihrer pH-abhängigen Löslichkeit als schlecht löslich eingestuft werden. Wirkstoffe, wie Flurbiprofen oder Naproxen, die zur Klasse II gehören, sind im zu prüfenden pH-Bereich schlecht löslich. Sie lösen sich jedoch bei neutralen pH-Werten im Dünndarm und verhalten sich dort wie Stoffe der Klasse I [23]. Es kann aber auch der umgekehrte Fall eintreten. Beispielsweise sind schwach basische Wirkstoffe im sauren Magen gut löslich. Sobald sich jedoch der pH-Wert der Umgebung ändert, z.B. beim Übergang des Wirkstoffs vom Magen in den Dünndarm, kann es zur Ausfällung des Wirkstoffs kommen. Je weniger Wirkstoff in gelöster Form vorliegt, desto weniger kann resorbiert werden, was wiederum die Bioverfügbarkeit verringert. Diese Problematik unterstreicht noch einmal, warum eine genaue Kenntnis der physiologischen Verhältnisse im GIT sowohl für die Formulierungsentwicklung als auch für die Prüfung unerlässlich ist.

Neben niedermolekularen Substanzen, so genannten *small molecules*, sind zunehmend Peptidarzneistoffe sowie verschiedenste Biopharmazeutika wie Antikörper, Proteine und Enzyme für die Arzneimittelentwicklung von Interesse [24]. Typische Anwendungsgebiete für Biopharmazeutika sind vor allem die Onkologie, Autoimmunerkrankungen und kardiovaskuläre Erkrankungen [25]. Diese Wirkstoffgruppe stellt hinsichtlich ihrer Bioverfügbarkeit andere Herausforderungen an Forschung und Entwicklung. Da sie hochspezifisch an biologische Rezeptoren binden, ist ihre Struktur entscheidend für ihre Wirkung. Aufgrund ihrer Proteinstrukturen unterliegen Biopharmazeutika stark dem Einfluss

der gastrointestinalen Umgebung. Dies führt dazu, dass die perorale Bioverfügbarkeit der meisten Biopharmazeutika bei 1-2 % liegt [26,27]. Die beiden wichtigsten biochemischen Barrieren für peroral verabreichte Biologika sind das saure Milieu im Magen und der enzymatische Abbau, der hauptsächlich im Dünndarm stattfindet [28]. Beides kann zu einer Denaturierung des Proteinarzneistoffs und einem damit verbundenen Funktionsverlust führen. Ist der Arzneistoff dennoch weitgehend unversehrt, muss er im nächsten Schritt die Schleimhautschicht überwinden, um in die Blutbahn aufgenommen zu werden. Dazu muss zunächst der Mukus passiert werden. Als Mukus bezeichnet man die viskoelastische, hydrogelartige Substanz, die auf allen Schleimhäuten vorhanden ist. Diese Substanz bildet eine netzartige Struktur und wirkt somit als physikalische Filterbarriere [29]. Unter der Schleimschicht befindet sich die Epithelschicht, die eine weitere Barriere für den Wirkstoff darstellt. Für die Aufnahme in die Blutbahn sind verschiedene Mechanismen möglich. Zum einen können Wirkstoffe durch passive Diffusion aufgenommen werden, wozu der parazelluläre und der transzelluläre Transport gehören. Voraussetzung für den transzellulären Transport ist eine hohe Lipophilie. Protein- und Peptidarzneistoffe weisen jedoch in der Regel log*P*-Werte < 0 auf, so dass dieser Transportmechanismus nicht möglich ist. Der parazelluläre Transport erfolgt über *Tight Junctions*, *Adherent Junctions* und Desmosomen. Diese weisen sehr enge Porenradien von 8-13 Å auf [30,31], wodurch eine Passage von großen Makromolekülen, zu denen Biologika in der Regel gehören, nicht möglich ist. Diese Problematik spiegelt sich auch in Bioverfügbarkeitsstudien wider. Ab einem Molekulargewicht von etwa 500-700 Da nimmt die orale Bioverfügbarkeit oft drastisch ab [32]. Als verbleibende Resorptionsmechanismen stehen der Carrier-vermittelte Transport, der aktive Transport oder die Endozytose und Pinozytose zur Verfügung.

Um den Herausforderungen der geringen peroralen Bioverfügbarkeit von schwerlöslichen Arzneistoffen und Biopharmazeutika zu begegnen, wurde in den letzten Jahrzehnten eine Vielzahl innovativer Darreichungsformen entwickelt. Dazu gehören unter anderem mukoadhäsive Arzneiformen [33,34] wie Tabletten, Filme, Pellets oder halbfeste Zubereitungen wie Gele. Das Phänomen der Mukoadhäsion beschreibt das Anhaften einer Darreichungsform an einer Schleimhaut [35]. Durch den direkten Kontakt mit der Schleimhaut sind mukoadhäsive Darreichungsformen in der Lage, die Verweildauer der Arzneiform auf der Schleimhaut zu verlängern und damit die Resorptionszeit zu erhöhen [36,37]. Ein weiterer Vorteil besteht darin, dass die lokale Wirkstoffkonzentration an der gut durchbluteten Mukosa erhöht wird und somit der Konzentrationsgradient ebenfalls die Wirkstoffresorption fördert [38]. Um den Mechanismus auf molekularer Ebene zu verstehen, muss die Struktur des Mukus betrachtet werden. Mukus ist der viskoelastische, hydrogelartige Schleim, der die inneren Hohlorgane auskleidet. Er besteht zum größten Teil aus Wasser, während die rheologischen Eigenschaften hauptsächlich von den Muzinen bestimmt werden. Dabei handelt es sich um eine Gruppe von Glykoproteinen, die aus einem Proteingerüst mit stark verzweigten glykosidischen Oligosacchariden bestehen [35]. Die Proteinstruktur enthält viele cysteinreiche Domänen, die in der Lage sind, Disulfidbrücken auszubilden und so die Struktur der Muzine zu beeinflussen. Durch Verknüpfung mit weiteren Muzinmolekülen entsteht ein

stark vernetztes Gel. Physiologisch dient dieses Gel dem Schutz der darunter liegenden Epithelzellen vor mechanischen und chemischen Einflüssen sowie der Befeuchtung der Schleimhaut. Aus Sicht der Formulierungsentwicklung stellen Muzine eine interessante Zielstruktur für die Formulierung mukoadhäsiver Arzneiformen dar. Die Bindung der Arzneiform an die Schleimhaut kann durch verschiedene chemische Wechselwirkungen erfolgen. Die stärksten Bindungen sind ionische Bindungen. Da Muzine aufgrund von Sialinsäureresten und Sulfatestern insgesamt negativ geladen sind, können ionische Bindungen mit kationischen Polymeren eingegangen werden. Kovalente Bindungen sind ebenfalls möglich und gehören zu den stärkeren Bindungen. Sekundäre chemische Bindungen wie Wasserstoffbrückenbindungen und van-der-Waals-Brückenbindungen stellen eine weitere Bindungsmöglichkeit dar. Sie sind in der Regel wesentlich schwächer als die primären Bindungen, können in Summe aber dennoch eine nicht unerhebliche Rolle spielen. Da das Phänomen der Mukoadhäsion sehr komplex ist, gibt es verschiedene Theorien, die Erklärungsansätze bieten und die Möglichkeit einer rationalen Formulierung eröffnen. An dieser Stelle sollen nur einige davon kurz vorgestellt werden.

a) Elektronentheorie: Durch einen Elektronentransfer zwischen zwei entgegengesetzt geladenen Schichten entsteht eine Ladungsdoppelschicht, die für die Adhäsion verantwortlich ist.
b) Adsorptionstheorie: Im Gegensatz zum Elektronentransfer spielen hier Wasserstoffbrückenbindungen und van-der-Waals-Brückenbindungen eine wichtige Rolle für die Bindung.
c) Diffusionstheorie: Angetrieben durch den Konzentrationsunterschied eines mukoadhäsiven Polymers zur Mukusschicht kommt es zur Interdiffusion der beiden Polymerketten. Das Ausmaß der Adhäsion wird durch die Beweglichkeit der Ketten, die Kettenlänge, die Kontaktzeit und den Diffusionskoeffizienten bestimmt.

Die Kenntnis der möglichen Adhäsionsmechanismen ermöglicht die gezielte Entwicklung von Darreichungsformen, die den *in vivo*-Bedingungen angepasst sind. Dazu muss ein *in vitro*-Testsystem entwickelt werden, das die physiologischen Bedingungen möglichst genau nachbildet und gleichzeitig ein Maß für die Mukoadhäsion liefert. Für die Messung der Mukoadhäsion gibt es keine standardisierte, in Arzneibüchern monographierte Methode [39]. Daher werden in der Literatur verschiedene Methoden beschrieben, welche grob in direkte und indirekte Methoden unterteilt werden können [35,40]. Indirekte Methoden zur Bestimmung der Mukoadhäsion umfassen die Messung von Parametern, die Aufschluss über die Wechselwirkungen zwischen der mukoadhäsiven Darreichungsform und der Schleimhaut geben. Dazu gehören z.B. rheologische Messungen, spektroskopische Methoden und die Bestimmung der Oberflächenenergie durch Kontaktwinkelmessungen [41]. Zu den direkten Methoden gehören quantitative Messungen wie die Messung der Verweildauer einer mukoadhäsiven Arzneiform auf einer Schleimhaut, einem biomimetischen Gel oder einem Muzin-Pressling. Ferner gehören dazu Methoden, bei denen die Kraft gemessen wird, die erforderlich ist, um die Arzneiform von einem Substrat zu

trennen. Diese Messungen werden in der Regel als Zugtest mit Texturprüfgeräten oder modifizierten Mikrowaagen durchgeführt. Bei der Messung werden die mukoadhäsive Darreichungsform und die Mukosa oder ein mukosamimetisches Material mit einer definierten Kontaktkraft zusammengepresst. Nach einer bestimmten Kontaktzeit werden die beiden Oberflächen mit einer definierten Abzugsgeschwindigkeit voneinander getrennt. Die dabei aufgenommenen Kraft-Weg-Diagramme geben Aufschluss über die maximale Ablösekraft F_{max}. Außerdem kann die Adhäsionsarbeit W_{ad} als Fläche unter der Kurve des Kraft-Weg-Diagramms berechnet werden. Ein Blick in die Übersichtsarbeiten zu Mukoadhäsionsmessungen zeigt, dass die einzelnen Parameter solcher Adhäsionsmessungen mit Texture Analysern stark variieren [39,40,42,43]. Nicht nur gerätespezifische Faktoren wie Anpresskraft, Kontaktzeit und Abzugsgeschwindigkeit unterscheiden sich zwischen den verschiedenen Arbeiten zum Teil erheblich. Auch das Substrat, auf dem die mukoadhäsive Darreichungsform haften soll, kann die Messergebnisse beeinflussen. *Ex vivo*-Untersuchungen an Schleimhautpräparaten können die tatsächliche makroskopische Struktur des angestrebten Applikationsortes am besten widerspiegeln. Tierische Gewebe, wie Schweine-, Ratten- oder Rinderschleimhaut werden häufig verwendet, um menschliches Gewebe zu imitieren [43]. Neben ethischen Fragen ist insbesondere die hohe Variabilität der Messergebnisse ein Nachteil der Verwendung biologischer Gewebe [44]. Auch die Vergleichbarkeit der Ergebnisse aus *ex vivo*-Versuchen an tierischer Schleimhaut mit dem zu erwartenden Verhalten an menschlicher Schleimhaut ist noch nicht ausreichend untersucht, um solche Präparate als validen Ersatz für menschliche Schleimhaut zu betrachten [40]. Erschwerend kommt hinzu, dass die Präparation der Gewebe in *ex vivo*-Versuchen unterschiedlich sein kann. Einige Autoren verwenden frisches, andere aufgetautes Gewebe [45]. Häufig werden Schleimhäute mit Wasser gereinigt [46] und lediglich auf die benötigte Größe zugeschnitten. Teilweise erfolgt eine weitergehende Präparation, indem die Schleimhaut vor Versuchsbeginn von der darunterliegenden Muskelschicht getrennt wird [47,48]. Häufig werden die Gewebeproben vor Versuchsbeginn auch mit einer bestimmten Menge Flüssigkeit benetzt, um die physiologischen Bedingungen am Applikationsort zu simulieren. Ethische Fragen, die Komplexität der Aufarbeitung und nicht zuletzt die Variabilität der Messergebnisse erschweren die Vergleichbarkeit der Ergebnisse. Die Entwicklung synthetischer oder halbsynthetischer Materialien, die als Ersatz für biologische Präparate dienen können, ist daher von großem Interesse [40,49]. Die Möglichkeiten für *in vitro*-Experimente reichen von komprimierten Muzin-Scheiben über einfache Hydrogele wie Gelatine oder Agar/Muzin-Gele [50–52] bis hin zu komplexeren biomimetischen Gelen. Darunter werden Gele zusammengefasst, die in ihrer Beschaffenheit eine Schleimhaut imitieren können. Hall et al. [49] beschrieben 2011 die Herstellung eines mukosamimetischen Gels, das aus der Copolymerisation von 2- Hydroxyethylmethacrylat (HEMA) und *N*-Acryloylglucosamin (AGA) hergestellt wurde. Dieses Gel zeigte in Mukoadhäsionstests Eigenschaften, die mit denen von porciner Mundschleimhaut vergleichbar waren.

1.2 Zielstellung

Ziel dieser Arbeit war es, eine Messmethode zur Bestimmung der Mukoadhäsion zu entwickeln, die auf rational begründeten und nicht auf willkürlichen Messparametern beruht. Als Zielstruktur für die Applikation des mukoadhäsiven Polyvinylalkohol-Films (PVA-Film) wurde der Dünndarm gewählt. Intestinale Filme werden in der Forschung eingesetzt, um die perorale Bioverfügbarkeit von Arzneistoffen zu verbessern, die über diesen Applikationsweg schlecht bioverfügbar sind. Dazu gehören vor allem Biopharmazeutika wie Proteine, Peptide und rekombinante Arzneistoffe. Durch eine verlängerte Verweildauer von mukoadhäsiven, intestinalen Filmen soll die für die Resorption dieser Substanzen zur Verfügung stehende Zeit erhöht werden [53,54]. Darüber hinaus bieten wirkstoffbeladene Filme den Vorteil, dass durch die Adhäsion an einer bestimmten Stelle lokal ein großer Konzentrationsunterschied erzeugt werden kann, was die Resorption ebenfalls begünstigt. Dieses Funktionsprinzip konnte bereits in Studien erfolgreich getestet werden [38,55].

Viele der bisher publizierten *in vivo*-Studien basieren auf *in vitro*-Mukoadhäsionsstudien, bei denen die entwickelte Darreichungsform mit zuvor willkürlich festgelegten Parametern an meist tierischen Schleimhäuten getestet wurde. Um einen *in vitro*-Test zu entwickeln, der das Verhalten der Darreichungsform *in vivo* möglichst gut vorhersagt, ist es notwendig, den Test auf der Grundlage physiologischer und methodischer Kenntnisse zu konzipieren. Daher sollte in einem ersten Schritt eine *in vitro*-Messmethode für die Prüfung von PVA-Filmen entwickelt werden, die mit der *solvent cast* Technologie hergestellt wurden. Die Methode sollte auf der Messung der zum Ablösen der Filme erforderlichen Kraft basieren, weshalb als Messgerät ein Texture Analyser eingesetzt wurde. Als mögliche Einflussfaktoren auf die Messergebnisse sollten gerätespezifische Parameter untersucht werden. In den *in vitro*-Versuchen wurden biomimetische Agar/Muzin-Gele verwendet. Gele, die als Ersatz für Gewebeproben in Mukoadhäsionsmessungen dienen, bieten den Vorteil, dass sie leicht herzustellen sind und reproduzierbare Ergebnisse liefern. Gleichzeitig können sie die zu erwartende Variabilität der Ergebnisse auf biologischen Präparaten nicht abbilden. Bei der Verwendung von Geweben gibt es eine Vielzahl von Faktoren, die potenziell Einfluss auf Mukoadhäsionsmessungen haben können. Der zweite Schwerpunkt dieser Arbeit lag deshalb auf den eingesetzten Substraten und deren Präparation. Basierend auf den Ergebnissen der Parameterfindung sollte ein Setup erstellt werden, welches in *ex vivo*-Experimenten an porcinem und humanem Dünndarmgewebe eingesetzt werden sollte. In diesen Experimenten sollten weitere Fragen beantwortet werden. Zunächst sollte der Einfluss der Präparation und Lagerung beider verwendeten Gewebearten auf die Messergebnisse untersucht werden. Im Rahmen der *ex vivo*-Humanstudie wurde daneben auch der Entnahmeort des Gewebes als möglicher Einflussfaktor untersucht. Schließlich wurde ein Vergleich der unter gleichen Messbedingungen erhaltenen Ergebnisse an biomimetischen Gelen sowie an Dünndarmgewebe von Schwein und Mensch durchgeführt. Damit sollte die Frage beantwortet werden, ob es für die hochkomplexe menschliche Darmschleimhaut geeignete Alternativen für *in vitro*-Versuche gibt.

2 Diskussion

Um ein grundlegendes Verständnis der potenziellen Einflussfaktoren auf die Messung der Mukoadhäsion zu erlangen, sollten in einem ersten Teil der Arbeit die Einflussfaktoren in *in vitro*-Versuchen mit einem Texture Analyzer an PVA-Filmen untersucht werden. Die Filme für diese Untersuchungen wurden jeweils am Vortrag im *solvent cast* Verfahren hergestellt. Hierbei wurden das mukoadhäsive Polymer PVA 18-88 (EMPROVE® ESSENTIAL PVA 18-88, Merck KGaA, Darmstadt, DE) und der Weichmacher Glycerol (AppliChem GmbH, Darmstadt, DE) in heißem Wasser gelöst, anschließend mithilfe eines automatischen Präzisions-Filmziehgerät (CX4, mtv messtechnick oHG, Erftstadt, DE) auf einem Liner ausgerakelt und bei Raumtemperatur getrocknet. Um die in den späteren Versuchen verwendeten Gewebe möglichst effizient nutzen zu können, musste das Messgerät modifiziert werden. Die verwendete Materialprüfmaschine Texture Analyser TA Plus (AMETEK Lloyd Instruments Ltd., Bognor Regis, UK) besteht normalerweise aus einem stationären Unterteil und einem vertikal beweglichen Teil. Der untere Teil wurde modifiziert, indem ein handelsüblicher Mikroskoptisch umgebaut wurde (Abbildung 1). Dies sollte eine freie horizontale Bewegung der Probe ohne wiederholtes Umpositionieren ermöglichen.

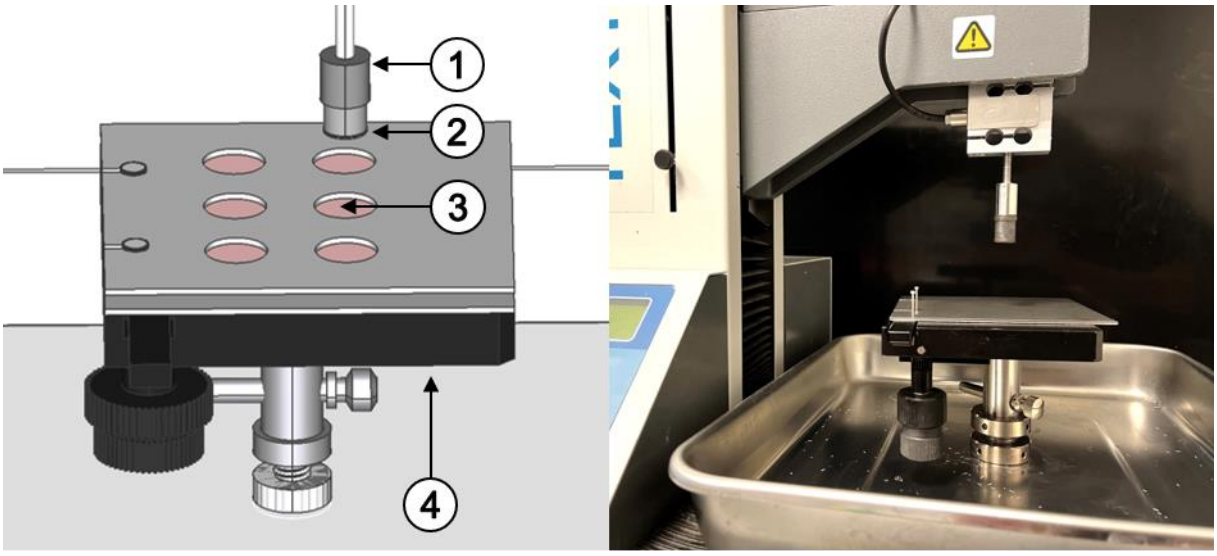

Abbildung 1: (a) Schematische Darstellung der am Texture Analyser vorgenommenen baulichen Veränderungen. 1: Messsonde, 2: Filmprobe, 3: Gewebe und 4: Mikroskoptisch. (b) Foto des Geräteaufbaus im Labor [52].

Veränderungen wurden ebenfalls an der oberen Messsonde vorgenommen. Um den Einfluss der Probenfläche untersuchen zu können, mussten Sonden mit unterschiedlichen Durchmessern verwendet werden. Dazu wurden Tablettenstempel mit den Durchmessern d=7 mm, d=10 mm und d=14 mm mit einem Gewinde versehen, um sie am Kraftaufnehmer der Apparatur zu befestigen.

Die Suche nach geeigneten Messparametern wurde mit biomimetischen Gelen aus 2 % Agar und 4 % Muzin durchgeführt. Der Muzingehalt der Gele sollte im physiologischen Bereich liegen. Im Rahmen der Versuche wurden drei gerätespezifische Faktoren untersucht:

a) Die Anpresskraft, mit der der Film auf das Gel gedrückt wurde,
b) die Kontaktzeit von Film und Gel sowie
c) die Abzugsgeschwindigkeit der Messsonde.

Weiterhin können Faktoren, die nicht mit dem Messgerät selbst zusammenhängen, die Messung beeinflussen. Hierzu gehören:

d) Die Fläche der Filmprobe,
e) Vorhandensein von Benetzungsflüssigkeit auf dem Gel und
f) das Alter des verwendeten Gels.

Ausgehend von einem Standardparametersatz, der sich an den in der Literatur üblichen Werten orientiert und mit einer Anpresskraft von 0,1 N, einer Kontaktzeit von 1 min und einer Abzugsgeschwindigkeit von 0,5 mm/s definiert ist, wurden die einzelnen Geräteparameter variiert, um deren Einfluss auf die Adhäsion beurteilen zu können. Bei den geräteabhängigen Faktoren zeigte sich, dass die Anpresskraft weder auf die maximale Abrisskraft F_{max} noch auf die Adhäsionsarbeit W_{ad} einen nennenswerten Einfluss ausübten (Abbildung 2a). Als Ausgangswerte für die Messungen wurden Kräfte von 0,05 bis 1,00 N gewählt. Diese entsprechen Drücken von etwa 3 bis 65 mbar, die nach einer hochkalorischen, fettreichen Mahlzeit im unteren physiologischen Bereich liegen, wie Daten aus SmartPill® -Studien von Koziolek et al. Zeigen [56]. Aus der Beobachtung, dass die Anpresskraft im untersuchten Bereich keinen relevanten Einfluss zeigte, kann geschlossen werden, dass dieser Parameter unter Berücksichtigung der Genauigkeit des Kraftaufnehmers frei wählbar ist. Neves et al. [57] untersuchten in ihrer Arbeit die Auswirkung verschiedene instrumentelle Parameter auf *in vitro*-Mukoadhäsionstests von halbfesten Vaginalia. In dem von ihnen getesteten Kraftbereich von 0,05 bis 0,25 N beobachteten sie zunächst einen Anstieg von F_{max} und W_{ad}. Beide Kurven zeigten eine Annäherung an ein Plateau, das bei F_{max} bereits ab 0,15 N erreicht wurde. Diese Beobachtungen stehen im Einklang mit den Daten, die auf Agar/Muzin-Gelen gewonnen wurden. Auch hier konnte im unteren Kraftbereich noch ein leichter Anstieg gemessen werden, während mit zunehmender Anpresskraft ein Plateau erreicht zu sein scheint.

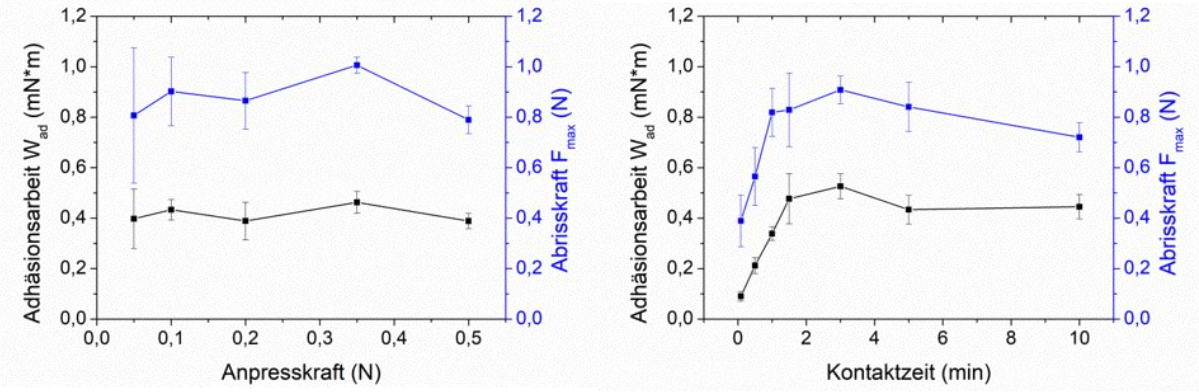

*Abbildung 2: (a) Auswirkung der Anpresskraft und (b) der Kontaktzeit auf die maximale Abrisskraft F_{max} (N) und die Adhäsionsarbeit W_{ad} (mN*m) von PVA-Filmen auf Gelen aus 2 % Agar und 4 % Muzin. MW ± SD, n=6.*

Die Kontaktzeit zwischen Film und Gel spielt bei den instrumentellen Parametern die größte Rolle. Nach einem steilen Anstieg der Kurven erreichten F_{max} und W_{ad} nach 3 min ihr Maximum (Abbildung 2b). Bis zu einer Versuchszeit von 10 min war eine langsame Abnahme beider Messgrößen zu beobachten. Diese Beobachtungen lassen sich durch die Struktur der mukoadhäsiven Filme erklären. Filme sind feste Arzneiformen, die dünn und flexibel sind [58]. Bei Kontakt mit Flüssigkeiten, wie z.B. den intraluminalen Flüssigkeiten im GIT, kommt es durch Wasseraufnahme zu einer Quellung der Polymerstruktur. Dadurch werden die Polymerketten gelockert und die Anzahl der freien funktionellen Gruppen, die zu einer mukoadhäsiven Bindung beitragen können, erhöht. PVA ist ein nichtionisches Polymer. Bei dieser Polymergruppe sind hauptsächlich Wasserstoffbrücken für die Ausbildung einer mukoadhäsiven Bindung verantwortlich [59]. Diese sekundären Bindungen bilden sich schnell aus [47]. Je mehr freie wasserstoffbrückenbildende Gruppen zur Verfügung stehen, desto stärker ist die gemessene Adhäsion. Durch eine zeitabhängige Quellung der Darreichungsform nimmt die Beweglichkeit der Polymerketten zu, es bildet sich ein Gel. Die Lockerung des Polymergerüstes führt auch zu einer Diffusion des PVA in die Muzinschicht. Dieses Phänomen lässt sich mit der Diffusions- oder Interpenetrationstheorie beschreiben [59,60]. Da die Diffusion neben dem Diffusionskoeffizienten vor allem von der Zeit abhängt, nimmt die Adhäsion mit zunehmender Kontaktzeit zu [61]. Ein Versagen der mukoadhäsiven Verbindung tritt immer an der schwächsten Stelle der beteiligten Komponenten auf, dabei sind grundsätzlich drei Bruchstellen denkbar. Die mukoadhäsive Bindung kann reißen, indem ein Bruch im Mukus selbst, in der Darreichungsform oder an der Kontaktfläche auftritt [35]. Bei festen Darreichungsformen ist die Kohäsion innerhalb der Darreichungsform hoch, so dass die schwächste Stelle innerhalb der Mukusschicht oder an der Grenzschicht liegt. Ein Hinweis auf einen Bindungsabbruch innerhalb des Gels oder Schleims kann makroskopisch durch ein Anhaften von Gelbestandteilen an der Messsonde nach deren Entfernung festgestellt werden [47]. Mit zunehmender Hydratation nimmt die Kohäsion des Polymergerüstes ab, wodurch ein Bruch innerhalb des Films wahrscheinlicher wird. Dabei kann das Wasser zur Quellung der festen Darreichungsform auch aus dem Mukus selbst stammen. Durch die Dehydratation des Schleims diffundiert das Wasser in das trockene

mukoadhäsive Objekt und fördert dort die Quellung [47,62]. Steht zusätzlich Wasser aus der Umgebung zur Verfügung, z. B. durch Befeuchtung des Gels, kann die Quellung schneller erfolgen, was wiederum die Kohäsionskräfte innerhalb der Darreichungsform reduziert und somit zu einer geringeren Mukoadhäsion führt.

Estrellas et al. [63] führen diese Thematik weiter, indem sie die Hypothese aufstellen, dass stark hydrophile Strukturen ein frühes Maximum ihrer Mukoadhäsion erreichen, bevor diese wieder abnimmt. Sie beobachteten an Dünndarmgewebe von Ratten, dass das schwach vernetzte Hydrogel Polycarbophil bei Kontakt mit wässriger Umgebung schnell quillt. Dies ermöglicht über physikalische Wechselwirkungen und Wasserstoffbrückenbindungen eine Adhäsion, die jedoch mit zunehmender Hydratation wieder abnimmt. Im Gegensatz dazu konnten hydrophobe Polymere wie Polybutadien/Maleinsäureanhydrid konjugiert mit L-Phenylalanin ihr mukoadhäsives Potential über den Beobachtungszeitraum von 91 min aufrechterhalten. Die Mukoadhäsion hängt also nicht nur von der Kontaktzeit, sondern auch von der chemischen Struktur der Darreichungsform ab.

Im Falle des hydrophilen PVA erfolgt die Quellung schnell. Neben der Zeit und der chemischen Struktur spielt aber auch die verfügbare Wassermenge eine Rolle. Diese Annahme konnte in Versuchen bestätigt werden, in denen die biomimetischen Agar/Muzin-Gele mit Phosphatpuffer pH 7,4 USP befeuchtet wurden (Abbildung 3a). Die Anwesenheit von frei verfügbarem Wasser führte bereits bei geringen Volumina zu einer starken Abnahme der Adhäsion. Die Menge der Befeuchtungsflüssigkeit schien dabei eine untergeordnete Rolle zu spielen.

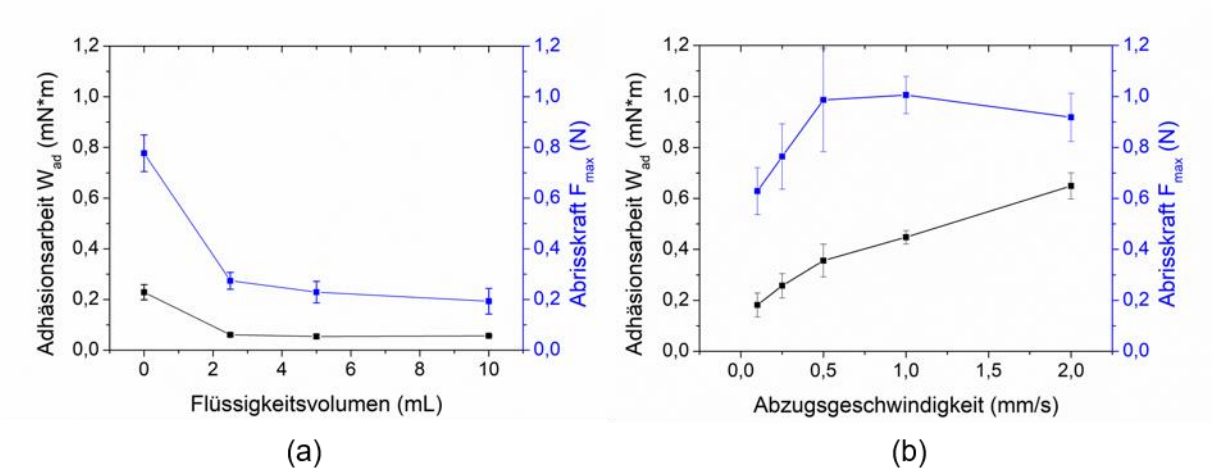

*Abbildung 3: (a) Auswirkung des Flüssigkeitsvolumens und (b) der Abzugsgeschwindigkeit der Messsonde auf die maximale Abrisskraft F_{max} (N) und die Adhäsionsarbeit W_{ad} (mN*m) von PVA-Filmen auf Gelen aus 2 % Agar und 4 % Muzin. MW ± SD, n=6.*

Der letzte frei wählbare Geräteparameter war die Geschwindigkeit, mit der die Messsonde vom Gel abgezogen wurde. Während des Ablösens muss der Film eine große Verformung erfahren können. Der Ablöseprozess eines weichen Polymergels ist in der Regel durch die Bildung von Fibrillen gekennzeichnet. Zunächst wird das gesamte Gel homogen verformt, bevor sich immer größere Hohlräume bilden, aus denen schließlich die Fibrillen entstehen. Diese können Energie speichern und

verteilen [64]. Die Abzugsgeschwindigkeit der Messsonde verändert die Zeit, in der sich das Polymergel verformen kann [65]. Dies wiederum kann das viskoelastische Verhalten des Gels beeinflussen. Bei langen Verformungszeiten, d.h. langsamen Abzugsgeschwindigkeiten, können sich die Polymerketten neu anordnen. Dadurch sinkt der innere Widerstand der Zubereitung, die Viskosität nimmt also ab. In Messungen der Abzugskraft und der Adhäsionsarbeit drückt sich dieses theoretische Konstrukt so aus, dass beide mit zunehmender Abzugsgeschwindigkeit ansteigen [66]. Die Ergebnisse der Adhäsionsversuche von PVA-Filmen auf Agar/Muzin-Gelen zeigten für die Adhäsionsarbeit eine Übereinstimmung mit dieser Annahme (Abbildung 3b). Allerdings erreichte die Abzugskraft bei 1,0 mm/s ein Maximum und nahm bei höheren Abzugsgeschwindigkeiten wieder ab. Wong et al. [44] kamen in ihren Versuchen zur Mukoadhäsion von Carbopol 974P Tabletten auf Hühnergewebe zu vergleichbaren Ergebnissen. Sie beobachteten außerdem eine Abnahme des Variationskoeffizienten bei höheren Geschwindigkeiten und folgerten daraus, dass Mukoadhäsionsmessungen bei höheren Geschwindigkeiten durchgeführt werden sollten, um eine höhere Sensitivität zu erreichen.

Auf der Grundlage dieser Vorüberlegungen wurde eine Messeinstellung (Tabelle 1) abgeleitet, die zu möglichst reproduzierbaren Ergebnissen führen sollte. In den Versuchen an menschlichem Gewebe wurde diese „optimierte“ Einstellung B mit der ursprünglichen Einstellung A verglichen.

Tabelle 1: Messparameter für die ex vivo-Mukoadhäsionsversuche, abgeleitet aus den vorangehenden in vitro Versuchen auf biomimetischen Agar/Muzin-Gelen.

	Einstellung A	**Einstellung B**
Probenfläche (mm^2)	154	154
Anpresskraft (N)	0,10	0,35
Kontaktzeit (min)	1	3
Abzugsgeschwindigkeit (mm/s)	0,5	1,0

Nicht nur die Messparameter können die Ergebnisse von Mukoadhäsionsstudien beeinflussen, sondern auch die Wahl einer geeigneten Schleimhaut oder eines Schleimhautersatzes. Die zweite Säule der systematischen Untersuchung einer Messmethode zur Messung der Mukoadhäsion von PVA-Filmen war daher die Untersuchung verschiedener Gewebe und deren Präparation. In einem ersten Schritt wurde dazu Dünndarmgewebe des Schweins untersucht. Das Schwein eignet sich für bestimmte Versuche als Versuchstier aufgrund der physiologischen Ähnlichkeit des GIT zum Menschen [67]. Darüber hinaus erleichtert die Verwendung als Schlachttier den Zugang zu porcinen Geweben. Der Schwerpunkt der Untersuchungen am Schweinedünndarm als Material lag auf der Präparation des Gewebes. Nach der Schlachtung und Probennahme des Gewebes wurde der Dünndarm der Versuchstiere in drei Teile geteilt. Ein Teil wurde im frischen Zustand ohne vorherige Reinigung des Darms verwendet. In diesen Abschnitten konnten einzelne Nahrungsbestandteile sowie intestinale Flüssigkeiten beobachtet werden. Gastrointestinale Flüssigkeiten wie Galle und Pankreassaft sind unabhängig von der Nahrungsaufnahme permanent im Dünndarm vorhanden, ihre Menge nimmt nach einer Mahlzeit allerdings zu [68]. Filme,

die im Dünndarm haften sollen, sind daher auch diesen Flüssigkeiten ausgesetzt, so dass eine Beeinflussung der Adhäsion möglich ist.

Zum Vergleich wurde ein anderer Abschnitt behutsam mit Wasser gereinigt, um Nahrungspartikel und intraluminale Flüssigkeiten zu entfernen. Dieser Schritt wird häufig in *ex vivo*-Mukoadhäsionsstudien durchgeführt [45,69,70]. Ebenso ist es üblich, die Präparate zur Lagerung einzufrieren und vor Versuchsbeginn aufzutauen [71,72]. Der Einfluss auf die Messergebnisse wurde bisher jedoch nicht untersucht. Aus diesem Grund wurden diese beiden Aufbereitungsweisen ebenfalls in den *ex vivo*-Versuchen berücksichtigt.Vergleicht man die Ergebnisse der drei Präparationsarten bei den drei Versuchstieren, so ist weder für W_{ad} noch für F_{max} ein eindeutiger Trend erkennbar. Bei den Schweinen 1 und 2 ist die Adhäsion am unaufbereiteten Gewebe am geringsten, während sie am gereinigten Tier nach einem Gefrierzyklus am höchsten ist (Tabelle 2).

Tabelle 2: Übersicht über die Messergebnisse (MW ± SD, n=6) aus den ex vivo und in vitro-Mukoadhäsionsstudien auf porcinem Darm und biomimetischen Agar/Muzin-Gelen. Dargestellt sind die Ergebnisse aus der Einstellung B.

	Aufbereitung	**Adhäsionsarbeit W_{ad}**	**Abrisskraft F_{max}**
		mN*m	N
Schwein 1	unaufbereitet	0,337 ± 0,058	0,147 ± 0,020
	gereinigt	0,553 ± 0,168	0,144 ± 0,041
	aufgetaut	1,046 ± 0,737	0,217 ± 0,168
Schwein 2	unaufbereitet	0,443 ± 0,090	0,111 ± 0,009
	gereinigt	0,599 ± 0,260	0,121 ± 0,050
	aufgetaut	1,190 ± 0,307	0,190 ± 0,068
Schwein 3	unaufbereitet	1,467 ± 0,380	0,292 ± 0,180
	gereinigt	0,402 ± 0,101	0,098 ± 0,025
	aufgetaut	0,766 ± 0,283	0,165 ± 0,054
Agar/Muzin-Gel		0,730 ± 0,122	1,104 ± 0,060

Makroskopisch konnten intraindividuelle Unterschiede festgestellt werden. Die typische Faltung (*Plicae circularis*) des Darms war bei frischem Gewebe deutlich zu erkennen. Durch das Einfrieren und Auftauen ging diese Struktur teilweise verloren, was durch eine Glättung des Gewebes gekennzeichnet ist. Eine glatte Oberfläche ermöglicht eine gleichmäßigere Haftung des Films auf dem Substrat, was zu einer besseren Mukoadhäsion führen kann.

Zusätzlich wurde beim Auftauen ein Flüssigkeitsaustritt festgestellt, der Rückschlüsse auf die mögliche Bildung von Eiskristallen während des Gefrierprozesses zulässt. Eiskristalle bilden sich, wenn Gewebe ohne Kryoprotektoren eingefroren wird [73]. Abhängig von der Einfriergeschwindigkeit können sie Zellen perforieren, wodurch Zellflüssigkeit austritt. Zusätzlich kann das Gewebe durch den osmotischen Druck, der durch die Eiskristallbildung entsteht, weiter geschädigt werden. Kälteeinwirkung kann sich auch auf die Mukus auswirken. Hägerström et al. [47] berichteten über strukturelle Veränderungen des nasalen Mukus von Schweinen während des Auftauens und kamen zu dem Schluss, dass Gewebe so frisch wie möglich verwendet werden sollte.

Ein Vergleich der *ex vivo*-Ergebnisse mit den *in vitro*-Ergebnissen zeigt, dass Agar/Muzin-Gele kein geeigneter Gewebeersatz sind. Zwar ist die Adhäsionsarbeit an den Gelen in etwa vergleichbar mit den Ergebnissen an gereinigtem Dünndarm, die mittlere Abrisskraft ist jedoch um den Faktor 5 bis 10 höher. Diese Beobachtung lässt sich vermutlich mit den Ergebnissen der *in vitro*-Experimente zum Einfluss von Flüssigkeit auf die Adhäsion erklären. Auf dem Dünndarmgewebe war visuell deutlich mehr Flüssigkeit zu erkennen als auf den vergleichsweise trockenen Gelen.

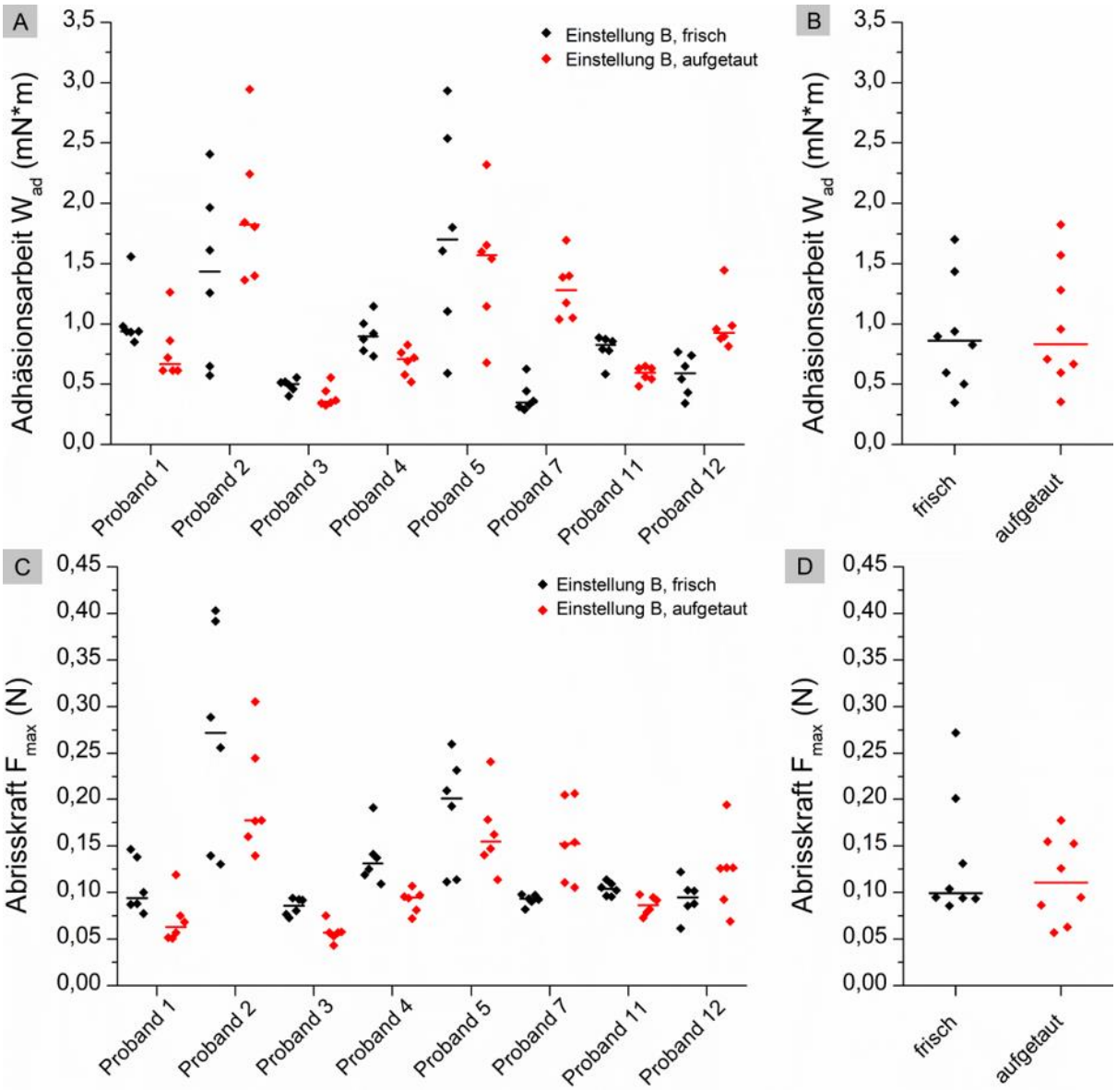

*Abbildung 4: Ergebnisse für Einstellung B. (A): Individuelle Daten der berechneten Adhäsionsarbeit W_{ad} (mN*m) mit Median (n=8); schwarz: frisches Gewebe; rot: aufgetautes Gewebe; (B): gepoolte Mediane aller Probanden mit Medianlinie; (C): Individuelle Daten der berechneten Abrisskraft F_{max} (N) mit Median (n=8); schwarz: frisches Gewebe; rot: aufgetautes Gewebe; (D): gepoolte Mediane aller Probanden mit Medianlinie. Ein signifikanter Unterschied zwischen W_{ad} und F_{max} wurde mit einem Wilcoxon Signed Rank Test überprüft.*

Die *ex vivo*-Experimente wurden auch an menschlichem Dünndarmgewebe durchgeführt [74]. Neben der Gewebelagerung standen hier der Vergleich der Daten vom Schwein mit denen vom Menschen und die Variabilität zwischen den Probanden im Vordergrund. Die Daten wurden statistisch ausgewertet, um

eine Aussage über statistisch signifikante Unterschiede treffen zu können. Das Studienkollektiv umfasste 12 Probanden, die sich aufgrund verschiedener gastrointestinaler Erkrankungen wie Krebs, Sigmadivertikulitis oder Morbus Crohn einem chirurgischen Eingriff unterziehen mussten. Bei diesen geplanten Operationen wurde neben dem pathogenen Gewebe aus technischen Gründen auch etwas gesundes Gewebe entfernt, das im Rahmen der Studie verwendet werden konnte.

Betrachtet man die Ergebnisse der *ex vivo*-Humanstudie, fällt zunächst die große Variabilität der Messwerte auf. Sowohl interindividuell als auch intraindividuell lässt sich die große Streuung der Messwerte erkennen. Wie bei den *ex vivo*-Versuchen an Schweinedünndarmgewebe wurde auch in der Humanstudie der Einfluss der Lagerung ausgewertet. Betrachtet man die mit Einstellung B erhaltenen Werte der Sechsfachbestimmungen, so zeigt sich, dass die Verwendung von aufgetautem Darmgewebe keinen signifikanten Unterschied in der Mukoadhäsion gegenüber frischem Gewebe aufweist (Abbildung 4). Dies gilt sowohl für die Adhäsionsarbeit W_{ad} als auch für die maximale Abrisskraft F_{max}. Bei Einstellung A, die durch eine geringere Anpresskraft, Kontaktzeit und Abzugsgeschwindigkeit charakterisiert ist, konnte ebenfalls kein signifikanter Unterschied in der Auswirkung auf die Abzugskraft beobachtet werden. Bei der Adhäsionsarbeit hingegen konnten signifikant höhere Werte ($p < 0{,}001$) bei Verwendung des aufgetauten Gewebes festgestellt werden. Während des Einfrierprozesses kann es zu strukturellen Veränderungen des Mukus kommen [47,75]. Diese strukturellen Veränderungen könnten im Fall von Präparat A auf aufgetautem Gewebe zu einer höheren Adhäsionsarbeit geführt haben. Da das Auftauen in Einstellung A jedoch keinen Einfluss auf die Abzugskraft hat, stellt sich die Frage, welches dieser beiden Messergebnisse als geeigneter Surrogatparameter für Mukoadhäsionsstudien geeignet ist. Dieser Frage gingen das Neves et al. [57] in ihrer Arbeit über die Adhäsion halbfester Zubereitungen an der Vaginalschleimhaut von Rindern nach. Sie argumentierten, dass die Adhäsionsarbeit die Summe aller adhäsiven Bindungen darstellt, während die Abrisskraft nur die maximale Kraft während des Ablösevorgangs widerspiegelt. Dies ermöglicht eine umfassendere Bewertung der Ablösemechanismen. Da Silva et al. [76] gelangten in ihren Untersuchungen zum gleichen Ergebnis und konnten darüber hinaus beobachten, dass W_{ad} stärker auf Änderungen der Testparameter reagiert als F_{max}. Diese Beobachtung konnte in der Studie zur Mukoadhäsion von PVA-Filmen auf humanem Dünndarmgewebe bestätigt werden. Beim Vergleich der Einstellungen A und B auf aufgetautem Gewebe konnte eine signifikant höhere Adhäsionsarbeit ($p < 0{,}01$) mit Einstellung B gemessen werden (Abbildung 5). Ein Effekt auf die Abzugskraft konnte jedoch nicht beobachtet werden. Diese Ergebnisse verdeutlichen, dass in zukünftigen Arbeiten diese Einflussfaktoren berücksichtigt werden sollten. Abhängig von den Messparametern kann auch die Gewebelagerung einen Einfluss auf die Messergebnisse haben.

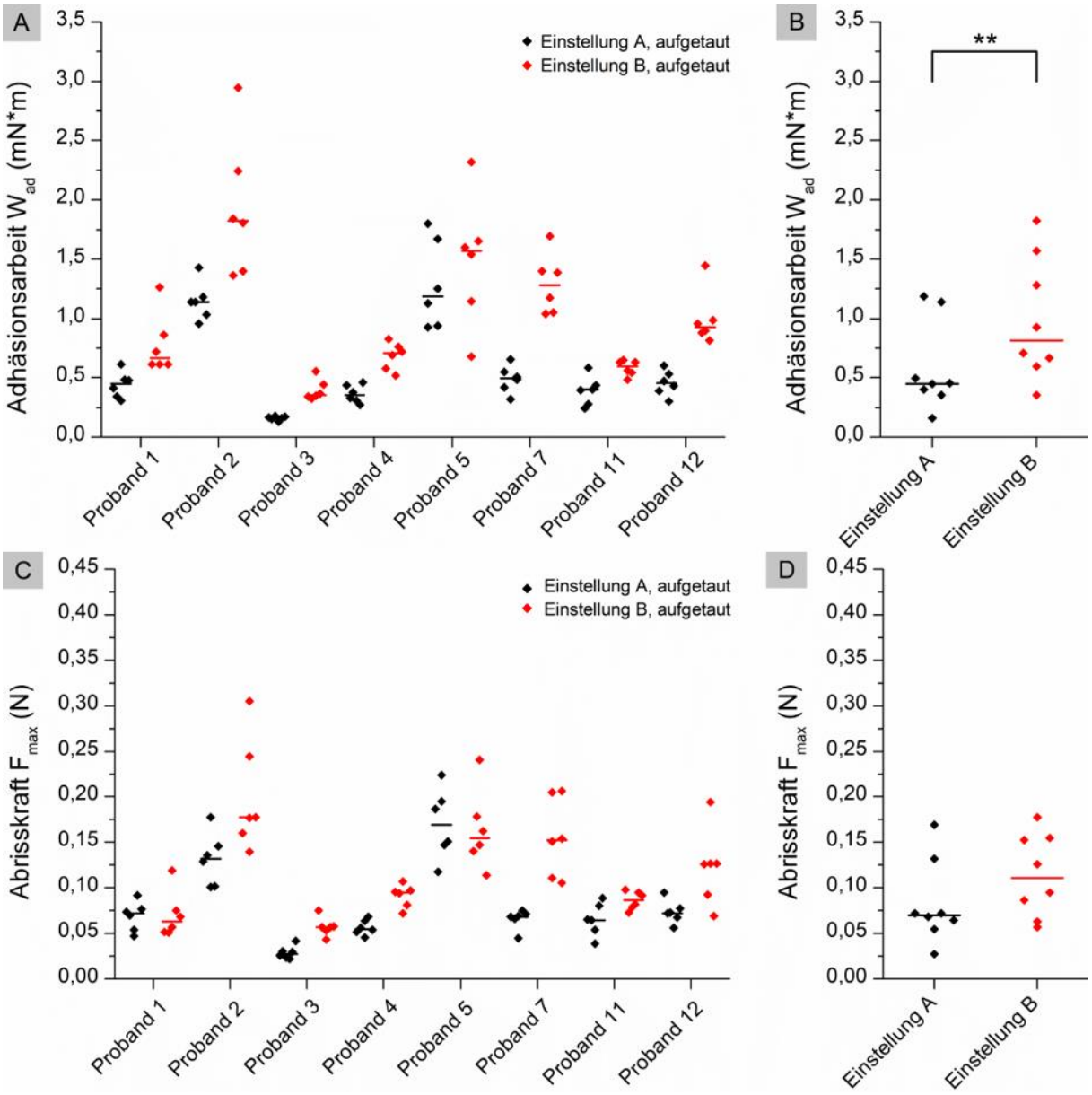

*Abbildung 5: Vergleich der Ergebnisse für Einstellung A und B an aufgetautem Gewebe. (A): Einzeldaten der berechneten W_{ad} (mN*m) mit Median (n=8); schwarz: Einstellung A; rot: Einstellung B; (B): gepoolte Mediane aller Probanden mit Medianlinie; (C): Einzeldaten der berechneten F_{max} (N) mit Median (n=8); schwarz: Einstellung A; rot: Einstellung B; (D): gepoolte Mediane aller Probanden mit Medianlinie. Ein signifikanter Unterschied zwischen W_{ad} und F_{max} wurde mit einem Wilcoxon Signed Rank Test überprüft: ** ($p < 0,01$).*

Das in den Versuchen verwendete gesunde Gewebe stammte von Patienten, die an unterschiedlichsten gastrointestinalen Erkrankungen litten. Der therapeutische Einsatz von mukoadhäsiven Filmen könnte prinzipiell sowohl zur Vorbeugung als auch zur Linderung von Erkrankungen erfolgen. Im Bereich der gastrointestinalen Erkrankungen sind hier beispielsweise chronisch entzündliche Darmerkrankungen zu nennen. Entzündliche Gewebe weisen andere Eigenschaften als gesunde Zellen auf, sodass hier möglicherweise eine Formulierungsanpassung an die erkrankte Applikationsstelle erfolgen muss. Insbesondere bei bereits erkrankten Patienten könnte neben histologischen Veränderungen auch die Medikation einen Einfluss auf die Adhäsion ausüben [77,78]. Medikamente wie Mukolytika beeinflussen die Struktur des Mukus, wodurch ein anderes Anhaftungsverhalten der Darreichungsform erwartbar wäre. Weitere Forschung auf diesem Gebiet sind notwendig, um den Einfluss von

Erkrankungen und der Begleitmedikation zu evaluieren und die mukoadhäsiven Darreichungsformen entsprechend den Anforderungen anzupassen.

Die Verwendung von menschlichem Gewebe ist ethisch wesentlich anspruchsvoller als die Verwendung von tierischem Gewebe. Aus diesem Grund werden Versuchstiere eingesetzt, um eine Annäherung an menschliche Präparate zu erreichen. Schweine werden in präklinischen Studien häufig als Modell für den menschlichen Gastrointestinaltrakt verwendet, da sie in bestimmten Grenzen eine ähnliche gastrointestinale Physiologie aufweisen [79,80]. Telemetrische Systeme wie die SmartPill® konnten bereits zur Messung physiologischer Zustände im GIT von Schweinen eingesetzt werden. Untersuchungen von Henze et al. [19] konnten zeigen, dass der pH-Wert des Dünndarms im Nüchternzustand im Duodenum zwischen pH 6,7 - 7,5 liegt, im Ileum wurden Werte zwischen pH 7,6 - 8,0 gemessen. Im Vergleich dazu lagen die Werte der menschlichen Probanden in der Studie von Schneider et al. [81] ebenfalls unter Nüchternbedingungen im Median bei pH 5,9 im Duodenum und pH 7,5 im Ileum. Die pH-Werte beim Schwein liegen also geringfügig höher, der pH-Gradient vom distalen zum proximalen Dünndarm ist jedoch bei beiden Spezies zu beobachten. Im nüchternen Zustand wurden beim Menschen mit Hilfe von Telemetriekapseln deutlich höhere Druckereignisse von 103 ± 65 mbar gemessen werden [82], beim Schwein berichten Henze et al. [83] von wesentlich geringeren Drücken, die maximal bei 99 mbar lagen. Als Modell für Untersuchungen, z.B. zur Beurteilung der Passagezeiten oder des Einflusses der Druckverhältnisse auf orale Darreichungsformen, ist das Schwein aufgrund seiner physiologischen Beschaffenheit nur bedingt geeignet. Hinsichtlich der makroskopischen und mikroskopischen Feinstruktur der tierischen Gewebe weisen diese eine sehr hohe histologische Ähnlichkeit zu physiologischem menschlichem Gewebe auf. Außerdem sind Gewebe des Schweins gut verfügbar, weshalb im Rahmen von *ex vivo*-Mukoadhäsionsstudien oft auf porcine Präparate zurückgegriffen wird [45,72,84,85]. Ob die Verwendung von porcinem Gewebe tatsächlich als Alternative zu humanem Gewebe dienen kann, sollte im letzten Schritt der systematischen Studie zur Mukoadhäsions-Messmethode evaluiert werden. Dazu wurden die Ergebnisse der beiden unterschiedlichen *ex vivo*-Experimente statistisch ausgewertet.

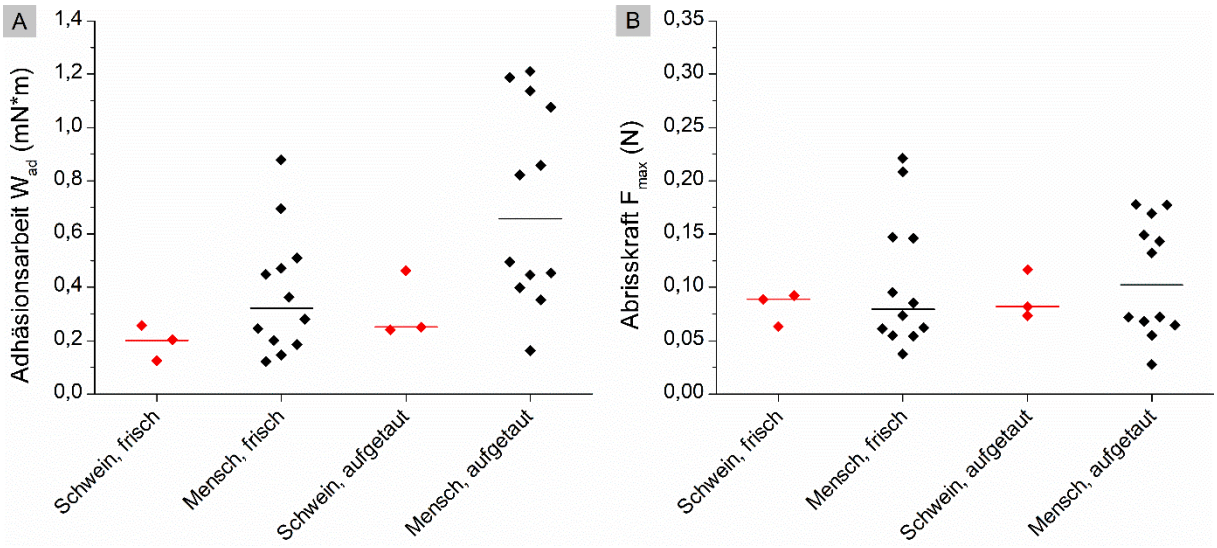

*Abbildung 6: Vergleich der Ergebnisse für Einstellung A an Dünndarmgewebe von Schweinen (n=3) und Menschen (n=12). (A): Gepoolte Mediane der berechneten W_{ad} (mN*m) mit Medianlinie; rot: Schwein; schwarz: Mensch; (B): Gepoolte Mediane der berechneten F_{max} (N) mit Medianlinie; rot: Schwein; schwarz: Mensch. Signifikante Unterschiede von W_{ad} und F_{max} wurden mit einem Mann-Whitney-U-Test überprüft.*

Obwohl die Anzahl der Versuchstiere bei den *ex vivo*-Versuchen an Schweinedünndarmgewebe mit n=3 zu gering war, um eine aussagekräftige Statistik zu erhalten, konnten erste Vermutungen abgeleitet werden. Weder für die Adhäsionsarbeit noch für die maximale Abzugskraft waren statistische Unterschiede zwischen menschlichem und Schweinegewebe erkennbar (Abbildung 6). Dies gilt sowohl für frische als auch für aufgetaute Präparate. Es konnte jedoch beobachtet werden, dass die Mediane der Adhäsionsarbeit bei menschlichem Gewebe höher waren als bei Schweingewebe. Möglicherweise ließe sich dieser Unterschied mit einer größeren Anzahl von Proben statistisch bestätigen. Die humanen Gewebeproben stammten von Patienten, die während der Operation nüchtern sein mussten, so dass diese Präparate frei von Nahrungsbestandteilen waren. Um einen möglichen Einfluss der Nahrung zu minimieren, wurden als „frische“ Schweinegewebeproben solche angesehen, die zuvor gereinigt worden waren. Der Reinigungsprozess könnte prinzipiell die Messergebnisse beeinflusst haben, indem der Mukus verdünnt oder sogar teilweise entfernt wurde. Jackson und Perkins [72] stellten in ihren Versuchen zur Mukoadhäsion von Cholestyramin an humaner und porciner Magenschleimhaut fest, dass die Adhäsion an porcinem Gewebe signifikant höher war als an humanem. Sie begründeten dieses Ergebnis mit der Beobachtung, dass die Schleimschicht des Schweinemagens makroskopisch dicker war als die der menschlichen Präparate. Einen Hinweis darauf, dass die Dicke der Mukusschicht einen Einfluss auf die Bioadhäsion hat, konnten auch Varum et al. finden [48]. Sie beobachteten eine stärkere Adhäsion von trockenen Carbopol 974P NF- und Ethylcellulose-Presslingen in Regionen des porcinen GIT, die mit einer dickeren Mukusschicht ausgekleidet waren. Sie beschreiben, dass eine dickere Schleimschicht auf eine tiefere Interdiffusion und Vernetzung zwischen Polymer- und Muzinketten und mehr verfügbare Gruppen für chemische Bindungen hindeutet. Gleichzeitig weisen die Autoren darauf hin, dass neben der Mukusdicke auch die Rheologie [86] und die Erneuerungsrate der Mukusschicht [87] die Adhäsion beeinflussen könnten.

Aus den durchgeführten Versuchen geht hervor, dass das Phänomen der Mukoadhäsion sehr komplex ist und von vielen Variablen abhängt. In einfachen *in vitro*-Versuchen mit biomimetischen Gelen können mögliche gerätespezifische Einflüsse untersucht werden. Die bisher sehr variablen Versuchsaufbauten und Messbedingungen zur Mukoadhäsion lassen eine Vergleichbarkeit der Daten zwischen verschiedenen Versuchen nicht zu, jedoch können die unterschiedlichen Messbedingungen und Parameter darreichungsspezifisch erforderlich sein, um eine relevante Aussage über die Leistungsfähigkeit der jeweiligen Darreichungsform zu erhalten. Welche physiologischen Faktoren *in vivo* oder *ex vivo* die Mukoadhäsion beeinflussen können, sollte in Zukunft durch geeignete Experimente evaluiert werden. Zur Generierung von Wissen über die physiologischen Bedingungen an den möglichen Applikationsorten können die bereits in der Einleitung beschriebenen telemetrischen Systeme eingesetzt werden. Aufgrund ihres nicht-invasiven Charakters wird als Vorteil die geringe Interferenz genannt [88]. Die meisten dieser Systeme bewegen sich passiv [89], d.h. sie werden durch peristaltische Bewegungen vorangetrieben [90]. Telemetrische Systeme haben jedoch auch Nachteile. Die Nachverfolgung der Systeme nach der Applikation ist oft nur teilweise gegeben, da die Ortung aufgrund von Druckereignissen oder pH-Änderungen oft ungenau ist und je nach System erst nach der Messung durchgeführt werden kann. Telemetrische Systeme sind große, unflexible Kapseln mit einer durchschnittlichen Größe von 20x10 mm [5]. Diese Eigenschaften beeinflussen die Magenentleerung des entsprechenden Objekts. Um sie durch den Pylorus zu befördern, sind starke Kontraktionen notwendig, wie sie während der Phase III des *Interdigestive Migrating Motor Complex* (IMMC) als zyklische Bewegungsmuster im Nüchternzustand auftreten [91,92]. Schluckbare Messsysteme können daher aufgrund ihrer Größe und Beschaffenheit nur im Nüchternzustand entleert werden und liefern somit keine Informationen über den physiologischen Zustand des Dünndarms im *fed state* [5]. Um diese Informationen zugänglich zu machen, muss bisher noch auf invasive Methoden wie die Verwendung von an Kathetern befestigten Sonden oder die Installation der Sensoren direkt im Dünndarm nach der Nahrungsaufnahme zurückgegriffen werden [93]. Die Entwicklung kleinerer, schluckbarer Messgeräte könnte in Zukunft helfen, diese Lücke zu schließen [94–96]. Gelingt es zudem, das Problem der Nachverfolgbarkeit der telemetrischen Systeme zu lösen, könnte neben der Gewinnung von *in vivo*-Daten auch die gezielte Freisetzung einer mukoadhäsiven Darreichungsform am vorgesehenen Applikationsort möglich werden. Mit IntelliCap®, IntelliSite und magnetisch getriggerten Kapseln stehen bereits intelligente Systeme für die gezielte Applikation von Wirkstoffen an spezifisch gewählten Abschnitten im GIT zur Verfügung [16,97–99]. Bisher sind die Reservoire dieser Kapselsysteme so ausgelegt, dass sie den Wirkstoff in flüssiger oder pulverförmiger Form aufnehmen können. Für die Applikation mukoadhäsiver Filme müsste daher entweder die Struktur des Systems oder die Form des Films verändert werden. Denkbar wäre hier z. B. die Verwendung vieler kleiner Filme, die im Reservoir gestapelt werden können, statt der Applikation eines großen Films [100].

3 Zusammenfassung

Innovative Wirkstoffe stellen die Entwickler und Entwicklerinnen von pharmazeutischen Darreichungsformen vor große Herausforderungen. Viele neue Arzneistoffe weisen eine unzureichende orale Bioverfügbarkeit auf. Die Gründe hierfür sind vielfältig und liegen unter anderem in der ortsabhängigen Löslichkeit und Permeabilität der betreffenden Substanzen. Dieser Problematik kann auf chemischer und technologischer Ebene begegnet werden. Auf der Seite der pharmazeutischen Technologie besteht beispielsweise die Möglichkeit, mukoadhäsive Darreichungsformen zu entwickeln. Darunter versteht man Systeme, die an der Schleimhaut haften und dadurch eine Retention des Arzneistoffes an der entsprechenden Stelle bewirken. Einerseits führt dies zu einer lokalen Erhöhung der Wirkstoffkonzentration, andererseits wird durch die Adhäsion die potenzielle Resorptionszeit verlängert. Beide Faktoren können sich positiv auf die Bioverfügbarkeit auswirken.

Zur Charakterisierung mukoadhäsiver Arzneiformen sind verschiedene Methoden beschrieben worden, von denen jedoch keine standardisiert ist. Mögliche Einflussfaktoren auf die Messung wurden in der Vergangenheit teilweise nicht ausreichend evaluiert, was die Konzeption einer geeigneten Messmethode erschwert. In der vorliegenden Arbeit wurde systematisch eine Methode zur Messung der Adhäsivität von Polyvinylalkohol-Filmen auf Basis der Messung der maximalen Haftkraft erarbeitet. Dazu wurde im ersten Teil der Arbeit ein *in vitro*-Test durchgeführt, der zur Aufklärung gerätespezifischer Einflussfaktoren diente. Es zeigte sich, dass die Adhäsion von PVA-Filmen an biomimetischen Agar/Muzin-Gelen in erster Linie zeitabhängig ist. Das Maximum der Adhäsion konnte nach 3 min beobachtet werden. In dieser Zeit kommt es zu einer Quellung des zuvor festen Films, wodurch die Beweglichkeit der Polymerketten zunimmt. Dies kann die Vernetzung von Polymer und Muzin begünstigen, wodurch die Adhäsion zunimmt. Nach Überschreiten eines Maximums kommt es zu einer Überhydratisierung des Systems und zu einer Abnahme der Kohäsion, so dass der Gelfilm beim Entfernen der Messsonde in sich reißt.

Die Geschwindigkeit, mit der die Sonde entfernt wurde, beeinflusste ebenfalls die Ergebnisse der Untersuchungen. Höhere Geschwindigkeiten führten zu höheren berechneten Adhäsionsarbeiten W_{ad}, während die maximale Abrisskraft F_{max} ein Plateau erreichte. Dieses Verhalten könnte durch die viskoelastischen Eigenschaften der beteiligten Bindungspartner beeinflusst werden.

Bezüglich der gerätespezifischen Parameter schien die Anpresskraft des Mukoadhäsivs an das biomimetische Gel den geringsten Effekt auszuüben. Unter Berücksichtigung der verwendeten Kraftmesszelle und der Integrität des Gels sollten höhere Anpresskräfte verwendet werden. Je nach Applikationsort sind physiologische Drücke zu berücksichtigen.

Ausgehend von den *in vitro* gewonnenen Erkenntnissen wurden anschließend zwei *ex vivo* Versuche durchgeführt. Diese Versuche konzentrierten sich auf die Eigenschaften der Substrate selbst, auf denen die PVA-Filme haften sollten. Der Einfluss der Präparation der verwendeten Gewebe auf die Adhäsion wurde sowohl an Dünndarmpräparaten vom Schwein als auch am Menschen untersucht. Die Gewebe

wurden zum einen im frischen Zustand unmittelbar nach der Entnahme für die Mukoadhäsionsmessungen verwendet, im zweiten Versuchsabschnitt wurden sie für eine Woche im Gefrierschrank gelagert und für die Experimente aufgetaut. Bei den Schweinedärmen stellte sich die Frage, ob die Reinigung der frischen Därme einen zusätzlichen Effekt haben könnte. Aufgrund der geringen Probenzahl konnte kein eindeutiger Trend festgestellt werden. Bei zwei von drei Versuchstieren erhöhte sich die errechnete Arbeit (W_{ad}) durch die vorsichtige Reinigung. Beim Auftauen der gereinigten Gewebe konnte bei allen Versuchstieren ein Anstieg der W_{ad} sowie der maximalen Abrisskraft beobachtet werden. Diese Daten wurden statistisch mit den Ergebnissen der *ex vivo*-Humanstudie verglichen. Das Probandenkollektiv, dessen Daten in die Untersuchungen einflossen, umfasste insgesamt 12 Teilnehmer, die sich aufgrund verschiedener gastrointestinaler Erkrankungen einer geplanten Operation unterziehen mussten. Gesundes Dünndarmgewebe, das aus operationstechnischen Gründen zusätzlich zum erkrankten Gewebe entfernt werden musste, wurde im Rahmen der Studie verwendet. Der statistische Vergleich der Gewebe unterschiedlicher Herkunft zeigte sowohl im frischen als auch im aufgetauten Zustand keine signifikanten Unterschiede. Bei Betrachtung der einzelnen Messwerte konnte jedoch festgestellt werden, dass die berechneten W_{ad} bei menschlichem Gewebe etwas höher lagen als bei Schweinepräparaten. Eine größere Probenzahl könnte einen möglichen statistisch signifikanten Unterschied aufzeigen. Bei menschlichem Gewebe konnte außerdem festgestellt werden, dass die Verwendung einer Messeinstellung mit höherer Anpresskraft, längerer Kontaktzeit und schnellerem Entfernen der Messsonde zu signifikant höheren W_{ad} führte (Einstellung A vs. Einstellung B). Bei allen Messungen wurde deutlich, dass die Adhäsionsarbeit empfindlicher auf Änderungen der Messparameter reagiert und daher möglicherweise der geeignetere Surrogatparameter für die Quantifizierung der Mukoadhäsion ist. Insgesamt zeigte sich bei den biologischen Präparaten eine deutlich größere Variabilität der Messwerte als bei den *in vitro*-Versuchen. Bei dem humanen Gewebe der *ex vivo*-Studie handelte es sich um Gewebe, welches von der für den Eingriff ursächlichen Erkrankung nicht betroffen war. In diesem Zusammenhang ist die große interindividuelle Variabilität der Messwerte auf der gesunden Schleimhaut hervorzuheben. Bei der Anwendung mukoadhäsiver Filme auf histologisch veränderter, pathogener Schleimhaut könnte die Mukoadhäsion möglichweise sogar weitaus variabler sein, da in diesem Fall neben der histologischen Veränderung auch eine mögliche Begleitmedikation eine Rolle spielen könnte. Diese Aspekte sollten bei dem Design einer Darreichungsform, aber auch eines geeigneten Testsystems zukünftig berücksichtigt werden.

Die in dieser Arbeit gewonnenen Erkenntnisse können zur weiteren Optimierung und Validierung einer Messmethode für die Mukoadhäsion beitragen, um die Genauigkeit und Zuverlässigkeit der Ergebnisse zu erhöhen. Um die biologische Relevanz dieser *in vitro*-Experimente zu verbessern, können physiologische Daten aus telemetrischen Systemen genutzt und in den experimentellen Aufbau implementiert werden.

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5 Veröffentlichungen

5.1 Ingestible devices for studying the gastrointestinal physiology and their application in oral biopharmaceutics

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Ingestible devices for studying the gastrointestinal physiology and their application in oral biopharmaceutics

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ABSTRACT

Ingestible sensor systems are unique tools for obtaining physiological data from an undisturbed gastrointestinal tract. Since their dimensions correspond to monolithic oral dosage forms, such as enteric coated tablets or hydrogel matrix tablets, they also allow insights into the physiological conditions experienced by non-disintegrating dosage forms on their way through the gastrointestinal tract. In this work, the different ingestible sensor systems which can be used for this purpose are described and their potential applications as well as difficulties and pitfalls with respect to their use are presented. It is also highlighted how the data on transit times, pH, temperature and pressure as well as the data from different animal models commonly used in drug product development such as dogs and pigs have contributed to a deeper mechanistic understanding of oral drug delivery.

Contents

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1. Introduction

Detailed knowledge of the physiological processes in the gastrointestinal tract is essential for the development of orally administered drugs. This is particularly true for the development of dosage forms, the development and implementation of biorelevant *in vitro* test procedures and the development of *in silico* models. In view of the fact that absorption from the gastrointestinal (GI) tract represents a challenge for the delivery of most modern drugs due to their molecular size, their low water solubility, and their tendency to food effects, it is becoming increasingly important to recognize and exploit opportunities arising from the physiology of the GI tract in drug development and also to identify problems at an early stage. The hope of developing oral dosage forms in the future for drugs previously considered non-oral, such as peptides or even proteins, may even depend on the extent to which we succeed in creating drug delivery systems that specifically exploit the physiology of the GI tract. Given the still great importance of animal models for drug development, it is not enough to know only the physiology of the human GI tract. Rather, a detailed knowledge of commonalities and peculiarities of the GI tract of different species that is of great relevance for drug development. Swallowable sensors nowadays represent an important tool for obtaining the physiological data required for successful drug product development from a gastrointestinal tract that is as undisturbed as possible. In the following, swallowable sensor systems will be presented, potential applications will be exemplified, major findings will be presented, and finally, difficulties and pitfalls will be highlighted.

2. A brief view in the history

The interest in being able to see inside the body and thus to understand the processes taking place in the body has driven scientists at least since the beginning of the Renaissance. Investigations in living organisms proved to be essential for understanding physiological processes. Pioneers such as René-Antoine Ferchault de Réaumur (1683–1757) and Lazarro Spallanzani (1729–1799) were able to show, for example, by means of food placed in small metal cages into animal stomachs, that digestion is a chemical process and not a mechanical one, as was previously mostly assume [1,2]. As experience grew, it also became clear that measurements on living organisms should be carried out as far as possible without any interference from the experimental design. Consequently, the importance of non-invasive measurement methods in studies of physiology as well as in medical diagnostics is still high today.

The invention of the transistor in 1948 by John Bardeen, William Shockley and Walter Brattain working at Bell Laboratories opened the door for the development of miniaturized electronic devices [3]. It was soon realized that this would allow the construction of self-contained radio transmitters so small that they could be swallowed like a "pill". In 1957, Vladimir Zworykin and John Farrar as well as Stuart Mackay and Bertil Jacobson independently reported about the first development and successful testing of ingestible measuring devices they called "radio pill" and "endoradiosonde", respectively [4,5].

The fundamental structure of swallowable telemetric measuring systems was already described at that time [6]. They consist of a miniaturized and thereby swallowable transmitter and an external receiver module. The ingestible transmitter contains a transducer, a power supply, and a transmitter unit with antenna. The receiver module consists of a receiving antenna, a receiver with data processing and an output unit or data memory.

From the beginning, the use of miniaturized medical devices as implantable or ingestible sensors and actuators was considered, either to obtain information from inside the body or for interventions such as the pacemaker [7]. In the case of ingestible devices, it was very soon recognized that measurements in regions of the gastrointestinal tract that were difficult or even impossible to reach by means of the then already established tubes were of particular interest [6]. Likewise, from the beginning, the focus was on the small intestine and the proximal colon. Also, the objectives were mainly temperature measurement, determination of chemical and enzymatic parameters, as well as pressure sensing [4–6].

Within a few years, a whole range of different telemetric sensors had been developed and used in studies. Most systems included a battery for power supply, but there were also inductively charged systems, as well as passive systems. Mainly either temperature or pressure was measured, occasionally also pH values. Frequencies between 350 and 2000 kHz were used as transmission frequencies [8].

Applications in the gastrointestinal tract were diverse; in addition to measuring gastrointestinal motility, measurements had already been made to determine core body temperature as well as the influence of food on pH in the stomach and on body temperature [6,9]. As early as 1958, the pH gradient between the stomach and duodenum was demonstrated [10]. Applications were not limited to the gastrointestinal tract. For example, pressure measurements were also made using a pressure probe inserted into the uterus during coitus as well as labor and fetal heartbeats were measured [11,12]. Furthermore, tooth contact patterns were determined using a small pressure sensor [13].

Since then, many other technical developments have taken place, microelectronics and computer technology have revolutionized our lives and also found their way into the further development of smart ingestible devices for diagnostics. Transmission technology has also evolved, with the question of which transmission frequency to use depending on a whole range of factors, such as the data transmission rate, the amount of data, the frequency-dependent attenuation due to the fabric, the required range, the power requirements, the transmission rate, the susceptibility to interference, the use by competing applications, the antenna geometry and others. There are quite a number of excellent reviews on the state of the art in telemetric systems, to which reference is made at this point [14–17].

3. State-of-the-art telemetric ingestible devices

3.1. Temperature sensors

Body temperature is controlled by the hypothalamus through a balance of heat loss, heat absorption and heat regeneration. The body can roughly be divided into a peripheral and a core compartment [21]. The core compartment contains vital organs such as those of the digestive tract. This compartment is characterized by a relatively stable temperature in a narrow range [22]. Normal core body temperature is in the range of 36.5–38.5 °C [23].

An increase in body temperature may indicate various diseases, especially infections [24]. Close monitoring of body temperature

Table 1
Ingestible sensors with wireless signal transmission.

Device	Company	Measured parameter (s)	Dimensions (mm)	Battery life (h)
e-Celsius	BodyCap	Temperature	18x9	480
CorTemp	Hqinc	Temperature	22x11	168–240
Equivital Life Monitor [a]	Equivital	Temperature	23x9	240
myTemp[b]	myTemp	Temperature	20x8	Infinite (no battery)
Heidelberg pH capsule	Heidelberg Medical	pH	20x8	>12 (>24)[c]
Bravo™ pH capsule	Medtronic	pH	25x6	up to 96 h
Proteus Pill	-[d]	pH	1x1x0,3	Electromagnetic actuation
Vibrant Capsule	Vibrant	Vibrational actuator	24x11	3 cycles per min total 240 cycles
SmartPill	Medtronic	Temperature, pH, pressure	27x12	>120
IntelliCap	-[e]	Temperature, ph, additional drug delivery reservoir	26x11	
Atmo Gas capsule	Atmo Biosciences	Temperature, H_2, O_2, CO_2, CH_4[f]	25x10	Temperature: up to 720 Gas sensing: up to 240

[a]Also known as VitalSense; [b]in development; [c]12 h medical version, 24 h pharmaceutical version; [d]discontinued (formerly Proteus Digital Health); [e]discontinued (formerly Medimetrics); [f]H_2S and short chain fatty acids (SCFA) announced.

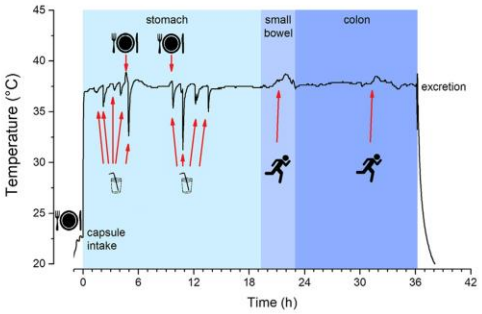

Fig. 1. Temperature profile obtained after intake of an ingestible sensor after breakfast.

plays an important role in the early detection of perioperative hypothermia [25,26]. Malignant hyperthermia triggered by anesthetic medication during surgery can also pose a risk to the patient which could be detected using a telemetric pill. Another field of application of ingestible temperature measurement systems can also be temperature control during acute patient care. Ingestible telemetric temperature sensors have also gained widespread use in ambulatory field-based applications like successfully in numerous sport and occupational applications such as the continuous measurement of body core temperature in deep sea saturation divers, distance runners and soldiers undertaking sustained military training exercises [27]. As ingestible temperature sensors have been found to show systematic bias, it is recommended to correct sensor temperature to a reference thermometer by linear function reaching an accuracy within ± 0.1 °C [28].

There are several methods to measure the body temperature. Among them, the most accurate measurement is achieved with a catheter in the pulmonary artery. Apart from this surgical method, there is the possibility to measure the temperature using rectal thermometers or an esophageal measurement at the level of the left atrium [23,29]. The esophageal method is invasive and both methods are critical in terms of patient compliance. Thus, ingestible systems for measuring gastrointestinal temperature represent a valuable alternative as a surrogate parameter for core body temperature. Furthermore, the tympanic membrane temperature measured via infrared thermometry is under discussion as a safe and least invasive method for the determination of the body core temperature. However, is not standard to-date [30]. A variety of swallowable temperature measurement systems have been developed. Available products are listed in Table 1. A typical temperature profile measured in a healthy volunteer that swallowed a telemetric capsule after breakfast is shown in Fig. 1, further examples are given in Figs. 6 and 7.

3.2. pH sensors

In 1964, the so-called Heidelberg pH capsule (Fig. 2A) was introduced by Nöller *et al.* [31], as a single use telemetric pH sensor system. The power supply is provided by means of an integrated battery, which consists of a silver chloride electrode and an outer antimony electrode separated by a membrane. The battery is activated by adding a sodium chloride solution as electrolyte. The voltage is approximately 1.5 V and was originally maintained for 6–8 h after activation. The effective voltage depends on the proton concentration at the outer antimony electrode. Changes in the output voltage result in frequency changes of the oscillator. After calibration the signal is linear in the range of pH 2.0–7.0 and measurement accuracy is in the range of 0.5 pH units [32]. The system is still available with modifications in a version for clinical use with at least 12 h battery life and a version for pharmaceutical research with a battery life of at least 24 h (Table 1). The clinical applications are predominantly gastric pH measurement mainly for the diagnosis of hyperchlorhydria, achlorhydria, hypochlorhydria and pyloric insufficiency (duodenal reflux). The Heidelberg pH capsule is also used to test for dumping syndrome, and delayed stomach emptying. Like all wireless transducer systems, the capsule is not a stand-alone device. The system also consists of a transceiver with antenna that is worn by the patient and a transceiver that receives the data and sends it via telemetry to a computer where the information is stored and displayed.

Another clinically applied ingestible pH sensor is the Bravo™ pH capsule (Fig. 2B) that is used for the investigation of esophageal reflux. The oblong single use sensor capsule is placed during endoscopy 5 cm proximally to the upper margin of the lower esophageal sphincter (LES). The attachment is achieved using a specialized delivery system. During the placement procedure, a piece of the esophageal sphincter is sucked into a well of the capsule using a vacuum and then attached by means of a spring-loaded stainless steel pin that is driven through the well of the pH capsule, tangential to the axis of the esophagus fixing the esophageal mucosa within the well [18]. As the esophageal mucosa heals, the capsule usually falls off within 5–10 days, passes through the gastrointestinal tract, and is eventually excreted. The capsule has an antimony pH electrode and a reference electrode located at its distal tip with an internal battery. Data security is accomplished by digital data transmission and by giving each capsule a unique identification code that is transmitted every 12 s along with two pH data points obtained at 6 s sampling intervals.

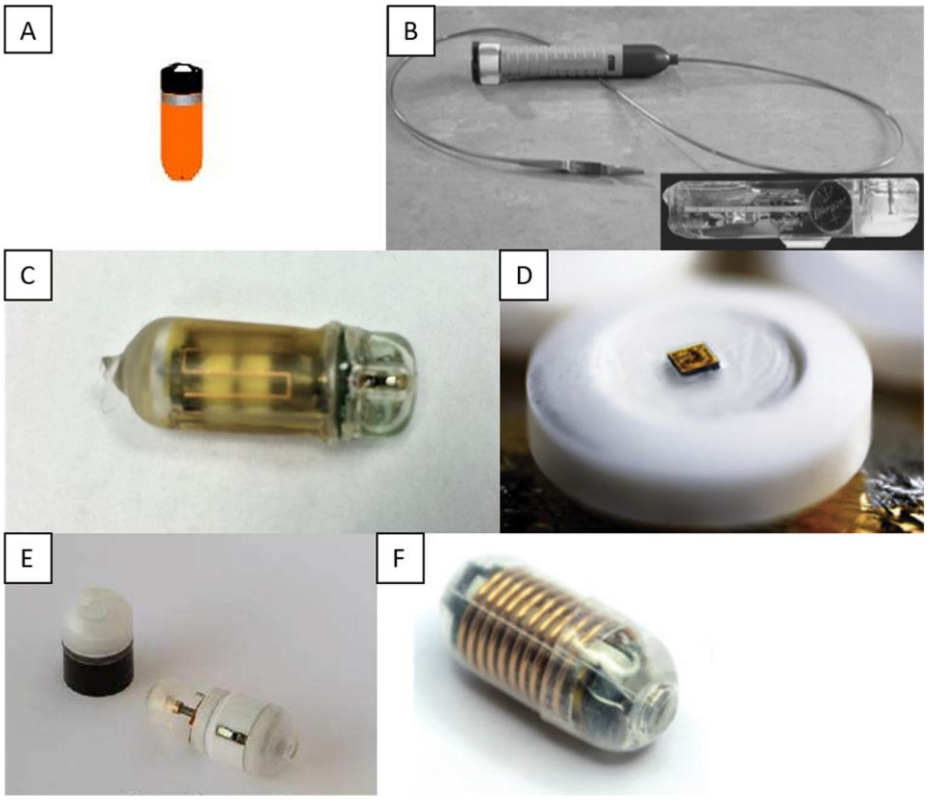

Fig. 2. A: Heidelberg pH capsule (copyright Heidelberg Medical, reprinted with permission), B: Bravo™ capsule (reprinted with permission from Pandolfino *et al.* [18]), C: SmartPill, D: Proteus capsule (reprinted with permission from Bettinger *et al.* [16]), E: IntelliCap (reprinted with permission from Söderlind *et al.* [19]), F: Atmo Gas (reprinted with permission from Kalantar-Zadeh *et al.* [20]).

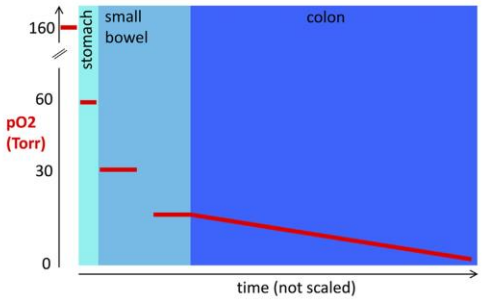

Fig. 3. Oxygen partial pressures in the different regions of the gastrointestinal tract (adapted from Kalantar-Zadeh et al. [15]).

3.3. Pressure sensors

The SmartPill® (Fig. 2C) is a single use capsule sensor for determining gastrointestinal pressure (Table 1). In addition to the pressure sensor (calibrated range 0–350 mmHg), it also contains a temperature sensor (range 25–49 °C) for detecting excretion and a pH sensor (range pH 0.05–9.0) for the determination of its location in the gastrointestinal tract [33]. Data is transferred in real time to a wearable external data receiver and can be displayed and analyzed using specialized software. Clinically, it finds particular application in the diagnosis of motility disorders such as delayed gastric emptying (gastroparesis) and chronic constipation by determining transit times through the stomach, small intestine and colon [34] which is predominantly based on the pH data. The investigation of gut motility by using the pressure data is difficult due to missing information on peristaltic wave propagation [35]. Although there is evidence of abnormal pressure patterns in some cases of gastroparesis [36] and chronic constipation [37], there is no clearly defined clinical application for pressure data to date [38].

3.4. Gas sensors

Atmo Biosciences is currently developing an ingestible gas sensor (Fig. 2F). The single use capsule includes a temperature sensor, gas sensors, a microcontroller, a transmission system and silver oxide batteries. The oxygen sensor is non-specific and responds to all oxidizing gases. Sensor data is encoded and transmitted every

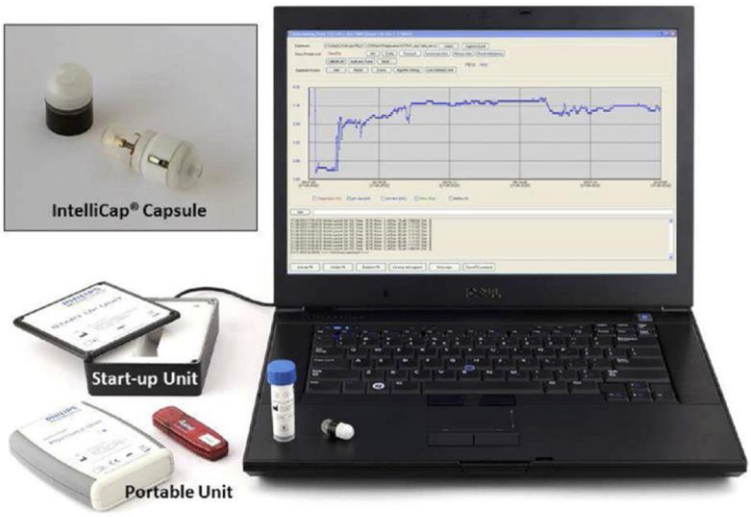

Fig. 4. The IntelliCap System (reprinted with permission from Söderlind et al. [19]).

5 min to a handheld monitor connected via Bluetooth to a cell phone application. To extend the functional life of the gas sensor system to more than 4 days, the microcontroller puts the capsule into a sleep mode when it is idle. The temperature sensor transmits independently for nearly 30 days so that excretion can be monitored [20,39,40]. In Fig. 3, a schematic representation of the oxygen partial pressures in the different regions of the gastrointestinal tract is shown.

3.5. Sensor devices with drug delivery unit

Medimetrics, a spin off from Phillips, developed an ingestible electronic drug delivery and monitoring system called IntelliCap (Fig. 2E). The first application in humans was reported in 2011 [41]. The electronic capsule consisted of a drug reservoir with a volume of 300 μL, a pH and temperature sensor, a microprocessor and wireless transceiver, a stepper motor, and batteries (Fig. 4) [41,42]. The pH data were used for the determination of the location of the capsule in the gastrointestinal tract, the temperature sensor for the determination of excretion. The stepper motor could be remotely actuated to either expel the contents of the drug reservoir or to suck probes into the reservoir. The liquid contents of the drug reservoir could be expelled through dispensing holes by the motion of a piston driven by the stepper motor via a screw-rod mechanism. Evidence that the IntelliCap is suitable for both prolonged gastrointestinal drug delivery and remotely targeted gastrointestinal drug delivery has been successfully demonstrated in humans [19]. The IntelliCap was the first commercialized ingestible electronic drug delivery device. It was discontinued in 2017.

3.6. Ingestible camera devices

With the great advances in microelectronics, it also became possible over time to realize miniaturized transmitters with the ability to transmit much higher amounts of data, as required for the transmission of image data. In 2001, the first swallowable capsule endoscope with wireless signal transmission was introduced to the market [43,44]. Since then, several different capsule endoscopes (“pill cameras”) have become available (Table 2, Fig. 5).

The images acquired during video capsule endoscopy (VCE) are mostly transferred to recording systems, where they are stored and evaluated at a later time. VCE is an established modality for the investigation of occult gastrointestinal bleeding and obscure iron deficiency anemia. It is also used for the diagnosis of Crohn’s disease and polyposis syndromes [45,46]. It is likely that diagnostic outcome will be further improved using artificial intelligence in data analysis [47]. For the investigation of the esophagus and the stomach, a magnetically controlled magnetically capsule endoscope called NaviCam is currently in clinical trials. The video capsule is attached to a detachable string to control the speed of esophageal transit. After arrival in the stomach the capsule endoscope is released and taken under external magnetic control in order to examine the lining of the stomach [48,49].

Compared to the use of ingestible sensor systems the number of applications of capsule endoscopes is much higher. This allows reliable statements to be made about the risks associated with the use of such ingestible devices. Capsule endoscopy is considered as a safe procedure, the most common complication is retention of the capsule in the gastrointestinal tract [50]. According to a general definition, one speaks of retention if the capsule has still not been excreted 2 weeks after its ingestion [51].

The incidence of retention is independent of patient age and capsule size, but is strongly influenced by gastrointestinal disease and is approximately 0.7–2% for capsule endoscopy in clinical applications [50,52,53]. In most cases of retention, spontaneous excretion of the capsule occurs, although this may take months. In cases of complications or discomfort, as well as obstructive disease, endoscopic or, if necessary, surgical removal of the capsule is performed [50]. In a clinical study with healthy subjects aged 18–70 years, retention of the capsule was not observed in any case in 773 applications [54]. This observation highlights the safety of using oral capsules in healthy subjects, as is often done to study physiology.

Fig. 5. PillCam SB3 (reprinted with permission from Medtronic), B: PillCam Colon 2 (reprinted with permission from Medtronic), C: PillCam Crohns (reprinted with permission from Medtronic), D: MiroCam (reprinted with permission from Intromedic), E: Endocapsule (reprinted with permission from Olympus) F: OMOM (reprinted with permission from Jinshan).

3.7. Patency devices

The risk of retention of the insoluble capsule endoscopes is particularly high in patients with Crohn's disease. To avoid this risk, special capsules of the same size as the capsule endoscopes have been developed to allow testing for intestinal patency in high-risk patients prior to capsule endoscopy. These capsules are referred to as patency capsules (PC). The Agile® patency capsule consists of a small (3 mm × 14 mm) radio frequency detectable identification tag (RFID) in a shell of lactose and barium sulfate as X-ray contrast agent [55]. At the ends are two caps that dissolve after 30 h. As soon as moisture enters through one of the two openings generated by the dissolved cap, the capsule disintegrates and the components, with the exception of the small RFID, finally dissolve. Due to its small size, the RFID can pass through the stenosis. The presence of the PC in the gastrointestinal tract can be verified by X-ray or with an RFID detector. The loss of signal from the radiofrequency identification tag, or absence of the device on radiological imaging, within less than 30 h after administration implies unimpeded intestinal transit. The procedure has been shown to be safe, efficient and cost-effective [56–58].

3.8. Further ingestible devices

A unique system is the Proteus pill (Fig. 2D), which has been developed to monitor the adherence of patients to their oral medication. The pill contains an integrated circuit that is powered by an Mg–Cu electrochemical couple. When activated by contact with gastric acid data is transmitted to the external receiver [16]. This makes it possible to monitor the intake of the labeled dosage form.

Another ingestible electronic device is the Vibrant capsule. It is not a sensor system and is listed here only for completeness. The

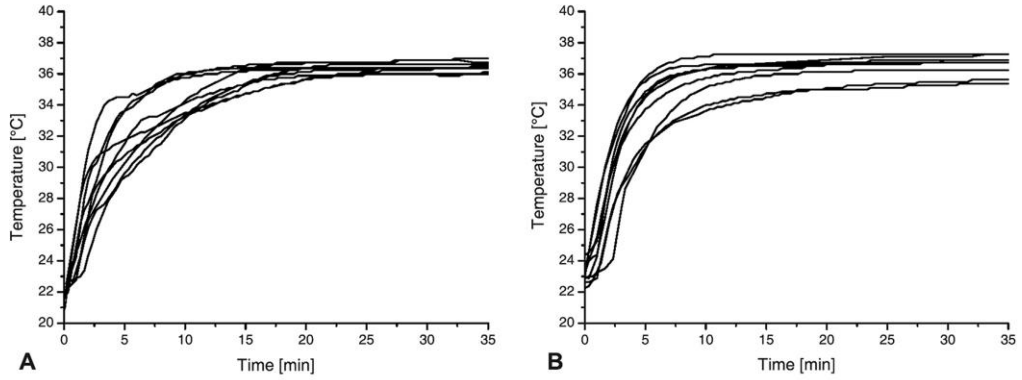

Fig. 6. Temperature profiles obtained after ingestion of a SmartPill under fasted (A) and fed state conditions (B) together with 240 mL of water (modified from Schneider *et al.* [72]).

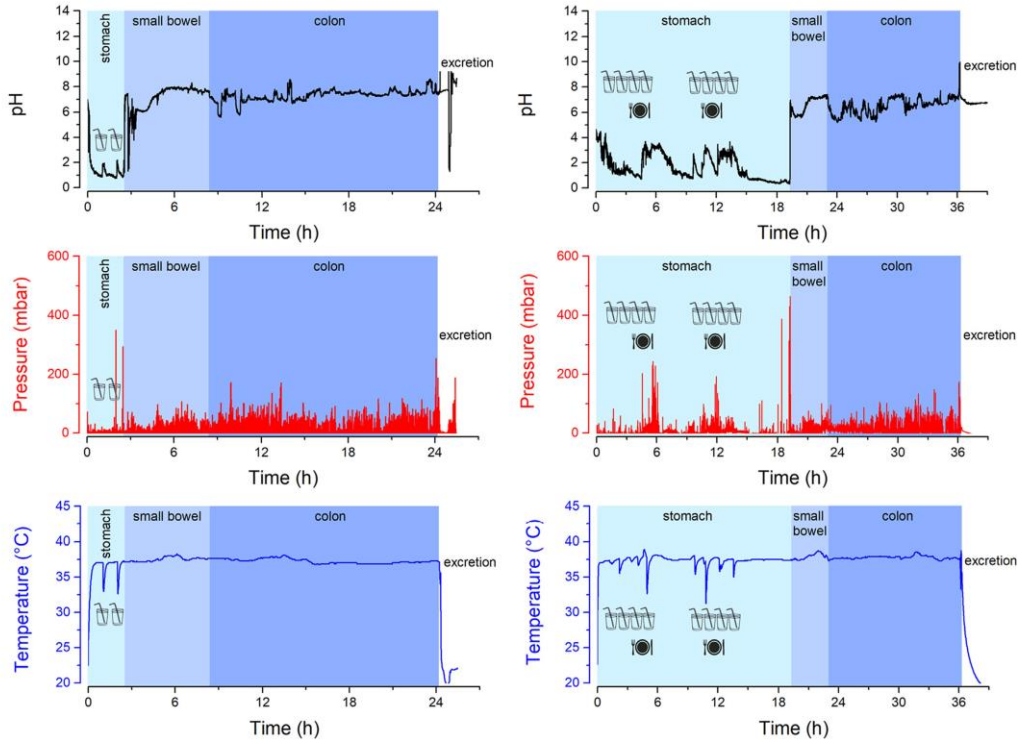

Fig. 7. Exemplary pH (top), pressure (middle) and temperature (bottom) profiles over time obtained after SmartPill administration fasted (left side) and 30 min after beginning of intake of the FDA standard breakfast (right side).

Vibrant capsule is designed to vibrate in the colon, inducing spontaneous bowel movements. The timing of vibration can be programmed, it is usually set to 6–8 h after ingestion [59]. According to the results of two clinical trials, it can potentially induce bowel movements, though the response rate is not different from placebo [60].

In addition to capsule endoscopy, other developments in ingestible sensors for medical diagnostics include ultrasound capsules,

Table 2
Available types of capsule endoscopes with wireless signal transmission.

Device	Company	Application	Frames per sec	Dimensions(mm)	Weight (g)	Battery life (h)
PillCam (SB3)	Medtronic	Small bowel	2–6	26x11	1,9	11
PillCam Colon 2	Medtronic	Large bowel	4–35*	32x12	2.9	10
PillCam Crohns	Medtronic	Small and large bowel	4–35*	32x12	2.9	10
MiroCam	Intromedic	Small bowel	3	24x11	3.25	12
Endocapsule	Olympus	Small bowel	2	26x11	3.3	12
OMOM	Jianshan	Small bowel	2	28x13	4,5	10
NaviCam SB	AnX Robotica; Ankon	Small bowel	0.5–6	27x12	–	8

*Depending on movement speed.

tactile capsules, thermometry capsules and non-white light imaging capsules [61–63].

There are a number of further interesting developments in the field of ingestible telemetric measurement devices and also in the field of interventional devices and of so-called intelligent or smart drug delivery systems, the presentation of which is beyond the scope of this review. Reference should therefore be made here to a number of reviews on these topics [15,17,64–71].

4. Human physiological data and examples for pharmaceutical applications

4.1. Temperature

The determination of core body temperature by means of ingested sensors is very reliable as long as it is ensured that the sensor capsule is in the intestine and not still in the stomach. If the probe is in the stomach, the measured temperature will be influenced by the temperature of the liquid or food ingested. When taking a dosage form together with 240 mL of water at room temperature (approx. 20 °C), as it is recommended in clinical studies, the temperature in the stomach drops to values between approx. 22 °C and 30 °C during fasting intake, depending on the volume of gastric juice present. On average, a temperature of 36 °C is reached in the stomach within about 20 min after fasting ingestion (Fig. 6A) [72]. When ingested after the high-calorie breakfast used in food effect studies [73], rewarming is faster due to the higher filling volume (Fig. 6B) [72].

The temperature effect of the co-ingested water is neglected in most disintegration and dissolution tests. Typically, the temperature is kept constant at 37 °C, which is obviously not biorelevant. However, the effect of temperature on the disintegration of dosage forms can be very pronounced as has been shown several times for gelatin hard capsules in release tests using realistic temperature gradients [74–76].

In combination devices with multiple sensors the temperature sensor is used to determine excretion, since the exit from the body can be easily identified as a rapid drop in temperature (Fig. 1).

4.2. pH

It was shown in previous chapters that various telemetric capsules (e.g. SmartPill, IntelliCap, Heidelberg pH capsule, Bravo pH capsule) are able to sense luminal pH values. In most systems, ion-sensitive field-effect transistor (ISFET) sensors are used for this purpose [77]. These sensors are small in size and allow the determination of luminal pH values in high temporal resolution. The data from these pH measurements therefore provide a detailed picture on luminal pH profiles. Apart from informing on pH changes caused by different prandial conditions, certain diseases or in certain populations, these values can also be used to determine gastric emptying time (GET) or colon arrival time (CAT). This is possible since distinct pH profiles are typically observed for stomach, small intestine and colon. For instance, the decision on transit times in the SmartPill GI monitoring system is mainly based on characteristic pH changes [78].

The luminal pH value is of paramount importance for drug release as many drugs as well as excipients are characterized by pH-dependent solubility. Especially if the luminal pH is around the pK_a value of a substance, even small changes in pH can have dramatic consequences on drug solubility (particularly for weak acids or bases) or formulation performance (e.g. enteric coated formulations) [79]. In pharmaceutical sciences, the view on luminal pH was rather simple for a long time: acidic conditions (pH 1–2) in stomach, neutral conditions (pH 6–7) in small intestine and slightly acidic conditions (pH 5–6) in colon. This view was also reflected in dissolution testing, in which rather static conditions were simulated in terms of pH. However, the broader application of telemetric capsules by biopharmaceutical scientists revealed that the pH value in the human GI tract is highly dynamic. One of the first studies, which had a broader impact in this respect, was a study published 1990 by Dressman and colleagues which was performed with the Heidelberg pH capsule [80]. This study nicely demonstrated the dynamic nature of luminal pH values in the upper GI tract. More than 20 years after this study, our group designed a SmartPill study to investigate the GI conditions in healthy volunteers under conditions, which should resemble the conditions present in bioequivalence and bioavailability studies. In Fig. 7, exemplary pH, pressure and temperature profiles from this study are depicted [81].

The first thing that is obvious from the figure is the long gastric transit time. It should be noted that large, indigestible objects can only be emptied from the stomach by forceful contractions associated with the Interdigestive Migrating Motor Complex (IMMC), a cyclic motility pattern present in fasted state. Thus, even if telemetric capsules are administered in fed state, they will stay in the stomach until recurrence of fasted conditions. Additional meal intake prolongs gastric transit as can be seen from the figure. Thus, telemetric capsules are not able to measure intestinal pH values present after food intake, since they only enter the small intestines in fasted state. Such data can only be obtained by techniques, in which a catheter or pH sensor is fixed in the small intestine [82].

It can further be seen from the figure that distinctive pH profiles are present in stomach, small intestine and colon. In the fasted stomach, a small volume of fluid with a pH value of pH 1–2 is present [83,84]. After the intake of drinks or food, the pH value can rise up to values of pH 6 [81]. This initial pH value is mainly dictated by the pH value of the meal, but is already strongly influenced by gastric secretions. Malagelada and co-workers have shown that gastric secretion can reach output rates of up to 10 mL/min [85]. The concentration of HCl in these secretions can reach values of about 160 mM [86]. Thus, the re-acidification of the stomach starts immediately after food intake. However, during this phase regions with different pH values are present in the stomach, which is often forgotten in *in vitro* and *in silico* tests. Due to the poor mixing in proximal stomach [87], it is not surprising to see high fluctuations in gastric pH data from telemetric capsules (Fig. 8).

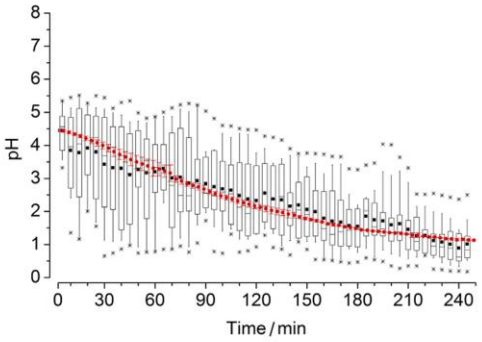

Fig. 8. Gastric pH values for the initial 5 h time frame after administration of telemetric capsule in fed state. Each box represents a 5 min interval. Box: 50%, whisker: 10–90%, square: mean, asterisks: max/min; n = 16. The red points represent the pH values simulated in GastroDuo, a biorelevant dissolution test device (reprinted with permission from Schick *et al.* [88]).

For solid meals like the FDA standard breakfast, it can be assumed that in regions close to the gastric wall, where the secreting cells are located, the pH is low even directly after food intake. In contrast, in the middle of food boli present inside the stomach, which are formed during mastication and swallowing [89], the buffering effect of different food components like amino acids or fatty acids will lead to higher pH. This explains why in recent SmartPill data fluctuations between pH 1 and 6 were observed [81].

As mentioned above, the intestinal pH profiles measured by telemetric capsules are in most cases fasted state profiles, in which pH values are around pH 6 in proximal parts and pH 7 – 8 in distal parts [84]. The reason for this increase in pH is unclear but could be due to the absorption of water during intestinal transit. The pH values in colon were shown to be highly dynamic with pH values ranging from pH 5 to pH 8. These fluctuations are typically explained by the colonization with different microbiota, which produce different organic substances like short chain fatty acids and other organic acids. However, the fluctuations may also result from the changing orientation of telemetric capsules during their colon transport, which may lead to the circumstance that the sensor is either facing towards the mucosa or the luminal contents.

In the past, there were different attempts to use these characteristic pH profiles observed in stomach, small intestine and colon for oral drug delivery. The most prominent example in this regard are enteric-coated formulations. In addition, there was also telemetric delivery device (IntelliCap), which tried to use this principle [41]. Based on pH measurement with certain sensors, the drug of interest could be released via a drug delivery unit. Although this system was technologically well-designed, it was commercially not successful and is currently no longer commercially available (June 2021). One limitation to the broader application of this system was the fact that a localization of the capsule inside the human GI tract solely based on pH can be misleading.

The information on luminal pH values from telemetric capsules are highly important for biopharmaceutical scientists as they allow us to gain a deeper understanding of the conditions and drug product performance within the human GI tract, but also to improve the *in vitro* and *in silico* models used to support drug product development. In this regard, the simulation of dynamic pH profiles in stomach and small intestine is key for a physiologically relevant simulation of drug release *in vitro*. For this purpose, biorelevant *in vitro* tools which either simulate these profiles in a simplified and stepwise manner (e.g. USP apparatus III) or in form of realistic profiles (e.g. GastroDuo, TNO TIM-1) can be applied [90]. Complex tools like TNO TIM-1 aim at the realistic simulation of the processes occurring in stomach and small intestine and therefore, they can simulate the interplay between gastric secretion, digestion and emptying. By this, they allow for a realistic simulation of gastric pH value. In another biorelevant dissolution tool, the GastroDuo, the information on luminal pH values from telemetric capsules were directly implemented [88]. Recently, Garbacz and co-workers have developed the pHysio-Grad, an *in vitro* device that allows the simulation of realistic intestinal pH profiles in more physiological bicarbonate buffers [91]. *In silico* models such as GastroPlus or SimCYP can also be used to simulate the effect of changing pH values throughout GI transit by considering both, GI pH profiles and pH-dependent solubility.

4.3. Pressure

The SmartPill is currently the only commercially available telemetric capsule that can measure pressures occurring during GI transit. From a diagnostic point of view, this system may not offer relevant advantages over techniques like high-resolution manometry since the localization of such a freely moving system cannot be controlled. However, for oral drug delivery the pressure data are highly interesting as they can inform about pressures potentially acting on larger oral dosage forms. Moreover, the pressure profiles can be used an input to *in vitro* tools aiming to simulate pressures occurring during GI transit. By having a closer look at the pressure profiles depicted in Fig. 7, it can be seen that especially in stomach and colon events of higher pressure (above 100 mbar) can be observed. These events are the result of forceful peristaltic contractions which are part of gastric and colonic motility. Particularly in the stomach strong contractions are present to enable the trituration of food particles in the so-called antral mill [89]. If pressure data from SmartPill are compared to data from manometry, the values measured by SmartPill are typically higher and a distinctive motility pattern is hardly observable. This deviation can be explained by the fact that the SmartPill is typically advanced by the contractions and only records a pressure event in the case of an obstruction such as a closed pylorus. This explains why it is difficult to identify motility patterns with telemetric capsules. In a recent SmartPill study, maximum pressures were often associated with food intake. It can be assumed that incoming food is pushing the telemetric capsule into distal parts of the stomach, where it can be exposed to strong antral contractions. However, gastric sieving in the prandial state prevents the emptying of large objects. This exposes them to the antral grinding process. Additionally, the reason for the higher pressures in comparison to high resolution manometry can also be related to the different diameters. The SmartPill has a diameter of 13 mm which is considerably higher than the diameter of most catheters used in high resolution manometry, which are mostly in the range of several millimeters. Since the closure of the antrum is not complete during the contraction wave [92], it is likely that larger objects experience larger pressures. In humans, pressure values of up to 500 mbar were measured by SmartPill.

For oral drug delivery, these pressure events can have dramatic consequences. It has been shown for hydrogel matrix tablets how pressure events of physiological fortitude can result in unwanted drug release profiles. In the worst case, the strong contractions in the stomach can destroy the integrity of the hydrogel matrix, which may result in dose dumping [93]. The availability of pressure data from telemetric capsules were highly important as they provided a physiological basis and could be implemented into *in vitro* tools such as the Dissolution StressTest device, the GastroDuo or the tiny-TIM [88,94,95]. In this regard, the application

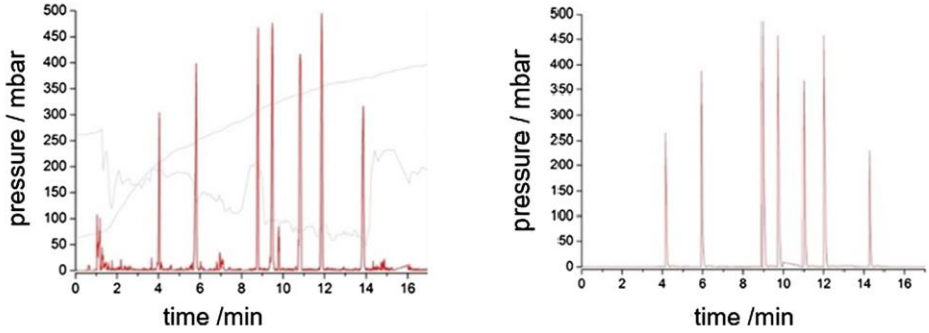

Fig. 9. In vivo (left) pressures by SmartPill and *in vitro* pressure profiles (right) simulated in a biorelevant dissolution device (reprinted with permission from Schneider *et al.* [95]).

of the Dissolution StressTest device developed by Garbacz and colleagues provided deeper insights into the effect of pressure on drug release from hydrogel matrix tablets and nowadays allows an early evaluation of the robustness of novel drug products towards physiological pressures (Fig. 9) [96,97].

4.4. Site specific absorption and permeability

Ingestible devices with drug release capability such as IntelliCap, InteliSite or magnetically triggered release capsules offer the opportunity to deliver drugs specifically within defined regions of the gastrointestinal tract [98–102]. This has already been applied for the investigation of the regional absorption capacity of drugs in the intestines of humans or large laboratory animals. In this respect, they represent an interesting alternative to the methodologically complex tube-based techniques (e.g. Loc-I-Gut) [103]. Regional differences in permeability and absorption are known for many drugs, which can significantly affect pharmacokinetics. This is related to the high variability of many parameters in the gastrointestinal tract, such as the composition of intestinal media, the differential charge of drug molecules due to local pH, differentially expressed intestinal uptake and efflux transporters or tight junctions [104]. Therefore, the assessment of site-specific permeability and absorption is highly relevant in oral biopharmaceutics. Localization of these devices is usually accomplished by online pH measurements, imaging techniques such as scintigraphy, magnetic tracking methods, or by considering typical transit times into the compartment of interest [99,100,102].

The accurate localization of these devices is critical for robust, site-specific release, but this is usually limited and hinders reproducible release of payloads at specific sites. This can be overcome by additional anatomical imaging [104]. Limitations of the applicability of ingested sensors for targeted delivery are discussed in more detail in Section 6.

4.5. Media composition and microbiota

Undoubtedly, knowledge of luminal fluid composition is a key factor in understanding oral biopharmaceutics. In addition to the established techniques for sampling by means of catheters, ingestible devices with a drug delivery unit can in principle also be used for sampling gastrointestinal media. In addition, special ingestible sampling devices are developed [105,106]. For detailed information on techniques, advantages, and challenges of sampling fluids from the gastrointestinal tract, see Sjögren *et al.* and Augustijns *et al.* [103,107].

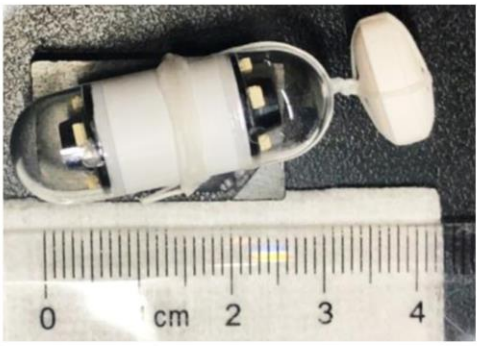

Fig. 10. Capsule endoscopic method for visualization of the behavior of solid dosage forms in the gastrointestinal tract (reprinted with permission from Blaabjerg *et al.* [116]).

In addition, swallowable devices can also be used for the sampling of the intestinal microbiota [108]. In addition to the many questions about the clinical significance and impact of the intestinal microbiome, the metabolism of drugs by intestinal bacteria is also of interest, as it can lead to their activation, deactivation, or toxification [109]. Furthermore, the intestinal microbiota and its interaction with excipients play a key role in colon targeting strategies [110]. Sampling can also be guided by pH changes, wireless external signals, or strong magnetic fields [105,106,111].

Contamination of samples during transport through more distal parts of the GI tract must be excluded [108]. The more proximal the samples are collected in the GI tract, the more likely changes in the sample will occur, as it may take several days for excretion and the sample will contain active enzymes, unstable digestion products, and active microbiota. These problems can be addressed by stabilizing agents carried in the capsule [111,112].

4.6. Capsule endoscopes

The possibility of directly visualizing the disintegration and dissolution behavior of drug forms in the stomach is fascinating. However, early studies with gastroscopes showed that the results obtained are significantly influenced by the examination technique [113,114]. By using capsule endoscopes, the problem of the invasiveness of the method can be avoided. When using ingestible

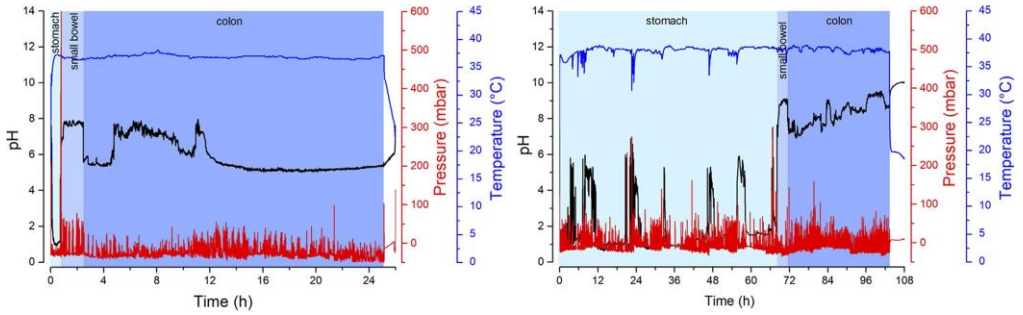

Fig. 11. Exemplary pH, pressure and temperature profiles obtained after administration of a SmartPill to a fasted dog (left) and a fasted pig (right).

cameras, however, the problem arises that the dosage form and the camera system move independently of each other and thus the dosage form to be examined is not necessarily in the field of view of the camera. Pedersen et al. solved this problem by fixing the dosage form in front of the lens of the capsule endoscope using a soft string [115]. The identical method was applied by Blaabjerg *et al.* for the comparison of two paracetamol tablet formulations in beagle dogs (Fig. 10) [116]. They also demonstrated that the attachment of the tablet to the capsule endoscope did not alter paracetamol absorption.

The gastrointestinal transit times measured using Pillcam SB and SmartPill have been compared [117], yielding longer gastric emptying times (GET) and longer small intestinal transit times (SITT) for SmartPill compared to Pillcam SB. However, in this study, both the study design and the patient groups were different. In particular, the Pillcam SB was administered in fasted state, whereas a meal (mixed meal with 260 kcal) was administered before the SmartPill was ingested. Thus, the observed differences are due to the sieving function of the food-filled stomach, at least in GET [118,119].

5. Comparison: Human and animal physiology

Apart from humans, telemetric capsules can also be applied in larger animals like dogs, pigs, cows or horses, which may be interesting from a veterinary perspective, for instance to study physiological function in healthy and diseased animals. For instance, in a recent SmartPill study, this telemetric capsule was applied to study abomasal emptying in calves suffering from naturally occurring diarrhoea as well as in healthy calves [120]. However, the large size of most telemetric capsules permits their application in smaller animals like mice, rats or rabbits.

From a pharmaceutical point of view, pigs and dogs represent the most relevant animal models with respect to formulation development and food effect predictions. In the last years, SmartPill was therefore applied in these animals to study similarities and differences in terms of GI physiology. Two exemplary profiles obtained by the application of SmartPill are shown in Fig. 11.

The application of telemetric capsules in dogs revealed that their intestinal transit time is typically shorter. Whereas in humans, small intestinal transit typically takes 2–6 h, it only takes 1–2 h in dogs [121]. For drugs with poor permeability in small intestine and colon, this aspect may be highly relevant. Moreover, higher pressures occur during GI transit in dogs with maximum pressures in stomach of up to 1000 mbar. Due to this circumstance, the dog represents a worst-case model for the robustness of formulations towards physiological pressures. In terms of luminal pH, dogs are quite comparable to humans and therefore, co-medication with histamine or histamine analogues like pentagastrin is not needed. This is in contrast to the common practice of "humanizing" the canine stomach by co-administering these drugs known to stimulate gastric acid secretion [122]. This practice is due to the assumption that variability in gastric pH is high because of low rates of unstimulated gastric acid secretion [123]. In a recent SmartPill study, we could show that the administration of pentagastrin does not provide an advantage with respect to simulating

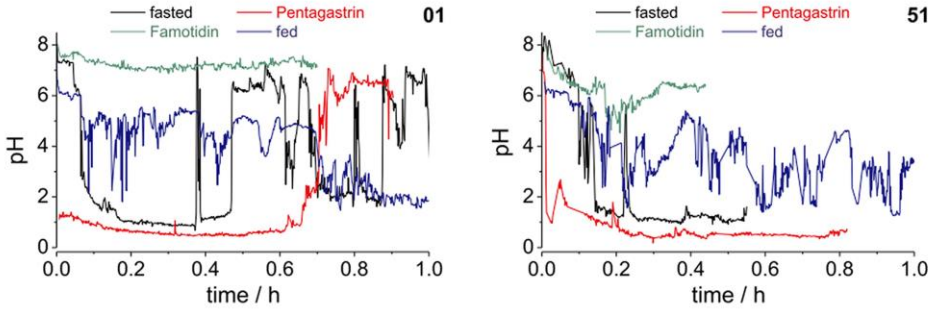

Fig. 12. Gastric pH values at different intake conditions in two exemplary dogs.

a pH profile closer to the situation in humans. In contrast, the co-administration of pentagastrin results in a highly acidic stomach, which represents a rather unrealistic scenario in terms of gastric pH (Fig. 12). For weakly basic compounds, gastric dissolution may be overestimated in fasted state.

Since the pig is commonly applied animal model in food sciences, it also received higher attention in terms of oral drug delivery in the last years [124]. However, considering data on gastric emptying as well as recent data from telemetric capsules, one must question the broader application of the pig model for predictions on oral drug delivery in humans [125,126]. Especially for monolithic, modified release formulations, the pig model should be omitted because of the very long gastric transit times. Nonetheless, since luminal pH and pressure values as well as intestinal transit times are comparable to those measured in humans, the pig model may be used to evaluate the *in vivo* behavior of immediate release formulations [127].

In general, recent studies with telemetric capsules have shown that significant differences exist in terms of human and animal GI physiology. Therefore, care must be taken when a decision is made on the application of animal models to predict the *in vivo* performance of oral drug products. Data from animal studies can be misleading if the characteristics of their GI function as well as their interplay with the properties of the drug and the formulation are ignored. Moreover, the study protocol (i.e. fluid administration, fasting period, type and composition of food) also plays a major role for the PK profile observed in animals. For example, simply adapting the caloric value of a test meal for food effect evaluation based on the body weight is not feasible since the gastric emptying rates differ in humans and animals.

6. Limitations and pitfalls

The data obtained by means of ingestible sensors have great importance for understanding and predicting the behavior of dosage forms in the gastrointestinal tract as well as the absorption of drugs from the gastrointestinal tract, as explained previously. However, there are also some methodological limitations. First, the determination of the localization of the sensors in the gastrointestinal tract is problematic. The use of pH measurements to determine gastric emptying as well as entry into the colon, which is usually performed, is often uncertain and cannot be reliably assessed directly while the measurement is being performed. In the case of the pH change during the passage from the stomach into the small intestine, this is due to the fact that the gastric emptying of the sensors, due to their size, is linked to the occurrence of sufficiently intense contractions within the IMMC, as they are preferably present during phase III of the IMMC (“housekeeping waves”) [128]. However, retropulsion of duodenal contents into the stomach also occurs frequently during phase III [129]. The stomach is mostly empty at this time (residual volumes of less than 5 mL are possible [83]), so that the entrance of bicarbonate rich intestinal fluid into the stomach can lead, at least for a short time, to a pH shift within the stomach, which can be mistakenly interpreted as a passage of the capsule into the small intestine (see Fig. 13). To avoid such misinterpretation, capsular passage into the duodenum is usually defined as the time at which pH rises abruptly from the lowest postprandial value by at least 2 pH units to a pH of at least 4 and does not fall to a value below 4 for more than 10 min at any subsequent time point in the recording [37]. In principle, this evaluation is only possible retrospectively. To com-

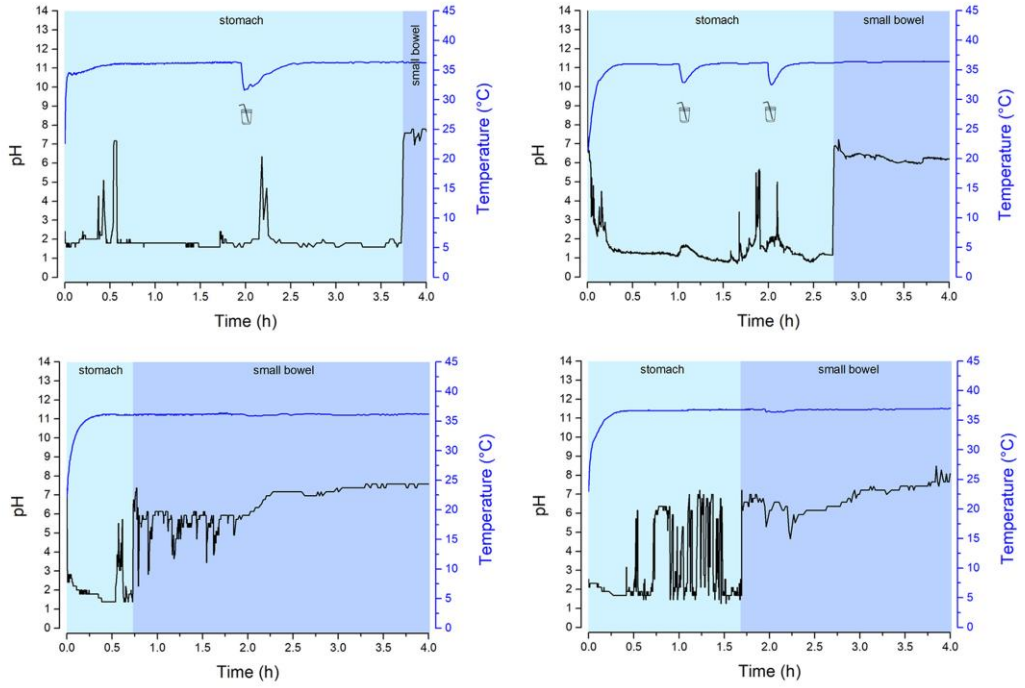

Fig. 13. Examples for the variability of gastric pH observed in studies using telemetric capsules (capsule intake under fasting conditions).

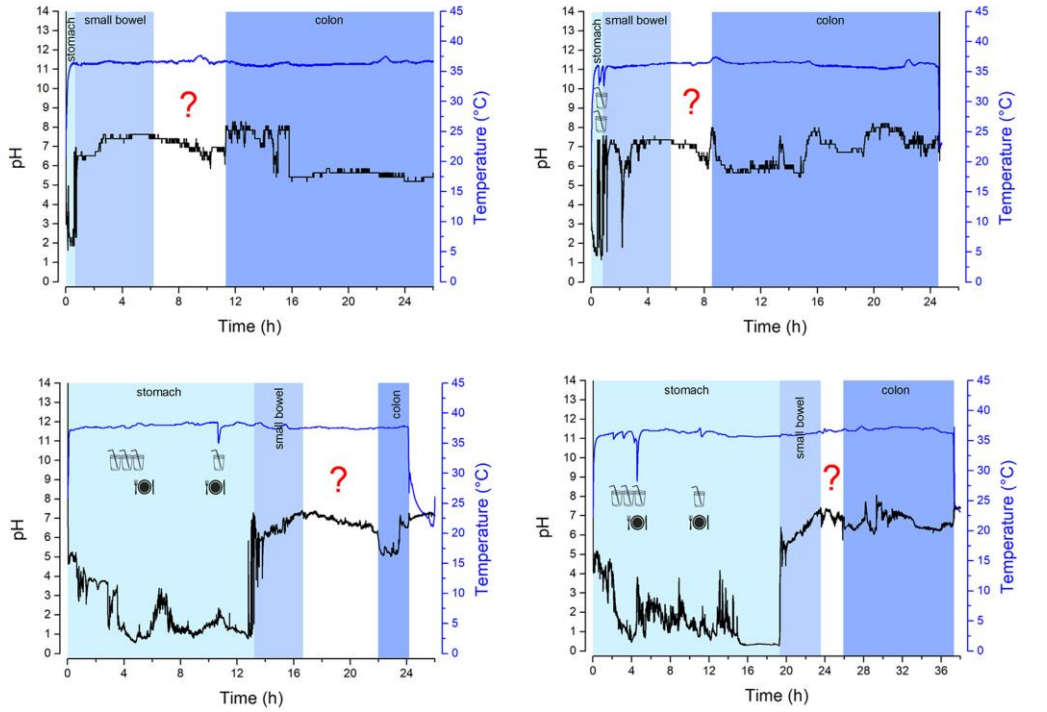

Fig. 14. Examples of a lack of pH lowering during the passage into the colon. Top: Intake under fasting conditions: bottom: Intake under fed conditions.

plicate matters, it is also possible that phase III in the stomach does not occur during each cycle of IMMC. Only 74% of phase III contractions measured in the proximal jejunum were found to also occur in the antrum of the stomach [130]. This may also explain the observation that ingested sensors as well as non-disintegrating monolithic dosage forms sometimes remain in the stomach for several hours after ingestion under fasting conditions [84,117,128,131] and thus significantly longer than one cycle of IMMC with a duration of typically 60–90 min [130].

In the course of clinical testing of the IntelliCap System for controlled drug release, a study was conducted with diltiazem as model drug that was continuously pumped out of the device over 24 h [132]. To ensure that the capsule actually left the stomach when a pH shift was observed, the study participants were asked to drink some cold water. If the administration of water resulted in a significant drop in temperature, it could be assumed that the capsule was still in the stomach. This method to verify gastric emptying is readily available in clinical studies, but not in clinical applications. The phenomenon of transiently high pH values within the stomach also leads to errors in the retrospective evaluation of pH profiles, at least when automated evaluation systems are used to determine gastric emptying time from ingested sensor data [117].

Another critical point is the determination of the entry into the colon. Typically, a pH drop of approximately one unit occurs during passage from the terminal ileum into the caecum, which is attributed to the presence of short-chain fatty acids as metabolites of colonic bacteria [133]. Capsule passage from the ileum into the cecum is therefore usually determined as an abrupt pH decrease of at least 1.0 pH unit at least 30 min after gastric emptying that persists for at least 10 min [37]. Such a pH drop is not necessarily present in all profiles, nor is it always easy to determine. In Fig. 14 examples with difficult determination of the time point of capsule arrival in the colon are given. Reasons for the absence of the pH drop are not understood but may be related to the bacteria in the caecum, and previous food intake [134]. For determining the time point of leaving the small intestine, as with using the rise in pH to determine gastric emptying, retrospective analysis of the entire data set to identify the time point of interest is much more feasible than ad hoc determination.

In terms of pH measurement by telemetric capsules, one further limitation needs to be considered. The ISFET sensor, which is used in most ingestible pH-sensing capsules, is prone to drift in the environment of the human GI tract [81,135,136]. Calibration data from a recent SmartPill study have shown that after excretion, the pH value recorded by the telemetric capsule can be 1–2 pH units above the real value. Therefore, care should be taken if absolute pH values are presented in publications on telemetric capsules. In our studies with the SmartPill, we assumed a drift based on a linear increase over time. Unless the mechanism behind this drift has been fully elucidated, this may be an acceptable approximation [28].

Taken together, it should be noted that pH data are insufficient to ensure reliable localization of ingestible devices within the different regions of the gastrointestinal tract as required for automated control of ingested electronic devices for targeted drug delivery or other functions like locoregional interventions.

There are also still considerable limitations with regard to the physiological data obtained. Since all devices available to date

are subject to gastric sieving due to their size and density and are thus not emptied together with caloric stomach contents, all existing data on pH values and pressures in the small intestine are data from the fasting and thus at least largely empty small intestine. Data on pH and pressures during digestion are not yet known from capsule sensor studies. It is conceivable that a study design could be created in which, after fasting, the subjects eat a meal immediately after gastric emptying of the capsule. However, in such a study design, it would first have to be clarified to what extent the chyme actually catches up with the sensor device or whether the device precedes the chyme.

A general disadvantage of ingestible sensors is that the systems are disposable, which leads to relatively high costs and is also not sustainable. The recorder must be worn quite close to the body so that the measured variable is transmitted correctly [27,137]. Furthermore, the participants must avoid being too close to each other to avoid interference with the radio signals [137]. Electromagnetic interference can occur, which can lead to data errors or even data loss [27,138,139]. Drifting sensor data, inaccurate transmission or a loss of data transmission due to a too far distance between the recorder and the sensor device can all lead to data losses or inadequate data interpretation. For example, it was reported that in patients with severe active Ulcerative Colitis the pH in proximal parts of the colon might be as low as pH 2–3 [140] or even below [141]. Such low pH values are quite surprising, as lactic acid as the strongest of the physiological acids in the colon has a pKa of 3.9. Later, it was demonstrated that the pH values in the proximal colon are not significantly lowered in patients with inflammatory bowel diseases as Morbus Crohn or Ulcerative Colitis compared to healthy persons [142,143]. Ewe et al. showed that one explanation for the discrepancy could be found in data transmission problems based on the position dependence of the signal transmission of the systems used at that time [142].

It should be further considered that the transit times of objects through the gastrointestinal tract may also be influenced by their size and density. While large particles are retained in the stomach during digestion, they pass through the colon faster than small particles [144]. Thus, a mean WGTT of 39.5 ± 8.6 h has been reported for small radio-opaque markers in young healthy subjects [145]. In our SmartPill studies, we observed a WGTT of 17.7 ± 8.9 h after ingestion of this large capsule in the fasting state in the morning and a WGTT of 35.9 ± 10.6 h when taken after a high-calorie meal [72,81]. The latter is strongly influenced by the prolonged gastric residence time. Accordingly, the applicability of intestinal transit times as observed with ingestible sensors may be misleading for particles of different size or density.

7. Outlook

The ongoing development of miniaturized electronics including new sensor technologies as well as innovations in the field of power supply offer much room for novel ingestible sensor systems [16,67,71,146]. These include, for example, sensors based on edible materials that offer the possibility of recording physiologically important parameters such as the concentration of bile acids or the activity of digestive enzymes [147]. Synthetic biology provides novel opportunities for the detection of multiple biomarkers and a first ingestible bio-electronic device combining microorganism and readout electronics has already been presented [148].

Spectroscopic methods based on NIR [149] or Raman [150] offer the possibility to identify substances and thus in the future even to measure drugs and metabolites in the intestine. It will also be very exciting to learn to what extent the combination of different measured variables, such as pH values and gas partial pressures, will enable reliable real-time localization. Progress in this area could significantly advance multiple applications of swallowable electronic systems for spatially targeted drug delivery and also for loco-regional intervention. If further technical development enables a substantial reduction in the size of the devices to below 3 to 4 mm, measurements could also be performed in the small intestine during the digestive phase in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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5.2 Characterization of an In Vitro/Ex Vivo Mucoadhesiveness Measurement Method of PVA Films

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Werner Weitschies	Erarbeitung der Fragestellung, Konzeption, Korrektur des Manuskripts

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Article

Characterization of an In Vitro/Ex Vivo Mucoadhesiveness Measurement Method of PVA Films

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Abstract: Transmucosal drug delivery systems can be an attractive alternative to conventional oral dosage forms such as tablets. There are numerous in vitro methods to estimate the behavior of mucoadhesive dosage forms in vivo. In this work, a tensile test system was used to measure the mucoadhesion of polyvinyl alcohol films. An in vitro screening of potential influencing variables was performed on biomimetic agar/mucin gels. Among the test device-specific factors, contact time and withdrawal speed were identified as influencing parameters. In addition, influencing factors such as the sample area, which showed a linear relationship in relation to the resulting work, and the liquid addition, which led to an abrupt decrease in adhesion, could be identified. The influence of tissue preparation was investigated in ex vivo experiments on porcine small intestinal tissue. It was found that lower values of F_{max} and W_{ad} were obtained on processed and fresh tissue than on processed and thawed tissue. Film adhesion on fresh, unprocessed tissue was lowest in most of the animals tested. Comparison of ex vivo measurements on porcine small intestinal tissue with in vitro measurements on agar/mucin gels illustrates the inter- and intra-individual variability of biological tissue.

Keywords: in vitro–ex vivo correlation; mucoadhesive films; tensile studies; porcine small intestine

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1. Introduction

The use of mucoadhesive polymers in the development of modern and innovative drug delivery systems is a common formulation step [1–3]. In this context, mucoadhesive polymers are used for various reasons. Particularly in the case of active ingredients that are poorly permeable, such mucoadhesive dosage forms can provide a high local concentration of active ingredient on the mucosa, which should lead to improved absorption [4,5]. Furthermore, mucoadhesive dosage forms are particularly interesting for active ingredients with a small absorption window in the upper small intestine. Suitably placed, such dosage forms can improve oral bioavailability through prolonged and consistent release [6–8]. However, local drug therapy can also be achieved by mucoadhesive dosage forms [9].

Many different mucoadhesive dosage forms, such as tablets, films, pellets, or even semi-solid preparations, such as gels, have already been developed with different targets [10,11]. For example, mucoadhesive films can be placed locally in the esophagus to treat esophageal diseases using the EsoCap platform technology [12]. Local drug therapy may also be of interest in the stomach, for example, due to Helicobacter pylori infection [13,14]. Srivastava et al., have developed a microparticulate system for this purpose, which consists of the mucoadhesive polymer thiolated polyacrylic acid and the two active ingredients famotidine and clarithromycin [13]. Another example are mucoadhesive matrix tablets for the therapy of ulcers, which contain the mucoadhesive polymers polyacrylic acid (Carbopol) and hypromellose (HPMC) in addition to the active ingredient ranitidine hydrochloride [15]. Intestinal mucoadhesive dosage forms for the delivery of protein drugs such as insulin [16–18] may also benefit from increased absorption due to a local high

drug concentration. A wide variety of delivery forms, such as mucoadhesive nanoparticles [16,19] or mucoadhesive patches [17,20] are the subject of research in this field.

Mucoadhesion is a complex phenomenon, and six theories are currently accepted to describe it [10,21]. These include electronic, wetting, adsorption, diffusion, mechanical, and fracture theory. The electronic theory assumes adhesion due to the formation of an electron double layer. In contrast, the adsorption theory assumes adhesion due to weak secondary bonds such as hydrogen or van der Waals bonds. The mechanic theory describes an adhesion to a rough surface by interlocking. The diffusion theory assumes that interaction between the mucus and the dosage form occurs through penetration and interaction of polymer chains of the dosage form and the mucin chains of the mucus. The wetting theory applies to liquids and describes adhesion as a function of surface and interfacial energies. Another approach is the fracture theory, which addresses the detachment force required to separate two surfaces after adhesion [21].

The extent of mucoadhesion depends on a lot of factors. On the one hand, mucoadhesion depends on the properties of the polymers, such as charge [22,23], mobility of the polymer chains, water absorption capacity, the extent of cross-linking, and molecular weight [24]. Furthermore, application site-specific factors such as the pH of the application site and the associated charge of the mucus, the presence and viscosity of wetting fluid such as saliva, gastric juice, or the amount of mucus, and mechanical stress on the dosage form at the application site [24] also influence mucoadhesion. In addition, there is high in vivo variability resulting from the particular site of application. Therefore, to estimate the mucoadhesion in vivo, biorelevant in vitro test systems can be highly interesting, especially in early development phases of innovative dosage forms or for the screening of different formulations. Various methods are reported in the literature, which can be divided into indirect and direct methods [25,26]. Indirect methods for determining adhesiveness include measuring parameters that provide information about the interactions of the mucoadhesive and the mucosa or mucus. These include, for example, rheological measurements, spectroscopic methods (e.g., Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy, Nuclear Magnetic Resonance (NMR)), and determination of surface energy by contact angle measurements [27]. On the other hand, direct methods include measuring the adhesion or residence time of the dosage form on the mucosa, biomimetic gel, or a mucin compact.

Another parameter that belongs to the direct measurement methods is the force that has to be applied to separate the dosage form from the mucosa. These measurements are mostly performed as tensile assays on texture analyzers or modified microbalances. During the measurement, the mucoadhesive dosage form and the mucosa or a mucosa-mimetic material are pressed together with a defined contact force. After a defined contact time, the two surfaces are separated from each other at a specified withdrawal speed. The force-distance diagrams recorded during this process provide information on the peak force, which is defined as the maximum detachment force F_{max}, and on the Area Under the Curve (AUC), which represents the work of adhesion W_{ad}. A brief look at the extensive literature reviews on mucoadhesion measurements shows that many different parameters within each measurement method could potentially influence the measurement result. Factors such as the contact force and time, as well as the withdrawal speed, are test equipment-specific variables that can be freely chosen by the respective authors [26,28–30]. There is also a wide variety in the choice of mucosa or mucosa-mimetic materials. Animal tissues such as porcine, rat, or bovine mucosa are often used to replicate human tissue [28]. Since the results obtained on animal tissue have poor reproducibility, there are alternative approaches [31–35]. These include a wide variety of gels (e.g., gelatin, agar/mucin gels, HEMA-AGA hydrogels), as well as pressed mucin discs, mucin dispersions, or mucin-soaked filters [28]. In addition, the animal tissues are prepared in different ways: Some authors separated the mucosa from the underlying muscle layer before starting the experiment [36,37], others did not use fresh but thawed tissue samples, and still, other authors processed the tissues before use [38]. The tissue samples are often wetted with a defined

amount of liquid before starting the experiment to better reproduce the physiological conditions at the application site. Depending on the tissue type, this can be, for example, simple buffer systems such as phosphate buffer pH 7.4 [17], but also more biorelevant wetting fluids such as Simulated Intestinal Fluid [39] or Simulated Saliva [40,41]. The simple wetting of the preparation can be further extended by a temperature-controlled measuring cell, where the mucoadhesive dosage form meets the mucosa in a humid and temperature-controlled environment [42–44].

Studies by Ivarsson et al., on the differences between ellipsometry, tensile strength, and rheology showed that these methods result in different conclusions regarding mucoadhesion when using the same polymers [45]. Therefore, the authors concluded that special attention must be paid to in vitro methods when comparing mucoadhesive dosage forms, as these do not lead to comparable results. However, even when using a single measurement method, such as tensile assays on the texture analyzer, the use of different measurement parameters [36,46] as well as different tissue types of different test animals [44] leads to different results, as various papers show.

These differences in the results show that it is important to comprehensively work out a measurement method for determining mucoadhesion. This includes the evaluation of device-specific parameters and sample-specific parameters. In the case of sample-specific parameters, the storage and wetting of in vitro gels and the treatment and storage of tissue in ex vivo experiments can have a decisive influence. To our knowledge, there has been no systematic investigation of different influencing parameters that includes both in vitro and ex vivo studies. In this work influencing factors such as sample area, contact force, contact time, and withdrawal speed on the adhesion of polyvinyl alcohol (PVA) films to biomimetic agar/mucin gels were investigated. In addition, the wetting of the gels and storage were to be investigated as gel-specific influencing factors. With the help of the results of these in vitro investigations, an optimized set of parameters for ex vivo measurements of small intestinal tissue of pigs was to be established. Furthermore, the effect that pretreatment of tissue has on the results of the mucoadhesion measurements was investigated in the ex vivo experiments.

2. Materials and Methods

2.1. Materials

The polymer polyvinyl alcohol EMPROVE® ESSENTIAL PVA 18-88 (Merck KGaA, Darmstadt, Germany) was used to prepare the films. Anhydrous glycerol (AppliChem GmbH, Darmstadt, Germany) served as plasticizer. Demineralized water was used as solvent.

Potassium dihydrogen phosphate (neoFroxx GmbH, Einhausen, Germany), sodium hydroxide (AppliChem GmbH, Darmstadt, Germany) and deionized water were used to prepare the phosphate buffer pH 7.4 USP. Both chemicals were used in analytical grade.

Agar for microbiology (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and mucin 75–95% for biochemistry (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) as well as demineralized water were used to prepare biomimetic gels.

For testing mucoadhesion on porcine intestine, porcine small intestine was obtained from a local slaughterhouse (female, 12–15 weeks of age, 35–50 kg, n = 3). The small intestine was examined after collection in the unprocessed and processed state. The preparation of the tissue is explained in more detail in Section 2.2.3 Preparation of animal tissue. Furthermore, prepared tissue sections were additionally deep-frozen at −20 °C, thawed for the measurements and subsequently examined.

2.2. Methods

2.2.1. Preparation of Mucoadhesive Films

The solvent cast method was used to prepare the films. For this purpose, 80.0 g demineralized water was suspended with 18.0 g PVA 18-88 and 2.0 g glycerol in a laboratory glass bottle at 500 rpm on a magnetic stirring plate. The mixture was then heated to 85 °C for 2 h with stirring at 100 rpm in a water bath. The stirring speed was then reduced to

50 rpm and the solution was stirred without adding heat until it had cooled down to room temperature. The solution was decanted into falcon tubes and centrifuged at 4400 rpm for 15 min at room temperature to remove air bubbles (Centrifuge 5702 R, Eppendorf SE, Hamburg, Germany). The films were cast at 12.0 mm/s on a polyamide-coated liner (POLY SILK 111/105, Loparex Deutschland GmbH & Co. KG, Forchheim, Germany) with a coating knife (mtv messtechnik oHG, Erftstadt, Germany) adjusted to 1000 µm on a coating bench (Automatic Precision Film Applicator CX4, mtv messtechnik oHG, Erftstadt, Germany). After drying at room temperature for 12 h, the films were stored in airtight aluminum multilayer bags (Ströbel GmbH, Langenzenn, Germany) until further use after one week. Film thickness was measured using a mechanical thickness gauge (n = 10, J15, Käfer Messuhrenfabrik GmbH & Co. KG, Villingen-Schwenningen, Germany) and residual moisture was determined (n = 3) using a Moisture Analyzer (MB35, OHAUS Europe GmbH, Nänikon, Switzerland) at 105 °C.

2.2.2. Preparation of Biomimetic Gels

2.0 g of agar was suspended in 94.0 g of demineralized water in a laboratory glass bottle and heated to 95 °C in a water bath on a magnetic stirring plate at 150 rpm for one hour to prepare the gels. The solution was then cooled to 55 °C and 4.0 g of mucin was added under stirring at 500 rpm. After a mixing time of 15 min, 15 mL of the mixture were transferred to Petri dishes, which were covered and cooled down to room temperature for 2 h. The gels were covered with parafilm, stored in the fridge at 5 °C, and were removed from refrigeration 60 min before use to investigate the effect of storage over time.

2.2.3. Preparation of Animal Tissue

Ex vivo mucoadhesion experiments were performed on porcine intestinal tissue. The tissue was removed immediately after slaughter and transported stored on ice. Investigations including transport were made at least within 2 h after slaughter. Since the effects of processing the tissue on mucoadhesion were to be investigated, the tissue was divided into three sections of approximately 7 cm each. The intestinal tube was cut longitudinally, resulting in sections of approximately 5 cm × 7 cm. One third was examined without processing, one third was carefully cleaned with deionized water to remove possible food particles, and the remaining third was cleaned with deionized water and frozen. The cleaned sections intended for freezing, were packed in sealable low-density polyethylene bags (Druckverschlussbeutel LDPE transparent, packpack.de GmbH, Jever, Germany) and frozen at −20 °C in the freezer for seven days. The samples were thawed in the polyethylene bags in a water bath with constant stirring at 37 °C for 2 h.

2.2.4. Adhesion Measurements with the Texture Analyzer

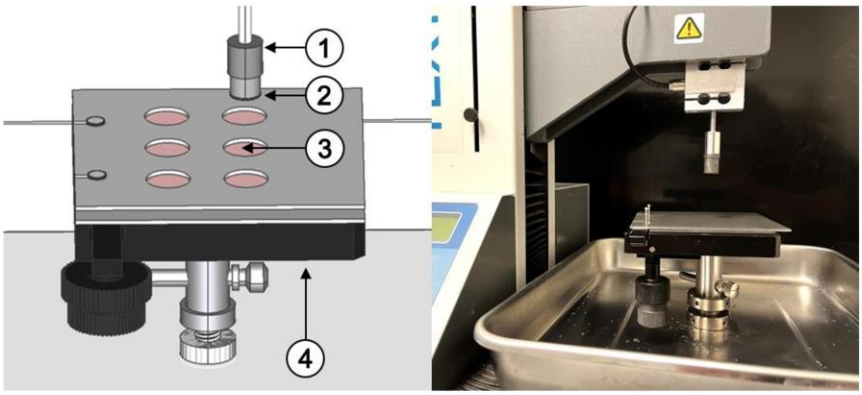
The investigations consisted of three main points:

- Investigation of the influence of the test equipment parameters on the maximum detachment force as well as the work of adhesion on agar/mucin gels;
- Characterization of further test parameters such as the area of the film used, wetting the gels with phosphate buffer pH 7.4 and storage of the agar/mucin gels;
- Comparative measurements of porcine intestinal tissue.

Influence of Instrument Parameters

Circular pieces with 14 mm diameter (A ≈ 153.94 mm^2) were punched out of the dried films using a punching iron to measure the adhesion of the prepared films to the respective surface. These were attached to the probe of a texture analyzer (TA Plus, LLOYD Instruments, Bognor Regis, UK) using double-sided adhesive tape (tesa® Doppelseitiges Klebeband universal, tesa SE, Norderstedt, Germany). Either biomimetic gels of agar and mucin or porcine small intestinal tissue were placed on the lower base. Instead of the commercially available stationary base, a microscope stage was converted to allow the most efficient use of samples, especially the tissue samples (Figure 1). The converted microscope

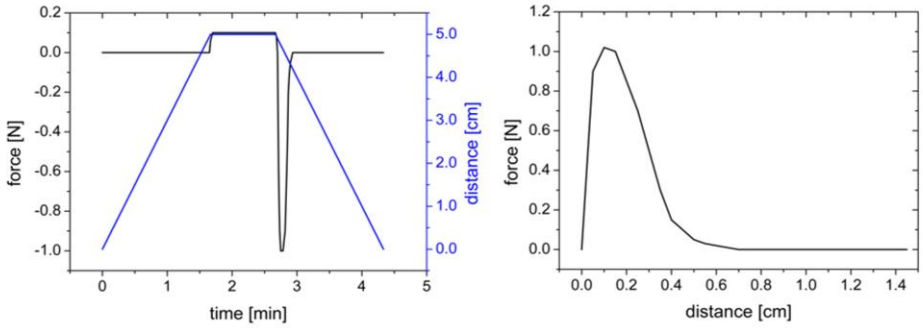
stage allows the test substrate to be moved along x and y directions so that the specimen only needs to be placed once on the lower base at the start of the experiment. A distance of 5 cm between the film and the respective substrate was set at the beginning of each measurement. The texture analyzer was equipped with a 10 N load cell.



(**a**) (**b**)

Figure 1. (**a**) Schematic illustration of the structural modifications made on the texture analyzer. 1: probe, 2: film sample, 3: porcine small intestine tissue and 4: microscope stage. (**b**) Photograph of the device setup in the laboratory.

The measurement followed the same routine: The probe with the film to be tested moved at a constant speed (0.5 mm/s) towards the lower base with the test substrate. If a counterforce of 0.10 N was measured, the upper probe remained in this position for 60 s and then moved back to the starting position at a withdrawal speed of 0.5 mm/s (Figure 2). The time sequence of the movement is shown as an example in the force-time-displacement diagram in Figure 2. During the upward movement when the film was pulled off of the test substrate, a force-distance diagram was recorded, from which the maximum detachment force F_{max} and the work of adhesion W_{ad} are evaluated as the area under the curve for the evaluation.



(**a**) (**b**)

Figure 2. (**a**) Schematic force−time−distance diagram of the upper probe movement. (**b**) Resulting force distance diagram.

Biomimetic gels were used to investigate the influence of test equipment parameters. The following parameters were varied starting from a standard setting: The contact force (f1–f5), the contact time of the film to the substrate (t1–t7), and the withdrawal speed (w1–w5), which are listed in Table 1.

Table 1. Measurement parameters of the in vitro experiments as well as the ex vivo experiments.

Sample	Settings	Contact Force	Contact Time	Withdrawal Speed	Sample Area	Liquid	Storage Time	Processing
		N	s	mm/s	mm^2	mL	d	
gel	standard	0.10	60	0.50	153.94	-	-	-
	f1	0.05						
	f2	0.10						
	f3	0.20	60	0.50	153.94	-	-	-
	f4	0.35						
	f5	0.50						
	t1		5					
	t2		30					
	t3		60					
	t4	0.10	90	0.50	153.94	-	-	-
	t5		180					
	t6		300					
	t7		600					
	w1			0.10				
	w2			0.25				
	w3	0.10	60	0.50	153.94	-	-	-
	w4			1.00				
	w5			2.00				
	a1				38.48			
	a2	0.10	60	0.50	78.54	-	-	-
	a3				153.94			
	l1					0.00		
	l2	0.10	60	0.50	153.94	2.50	-	-
	l3					5.00		
	l4					10.00		
	s1						-	
	s2	0.10	60	0.50	153.94	-	1	-
	s3						7	
	s4						14	
	optimized *	0.35	180	1.00	153.94	-	-	-
tissue	optimized1 *						-	unprocessed
	optimized2 *	0.35	180	1.00	153.94	-	-	cleaned
	optimized3 *						7	thawed

* Optimized settings resulted from studies conducted on agar/mucin gels which were carried out to investigate influencing parameters. For detailed information on the origin, the reader is referred to Section 3.1.6.

Influence of Further Test Parameters

Furthermore, film sections of different sizes were applied to agar/mucin gels. The circular areas d1–d3 had a diameter of d1 = 7 mm, d2 = 10 mm and d3 = 14 mm, resulting in sample areas of A1 ≈ 38.48 mm^2, A2 ≈ 78.54 mm^2 and A3 ≈ 153.94 mm^2. The influence of different amounts of phosphate buffer pH 7.4 USP (T = 22.5 ± 1.0 °C) as wetting liquid (l1–l4) was also investigated. The gels were additionally stored at 5 °C in the refrigerator for a certain time (s1–s4) after preparation to investigate the effect of storage conditions on adhesion.

The detailed measurement parameters for the measurements on the agar/mucin gels can be found in Table 1.

Comparative Measurements on Porcine Tissue

In addition to the investigation of test equipment parameters, the influence of tissue preparation and storage was also characterized. For this purpose, measurements were performed at measurement parameters resulting from the initial investigations on biomimetic agar/mucin gels. The reader is referred to Section 3.1.6. for the description of these measurement conditions.

During the measurements, the tissues were additionally weighted with a perforated plate made of stainless steel to prevent the tissue from lifting off when force was applied. For all tests performed on the texture analyzer, the number of samples was $n = 6$. 1000 data points were recorded in each case. The maximum detachment force F_{max} was evaluated as the peak force during detachment and the work of adhesion W_{ad} as the area under the force-distance diagram.

3. Results and Discussion

3.1. Influence of Test Equipment Parameters on the Adhesiveness of Films on Biomimetic Gels

In order to evaluate the optimal measurement parameters for subsequent investigations of the tissues, experiments were carried out with biomimetic gels based on agar and mucin. The PVA films used for this purpose had an average film thickness of 128 (±1.5) µm and residual moisture of 10.48 (±3.2) % in the dried state.

3.1.1. Influence of the Sample Area

With increasing film sample area, the adhesion force increased from 0.234 N for a sample area of A = 38.48 mm^2 to 0.866 N for a sample area of A = 153.94 mm^2, and the work of adhesion increased from 0.072–0.327 mN×m (Figure 3). As expected, the adhesion force and work increased approximately linear with the area of the film used. The linear relationship between the sample area and the measured values can be confirmed by the regression coefficient of $R^2(F_{max})$ = 0.9996 and $R^2(W_{ad})$ = 0.8164. An approximately linear relationship between the sample area with the adhesion work and the maximum detachment force has also been observed by Göbel et al. [47] when they investigated the influence of the sample area of circular HPMC and PVA films with a diameter of 5–20 mm on adhesion to gelatin type A gels.

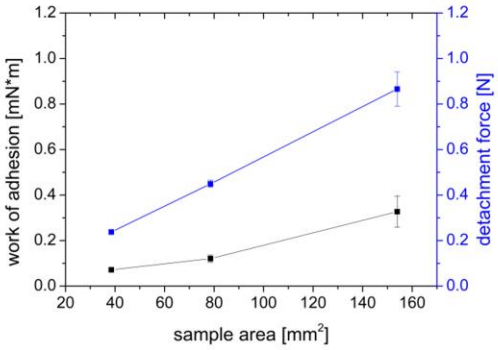


Figure 3. Effect of sample area of a PVA film on the work of adhesion (mN×m) and the detachment force (N) measured on a gel of 2% agar and 4% mucin. Mean ± SD, $n = 6$.

3.1.2. Influence of the Contact Force

A range of 0.05–0.50 N was selected to investigate the influence of adhesion contact force. The results shown in Figure 4, point out that the work of adhesion measured at constant parameters varied from 0.389–0.463 mN×m, as did the adhesion force, which ranged from 0.790–1.010 N. A trend of the measured values over the changed parameter of

the contact force could not be observed. Macroscopic observation of the gels also showed no defects due to a damaged gel structure in any case. However, it can be seen from the standard deviations that the variability of the measured values decreases with increasing contact pressure. The lower variability could be related to a higher precision of the load cell at higher forces.

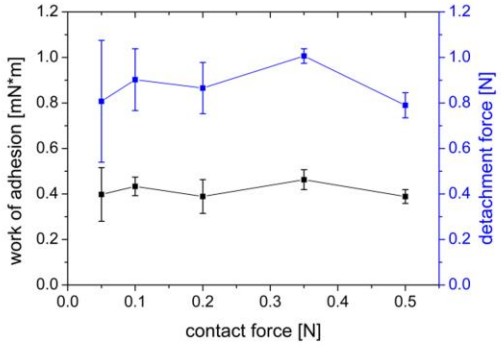


Figure 4. Effect of contact force of a PVA film on the work of adhesion (mN×m) and the detachment force (N) measured on a gel of 2% agar and 4% mucin. Mean ± SD, $n = 6$.

Comparable observations were also made by Wong et al. [48] when they examined mucoadhesive tablets of Carbopol 974P and Methocel K4M on chicken pouch. The authors did not observe any significant effect of contact force in the range of 0.05–0.10 N and from 0.5–1.0 N on adhesion at low contact times below one minute. For contact times exceeding one minute, a statistically significant difference was only observed in percentage more extensive of contact force from 0.05–0.50 N. Adhesion requires intimate contact of the dosage form to the respective substrate (tissue or biomimetic gel). The more completely the dosage form rests, the better it can interact with the substrate. At the same time, however, the contact force must not be so high that it could damage the tissue.

3.1.3. Influence of Contact Time

When measuring the influence of the contact time of the film on the gel, times in the range of 5–600 s were chosen. After 180 s of contact time, a maximum was observed in the measured work (W_{ad} = 0.5261 mN×m) and the detachment force (F_{max} = 0.9083 N) (Figure 5). With longer contact time, the measured values decreased again, but not as fast as they increased. After a contact time of 600 s, the measured work decreased to W_{ad} = 0.4451 mN×m and the detachment force decreased to F_{max} = 0.7201 N.

These observations may be explained by the PVA's chemical structure and the mucoadhesion diffusion theory. PVA is a nonionic polymer that swells slowly in water. Due to this slower swelling, it takes time for the polymer chains to be able to diffusely interact with the mucin chains of the agar/mucin gel. As the swelling of the PVA progresses, however, adhesion again decreases as the previously dry and hydrophilic film decomposes over time to a gel-like structure that adheres more poorly to the agar/mucin gel. Göbel et al. [47] investigated the influence of the contact time of PVA films on gelatin type A gels in vitro. They could not observe a clear trend in the observed time from 3–120 s of contact time. With a longer contact time as in our study, a trend could have possibly been observed.

Solid dosage forms tend to break at the contact surface when the adhesive bond is released from a surface, as this is where the weakest bond is found. In gel-like preparations, however, the weakest bond is often found within the gel, so that the mucoadhesive dosage form tears when detached from the respective test substrate [36].

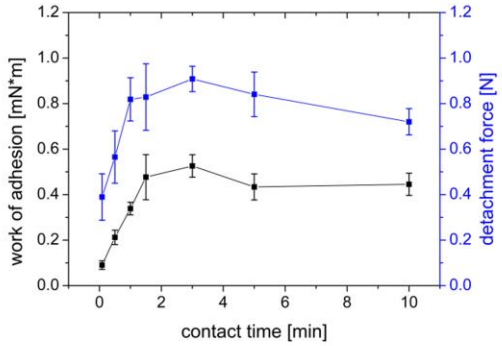


Figure 5. Influence of contact time of a PVA film on work of adhesion (mN×m) and detachment force (N) measured on a gel of 2% agar and 4% mucin. Mean ± SD, $n = 6$.

Estrellas et al. [49] studied the adhesion of different polymers to small pieces of intestinal tissue ex vivo and obtained similar results. They concluded that hydrophilic polymers such as polycarbophil, which can swell rapidly, adhere to tissue quickly but also lose their bioadhesion to rat small intestinal tissue quickly. In contrast, polymers with more hydrophobic backbones exhibit higher adhesion. However, transferring their results to our in vitro study is problematic due to the different experimental conditions. In the study of Estrellas et al., different amounts of liquid can be assumed on the small intestine tissue than on the biomimetic agar/mucin gels. The amount of fluid may affect swelling and, thus, mucoadhesion of the PVA polymer. Preconditioning of the biomimetic gels with wetting fluids could be performed to reproduce these conditions in in vitro experiments.

3.1.4. Influence of the Withdrawal Speed

When varying the withdrawal speed of the PVA film from the agar/mucin gel, it can be observed that the work of adhesion increased with increasing withdrawal speed (Figure 6). The maximum detachment force was observed with a value of $F_{max} = 1.006$ N at a withdrawal speed of 1.0 mm/s and decreased again with further increasing withdrawal speed. In experiments with chicken pouch and Carbopol 974P and Methocel K4M tablets, Wong et al. [48] observed an overall increase in the work of adhesion and maximum detachment force the faster the specimen was removed from the tissue.

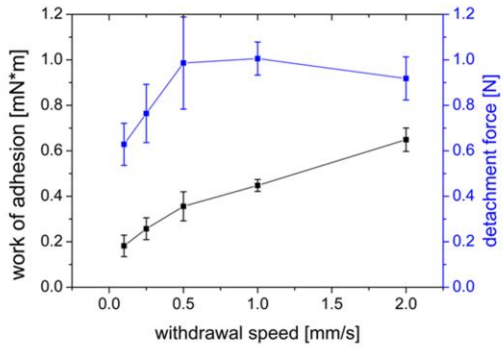


Figure 6. Influence of the withdrawal speed of a PVA film on the work of adhesion (mN×m) and detachment force (N) measured on a gel of 2% agar and 4% mucin. Mean ± SD, $n = 6$.

The withdrawal speeds studied in their work ranged from 0.1 to 1.0 mm/s. A slight decrease in the maximum detachment force from 0.5 mm/s to 1.0 mm/s was observed for the tablets made of Carbopol 974P. In our study, the maximum detachment force decreases from a velocity of 1.0 mm/s. These differences may be because the polymers are different and the tissue used by Wong et al. [48] was wetted, unlike the biomimetic agar/mucin gels.

A slow withdrawal speed may decrease adhesion as there may be a lack of dissipation in the gel [50]. In their study, Baus et al. [41] investigated withdrawal speeds from 0.1–2.0 mm/s and concluded that both F_{max} and W_{ad} are lower at lower withdrawal speeds. The lower forces and reduced work are thought to be caused by the elastic properties of the gel that occur when the film contacts the respective substrate [36]. Due to higher stress, which results from higher withdrawal speeds, the time for bond deformation is reduced, resulting in higher measurable adhesion [51].

3.1.5. Addition of Wetting Liquid

Adding even small amounts (2.5 mL) of phosphate buffer pH 7.4 USP to a freshly prepared agar/mucin gel abruptly decreased the adhesion of the PVA film from F_{max} = 0.777 N and W_{ad} = 0.229 mN×m to F_{max} = 0.274 N and W_{ad} = 0.061 mN×m (Figure 7).

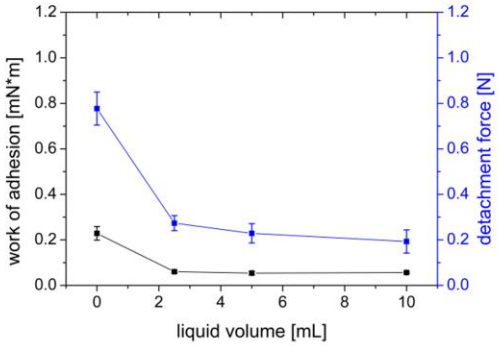


Figure 7. Effect of adding wetting liquid (PBS buffer pH 7.4) of a gel of 2% agar and 4% mucin on work of adhesion (mN×m) and detachment force (N) measured with a PVA film. Mean ± SD, n = 6.

One reason for better adhesion to drier surfaces could be the movement of water from the mucus layer into the film as described by Baus et al. [41]. Due to this osmotic effect, the PVA film adheres well to comparatively dry surfaces. As a nonionic and hydrophilic polymer, the PVA mainly binds to the agar/mucin gel through secondary bonds. These secondary bonds are mostly hydrogen bonds. When a wetting liquid is added, the hydrogen bonds preferentially interact with it rather than with the underlying agar/mucin gel. Moreover, the additional presence of solvent can lead to faster swelling of the PVA film. The consequences of faster swelling have already been discussed in Section 3.1.3.

3.1.6. Influence of Gel Storage

When investigating the effect of storage time on the adhesion, it is clear that there was a change in the adhesion of the PVA films to the gel surface over time (Figure 8). On the production day, the measured work of adhesion was lowest (0.352 mN×m), increased with time until day 7 (0.421 mN×m), and decreased back to the initial level after 14 days of storage (0.356 mN×m). However, the influence of the storage time is more pronounced in terms of the maximum detachment force. The detachment force increased from the day of manufacture (0.717 N) to the following day (0.981 N) and remained essentially constant after (1.028–0.996 N). Agar gels are known to be subject to the phenomenon of syneresis. This involves spontaneous shrinkage of the gel and separation of the bound water [52]. The water collects in tiny droplets on the surface of the gel. At the same time, there is an increase

in the concentration of the polymers in the gel. The increase in mucin concentration due to water separation during storage is possibly the reason for the increased adhesion of the PVA film. With increasing concentration of a mucoadhesive polymer, more polymer chains are available for crosslinking with the mucin chains, resulting in greater adhesion [24]. Similarly, it is also conceivable that increasing mucin concentration, increases adhesion as more mucin chains can interact.

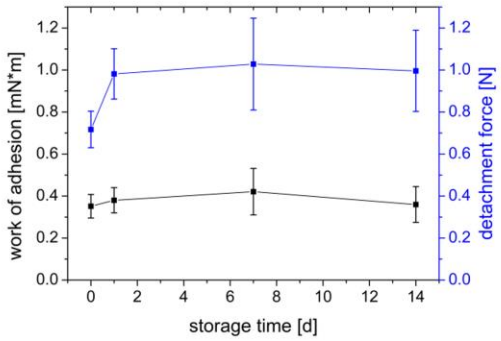


Figure 8. Influence of storage time of a gel of 2% agar and 4% mucin on work of adhesion (mN×m) and detachment force (N) measured with a PVA film. Mean ± SD, $n = 6$.

The experiments to assess adhesion using PVA films and agar/mucin gels showed that using a larger sample area can lead to stronger adhesion. At the same time, adding a wetting liquid can reduce the adhesion of a PVA film. Furthermore, the storage time of biomimetic gels also plays a role. Therefore, they should always be freshly prepared before starting the experiment to obtain reproducible results.

Of the test equipment-specific parameters, the contact force has the most negligible influence on the adhesion of the films to the biomimetic gels. The contact time, on the other hand, influences the adhesion to the extent that when using 128 (±1.5) µm thick PVA films, a maximum can be observed after a time of 180 s for both the measured maximum detachment force and the calculated W_{ad}. For the withdrawal speed, it can be observed that the work of adhesion increases with increasing withdrawal speed, while the tear-off force is at its maximum at 1 mm/s.

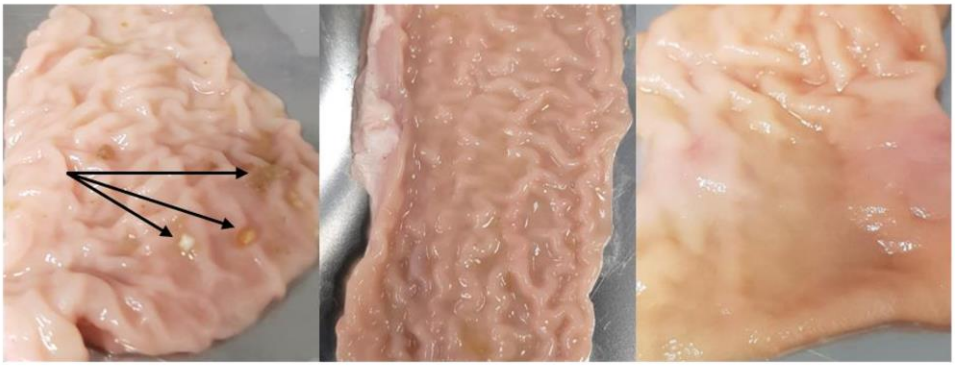
From the results of the tests on the agar-mucin gels, it was possible to derive an optimized test equipment parameter, which is favoring the highest possible detachment force F_{max} and work of adhesion W_{ad} of PVA films on the agar/mucin gels. For example, the contact force was adjusted to include the structural makeup of the tissue used below. The small intestinal tissue of pigs is partially compressible. Higher contact forces could compress the tissue more, with the tissue losing integrity as a result [48]. At the same time, the sample should adhere as completely as possible to the tissue, which is textured relative to the gel, because adhesion is usually higher on smooth surfaces than on uneven surfaces [53]. A higher contact force and a resulting relaxation of the tissue can lead to a smoothing of the surface of the tissue. The influence of the contact force does not seem to have a great impact on the measurement results in the measured range, which is why a force of 0.35 N was chosen due to the minimization of damage to the tissues. The optimum contact time of the PVA film for the highest possible tear-off force and WoA was 180 s. The influence of the withdrawal speed on the tear-off force has its optimum at 1.0 mm/s, while the influence on the WoA increases further with increasing withdrawal speed. In favor of a controlled film peeling, a withdrawal speed of 1.0 mm/s was chosen for the optimized measurement conditions. The diameter of the circular PVA film section was also 14 mm ($A \approx 154$ mm^2) for the adhesion measurements on porcine small intestinal tissue.

3.2. Comparative Measurements on Porcine Small Intestine Tissue

To investigate the influence of small intestine tissue processing on mucoadhesion, tissue from three pigs was measured using the optimized test equipment parameters described previously. Three measurements were performed for each experimental animal:

- First, the tissue was used in fresh and uncleaned condition;
- Second series of measurements were performed on fresh, cleaned tissue;
- Third series of measurements consisted of cleaned tissue frozen for seven days.

As shown in Figure 9, visible differences were already evident between the samples of the individual test animals. While in pig #1, food components adhered to the mucosa, only liquid components were visible in pig #2 and pig #3.



(**a**) (**b**) (**c**)

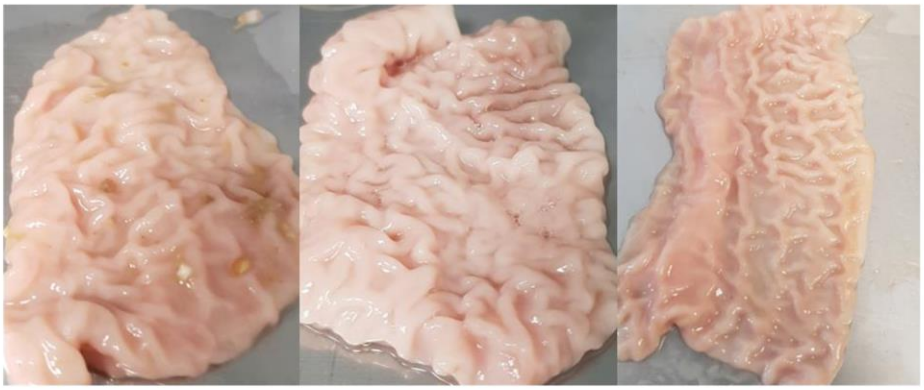
Figure 9. Differences between the individual tissue samples of the pig small intestines. (**a**) pig #1; (**b**) pig #2; (**c**) pig #3. The intestinal tube was cut longitudinally and divided into approx. 5 cm × 7 cm sections. Food components (about 3 mm) present in pig #1 are marked with arrows.

Since the test animals had food and water available before slaughter and tissue removal, this explains the different filling states of the small intestine. On the one hand, this can lead to poor interindividual comparability and reproducibility [31]. In vivo, fluids are always present in the small intestine regardless of the prandial state. In the fasted state, a constant secretion of mucus as well as small amounts of gastric acid, bile and pancreatic juice can be expected. In the fed state, the number of components emptied from the stomach increases, as does the production of bile and pancreatic juice [54]. During careful cleansing of the tissues, these solid and liquid components can be removed. This suggests that the uncleaned intestine may better represent the fed state in vivo.

The effects of processing on the tissue were also macroscopically visible as exemplified by the tissue of pig #1 in Figure 10. While individual food components are still visible on the fresh, unprocessed tissue, they are no longer visible on the section of the small intestine washed with deionized water. The thawed washed tissue lost notable firmness during thawing, and the folding (plicae circularis) was less pronounced. In addition, tissue fluid leaked during the thawing of the tissue samples.

The fact that the structure of the mucus layer of the porcine nasal mucosa is also changed during thawing was also observed by Hägerström et al. [36]. They concluded that the tissue should be used as fresh as possible. During the thawing of the specimens, there was a leakage of tissue fluid. This can be attributed to the presence of ice crystals. Ice crystals are formed when tissue is frozen without antifreeze agents [55]. Depending on the speed of the freezing process, these ice crystals may damage the cells by perforation. In addition, ice formation can lead to osmotic processes, which in turn can damage the integrity of the tissue [55]. Baraibar et al., observed in studies on canine small intestines

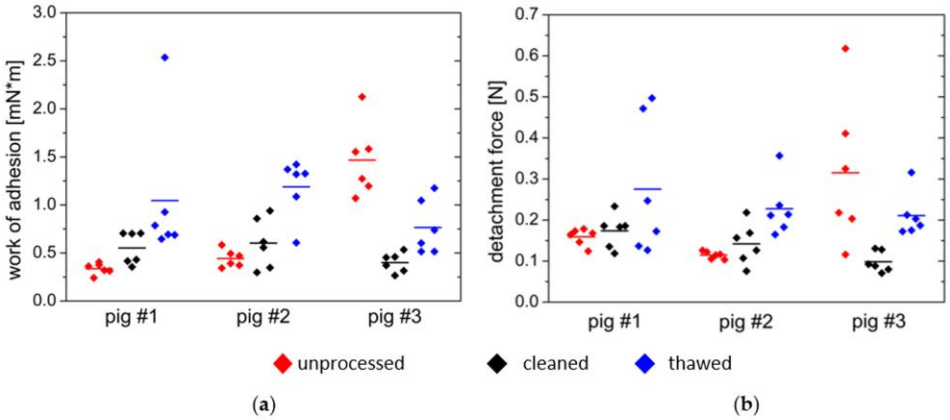
that there was an increase in autolysis of the mucosa during freezing for seven days and subsequent thawing. They also observed that no mucus was detectable after thawing.



(**a**) (**b**) (**c**)

Figure 10. Intraindividual differences between processed tissue samples from pig #1. (**a**) Unprocessed tissue; (**b**) cleaned tissue; (**c**) thawed tissue.

The effect of these intra- and interindividual differences can not only be seen macroscopically but also in the mucoadhesion measurements. Figure 11 shows that the maximum detachment force F_{max} and the work of adhesion W_{ad} differ between individuals and interindividual with tissue processing.



(**a**) (**b**)

Figure 11. (**a**) Work of adhesion in mN×m and (**b**) maximum detachment force in N required to detach PVA films from differently prepared tissue samples. Shown are the individual data and median, $n = 6$.

A trend can be seen in the tissue samples from pig #1 and pig #2, as can be seen in Figure 11. The work of adhesion and maximum detachment force were both low on the uncleaned tissue and showed a low variation of measured values. The thawed, cleaned tissue showed a higher adhesion work and maximum detachment force in both cases. The variation of the measured values was also higher within one test animal. In pig #3, the uncleaned tissue differed from the observations of the measurements of pig #1 and pig #2.

The adhesion appeared to be very high on this tissue, which is illustrated by a mean work of adhesion of W_{ad} = 1.467 mN×m and a mean maximum detachment force of F_{max} = 0.292 N in contrast to W_{ad} = 0.402 mN×m and F_{max} = 0.098 N for the cleaned tissue. At the same time, the variation of the measured values was high for the fresh, uncleaned fabric.

The most notable interindividual differences could be found with a value range of approximately W_{ad} = 0.3–1.5 mN×m in the fresh, uncleaned tissue (Table 2). For the fresh, cleaned tissue, the work of adhesion values ranged from W_{ad} = 0.4 mN×m to W_{ad} = 0.6 mN×m and were thus closer to each other. Adhesion on thawed tissue ranged from W_{ad} = 0.77 mN×m to W_{ad} = 1.2 mN×m.

Table 2. Overview of the results (mean ± SD) of the mucoadhesion in vitro and ex vivo tests at the test parameters Fcontact = 0.35 N; tcontact = 180 s and withdrawal = 1.0 mm/s.

		W_{ad}	F_{max}
		mN×m	**N**
pig #1	unprocessed	0.337 ± 0.058	0.147 ± 0.020
	cleaned	0.553 ± 0.168	0.144 ± 0.041
	Thawed	1.046 ± 0.737	0.217 ± 0.168
pig #2	unprocessed	0.443 ± 0.090	0.111 ± 0.009
	cleaned	0.599 ± 0.260	0.121 ± 0.050
	thawed	1.190 ± 0.307	0.190 ± 0.068
pig #3	unprocessed	1.467 ± 0.380	0.292 ± 0.180
	cleaned	0.402 ± 0.101	0.098 ± 0.025
	thawed	0.766 ± 0.283	0.165 ± 0.054
agar/mucin gel		0.730 ± 0.122	1.104 ± 0.060

Adhesion can be influenced by a wide variety of parameters that differ interindividual and are challenging to evaluate. Especially the amount of fluid can influence mucoadhesion. This can vary depending on the filling state of the small intestine. Additionally, the thickness and viscosity of the mucus layer can influence mucoadhesion as shown by Varum et al. [37]. The authors observed that to detach a pellet of Carbopol 974P NF from gastric mucosa, a significantly higher adhesion work W_{ad} had to be applied than when detaching mucosa from the jejunum. As a possible reason, the different thickness of the respective mucus layer is mentioned, which in pigs is significantly thicker in the stomach (about 51–68 µm) than in the jejunum (about 29 µm). Due to the thicker mucus layer, a more substantial and deeper chain diffusion could take place, which leads to stronger adhesion, according to the authors.

When carefully cleaning the tissue samples, damage to the mucus layer may occur. Depending on the cleaning intensity, there may be dilution or even washing away of the mucus. From this consideration, one would expect adhesion to be lower after cleaning. In the present results, this was the case only in one of three samples (pig #3). The amount of liquid after washing may be lower in pig #1 and pig #2 than in the uncleaned state, which could result in higher adhesion. In contrast, Mortazavi and Smart observed in their studies that the presence of mucus decreased the adhesive forces [56]. The thickness and texture of the mucosa could have been determined using histological sections to conclude the effect of cleaning and thawing on the mucosa. This should be part of further investigations. Since mucoadhesion is a highly complex process that can be influenced by many factors, some of which are also mutually dependent, the reasons for the observations can only be speculated.

Comparing the measurement results on the tissues with the mucosa-mimicking agar/mucin gels (Table 2), it is noticeable that the maximum detachment force of 1.104 N is much higher for the gel than for the tissues (0.098–0.292 N). For the work of adhesion, the value for the gels of 0.730 mN×m is in the range of 0.337–1.467 N measured on the tissue. Because the variability of the tissue is so high, the agar/mucin gel used can only replicate

mucoadhesion to tissue to a limited extent. For example, gels for the different prepared tissues could be investigated in further studies to represent a worst-case and a best-case of adhesion.

When characterizing and deriving the measurement parameters considered optimal, it should be noted that they do not have general validity but represent the optimal measurement conditions for PVA films on agar/mucin gels in the texture analyzer. Other parameters may be necessary depending on the dosage form or polymer used. For polymers that swell quickly, a shorter contact time may be necessary. On the other hand, solid dosage forms such as tablets sometimes require more time to absorb and swell water from the mucosa-mimicking gel. In these cases, wetting the gel with liquid or preswelling the dosage form may be beneficial to simulate physiological conditions [37]. Thus, many variables should be considered in mucoadhesion measurement.

4. Conclusions

The presented study successfully characterized a measurement method for determining the mucoadhesion of PVA films. Various factors affect the measurement results when measuring the mucoadhesion of PVA films on agar/mucin gels. For the test equipment-specific parameters of contact force, contact time and withdrawal speed, the influence of contact time in particular was observed as an influencing variable. The optimum contact time for the PVA films investigated was 180 s. The withdrawal speed also influences the test results—the adhesion work increases with a higher withdrawal speed. Less influence on the measured variables was observed for the contact force. The sample area, the wetting liquid's presence, and the gels' age were identified as further influencing variables.

An optimized set of parameters was derived from the experiments on the mucosa-mimicking agar/mucin gels, which were used to perform ex vivo experiments on small intestinal tissue from pigs. In the ex vivo experiments, the effect of tissue preparation was investigated. Intraindividual differences were found depending on whether the tissue was used in the uncleaned fresh state, cleaned fresh state, or cleaned and thawed state. In two out of three pigs, an increase in the maximum detachment force F_{max} and the work of adhesion W_{ad} could be observed from the uncleaned fresh to the cleaned fresh and the cleaned thawed tissue. Notable interindividual variability could also be observed. In addition to reducing the use of experimental animals, the findings obtained in this study also highlight the need for mucosa-mimicking gels with high reproducibility of results. Ideally, these should be able to cover a wide range of tissue types to represent interindividual differences as well. Further studies should be performed on human tissues to investigate the comparability of mucosa-mimicking gels, potential animal tissues used ex vivo, and human tissues. In particular, human tissues suggest even more pronounced interindividual variability due to age, sex, and potential disease of the tissue.

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5.3 Determination of Mucoadhesion of Polyvinyl Alcohol Films to Human Intestinal Tissue

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Adrian Rump	statistische Auswertung, Korrektur des Manuskripts
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Werner Weitschies	Erarbeitung der Fragestellung, Konzeption, Korrektur des Manuskripts

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Article

Determination of Mucoadhesion of Polyvinyl Alcohol Films to Human Intestinal Tissue

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Abstract: The absorption of drugs with narrow absorption windows in the upper small intestine can be improved with a mucoadhesive drug delivery system such as enteric films. To predict the mucoadhesive behaviour in vivo, suitable in vitro or ex vivo methods can be performed. In this study, the influence of tissue storage and sampling site on the mucoadhesion of polyvinyl alcohol film to human small intestinal mucosa was investigated. Tissue from twelve human subjects was used to determine adhesion using a tensile strength method. Thawing of tissue frozen at −20 °C resulted in a significantly higher work of adhesion ($p = 0.0005$) when a low contact force was applied for one minute, whereas the maximum detachment force was not affected. When the contact force and time were increased, no differences were found for thawed tissue compared to fresh tissue. No change in adhesion was observed depending on the sampling location. Initial results from a comparison of adhesion to porcine and human mucosa suggest that the tissues are equivalent.

Keywords: mucoadhesion; site-specific application; intestinal application; ex vivo measurements; human intestinal mucosa

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1. Introduction

An ideal drug substance should be absorbed uniformly throughout the small intestine. Some drugs are poorly absorbed due to narrow absorption areas, also known as absorption windows. These are usually located in the upper part of the small intestine. Poor absorption may be caused by specific transport mechanisms such as active transport or active excretion. Several drug delivery approaches have been developed to overcome this challenge, such as mucoadhesive films, which can be a highly beneficial drug delivery system (DDS) for site-specific applications, such as in the upper intestine. Examples of drugs that are only absorbed in the upper small intestine are furosemide [1], acyclovir [2,3] and gabapentin [4]. Other possible drugs that could benefit from prolonged residence time through mucoadhesion are therapeutic peptides and proteins [5,6]. These macromolecules mostly have very low oral bioavailability due to their high molecular weight and vulnerable structure. The specific amino acid sequence essential for drug activity can be destroyed by the chemical, physical and proteolytic nature of the gastrointestinal tract [7]. An ideal DDS for peptides and proteins should protect and preserve the drug structure and release it at the highly vasculated specific absorption site [8].

To predict the adhesion of the dosage form in vivo during formulation development, appropriate in vitro methods can be useful. In vitro methods have the advantage of good reproducibility and avoidance of biological tissues. Many biomimetic materials have been described in literature to mimic and replace tissue. They include, for example, simple hydrogels such as gelatin [9] or agar gels [10] and more complex hydrogels such as HEMA-AGA hydrogels [11] or mucin compacts [12]. The disadvantage of these biomimetic

materials is that they may not adequately represent the inter-individual variability of ex vivo and in vivo studies. This can lead to biased prediction of in vivo behavior by in vitro methods. Therefore, ex vivo methods using tissue can be very helpful to get an idea of the variability in vivo. Ideally, the tissue used should represent as closely as possible the application site of the DDS under development.

Tissues from animal sources are mainly used as ex vivo substrates, such as chicken pouch [13], porcine tissue [14] or bovine tissue [15]. Although animal tissues are often used in ex vivo studies, there is the ethical drawback that animals have to be slaughtered to obtain the tissue. Along with these ethical concerns, the choice of suitable animal tissue is another issue. When it comes to mucoadhesion studies in the small intestine, rodents are known to be poor model animals. Not only the anatomy and physiology have been found to be different from humans [16,17] but also the pH and water content [18]. Therefore, large animal models such as pigs are often used to study the small intestine. However, although the pig anatomy is quite similar to that of humans, there are still some differences. Mucus thickness and composition are known to influence mucoadhesion [19]. In pigs, the average thickness of the small intestinal mucus is about 26 to 31 µm [19], whereas in humans the gastroduodenal mucus layer is of variable thickness [20]. These differences may affect mucoadhesion and the in vitro-in vivo correlation of mucoadhesion studies. Therefore, the ideal tissue for mucoadhesion studies is potentially human tissue. Patients taking medicines are usually elderly people suffering from more than one disease [21]. Their gastrointestinal tract may further differ from that of animals used in animal models. Theoretically, tissue from the target patient population should ideally be used to obtain the most predictive results.

Despite the choice of tissue, tissue preparation and storage may also affect the outcome of studies. In previous studies, mucoadhesion was found to be higher on thawed porcine small intestine tissue than on fresh tissue [22]. As these results may not be applicable to human tissue, further mucoadhesion studies on human small intestinal mucosa are needed. To the best of our knowledge, there is no ex vivo mucoadhesion study on human intestinal tissue. In this study, several questions will be addressed, the first of which is whether tissue storage has an effect on mucoadhesion in two different test setups. Secondly, the effect on the sampling site was investigated. Finally, a comparison was made with results on porcine tissue obtained in previous studies [22] using the same methodology. The results should indicate that the choice and storage of the tissue and the experimental design of each mucoadhesion study are very important variables that need to be investigated in order to understand the underlying mechanisms of mucoadhesion and to achieve predictive results for respective DDS.

2. Materials and Methods

2.1. Study Materials

The water-soluble polyvinyl alcohol quality EMPROVE® ESSENTIAL PVA 18–88 (PVA 18–88, $M_w \approx 96{,}000$ g/mol, Merck KGaA, Darmstadt, Germany) with a degree of hydrolysis of 88% was used as the mucoadhesive polymer for the preparation of the mucoadhesive films. Anhydrous glycerol (AppliChem GmbH, Darmstadt, Germany) was used as a plasticizer. The chemicals were dissolved in demineralized water.

2.2. Preparation of Mucoadhesive Films

Mucoadhesive films were prepared using the solvent casting technique on the day before the planned surgery. A total of 80.00 g demineralised water and 2.00 g anhydrous glycerol were mixed on a magnetic stirring plate (IKA® RCT basic, IKA®-Werke GmbH & CO. KG, Staufen, Germany) at 250 rpm. Then, 18.00 g ground PVA 18–88 was added at 500 rpm. The dispersion was heated to 85 °C under continuous magnetic stirring at 150 rpm for 1 h until a clear solution was obtained. The solution was centrifuged at 4400 rpm for 15 min to remove air bubbles (Centrifuge 5702 R, Eppendorf SE, Hamburg, Germany). The solution was cast on a liner at 12.0 mm/s with a coating knife set to 1000 µm

(mtv messtechnik oHG, Erftstadt, Germany) using an automatic coating bench (Automatic Precision Film Applicator CX4, mtv messtechnik oHG, Erftstadt, Germany). The cast film was dried at room temperature.

2.3. Study Participants

A positive ethical vote was obtained from the Ethics Committee of the University Medicine of Greifswald for the mucoadhesion study on human tissue (Ethical Protocol No. BB 027/21, date of approval: 2 March2021). A total of 13 patients (10 male, 3 females; BMI = 24.5 ± 5.5 kg/m^2) aged 36 to 84 years (68 ± 13 years) was included. The patients suffered from various diseases of the gastrointestinal tract, such as cancer, sigmoid diverticulitis or Crohn's disease. The operations during which the samples for the study were taken were directly related to these diseases. Written informed consent was obtained from all subjects and included information about the tissue sampling, the experimental plan, the handling of personal data and the data protection laws of Germany. During medically necessary surgery for Whipple procedure (n = 4), right hemicolectomy (n = 4), ileostomy (n = 4) or pancreatectomy (n = 1), a portion of healthy small bowel was also removed for technical reasons. This tissue was the proximal jejunum (n = 5) or the distal ileum (n = 8). In addition to demographic data, premedication data were also collected from the study participants.

2.4. Mucoadhesion Study

Mucoadhesion was determined using the same texture analysis method described in a previous study [22]. Briefly, a texture analyser (TA plus, AMETEK Lloyd Instruments Ltd., Hampshire, UK) equipped with a 10 N load cell was used to measure the maximum detachment force (F_{max}) and the work of adhesion (WoA). Circular pieces (d = 14 mm, A ≈ 1.54 cm^2) of the PVA films were punched out using a punching iron. The films were attached to the upper probe using double-sided adhesive tape (tesa® Doppelseitiges Klebeband universal, tesa SE, Hamburg, Germany). The tissues were placed on the lower base of the apparatus. They were collected at the time of removal during surgery and transported to the laboratory in a polystyrene cooler filled with ice. To avoid direct contact, a bag filled with water was placed between the tissue placed in another bag and the ice. The time between the collection of the samples and the start of the experiments was a maximum of 30 min. The intestinal tissue was cut into four pieces, two of which were used immediately. The other two were placed in sealed PE bags and frozen at −20 °C in a freezer. After one week of storage, the tissues in the PE bags were thawed in a water bath at 37 °C.

F_{max} and WoA were measured in two settings based on a previous study [22]. In brief, a standard setting (setting A) was used as a starting point to investigate the influence of contact force, contact time and withdrawal speed on the mucoadhesion of PVA films to agar/mucin gels. Setting A was chosen on the basis of literature values. A low contact time and low contact force were used. In the following investigations, setting B was found to be the best compromise between the highest F_{max} and WoA and gel integrity. The experimental setups for both are described in Table 1. Each setting was performed on fresh and thawed tissue. The upper probe with the polymer film was lowered at a constant speed to the tissue from a distance of 5 cm until a specified contact force was detected. The probe remained in this position during the contact time and was then removed at a defined withdrawal speed. During removal from the tissue, load and machine extension were measured using NEXYGEN Plus software (AMETEK Lloyd Instruments Ltd., Hampshire, UK).

Table 1. Instrument settings for Setting A and B.

Variable	Setting A	Setting B
contact force [N]	0.1	0.35
contact time [s]	60	180
withdrawal speed [mm/s]	0.5	1.0

2.5. Statistical Analysis

F_{max} and WoA were calculated using Microsoft® Excel® 2019 (Microsoft Corporation, Redmond, WA, USA) and reported as individual data and medians. F_{max} was the maximum force measured during film detachment. WoA describes the area under the curve (AUC) and was calculated using the linear trapezoidal rule. Statistical analysis was performed using GraphPad Prism 5 (v. 5.01; GraphPad Software, Boston, MA, USA). F_{max} and WoA were tested for normal distribution using the D'Agostino and Pearson omnibus normality test. If the data were normally distributed and paired (e.g., derived from same subject), a paired t-test was used. Data that were not normally distributed were compared non-parametrically. A Wilcoxon signed rank test was used for paired data and a Mann-Whitney U test was used for unpaired data.

3. Results

A total of 13 subjects was initially part of the study. One subject (female, age = 84 years, BMI: 18 kg/m^2) had to be excluded during the study because the amount of tissue removed for clinical reasons of the main indication was too small to perform mucoadhesion measurements with a sufficient number of samples. Data from this subject are excluded below. The demographics of the subjects are shown in Table 2.

Table 2. Demographic data of the remaining 12 study participants.

Parameter	Median (Range)	Mean ± SD
sex	m = 10; f = 2	m = 83%; f = 17%
age/y	70 (36–80)	67 ± 12
height/m	1.76 (1.59–1.87)	1.74 ± 0.09
weight/kg	75.6 (43.0–105.0)	75.6 ± 16.8
BMI/kg/m^2	23.9 (16.4–37.6)	25.0 ± 5.4

In the remaining 12 subjects, both settings were to be performed on fresh and thawed tissue. In four subjects the tissue was too small to try both settings. Therefore, setting A with lower contact force, contact time and withdrawal speed was preferred on fresh and thawed tissue. The data have been checked for normal distribution. As not all data were normally distributed, a Gaussian distribution was not assumed for statistical comparison. The individual medians of the subjects can be found in Table S1.

3.1. Processing of the Tissue

The intestinal segments were divided into four parts, two of which were frozen and thawed for the experiments after a one-week storage period, and the other two were used fresh. As setting A was the preferred setting, a comparison of fresh and thawed tissue could be made for all 12 subjects.

Significant differences were found for WoA ($p = 0.0005$) in setting A (Figure 1), whereas no significant difference was found for F_{max} ($p = 0.6221$). For individual data, WoA was higher on thawed tissue than on fresh tissue. No clear trend can be seen for F_{max}.

For setting B, where a higher contact time and force are applied, no significant differences can be found for either WoA ($p = 0.7422$) or F_{max} ($p = 0.3125$) (Figure 2). In contrast to setting A, no trend can be seen in the individual data for either of the calculated mucoadhesion values. Overall, the measured and calculated results were higher in setting B than in setting A.

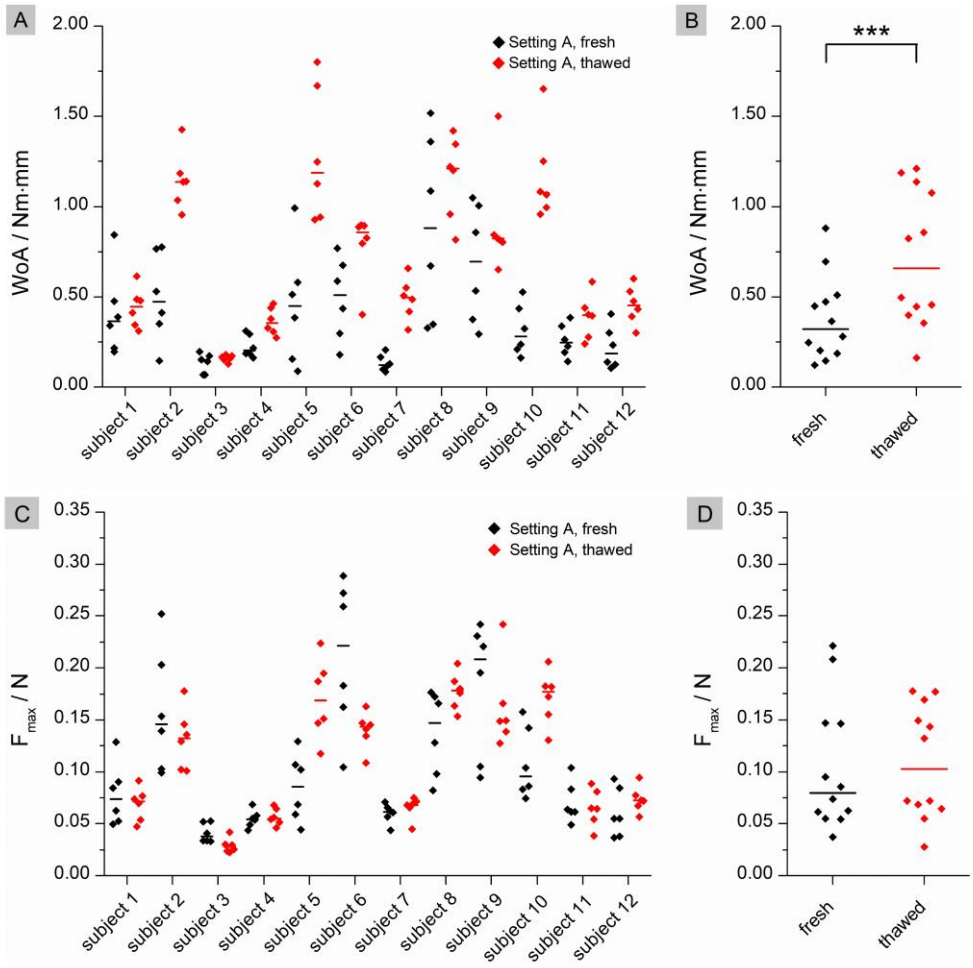

Figure 1. Results for Setting A. (**A**): Individual data of the calculated WoA (Nm × mm) with median (n = 12); black: fresh tissue; red: thawed tissue; (**B**): pooled medians of all subjects with median line; (**C**): Individual data of the calculated F_{max} (N) with median (n = 12); black: fresh tissue; red: thawed tissue; (**D**): pooled medians of all subjects with median line. Significant difference of WoA and F_{max} was checked by using a Wilcoxon signed rank test: *** ($p < 0.001$).

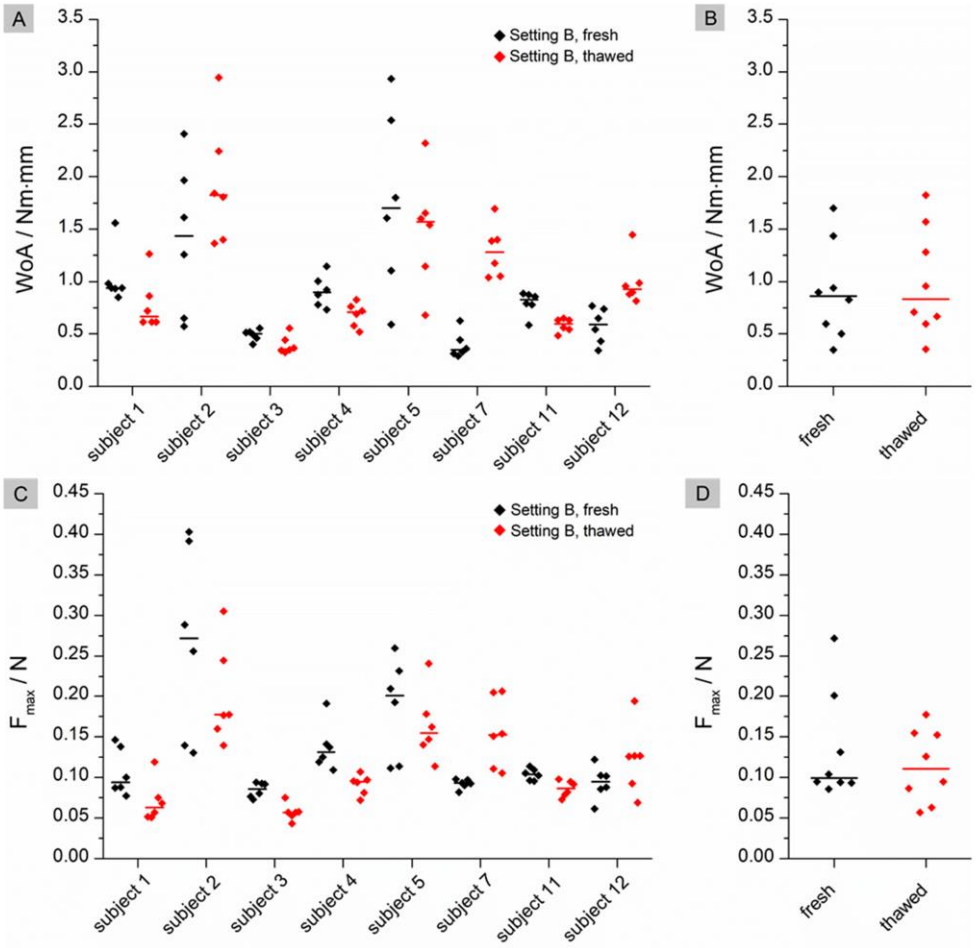

Figure 2. Results for Setting B. (**A**): Individual data of the calculated WoA (Nm × mm) with median (n = 8); black: fresh tissue; red: thawed tissue; (**B**): pooled medians of all subjects with median line; (**C**): Individual data of the calculated F_{max} (N) with median (n = 8); black: fresh tissue; red: thawed tissue; (**D**): pooled medians of all subjects with median line. Significant difference of WoA and F_{max} was checked by using a Wilcoxon signed rank test.

3.2. *Comparison of Different Test Settings*

Fresh and thawed tissues were also compared for both settings to investigate the influence of the test parameters. Setting A used a lower contact time, lower contact force and lower withdrawal speed. Only data from participants who were able to use both settings were included in the comparison, resulting in eight measurements.

As shown in Figure 3, the fresh tissue showed significant differences between setting A and setting B for WoA ($p = 0.0078$) and F_{max} ($p = 0.0078$). For both WoA and F_{max}, the median of each individual data set was significantly higher in setting B than in setting A.

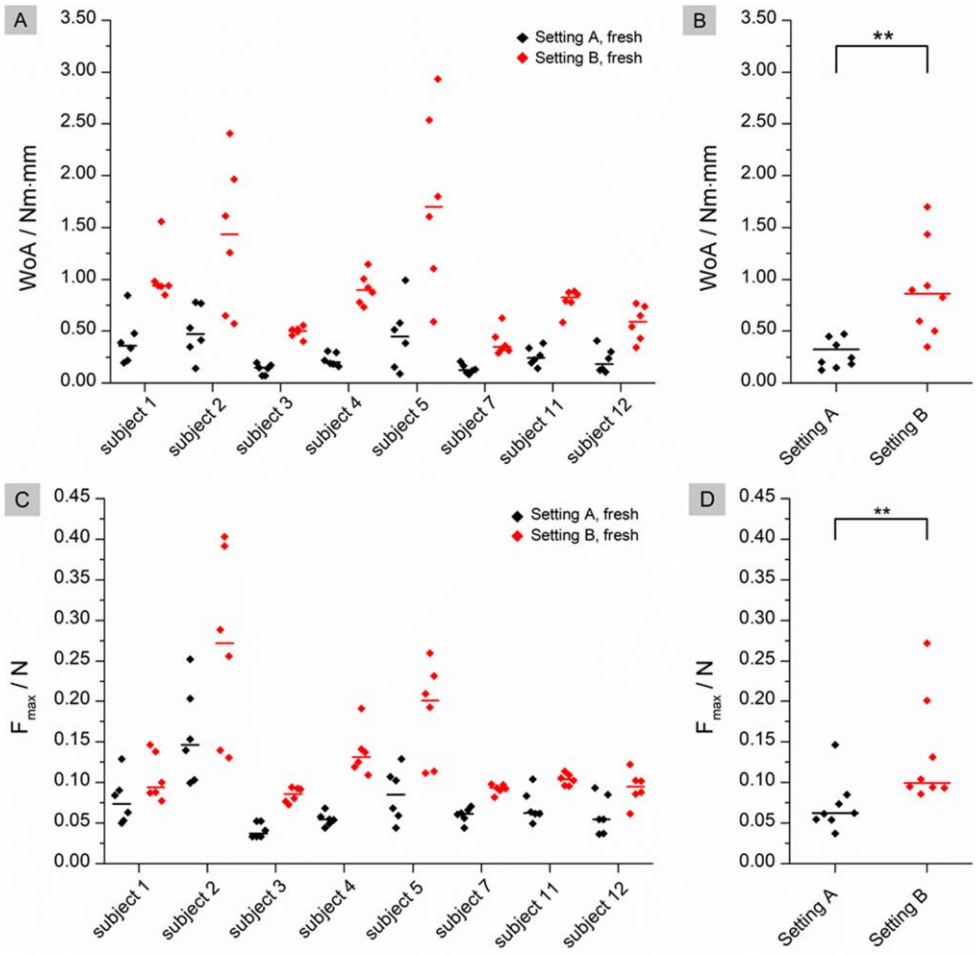

Figure 3. Comparison of the results for Setting A and B on fresh tissue. (**A**): Individual data of the calculated WoA (Nm × mm) with median (n = 8); black: Setting A; red: Setting B; (**B**): pooled medians of all subjects with median line; (**C**): Individual data of the calculated F_{max} (N) with median (n = 8); black: Setting A; red: Setting B; (**D**): pooled medians of all subjects with median line. Significant difference of WoA and F_{max} was checked by using a Wilcoxon signed rank test: ** ($p < 0.01$).

The same comparison was made for thawed tissue. As shown in Figure 4, statistically significant differences were also found for WoA ($p = 0.0078$), but not for F_{max} ($p = 0.3828$).

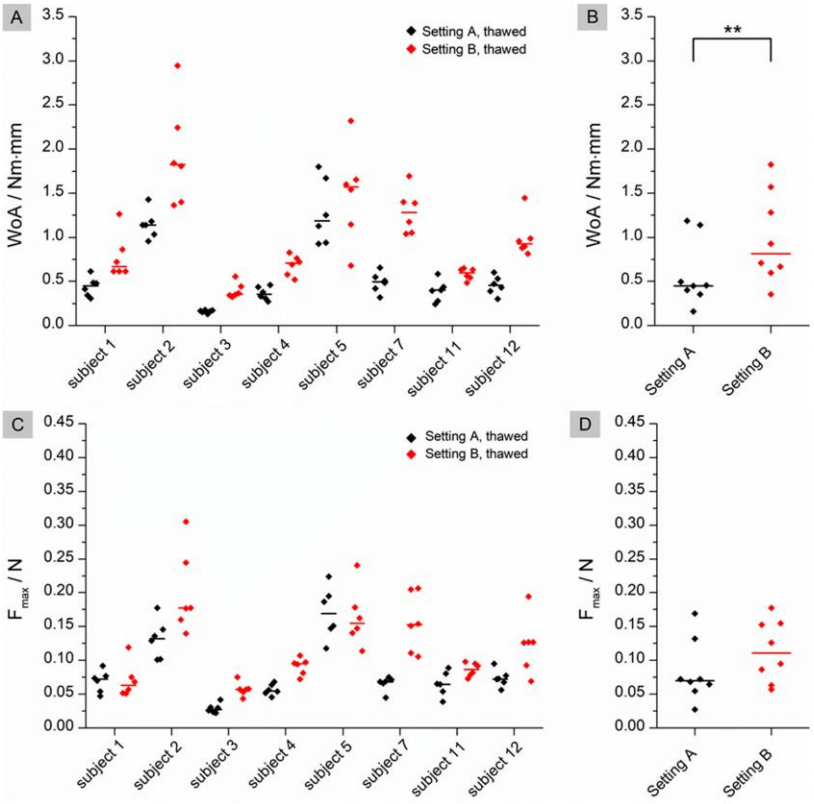

Figure 4. Comparison of the results for Setting A and B on thawed tissue. (**A**): Individual data of the calculated WoA (Nm × mm) with median (n = 8); black: Setting A; red: Setting B; (**B**): pooled medians of all subjects with median line; (**C**): Individual data of the calculated F_{max} (N) with median (n = 8); black: Setting A; red: Setting B; (**D**): pooled medians of all subjects with median line. Significant difference of WoA and F_{max} was checked by using a Wilcoxon signed rank test: ** ($p < 0.01$).

3.3. Sampling Location

Another issue was the importance of the sampling site, as there may be differences in adhesion in the proximal jejunum compared with the distal ileum. A Mann-Whitney U test was performed as the data were not paired and the number of samples was too small to assume a normal distribution. The test was performed on fresh and thawed tissue for setting A only, as the number of samples in this case was 12. No statistical differences were found for either WoA ($p_{fresh} = 0.5303$; $p_{thawed} = 0.2020$) or F_{max} ($p_{fresh} = 0.2677$; $p_{thawed} = 0.1490$).

3.4. Comparison of Mucoadhesion on Porcine Versus Human Intestinal Tissue

The data obtained in this study were compared with those of a previous study carried out on porcine small intestine tissue [22]. In the previous study, the identical test setup A was used to measure mucoadhesion. The only difference, apart from the origin of the tissue, was that the number of samples was much smaller (n = 3) compared to the new study (n = 12). Cleaned porcine tissue was used as a reference because, unlike the participants' tissue, it was not free of food residues due to the surgical specifications. As a result, the statistical analysis presented below may only give an indication of the difference. A Mann-

Whitney U test was performed to evaluate possible differences in WoA and F_{max} between fresh and thawed tissue. The results are shown in Figure 5.

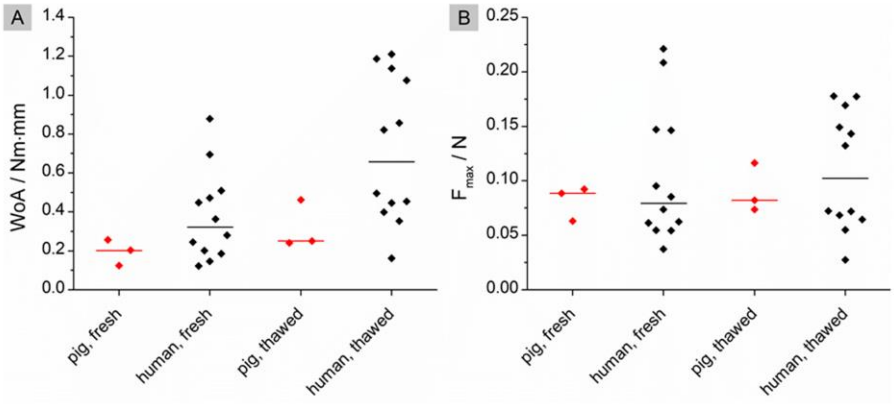

Figure 5. Comparison of the results for Setting A on fresh tissue of pigs (n = 3) and humans (n = 12). (**A**): Pooled medians of the calculated WoA (Nm × mm) with median line; red: pig; black: human; (**B**): Pooled medians of the calculated F_{max} (N) with median line; red: pig; black: human. Significant difference of WoA and F_{max} was checked by using a Mann-Whitney U test.

No significant differences could be found for the WoA (p_{fresh} = 0.2790; p_{thawed} = 0.1296) nor the F_{max} (p_{fresh} = 0.9425; p_{thawed} = 0.9425). The data presented in Figure 5A indicate a trend towards a slightly higher WoA on fasted human tissue compared to washed porcine tissue. However, the sample numbers of porcine tissues are too small to state this with certainty.

4. Discussion

Ex vivo mucoadhesion measurements of PVA-films on human small intestine tissue show that F_{max} and WoA are highly variable inter-individually and intra-individually. Possible influences on the measurement results were investigated. Statistical analysis of the mucoadhesion values in two settings and on tissues prepared in different ways showed that the WoA appears to be sensitive to storage and test parameters. WoA was significantly higher on thawed tissue in setting A, where a lower contact force is applied for a shorter time, but surprisingly not when a higher force is applied for a longer contact time, as in setting B. If tissue is frozen without a cryoprotectant, ice crystals may form. This depends on the rate at which the tissue is frozen. A slow freezing rate often results in the formation of sharp crystals that can damage tissue cells by perforating them [23]. In addition, cells can be further damaged by the osmotic pressure that can result from ice formation [23]. Signs of possible tissue damage were observed after storage of the respective tissue samples. The appearance of the tissue changed during storage. The macrostructure of the tissue appeared flatter than in the fresh condition. There was also some leakage of fluid from the tissue as can be seen in Figure 6.

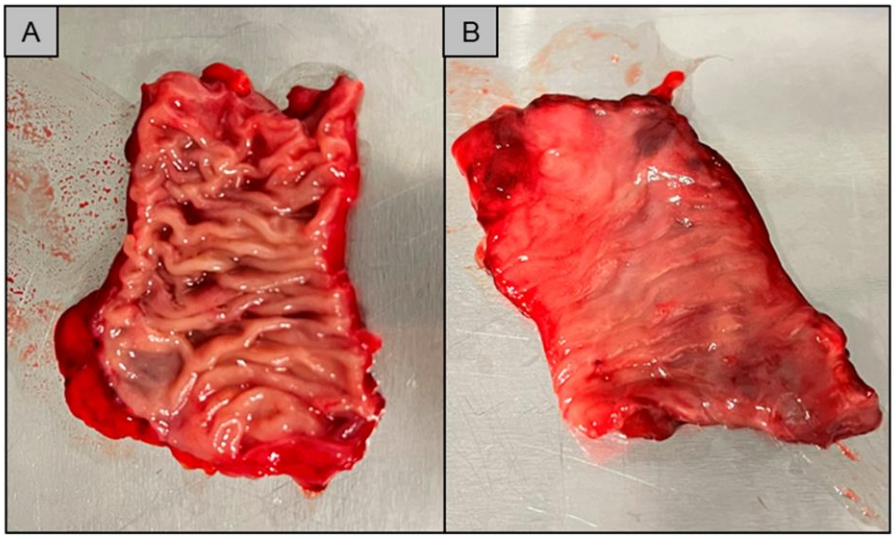

Figure 6. (**A**): Fresh tissue; (**B**): thawed tissue after a storage time of 7 days at T = −20 °C in a freezer.

The flattened structure of thawed tissue may explain the higher observed WoA. When a low force is applied in the fresh state, the mucoadhesive film may not be in contact with the entire tissue due to the macroscopically visible folded structure. The contact forces of 0.1 N and 0.35 N correspond to biorelevant pressures of 6.5 mbar and 22.7 mbar, respectively. These are within the physiological range of the small intestine as determined in telemetric studies with the SmartPill [24]. Higher contact forces may result in a flatter structure due to tissue compression and therefore more even contact between the film and the mucosa. As a result, WoA may increase when higher forces are applied (setting A versus setting B) or when the tissue loses structure due to thawing. No statistical differences can be found for WoA on fresh versus thawed tissue in setting B. A possible reason for this finding could be that not only the macrostructure but also the microstructure of the mucus changes during storage, as observed by Hägerström et al. [25]. Negatively charged glycoproteins called mucins, which make up approximately 0.5–5% of mucus [26], play an essential role in mucoadhesion. Mucoadhesive polymers can bind to mucins either through chemical bonds, such as ionic, covalent or secondary bonds, or through physical bonds. These include interpenetration and entanglement of polymer structures and mucin chains [27]. Polyvinyl alcohol, which was used in our study, is a non-ionic polymer. This group of polymers is known to bind to mucus through secondary chemical bonds such as hydrogen bonds and chain entanglements. Typically, the mucoadhesion of non-ionic polymers is lower than that of cationic polymers such as chitosan, which bind by electrostatic attraction to negatively charged mucins [28]. If the structure of the mucus changes during freezing and thawing [29], it may loosen, resulting in a looser structure that potentially facilitates interpenetration and chain entanglement. This may have a positive effect on the mucoadhesion of non-ionic polymers, as observed for thawed tissue in setting A (Figure 1B). The influence of a higher contact force (setting B) appears to have a greater effect on mucoadhesion than the thawing process, as there are no statistical differences between fresh and thawed tissue in setting B. However, the loss of the microstructure of mucus may influence the adhesion of charged polymers. When mucus hydrogels are frozen and thawed, there is a phase separation between the aqueous phase and the hydrogel former, resulting in a concentration of mucins. This in turn can lead to an increased number of possible electrostatic interactions, resulting in a higher mucoadhesive work. In contrast

to WoA, F_{max} is not influenced by storage (Figures 1D and 2D). The question therefore arises as to whether WoA or F_{max} is the better surrogate for the measurement of mucoadhesion. In their paper, das Neves et al. [30] discussed whether WoA or F_{max} is more suitable for evaluating the mucoadhesion of semi-solids to bovine vaginal mucosa. WoA represents the sum of all adhesive forces, whereas F_{max} represents only the peak force during detachment. Therefore, the authors consider WoA to be the more accurate parameter for mucoadhesion. Da Silva et al. [12] also confirmed in their work that the WoA is more sensitive to changes in the test parameters and therefore the better surrogate for mucoadhesion studies in texture analyser studies. These results are confirmed by our study. For future mucoadhesion measurements with the texture analyser, it should be noted that both the test parameters and, in particular, the storage of the tissues have an influence on the measurement results, making it difficult to compare different studies.

In addition to the storage and test setup, the influence of the sampling site was investigated. No statistical differences were found between the results obtained from the proximal jejunum and the distal ileum. Another patient-specific parameter that may influence the results of the study is the amount of aqueous medium (e.g., mucus and/or bile acid) present on the tissue. A higher amount of water can cause faster hydration of the solid polymer in the mucoadhesive film. This effect is advantageous in the contact stage, as chain disentanglement of the former solid polymer occurs upon hydration [31]. The detrimental effect begins as soon as the polymer hydrogel is diluted. If the amount of water in the polymeric gel becomes too high, the cohesiveness of the gel will decrease. This results in a failure of adhesion within the gel as the test preparation is detached from the mucosa which is represented by lower values for F_{max} and WoA. To minimise the influence of intestinal fluids, the tissues can be washed [32] or wettened with a specified amount of liquid [33] prior to the experiment. The disadvantage of these methods is that the tissues may no longer represent the actual in vivo state.

Another patient-related factor to be considered is age. Intestinal morphology does not appear to change in older people [34,35]. There is some evidence that there may be changes in the structure of mucus with age. Elderman et al. [36] reported that the age of mice can influence the thickness of their colonic mucus, with older mice having a thinner layer of mucus compared to young mice. As mentioned above, mucus thickness may influence mucoadhesion, so it would be interesting to investigate age-related changes in mucus in humans. Other important changes that occur with ageing are increased illness and polymedication [21]. Drugs and inflammatory bowel diseases could also affect mucoadhesion, as they can affect pH and mucus [37]. It is important to note that there are many factors that can influence mucoadhesion in vivo, especially in the elderly. These factors are less likely to have a visible effect on ex vivo mucoadhesion studies as their influence may be small. However, when it comes to in vivo performance, they should be considered.

The final point evaluated in our study was the comparison of mucoadhesion to porcine versus human small intestinal tissue. The data for porcine tissue were taken from a previous study carried out in our laboratory [22] under the same conditions. It should be noted that the results of this comparison can only indicate a possible trend, as the number of samples for the porcine tissue was too small. This is related to the fact that the porcine experiments were aimed at a broader screening with more different setups and variables, thus limiting the sample size of measurements comparable to human ex vivo measurements from this study. No significant differences were found for either WoA or F_{max} on fresh and thawed porcine or human tissue. The individual data may suggest that the WoA is slightly higher on human mucosa compared to porcine mucosa, especially when thawed tissue is used. This again might lead to the conclusion that the WoA is the better surrogate for mucoadhesion as it seems to be more sensitive to changes in the tested mucosal sample.

Pigs are often used as model animals for studies involving the gastrointestinal tract because their gastrointestinal physiology is very similar to that of humans [16]. In addition, the availability of tissues is usually good, as pigs are common farm animals, and intestinal

tissues are most often slaughterhouse waste. Jackson and Perkins reported that the mucoadhesion of cholestyramine on porcine gastric mucosa was found to be higher than with human mucosa [38]. They explained this result with a possibly thicker mucus layer in pigs compared to humans. This is contrary to the results obtained in our study, but the limited number of porcine tissue samples and a different mucosa may influence the outcome of these studies.

5. Conclusions

The purpose of this ex vivo study was to highlight the inter-individual variability of mucoadhesion to human small intestine tissue. The study data showed the range of individual results, highlighting the high variability of biological materials. The results show that an ideal mucoadhesive DDS should be able to demonstrate good adhesion despite the high inter-individual variability. PVA mucoadhesive films were used to investigate the test-related factors influencing this variability.

Storage is an important factor influencing the study results and should be considered when performing a mucoadhesion test. The WoA seems to be more sensitive to the storage of tissue when a force of 0.1 N is applied for 60 s. The effect on F_{max} is less pronounced. No statistical differences for both can be found if a higher force of 0.35 N is applied for 180 s. Comparing setting A (lower force and contact time) to setting B (higher force and contact time) shows that there is a significant difference in the measurement results of WoA and F_{max} on fresh tissue. Again, no difference was found on thawed tissue for the F_{max}. Therefore, WoA is assumed to be the better surrogate for mucoadhesion. The results show that the adhesion is dependent on both the test setup and the sample preparation. An ideal test setup and storage of the sample to which the dosage form is to adhere must be individually tested prior to each test.

Although the data available were limited, a comparison of mucoadhesion on porcine and human mucosa was made. The initial impression is that the two tissues are comparable. If further studies confirm the hypothesis that porcine intestinal tissue could replace human intestinal tissue, this would facilitate ex vivo mucoadhesion studies. Tissue of animal origin can be obtained in larger quantities and without the regulatory requirements of ex vivo human tissue studies.

Despite the test-related factors, the sampling site was examined as a patient-related factor. No difference was found between proximal jejunum and distal ileum. Other patient-related factors need to be investigated in the future, as mucoadhesion is a complex phenomenon and the understanding of all factors affecting mucoadhesion in vivo is still limited. Gender, mucus thickness, gastrointestinal fluids, diseases and medications may be other parameters to consider. A larger number of subjects would be needed to gain a deeper understanding of the physiological effects on mucoadhesion and to design an ideal DDS that is minimally affected by these variables. A simple way to address the variability and allow comparability between different test devices, tissue preparations and possible new innovative delivery forms could be to measure adhesion against a simple and reproducible manufacturable standard, such as a polyvinyl alcohol film.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmaceutics15061740/s1, Table S1. Individual medians of F_{max} (N) and WoA (Nm × mm) of all subjects in both test setups (setting A and B) and different tissue preparation. All tests were performed in six replicates.

Author Contributions: Conceptualization, L.M., C.R., M.G., A.B. and W.W.; methodology, L.M. and C.R.; formal analysis, L.M., A.R. and M.G.; investigation, L.M. and F.K.; resources, A.K. and A.B.; data curation, L.M., A.K. and A.B.; writing—original draft preparation, L.M.; writing—review and editing, C.R., A.R., M.G., A.B. and W.W.; visualization, L.M.; supervision, A.B. and W.W.; project administration, M.G., A.B. and W.W. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of the Greifswald University Medicine (Ethical Protocol No. BB 027/21).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Subjects are not identifiable in these data; nonetheless, written informed consent has been obtained from the subjects to publish this paper.

Data Availability Statement: Data are available within the paper and its supplementary material.

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Folgende weitere Publikationen entstanden während der Promotionszeit, sind jedoch nicht Gegenstand der vorliegenden Dissertation:

Krause, J.; Müller, L.; Sarwinska, D.; Seidlitz, A.; Sznitowska, M.; Weitschies, W. 3D Printing of Mini Tablets for Pediatric Use. *Pharmaceuticals* **2021**, 14, 143, doi:10.3390/ph14020143.

Vorträge

“Effect of disintegrants and tablet infill on release behavior of 3D printed tablets”, Additive Manufacturing Meets Medicine, Lübeck, Deutschland, **2021**

Posterpräsentationen

“Effect of L-HPC 21 addition on the release behavior of 3D-printed tablets”, 4th World Meeting on Pharmaceutics. Marseille, Frankreich, **2023**

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

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Laura Müller

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