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Antimikrobielle Beschichtungen für resorbierbare, chirurgische Nahtmaterialien zur lokalen Infektprophylaxe

- Chlorhexidin- und Oktenidinbeschichtungen auf Fettsäurebasis zur verzögerten Wirkstofffreisetzung

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Dissertation
zum Erwerb des Doktorgrades der Humanbiologie
(Dr. rer. biol. hum.)
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von

Andreas Karl-Werner Obermeier

aus

München

2018

Mit Genehmigung der Medizinischen Fakultät
der Universität München

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Tag der mündlichen Prüfung:	20.12.2018

Für Ingrid

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meiner Mutter

mit Rudi

Eidesstattliche Versicherung

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Ich erkläre hiermit an Eides statt,
dass ich die vorliegende Dissertation mit dem Thema:

Antimikrobielle Beschichtungen für resorbierbare, chirurgische Nahtmaterialien zur lokalen Infektprophylaxe

- Chlorhexidin- und Oktenidinbeschichtungen auf Fettsäurebasis zur verzögerten Wirkstofffreisetzung

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München, den 22.12.2018

Ort, Datum	Andreas Obermeier

Inhaltsverzeichnis

Abkürzungsverzeichnis

Ag-BG	silberdotiertes Bioglas
Ag-NP	Silbernanopartikel
AMP	antimikrobielle Peptide
ATCC®	American Type Culture Collection
BAI	biomaterial-assoziierte Infektion
bzw.	Beziehungsweise
CDC	Centers for Disease Control in der USA
d.h.	das heißt
DOI	Digital Object Identifier
DOPA	3,4-Dihydroxyphenylalanin
engl.	Englisch
etc.	et cetera
Fa.	Firma
FDA	Food and Drug Administration in der USA
ggf.	Gegebenenfalls
ggü.	Gegenüber
GRF	Gelatine-Resorcin-Formaldehyd
GSE	grapefruit-seed extrakt
ISO	International Organization for Standardization
MRSA	methicillin-resistenter *Staphylococcus aureus*
NO_x	Stickoxide
nSnH	Nanosilber in Nanohydrogel
PGA	Polyglykolsäure (engl. polyglycolic acid)
Ph. Eur.	European Pharmacopoeia
REM	Rasterelektronenmikroskop
sog.	so genannt
SSI	surgical site infections (chirurgische Wundinfektion)
u.w.	und weitere
US	United States of America
USP	United States Pharmacopoeia
v. Chr.	vor Christus
VP	Vicryl® Plus
WHO	World Health Organization
z.B.	zum Beispiel

1 Publikationen für die kumulative Dissertation

Novel high efficient coatings for anti-microbial surgical sutures using chlorhexidine in fatty acid slow-release carrier systems.

Obermeier A, Schneider J, Wehner S, Matl FD, Schieker M, von Eisenhart-Rothe R, Stemberger A, Burgkart R.

PLoS One. 2014 Jul 1; 9(7):e101426. doi: 10.1371/journal.pone.0101426. eCollection 2014.

Journal: PloS One (accepted: 5. Juni 2014)
JCR 2014: Kategorie: MULTIDISCIPLINARY SCIENCES, Ranking: 9/57 (Q1); IF (2014) = **3,23**
DOI: 10.1371/journal.pone.0101426

In vitro evaluation of novel antimicrobial coatings for surgical sutures using octenidine.

Obermeier A, Schneider J, Föhr P, Wehner S, Kühn KD, Stemberger A, Schieker M, Burgkart R.

BMC Microbiol. 2015 Sep 24; 15:186. doi: 10.1186/s12866-015-0523-4.

Journal: BMC Microbiology (accepted: 18. September 2015)
JCR 2015: Kategorie: MICROBIOLOGY, Ranking: 58/123 (Q2); IF (2015) = **2,58**
DOI: 10.1186/s12866-015-0523-4

Viable adhered *Staphylococcus aureus* highly reduced on novel antimicrobial sutures using chlorhexidine and octenidine to avoid surgical site infection (SSI).

Obermeier A, Schneider J, Harrasser N, Tübel J, Mühlhofer H, Pförringer D, Deimling CV, Foehr P, Kiefel B, Krämer C, Stemberger A, Schieker M, Burgkart R, von Eisenhart-Rothe R.

PLoS One. 2018 Jan 9; 13(1):e0190912. doi: 10.1371/journal.pone.0190912. eCollection 2018.

Journal: PloS One (accepted: 22. Dezember 2017)
JCR 2016: Kategorie: MULTIDISCIPLINARY SCIENCES, Ranking: 15/64 (Q1); IF (2016) = **2,81**
DOI: 10.1371/journal.pone.0190912

Übergeordnete Einleitung zur schriftlichen, kumulativen Promotion

gemäß § 4a der Promotionsordnung für die Medizinische Fakultät der LMU
vom 1. Juni 1983
in der konsolidierten Fassung
der 11. Änderungssatzung vom 15. September 2016

2 Einleitung

Biomaterialien werden heute zahlreich in der Medizin im menschlichen Körper eingesetzt, um Körperfunktionen zu erhalten oder Krankheiten zu behandeln [1]. Deren Definition entwickelte sich dabei mit den Materialien selbst bis heute ständig weiter.

In den 1950er Jahren wurden mit diesem Begriff lediglich avitale, synthetische Materialien bezeichnet, die mit einem biologischen System in Wechselwirkung stehen und sich dabei bioinert verhalten (Biomaterialien der sog. 1. Generation). Der Begriff der Biomaterialien umfasst seit den 70er und 80er Jahren, auch natürliche Materialien mit bioaktiven und resorbierbaren Eigenschaften für bekannte Gewebereaktionen (2. Generation). Der aktuellste und breitgefassteste Begriff für Biomaterialien umfasst seit dem Jahr 2000 bis heute, natürliche und synthetische Materialien sowie Kombinationen hieraus in den verschiedensten Variationen und Zusammensetzungen (3. Generation). Dabei kommen zusätzlich Trägermaterialien (sog. Scaffolds) in Kombination mit lebenden Zellen für den Gewebsersatz bzw. für die Stimulation des Gewebeaufbaus mit Hilfe von „Tissue Engineering“ zum Einsatz. [2]

Diverse Biomaterialien finden somit breit gefächerte Anwendung so z.B. als hoch differenzierte Implantatsmaterialien, mit Zellen bestückte Gerüste zur Geweberegeneration in einer Vielzahl von Medizinprodukten, die mit dem menschlichen Körper kurz- oder langfristig interagieren. Eine weitere Unterteilung der Implantate und Medizinprodukte erfolgt in aktive Produkte, wie z.B. Herzschrittmacher und sog. passive Produkte, wie z.B. Gelenkendoprothesen, Knochenersatzmaterialien, Drainagen, Katheter, Nahtmaterialien sowie eine Vielzahl von Materialien zur Wundversorgung.

Insbesondere Nahtmaterialien spielen in der Chirurgie eine bedeutende Rolle, da diese bei vielen chirurgischen Eingriffen als temporäre oder endgültige Wundverschlussmethode zum Einsatz kommen. Einer aktuellen Studie der WHO zufolge stieg die Gesamtzahl an chirurgischen Eingriffe in 66 Ländern im Verlauf von 2004 bis 2012 um 38 % auf etwa 313 Millionen Eingriffe pro Jahr an [3]. Im Zuge dessen stieg auch der Verbrauch an Nahtmaterialien bis zum Jahr 2013 deutlich an und erreichte allein im Segment der resorbierbaren Nahtmaterialien ein Marktvolumen von 649 Millionen US-Dollar weltweit [4, 5]. Hier wird ein weiterer Anstieg auf 709 Millionen US-Dollar bis

zum Jahr 2018 prognostiziert [5]. Aufgrund dieser Zahlen lässt sich die Relevanz von Nahtmaterial als einem der wichtigsten chirurgischen Verbrauchsgüter erkennen.

2.1 Chirurgisches Nahtmaterial

2.1.1 Historie des Nahtmaterials

Bereits im alten Ägypten wurden ab etwa 3.000 v. Chr. eine Art Nadel sowie ein nahtähnliches Material zum Zwecke des Wundverschlusses verwendet. Ebenfalls waren bei den antiken Griechen und zu Zeiten des Römischen Reiches bereits Nahttechniken bekannt, um Wunden zu verschließen. Die verwendeten Nahtmaterialien bestanden zur damaligen Zeit vorwiegend aus Naturstoffen, wie Pflanzenfasern, Haaren, Leinen, Sehnen oder Darmsaiten und besaßen somit teilweise bereits die Eigenschaft der Resorbierbarkeit. Im Laufe des 16. Jahrhunderts tauchte der Begriff „*Catgut*“, für ein aus Schafsdarmsaiten hergestelltes und noch heute bekanntes Nahtmaterial, zum ersten Mal auf. [6]

Allerdings war die Verwendung von „*Catgut*“ stets verbunden mit den Problemen einer unzureichenden Sterilisationsmöglichkeit. Um Infektionen bei der Anwendung zu vermeiden, sollten die innerhalb des natürlichen „*Catguts“* befindlichen Bakterien abgetötet werden, ohne das Material selbst zu zerstören. Es stellte sich also die Frage nach einer neuen ubiquitär im Material wirksamen Sterilisationsmethode. [7] Erst die Etablierung einer zuverlässigen Erregerabtötung für Darmsaiten ermöglichte den sicheren medizinischen Gebrauch industriell gefertigter naturstoffbasierter Nahtmaterialien. Diese wurde um 1908 von dem Mediziner Franz Kuhn und dem Apotheker Carl Braun, dem Stammvater der heutigen B. Braun AG, entwickelt. [6, 7] Dies verhalf den Nahtmaterialien zum weit verbreiteten, sicher einsetzbaren und kommerziell erfolgreichen Medizinprodukt zu werden, wie wir es heute kennen.

In den 1930er und 1940er Jahren kamen die ersten nicht-resorbierbaren Kunststoff-Nahtmaterialien auf den Markt, angefangen von Polyvinylalkoholen über perlonbasierte Fäden bis hin zu Polyestern und Polypropylen. 1968 entstanden die ersten synthetisch hergestellten resorbierbaren Nahtmaterialien aus Polyglykolsäure (PGA) für

den Medizinmarkt. [8] Daraufhin folgte eine rasante Entwicklung der Nahtmaterialien auf Kunststoffbasis. Die Einführung von synthetischen Materialien aus nicht-resorbierbaren und resorbierbaren Kunststoffen verhalf den Nahtmaterialien zum Durchbruch als weltweites Massenprodukt, die aus der heutigen Chirurgie nicht mehr wegzudenken sind. Insbesondere bei den resorbierbaren, synthetischen Nahtmaterialien sind in Zukunft weitere Entwicklungen durch Variation und Kombination der polymeren Monomerbausteinen zu erwarten [9]. Aus diesem Grund ist die Weiterentwicklung von Nahtmaterialien nicht als abgeschlossen zu betrachten, zumal die Ansprüche der Anwender stetig wachsen [6].

Industriell hergestellte Nahtmaterialien zeichnen sich heute durch eine geringe Anzahl an allergischen Reaktionen, hohe reproduzierbare Qualitäten und produktionstechnisch einstellbare Eigenschaften aus. Anhand der technischen Merkmale Material, Struktur und Fadenstärke können Nahtmaterialhersteller relevante Parameter, wie maximale Reißkraft, Resorptionszeit, sowie Dauer bis zur 50 %-Reißkraft aufgrund von Hydrolyse für die klinische Anwendung anpassen. Dies führt in der Regel zu einer Vielzahl von Nahtmaterialvarianten aus denen der behandelnde Arzt je nach gewünschter Wundversorgung wählen kann. [10]

Die Auswahl einer Nahttechnik erfolgt anhand von Operations-Standards oft in Abhängigkeit vom chirurgischen Eingriff und des anatomischen Einsatzortes [11]. Grundlage der Nahtmaterialauswahl sind meist Erfahrungswerte der jeweiligen Chirurgen aus Behandlungszentren und Arztpraxen. Die Hersteller bieten zudem unterstützende Empfehlungen in Form von Tabellen oder sog. Indikationsfibeln an. [10, 12]

Speziell der Fadendurchmesser von Nahtmaterialien wird gemäß den internationalen Arzneibüchern (Pharmacopoeia) in standardisierten Maßeinheiten angegeben, in Europa, metrisch nach Ph. Eur. und weltweit, gemäß amerikanischer USP-Klassifikation [11]. Die europäische Ph. Eur. enthält zudem standardisierte Prüfvorschriften in Form einer Monographiegruppe: „*Nahtmaterial für Menschen*“. Hierdurch sollen vorgeschriebene Mindestreißfestigkeiten im Knotenzug für eine einheitliche Qualität und eine sichere Verwendbarkeit als Medizinprodukt, gemäß Richtlinie 93/42/EWG belegt werden. [13]

2.1.2 Einteilung und Aufbau moderner Nahtmaterialien

Chirurgisches Nahtmaterial wird heute grundsätzlich in resorbierbares und nicht-resorbierbares Nahtmaterial unterteilt, was einen entscheidenden Einfluss auf die jeweilige Anwendung zur Wundversorgung hat [8]. So kommen resorbierbare Nahtmaterialien häufig bei intrakorporalen Nähten zum Einsatz, bei denen die Nahtfestigkeit nur temporär gegeben sein muss, wie z.B. am Darm, bei Muskeln oder Faszien [14], wobei kein erneuter Eingriff zur Entfernung des Nahtmaterials gewünscht ist. Im Gegensatz dazu werden chirurgische Wunden im Hautbereich oft mit nicht-resorbierbaren Nahtmaterialien versorgt und dieses in der Regel meist nach 8 bis 14 Tagen, abhängig von der Lokalisation des Eingriffes und der erfolgter Wundkonsolidierung, entfernt [10].

Eine weitere Einteilung des Nahtmaterials erfolgt nach dessen Struktur im Querschnitt, man unterscheidet grundsätzlich monofile, multifile und pseudomonofile Strukturen. Monofiles Nahtmaterial ist zylinderförmig mit kreisrunder Querschnittsfläche, was den Vorteil hat, dass es mit relativ geringem Widerstand auf Zug durch das Gewebe gleitet. Multifile Fäden werden hingegen aus vielen kleineren Einzelfasern verdrillt oder geflochten, woraus sich eine unebene Oberfläche ergibt, welche beim Nähen ein Gewebetrauma aufgrund der sog. „Sägewirkung“ erzeugt. Zudem existiert für diese Fäden eine sog. „Kapillarität mit Dochtwirkung“, was zu einer erhöhten Infektionsgefahr durch den Transport von Mikroorgansimen beitragen kann. [8, 15] Insgesamt kommen geflochtene, multifile Fäden häufig in der Chirurgie zum Einsatz, da sie sich durch eine hohe Zugfestigkeit, geschmeidige Handhabung und einen festen Knotensitz auszeichnen [8, 16]. Zur Kompensation der genannten Nachteile von multifilen Fäden existieren sog. pseudomonofile Fäden, welche multifile Fäden mit einer glättenden Ummantelung oder Beschichtung darstellen, die sich beim Gewebedurchzug fast wie monofile Fäden verhalten, allerdings die haptischen Vorteile multifiler Fäden aufweisen [8, 10, 16]. Aus diesem Grund sind heutzutage käuflich erhältliche multifile Fäden häufig bereits mit resorbierbaren Copolymeren beschichtet, teilweise unter Verwendung diverser Zusätze, wie z.B. des Fettsäuresalzes Calciumstearat, um die Gleiteigenschaften zu verbessern [9, 17]. In der nachfolgenden *Abbildung 1* sind heute gängige Nahtmaterialstrukturen prinzipiell dargestellt:

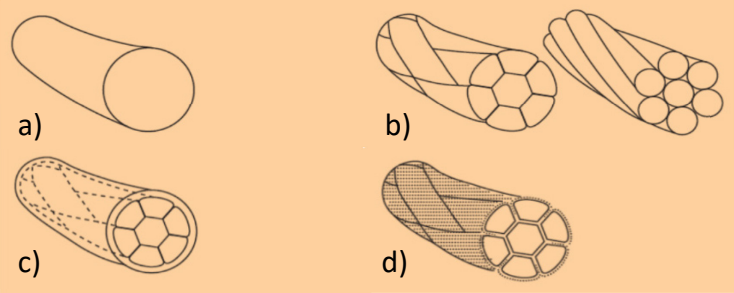

Abbildung 1: Prinzipieller Aufbau moderner Nahtmaterialien a) monofil, b) multifil (links: geflochten; rechts: verdrillt), sowie pseudomonofiler Aufbau c) ummantelt oder d) beschichtet. [8]

An Nahtmaterialien neuester Generation werden aufgrund zunehmender Komplikationen nach chirurgischen Eingriffen höhere Anforderungen gestellt. Deshalb gibt es seit einigen Jahren eine weitere Einteilungskategorie für sog. biologisch aktive Nahtmaterialien. Die bisher verwendeten Nahtmaterialien waren stets rein passiv zum mechanisch festen Wundverschluss verwendet worden und enthielten keinerlei Wirkstoffe. Neuere Nahtmaterialien hingegen enthalten u.a. pharmazeutische Wirkstoffe, wie Antiseptika zur lokalen Infektprophylaxe, sei es in Form der Inkorporation in das Grundmaterial oder als Beschichtung auf den Oberflächen. [4, 18]

2.1.3 Aktuelle Entwicklungen und Stand der Technik

In den letzten Jahren stieg die Anzahl an neuen Entwicklungen im Bereich der chirurgischen Nahtmaterialien deutlich an. Ziel ist es bestehende klinische Probleme in Verbindung mit postoperativen Wundheilungsstörungen künftig besser beherrschen zu können. Das Spektrum der Entwicklungen umfasst gegenwärtig die Ausstattung von Nahtmaterialien mit antimikrobiellen und wirkstofffreisetzenden Eigenschaften, Stammzellbesiedelung zur Gewebsregeneration und sogar sog. „smarte“ Nahtmaterialien mit integrierten Sensoren. [4]

Ein wesentlicher Schwerpunkt aktueller Entwicklungen liegt dabei in der bioaktiven Ausrüstung von Nahtmaterialien mit Hilfe von bioaktiven Molekülen/Proteinen, teils synthetischen teils natürlichen Ursprungs. Dies sind vor allem pharmazeutische Stoffe, wie z.B. Antibiotika, Antiseptika, nichtsteroidale Schmerzmittel, ebenso wie natürliche

vorkommende Stoffe, u.a. Antimikrobielle Peptide (AMPs), Antikörper, Wachstumsfaktoren sowie darüber hinaus Stammzellen und Silbernanopartikel. Die bioaktive Ausrüstung von Nahtmaterialien kann dabei je nach Zielindikation und Art der bioaktiven Stoffauswahl verschiedene Funktionalitäten adressieren. So werden antimikrobielle Nahtmaterialien unter Verwendung von antimikrobiell wirkenden Substanzen, wie Antibiotika, Antiseptika, Silbernanopartikeln, AMPs und speziellen mikrostrukturierten Oberflächen hergestellt. Nichtsteroidale Arzneistoffe werden zur Ausrüstung mit antiinflammatorischen und schmerzlindernden Eigenschaften verwendet, corticoide Substanzen hingegen zur Reduzierung der Narbenbildung. Wachstumsfaktoren und Stammzellbesiedelungen wurden in Studien bereits erfolgreich zur Anregung der Wundheilung und Geweberegenerierung z.B. bei Achillessehnen angewendet. Eingesetzte bioaktive Substanzen bzw. Nahtmaterialausrüstungen können prinzipiell synthetisch oder biologisch-natürlichen Ursprungs sein und es kann sich dabei sowohl um passiv oder aktiv wirkende Substanzen/Modifikationen handeln. Zudem unterscheidet man die Art und Weise des technologischen Aufbringens bzw. der Einarbeitung der aktiven Substanzen dahingehend, ob die Stoffe ins Grundmaterial inkorporiert, chemisch gebunden oder in einer anderen Weise auf dieses aufgebracht sind. Hierbei wird unterschieden zwischen oberflächenaktiven (non-releasing) und wirkstofffreisetzenden (drug-releasing) Nahtmaterialien. Über Dosis und Technologie kann die gewünschte Drug-Release Kinetik eingestellt werden. [4, 18]

Ein verbesserter Wundverschluss mit Hilfe von selbstsichernden, knotenfreien Nahtmaterialsystemen soll mit sog. *„barbed sutures“* erreicht werden. Diese Nahtmaterialien weisen mikroskopisch kleine „Stacheln“ (engl. barbed) auf den Oberflächen auf, welche sich beim Durchzug durch das Gewebe darin verhaken und einen festen Sitz und Zug auf die Wundränder ermöglichen, ohne Knoten zu erfordern. [9] Dies spart teure Operationszeit beim Wundverschluss, reduziert den Materialbedarf im Gewebe und senkt dabei die Kosten und gleichzeitig die Komplikationsraten [19]. Derartiges Nahtmaterial ist heute bereits seit mehreren Jahren als sog. knotenfreies Nahtmaterialsystem (Stratafix™, Fa. Ethicon) mit uni- und bidirektionalen Stachelausrichtungen im klinischen Einsatz [10].

„Smarte Nahtmaterialien“ sollen in Zukunft die Erfassung von biochemischen und biomechanischen Wundparametern ermöglichen. Hiermit sollen u.a. Infekte frühzeitig

aufgrund von lokalen Temperatur- und pH-Messungen erkannt werden. Ebenso stellt die Messung von Nahtmaterial-Zugkräften einen vielversprechenden Ansatz für die Erfassung des idealen Zeitpunkts zur Fadenentfernung nach ausreichend fester Wundheilung dar. [4]

Ein kurzer Überblick über den aktuellen Stand der Entwicklungen im Bereich der chirurgischen Nahtmaterialien ist in *Abbildung 2* dargestellt:

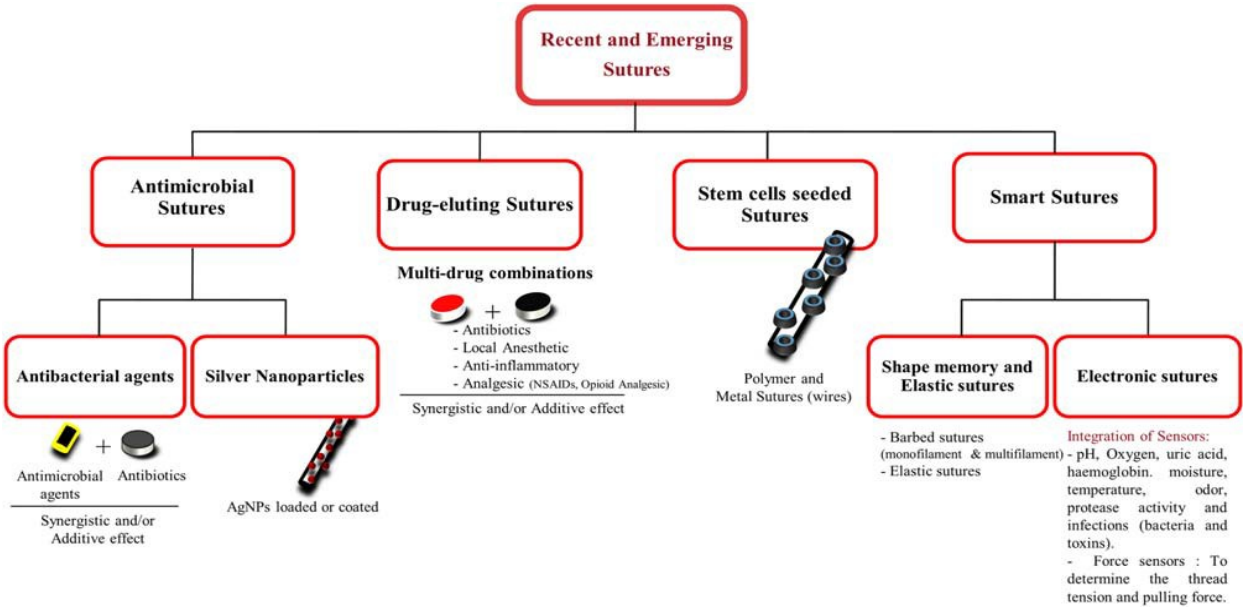

Abbildung 2: Aktuelle Entwicklungen im Bereich chirurgischer Nahtmaterialien. [4]

Speziell die Ausstattung mit antimikrobiellen Eigenschaften stellt heute den größten Bereich innerhalb der Nahtmaterialentwicklungen dar. Beschleunigt durch die zunehmende klinische Infektproblematik gibt es eine Vielzahl an technologischen Lösungsansätzen, um Infektraten zu senken und infektresistente Nahtmaterialien auf den Markt zu bringen. In *Abbildung 3* sind gegenwärtig kommerziell erhältliche und neueste Entwicklungen hierfür dargestellt. Hierbei finden zahlreiche Stoffe Anwendung, insbesondere Pharmazeutika wie Antibiotika z.B. Tetrazyclin, Vancomycin und Levofloxacin, sowie Antiseptika, wie z.B. Chlorhexidin, Oktenidin, Triclosan und Jod. Antiseptika besitzen den Vorteil von meist unspezifischen Wirkmechanismen, was eine Resistenzbildung erschwert. Eine weitere große Stoffgruppe zur antimikrobiellen Ausrüstung sind die natürlichen Stoffe, meist aus der Natur oder von der Natur zur Nutzbarmachung der natürlichen Stoffwirkung biotechnologisch imitiert. Hierunter fallen beispielsweise

Stickoxide (NO_x), die im menschlichen Körper vorkommen, antimikrobiell wirken und z.B. in Polycaprolacton-Träger eingearbeitet werden können, um deren Freisetzung zu verzögern. Ebenso zeigen Grapefruitkernextrakte (GSE), antimikrobielle Peptide (AMPs) und Chitosan als Nahtmaterialbeschichtungen vielversprechende hochwirksame, natürliche Lösungsansätze. Die antimikrobielle Wirkung von bestimmten Metallen und Metallionen ist historisch schon länger bekannt. So werden aktuell auch Silber (Ag) und Silbernanopartikel (Ag-NP) beinhaltende Beschichtungen in verschiedensten Formulierungen erforscht und erprobt. Diese zeigen gute antimikrobielle Wirkungen und langanhaltende Freisetzungen, sei es in einer Polymermatrix, innerhalb von Citrathüllen, mit Alginat- bzw. Hydrogel-Verbindungen (nSnH: Nanosilber in Nanohydrogel), oder als Dotierungen in sog. bioaktiven Gläsern (Ag-BG). Des Weiteren zeigen neue „passive Oberflächenstrukturen“ z.B. durch Plasmaätzung von Mikrolamellen, Anbindung von kurzkettigen Polymeren (sog. Nanobrushes) teilweise mit zusätzlichen amphiphilen Polymeranbindungen, stark reduzierte bakterielle Adhärenz und stellen somit vielversprechende, wirkstofffreie Modifikationen zur Infektprophylaxe dar. [4, 18, 20-23]

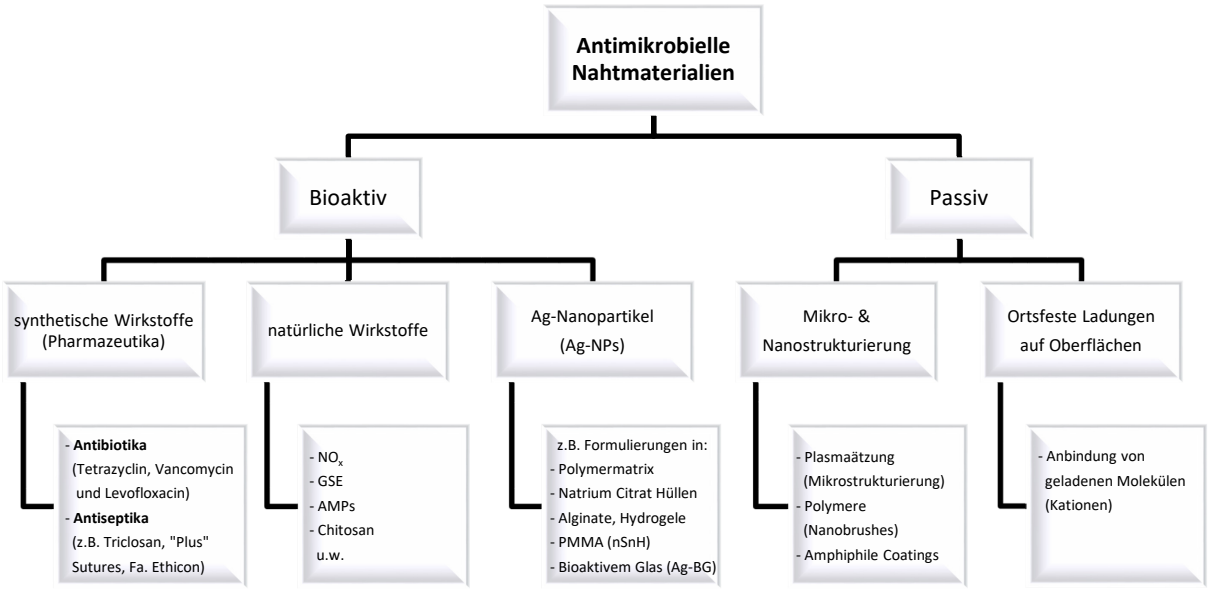

Abbildung 3: Entwicklungen im Bereich antimikrobieller Nahtmaterialien. (modifiziert nach [4, 18])

Gegenwärtig kommerziell erhältliche antimikrobielle Nahtmaterialien für den Humaneinsatz enthalten ausschließlich Triclosan als antimikrobiellen Wirkstoff [22]. Dies sind die seit 2002 auf dem Medizinproduktemarkt erhältlichen Nahtmaterialien unter der Bezeichnung „Plus“-Sutures (Fa. Ethicon) [18], wie z.B. das multifil-resorbierbare Vicryl® Plus, das monofil-resorbierbare Monocryl® Plus, sowie das monofil-langsamresorbierbare PDS® Plus. Des Weiteren, das käuflich erwerbbare knotenfreie antimikrobielle Nahtmaterialsystem (Stratafix™) in Kombination mit Triclosanausrüstung. [10]

2.1.4 Alternative Wundverschlusstechniken

Nahtmaterialien werden mit dem Ziel einer schnellen und komplikationslosen Wundheilung zum Wundverschluss eingesetzt. Dabei werden die Wundränder physisch in Kontakt gebracht und solange zusammengehalten bis eine ausreichende Festigkeit der heilenden Wunde gegeben ist. Die Wundrandadaption sollte bis mindestens zum vierten postoperativen Tag allein durch die entsprechende Wundverschlusstechnik gewährleistet sein. Nach weiteren 10 Tagen und der Bildung einer mechanisch festen Wunde kann diese Stützfunktion vollständig nachlassen. [10]
Die wichtigsten Ergänzungen zum Wundverschluss mittels Nahtmaterial stellen Klammern, Gewebeklebstoffe sowie chirurgische Klebestreifen (sog. Steri-Strips) dar [9, 24]. Klammerungen haben heutzutage einen festen Stellenwert in Teilen der Chirurgie, aufgrund der Möglichkeit des zeitsparenden und reproduzierbaren Wundverschlusses. Prinzipiell unterscheidet man die zirkulären (z.B. für Darmanastomosen) von den linearen Klammerungen bei geradlinigen, längeren Wunden (wie z.B. in der Orthopädie, Unfall- und Viszeralchirurgie). [14] Bei den Gewebsklebstoffen unterscheidet man stoffabhängig zwischen Cyanoacrylaten, Fibrinklebern, Muschelsekreten (3,4-Dihydroxyphenylalanine, sog. DOPA) und Gelatine-Resorcin-Formaldehyden (GRF). Die Anwendung von Gewebsklebstoffen zum Wundverschluss hat sich heute lediglich in speziellen Nischen bewährt. [25] Zum einen werden Cyanoacrylate bei oberflächlichen Hautwunden bei Kindern oder in der ästhetisch plastischen Chirurgie aufgrund der geringen Narbenbildung eingesetzt [24, 26]. Fibrinkleber dagegen werden aufgrund ihrer hämostyptischen Eigenschaft in Verbindung mit mechanisch gering belastbaren Wunden vorwiegend bei Eingriffen an parenchymatösen Organen (Leber, Milz, Pankreas und Niere) eingesetzt. [27, 28] GRF-Klebstoffe zeigen vielversprechende Resultate bei

Eingriffen zur Behandlung von Aortendissektionen [29]. Insgesamt erreichen Gewebekleber bisher nicht die gleichen Wundverschlussstabilitäten wie Nahtmaterialien und zeigen häufig eine gewisse Toxizität sowie Neigung zu Abstoßungsreaktionen [25]. Im Gegensatz dazu sind Nahtmaterialien ubiquitär einsetzbar und stellen gegenwärtig den überwiegenden Teil der Materialien zum Wundverschluss dar [9, 24]. Insbesondere synthetische Nahtmaterialien sind in großer Variation verfügbar und gehören zur Standardausrüstung jeder chirurgischen Versorgungseinrichtung. In der Chirurgie haben sich verschiedene Nahttechniken etabliert, um je nach Nahtlokalisation eine stabile Wundnaht zu erreichen. Dabei ist die Balance zwischen hoher Nahtfestigkeit mit möglichst geringem Gewebetrauma und vor allem dem Erhalt der Gewebsdurchblutung für die anschließende Wundheilung von entscheidender Bedeutung. [9, 10, 15]

2.2 Wundheilung und Wundheilungsstörungen

2.2.1 Der ungestörte Wundheilungsverlauf

Die Wundheilung nach einem chirurgischen Eingriff oder einer sonstigen Verletzung, bei denen die äußeren Hautschichten eröffnet und Gefäße verletzt wurden, beginnt bereits unmittelbar nach dem Gewebetrauma. Im Rahmen der physiologischen Hämostase wird dabei schon nach wenigen Sekunden die Blutgerinnung im Verletzungsbereich auf zwei Wegen angeregt: Zum einen, die sofort einsetzende thrombozytäre (primäre) Gerinnung, bei der es zur Anlagerung und Aggregation von Thrombozyten an den von-Willebrand-Faktor (vWF) kommt. Der vWF ist eine Art „Andockbrücke“ für Thrombozyten an offengelegte Strukturen von zerstörten Gefäßzellen. Durch die weitere Aktivierung der Thrombozyten kommt es zum zellulären Thrombus und zur ersten Blutstillung. Ferner wird die plasmatische (sekundäre) Gerinnung durch zerstörte Gewebszellen und den dabei freigesetzten sog. Tissue-Faktor (TF) aktiviert. Zusammen mit dem aktivierten Hagemann-Faktor (FXIIa) des Blutes triggert der TF-FXIIa-Komplex die Thrombin- und schließlich die Fibrinbildung, was letztlich über ein stützendes Fibrinnetzwerk zum stabilen Wundverschluss führt. [30] Dieser Vorgang nimmt einige Minuten in Anspruch und ist durch die Gerinnungszeit gegeben [31].

Im Anschluss daran folgt die eigentliche Wundheilung. Diese wird häufig in drei Phasen über einen Zeitraum von mehreren Tagen unterteilt. Während der anfänglichen **Exsudationsphase** (I) ist der Wundspalt mit Blut- und Lymphflüssigkeit gefüllt, die Gerinnung erzeugt einen stabilen Wundverschluss und Thrombozyten geben darüber hinaus Wachstumsfaktoren ab. Überflüssige Zellbestandteile werden in der manchmal separat aufgeführten Resorptionsphase durch eine natürliche Entzündungsreaktion durch Granulozyten zersetzt und eine wundreinigende Flüssigkeitsabsonderung findet statt. In der darauffolgenden **Proliferationsphase** (II) wandern Fibroblasten ein und überbrücken durch weitere Kollagenproduktion die Wundlücke, es entsteht das sog. Granulationsgewebe und die **Regenerationsphase** (III) beginnt. Dabei vernetzen sich im Granulationsgewebe die Kollagenfasern zu einem mechanisch stabilen Netzwerk und bilden dadurch das Narbengewebe. Dabei zieht sich das Gewebe aufgrund von Gefäßverengungen im neu gebildeten weißlich verfärbten Narbengewebe zusammen. Abschließend erfolgt die erneute Epithelialisierung. [14, 32]

2.2.2 Komplikationen der Wundheilung

Bei komplikationslosem Heilungsverlauf mit einer direkt stabil geschlossenen Wunde spricht man von der primären Wundheilung. Wenn jedoch ein direkter Wundverschluss z.B. bei großflächigen Wunden nicht möglich ist oder eine Eröffnung der Wunde aufgrund einer Wundkomplikation notwendig wird, spricht man von einer sekundären Wundheilung. Wundheilungsstörungen können aseptische und septische Ursachen haben. Bei infizierten Wunden kommt eine Wunderöffnung bewusst zur Anwendung, um eine lokale Infektbehandlung, wie Reinigung und Desinfektion der Wunde zu bewerkstelligen. Die Abheilung der eröffneten, nun großflächigen Wunde verzögert sich je nach Größe meist um mehrere Wochen. [14, 32] Weitere Ursachen für komplikative Wundheilungsverläufe stellen lokale und systemische Störfaktoren dar. Grundsätzlich sollte jede einzelne Wundheilungsphase ungestört durchlaufen werden, um eine Gewebsneubildung für eine stabile Wunde zu erreichen. Lokale Störfaktoren betreffen die Wunde selbst, systemische Faktoren hingegen den Allgemeinzustand des Patienten. So kann eine lokale Wunddehiszenz oder Infektion, ebenso wie ein schlechter Gesundheitszustand mit Durchblutungsstörungen aufgrund von Diabetes zu Komplikationen der Wundheilung führen. Störungen der Wundheilung bedürfen einer adäquaten

Behandlung, da diese stets die Gefahr einer Chronifizierung der Wunde bergen. Bei chronischen Wunden handelt es sich oft um eine Stagnation innerhalb einzelner Heilungsphasen. Diese müssen konsequent z.B. durch lokale Debridements und Behandlung der systemischen Grunderkrankung therapiert werden. Dabei kommt es meist zu langwierigen Behandlungsverläufen verbunden mit höheren Kosten durch Mehraufwand aufgrund des entsprechenden Wundmanagements sowie zusätzlichen chirurgischen Eingriffen. Daher gibt es eine Vielzahl von Entwicklungen, um die Wundheilung positiv zu beeinflussen, u.a. feuchthaltende Wundauflagen (z.B. Hydrokolloide, Hydrogele), Vakuumsysteme zur Exsudatabsaugung, wirkstoff- bzw. silberhaltige Auflagen zur Infektprophylaxe. Des Weiteren gibt es neue bioaktive Wundauflagen, welche durch Abgabe von Wachstumsfaktoren die Gewebsneubildung anregen. [33] Zudem sollen neueste bioaktive Nahtmaterialien je nach Funktionalität lokalen Infektionen vorbeugen, Entzündungs- und Schmerzreaktionen lindern, Narbenbildungen reduzieren und Wundheilungs- und Regenerationsprozesse anregen. [4] Die häufigste und mitunter schwerwiegendste Wundheilungsstörung stellt bis heute die postoperative Wundinfektion dar. [34]

2.3 Postoperative Wundinfektionen (SSI)

Von einer chirurgischen Wundinfektion (engl. surgical site infection, kurz SSI) spricht man heute laut *Centers for Disease Control* (CDC), wenn das Operationsgebiet innerhalb von 30 Tagen nach dem Eingriff von einem Infekt betroffen ist. Im Falle einer Implantatsbeteiligung gilt dies sogar noch bis zu einem Jahr nach dem Eingriff. Chirurgische Wundinfektionen teilt man prinzipiell in drei Kategorien je nach vom Infekt betroffener Körpertiefe ein. Je tiefer ein Infekt sich ausbildet, umso höher ist dessen Schweregrad und daraus resultierend das unmittelbare Gesundheitsrisiko für den Patienten. Es werden an der Oberfläche liegende, tiefere Regionen betreffende und organbetreffende Infektionen voneinander unterschieden. [33, 35] Chirurgische Wundinfektionen treten abhängig von der Lokalisation des Eingriffs in unterschiedlicher Häufigkeit auf. So führen chirurgische Eingriffe insgesamt im Durchschnitt gegenwärtig zu Infektionsraten von 2-5 % [36]. Nimmt man die Bereiche gastrointestinale, hepatobiliären und pankreasbezogene Chirurgie zusammen geht man sogar von 11-36%igen

Infektionsraten aus [37]. Es besteht außerdem ein Zusammenhang zwischen der Wundlokalisation eines chirurgischen Eingriffes und dem infektionsauslösenden Erregerspektrum. Insgesamt ist *Staphylococcus aureus* der häufigste Erregertyp von Wundinfektionen in der Chirurgie, der Traumatologie und Orthopädie. Fachspezifisch gibt es allerdings Unterschiede, so lassen sich bei viszeralchirurgischen Infektionen *E. coli* und *Enterococcen* als häufigste Erregertypen ausmachen. [33, 35]

Postoperative Wundinfektionen können sowohl durch körperfremde, als auch körpereigene Erreger z.B. aus der eigenen Hautflora bei immunsupprimierten Patienten ausgelöst werden. Wundinfektionen können zudem auf exogenem oder hämatogenem Wege entstehen. Hauptinfektionsweg stellt der exogene Pathogeneintrag, meist durch Bakterien dar, d.h. der Erreger wird von außen in die Wunde eingebracht. Dies geschieht z.B. durch ein Trauma mit offener Wunde oder trotz aller Vorsichtsmaßnahmen häufig durch den chirurgischen Eingriff selbst, obwohl diese unter aseptischen Bedingungen erfolgen. Selbst eine Kontamination mit einer geringen Anzahl an vermehrungsfähigen Erregern kann dann je nach Virulenz ausreichen, um den Grundstein für eine lokale Infektion zu einem späteren Zeitpunkt zu legen. [33] Eine gängige Behandlungsmöglichkeit stellt die Eröffnung und Reinigung der Wunde (sog. Wundtoilette), verbunden mit einem sekundären Wundheilungsprozess dar. Hinzu kommt die systemische Antibiose, idealerweise nach Wundabstrich und mikrobiologischer Abklärung des Erregerspektrums in der klinischen Mikrobiologie. Hiermit soll eine Infektausbreitung im Körper und ein Übergreifen auf das Blutkreislaufsystem mit der daraus resultierenden Gefahr einer lebensbedrohlichen Sepsis vermieden werden. [33]

Wundinfektionen stellen ein gesundheitliches Risiko für die betroffenen Patienten und eine enorme finanzielle Belastung für das Gesundheitssystem dar. Dies wird vor allem durch wiederkehrende Eingriffe zur Wundsanierung und damit verbundene verlängerte Krankenhausaufenthalte begründet [36]. Die Zunahme von antibiotikaresistenten Erregern verschärft die Situation für die Patienten, das behandelnde Personal und die Kliniken selbst. Im Falle des Auftretens von multi-resistenten Wunderregern (wie z.B. MRSA, MRSE, ESBL) müssen betroffene Patienten gemäß den Empfehlungen des Robert-Koch Instituts umgehend isoliert werden und dürfen nur unter Berücksichtigung spezieller Hygieneschutzmaßnahmen weiter behandelt werden. Dies beinhaltet die

komplette Einkleidung mit Einweg-Schutzkleidung vor jeder pflegerischen, sowie diagnostisch/therapeutischen Tätigkeit, stets verbunden mit dem Risiko der eigenen Infektion. Das oberste Ziel ist hierbei die Vermeidung der Infektionsverbreitung, sowie wenn möglich die Dekolonisierung des Patienten mittels einer kontrollierten und kalkulierten Antibiotikatherapie. [38]

Einer der wichtigsten Faktoren, der eine Wundinfektion begünstigen kann, ist die Gegenwart von Fremdmaterialien im menschlichen Körper wie z.B. Nahtmaterialien oder Implantate. So geht man von einem erhöhten Infektionsrisiko erst über 10^7 Bakterienkolonien aus, wohingegen sehr viel geringere Keimzahlen von nur 100 (10^2) Kolonien für eine Infektauslösung in Gegenwart eines Fremdmaterials genügen. Dies wird auf eine örtlich stark eingeschränkte Wirkung des Immunsystems an der Oberfläche des Implantats zurückgeführt. Das infizierte Gewebe wird hier nun nicht mehr von allen Seiten vaskularisiert und ist somit den Immunabwehrzellen nicht mehr vollständig zugänglich. [39]

Zudem adhärieren Bakterien nach Oberflächenkonditionierung mit Proteinen verstärkt an den Fremdkörperoberflächen. Adhärierte Bakterien ändern ihren Wachstumsmodus in eine sessile Form und produzieren extrazellulären Schleim, sog. Biofilme. Mikroorganismen in Biofilmen auf den Fremdmaterialoberflächen vermehren sich daraufhin weiter und es beginnt eine Ausbreitung der Infektionen in wiederkehrenden akuten Schüben, bedingt durch planktonische Bakterienfreisetzungen. Der Biofilm schützt die Mikroorgansimen zudem vor Angriffen des Immunsystems sowie vor systemisch verabreichten Antibiotika. Diese werden von Biofilm abgeschirmt und erzeugen lokale Konzentrationsgradienten für die Mikroorganismen was letztlich auch zu Resistenzbildung führt. Aus diesem Grund sind derartige implantat/biomaterial-assoziierte Infektionen nur schwer in der Klinik zu behandeln und verlaufen oft chronisch. In letzter Konsequenz muss daraufhin meist das Fremdmaterial zur Erzielung einer Infektberuhigung chirurgisch entfernt werden. [39, 40]

Eine Vielzahl von Faktoren, die Einfluss auf das Wundinfektionsrisiko nehmen sind ggw. nicht zu beeinflussen. Hierzu zählt die Kontaminationsklasse des jeweiligen Eingriffes, die Dauer des Eingriffes, sowie allgemeine den Patienten betreffende Faktoren, wie z.B. Alter und Nebenerkrankungen. Aktuell sind als prophylaktische Maßnahmen

zur Vermeidung von chirurgischen Wundinfektionen vor allem präoperative antiseptische Waschungen, perioperative systemische Antibiotikagaben, die lokale Wunddesinfektion, die Verwendung von sterilen Instrumentarien sowie aseptische Umgebungsbedingungen während des operativen Eingriffs zu nennen. [32, 33, 35, 41]

Ergänzend kommen lokal wirkende antimikrobielle Materialien zur Wundversorgung, wie Wundauflagen und Nahtmaterialien zum Einsatz. [32, 33] Antimikrobielle Oberflächen können sogar für Implantatsmaterialien eine entscheidende Unterstützung zur Infektprophylaxe darstellen. So haben sich gentamicinhaltige Knochenzemente, Kollagenschwämme und Knochenersatzmaterialien in der klinischen Praxis bewährt, dies hat zur Entwicklung von Gentamicin-Polylaktid-Beschichtungen für Tibiamarknägel geführt. [42, 43] Antimikrobielle Oberflächen unterscheidet man prinzipiell nach den Mechanismen der Mikroorganismenabwehr Abstoßung und Abtötung, sowohl in Kontakt mit, oder in der Umgebung der Oberfläche durch Freisetzung antimikrobieller Wirkstoffe [44].

Chirurgische Nahtmaterialien sind ebenfalls in der Lage fremdmaterial-assoziierte Wundinfektionen auszulösen [35, 45, 46]. Die Ausrüstung von Nahtmaterialien mit antimikrobiellen Wirkstoffen kann die bakterielle Adhärenz bzw. Biomaterialinfektion und die damit verbundenen chirurgischen Wundinfektionen reduzieren [47-49]. Aus diesem Grund werden gegenwärtig antimikrobielle Nahtmaterialien mit dem Wirkstoff Triclosan (Fa. Ethicon) zur Vermeidung von postchirurgischen Wundinfektionen von der WHO empfohlen [50, 51]. Gegenwärtig sind als wirkstoffhaltige Nahtmaterialien zur Infektprophylaxe allerdings nur triclosanhaltige Nahtmaterialien zugelassen [18, 22].

Die alleinige Verwendung von Triclosan zur Infektprophylaxe auf Nahtmaterialien bringt allerdings auch Probleme mit sich. Ursache hierfür ist vermutlich der leichtfertige Einsatz von Triclosan in einer Vielzahl von Konsumgütern, wie z.B. in Seifen, Shampoos, Zahnpasten, Reinigungsmitteln bis hin zu Kleidungsstücken. [20, 52] Es wurden bereits Triclosanresistenzen sowie in Gegenwart von Triclosan Kreuzresistenzen zu Chinolon-Antibiotika nachgewiesen, ebenso wie einer erhöhten Gewebsbesiedelung mit Staphylokokken berichtet. [53-55]

2.4 Lösungsansatz: Neuartige antimikrobielle Beschichtungen für Nahtmaterialien unter Verwendung von Antiseptika und Fettsäure-Wirkstoffträgern

Die vorliegende Arbeit beschreibt eine neuartige Alternative für den Wundverschluss mit Nahtmaterialien bei Triclosanresistenz zur Wundinfektionsprophylaxe mit Hilfe antiseptischer Wirkstoffe. In dem angestrebten neuen Lösungsansatz wurden resorbierbare, geflochtene Nahtmaterialien mit Hilfe von Chlorhexidin und Oktenidin antimikrobiell beschichtet. Diese Antiseptika stellen klinisch etablierte Substanzen in der Mund- und Hautdesinfektion dar, besitzen ein breites Wirkspektrum, eine geringe Neigung zur Resistenzentwicklung und werden zur Eradikation von Biofilmen und MRSA Infektionen in bestimmten Fällen empfohlen [56-58]. Experimentell wurden Fettsäurewirkstoffträger in Formulierungen für Beschichtungslösung verwendet, um den Wirkstoffrelease durch gezielte Reduktion der Wirkstofflöslichkeiten zu verzögern. Das neuartige Nahtmaterial sollte dabei für Zeiträume von min. 3-4 Tage postoperativ, die Zeitspanne bis zur primären Wundheilung, wirksame Stoffmengen abgeben können. Die Wirkstofffreisetzungen, antimikrobielle Wirkung gegenüber gängigen Wunderregern, vor allem *Staphylococcus aureus* und die Biokompatibilität der antimikrobiell neuartig beschichteten Nahtmaterialien wurden dosisabhängig getestet und anschließend systematisch für eine mögliche klinische Anwendung evaluiert.

2.4.1 Wissenschaftliche Fragestellungen

Neue Formulierungen für antimikrobielle Beschichtungen auf Fettsäurebasis für resorbierbare Nahtmaterialien sollten unter Verwendung von Chlorhexidin und Oktenidin eine antimikrobielle Wirkung über den initialen postoperativen Zeitraum von mehreren Tagen bzw. einer Woche erreichen. Gleichzeitig sollte die Grundvoraussetzung für den späteren klinischen Einsatz, eine biokompatible Beschichtungsformulierung gefunden werden, welche antimikrobiell hochwirksam gegenüber dem relevanten Wunderregerspektrum ist. Als Maß der Effektivität sollten Wirkstofffreisetzungskinetiken, antimikrobielle Hemmhofverläufe und vital adhärierte Mikroorgansimen im Vergleich zu Biokompatibilitätsvorgaben der ISO 10993-5 herangezogen werden. Neben der

antiseptischen Wirkstoffvariation sollte die Wirkstoffmenge in den Beschichtungslösungen zur Dosisoptimierung, sowie der Fettsäureträger selbst zwischen Palmitin- und Laurinsäure variiert werden. Speziell die Wirkstoffdosis- und Fettsäurevariation sollte Unterschiede beim verzögerten Drug-Release und der antimikrobiellen Wirkung im zeitlichen Verlauf erkennen lassen.

2.4.2 Inhaltliche Aspekte der einzelnen Veröffentlichungen

Zur Erfüllung der Zielstellung und der Beantwortung der genannten wissenschaftlichen Fragestellungen wurden einzelne inhaltliche Aspekte in die nachfolgenden drei Teilstudien separiert und diese entsprechend publiziert.

2.4.2.1 Publikation I: Chlorhexidin-Beschichtungen für Nahtmaterialien (Drug-Release, antimikrobielle Hemmhöfe und Biokompatibilität)

Im Rahmen der ersten Publikation wurde die Frage beantwortet, ob mit Chlorhexidin-Beschichtungsformulierungen auf Fettsäurebasis ein Wirkstoffrelease über mehrere Tage zum Schutz vor chirurgischen Wundinfektionen erreicht werden kann. Im Anschluss daran erfolgte die Auswahl einer Wirkstoffkonzentration und Trägerfettsäure zur Nahtmaterialbeschichtung für den späteren klinischen Einsatz, welche sowohl antimikrobiell wirksam, als auch biokompatibel sind.

Inhalte:

- Es erfolgte die Formulierung von Chlorhexidin-Beschichtungslösungen mit abgestuften Wirkstoffkonzentrationen in organischen Lösungsmitteln und zweierlei Fettsäure-Wirkstoffträgern (Palmitin- und Laurinsäure). Es wurde eine reproduzierbare Methode zur aseptischen Nahtmaterialbeschichtung etabliert.
- Mechanische Testungen wurden durchgeführt zur Bestimmung der vorgeschriebenen Mindest-Knoten-Zugfestigkeit für Nahtmaterialien gemäß Ph. Eur. 7.0 Monographie 0667 mit dem Titel: *„Sterile synthetische resorbierbare geflochtene Nahtmaterialien“*, um negative Einflüsse durch das Beschichtungsverfahren auf deren Zugfestigkeit zu untersuchen.
- Nachweis der antimikrobiellen Wirkung mit Hilfe einer modifizierten Hemmhoftestung über einen längeren Zeitraum ggü. *Staphylococcus aureus* (ATCC® 49230™) als Haupterregerstamm von implantat-assoziierten Infektionen.

- Ebenfalls erfolgten konzentrationsabhängige Biokompatibilitätsprüfungen gemäß ISO 10993-5 anhand eines WST-1 Tests zur Erfassung der metabolischen Aktivität von L929-Mausfibroblasten in Gegenwart von Eluaten der wirkstoffbeschichteten Nahtmaterialien.
- Schließlich wurde zur Erfassung der Drug-Release-Kinetik eine Wirkstoffdetektionsmethode für Chlorhexidin mittels UV/VIS-Absorptionsmessung etabliert. Mit Hilfe erstellter Kalibriergeraden bei 280 nm erfolgte die quantitative Wirkstoffbestimmung *in vitro* an Nahtmaterialeluaten. Die kumulative Auftragung ergab wirkstoffmengen- und fettsäureträgerabhängige Drug-Release-Kinetiken im zeitlichen Verlauf der Elutionsdauer.

2.4.2.2 Publikation II: Oktenidin-Beschichtungen für Nahtmaterialien (Drug-Release, antimikrobielle Hemmhöfe und Biokompatibilität)

In der zweiten Publikation wurde die Frage beantwortet, ob auch mit Oktenidin-Beschichtungsformulierungen auf Fettsäurebasis ein Wirkstoffrelease über mehrere Tage zum Schutz vor chirurgischen Wundinfektionen erreicht werden kann. Zudem wurde geklärt, welchen Einfluss der jeweilige Fettsäureträger auf den Drug-Release hat. Insgesamt konnte hiermit das Wirkstoffspektrum für antimikrobielle Nahtmaterialien erweitert werden. Es wurde ebenfalls eine Wirkstoffkonzentration für Oktenidinbeschichtungen auf Nahtmaterialien bestimmt, welche sowohl antimikrobiell wirksam als auch gleichermaßen biokompatibel ist.

Inhalte:

- Formulierung von Oktenidin-Beschichtungslösungen mit abgestuften Wirkstoffkonzentrationen in zweierlei Fettsäure-Wirkstoffträgern in Anlehnung an die neuen Formulierungen für Chlorhexidin-Beschichtungen der ersten Publikation.
- Nachweis der antimikrobiellen Wirkung mittels Langzeit-Hemmhoftestung ggü. *Staphylococcus aureus* (ATCC® 49230™).
- Biokompatibilitätsprüfung gemäß ISO 10993-5 mittels WST-1 Test in Abhängigkeit der Wirkstoffkonzentrationen beschichteter Nahtmaterialien.
- Etablierung einer Wirkstoffdetektionsmethode für Oktenidin, Erstellung einer Kalibriergeraden zur quantitativen Oktenidinbestimmung.
- Elutionsexperimente zur Erfassung der Drug-Release-Kinetiken neuartig beschichteter Nahtmaterialien.

2.4.2.3 Publikation III: Chlorhexidin & Oktenidin-Beschichtungen (Mikrobielles Wirkspektrum, Adhäsion & Inhibierung vital adhärierter *S.aureus*)

In der dritten Publikation wird die Frage der mikrobiellen Breitenwirkung der neuartigen chlorhexidin- und oktenidinbeschichteten Nahtmaterialien gegenüber dem Hauptmillieu von Wundinfektionserregern beantwortet. Die Oberflächenstruktur der Coatinglayer und die zahlenmäßige Adhäsion von *S. aureus* auf antimikrobiell beschichteten Nahtmaterialien wurde in Rasterelektronenmikroskopie(REM)-Aufnahmen untersucht. Die inhibierende Wirkung gegenüber vitalen oberflächenadhärenten Mikroorganismen, wurde speziell mit dem Haupterreger *S. aureus* getestet. Alle Untersuchungen erfolgten stets im Vergleich zu kommerziell erhältlichen wirkstofffreien und triclosanhaltigen Nahtmaterialien (Vicryl® Plus). Die vital adhärierten Bakterien auf den neuartigen antimikrobiellen Nahtmaterialen waren signifikant reduziert im Vergleich zu Vicryl® Plus. Die Besiedelung mit vitalen Staphylokokken (Biofilmbildnern) stellt ein potentielles Infektionsrisiko dar. Die vorhandenen Daten deuten auf eine höhere Wirksamkeit der Chlorhexidin- und Oktenidinbeschichtungen zur Wundinfektionsprophylaxe hin, als sie bisher kommerziell erhältliche Nahtmaterialien leisten können.

Inhalt:

- Zunächst wurde das mikrobielle Wirkspektrum der neuen antimikrobiellen Nahtmaterialien mittels Hemmhoftestungen gegenüber den fünf gängigsten klinischen bakteriellen Erregern (*S. aureus, MRSA, S. epidermidis, E. faecalis* und *E. coli*) untersucht. Zum einen konnte hiermit die Effizienz gegenüber einer Biofilmbildung gezeigt werden, welche klinisch vorwiegend aus multiplen Bakterienspezies besteht. Zum anderen konnte dadurch die antimikrobielle Wirkung der getesteten Bakterienspezies relativ zum Haupterreger *S. aureus* gezeigt werden. Es ergaben sich vergleichbar gute antimikrobielle Wirksamkeiten über den klinisch entscheidenden Zeitraum von 48 h postoperativ. Deshalb wurden weitere detaillierte Untersuchungen zu Inaktivierungen von oberflächenadhärenten Bakterien mit *S. aureus* als einzigem Testkeim durchgeführt.
- REM-Aufnahmen der Oberflächenstrukturen zeigten gleichmäßige Coatinglayer auf den Nahtmaterialen, sowie keine erkennbaren negativen strukturellen Beeinflussungen durch den Coatingprozess. Weitere REM-Aufnahmen in höheren Vergrößerungen zum Nachweis zahlenmäßiger bakterieller Adhärenz, wiesen *S. aureus*-Bakterien auf allen getesteten Nahtmaterialoberflächen auf.

- Ein bakterieller Adhäsionsassay mit *S. aureus* belegt, dass die vitalen Keimzahlen nach Oberflächenkontakt an den neuen antimikrobiellen Nahtmaterialien im Vergleich zu Vicryl® Plus (0,5 log) signifikant reduziert werden. Dies gilt insbesondere für die niedrigdosierten 11 µg/cm-Wirkstoffbeschichtungen mit biokompatiblen Eigenschaften. Hierbei zeigen Chlorhexidin-Coatings im Mittel eine 1,1 log und Oktenidin-Coatings eine 0,3 log höhere Reduktionen vital adhärenter Bakterien.
- Mit einem Suspensionsassay wurde das planktonische Wachstum von *S. aureus* in Gegenwart von antimikrobiell beschichteten Nahtmaterialien näher untersucht. Bakterien in der flüssigen Umgebung antimikrobiell beschichteter Nahtmaterialien werden generell reduziert, wie z.B. für Vicryl® Plus (0,3 log). Chlorhexidin-Coatings zeigen im Mittel eine 0,7 log und Oktenidin-Coatings eine 0,1 log höhere Reduktion planktonischer Bakterien als Vicryl® Plus.
- Zusammenfassende Evaluierung der chlorhexidin- und oktenidinbasierenden Nahtmaterialien anhand der biokompatiblen Wirkstoffkonzentrationen (11 µg/cm) im Vergleich zum kommerziellen triclosanhaltigen Vicryl® Plus. Unabhängig vom Fettsäureträger werden Chlorhexidinbeschichtungen CL11 und CP11 zur Prophylaxe empfohlen, um gegenwärtige Infektraten von postoperativen Wundinfektionen weiter senken zu können. Insbesondere CL11 zeigt innerhalb der ersten 48 h eine hochwirksame lokale mikrobielle Inhibierung von oberflächenadhärenten Bakterien, eine biokompatible Oberfläche, geringe bakterielle Adhärenz und eine effektive Wirkstofffreisetzung *in vitro*.

3 Diskussion und Schlussfolgerungen

Chirurgische Wundinfektionen (SSI) stellen eine wachsende Herausforderung im klinischen Alltag dar. Die Infektionsraten liegen aktuell je nach Wundlokalisation zwischen 5 % im Jahresdurchschnitt aller chirurgischer Eingriffe [36] und können auf bis zu 36 % bei gastrointestinalen und hepatobiliären/pankreatischen Eingriffen ansteigen [37]. Nahtmaterialien zum Wundverschluss können derartige Infektionen auf dreierlei Art begünstigen. Erstens stellen Nahtmaterialien eine Besiedelungsgrundlage für Bakterien zur Biofilmbildung dar und zweitens eine Leitschiene für den aktiven und passiven Transport für Mikroorganismen in die Wunde aufgrund von „Kapillareffekten“. Dies ist insbesondere bei den häufig eingesetzten geflochtenen Nahtmaterialien im Sinne einer Dochtwirkung der Fall. Drittens kann bereits deren Eigenschaft als Fremdmaterial die Infektentstehung begünstigen. Dabei wird das an den Fremdmaterialoberflächen befindliche Gewebe nicht mehr von allen Seiten mit Gefäßen und Immunabwehrzellen aus dem Blutkreislauf versorgt. Das Immunsystem kann somit seine volle Abwehrkraft lokal nicht mehr entfalten und es kommt wesentlich leichter zu einer Infektausbildung selbst bei geringeren Keimzahlen. Wundinfektionen stellen ein Gesundheitsrisiko für die Patienten und einen erheblichen Kostenfaktor für das Gesundheitssystem dar. Es ist wünschenswert, zunehmende Resistenzen durch effektive und frühzeitige Infektprophylaxe zu unterbinden. Antimikrobielle Nahtmaterialien stellen eine adjuvante Maßnahme zur Infektprophylaxe dar. Derzeit sind nur triclosanhaltige Nahtmaterialien (Fa. Ethicon) für die Anwendung am Menschen zugelassen. Aktuell tauchen allerdings gegen den Wirkstoff Triclosan bereits erste Resistenzen auf, weshalb Alternativen zum antimikrobiellen Wundverschluss sinnvoll sind. Eine mögliche Abhilfe stellen neuartige antimikrobielle Nahtmaterialien dar, die mit antiseptischen Wirkstoffen wie Chlorhexidin oder Oktenidin beschichtet sind. Ziel ist es in Abhängigkeit von Wirkstoff, Dosis und Wirkstoffträger auch künftig im postoperativen Zeitraum von 48 h nach Wundverschluss vor Infektionen schützen zu können. In Anbetracht aller erhobener Daten zur mikrobiologischen Effizienz, Biokompatibilität und Freisetzungskinetik wurde eine vergleichende Evaluierung durchgeführt, um das Optimum der neuen Beschichtungssysteme für die spätere klinische Anwendung zu bestimmen. [59-61]

Neuartige antimikrobielle Nahtmaterialien konnten reproduzierbar mit Wirkstoffbeladung hergestellt werden, ohne negative Beeinflussung der Zugfestigkeit bei gleichmäßen Beschichtungen. Damit dürfte sich dieses Verfahren für einen größeren industriellen Maßstab eignen. Hinsichtlich der Wirkstofffreisetzungen zeigen Chlorhexidinbeschichtungen vermutlich aufgrund der höheren Löslichkeit einen schnelleren Release im Vergleich zu solchen mit Oktenidin. Zusätzlich beeinflusst die Art des Fettsäurewirkstoffträgers wesentlich die Dauer der Freisetzung. So kann unter Verwendung von Palmitaten eine deutliche Verlangsamung der Wirkstofffreisetzung erreicht werden, was sich u.a. an den prozentual freigesetzten Wirkstoffmengen nach 96 h bemerkbar macht. Somit ist nicht nur eine Verzögerung, sondern auch eine Niveauabsenkung der Stoffmengen ohne begleitenden Wirksamkeitsverlust zu erreichen. Je nach Applikation könnte dies als sog. „Toolbox“ verwendet werden, um Nahtmaterialsysteme optimiert auf die jeweiligen Bedürfnisse zusammenzustellen. Die breite antimikrobielle Wirkung *in vitro* sowohl für Chlorhexidin-, als auch Oktenidinbeschichtungen lässt auf eine Hemmwirkung von klinischen Biofilmen in Wunden *in vivo* schließen. Klinisch wird vor allem eine Infektprophylaxe für die ersten 48 h nach dem chirurgischem Eingriff gefordert [62]. Mit neuartig beschichteten Nahtmaterialien kann vermutlich die Rate von Wundinfektionen weiter gesenkt werden, als dies bisher mit kommerziell erhältlichem triclosanhaltigen (Vicryl® Plus) möglich ist. Zum einen, werden für die getesteten neuen Nahtmaterialien Bakterien über Zeiträume von >48 h effektiv gehemmt und zum anderen sind signifikant weniger vermehrungsfähige Staphylokokken auf den Nahtmaterialoberflächen nachweisbar. Die Voraussetzung für eine ausreichende Biokompatibilität grenzt die potenziell klinisch einsetzbaren Nahtmaterialien auf die mit der geringsten Dosis (11 µg/cm) ein. Diese wurden vergleichend für die Anwendung evaluiert, mit der Empfehlung zu Chlorhexidin-Laurat-Coatings (CL11), da diese die oberflächenadhärierten und planktonischen Bakterien am stärksten inaktivieren, eine hochkonzentrierte Wirkstofffreisetzung zur Keimeradikation innerhalb der ersten 48 h besitzen und damit am besten zur Senkung der Wundinfektionsraten beitragen können. [59-61]

Schlussfolgerungen

Antimikrobielle Nahtmaterialien für den chirurgischen Wundverschluss werden zur Infektprophylaxe eingesetzt. Gegenwärtig gibt es nur triclosanhaltige Nahtmaterialien für den klinischen Einsatz, wobei sich mittlerweile eine Tendenz zur Resistenzentwicklung zeigt. Neuartige chlorhexidin- bzw. oktenidinhaltige Nahtmaterialien mit Fettsäurewirkstoffträgern besitzen ein hohes Potential die gegenwärtigen Wundinfektionsraten und die damit verbundenen Kosten für das Gesundheitssystem weiter zu senken. Dies ist aufgrund der starken Inhibierung von bakteriellen Erregern sowohl auf den Nahtmaterialien, als auch in deren Umgebung möglich. Hiermit kann die Besiedelung von Nahtmaterialien bzw. der Transport entlang der Nahtmaterialien vorwiegend unterbunden und das verwendete Nahtmaterial als Ursache für die Ausbildung einer Wundinfektion (SSI) ausgeschlossen werden. Prä-klinische Studien sind notwendig, um deren Wirksamkeit zur Infektionsvermeidung und sichere Anwendung *in vivo* zu belegen. Das vorliegende Beschichtungssystem hat seine Effizienz mit einstellbarer Drug-release-Kinetik *in vitro* belegt, so dass die Erweiterung der Wirkstoffe für den klinischen Einsatz ebenso wie für die Übertragung auf weitere Implantat-/Biomaterialsysteme angedacht wird. [59-61]

Disclaimer

Teile der vorliegenden Arbeit wurden in abgewandelter Form bereits im Englischen in wissenschaftlichen peer-reviewed Fachorganen publiziert [59-61]. Es handelt sich dabei um originäre Arbeiten von Andreas Obermeier, welche im Literaturverzeichnis entsprechend aufgeführt sind.

4 Zusammenfassung

Hintergrund: Chirurgische Wundinfektionen (surgical site infections, SSI) stellen eine wachsende Herausforderung im klinischen Alltag dar. Die Infektionsraten werden je nach Wundlokalisation zwischen 5-36 % beschrieben [36, 37]. Nahtmaterialien können derartige Infektionen begünstigen, da diese eine Besiedelungsgrundlage zur Biofilmbildung und einen Transportweg für bakterielle Erreger durch Kapillareffekte darstellen. Antimikrobielle Nahtmaterialien können zur Infektprophylaxe eingesetzt werden. Aktuell sind nur triclosanhaltigen Nahtmaterialien für die Anwendung am Menschen zugelassen. Allerdings tauchen gegen Triclosan bereits erste Resistenzen auf, weshalb Alternativen zum antimikrobiellen Wundverschluss angeraten sind. Aus diesem Grund werden neue antimikrobielle Nahtmaterialien mit Chlorhexidin und Oktenidin als Wirksubstanzen mittels Tauchbeschichtung entwickelt. Ziel ist es in Abhängigkeit von Wirkstoff, Dosis und Wirkstoffträger auch künftig im postoperativen Zeitraum von 48 h nach Wundverschluss vor Infektionen schützen zu können. In Anbetracht aller erhobener Daten zur mikrobiologischen Effizienz, Biokompatibilität und Freisetzungskinetik wurde eine vergleichende Evaluierung durchgeführt, um das Optimum der neuen Beschichtungssysteme für die spätere klinische Anwendung zu bestimmen. [59-61]

Methoden: Antimikrobielle Nahtmaterialien wurden im Tauchverfahren hergestellt. Die Beschichtungslösungen enthielten einen Wirkstoff- (Chlorhexidin/Oktenidin) und einen Fettsäureträgeranteil (Laurat/Palmitat). Damit ergaben sich vier Beschichtungssysteme: Chlorhexidin-Palmitat oder -Laurat (CL, CP) und Oktenidin-Palmitat oder -Laurat (OL, OP). Für Testreihen wurde die Wirkstoffmenge jeweils zwischen 11 µg/cm, 22 µg/cm und 33 µg/cm variiert. Zunächst wurde die maximale Zugfestigkeit im Knotenzug dieser Nahtmaterialien vor und nach Beschichtung gemäß Ph. Eur. 0667 im Vergleich zu unbehandeltem Fadenrohmaterial (PGA Gunze) geprüft. Drug-Release Kinetiken wurden mit Nahtmaterialeluaten in PBS bei 37°C durch UV/VIS-Absorptionsmessung bei 280 nm bestimmt. REM-Aufnahmen wurden zur Oberflächeninspektion der Beschichtungen und zur Beurteilung adhärenter Mikroorgansimen durchgeführt. Die antimikrobielle Wirksamkeit beschichteter Nahtmaterialien wurde in Agardiffusionstests mit dem klinischen Erregermillieu für Wundinfektionen (*S. aureus, MRSA, S. epidermidis, E. faecalis* und *E. coli*) über 48 h und zur Erfassung der Langzeitwirkungen mit *S. aureus* getestet. Zusätzlich wurde die mikrobielle Adhäsion beimpfter Nahtmaterialien anhand vitaler *Staphylococcen* nach Ultraschallbehandlung quantitativ bestimmt. Demgegenüber wurde die Biokompatibilität via WST-1 Test an L929-Mausfibroblasten gemäß ISO 10993-5

mit Nahtmaterialproben durchgeführt. Als Kontrolle wurde kommerziell erhältliches wirkstofffreies (PGA Gunze, PGA Resorba, Vicryl®) und das triclosanhaltige Nahtmaterial (Vicryl® Plus) herangezogen. [59-61]

Ergebnisse: Zugfestigkeitsprüfungen wiesen im Mittel 78,1±1,9 N für neuartig beschichtete Nahtmaterialien und 73,7±3,0 N für unbehandeltes Fadenmaterial (PGA Gunze) auf. Drug-Release Kinetiken zeigten stetige Freisetzungen bis zum Sättigungszeitpunkt in Abhängigkeit vom Wirkstoff, Wirkstoffträger und der jeweiligen Konzentration. Anhand der freigesetzten Wirkstoffmengen nach 96 h und der maximalen Freisetzungsdauer ließ sich das Freisetzungsverhalten für jeden Beschichtungstyp erkennen: CL11 (87 %, 48 h), CP11 (40 %, 96 h), OL11 (81 %, 72 h), OP11 (6 %, 168 h). REM-Aufnahmen zeigten gleichmäßige Beschichtungen mit raueren Strukturen bei Palmitaten im Vergleich zu Lauraten. Zudem konnten adhärente Bakterien auf den Oberflächen aller untersuchten Nahtmaterialien nachgewiesen werden. Agardiffusionstests zeigten vergleichbare Hemmhofgrößen (in Millimeter) innerhalb eines Beschichtungstyps gegenüber den 5 Teststämmen von Wunderregern, gemittelt nach 48 h: CL22 (7,6), CP22 (8,8), OL22 (1,6) und OP22 (1,7). Langzeitverläufe mit *S. aureus* zeigten für Chlorhexidin- eine bis zu 5 Tage anhaltende und für Oktenidin-Nahtmaterialien eine bis zu 9 Tage anhaltende antimikrobielle Wirkung. Adhärierte *S. aureus*-Bakterien konnten mit Vicryl® Plus und OL11-coatings um 0,5 Log reduziert werden. Signifikant höhere Reduktionen (Log-Stufen) wurden mit CL11 (1,7), CP11 (1,4) und OP11 (1,0) erreicht. Mit höheren Wirkstoffkonzentrationen konnten bis zu 6,1 Log erreicht werden. Zytotoxizitätsprüfungen mittels WST-1 Test ergaben nur für die 11 µg/cm haltigen beschichteten Nahtmaterialien noch ausreichende metabolische Aktivitäten: CL11 (69 %), CP (74 %), OL11 (77 %) und OP11 (85 %). [59-61]

Diskussion: Neuartige antimikrobielle Nahtmaterialien konnten reproduzierbar mit Wirkstoffbeladung hergestellt werden, ohne negative Beeinflussung der Zugfestigkeit bei gleichmäßen Beschichtungen. Damit dürfte sich dieses Verfahren für den industriellen Maßstab eignen. Hinsichtlich der Wirkstofffreisetzungen zeigen Chlorhexidinbeschichtungen vermutlich aufgrund der höheren Löslichkeit einen schnelleren Release im Vergleich zu solchen mit Oktenidin. Zusätzlich beeinflusst die Art des Fettsäurewirkstoffträgers wesentlich die Dauer der Freisetzung. So kann unter Verwendung von Palmitaten eine deutliche Verlangsamung der Wirkstofffreisetzung erreicht werden, dies ist ebenso an den prozentual freigesetzten Wirkstoffmengen nach 96 h zu erkennen. Somit ist nicht nur eine Verzögerung, sondern auch eine Niveauabsenkung der Stoffmengen ohne begleitenden Wirksamkeitsverlust zu erreichen. Je nach Applikation könnte dies als sog. „Toolbox“ verwendet werden, um angepasste Nahtmaterialsysteme zusammenzustellen. Die antimikrobielle breite Wirkung *in vitro* sowohl für Chlorhexidin-,

als auch Oktenidinbeschichtungen lässt auf eine Hemmung von klinischen Biofilmen in Wunden schließen. Klinisch wird vor allem eine Infektprophylaxe für die ersten 48 h nach dem chirurgischem Eingriff gefordert [62]. Mit den neuartig beschichteten Nahtmaterialien kann vermutlich die Rate von Wundinfektionen weiter gesenkt werden, als dies bisher mit kommerziell erhältlichem triclosanhaltigen (Vicryl® Plus) möglich ist. Zum einen, werden für die getesteten neuen Nahtmaterialien Bakterien über Zeiträume von >48 h effektiv gehemmt und zum anderen sind signifikant weniger vermehrungsfähige *Staphylococcen* auf den Nahtmaterialoberflächen nachweisbar. Die Voraussetzung zur Biokompatibilität grenzt die potentiell klinisch einsetzbaren Nahtmaterialien auf die 11 µg/cm Varianten ein. Diese wurden vergleichend für die Anwendung evaluiert, mit der Empfehlung zu Chlorhexidin-Laurat-Coatings (CL11), da diese die oberflächenadhärierten und planktonischen Bakterien am stärksten inaktivieren, eine hochkonzentrierte Wirkstofffreisetzung zur Keimeradikation innerhalb der ersten 48 h besitzen und damit am besten zur Senkung der Wundinfektionsraten beitragen können. [59-61]

Schlussfolgerungen: Antimikrobielle Nahtmaterialien für den chirurgischen Wundverschluss werden zur Infektprophylaxe eingesetzt. Gegenwärtig gibt es nur triclosanhaltige Nahtmaterialien für den klinischen Einsatz, mit Tendenz zur Resistenzentwicklung. Neuartige Chlorhexidin- bzw. Oktenidin-Nahtmaterialien besitzen ein hohes Potential die gegenwärtigen klinischen Wundinfektionsraten weiter zu senken. Sie könnten künftig Alternativen zum antimikrobiellen Wundverschluss für die Infektprophylaxe darstellen. Des Weiteren sind prä-klinische Studien notwendig, um deren Wirksamkeit zur Infektionsvermeidung und sichere Anwendung *in vivo* zu belegen. [59-61]

5 Abstract

Background: Surgical site infection (SSI) poses a growing challenge within the clinical daily routine. Infection rates are described in literature in the range of 5-36 % dependent on wound localization [36, 37]. Surgical sutures are able to promote such infections, as they represent a basis for bacteria to colonize and form biofilms. Additionally, sutures can be a route of transport for microorganisms via capillary mechanism. Antimicrobial sutures can also be employed for wound closure as infection prophylaxis. Currently, only triclosan-containing surgical sutures are CE-certified for the human use. However, first resistances against triclosan are on the rise, therefore, novel alternatives to antimicrobial wound closures are advised. For this purpose, novel antimicrobial surgical sutures using chlorhexidine and octenidine as active substances were developed in a dip coating process. The aim of this study is to allow dependent on active agent, dose and drug carrier also be able to protect against infections during the postoperative period of 48 hours after wound closure. To determine the optimum of the novel coating system for the future clinical use, all collected data regarding microbiological efficacy, biocompatibility and drug-release kinetics were comparative evaluated. [59-61]

Methods: Surgical sutures were prepared via a dip coating process. Coating solutions consist of one drug (chlorhexidine/octenidine) and one fatty acid carrier (laurate/palmitate) component each. Resulting in four types of coatings: Chlorhexidine laurate or palmitate (CL, CP) and octenidine laurate or palmitate (OL, OP). Drug contents varied in the range of 11 µg/cm, 22 µg/cm and 33 µg/cm for each coating type. After dip coating, tensile strength of sutures was measured according to Ph. Eur. 0667 to compare uncoated raw sutures (PGA Gunze) with novel coated sutures. Drug-release kinetics were determined via suture elutions in PBS at 37°C, followed by absorption measurements at 280 nm. SEM pictures were taken to inspect coatings on sutures' surfaces, as well as to evaluate adhered microorganisms. Antimicrobial efficacies of novel coated sutures were tested by using the agar diffusion assay. Determinations were made against a broad pathogen spectrum of wound infections (*S. aureus, MRSA, S. epidermidis, E. faecalis* and *E. coli*) over 48 h, as well as against *S. aureus* the major cause of such infections to detect the antimicrobial long term efficacy. Moreover, microbial adhesion was measured quantitatively with staphylococcus species after sonication of inoculated surgical sutures. On the other hand, biocompatibility was detected according to ISO 10993-5 measuring the metabolic activity of L929 mouse fibroblasts in contact with suture eluates. As test controls in this study, commercially available sutures without drug (PGA Gunze, PGA Resorba, Vicryl®) and triclosan-containing sutures (Vicryl® Plus) were used. [59-61]

Results: Novel coated sutures showed an average tensile strength at 78.1±1.9 N and uncoated raw sutures (PGA Gunze) at 73.7±3.0 N. Drugs are continuously released until reaching the time point of saturation in the release kinetics. Duration until saturation is strongly dependent on the kind of drug, drug carrier and the drug concentration inside the coating. Amounts of released drugs after 96 h and duration of drug release at whole revealed the release properties for each coating type: CL11 (87 %, 48 h), CP11 (40 %, 96 h), OL11 (81 %, 72 h), and OP11 (6 %, 168 h). SEM pictures demonstrated consistent coating layers, showing rougher structures when using palmitates compared to laurate carriers. Additionally, adhered bacteria could be detected on surfaces of all investigated sutures. Agar diffusion tests revealed comparable inhibition zones (in millimeters) within each type of coating for the used bacterial test spectrum from wound infections, in the middle 48 h: CL22 (7.6), CP22 (8.8), OL22 (1.6) und OP22 (1.7). Long-term efficacy test using *S. aureus* resulted for chlorhexidine- in up to 5 days and for octenidine sutures in up to 9 days sustaining antimicrobial activity. Vicryl® Plus, as well as OL11 sutures reduced adhered *S. aureus* bacteria to 0.5 Log. In contrast, significantly higher Log-reductions were reached by CL11 (1.7), CP11 (1.4) und OP11 (1.0) sutures. The higher drug concentration containing sutures were able to reach reductions up to 6.1 Log. On the other hand cytotoxicity tests via WST-1 assay showed that only 11 µg/cm coated sutures obtained sufficient metabolic cell activities: CL11 (69 %), CP (74 %), OL11 (77 %) and OP11 (85 %). [59-61]

Discussion: Novel antimicrobial sutures could be reproducibly coated with drugs, without negative effects on tensile strength and reaching uniform coating layers. Therefore, the used coating procedure should be suitable for an industrial upscaling. Chlorhexidine showed generally higher quantities of drug release, presumably because of its higher solubility compared to octenidine. Additionally, the type of fatty acid carrier has a relevant impact on release and release duration. Thus, a clear slowdown of the drug release, as well as the percentage of released drugs after 96 h is detectable when using palmitates. In this manner not only a delay of drug release is achievable, but rather a decrease of the releasing level of drugs without loosening antimicrobial efficacy. Dependent on a clinical application this could be used as a kind of “tool box” to adjust proper suture coating systems. The antimicrobial broad efficacy *in vitro* for chlorhexidine- and octenidine coatings suggests an inhibition of bacterial biofilms inside clinical wounds. Infection prophylaxis is clinically postulated for the first 48 h after surgical intervention [62]. Presumably, the novel coated surgical sutures are able to further reduce the rate of wound infections as it could be reduced by triclosan-coated sutures (Vicryl® Plus) nowadays. The novel sutures effectively inhibit surrounding bacteria for more than 48 h. Additionally, there are significantly less viable adhered bacteria on sutures’ surfaces detectable. Biocompatibility as

precondition narrows down the potential clinically usable novel sutures to the 11 µg/cm variants. These sutures were evaluated in regard to their efficiencies; resulting in the recommendation of chlorhexidine laurate sutures (CL11), as these sutures inhibit adherent and planktonic bacteria most efficiently, releasing sufficient drug concentrations to eradicate bacteria within the first 48 h. Therefore, this type of suture fits the medical need for a fast bacteria eradication of adhered bacteria best and may contribute to reduce infection rates. [59-61]

Conclusions: Antimicrobial sutures were employed for infection prophylaxis in surgical wound closure. Currently, only triclosan-coated sutures are certified for the clinical use, showing a tendency to develop resistances. Novel chlorhexidine or octenidine sutures indicate a high potential to further reduce the wound infection rates. These sutures may represent alternatives for future antimicrobial wound closure techniques. Further pre-clinical studies are necessary to prove their efficacy in infection prevention as well as safety *in vivo.* [59-61]

6 Originalarbeiten

6.1 Eigenanteil an den vorgelegten Arbeiten

Das Thema der Dissertation sowie das **Konzept und das Design der Studien** entwickelte Herr Obermeier in enger Zusammenarbeit mit den Supervisoren Herr Prof. Dr. R. Burgkart, Herr Prof. Dr. Dr. A. Stemberger, Herr Prof. Dr. R. v. Eisenhart-Rothe und Herr Prof. Dr. M. Schieker. Herr Obermeier hat die verwendeten mikrobiologischen, zellbiologischen und pharmazeutischen **Methoden** selbstständig eingeführt, weiterentwickelt und hierfür standardisierte Versuchsprotokolle etabliert. Anschließend führte Herr Obermeier die **Experimente** teilweise mit der Unterstützung von durch ihn angelernte Studenten zur Datenreproduktion durch: Hierzu zählen Versuchsreihen zur Testung der antimikrobiellen Wirksamkeit beschichteter Nahtmaterialien gemeinsam mit Herr S. Wehner. Ebenso unterstützte Frau B. Kiefel die Reproduktion gemessener Daten der mikrobiellen Adhäsion auf diversen Nahtmaterialien und Frau A. Kalaai half bei der Reproduktion von Messdaten zur Oktenidin-Freisetzung. Außerdem, stand Herr Dipl.-Ing. P. Föhr unterstützend bei der Durchführung von biomechanischen Zugversuchen zur Seite. Mit seiner Unterstützung wurden Reißfestigkeiten von Nahtmaterialien gemäß der Europäischen Pharmakopöe bestimmt. Frau J. Tübel half beim Umgang mit Zellkulturen und unterstützte die selbstständige Durchführung von *in vitro* Zytotoxizitätsbestimmungen gemäß DIN ISO 10993-5. Herr Dipl.-Ing. C. von Deimling beriet bei statistischen Fragestellungen. Frau S. Schnell-Witteczek unterstützte die Durchführung von REM-Aufnahmen und Probenpräparation für diese Arbeit. Für Fragen zur Mikrobiologie standen Herr Dr. J. Schneider und Frau C. Krämer kompetent zur Seite. Das Thema klinische Infektsituation und Pathogenese wurde praxisnah von den Herren Dres. H. Mühlhofer, D. Pförringer und N. Harrasser unterstützt. Herr Dr. F. Matl und Herr Prof. A. Stemberger standen bei pharmazeutischen Fragen, wie Arzneimittelwirkung, -nachweis und Drug-Release-Bestimmung jederzeit beratend zur Seite. Die **Auswertung der Ergebnisdaten** erfolgte durch Herr Obermeier ohne fremde Hilfe, er evaluierte diese und stellte diese zusammenfassend dar. Die **Interpretation der Daten** erfolgte in enger Zusammenarbeit mit den Supervisoren. Nach eigenen **Literaturrecherchen** wurden von Herr Obermeier, der in allen drei Publikationen alleiniger Erstautor ist, die wissenschaftlichen **Manuskripte selbstständig verfasst.** Daraufhin wurden zusammen mit allen beteiligten Co-Autoren jeweils die **Manuskripte überarbeitet**. Schließlich erfolgte die **Freigabe der finalen Manuskriptversionen** zur Veröffentlichung durch alle beteiligten Autoren vor Einreichung und jeder weiteren Revision.

6.2 Originalarbeiten im Volltext

6.2.1 Publikation I

Title: **Novel high efficient coatings for antimicrobial surgical sutures using chlorhexidine in fatty acid slow-release carrier systems.**

Autoren: Obermeier A, Schneider J, Wehner S, Matl FD, Schieker M, von Eisenhart-Rothe R, Stemberger A, Burgkart R.

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PLOS ONE

Novel High Efficient Coatings for Anti-Microbial Surgical Sutures Using Chlorhexidine in Fatty Acid Slow-Release Carrier Systems

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Abstract

Sutures can cause challenging surgical site infections, due to capillary effects resulting in bacteria permeating wounds. Anti-microbial sutures may avoid these complications by inhibiting bacterial pathogens. Recently, first triclosan-resistances were reported and therefore alternative substances are becoming clinically relevant. As triclosan alternative chlorhexidine, the "gold standard" in oral antiseptics was used. The aim of the study was to optimize novel slow release chlorhexidine coatings based on fatty acids in surgical sutures, to reach a high anti-microbial efficacy and simultaneously high biocompatibility. Sutures were coated with chlorhexidine laurate and chlorhexidine palmitate solutions leading to 11, 22 or 33 µg/cm drug concentration per length. Drug release profiles were determined in aqueous elutions. Antibacterial efficacy against *Staphylococcus aureus* was assessed in agar diffusion tests. Biocompatibility was evaluated via established cytotoxicity assay (WST-1). A commercially triclosan-containing suture (Vicryl Plus), was used as anti-microbial reference. All coated sutures fulfilled European Pharmacopoeia required tensile strength and proved continuous slow drug release over 96 hours without complete wash out of the coated drug. High anti-microbial efficacy for up to 5 days was observed. Regarding biocompatibility, sutures using 11 µg/cm drug content displayed acceptable cytotoxic levels according to ISO 10993-5. The highest potential for human application were shown by the 11 µg/cm chlorhexidine coated sutures with palmitic acid. These novel coated sutures might be alternatives to already established anti-microbial sutures such as Vicryl Plus in case of triclosan-resistance. Chlorhexidine is already an established oral antiseptic, safety and efficacy should be proven for clinical applications in anti-microbial sutures.

Citation: Obermeier A, Schneider J, Wehner S, Matl FD, Schieker M, et al. (2014) Novel High Efficient Coatings for Anti-Microbial Surgical Sutures Using Chlorhexidine in Fatty Acid Slow-Release Carrier Systems. PLoS ONE 9(7): e101426. doi:10.1371/journal.pone.0101426

Editor: Ali Al-Ahmad, University Hospital of the Albert-Ludwigs-University Freiburg, Germany

Received February 27, 2014; **Accepted** June 5, 2014; **Published** July 1, 2014

Funding: This work was financially supported by the Heraeus Medical GmbH, Wehrheim, Germany (http://www.heraeus-medical.com/de/home_15.html). Within the Klinikum rechts der Isar, Technische Universität München the external funding number was 8895117_HeraeusCoating. This work was supported by the German Research Foundation (DFG) and the Technische Universität München within the funding programme Open Access Publishing. The funder's financial support does not alter the authors' adherence to PLOS ONE policies on sharing data and materials. The funding organization didn't play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript and only provided financial support and research materials.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Surgical site infections (SSI) are still challenging complications after operations despite of established systemic antibiotic prophylaxis today [1]. Reported rates for SSI are usually in the range of 2–5% [2,3], but can even rise up to 25% e.g. for colorectal surgery [4]. In case of infection, further surgical interventions become necessary followed by a prolongation of hospitalization up to 10 days [2]. In conclusion, this means higher treatment costs of about 50,000 US$ per case on average [5] for the healthcare system as well as an elevated risk profile for the individual patient.

The presence of foreign material increases the risk of infections [6], especially surgical sutures can act as a wick for SSI [7]. So pathogens from the natural skin flora can easily enter wounds via capillary action. After having attached to suture surfaces the pathogens proliferate and are then able to form biofilms difficult to treat [8,9]. One potential solution approach to prevent this process is the use of anti-microbial coated sutures. At present, all commercially available anti-microbial sutures are exclusively coated with triclosan such as Vicryl Plus, Monocryl Plus, and PDS Plus [10].

In vitro studies using triclosan for anti-microbial sutures, e.g. Vicryl Plus, have resulted in a highly efficient defense against various bacterial pathogens [11,12]. *In vivo* studies for sternum surgery [13], abdominal wall closure [14], and cerebrospinal fluid shunting procedures [15] indicate significantly lower infection rates for surgical interventions using triclosan coated anti-microbial sutures [16]. In contrast, for some indications there is an ongoing controversial discussion [10,17], such as appendicitis, breast cancer and colorectal surgery [4,18–20].

The main disadvantage of the so far predominantly used antimicrobial triclosan is its wide non-medical use in cosmetics, hygiene- and household products [21]. First research groups reported resistances of bacteria against triclosan and warnings of

Table 1. Prepared anti-microbial sutures and normalized weights of chlorhexidine.

ratio of chlorhexidine in fatty acid (%)	weight of chlorhexidine (mg)	weight of lauric-/ palmitic acid (mg)	normalized drug weight (µg/cm)	coating type and abbreviation for coated sutures	
20	0.44	1.76	**11**	chlorhexidine-lauric acid	**CL11**
				chlorhexidine-palmitic acid	**CP11**
40	0.88	1.32	**22**	chlorhexidine-lauric acid	**CL22**
				chlorhexidine-palmitic acid	**CP22**
60	1.32	0.88	**33**	chlorhexidine-lauric acid	**CL33**
				chlorhexidine-palmitic acid	**CP33**

Amount of chlorhexidine on anti-microbially coated sutures after preparation. The mean coating weight of 40 cm suture samples were determined at 2.2±0.2 mg. The same amounts of chlorhexidine were calculated for the coating types (CL, CP) inside coating solutions (n = 10). The weights on coated sutures for drug contents, fatty acid carriers and normalized mean drug weight per cm suture are given above.
doi:10.1371/journal.pone.0101426.t001

potential pathogen selections [22,23]. Therefore, we were looking for new alternative substances and chose chlorhexidine to equip surgical sutures anti-microbially. This antiseptic is effective against a broad spectrum of relevant pathogens including clinically problematic bacteria like *Staphylococcus aureus* [24]. Besides the known spectrum chlorhexidine is already approved in a variety of medical applications as coating for medical devices [25–27], skin antiseptic [28,29] or as well as oral antiseptic [30].

In a first feasibility study we could show that chlorhexidine coated sutures demonstrated high efficacy against *S. aureus* with the disadvantage of drug concentration related cytotoxicity [31]. Anti-microbial sutures must fulfill a balancing act between inhibiting bacteria and sustaining biocompatibility to the surrounding eukaryotic tissue cells.

The aim of this study was therefore the optimization of new chlorhexidine fatty acid coating formulations for anti-microbial sutures in regard to their drug concentration. We achieved high anti-microbial efficacy over several days combined with ISO 10993-5 required biocompatibility by variation of drug concentrations at 11, 22 or 33 µg per cm suture. Anti-microbially coated sutures were evaluated by measuring tensile strength, inhibition zones, and drug release profiles. These investigations were compared to commercially available plain sutures and triclosan containing Vicryl Plus, as anti-microbial reference.

Materials and Methods

Surgical sutures

Plain braided sutures (Gunze Ltd., Japan) made of polyglycolic acid (PGA) without the usual fatty acid coating to avoid sewing effects were used for the preparation of the anti-microbial sutures. As reference one triclosan-containing absorbable suture Vicryl Plus (Ethicon GmbH, Germany) and two standard absorbable sutures PGA Resorba (Resorba GmbH, Germany) and Vicryl (Ethicon GmbH, Germany) were used. All sutures used in experiments corresponded to United States Pharmacopeia standard 1 (USP 1).

Anti-microbial coating solutions

Anti-microbial coating solutions were prepared by dissolving a fatty acid (palmitic or lauric acid) and chlorhexidine in 99.8% ethanol (C. Roth GmbH, Germany (CR)). In order to prepare the solutions for suture coatings having a mass content of 5% (w/w), 395.0 mg of the corresponding fatty acid and the antiseptic drug were dissolved in 10.0 ml (7.9 g) ethanol. In particular, two groups of coating types were produced. Group I (**CL**): Chlorhexidine diacetate (Sigma-Aldrich GmbH, Germany) in lauric acid as drug carrier (CR). Group II (**CP**): Chlorhexidine diacetate in the drug carrier palmitic acid (CR). For each type of coating three solutions with 20%, 40% or 60% (w/w) drug concentration were formulated. The percentage of drug was related to the 5% total mass of substances (drug plus drug carrier) dissolved in coating solutions. Components were prepared by weighing, using the precision balance Atilon ATL-224 (Acculab Inc., Massachusetts, USA).

Preparation of anti-microbial sutures

Sutures were cut into 40 cm pieces followed, by coating them in a dip process in flasks under aseptic conditions. The closed flasks were placed on a thermo-shaker (Heidolph Instruments GmbH, Germany) for 2 minutes at 35°C and 150 rpm, in order to achieve reproducible coating weights. After drying for at least 2 hours the coating weight was determined by using the precision balance related to the weight of uncoated sutures. The amount of drug normalized per length of suture (µg/cm) was calculated for each concentration (Table 1).

Tensile strength test

For tensile strength testing the uncoated plain PGA sutures (Gunze), PGA Resorba, Vicryl, Vicryl Plus, and exemplarily novel coated sutures CL22, CP22 were measured. The mechanical strength of individual surgical sutures (n = 5) was determined according to the European Pharmacopoeia (Ph. Eur. 7.0/0667: required minimum for USP 1 sutures is 50.8 N) using the tensile testing instrument Zwicki 8253 (Zwick GmbH, Germany).

Drug release from fatty acid drug delivery coatings

Drug release kinetics of coated sutures were measured over a time period of 96 hours in phosphate-buffered saline (PBS). For this purpose sutures of 2 cm length (n = 3) were eluted in 1 ml PBS inside a thermomixer MHR 23 (HLC-Biotech, Germany) at 37°C and 200 rpm. After 1 h, 3 h, 5 h, 7 h, 24 h, 48 h, 72 h und 96 h, elution media was taken out and replaced by fresh PBS. Amount of released chlorhexidine was measured by absorption at 280 nm in a microplate spectrophotometer Multiskan Go (Thermo Fisher Scientific GmbH, Germany). Measured drug concentrations were normalized to length of suture samples and drug elution profiles were recorded by cumulating the released drug amounts over time. The percentage of released chlorhexidine was calculated referring to loaded drug per cm length.

Table 2. Mean tensile strength values of surgical sutures (USP 1).

Tensile strength	Gunze	PGA Resorba	CL22	CP22	Vicryl	Vicryl Plus
mean F_{max} (N)	73.7	81.6	80.3	75.9	78.3	72.2
± standard deviation (N)	3.0	1.6	1.5	2.2	1.0	2.2

Mean values of the maximum force F_{max} (n = 5) for uncoated, commercial and novel coated sutures CL22 and CP22 in example are given above. The required minimum for USP 1 surgical sutures at 50.8 N according to European Pharmacopoeia, was reached for all tested sutures.
doi:10.1371/journal.pone.0101426.t002

Anti-bacterial efficacy of coated sutures via zones of inhibition

Anti-microbial efficacy was determined in Agar plate diffusion tests by using the strain *Staphylococcus aureus* (ATCC 49230), the main pathogen of implant-associated infections [32,33]. According to CLSI criteria, bacterial suspensions were prepared to 0.5 McFarland standard [34]. On each Agar plate 1 ml of this suspension was uniformly distributed. Coated sutures with 3 cm length (n = 3) were placed onto these inoculated Agar plates and incubated at 37°C for 24 hours. Inhibition zones were measured (in mm), with a calliper perpendicular to the middle of the threads. Novel coated sutures and Vicryl Plus were put on Agar plates every 24 hours with fresh bacterial lawns and incubated overnight. Over the following days we measured the inhibition zones. Analogous to Ming et al. [12] this procedure was repeated by using the same samples for several days to recognize the remaining anti-bacterial activity until no detectable inhibition zone was left.

Cytotoxicity study

According to ISO 10993-5:2009, mouse fibroblasts L-929 (DSMZ, Germany) were used for in vitro cytotoxicity testing of coated sutures. The evaluation was performed by measuring the metabolic activity of cells in the presence of eluates from coated sutures via a WST-1 cell proliferation assay (Roche Diagnostics GmbH, Germany). Cells were cultivated in the corresponding media (D-MEM 4.5 g/l D-glucose, Biochrom AG, Germany) containing 10% fetal bovine serum at 37°C and 5.0% CO2 in a humidified atmosphere. Pre-cultures started at 10.000 cells/well inside 96 well microtiter plates, incubated for 24 hours in 200 µl D-MEM. Simultaneously, eluates were generated by eluting coated sutures of 1 cm length (n = 7) in 1.5 ml D-MEM for 24 hours on a thermomixer at 37°C and 300 rpm. After 24 hours cell media was swapped with eluates. Finally, after 48 hours referred to the WST-1 protocol the metabolic activity was measured by quantitative detection of formazan at 405 nm in a spectral photometer. Metabolic activities obtained were referred to activity of pure L-929 culture. A metabolic activity of 70% represents the limit to claim "biocompatibility" for medical devices.

Statistics

Statistical methods were performed by using the student's t-test (Microsoft Excel 2013) with significant level $p<0.05$. Mean values and standard deviations were calculated from at least 3 measurements. Calculation of mean values from several measurements was accompanied by the Gaussian error propagation law.

Results

Reproducibility of anti-microbial sutures

Coating weight of prepared anti-microbial sutures was an important parameter to estimate weights of drugs per unit length. The weight difference of 40 cm sutures in length between uncoated and coated sutures resulted in a mean coating weight at 2.2 mg±0.2 mg (n = 10) independent from drug concentrations used. The triclosan content on Vicryl Plus sutures was 2.7 µg/cm [35,36].

Tensile strength test

The quasi-static tensile strength values of all sutures tested - uncoated, commercially and novel coated sutures - proved mean maximum tensile strength values higher than the Ph. Eur. required minimum of 50.8 N for USP1 absorbable surgical sutures (Table 2). The strength values showed a moderate but significant increase between Gunze and PGA Resorba ($p<0.05$), similar to novel coated sutures CL22 and CP22 exemplarily. Vice versa for Vicryl Plus sutures, a moderate reduction of tensile strength ($p< 0.05$) was observed compared to Vicryl sutures.

Drug release from fatty acid drug delivery coatings

Released drug concentrations in PBS eluates normalized by length were determined. All coated sutures demonstrated continuous drug release within four days of experiment. Chlorhexidine was continuously released from lauric acid (Figure 1A) resulting in a drug concentration at 96 hours of 9.6 µg/ml (CL11), 16.6 µg/ml (CL22) and 23.2 µg/ml (CL33). The coating type chlorhexidine in palmitate achieved a drug concentration of 4.4 µg/ml (CP11), 5.8 µg/ml (CP22) and 15.2 µg/ml (CP33) after 96 h (Figure 1B). Percentage of drug release, the ratios between released drugs after 96 hours and amount of drugs on sutures per cm length were compared to each other for chlorhexidine laurate coatings (Figure 2A) and chlorhexidine palmitate coatings (Figure 2B).

Anti-bacterial efficacy of coated sutures via zones of inhibition

Anti-microbial efficacy of coated sutures was daily assessed by using an Agar diffusion assay over several days (Figure 3A). Sutures coated with chlorhexidine in lauric acid (Figure 3B, a) revealed large *S. aureus* inhibition zones after 24 hours of 7.1 mm (CL11), 8.2 mm (CL22) and 8.7 mm (CL33). Inhibition zones during the first three to four days of experiments averaged up to 1.7 mm for CL11, 3.4 mm for CL22 and 2.5 mm for CL33. After the fifth day no inhibition zones were detectable. Chlorhexidine in palmitic acid coatings showed similar results (Figure 3B, b). Inhibition zones after 24 hours were assessed at 4.9 mm (CP11), 7.1 mm (CP22) and 8.9 mm (CP33). The dimensions and durations of the zones of inhibition were dependent on drug concentrations. CP11 ended up after fourth day of experiment, whereas CP22 and CP33 ended up after the fifth day of experiment. The triclosan containing Vicryl Plus showed large inhibition zones after 24 hours on Agar plates with 19.8 mm (Figure 3B, c) lasting for at least nine days and ending up with 1.7 mm zones. The bioavailability of the antiseptics from the fatty

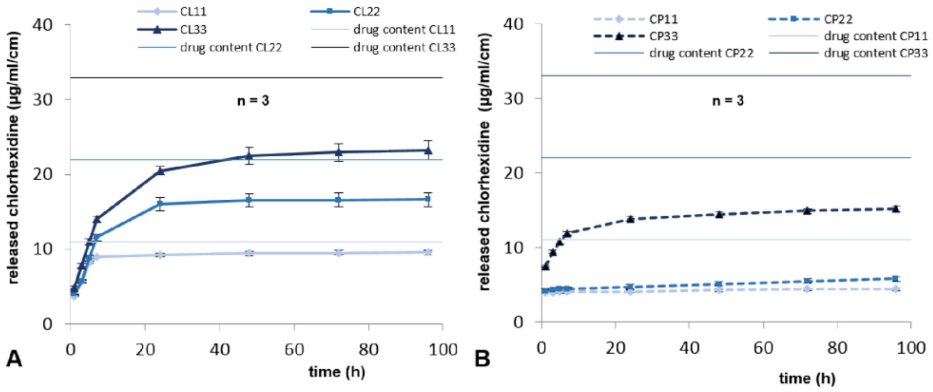

Figure 1. Elution profiles of chlorhexidine coated sutures. Released chlorhexidine in PBS buffer at 37°C for A) chlorhexidine-laurate coatings and B) chlorhexidine-palmitate coated sutures. Elution profiles were determined for each carrier at drug content 11, 22 or 33 µg/cm. The horizontal lines depict the limit of drug release for each concentration, the normalized content of chlorhexidine on coated sutures.
doi:10.1371/journal.pone.0101426.g001

acid delivery systems was corroborated by the microbial experiments.

Cytotoxicity study

Cytotoxicity tests were performed via eluates from individual coated sutures and references. All metabolic activities were referred to L-929 cell samples used as growth reference without sutures. Coatings with chlorhexidine in lauric acid ended with metabolic cell activities at 69.1±7.0% (CL11), 0.9±0.5% (CL22) and 0.3±0.3% (CL33). Chlorhexidine in palmitic acid coatings showed activities at 74.5±29.3% (CP11), 1.1±1.9% (CP22) and no more cell activity for CP33 sutures. The fatty acid coated references identified metabolic activities at 87.8±13.2% and 80.4±13.4% (lauric acid, palmitic acid). Eluates from uncoated sutures reached a metabolic activity at 100.8±9.7% (Gunze). Vicryl Plus eluates demonstrated activities at 98.7±7.1% (Figure 4).

Discussion

Surgical site infection still poses a major complication in surgery. Sutures can cause so called suture-associated infections, induced by proliferation of adhering pathogens. Adhering bacteria enter wounds by capillary action and form infamous biofilms, leading to chronic infections [8]. Anti-microbial coatings for surgical sutures can solve that problem via protecting sutures by inhibiting bacterial growth.

In the present study we developed new anti-microbial suture coatings based on fatty acid carriers using chlorhexidine and adjusting their drug concentrations. The aim was to identify anti-microbial sutures posing effective protection against microbes while being biocompatible in regard to eukaryotic cells. Fatty acids constitute a lubricating film and are state of the art in order to reduce the unwanted sewing effect of sutures. This kind of drug-release system still allows slow-release properties, because of low solubility of fatty acid carriers in aqueous environments [37,38].

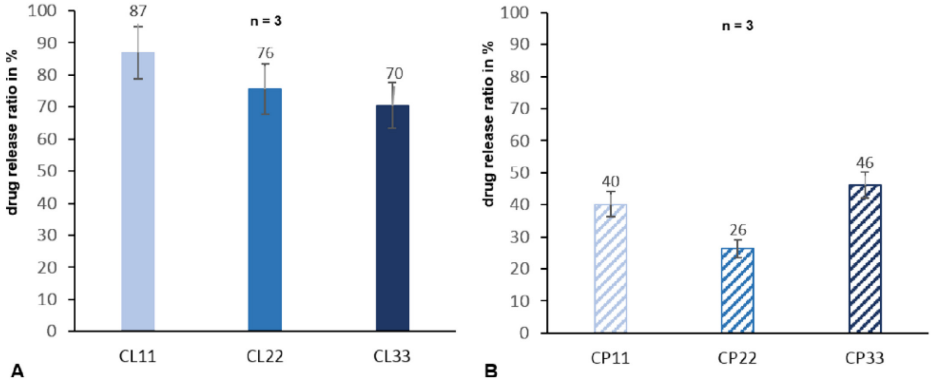

Figure 2. Percentage of chlorhexidine released after 96 hours related to the amount of drug in coated samples. Percentage of chlorhexidine release related to the drug content on coated sutures per cm length at 96 hours of elution in PBS buffer for A) chlorhexidine-laurate and B) chlorhexidine-palmitate coated sutures.
doi:10.1371/journal.pone.0101426.g002

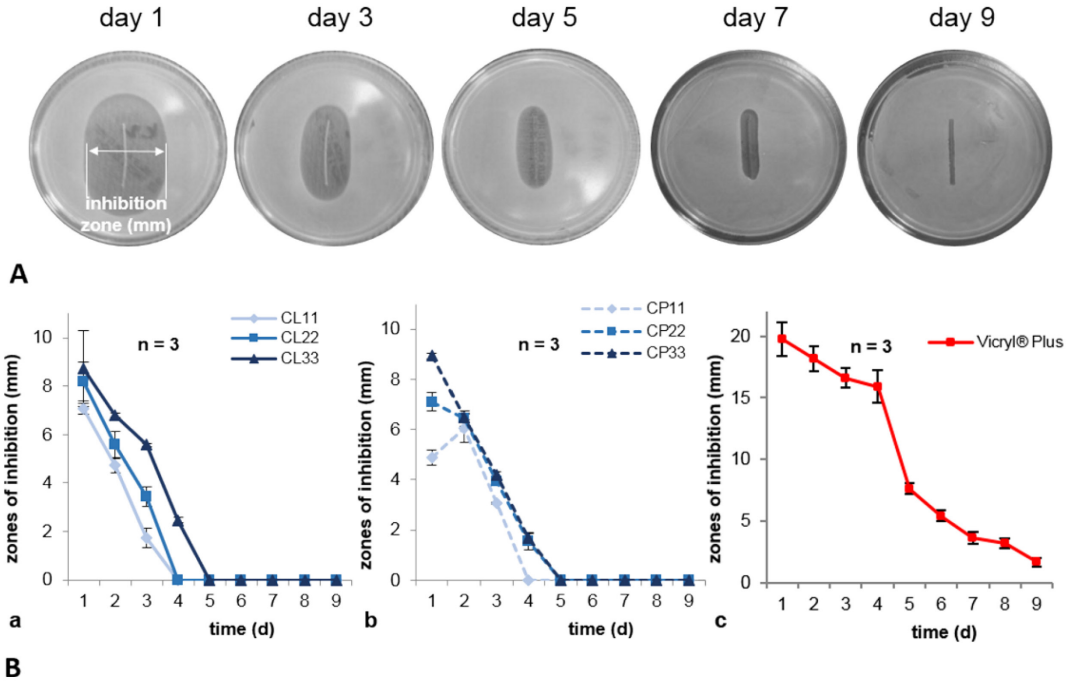

Figure 3. Measuring anti-bacterial efficacy of coated sutures via zones of inhibition. A) Principle of measuring inhibition zones in a longitudinal analysis on the example of a Vicryl Plus suture, in order to achieve anti-microbial effectiveness over several days. B) Anti-microbial efficacies on Agar plates with *S. aureus* lawns (2×10^8 cfu/ml) for a) chlorhexidine lauric acid and b) chlorhexidine palmitic acid coated sutures. Coated suture samples with three different chlorhexidine concentrations 11, 22 and 33 µg/cm. c) Vicryl Plus as reference for commercial anti-microbial sutures.
doi:10.1371/journal.pone.0101426.g003

In a reproducible dip coating process we developed several coating types at various concentrations based on chlorhexidine with lauric or palmitic acid (CL, CP). Coated sutures obtained were tested systematically regarding their tensile strength, drug release, anti-microbial efficacy against *S. aureus*, and cytotoxicity using a WST-1 assay.

Tensile strength of coated PGA sutures was just negligibly influenced by the dip coating process using ethanol. All coated sutures undergoing this process showed much higher maximum strength values than required by the Ph. Eur. standards for USP1 resorbable sutures. The mean strength values of novel coated sutures were comparable to commercially available PGA sutures (PGA Resorba, Vicryl, Vicryl Plus). Therefore, no negative influence of coatings on in vivo degradation time for anti-microbial coated PGA sutures is to be expected. Flexibility of coated sutures remained steady, no delamination was observed after mechanical stress tests. Consequently, no delamination is to be expected while pulling them through the tissue.

Released drug concentration in PBS for chlorhexidine coated sutures showed a continuous drug release for 96 hours with initial rapid release slowing down significantly after 7 hours. In general, drug release should be as slow as possible, however, anti-microbially effective to inhibit pathogens as long as possible, to achieve long-term protection of the coated biomaterial. Drug release is strongly dependent on drug concentration inside coatings. Referring to drug carriers, palmitic acid coatings showed slower drug release values over time than lauric acid coatings with similar anti-microbial efficacies. Moreover, lesser amounts of chlorhexidine were released from palmic acid coatings. Therefore, anti-microbial effects of coated sutures using palmitic acid carrier should have a higher potency for long-term protection in vivo than coatings using lauric acid carriers.

Novel coated sutures showed high anti-microbial efficacy in agar diffusion tests against *S. aureus*. Anti-bacterial tests on agar plates mimic the tissue contact transferring substances by diffusion. All coated sutures generated inhibition zones for more than 24 hours and documented efficacies over several days, similar to Vicryl Plus. Inhibition zones of CP11, on the second day, showed a little increase, presumably a consequence of non-uniform contact of suture samples on Agar surfaces and therefore diffusion problems. The release of chlorhexidine indicated by the inhibition zones is faster during the first days compared to triclosan, because of its much higher solubility in aqueous environments like PBS or Agar. This faster consumption of substances on sutures leads to earlier leaching of inhibition zones. On the one hand, this could be a benefit, because a sufficient release of antiseptics in the wound area in the first days might be an important factor to prevent a potential early wound infection. The long-term efficacy of chlorhexidine coated sutures against *S. aureus* lasted up to 5 days at high levels. Regarding drug concentrations, the dimension of inhibition zones over time did not differ greatly. Thus, even the low drug content of 11 µg/cm can almost be as effective as 22 or

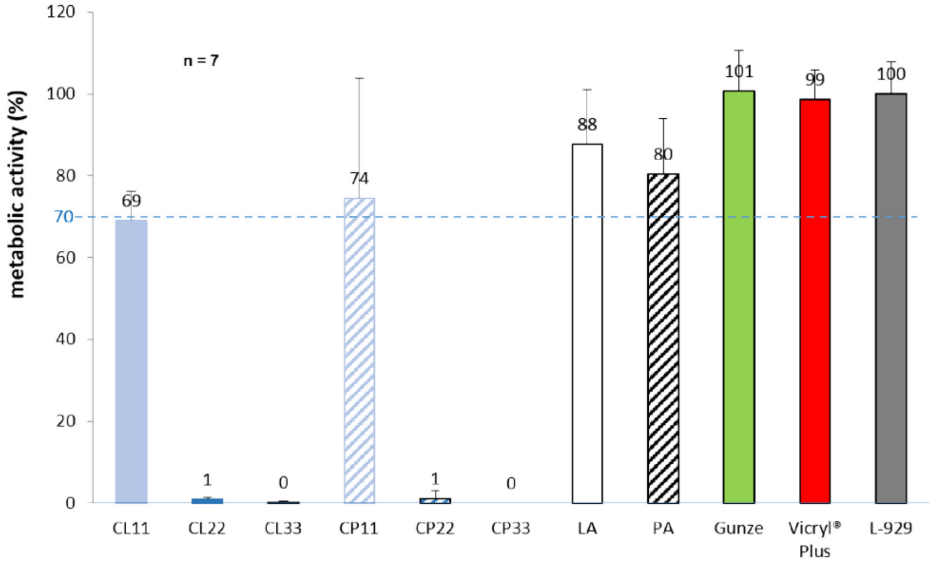

Figure 4. Evaluation of biocompatibility of coated sutures in cytotoxicity tests via WST-1 assay. Metabolic cell activity of fibroblasts in the presence of eluates from coated sutures measured with the WST-1 proliferation assay. Cells were incubated with eluates from coated sutures, suture references: lauric acid (LA), palmitic acid (PA), uncoated suture (Gunze), and Vicryl Plus. All values referred to cellular growth control, pure L-929 mouse fibroblast cultures. Dashed line at 70% pictures the level for acceptable lowering of metabolic activity according to ISO 10993-5:2009 in order to declare biocompatibility of medical devices.
doi:10.1371/journal.pone.0101426.g004

rather 33 µg/cm in protecting surgical sutures, without depletion of the anti-microbial drug on sutures.

Biocompatibility studies on coated sutures demonstrated acceptable cytotoxicities only for the lowest drug concentrations at 11 µg/cm independent from the fatty acid used. Such sutures fulfilled the at least required 70% remaining metabolic activity of L-929 cells to claim non-cytotoxicity according to ISO 10993-5:2009. Nevertheless, those sutures still have a high anti-microbial efficacy. Therefore, 11 µg/cm chlorhexidine coated sutures are potential candidates for further pre-clinical and human in vivo studies. In general, a strong dose-dependent effect for anti-microbial coated sutures was recognized regarding cytotoxicity. To improve biocompatibility a fine tuning with reduction of drug concentration, i.e. from 11 to 9 µg/cm without sacrificing the high anti-microbial efficacies seems promising.

There are limitations of our study, at first, the use of only one bacterial strain, *S. aureus*, for testing efficacy of coated anti-microbial sutures. In vitro tests should be performed to further prove efficacy against other relevant types of pathogens. Second, no effects on biofilms by anti-microbial coated sutures were investigated but bacterial cultures on agar and in suspensions are most common for first evaluation. For that purpose in vitro experiments with microbiological biofilm models are necessary. Third, other antiseptic substances should be identified and investigated regarding biocompatibility and anti-microbial efficacy.

To sum up, we demonstrated a prototypical coating process to provide anti-microbial sutures at high reproducible quality. Mechanical strength tests indicated negligible influences of the coating process comparable to commercial sutures and beyond the Ph. Eur. required strength values for resorbable sutures. Drug release from the novel coated sutures in aqueous media revealed to be dependent on dose, and the entity of the fatty acid carrier. We identified that all used novel chlorhexidine coated sutures proved high anti-bacterial efficacy. Duration of inhibition zones on Agar plates was dependent on the chlorhexidine dose, however there seemed to be no influence from fatty acid carriers. Biocompatibility testing of coated sutures also indicated strong dose dependency.

Conclusions

In this study we developed novel chlorhexidine coatings for anti-microbial surgical sutures with three different antiseptic concentrations based on palmitic and lauric acid carriers. We demonstrated their high anti-microbial efficacy against *S. aureus* in vitro. In particular, chlorhexidine coated sutures with 11 µg/cm concentration proved acceptable cytotoxicity according to ISO 10993-5 and simultaneously high anti-microbial protection over several days. Such coated sutures represent an alternative in the case of triclosan-resistance for prophylactic sutures. The aim is to support surgeons with an effective weapon to reduce suture-associated surgical site infections. However, further pre-clinical and clinical trials are necessary to confirm safety and efficacy in vivo.

Acknowledgments

We would like to express our great appreciation to Dr. K.-D. Kühn (Heraeus Medical GmbH) and Dr. K.-H. Sorg (Resorba GmbH) for their kind supply of materials. Also, many thanks to J. Hintermair (Zentralin-

stitut für Medizintechnik IMETUM, TU München) for supplying the mouse fibroblastic cell line L-929. Many thanks to J. Tübel (cell culture laboratory, Klinikum rechts der Isar, Klinik für Orthopädie, TU München) for her great assistance in cell biological experiments. Also, many thanks to P. Föhr (biomechanics laboratory, Klinikum rechts der Isar, Klinik für Orthopädie, TU München) for his great guidance in biomechanical testing.

Author Contributions

Conceived and designed the experiments: AO JS SW FDM MS RvER AS RB. Performed the experiments: AO SW JS FDM. Analyzed the data: MS RvER AS RB. Contributed reagents/materials/analysis tools: MS RvER AS RB. Wrote the paper: AO JS SW FDM MS RvER AS RB.

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6.2.2 Publikation II

Title: **In vitro evaluation of novel antimicrobial coatings for surgical sutures using octenidine.**

Autoren: Obermeier A, Schneider J, Föhr P, Wehner S, Kühn K-D, Stemberger A, Schieker M, Burgkart R.

Obermeier *et al. BMC Microbiology*
DOI 10.1186/s12866-015-0523-4

BMC Microbiology

RESEARCH ARTICLE Open Access

In vitro evaluation of novel antimicrobial coatings for surgical sutures using octenidine

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Abstract

Background: Sutures colonized by bacteria represent a challenge in surgery due to their potential to cause surgical site infections. In order to reduce these type of infections antimicrobially coated surgical sutures are currently under development. In this study, we investigated the antimicrobial drug octenidine as a coating agent for surgical sutures. To achieve high antimicrobial efficacy and required biocompatibility for medical devices, we focused on optimizing octenidine coatings based on fatty acids. For this purpose, antimicrobial sutures were prepared with either octenidine laurate or octenidine palmitate at 11, 22, and 33 µg/cm drug concentration normalized per length of sutures. Octenidine containing sutures were compared to the commercial triclosan-coated suture Vicryl® Plus. The release of octenidine into aqueous solution was analyzed and long-term antimicrobial efficacy was assessed via agar diffusion tests using *Staphylococcus aureus*. For determining biocompatibility, cytotoxicity assays (WST-1) were performed using L-929 mouse fibroblasts.

Results: In a 7 days elution experiment, octenidine-palmitate coated sutures demonstrated much slower drug release (11 µg/cm: 7 %; 22 µg/cm: 5 %; 33 µg/cm: 33 %) than octenidine-laurate sutures (11 µg/cm: 82 %; 22 µg/cm: 88 %; 33 µg/cm: 87 %). Furthermore sutures at 11 µg/cm drug content were associated with acceptable cytotoxicity according to ISO 10993–5 standard and showed, similar to Vicryl® Plus, relevant efficacy to inhibit surrounding bacterial growth for up to 9 days.

Conclusions: Octenidine coated sutures with a concentration of 11 µg/cm revealed high antimicrobial efficacy and biocompatibility. Due to their delayed release, palmitate carriers should be preferred. Such coatings are candidates for clinical testing in regard to their safety and efficacy.

Keywords: Surgical sutures, Antimicrobial, Coating, Octenidine, Slow-release, Fatty acid, Staphylococcus aureus, Surgical site infection, Biocompatibility, *In vitro*

Background

Surgical site infection (SSI) is a common complication after surgical intervention and incidence for SSI can be as high as 25 %, depending on the anatomical location of the surgical site [1–5]. The onset of SSI has been associated with a variety of factors, including surgical sutures [6]. The property of surgical suture for adhering bacteria promotes the occurrence of such infections [7]. The use of antimicrobially coated sutures poses one possible approach to prevent or reduce suture-associated infections. At present, the only commercially available antimicrobial sutures are coated with triclosan, such as the resorbable multifil Vicryl® Plus [8]. Several *in vitro* studies reported that triclosan-incorporated sutures showed high antimicrobial activity against a broad spectrum of pathogens [9–11]. Dependent on the surgery site, the clinical benefit of such coated sutures varies. On the one hand, no benefit was reported in studies for appendicitis, breast cancer or colorectal surgery [3, 12–14]. In contrast, the use of antimicrobial coated sutures in sternum surgery [15], abdominal wall closure [16, 17] and cerebrospinal fluid shunting procedures [18] was associated with significantly lower

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wound infection rates. Moreover, a recent meta-analysis showed a significant benefit of triclosan coated sutures to prevent surgical site infection [19]. However, due to its wide use in health-care, household and cosmetic items triclosan resistance is frequently reported for *S. aureus* a common pathogen in wound infections [20]. Furthermore the promotion of multi drug resistances in the presence of triclosan via increased activity of efflux pump system is a major concern [21]. Screening tests performed between 2003 and 2004 already demonstrated detection of triclosan in the urine of 74.6 % of US citizens [22, 23]. Therefore, the development of sutures with different antimicrobial drugs is urgently required.

In a previous study, we established a coating process to render surgical sutures antiseptic, while meeting the European Pharmacopoeia requirements for the material strength of surgical sutures [24, 25]. We showed that sutures coated with the antimicrobial drug chlorhexidine pose a highly efficient alternative with low cytotoxicity to the established antimicrobial sutures using triclosan, such as Vicryl® Plus. Furthermore we demonstrated a strongly dose dependent efficacy and biocompatibility. However, the antimicrobial effect lasted up to 4 days and biocompatibility was also limited. Therefore, we chose the antimicrobial drug octenidine for further development of suture coatings. Octenidine is well established in skin and wound antiseptic solutions, and even recommended as potential alternative in case of triclosan resistance [26]. Further, this antiseptic drug has a broad-spectrum activity, including common pathogens of wound infections such as multiresistant bacteria [27]. In addition, the lower solubility in aqueous medium may result in slower drug release, with longer efficacy against pathogens and lower cytotoxicity compared to chlorhexidine coatings.

The aim of the present study was the development of antimicrobial octenidine containing formulations based on fatty acids. We focused on optimizing drug concentrations in order to achieve improved long-term antimicrobial efficacy and biocompatibility concerning resorbable sutures. Both antimicrobial efficacy over several days, as well as cytotoxicity of novel coated octenidine containing sutures were compared to commercially available plain polyglycolic acid (PGA) and triclosan containing sutures like Vicryl® Plus.

Methods

Surgical sutures

Sutures in our study had diameters according to United States Pharmacopeia (USP 1). The used suture consists of polyglycolic acid (PGA, Gunze Ltd., Japan), free of fatty acids to avoid sewing effect. Our investigations were compared with reference sutures (PGA Resorba®, Resorba Medical GmbH, Germany; Vicryl® and Vicryl® Plus, Ethicon GmbH, Germany).

Antimicrobial coating solutions

Antimicrobial coating solutions were prepared as follows: Either palmitic or lauric acid together with octenidine were dissolved in 99.8 % ethanol (Carl Roth GmbH, Germany). The solutions contained 395.0 mg of both components fatty acid and octenidine in 7.9 g (10.0 ml) ethanol, which corresponds to a mass content of 5 % (w/w). Under aseptic conditions, the solutions had to be homogenized and filtered (minisart, sartorius AG, Germany, pore size 0.2 μm). Two types of coating were produced: Octenidine dihydrochloride (Dishman Pharmaceuticals & Chemicals Ltd., India) in lauric acid (**OL**) and Octenidine dihydrochloride in palmitic acid **(OP)**. Both for OL and OP, three different antiseptic drug concentrations inside coating solutions were chosen: 20 %, 40 % and 60 % (w/w), respectively (Table 1 a).

Preparation of antimicrobial sutures

The sutures (40 cm in length, $n = 7$) were coated in a dipping process with the prepared antimicrobial coating solutions using a thermo-shaker (Heidolph Instruments GmbH, Germany) for 2 min at 35 °C at 150 rpm. Subsequently, sutures were fixed on a device and dried for 2 h (h) at room temperature. After this drying process, the coating weight was determined by using a precision

Table 1 Octenidine fatty acid coating of sutures with 40 cm in length and the resulting concentrations

		a) Coating solutions			b) Resulting antimicrobial suture preparation		
Coating type		Ratio of octenidine in fatty acid carrier	Drug weight (mg)	Fatty acid weight (mg)	Weight of octenidine (mg)	Weight of lauric or palmitic acid (mg)	Normalized drug weight (μg/cm)
octenidine-laurate	OL11	20 %	79.0	316.0	0.44	1.76	11
octenidine-palmitate	OP11						
octenidine-laurate	OL22	40 %	158.0	237.0	0.88	1.32	22
octenidine-palmitate	OP22						
octenidine-laurate	OL33	60 %	237.0	158.0	1.32	0.88	33
octenidine-palmitate	OP33						

a) Drug and fatty acid components were applied at given ratios above and dissolved in 10.0 ml ethanol to produce the specific coating solutions with 5 % mass (w/w). **b)** Octenidine content of coated sutures after preparation. The mean coating weight of 40 cm suture samples were determined at 2.2 ± 0.2 mg ($n = 7$). Weights on coated sutures for octenidine, fatty acid carrier and normalized mean drug weight per cm thread are given above

balance (Atilon ATL-224; Acculab Inc., Massachusetts, USA). The drug amount normalized per length of sutures (μg/cm) was calculated for each drug concentration (20 %, 40 %, 60 %) via the measured coating weight (Table 1 b). Finally, coated sutures of 10 cm length were vacuum-sealed in sterile polyethylene bags and stored at room temperature. At the beginning of the experiments, coated sutures were cut into 1 cm, 2 cm and 3 cm long samples.

Antimicrobial efficacy of coated sutures via agar diffusion test

Antimicrobial efficacy of sutures was tested via the agar diffusion test ($n = 3$) compared to Vicryl® Plus. According to CLSI criteria, suspensions of *Staphylococcus aureus* (ATCC® 49230™) were prepared to an optical density of 0.5 McFarland standard. Then, 1 ml of this suspension was plated uniformly on Mueller Hinton II Agar plates with 90 mm standard size. After removal of the supernatant and drying the petri dishes, suture samples were placed on the inoculated Agar plates and incubated at 37 °C overnight. After 24 h, zones of inhibitions were measured in millimeter (mm) by using a calliper perpendicular to the sutures. According to Ming et al. [10], this procedure was repeated daily by using the same suture samples for several days to recognize the remaining anti-bacterial activity until no detectable inhibition zone remained.

Octenidine release from laurate and palmitate coatings

The octenidine release kinetics of the coated sutures were analysed over a period of 168 h in phosphate-buffered saline (PBS) at pH = 7.4. Sutures of 2 cm length ($n = 6$) were put in 1.5ml-cups (Eppendorf AG, Germany) with 1 ml PBS at 37 °C in a thermomixer MHR 23 (HLC-Biotech, Germany) at 200 rpm. Elution media was replaced by fresh PBS at fixed time intervals (after 0.5 h, 1.5 h, 3.5 h, 5.5 h, 7.5 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h). The release of octenidine was measured by absorption at a wavelength of 280 nm in a microplate photometer (Multiskan Go; Thermo Fisher Scientific GmbH, Germany). The amount of measured octenidine was normalized to the length of suture samples. Drug elution profiles were recorded by cumulating the released drug amounts over time. Ratios of the released octenidine were calculated relating to the drug content on suture samples at 168 h, depending on octenidine concentration of coated sutures.

Biocompatibility study

In accordance with both the ISO 10993–5 guideline and the WST-1 assay instruction, analysis of *in vitro* cytotoxicity of coated sutures was performed by using mouse fibroblasts L-929 (ACC 2; DSMZ, Germany) and measuring the metabolic activity of cells in the presence of eluates from coated sutures. Cell cultures grew in the corresponding Dulbecco's Modified Eagle Medium (DMEM with 4.5 g/l D-glucose, Biochrom AG, Germany) consisting of 10 % fetal bovine serum at 37 °C and 5.0 % CO_2 in a humidified atmosphere. Each well of a 96 well microtiter plate was filled with 10.000 cells and 200 μl DMEM, followed by incubation over 24 h. Eluates were generated simultaneously by placing coated sutures ($n = 7$) of 1 cm in length in 2 ml tubes containing 1.5 ml DMEM, for 24 h on a thermomixer at 37 °C and 300 rpm. Subsequently, cell culture's media were replaced by the eluates after 24 h. After 48 h, each well was supplemented with 20 μl WST-1 reagent to commence cell reaction to generate formazan salts by cellular mitochondrial dehydrogenases. After a reaction time of 2 h at 37 °C, the amount of formazan salts was detected at 450 nm in an absorption reader. Metabolic activities were referred to L-929 cells used as growth reference without sutures. A threshold of 70 % according to ISO 10993–5 standard was used to claim "biocompatibility" for drug eluting sutures.

Statistics

Statistical analysis was conducted by using the *student's t-test* with significant level $p < 0.05$. Measurements based on mean values and standard deviations from at least three values. The Gaussian error propagation law was used to correct mean value calculations from several measurements.

Results

Reproducibility of antimicrobial suture coatings

Independently from the drug concentrations used, the mean coating weight - the difference between the uncoated and coated sutures of 40 cm in length was 2.2 mg ± 0.2 mg ($n = 7$). The amount of octenidine, fatty acid and the normalized amount of drug per length of thread was calculated for each drug concentration relative to this mean coating weight; 11, 22 and 33 μg/cm, respectively (Table 1). The weight of the triclosan containing Vicryl® Plus sutures was 2.7 μg/cm [28, 29], as declared by the manufacturer.

Antimicrobial efficacy of coated sutures via agar diffusion test

Lauric and palmitic coating of octenidine kept zones of inhibition relatively stable from the second to the ninth day of experiments from 1.9 mm to 1.6 mm at day 9 (Fig. 1). On the tenth day, experiments were discontinued because the threads lost stability by humidity on agar plates and could not be transferred anymore. Long-term protection was detected for the three drug concentrations, without depletion of the antimicrobial efficacy.

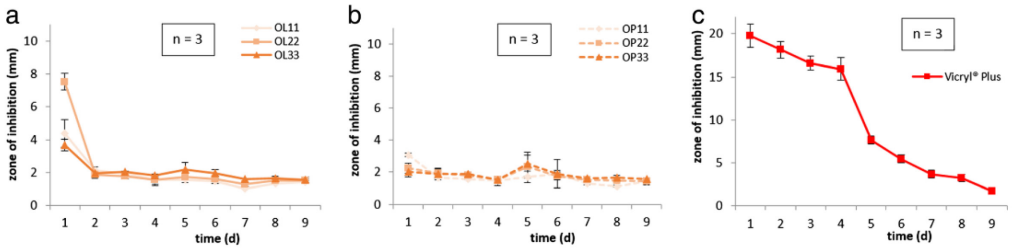

Fig. 1 Antimicrobial long-term efficacies via agar diffusion tests using *S. aureus* lawns (2×10^8 cfu/ml) showed inhibition zones over time for **a** octenidine-laurate coated sutures, **b** octenidine-palmitate coated sutures. Each coating type with three different octenidine concentrations 11, 22, and 33 µg/cm. **c** Vicryl® Plus as reference for commercial antimicrobial sutures

Triclosan coated sutures (Vicryl® Plus) demonstrated zones from 19.8 mm to 1.7 mm of microbial inhibition for the same observation period (Fig. 1c).

Octenidine release from laurate and palmitate coatings

The release of octenidine from fatty acid coatings was measured over a period of 168 h via elution in PBS. As demonstrated, octenidine release (Fig. 2) depends on the used fatty acid as a coating component. Octenidine values cumulated over 168 h for octenidine-laurate coated sutures showed released concentrations of 9.0 µg/ml/cm (OL11), 19.4 µg/ml/cm (OL22), and 28.6 µg/ml/cm (OL33). Elution data with palmitate as retarding agent showed excellent delayed action. After 168 h, palmitate coated sutures reached 0.8 µg/ml/cm (OP11), 1.0 µg/ml/cm (OP22) and 10.8 µg/ml (OP33). The released amounts at 168 h were referred to the absolute amount of octenidine on coated sutures in percent (Fig. 3). The degree of octenidine release calculated for octenidine-laurate coatings results in 82 ± 10 % (OL11), 88 ± 17 % (OL22), and 87 ± 11 % (OL33). In comparison, octenidine-palmitate coatings released only 7 ± 1 % (OP11), 5 ± 1 % (OP22), and 33 ± 3 % (OP33) of the coated octenidine.

Biocompatibility study

L-929 cells exposed to eluates from octenidine-laurate coatings showed metabolic activities of 77 ± 8 % (OL11), 48 ± 34 % (OL22), and 3 ± 1 % (OL33). Whereas, eluates from octenidine-palmitate resulted in metabolic activities of 85 ± 27 % (OP11), 23 ± 22 % (OP22), and 1 ± 0.3 % (OP33). The fatty acid coated references identified metabolic activities at 88 ± 13 % and 80 ± 13 % (lauric acid, palmitic acid). The control group with uncoated sutures reach a metabolic activity at 101 ± 10 % (Gunze) and Vicryl® Plus eluates demonstrated activities at 99 ± 7 % (Fig. 4).

Discussion

Surgical site infections caused by sutures represent a serious problem in clinics. Antimicrobially coated sutures may reduce suture-associated surgical site infections. Bacterial contamination of the wound associated with sutures occurs through the exogenous pathway. Pathogens are introduced either during surgery or from natural skin flora via wicking effect. In the presence of suture materials a smaller amount of pathogens is often sufficient to cause an infection compared to wounds without foreign material [30]. In this study, we coated

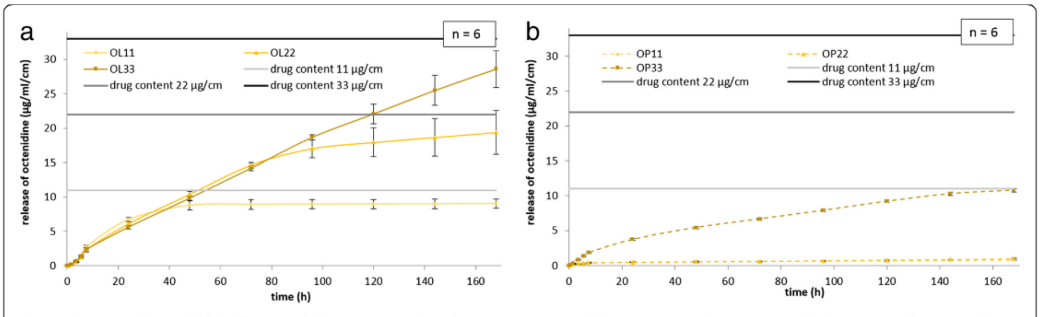

Fig. 2 Elution profiles in PBS buffer at 37 °C from **a** octenidine-laurate coatings and **b** octenidine-palmitate coated PGA sutures. Elution profiles were determined for each coating type at 11, 22, and 33 µg/cm containing sutures. Horizontal lines depict the normalized drug contents per cm coated suture, representing the limit of drug release for each drug concentration

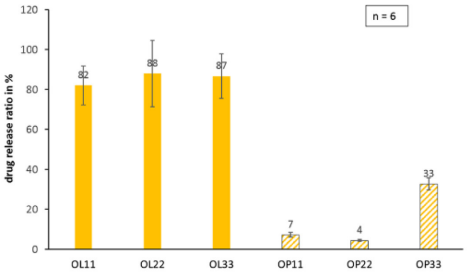

Fig. 3 The percentage of drug release related to the drug content on coated sutures per cm length for each coating after 168 h of elution in PBS buffer

surgical sutures with 11, 22, or 33 µg octenidine per cm length either in a palmitate or laurate fatty acid as retarding carrier. The coated sutures were tested regarding their antimicrobial efficacy, octenidine release and biocompatibility. We found that the antimicrobial activity of octenidine coated sutures was as long as for triclosan coated sutures, like Vicryl® Plus. The favorable antimicrobial suture coating at 11 µg/cm was associated with high biocompatibility.

According to antimicrobial efficacy, inhibition zones of octenidine coated sutures on bacterial lawns were detected for up to 9 days. In comparison to the triclosan control group (Vicryl® Plus), the zones of inhibitions of octenidine were smaller, although the amount of octenidine on the sutures was significantly more than used with triclosan. This discrepancy in inhibition zones can be related to the presumably slower drug release by diffusion of octenidine over time compared to triclosan. This study was aimed to evaluate concentrations of octenidine on sutures in the balance of antimicrobial efficacy and biocompatibility. Released octenidine concentrations are thus low, but that may be desirable to protect the suture material, ensuring drug release for the first critical week of wound healing. Both octenidine- and triclosan coated sutures revealed a long lasting antimicrobial effect, but degradation of absorbable suture samples by hydrolysis caused termination of experiments after the ninth day. Despite this degradation process, no increase of inhibition zone could be detected as a possible indication of a sudden burst of octenidine release. Nevertheless, the results of this *in-vitro* model must be validated *in-vivo* prior to the application in humans, in order to dismiss concerns for acute toxicity as a consequence of the suture degradation process of octenidine coated sutures. The time dependency of inhibition zones did not differ greatly regarding the loaded drug concentrations of octenidine, so the three drug concentrations are equally effective absorbable sutures, as the essential protection is at least ensured for 9 days. Since the mechanism of wound healing provide an unaltered germ free de novo synthesis of tissue, this time period of antimicrobial protection should be sufficient to prevent infection during the first essential phase of wound healing.

The assessment of octenidine kinetics showed a slow continuous release over the first days of the experiment for each coated suture. The release of octenidine was delayed by the used fatty acid carriers and the drug's low solubility in aqueous media. Triclosan coated sutures in such media, like Vicryl® Plus assessed similar durations of drug release [31]. Additionally, these data confirm our measured efficacy over at least 9 days for Vicryl® Plus sutures, considering the fact that our tested sutures from the EU containing less triclosan (max. 2.7 µg/cm), than

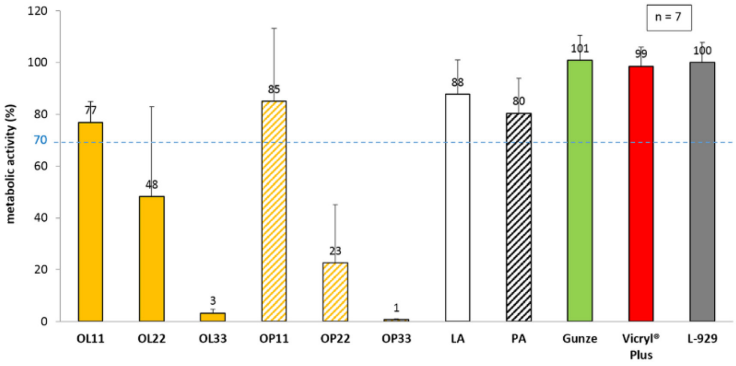

Fig. 4 Metabolic cell activity of fibroblasts in the presence of eluates from octenidine coated sutures via WST-1 proliferation assay. L-929 mouse fibroblasts were incubated with suture eluates and used references: lauric acid (LA), palmitic acid (PA), uncoated sutures (Gunze), and Vicryl® Plus. All values were referred to cellular growth control, pure L-929 cells with culture media. Dashed line at 70 % pictures the level for acceptable lowering of metabolic activity according to ISO 10993–5:2009 in order to declare biocompatibility of medical devices

Vicryl® Plus sutures available in the US (max. 4.7 μg/cm) [29]. Drug release kinetics over 7 days for octenidine coatings showed, that all type of coatings retarded the attached octenidine without full depletion. It is accepted that the antimicrobial coating remains between the suture filaments. Thus, the so-called wick effect of multifilament thread can be interrupted.

Referring to the drug carriers, palmitic acid in coatings showed a much slower drug release characteristic in comparison to lauric acid coatings demonstrating comparable antimicrobial efficacies. The ratio between released drug and loaded drug amounts on sutures after 7 days indicates that no coating type had fully washed-out the antimicrobial agent octenidine. Similar to our previous findings [25], we found that palmitic acid carriers delay the drug release more effectively than lauric acid carriers do. As anticipated, the octenidine release was dependent on the initial drug concentration in the suture coatings. Antimicrobial action could be demonstrated even for the lowest octenidine concentration (11 μg/cm) over 9 days. Especially, the palmitic acid coatings may guarantee a longer antimicrobial effect than lauric acid carriers may. Therefore, we recommend a combination of the drug octenidine with palmitic acid as carrier for long-term antiinfective protection of surgical sutures to avoid surgical site infections.

Biocompatibility, as defined by the ISO 10993–5:2009 guideline was reached for octenidine at the lowest concentration (11 μg/cm) with both coatings consisting of palmitic acid and lauric acid. Cytotoxicity tests of coated octenidine sutures showed a strong dose dependency. An increased drug concentration on loaded sutures, revealed an increased cytotoxic reaction. Octenidine-palmitate and -laurate sutures at 11 μg/cm showed similar metabolic activities in comparison to pure lauric and palmitic acid coated sutures. Compared to commercially available triclosan sutures (Vicryl® Plus), octenidine-palmitate coated sutures also met the ISO standard for biocompatibility.

The comparison of octenidine coated sutures to chlorhexidine coatings, tested in a previous study [25], showed different results in antimicrobial efficacy, drug release and biocompatibility by using the same antiseptic drug quantities. Octenidine sutures in lauric or palmitic acid showed initial slightly smaller inhibition zones than of chlorhexidine coatings during the first days of experiment. However, the duration of the inhibition zones lasted substantially longer for octenidine coatings, at least up to 9 days. Octenidine-laurate coated sutures compared to chlorhexidine-laurate coatings showed similar released drug amounts at 96 h, but the time span until flat drug kinetics were reached were much longer. In addition, the drug release of octenidine-palmitate sutures lasted longer and showed smaller released amounts at 96 h, referring to slower release compared to chlorhexidine-palmitate coatings. The reason for the longer drug release of octenidine can be related to the lower solubility of palmitic acid carrier and octenidine itself in aqueous media, such as PBS. Additionally, we observed that octenidine coatings were less cytotoxic by using the same drug doses compared to chlorhexidine coatings. Therefore, the drug release kinetics, antimicrobial activities and biocompatibility of octenidine coated sutures are superior compared to chlorhexidine coatings.

Our study has two main limitations: First, antimicrobial coated sutures were exposed to only one pathogenic strain, *S. aureus*. Herewith we wanted to primarily demonstrate the feasibility of effective triclosan alternatives. The activity of octenidine against other relevant bacteria as well as biofilm inactivation against *S. aureus* has been published [32]. Therefore, antimicrobial efficacy of coated sutures against other common pathogens of wound infections should be tested in the future. Second, the influence of the biofilm formation on the antimicrobial efficacy of the coated sutures was not simulated in our experiments. Regardless, the use of octenidine is likely to circumvent any of the acquired resistances which are limiting the extended use of triclosan.

Conclusions

In the current study, novel octenidine coatings to render surgical sutures antimicrobial with three different concentrations based on palmitic/lauric acid were developed and analyzed. All novel coatings proved high long-term antimicrobial efficacy against *S. aureus*. Octenidine coatings with drug concentration of 11 μg/cm on sutures combine long-term antimicrobial efficacy up to 9 days and slow drug release with demonstrated high biocompatibility. The drug release was dependent on the fatty acid carrier and optimized delay was represented for palmitate. Compared to the commercially available Vicryl® Plus the antimicrobial efficacy of octenidine coated sutures was only slightly reduced. The potential disadvantages of triclosan are severe toxic side products such as dioxide and the promotion of multi drug resistance, which justifies the search for alternative substances for future use. However, despite these promising results, it needs to be clearly pointed out that it cannot be concluded from our findings that octenidine coated sutures are superior to the triclosan coated sutures. Therefore, further studies are necessary to prove that octenidine coated sutures represent a serious alternative to the currently commercially available triclosan coated sutures.

Abbreviations

SSI: Surgical site infection; PGA: Polyglycolic acid; S. aureus: Staphylococcus aureus; USP: United States Pharmacopeia; OL: Octenidine in lauric acid coating; OP: Octenidine in palmitic acid coating; OL11: Octenidine-laurate

coating at 11 μg/cm; OL22: Octenidine-laurate coating at 22 μg/cm; OL33: Octenidine-laurate coating at 33 μg/cm; OP11: Octenidine-palmitate coating at 11 μg/cm; OP22: Octenidine-palmitate coating at 22 μg/cm; OP33: Octenidine-palmitate coating at 33 μg/cm; rpm: Revolutions per minute; h: Hour; CLSI: Clinical and laboratory standards institute; mm: Millimeter; PBS: Phosphate-buffered saline; DMEM: Dulbecco's modified eagle medium; EU: European Union; US: United States.

Competing interests
This work was partially financially supported by the Heraeus Medical GmbH, Wehrheim, Germany.

Authors' contributions
Conceived and designed the experiments: AO JS PF SW KDK AS MS RB. Performed the experiments: AO JS PF SW. Analyzed the data: AO SW KDK AS MS RB. Contributed reagents/materials/analysis tools: KDK AS MS RB. All authors take part in both writing this manuscript, read and approved the final manuscript.

Authors' information
Not applicable.

Acknowledgements
We would like to thank Dr. K.-H. Sorg (Resorba Medical GmbH, Germany) for his supply of PGA Resorba® and PGA raw suture material, without whose kind support, this project would not have been possible. In addition, many thanks to J. Hintermair (Zentralinstitut für Medizintechnik IMETUM, TU München) for supplying the mouse fibroblastic cell line L-929. We are also grateful to J. Tübel (Klinikum rechts der Isar, Klinik für Orthopädie, Zellkulturlabor, TU München) for her great assistance. Finally, many thanks to Dr. P. Mayer-Kuckuck for his fruitful discussions (Klinikum rechts der Isar, Klinik für Orthopädie, bone cell biology and imaging laboratory).

Funding
This work was financially supported by the German Research Foundation (DFG) and the Technische Universität München within the funding programme Open Access Publishing.

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Received: 9 May 2015 Accepted: 18 September 2015
Published online: 24 September 2015

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6.2.3 Publikation III

Title: **Viable adhered *Staphylococcus aureus* highly reduced on novel antimicrobial sutures using chlorhexidine and octenidine to avoid surgical site infection (SSI).**

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PLOS ONE

RESEARCH ARTICLE

Viable adhered *Staphylococcus aureus* highly reduced on novel antimicrobial sutures using chlorhexidine and octenidine to avoid surgical site infection (SSI)

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OPEN ACCESS

Citation: Obermeier A, Schneider J, Harrasser N, Tübel J, Mühlhofer H, Pförringer D, et al. (2018) Viable adhered *Staphylococcus aureus* highly reduced on novel antimicrobial sutures using chlorhexidine and octenidine to avoid surgical site infection (SSI). PLoS ONE 13(1): e0190912. https://doi.org/10.1371/journal.pone.0190912

Editor: Dermot Cox, Royal College of Surgeons in Ireland, IRELAND

Received: October 7, 2016

Accepted: December 22, 2017

Published: January 9, 2018

Data Availability Statement: All relevant data are within the paper.

Funding: This study was partially funded by the Heraeus Medical GmbH, Wehrheim, Germany (www.heraeus.com). At the Klinikum rechts der Isar, Technische Universität München the external funding number was 8895117_HeraeusCoating. The funder had no role in study design, data collection and interpretation, nor the decision to submit the work for publication. Additionally, this

Abstract

Background

Surgical sutures can promote migration of bacteria and thus start infections. Antiseptic coating of sutures may inhibit proliferation of adhered bacteria and avoid such complications.

Objectives

This study investigated the inhibition of viable adhering bacteria on novel antimicrobially coated surgical sutures using chlorhexidine or octenidine, a critical factor for proliferation at the onset of local infections. The medical need, a rapid eradication of bacteria in wounds, can be fulfilled by a high antimicrobial efficacy during the first days after wound closure.

Methods

As a pretesting on antibacterial efficacy against relevant bacterial pathogens a zone of inhibition assay was conducted with middle ranged concentrated suture coatings (22 µg/cm). For further investigation of adhering bacteria in detail the most clinically relevant *Staphylococcus aureus* (ATCC®49230™) was used. Absorbable braided sutures were coated with chlorhexidine-laurate, chlorhexidine-palmitate, octenidine-laurate, and octenidine-palmitate. Each coating type resulted in 11, 22, or 33 µg/cm drug content on sutures. Scanning electron microscopy (SEM) was performed once to inspect the coating quality and twice to investigate if bacteria have colonized on sutures. Adhesion experiments were assessed by exposing coated sutures to *S. aureus* suspensions for 3 h at 37˚C. Subsequently, sutures were sonicated and the number of viable bacteria released from the suture surface was

work was supported by the German Research Foundation (DFG) and the Technical University of Munich (TUM) in the framework of the Open Access Publishing Program (www.ub.tum.de). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The partial financial support by the Heraeus Medical GmbH, does not alter our adherence to PLOS ONE policies on sharing data and materials.

determined. Furthermore, the number of viable planktonic bacteria was measured in suspensions containing antimicrobial sutures. Commercially available sutures without drugs (Vicryl®, PGA Resorba®, and Gunze PGA), as well as triclosan-containing Vicryl® Plus were used as control groups.

Results

Zone of inhibition assay documented a multispecies efficacy of novel coated sutures against tested bacterial strains, comparable to most relevant *S. aureus* over 48 hours. SEM pictures demonstrated uniform layers on coated sutures with higher roughness for palmitate coatings and sustaining integrity of coated sutures. Adherent *S. aureus* were found via SEM on all types of investigated sutures. The novel antimicrobial sutures showed significantly less viable adhered *S. aureus* bacteria (up to 6.1 log) compared to Vicryl® Plus (0.5 log). Within 11 μg/cm drug-containing sutures, octenidine-palmitate (OL11) showed the highest number of viable adhered *S. aureus* (0.5 log), similar to Vicryl® Plus. Chlorhexidine-laurate (CL11) showed the lowest number of *S. aureus* on sutures (1.7 log), a 1.2 log greater reduction. In addition, planktonic *S. aureus* in suspensions were highly inhibited by CL11 (0.9 log) represents a 0.6 log greater reduction compared to Vicryl® Plus (0.3 log).

Conclusions

Novel antimicrobial sutures can potentially limit surgical site infections caused by multiple pathogenic bacterial species. Therefore, a potential inhibition of multispecies biofilm formation is assumed. In detail tested with *S. aureus*, the chlorhexidine-laurate coating (CL11) best meets the medical requirements for a fast bacterial eradication. This suture coating shows the lowest survival rate of adhering as well as planktonic bacteria, a high drug release during the first–clinically most relevant– 48 hours, as well as biocompatibility. Thus, CL11 coatings should be recommended for prophylactic antimicrobial sutures as an optimal surgical supplement to reduce wound infections. However, animal and clinical investigations are important to prove safety and efficacy for future applications.

Introduction

Surgical site infection (SSI) rates vary in the range of 2% to 20% depending on the chosen type of surgical procedure [1–4]. SSI generally poses a risk for patients due to an increased morbidity and even mortality [4]. Affected patients often need further surgical intervention leading to higher cost for the health care system [1, 5]. Several factors are involved in the onset of SSI, one of which is the surgical suture itself. The presence of foreign material highly reduces the critical number of bacteria facilitating a clinically relevant infection [6–8]. Furthermore, the capillarity of sutures supports the path of bacteria into wounds by soaked fluids. This so-called "wicking effect" triggers such infections. [9] Especially, the type of material and structure of surface determine the ability of bacteria to adhere and induce infections [9]. In this context, the number of viable adhered bacteria is considered an essential trigger for SSI related to suture material. The main issues are proliferation of attached bacteria and formation of persistent biofilms [9–11]. Once a biofilm has developed, it protects bacteria against the host's immune system as well as systemically [12, 13] and locally applied antibiotics.

A possible solution to prevent suture-associated site infections is the use of antimicrobially coated sutures. These sutures can be used to inhibit viable adhered microbes and thus prevent biofilm formation. Clinical indications for antimicrobial sutures may be infection prophylaxis in susceptible patients (e.g. immunosuppression) and especially in surgical procedures with elevated risk of infection (e.g. contaminated surgical site). To our knowledge, so-called "Plus" sutures containing triclosan are the only antimicrobial sutures currently available on the European market.

A systematic literature review on antimicrobial sutures by Chang et al. identified seven randomized clinical trials finding no significant reduction of local infections by means of these materials [14]. However, these studies did not fulfill the recommended standards for meta-analyses [15]. In contrast, the latest independent meta-analyses indicate a beneficial use of antimicrobial sutures for wound closure containing triclosan [16, 17]. Due to this data, antimicrobial sutures are highly recommended as a supplementary step to reduce the risk of SSI [15]. Further studies showed high efficacy and cost reduction of antimicrobial sutures for infection prevention [18–22] and could clarify which indication benefits most from the use of available antimicrobial sutures [23, 24].

Apart from promising study results of triclosan-coated sutures, triclosan also has drawbacks including formation of toxic side products (e.g. chlorinated phenols, methyl triclosan) [25] and antibiotic resistances [26, 27], likely due to its prevalence in cosmetics and soap products [28, 29]. Additionally, triclosan promotes the protein mediated binding of staphylococci to host cells with the consequence of an increased number of nasal infections caused by *S. aureus* colonization in the presence of triclosan [30]. Due to these restrictions in the use of triclosan, alternatives are urgently needed. Chlorhexidine and octenidine are highly effective alternatives, inhibiting relevant pathogens of wound infections. Both antiseptics have a broad antibacterial spectrum as well as high biocompatibility indices [31–33]. Chlorhexidine is routinely used in oral surgery [31]. In combination with silver, chlorhexidine is also used for the antimicrobial protection of hernia meshes. These chlorhexidine meshes show antibacterial efficacy, safety and high tissue integration [34]. Octenidine is a clinically well-established skin and wound antiseptic solution and does not seem to select for resistance [35].

Chlorhexidine and octenidine have similar mechanism of action: Positively charged drug molecules bind to negative charges on bacterial cell walls, leading to membrane leakages and finally cell death [36, 37]. Both antiseptics are effective against the most gram-negative and gram-positive bacteria [37, 38], including the most clinically relevant pathogen genus staphylococci, causing wound and nosocomial infections [39, 40]. In order to support wound healing, a fast and if possible complete eradication of bacteria inside wounds after surgery is necessary. Therefore, administration of antimicrobial agents is recommended at high dosages and short time periods for prophylaxis to avoid formation of resistant bacteria [41, 42].

Antimicrobial sutures must fulfill a balancing act between inhibiting bacteria and sustaining biocompatibility to the healing wound consisting of eukaryotic tissue. In former studies, we adjusted the drug concentration dependent on efficacy and biocompatibility of novel antimicrobial suture coatings containing chlorhexidine diacetate [43] or octenidine dihydrochloride [44]. These studies used coatings based on fatty acid carriers to achieve delayed drug release systems and to sustain bacterial inhibition zones *in vitro*.

The aim of the present study was to investigate the effectiveness of novel chlorhexidine- or octenidine-coated sutures against adherent bacteria. At first, a zone of inhibition assay was conducted to determine the efficacy against several relevant pathogenic bacteria. Then, in order to investigate the effects of novel antimicrobially coated sutures on viable adhering bacteria in detail the clinically most relevant *S. aureus* was used. Therefore, coated suture samples were exposed to *S. aureus* suspensions. Scanning electron microscopy (SEM) was performed to inspect suture coatings before and adherent bacteria after *S. aureus* exposure. The viability

of bacteria adhered on sutures was investigated after sonication. In addition, the viability of planktonic bacteria in the surrounding of coated sutures was measured. The tested novel coated sutures were compared to commercially available absorbable sutures without any drug cover as well as triclosan-containing Vicryl® Plus sutures.

Materials and methods

Surgical sutures

In this study, uncoated braided absorbable—polyglycolic acid—suture Gunze (G: Gunze PGA, Kyoto, Japan) of 0.4 mm in diameter, corresponding to the United States Pharmacopeia standard USP1, was used to produce antimicrobial sutures by coating. Suture controls were commercial PGA Resorba® (R: Resorba, Nürnberg, Germany), Vicryl® and triclosan-containing Vicryl® Plus (V and VP, respectively: Ethicon, Norderstedt, Germany). Furthermore, Gunze PGA sutures only coated with fatty acids—palmitic acid (PA80) and lauric acid (LA80)—were tested to investigate potential effects of drug carriers only.

Antimicrobial suture preparation using chlorhexidine and octenidine in fatty acid carriers

The formulation of coating solutions and the reproducibility of the dip coating process for antimicrobial coating of absorbable PGA sutures (Gunze) was described earlier in one of our studies for chlorhexidine diacetate [43] and octenidine dihydrochloride [44] based on fatty acids as drug carriers. These coating procedures resulted in an average coating weight of 2.2 mg ± 0.2 mg (n = 10) for 40 cm braided, absorbable sutures (USP1) [43, 44].

In the present study, four coating types were compared: Chlorhexidine in lauric acid (CL) or palmitic acid (CP) and octenidine in lauric acid (OL) or palmitic acid (OP). For each type of suture coating, three different solutions with defined concentrations of active agents were formulated. To obtain preparation solutions, antiseptic drugs and fatty acid carriers (palmitate or laurate) were dissolved in 99.8% ethanol with a total mass content of 5% (w/w). Sutures were dipped in these sterile coating solutions for 2 min, followed by a drying period of 2 hours. Then, the weight of coatings on sutures was measured via a precision balance (Atilon ATL-224, Acculab, Bradford, USA) and the resulting drug concentration per unit of length was calculated. This procedure generates antimicrobial sutures with 11 μg/cm, 22 μg/cm and 33 μg/cm for both chlorhexidine- and octenidine-containing sutures. An overview of the tested novel antimicrobial sutures and their coating composition for this study is given in Table 1.

Table 1. Overview of the prepared novel antimicrobially coated sutures.

A) chlorhexidine-coated sutures			B) octenidine-coated sutures			C) fatty acid carrier	
types of chlorhexidine coating		drug content (μg/cm)	types of octenidine coating		drug content (μg/cm)	content (μg/cm)	ratio (%)
chlorhexidine-laurate	CL11	11	octenidine-laurate	OL11	11	44	80
chlorhexidine-palmitate	CP11		octenidine-palmitate	OP11			
chlorhexidine-laurate	CL22	22	octenidine-laurate	OL22	22	33	60
chlorhexidine-palmitate	CP22		octenidine-palmitate	OP22			
chlorhexidine-laurate	CL33	33	octenidine-laurate	OL33	33	22	40
chlorhexidine-palmitate	CP33		octenidine-palmitate	OP33			

Chlorhexidine-coated sutures (A) and octenidine-coated sutures (B) and their coating compositions are shown in detail. For both types of sutures, the amount of antimicrobial substance per length of sutures after preparation resulting from a mean coating weight of 40 cm suture samples at 2.2 ± 0.2 mg (n = 7) is given. Additionally, the fatty acid content and ratio (C) is referred to the total weight of coating mass per cm length of the sutures.

https://doi.org/10.1371/journal.pone.0190912.t001

In comparison to our chlorhexidine- or octenidine-containing antimicrobial sutures, the Vicryl® Plus control group suture contains 2.7 μg/cm triclosan within the European Union [29].

Antimicrobial efficacy against multiple relevant pathogenic bacteria

In order to achieve information about a multispecies efficacy *Staphylococcus aureus* (ATCC®49230™), a methicillin-resistant *S. aureus* strain—short *MRSA* (ATCC®43300™), *Staphylococcus epidermidis* (ATCC®35984™), *Enterococcus faecalis* (ATCC®29212™) and *Escherichia coli* (ATCC®25922™) was used for general antibacterial suture tests. The zone of inhibition assay was conducted over a period of 48 hours to compare the species-dependent efficacy of each suture type using the middle ranged drug concentration in the amount of 22 μg/cm. Therefore, coated suture samples were placed on bacterial lawns on Agar plates (Mueller Hinton II), inoculated with a bacterial suspension at an optical density of 0.1 at 600 nm. Plates with samples were incubated over night at 37˚C, then zones of inhibition were measured in tenths of a millimeter and coated suture samples were transferred to newly inoculated Agar plates. This process was repeated twice for two days. A more detailed description of the zones of inhibition assay is given in literature [43].

Scanning electron microscopy (SEM) for structural analysis of coated sutures

In order to inspect quality of suture coatings as well as integrity, SEM pictures were taken without bacterial exposure at lower magnifications (up to 200x) to achieve an overview perspective. For this purpose, novel antimicrobially coated sutures, as well as uncoated and commercially available suture samples were prepared for common SEM. During preparation of suture samples, gold was sputtered on the suture samples at 5 x 10^{-2} mbar two times for 40 sec each with a Bal-tec Med020 coating system (Bal-tec, Balzers, Liechtenstein). Hereby, a thin gold layer of approximately 28 nm was generated improving image quality by generating conductive surfaces and protecting biological objects [45]. Pictures were taken for this investigation using a low vacuum SEM type JSM 6060LV (JEOL, Freising, Germany). Regarding the thermally labile suture—consisting of PGA—a low acceleration voltage of 5 kV was chosen.

Scanning electron microscopy (SEM) for visualization of adherent bacteria

Additionally, SEM inspections were executed at higher magnification (2,500x) to investigate bacteria adherence on coated suture samples after bacterial exposure. SEM investigations were performed after washing of inoculated sutures, and before sonication. The number of adhered bacteria was estimated by using the field of view from SEM pictures (approximately 50 x 50 μm^2) and counting visible adhering bacteria. The mean of three pictures from three sutures was calculated. Semi-quantitative levels for adhered bacteria were defined (low: up to 50 bacteria, moderate: 50 to 200 bacteria, and high: > 200 bacteria). Suture samples exposed to bacteria were treated with 4% paraformaldehyde in 0.01 M pbs solution for at least 1 h. This fixation step stabilizes the biological structure of the attached bacteria by cross-linking of proteins [46] and simultaneously inactivating bacteria. Subsequently, bacteria-containing suture samples were dried, gold sputtered as described and investigated by SEM.

Viability of adhered bacteria on coated sutures (bacterial adhesion assay)

To quantitatively investigate the influence of antiseptic suture coatings on the viability of adhered bacteria, coated and uncoated suture samples were inoculated in bacterial suspensions

for 3 h at 37˚C using *Staphylococcus aureus* (ATCC®49230™). Attached viable *S. aureus* numbers on suture samples were measured after sonication and incubation of detached bacteria. Viable bacteria were determined by growth on Mueller Hinton II Agar plates (MHA; BD Diagnostic Systems, Heidelberg, Germany) and counting of colony-forming units (cfu). The bacterial adhesion assay described by Gollwitzer et al. [18, 47] was modified using the following procedure:

Mueller Hinton Broth (MHB; BD Diagnostic Systems, Heidelberg, Germany) was used to cultivate bacteria in suspension. Bacterial concentration was adjusted with a biophotometer (Eppendorf, Hamburg, Germany) at a wavelength of 600 nm. Suture samples of 1 cm in length (n = 10) were put in 1.5 ml-tubes filled with 1 ml *S. aureus* suspension at an initial concentration of 1.3 x 10^8 cfu/ml (OD600 = 0.1). The tubes were incubated in a thermo-shaker (Unimax 1010, Heidolph Instruments, Schwabach, Germany) for 3 h at 37˚C while shaking at 200 rpm. To remove weakly adhered bacteria from sutures, a washing process involving dipping the sutures 3 times in 1 ml sterile isotonic saline (0.9%) was performed. Subsequently, to remove strongly adhered bacteria from suture surfaces, samples were put into tubes with 1 ml sterile 0.01 M phosphate buffered saline (pbs: NaCl 0.138 M, KCl 0.0027 M; pH 7.4; P3818, Sigma-Aldrich, Germany) and treated using a 3-step procedure: (1) vortexing for 10 sec, (2) sonication for 1 min using an ultrasound at 35 kHz/280W (Sonorex RK255H, Bandelin, Berlin, Germany), and (3) vortexing for 10 sec. The obtained bacterial suspension was diluted to 1:10, 1:100, 1:1,000, and 1:10,000 with sterile 0.01 M pbs, and 100 μl of each dilution were plated in double on MHA plates. After 24 h of incubation at 37˚C, colony-forming units were counted and the number of viable adhered bacteria on the suture surfaces was calculated. The numbers obtained were compared to those obtained from the following references: uncoated PGA suture (Gunze), palmitic and lauric acid coatings (PA80, LA80), commercially available absorbable sutures (Vicryl® and PGA Resorba®) with fatty acid coating, and triclosan-coated sutures (Vicryl® Plus). For all determined numbers of viable adhered bacteria a logarithmic reduction was calculated referred to the uncoated Gunze suture (G). Significance tests were compared in general to uncoated Gunze (G) and especially for antimicrobially coated sutures to the commercial antimicrobial suture Vicryl® Plus (VP).

Viable bacteria of suspensions after suture incubation

To investigate potential growth inhibition on suture-surrounding bacteria in planktonic form, antimicrobial sutures were incubated for 3 h in *S. aureus* suspensions followed by detecting viable numbers of bacteria. Therefore, suture samples at 1 cm length were incubated in *S. aureus* suspensions during the *viability adhesion assay* experiments as described above. The turbidity of the bacterial suspension was measured at 600 nm (OD600) using a biophotometer (Eppendorf, Hamburg, Germany) for each inoculated suture at the beginning and at the end of experiment after 3 h. Numbers of viable bacteria were determined via a calibration curve for the bacterial test strain. The number of viable planktonic bacteria was compared to the bacterial growth in the presence of uncoated Gunze suture (G) and a logarithmic reduction was calculated after 3 hours of incubation for each suture sample.

Evaluation of results of 11 μg/cm drug-containing sutures in regard to former studies

To determine the best novel antimicrobial suture for medical need, we evaluated the 11 μg/cm drug-containing novel chlorhexidine- and octenidine-coated sutures (CL11, CP11, OL11, and OP11) in comparison to the antimicrobial control Vicryl® Plus (VP). Results of the present study as well as from former studies [43, 44] were taken into account for comparative

evaluation. Thus, for each relevant aspect (viability of bacteria adhered or in suspension, numbers of bacteria detected via SEM, biocompatibility, drug release kinetics and efficacy in zone of inhibition tests), semi-quantitative levels were defined.

Statistics

Mean values and standard deviations were calculated from at least five independent measurements. Student's t-test was performed for testing on equality of data sets at significance levels $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). The distribution of data was checked for each group referred to the mentioned controls via F-Test in Microsoft Excel® 2013. These results were taken into account during the student's t-test. The Gaussian error propagation law was used for the subsequent use of flawed values. GraphPad Prism 6.0 (GraphPad® Software, La Jolla, CA, USA) was used for data evaluation and visualization of the result graphs.

Results

Antimicrobial efficacy against multiple relevant pathogenic bacteria

In general, high antimicrobial efficacy was found for all of the tested bacterial strains over the relevant test period of 48 hours (Fig 1). The type of coating affected the sizes of inhibition zones, especially the type of coated drug. On average, chlorhexidine-coated sutures inhibited bacteria at 8.3(±1.4) mm and octenidine-coated sutures at 2.3(±0.5) mm after 24 hours. After 48 hours, the inhibition zones were on average 8.2(±1.7) mm and 1.7(±0.4) mm for chlorhexidine and octenidine coatings, respectively. The antibacterial efficacy of novel coated sutures against tested bacterial strains was comparable to the most relevant bacterial strain *S. aureus*, used for further detailed investigations on bacterial adhesion.

Scanning electron microscopy (SEM) for structural analysis of coated sutures

Novel coated sutures show sustaining integrity and uniformly covered surfaces by drug-containing coating layers. There are hardly detectable differences via SEM for suture coatings using concentrations at 11 μg/cm, 22 μg/cm and 33 μg/cm. Therefore, sutures with the lowest and highest drug concentrations (11 μg/cm and 33 μg/cm) are presented (Fig 2, left). The reference suture (G) shows the structure of the uncoated suture material used for preparing antimicrobial sutures. Both drug carrier preparations (palmitic acid and lauric acid) completely covered the suture surface. The lauric acid-containing coatings CL11, OL11, CL33, and LA80 sutures showed smooth surface layers around each single filament. In contrast, a rougher structure of palmitic acid-containing coatings CP11, OP11, CP22, and PA80 was a characteristic feature. In general, the surface roughness of palmitate using novel coated sutures was comparable to commercially available sutures such as Vicryl® Plus, Vicryl®, and PGA Resorba® (Fig 2, right).

Scanning electron microscopy (SEM) for visualization of adherent bacteria

In particular, the antimicrobial control (VP), the commercial triclosan-coated suture Vicryl® Plus showed relatively high numbers of adhering bacteria. All tested novel antiseptic coated sutures (CL11, CP11, OL11, and OP11) showed numerous bacteria on their surfaces (Fig 3: left), even for sutures at higher drug concentrations (CL33, CP33, OL33, and OP33). Numerous adhering bacteria were detectable on the non-antimicrobial suture control (G) and other sutures without antimicrobial substances (Fig 3: right; LA80, PA80, V, R, and G). Especially, inside gaps between single filaments, a high accumulation of *S. aureus* colonies was found on

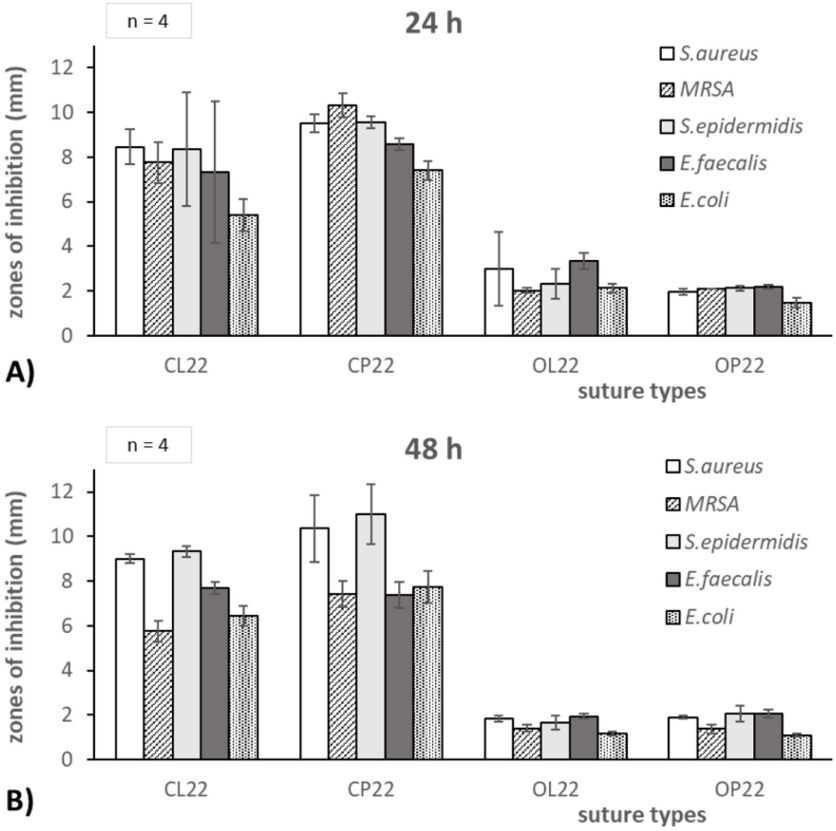

Fig 1. Zone of inhibition assay for five bacterial species over 48 hours. Zones of inhibition in millimeter for each coating type at 22 μg/cm drug content (CL22, CP22, OL22, OP22) on sutures. Test strains used were *S. aureus, MRSA, S. epidermidis, E. faecalis* and *E. coli* after A) 24 hours and B) 48 hours test period.

https://doi.org/10.1371/journal.pone.0190912.g001

top of the fine coatings of lauric acid components as well as on the rough lumps of palmitic acid coatings.

Viability of adhered bacteria on coated sutures (bacterial adhesion assay)

Chlorhexidine-laurate suture (CL11) shows the lowest numbers of viable adhered bacteria within the 11 μg/cm drug-containing novel coated sutures. Compared to the antimicrobial control Vicryl® Plus, CL11 shows a 1.2 log greater reduction. In general, chlorhexidine and octenidine coatings exhibit lower colony numbers of viable adhered *S. aureus*, as compared to the non-antimicrobial control (G). The number of viable adhered bacteria of each novel antimicrobially coated suture type (CL, CP, OL, OP) and Vicryl® Plus (VP) was statistically significantly reduced ($p < 0.001$: ***; Fig 4) compared to sutures without active substances (PA80, LA80, V, R, and G). In comparison to the triclosan-containing suture (VP), representing the antimicrobial suture control, the novel sutures showed an even more significant reduction of viable adhered bacteria ($p < 0.001$: ***; CL11-CL33, CP11-CP33, OL11, OL33, OP22,

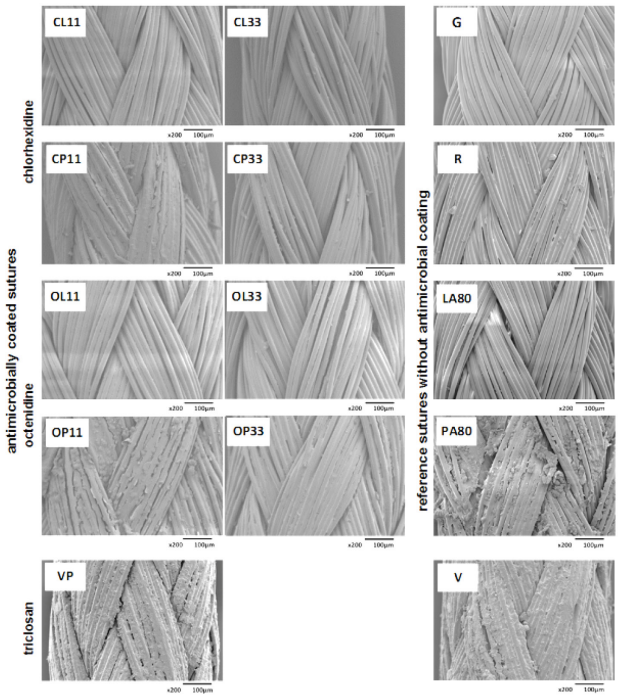

Fig 2. SEM pictures prior to bacterial exposure to inspect coating quality and suture integrity (magnification 200x). Left: Chlorhexidine- and octenidine-coated sutures for the lowest and highest drug concentrations used. Chlorhexidine sutures (CL11, CL33, CP11, and CP33) and octenidine-coated sutures (OL11, OL33, OP11, and OP33) are shown for laurate or palmitate carriers. Commercial antimicrobial sutures Vicryl® Plus (VP). **Right**: Reference sutures without antimicrobial drugs. Plain PGA suture material Gunze used for preparations (G) and commercially available resorbable sutures PGA Resorba® (R) and Vicryl® (V). Furthermore, sutures coated solely with fatty acid lauric acid (LA80), or palmitic acid (PA80) were investigated. Images are representative of three numbers of fields from three suture replicates.

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and OP33) and ($p < 0.05$: *; OL22). Adhered bacteria were slightly inhibited by OP11 (n.s.), comparable to Vicryl® Plus. The reduction of viable adhered bacteria (Fig 4) was expressed by a logarithm of the basis 10, calculated for each tested suture. Bacterial log reductions were calculated referred to uncoated Gunze suture (G) without any drug. Significance tests were referred to the control (G), and on the other hand to triclosan-coated Vicryl® Plus suture (VP). Chlorhexidine- and octenidine-coated sutures demonstrated a high log10 reduction of adhered *S. aureus* colonies in the range of 0.5 (OP11) up to 6.1 (OL33) compared to uncoated Gunze suture (G). In contrast, triclosan-containing Vicryl® Plus suture demonstrated a small 0.5 log reduction against adhering bacteria.

Viable bacteria in suspensions after suture incubation

The reduction of planktonic bacteria in suspensions within the 11 μg/cm drug-containing novel antimicrobial sutures is lowest for the chlorhexidine-palmitate (CP11) and laurate sutures (CL11). Compared to the antimicrobial control Vicryl® Plus, CP11 and CL11 showed a greater bacterial reduction of 0.7 log and 0.6 log, respectively. In general, suspension bacteria were highly inhibited by the novel bactericidal sutures (Fig 5), whether coated with chlorhexidine or octenidine for each used concentration. Bacterial reductions were referred to the non-

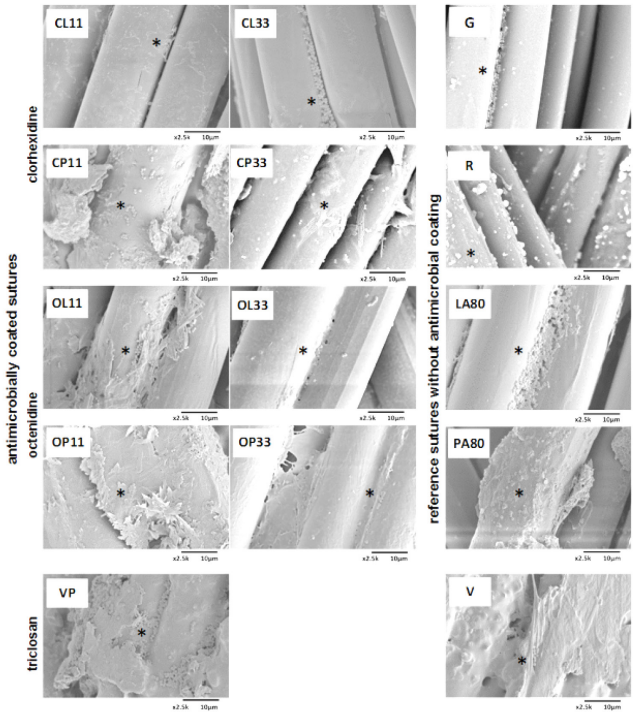

Fig 3. SEM pictures following bacterial exposure of coated sutures to visualize adhered bacteria and estimate their number semi-quantitatively (magnification 2,500x). Sutures were incubated in *S.aureus* suspension at 1.3 x 10^8 cfu/ml for 3 hours. **Left**: Novel antimicrobially coated sutures are shown for the lowest and highest drug concentrations at 11μg/cm and 33μg/cm, respectively. Chlorhexidine coated sutures (CL11, CL33, CP11, and CP33) and octenidine coated sutures (OL11, OL33, OP11, and OP33) depicted for laurate or palmitate carriers. The commercial antimicrobial triclosan reference Vicryl® Plus (VP) is also shown in the last row. **Right**: Suture references without antimicrobial substances (G, R, LA80, PA80, and V). Adhered bacteria were exemplarily marked with an asterisk (*). Images are representative of three numbers of fields from three suture replicates.

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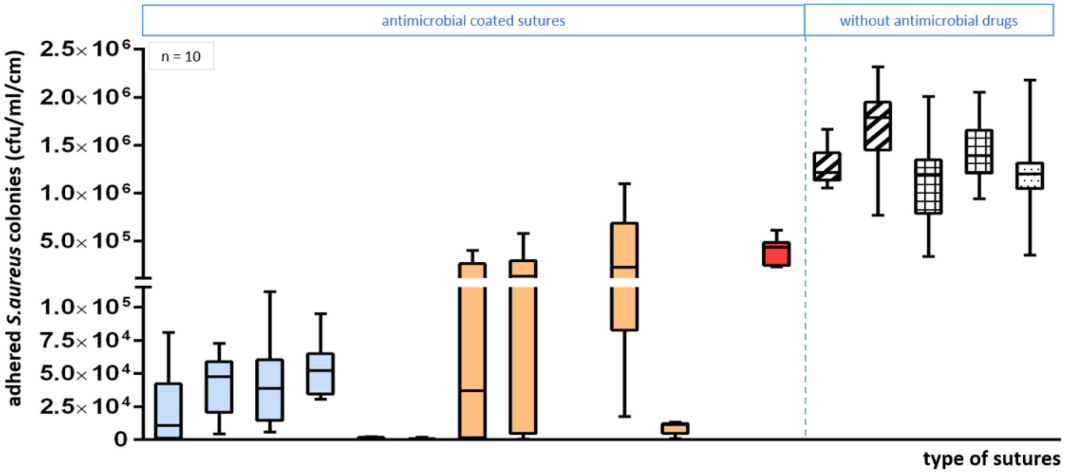
antimicrobial suture control (G), showing similar unaltered bacterial growth in suspensions similar to other tested sutures without antimicrobial substances (PA80, LA80, V, and R). In comparison to uncoated Gunze (G), Vicryl® Plus (VP) showed a bacterial reduction of 0.3 log, comparable to OL11 and OL22. Tested chlorhexidine-coated sutures decreased bacteria in suspension even further ($p < 0.001$: ***; CL11-CL33 and CP11-CP33) with at least 0.9 log. In addition, most of the octenidine sutures also showed a higher reduction of suspension bacteria compared to (G): OP11 (0.5 log), OL33 (1.0 log), OP22 (1.0 log), and OP33 (0.9 log).

Evaluation of results of 11 μg/cm drug-containing sutures in regard to former studies

The chlorhexidine-laurate sutures (CL11) best met medical requirements. CL11 shows the lowest number of viable bacteria on sutures, a high drug release within the first 48 h, as well as good biocompatibility. Potentially, each of the four novel coated sutures using 11 μg/cm drug concentration can be clinically applied, since they are antimicrobial effective over several days and biocompatible [43, 44]. The experimental data from the current study (Table 2: white background) and earlier studies [43, 44] (Table 2: blue, orange and light gray background)



	chlorhexidine sutures						octenidine sutures							non-antimicrobial				
	CL11	**CL22**	**CL33**	**CP11**	**CP22**	**CP33**	**OL11**	**OL22**	**OL33**	**OP11**	**OP22**	**OP33**	**VP**	**PA**	**LA**	**V**	**R**	**G**
mean cfu (cfu/ml/cm)	2E+04	4E+04	4E+04	5E+04	2E+03	6E+02	1E+05	2E+05	1E+00	4E+05	9E+03	4E+01	4E+05	1E+06	2E+06	1E+06	1E+06	1E+06
log reduction (compared to G)	1.7	1.4	1.4	1.4	2.9	3.3	1.0	0.8	6.1	0.5	2.1	4.5	0.5	0.0	-0.2	0.0	-0.1	0.0
significance (compared to G)	***	***	***	***	***	***	***	***	***	***	***	***	***	n.s.	**	n.s.	*	n.a.
significance (compared to VP)	***	***	***	***	***	***	***	*	***	n.s.	***	***	n.a.	-	-	-	-	-

Fig 4. Numbers of adhered *S. aureus* colonies on sutures' surfaces per cm sample after 3 hours of incubation in on average 1.3 x 10^8 cfu/ml bacterial suspension. Viably adhered numbers of bacteria and their reductions compared to uncoated Gunze (G) suture. **Left** (up to dashed line): Sutures coated with antimicrobial substances, such as chlorhexidine-laurate (CL), chlorhexidine-palmitate (CP), octenidine-laurate (OL), and octenidine-palmitate (OP) each with the drug concentration 11, 22, and 33 μg/cm. Novel coated sutures were also compared to commercially available triclosan containing Vicryl® Plus (VP) suture. **Right**: Groups of sutures without active antimicrobial agents, uncoated Gunze (G), coated with fatty acids (PA80, LA80) and commercially available common resorbable sutures (V: Vicryl®, R: PGA Resorba®). Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***); n.s.: not significant, n.a.: not applicable.

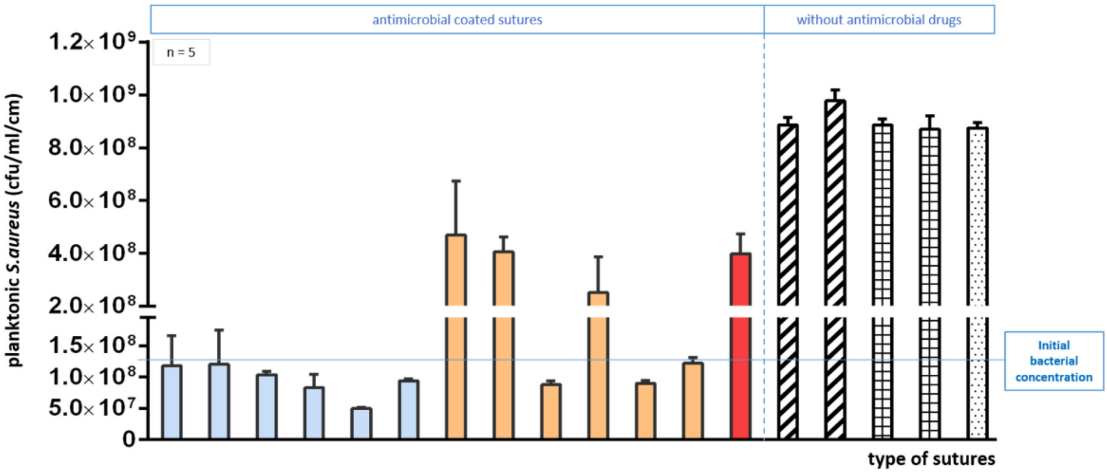
https://doi.org/10.1371/journal.pone.0190912.g004

were compared to each other. The rating levels (+, ++ and +++) used for the comparison of antimicrobial sutures are declared in Table 3.

The recent data demonstrated that chlorhexidine-laurate suture (CL11) shows the most efficient inhibition of adhered bacteria, which is critical for local infections. Therefore, we recommend the CL11 suture compared to Vicryl® Plus as an optimal surgical supplement to reduce wound infections. Nevertheless, octenidine-containing sutures at 11 μg/cm can also be helpful in applications where a slower and longer lasting drug release should be necessary.

Discussion

In this study, we found that novel antimicrobial sutures using chlorhexidine or octenidine coatings were effective against multiple bacterial pathogens. Especially, viable adhering and surrounding planktonic *S. aureus* were strongly inhibited. Additionally, we found that reduction of adherent bacteria via novel sutures could be up to 12-fold higher than achievable with commercial antimicrobial suture Vicryl® Plus using triclosan. Scanning electron microscopy (SEM) pictures were used to investigate suture coatings and to demonstrate bacterial adhesion. The main finding of the present study was that novel antimicrobially coated sutures show



	chlorhexidine sutures						octenidine sutures							non-antimicrobial				
	CL11	CL22	CL33	CP11	CP22	CP33	OL11	OL22	OL33	OP11	OP22	OP33	VP	PA80	LA80	V	R	G
mean cfu (cfu/ml/cm)	1E+08	1E+08	1E+08	8E+07	5E+07	9E+07	5E+08	4E+08	9E+07	3E+08	9E+07	1E+08	4E+08	9E+08	1E+09	9E+08	9E+08	9E+08
log reduction (compared to G)	0.9	0.9	0.9	1.0	1.2	1.0	0.3	0.3	1.0	0.5	1.0	0.9	0.3	0.0	-0.1	0.0	0.0	0.0
significance (compared to G)	***	***	***	***	***	***	**	***	***	***	***	***	***	n.s.	*	n.s.	n.s.	n.a.
significance (compared to VP)	***	***	***	***	***	***	n.s.	n.s.	***	n.s.	***	**	n.a.	-	-	-	-	-

Fig 5. Numbers of viable bacteria in suspension incubated for 3 hours with novel antimicrobial sutures. An initial *S. aureus* concentration of 1.3 x 10^8 cfu/ml was used for bacterial suspensions. Chlorhexidine- or octenidine-coated sutures showed a strong inhibition of pathogens in the surrounding suspensions. The triclosan-coated suture Vicryl® Plus (VP) and the uncoated Gunze suture (G) were used as controls. Fatty acid-coated sutures (PA80, LA80) and commercial sutures without any drug content (V: Vicryl®, R: PGA Resorba®) were tested within the non-antimicrobial suture group. Significance levels are $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***); n.s.: not significant, n.a.: not applicable.

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Table 2. Evaluation of the novel antimicrobial sutures using chlorhexidine or octenidine at 11 μg/cm drug compared to commercial triclosan-containing Vicryl® Plus.

Type of antimicrobial sutures	Viability of bacteria		SEM investigation	Biocompatibility	Delayed drug release	Zones of inhibition	
	log reduction of adhered bacteria	log reduction of planktonic bacteria	number of adhered bacteria	metabolic activity (%)	residual content (%)	duration (d)	initial size (mm)
CL11 [43]	+++	+++	+	+	+	++	++
CP11 [43]	+++	+++	++	++	+++	++	++
OL11 [44]	+++	+	++	++	+	+++	+
OP11 [44]	+	+	++	+++	+++	+++	+
Vicryl® Plus [43, 44]	+	+	+++	+++	n. d. [a]	+++	+++

[a] No determination of drug release, because of triclosan's extremely low solubility in aqueous media. Referred to other *in vitro* studies by Ming et al. [48] and Edmiston et al. [18], the triclosan release was rated as +++ level.

Data from our recent study (white background) concerning reduction of viable adhered, as well as bacteria in suspension, and SEM investigations were arranged next to each other. Additionally, data from earlier studies [43, 44] are considered for evaluation regarding cytotoxicity, antimicrobial efficacy via zone of inhibition assay over time, and the slow drug release properties (dark blue background: chlorhexidine sutures, orange background: octenidine sutures, and light gray background: Vicryl® Plus suture control).

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Table 3. Rating levels used for comparative antimicrobial suture evaluation.

Sutures properties that are compared		Rating levels		
		+	++	+++
Viability of bacteria	log reduction of bacteria	≥ 0.3	≥ 0.6	≥ 0.9
SEM investigation	number of adhered bacteria	< 50	50–200	> 200
Biocompatibility	metabolic activity	≥ 60%	≥ 70%	≥ 80%
Delayed drug release	residual content after 96 h	≥ 10%	≥ 40%	≥ 60%
Zones of inhibition	initial size after 24 h	≥ 1 mm	≥ 4 mm	≥ 10 mm
	days of duration	≥ 1 d	≥ 4 d	≥ 8 d

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considerably less viable bacteria on suture surfaces than triclosan-containing suture Vicryl® Plus. Therefore, these novel coated sutures may reduce suture-associated surgical site infection (SSI) more effectively than otherwise possible today. SSI is still an issue in medical daily routine. Sutures can promote such infections via the so-called "wicking effect" as well as by enabling bacteria to colonize. Sutures themselves affect bacterial adhesion, especially due to the chemical composition of suture material, surface structure, as well as capillarity. It has been shown that the property of sutures acts as a substrate for adhering bacteria can be correlated with the rate of infection [9]. Viable adhering bacteria form biofilms on suture surfaces by proliferation. These biofilms are even detectable on sutures in "culture-negative" SSI, a special form of wound infection in which no bacterial pathogens could be cultured using conventional diagnostic methods. [11] Antimicrobial-coated sutures also inhibit adhering bacteria and can be an established adjunctive aspect in reducing SSI [15] and thus interrupt this infection pathway.

The zone of inhibition assay showed a multispecies efficacy of novel coated sutures against the five tested relevant bacterial species. Therefore, a potential inhibition of clinical relevant pathogens is assumed. The efficacy is mainly dependent on the type of drug used for coating (chlorhexidine or octenidine) but also–to minor degree–on the drug carrier (laurate or palmitate). Overall, the antibacterial efficacy of coated sutures was comparably to the clinically most relevant *S. aureus* species. Therefore, *S. aureus* was used to investigate the bacterial adhesion in further detail. The inhibition zones indicated a sustaining broad-activity over the tested 48 hours. The amount of drug release is directly indicated by the size of inhibition zones. The suture's drug release persists for more than two days. That "fits" well with the description in literature [43, 44] antimicrobial efficacy lasting for nine days using octenidine coatings and for up to five days with chlorhexidine coatings. Novel coated suture materials protected broadly against microbes for the critical period of 48 hours after surgery, which is necessary to avoid SSI. Moreover, there is a high local efficacy against problematic MRSA infections.

Structural investigations by SEM of coated sutures showed uniformly distributed antimicrobial coatings on surfaces around the multifilament structure. Dependent on the type of fatty acid carrier, there was a detectable difference concerning the level of roughness. For lauric acid coatings, the fine structure of suture filaments was preserved. Coatings containing palmitic acid seemed to laminate filament strands resulting in a high degree of roughness, probably an effect of the presence of longer hydrocarbon chains. This observation is comparable to commercial sutures, such as Vicryl®, PGA Resorba®, and Vicryl® Plus. Especially, absorbable braided sutures are using coatings consisting of calcium stearate formulations to improve handling [49] and to smoothen the surface. Thus, the tissue damage by braided sutures during suturing, the so-called "sawing action" is reduced [50]. Calcium stearates consist of stearic acid, a fatty acid with an 18-carbon chain that is comparable to palmitic acid.

SEM pictures of inoculated sutures with 1.0 x 10^8 cfu/ml *S. aureus* suspension over 3 h indicated numerous adhering bacteria on all suture surfaces whether coated with antimicrobial agents or not. Bacterial adherence seems to be independent from substance, drug carrier, surface roughness, or drug concentration. Especially, the gaps between single filaments of uncoated or laurate-containing sutures represent areas which were colonized by bacteria leading to a pearl chain arrangement (Fig 3, e.g. Gunze, CL33 and LA80). Adhering bacteria on surgical sutures represent a potential risk for wound infections and need effective inactivation to counteract infections. The sutures' capillarity acts as a door opener for pathogens to penetrate into wounds as these microorganisms may trigger infection [51].

Some authors detected biofilm formation of bacteria grown on suture surfaces [52]. Further SEM pictures of inoculated suture samples also potentially demonstrated the production of little extracellular matrix around adhered bacteria, indicating the beginning of biofilm formation on sutures. Adhered bacteria were detached by sonication and viable bacteria were quantified afterwards. A strong inhibition of initially adhered bacteria during a short period of incubation was detected for the novel antimicrobial sutures. Therefore, an inhibiting effect on biofilm formation on sutures can be strongly expected. Sutures using lauric acid showed a higher number of adhering bacteria than those using the palmitic acid carriers. This conspicuousness was confirmed by the bacterial adhesion assay, proving higher numbers of viable adhering bacteria for lauric acid containing sutures.

The bacterial adhesion assay indicated a drastic log reduction of viable adhered bacteria on novel antimicrobially coated sutures. Compared to the weak log reduction of Vicryl® Plus the bacterial inhibition by contact with the novel antimicrobial sutures can be up to 12-times higher dependent on the kind of substance, drug carrier, and drug concentration employed. We suggest that the adhesion of bacteria could not be avoided by numbers via antimicrobial coatings. However, antimicrobial agents inside novel coatings significantly reduced the number of viable adhering bacteria in our experiments. Novel coated sutures may inhibit bacterial proliferation on suture surfaces and thus inhibit the initial biofilm formation. Consequently, novel chlorhexidine- and octenidine-coated sutures may have a higher ability to prevent SSI related to suture material than Vicryl® Plus. This effect could be limited by the numbers of microbes inside the incision or on the threads, and the sensitivity of bacteria against the type of drugs used in the coating layers. Sutures coated with fatty acid carriers only showed a slightly higher number of viable adhered bacteria compared to Gunze sutures without any coating (G). Especially lauric acid coatings (LA80) seem to attract adhering bacteria more than palmitic acid suture (PA80). Thus, laurates are presumably more suitable as drug carriers than palmitates to achieve low bacterial adherence.

Regarding the ultrasound treatment to release adhered bacteria, sonication is a competitive process between releasing and killing adhering bacteria, dependent on the duration of sonication [53]. Therefore, a short sonication time of 1 min was chosen, resulting in a low killing rate versus a detectable viable bacterial release. In combination with vortexing an increased soft release could be achieved [54]. Since all sutures were treated equally, sonication and vortexing is a meaningful process to release bacteria. Antimicrobial coatings are not able to reduce bacterial adhesion in general. However, they are an effective method to inactivate viable adhering bacteria [47].

Bacterial growth was investigated by incubation of *S. aureus* suspensions including coated and uncoated suture samples. Planktonic bacteria were highly inhibited by chlorhexidine- or octenidine-coated sutures compared to sutures without antimicrobial coating. In comparison to the commercially available triclosan suture Vicryl® Plus, a much higher effectiveness of bacterial reduction was demonstrated for all types of tested chlorhexidine sutures, as well as for the higher concentrated octenidine sutures. In addition, lower concentrations of octenidine

in sutures showed a similar inhibition effect to that obtained by Vicryl® Plus. The efficacy was strongly dependent on the drug type, presumably in regard to its individual solubility and therefore differing drug release from suture coatings. Solubility in aqueous media was extremely low for triclosan, higher for octenidine and highest for chlorhexidine. We hypothesize that free drug molecules combined with a certain drug concentration would be necessary for an effective antimicrobial activity against bacteria.

Siedenbiedel and Tiller described multiple mechanism for antimicrobial surfaces, the killing effect on surrounding pathogens by drug releasing surfaces and the direct contact inactivation on surfaces, as well as a repelling effect on microorganisms [55]. We hypothesize that antimicrobial sutures based on fatty acid drug carriers inhibit surrounding pathogens by drug release, and on the other hand inhibit viable pathogens by direct contact with drug molecules during attachment on surfaces. We also presume that bacterial inhibition is dominated by surface inactivation or rather bactericidal effects on surfaces, due to the highly reduced number of viable adhered bacteria.

The present study has some important limitations: Although, the broad antibacterial efficacy of novel coated sutures has been shown in a zone of inhibition assay, only the clinically most relevant pathogen *Staphylococcus aureus* (*S. aureus* ATTC®49230™) was used for the bacterial adhesion assay. Staphylococci species are the most common pathogens responsible for wound infections and a variety of implant-associated infection [40, 56]. However, to identify the full potential of this approach, further research has to investigate bacterial adhesion by using other relevant strains. Nevertheless, the high multispecies antimicrobial efficacy via inhibition zones presumably also indicates high degrees of inactivation of other adhering bacteria. Our monospecies microbiological setup for investigation of bacterial adhesion is able to provide answers to the potential effect on initially adhered staphylococci and thus results in the potential to inhibit the following biofilm formation. Data was collected advantageous without any interference by interactions between different species. A further limitation is the sonication itself as a competitive process between detachment of bacteria and potential harm. Due to methodological constraints, the absolute number of bacteria adhering to suture surfaces could not be detected. The counts of cfu from surface-released bacteria only represent the viable content of adhering bacteria. Moreover, when using SEM pictures for visualization of adhering bacteria, it is not possible to distinguish between viable and inactivated bacteria. The fluorescence microscopy technique combined with a live/dead bacterial staining assay could solve this problem in future studies.

In summary, the zone of inhibition assay documented a bacterial multispecies efficacy over 48 hours. SEM investigations showed uniformly covered suture surfaces by coating and different roughness dependent on the type of fatty acid carrier. Furthermore, adhering *S. aureus* were found on each kind of tested suture, whether coated with antimicrobial substances or not. The number of viable *S. aureus* adhering on suture control groups was extremely high, without any drug and on the antimicrobial control Vicryl® Plus. Therefore, coated sutures presumably could not avoid bacterial adhesion itself. At the same time, novel coated sutures using chlorhexidine or octenidine inhibited adhered *S. aureus* significantly. Chlorhexidine-laurate suture (CL11) shows the lowest remaining number of viable adhered bacteria, despite an extremely high concentration of *S. aureus* inoculation. These bacteria are critical for the onset of local infections, thus this suture has the highest potential to further reduce the rates of SSI. Consequently, CL11 best fulfills the medical need and we recommend this suture type compared to Vicryl® Plus as an optimal surgical supplement to reduce wound infections. Furthermore, planktonic bacteria in suspension were also drastically inhibited by the novel coated sutures chlorhexidine-laurate, chlorhexidine-palmitate and octenidine-palmitate at 11 µg/cm. Octenidine-laurate at 11 µg/cm exceptionally showed a similar number of adhered bacteria to

Vicryl® Plus, which nevertheless represents a reduction of 0.5 log compared to uncoated Gunze sutures. Octenidine-containing sutures at 11 μg/cm (OL11) can also be helpful in applications where a longer-lasting drug release may be necessary, e.g. when infections already exist during septic surgery.

Novel coated sutures in former studies showed excellently adjustable antimicrobial efficacy and release kinetics, lasting some days for chlorhexidine formulations and up to nine days for octenidine coatings. Dependent on the kind of drug, it could therefore possibly be useful to distinguish between two fields of application: on the one hand, a long-term drug release, e.g. for wound closure during septic surgery. On the other hand, applications with a shorter drug release, e.g. for infection prophylaxis in common surgery.

The present study fundamentally demonstrated a much higher inactivation of viable adhering bacteria through novel antimicrobially coated sutures and thus, presumably, a much higher potential to interrupt the"wicking effect" compared to Vicryl® Plus. Therefore, we suppose that the novel sutures have a higher potential to avoid suture-associated SSI. Pre-clinical studies, followed by clinical investigations are necessary to demonstrate their ability to avoid SSI in vivo and prove their safety. Novel antimicrobial sutures using chlorhexidine or octenidine at 11 μg/cm drug content may pose an alternative in case of triclosan resistance, or to extend the active substances clinically used on antimicrobial sutures. This should give surgeons an additional effective tool to react to complex pathogen milieus and resistances.

Conclusions

In this study, we found that the novel chlorhexidine- and octenidine-coated sutures are effective against multiple bacterial species over the critical period of 48 hours after surgery. The analysis in detail for *S. aureus* revealed that antimicrobial sutures at 11 μg/cm drug content demonstrate superior bactericidal properties against adhering *S. aureus* compared to commercial triclosan-containing Vicryl® Plus. Especially, the chlorhexidine-laurate coating (CL11) shows the highest efficacy to minimize the number of adhered as well as planktonic bacteria. This coating provides a high drug release in the first, clinically most relevant 48 h after suture application and is–in addition–highly biocompatible. Therefore, this coating type best meets the medical needs and should be recommended for potential clinical application. The high reduction of viable adhering bacteria on this novel coated suture is a promising approach to improve prevention of surgical site infections in routine surgery. These results encourage further pre-clinical and clinical trials to confirm safety and efficacy of this coating technology *in vivo*.

Acknowledgments

We would like to thank Dr. K.-H. Sorg (Resorba Wundversorgung GmbH, Germany) for his supply with PGA Resorba® sutures and raw PGA material (Gunze Limited, Japan). We would like to express our special thanks to S. Schnell-Witteczek (Institute of Medical Engineering, Technical University of Munich (IMETUM), Garching) for her great assistance in scanning electron microscopy. Many thanks to Mr. F. Seidl (M.A. Interpreting and Translating) for his kind support in scientific English.

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Danksagung

Als erstes möchte ich Herrn Prof. Dr. med. Rainer Burgkart ganz herzlich Danke sagen für die Möglichkeit der Bearbeitung des Themas in einem sehr motivierenden und stets freundlichen Arbeitsumfeld der orthopädischen Forschung am Klinikum rechts der Isar, der TU München. Er stand mir stets mit Rat, Tat und einer offenen Tür vor Ort zur Seite. Ebenso möchte ich Herrn Prof. Dr. med. Rüdiger von Eisenhart-Rothe als Klinikdirektor der Orthopädischen Klinik an der TU München danken.

Herrn Prof. Dr. med. Matthias Schieker möchte ich danken für die fachliche Betreuung dieser Promotion und für die Hilfe bei diversen Fragestellungen. Ebenfalls möchte ich Herrn Prof. Dr. med. Wolfgang Böcker sehr danken für die direkte Ermöglichung dieser Promotion an der Klinik für Allgemeine, Unfall- und Wiederherstellungschirurgie des Klinikums der Universität München.

Insbesonderer Dank gebührt Herrn Prof. Dr. Dr. Axel W. Stemberger für sein persönliches Engagement mit fachlicher Betreuung bei pharmazeutischen und sonstigen Fragestellungen, darüber hinaus für das über die Jahre gewachsene freundschaftliche Verhältnis und Mentoring.

Viel lieber Dank geht an Frau Jutta Tübel und Carmen Marthen (Zellkulturlabor, Klinik für Orthopädie am Klinikum rechts der Isar der TUM) für deren großartige Unterstützung nicht nur bei zellbiologischen Experimenten zur Biokompatibilitätsbeurteilung. Ebenso gilt mein Dank Herrn Peter Föhr (Biomechanik, Klinik für Orthopädie am Klinikum rechts der Isar der TUM) für seine stete Hilfsbereitschaft u.a. bei biomechanischen Materialanalysen und spannenden Diskussionen. Vielen Dank auch an Herrn Constantin von Deimling für seine Hilfe bei u.a. statistischen Auswertungen und Unterstützungen bei Computer- und Softwarefragestellungen. Zudem möchte ich den ehemaligen Studenten Frau Ahlem Kalaai, Frau Barbara Kiefel und Herrn Steffen Wehner für deren Engagement und Mitarbeit danken.

Ein spezieller Dank geht an Frau Susanne Schnell-Witteczek (Institute of Medical Engineering der TU München, IMETUM in Garching), für ihre Unterstützung bei der Durchführung von Rasterelektronenmikroskopie-Aufnahmen und der damit verbundenen Probenpräparation. Ebenso möchte ich Hr. Dr. med. Dominik Pförringer für seine fruchtbaren Korrekturen und leidenschaftliche Hingabe zur deutschen Sprache danken.

Danken möchte ich des weiteren Herrn Prof. Dr. Klaus-Dieter Kühn (Heraeus Medical GmbH) mit Hilfe dessen Anschubfinanzierung das Projekt erfolgreich durchgeführt werden konnte. Zudem möchte ich Herrn Dr. Karl-Heinz Sorg (Resorba Medical GmbH) für die freundliche Bereitstellung von Nahtmaterialien, wie PGA Resorba® und PGA Rohmaterial für die eigenen Nahtmaterialbeschichtungen danken.