

Accessible Agent-Fatty Acid Coatings of Titanium Prostheses for Local Prevention and Treatment of Anti-Microbial Infections

Klemens Vertesich^{1,*}, Thomas Mayrhofer¹, Reinhard Windhager¹, Klaus-Dieter Kühn²

¹Department of Orthopaedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria

²Department of Orthopaedics and Trauma Surgery, Medical University of Graz, Graz, Austria

Email address:

klemens.vertesich@meduniwien.ac.at (K. Vertesich)

*Corresponding author

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Abstract: Prosthetic joint infection represents a major issue in arthroplasty. Local anti-infective treatment is not established in cementless prosthetic surgery. The aim of this study was to perform simulate a perioperative application of agent-fatty acid complexes on surfaces of primary and revision prosthetic material. Further, it was aimed to investigate the efficacy of these coatings by in vitro microbiological tests. Coating of cementless titanium prostheses with gentamicin-palmitate and octenidine-laurate was performed by using a spray gun system. Coating with vancomycin eluted in trilaurin was performed by dipping of the prostheses in the solution. The prostheses were incubated in phosphate buffered saline for 7 days. Microbiological testing was performed with inhibition areolae testing for *S. aureus*, *S. epidermidis*, MRSA and *C. albicans*. Coating of prosthetic material was reliable and reproducible with two different techniques, dipping and spraying. The surface-concentrations of agents have reached 195µg/cm² for gentamicin, 460µg/cm² for octenidine and 323µg/cm² for vancomycin. Agents inhibited *S. epidermidis* and *S. aureus* growth for seven days, *C. albicans* for three days and MRSA for two days. Agent-fatty acid coatings used in this study represent a biodegradable layer with good biocompatibility and comparable anti-infective efficacy as in cemented surgery due to the use of established agents, even if low concentrations are used. Modular and individual anti-infective coating was reproducibly and reliably performed by dipping coating, which may represent a potential perioperative coating approach.

Keywords: Anti-Infective Coating, Biodegradable Coating, Prosthetic Joint Infection, Gentamicin, Vancomycin, Octenidine

1. Introduction

Prosthetic joint infection (PJI) represents a major problem in total arthroplasty. Infection rates for total hip arthroplasty (THA) amount 1% and range from 1% to 2% in total knee arthroplasty (TKA) [1-3]. Further, PJI is a major reason for revision. 14.8% of revisions performed after THA are due to infection [4, 5]. In TKA, with 27% the risk of revisions performed due to PJI is even higher [3, 5].

PJI is usually caused by microorganisms that colonize the surface of prosthetic material. Bacteria cluster together in biofilms, thereby getting up to 1000 times more resistant to antimicrobial agents, which makes pharmacological

intervention very challenging [6, 7].

Methicillin-sensitive *S. aureus* (MSSA, 19%) and methicillin-sensitive *Staphylococcus epidermidis* (MSSE, 11%) are the most frequently isolated microorganisms [8]. PJI caused by methicillin-resistant *Staphylococcus aureus* (MRSA), isolated in 19% of cases [8], constitute a major additional difficulty.

Treatment options available for PJI can be divided by interventional and non-interventional treatment [9]. Surgical treatment outranges from irrigation and debridement, over one-stage and two-stage revision to permanent explantation of the prosthetic devices [10].

Two-stage revision, with explantation of the infected

device and implantation of a new prosthetic device after a period of antibiotic treatment for up to 8 weeks, is most commonly used in PJI treatment [11].

Prevention of PJI in clinical routine is done by prophylactic antibiotic medication, which represents the most important factor of infection prophylaxis [12, 13].

Local anti-infective methods in cemented joint surgery were first performed in the 1970s, resulting in improvement of PJI rates [14]. Antibiotic agents are suspended in PMMA bone cement, resulting in the so-called antibiotic loaded bone cement (ALBC), which causes high local antibiotic concentrations after implantation. ALBC is a commonly used local anti-infective method in prevention and treatment of PJI [15]. However, in cementless arthroplasty local anti-infective methods are challenging due to non-availability of agent-carrier [16].

Prosthetic surface modifications using cross linked albumin [13] or titanium nanotubes [17, 18] prevent bacterial attachment due to their anti-adhesive properties.

Coating of prostheses using silver and other metals leads to an anti-microbial effect [19-21]. Metals interfere with bacterial replication and cell wall formation, due to their nanostructure. Further non-antibiotic anti-microbial agents like Povidon iodine can be used for anti-infective coating of prostheses as well, resulting in infection prevention and successful treatment in PJI patients [22, 23].

Coating of prosthetic joints with antibiotic or antiseptic agents can be achieved by using fatty acids. If agents are suspended in fatty acids, the lipids act as a biodegradable agent carrier. Further, the physical properties of the agent change by ionic bonding of lipids to antibiotic or antiseptic agents like gentamicin or octenidine, resulting in advanced adhesion [24]. Increased hydrophobia and adhesion of antibiotics or antiseptics combined with lipids provide a suitable coating of prostheses. After implantation, ionic bonds between lipid carriers and anti-infective agents dissolve, agents diffuse into bone and surrounding tissue, thereby causing an anti-infective local effect [25, 26]. Efficacy of these agents utilized in coatings of titanium prostheses was shown *in vitro* and *in vivo* [27-29].

In the present study, we aimed to assess the application of three different agent-fatty acids to surfaces of prosthetic devices for primary and revision orthopedic surgery, in specifically low active agent concentrations. Therefore, we used two different coating strategies. Furthermore, we investigated agents' efficacy by microbiological effects *in vitro*.

2. Methods

2.1. Prosthetic Material

Two types of cementless prosthetic material was chosen. To represent primary prosthetic devices cementless hip-stem (PP) (Alloclassic[®] Zweymüller[®], Zimmer Biomet Holdings, Warsaw, ID, USA) were chosen, as well as flat titanium discs (TD) with rough blasted surface, non-coated

with diameter of 1,56cm (Waldemar Link GmbH & Co KG, Hamburg, Germany). Titanium alloy used for this TD is commonly used in producing Link C. F. P[®], MP[®]-reconstruction prosthesis and Gemmin knee[®].

To represent the group of revision prosthetic material megaprosthetic devices (MP) from modular reconstruction prostheses (GMRS[®], Stryker Corporation, Duisburg, Germany) were chosen. Further, osteosynthetic molded body (OS) generated by cutting of grinded, anodized titanium osteosynthetic plate (Litos GmbH, Ahrensburg, Germany) were used as well as segments of medullary nails (MN) (Depuy Synthes Companies, Warsaw, ID, USA).

2.2. Antibiotic-Fatty Acid Agents

Gentamicin-palmitate (GP) (Heraeus Medical GmbH, Wehrheim, Germany), an agent-fatty acid complex, ionic bonded, consisting of 26% Gentamicin and 74% palmitate acid. For coating procedures 4% gentamicine-palmitate was dissolved in methanol.

Vancomycin suspended in trilaurin (Heraeus Medical GmbH, Wehrheim, Germany). Due to the hydrophilic properties of vancomycin, ionic bonding to fatty acid carrier was not feasible, therefore 3% vancomycin was suspended in 80°C heated trilaurin as carrier agent.

Octenidine-laurate (OL) (Heraeus Medical GmbH, Wehrheim, Germany), agent-fatty acid complex, consisting of 60% octenidine ionic bonded to 40% laurate acid. For application 8.5% octenidine-laurate was dissolved in ethanol.

2.3. Coating

Application of anti-infective coatings to dedicated areas of prosthetic surfaces was performed under simulated operation theater conditions using two different coating procedures.

For GP and OL application, an airbrush spraying system was used. Dissolved agent-fatty acid complexes were applied by using a conventional airbrush pistol connected to a static air pressure system using pre-set two bar pressure. Application was performed by a single operator, with optimized application skill through preliminary coating tests.

Application of VT was performed by dipping coating procedure. This was performed due to viscosity and chemical properties of the coating. It could not be eluted in alcohol and prepared for spraying. Vancomycin and trilaurin solvent were heated to a temperature of 80°C. Test objects were then coated by single dipping into VT solvent (<0.5seconds). Dipping process was performed by a single operator, with optimized coating skills due to preliminary coating tests.

Surface concentration of applied agents was detected by weighting. The prosthetic material was weighted before and after weighting using micro scales. The agent concentration was calculated subsequently considering the molecular compound of the coatings. Prosthetic surfaces after anti-infective coating were investigated by digital light microscopy.

2.4. Microbiological Testing

Efficacy of anti-infective coated prosthetic implants was investigated by tests based on inhibiting areola tests.

Prosthetic objects were incubated in 10% phosphate buffered saline (PBS) over a period of 168h. Objects were transferred in fresh PBS after one, two, three, four and seven days. After each incubation step, eluates were retained.

After completion of incubation period, bacterial colonies were streaked on blood agar, using a germinal suspension of 1×10^6 cfu/ml. Mycotic colonies in a suspension of 1×10^6 cfu/ml were streaked on Sabouraud dextrose agar. Implants

coated with GP were tested against MSSA and MSSE, implants coated with OL were tested against MSSA and *C. albicans* and implants coated with VT suspension were tested against methicillin-resistant MRSA.

Based on agar diffusion tests, 50 μ l of each eluate was pipetted on designated agar plate and then stored in an incubator for 24 h.

Results were evaluated by using image-processing applications raising the resulting areolas of inhibition. An overview on the experimental set-up is provided by Table 1.

Table 1. Overview on test schedule, emphasizing the application anti-infective coatings to primary and revision prosthetic devices with the dedicated method of application and testing against respective microorganisms.

Coating solution	Primary prostheses	Revision prostheses	Application method	Tested microorganisms
GP	PP TP	OS MN	Spraying	MSSE MSSA
VT	TP	MP OS MN	Dipping	MRSA
OL	PP TP	MP OS	Spraying	MSSA <i>C. albicans</i>

2.5. Data Analysis

Antibacterial efficacy and performance was analyzed using descriptive statistical methods and illustration was performed using appropriate charts and graphs. Further statistical testing was used to perform data analysis. Due to multiple measurements in each sample a multivariate analysis of variance (ANOVA) was performed to detect a potential effect of the concentration of agent on the efficacy as well as the effect of different agents on the efficacy of coatings. Confidence intervals of 95% were used for entire statistical analysis. Statistical testing was performed using SPSS 24.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Anti-Infective Coating of Prosthetic Material

Coating performance of prosthetic material with the spraying technique was reproducible in our tests. Reproducible and continuous coating results were achieved

by spraying for less than three seconds onto the dedicated surface area. Spraying distance of 3cm showed prime coating results, as well as application to 80°C preheated prosthetic surfaces. Spraying process showed contamination of laboratory area. Due to safety reasons spraying was performed using an air exhausting system.

The prosthetic surface showed substantial darker color areas after application of anti-infective coating with the spraying technique compared to uncoated prosthetic surfaces (Figure 1).

Coating with the dipping technique was reproducibly and reliably performed under simulated operation theater conditions. Dipping of prosthetic material into anti-infective coating solution for less than one second showed prime coating results. Further, application of 82°C heated solution to pre-cooled surfaces (4-6°C) resulted in the most reproducible coatings.

Surfaces roughness of MP was examined after dipping coating with VT by digital light microscopy. Initial surface roughness of 200-600 μ m was reduced to 70-200 μ m after coating process (Figure 2).



Figure 1. Prosthetic surface after anti-infective coating with spraying technique. Dark-grey represents coated areas, light-grey uncoated areas.

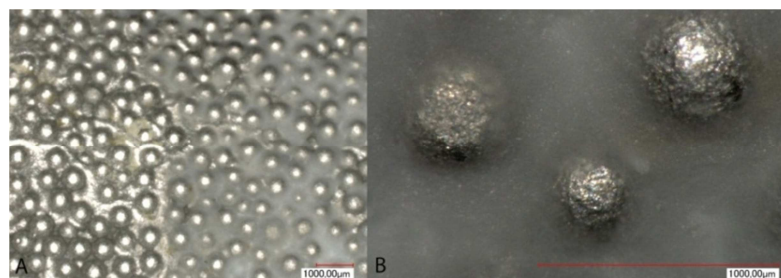


Figure 2. Light microscopy image of MP after dipping coating with VT. A – Surface-roughness of 200-600 μ m was detected (left). A roughness of 70-200 μ m was detected after coating (right). B – Close-up of coated megaprosthesis surface.

3.2. Agent Concentrations

The agent concentrations within the anti-infective coatings were exceptionally low. After spraying-coating with GP, the mean pure agent concentrations, controlled by weighting, were $195\mu\text{g}/\text{cm}^2$ (SD: $122.98\mu\text{g}/\text{cm}^2$, range: $45\text{--}557\mu\text{g}/\text{cm}^2$). Dipping-coating with VT resulted in a mean vancomycin concentration of $323\mu\text{g}/\text{cm}^2$ (SD: $375.18\mu\text{g}/\text{cm}^2$, range: $116\text{--}1299\mu\text{g}/\text{cm}^2$). Spraying-coating with OL resulted in mean pure octenidine concentrations of $460\mu\text{g}/\text{cm}^2$ (SD: $162.97\mu\text{g}/\text{cm}^2$, range: $306\text{--}857\mu\text{g}/\text{cm}^2$) (Table 2).

Table 2. Agent concentrations after spraying-coating (GP, OL) or dipping-coating (VT).

	Agent concentration ($\mu\text{g}/\text{cm}^2$)			
	Mean	SD	Minimum	Maximum
Gentamicin	195	122.98	45	557
Vancomycin	323	375.18	116	1299
Octenidine	460	162.97	306	857

3.3. Efficacy of Anti-Infective Coatings

Microbiological testing of inhibition revealed an anti-microbiological efficacy over a period of 48–168h, with GP being the most potent agent. OL showed potent inhibition over the entire period of testing. VT showed prime inhibition

after 24h with decreasing effect over the period (Figure 3, Figure 4).

Inhibition of MSSE showed the most potential effect according to every anti-infective coating tested, resulting in mean inhibition areola of 2.12cm diameter after 24h and 0.50cm after 168h. Inhibition of MSSA showed comparable effects with maximum areola of inhibition (mean: 1.60cm) after 24h and 0.35cm after seven days. MRSA inhibition showed initial effects after 24h with 1.86cm inhibition areolae in mean. MRSA inhibition showed just minimal effects after three to seven days, resulting in minimal inhibition areolae at the last point of testing.

Inhibition of *C. albicans* showed good initial effects as well, with inhibition areolae of 0.54cm after one day and 0.52cm after two days. The inhibition effect decreased after three and seven days until areolae of inhibition could no longer be detected. However, zones of decreased microorganism-growth were visible (Figure 3). Detailed course of bacterial inhibition is provided in Figure 5.

Statistical testing of variance detected a significant affectation of anti-microbial efficacy by the type of agent ($p=.007$) as well as different germ ($p=.008$). Further a significant affectation of surface concentration of agent on the efficacy could be detected ($p=.002$).

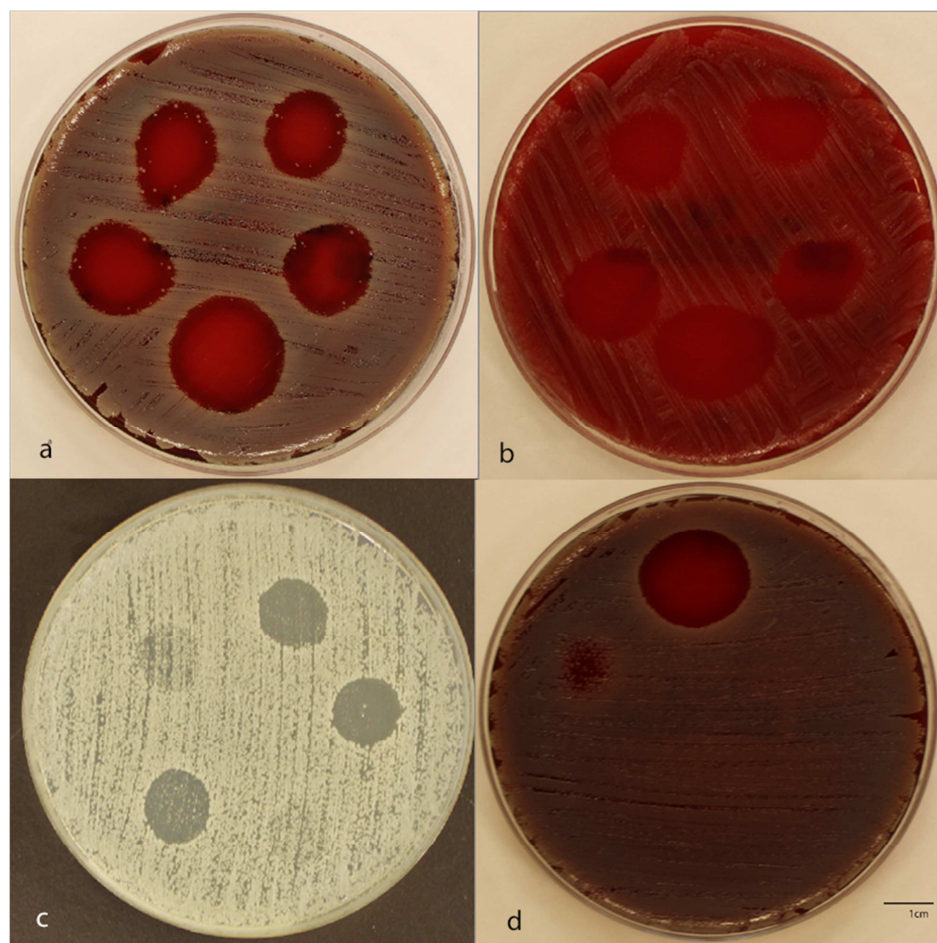


Figure 3. Inhibition areola testing after 24 of germinal growth; a – efficacy testing of GP against MSSA; b – efficacy testing of GP against MSSE; c – efficacy testing of GP against *C. albicans*; d – efficacy testing of VT against MRSA.

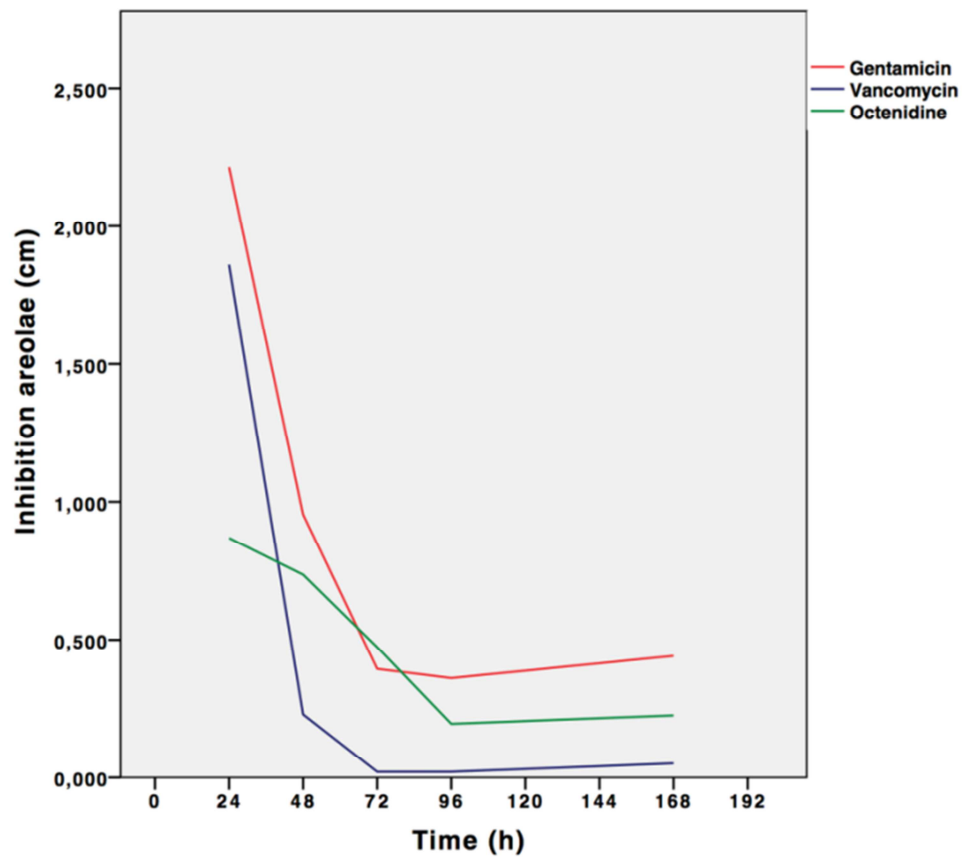


Figure 4. Anti-microbiological efficacy separated by agents (GP, OL and VT) over a period of one to seven days.

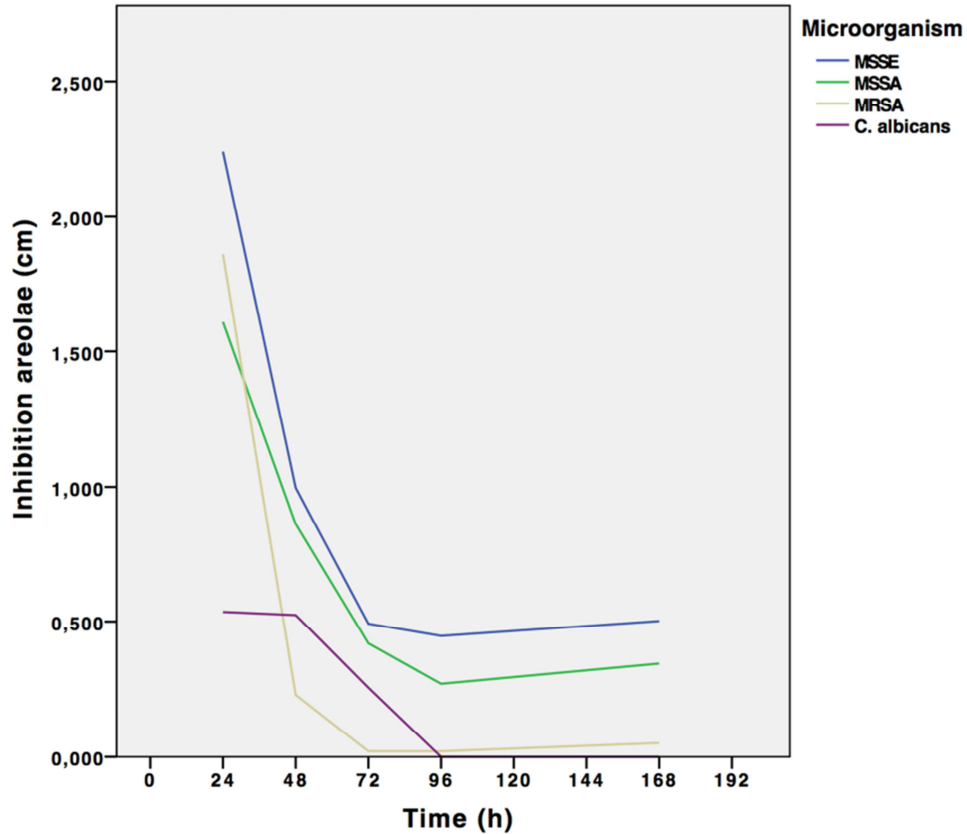


Figure 5. Microbiological testing of growth inhibition of microorganisms (MSSE, MSSA, MRSA, C. albicans) over a period of one to seven days.

4. Discussion

In this study, it was aimed to assess a perioperative application of antibacterial-coating to orthopedic prostheses for prevention and treatment of PJI, which represents a major issue in orthopedic surgery. According to projections of demography, total joint replacements will continue to increase in future years [30]. In turn, this will lead to more complications, especially regarding the rates of PJI, and to a massive burden for patients and healthcare systems. Increasing costs for PJI-treatment are estimated over more than 1.6 billion dollars by 2020 in the USA [31]. Functional, secure and reliable local anti-infective methods in cementless arthroplasty could provide mutual benefit.

Modification of prosthetic surfaces should provide functional bone ingrowth and adequate biocompatibility [16]. Various concepts for anti-infection in uncemented prosthetic surgery have been pursued, to potentially prevent or treat PJI. Anti-adhesive surface modification using cross-linked albumin, or titanium nanotubes, may prevent bacterial colonization, but may also affect bone ingrowth [16, 32]. The nano-structure of metals, especially silver, inhibits bacteria due to unspecific interaction with cell walls. Inhibition of human cell growth is referred to the same mechanism leading to reduced biocompatibility [21, 33, 34]. Antibiotic or antiseptic agents bonded to or substituted in fatty acids can restore adequate local anti-infective function with minor risk of reduced biocompatibility. Various studies show adequate in vitro and in vivo biocompatibility, as well as comparable and safe anti-infective function as ALBC [24, 25, 27-29, 35].

In the present study in vitro testing of anti-infective coatings shows anti-infective efficacy over a period of two to seven days of testing. Anti-infective agents bonded to fatty acids show prolonged emission behavior. Proved by the present findings, related studies show an initial agent boost, followed by a slower emission of agent over a longer period by degradation of the lipid layer [27, 35]. This behavior can provide a reduction of perioperative local bacterial contamination that may be a cause for early PJI [25].

ALBC supports modularity in local anti-infective prevention and treatment. Special requirements, like potential antibiotic resistance of detected bacteria, or high infection risk of patients through obesity or extended operation time, can be fulfilled by adjusting agent and concentration of ALBC [15]. In this study application of anti-infective coating to non-cemented prostheses was performed under simulated operative conditions, to test a potential and modular perioperative application. Dipping coating provided safe and reproducible usage. Spraying-coating provided reproducible results with minor safety of application through contamination of area by spreading of coating solution. According to these findings, and findings of comparable studies, dipping can potentially support perioperative coating in uncemented prosthetic surgery, with the necessity of a standardized coating procedure [36].

Pure antibiotic concentrations used in this study were comparably lower than in prior studies analyzing anti-microbiological efficacy in vitro. In the present study, coating of dedicated areas of prosthetic surfaces provided correct detection of concentration. Testing of GP showed an efficacy of the entire period of testing, as described in comparable prior studies. VT showed an anti-microbiological effect of two days in particular low concentrations. This effect may refer to low concentrations as well as the molecular structure of vancomycin, which, due to its hydrophilicity, refuses ionic bonding to fatty acids [24, 37].

According to current literature, this study could prove the first ever in vitro efficacy of OL in coating of non-cemented prostheses. Efficiency could be detected over a period of seven days against MSSA. Under the aspect of a potential co-infection with fungal microorganisms in complicated PJI, testing against *C. albicans* showed efficacy over a period of three days. Further detection of inhibition areolae after three days showed areas of reduced microorganism growth.

Coating of diverse uncemented primary and revision prosthetic material in this study represents a broad potential field of use. A limitation of this study represents the lack of standardized microbiological assessment. Due to the pilot character of the study and the variety of tested material, different coatings and different pathogens a standardized procedure was not feasible. The chosen areola inhibition testing represents and monitors a qualitative and quantitative result of in vitro activity of used agents. This method does not provide standardized quantitative results of antimicrobial activity and therefore represent a limitation of the study. Further, there is a lack of application of every coating to every surface. It was aimed to selected an application of GP and OL to primary prosthetic material and VT to revision prosthetic material, referring to the agent's indications in primary and revision prosthetic surgery. This fact limits the comparability of the resulted microbiological inhibition. The setup of the study with in vitro testing and the use of PBS-diffusion series may not represent in vivo tissue properties. Further, for detection of surface concentration of agent, we coated only predefined areas of the prostheses. These test settings could potentially limit the result of coating efficiency and may not represent the antimicrobial effect in vivo.

5. Conclusion

Anti-infective coatings using anti-microbiological agents bonded to or diffused in fatty acids can reproducibly be performed using dipping or spraying method. Dipping coating method shows superior results compared to spraying in application due to safe and reliable application and may potentially be chosen for perioperative coating.

Further, low concentration of anti-infective agents in fatty acid coating shows in vitro inhibition of microorganisms over a period of two to seven days.

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