

Cite this: *Mater. Adv.*, 2025,  
6, 2423

# A biocompatible polylactide- $\epsilon$ -caprolactone polymer coated with poly(hexamethylene biguanide) displays antibacterial properties against slime-producing *S. epidermidis*<sup>†</sup>

Paula Zwicker,<sup>†</sup> Melanie Hornschuh,<sup>‡</sup> Thomas Schmidt,<sup>a</sup> Jan Schäfer,<sup>†</sup> Carsten Becker-Willinger,<sup>d</sup> Marlon Jochum,<sup>d</sup> Axel Kramer<sup>a</sup> and Gerald Müller<sup>a</sup>

**Introduction:** produced by renewable resources, biodegradable polymers with their competitive mechanical properties, thermal stability and biocompatibility are important alternatives to other synthetic materials for use in medical devices, *i.e.* endotracheal suction catheters. However, infected catheters may lead to nosocomial infections, such as lower respiratory tract infections, with mechanical ventilation being a major risk for these. Antimicrobially coated endotracheal suction catheters may be one measure to reduce this risk. **Methods:** two procedures using ethanol and sodium hydroxide were tested to immobilize poly(hexamethylene biguanide) (PHMB) to polylactide- $\epsilon$ -caprolactone (PLA- $\epsilon$ -CL). The cytocompatibility of the coating was verified *via* the MTT assay and cytokine analysis in a cell monolayer and in a 3D mucosa model. The antimicrobial efficacy was tested using *S. epidermidis*; after this bacterial contamination and the adherence and viability of cells were tested. Chemical surface analysis has been performed with pristine and PHMB-coated specimens by means of infrared spectroscopy (ATR-FTIR). **Results:** with both applied coating procedures, PHMB could be immobilized onto the PLA- $\epsilon$ -CL surface. The biocompatibility of PLA- $\epsilon$ -CL was not impaired by the PHMB coating. IL-1 $\alpha$  was slightly but significantly increased. Reduction of *S. epidermidis* was about 4 lg-levels after 6 h of incubation. Contamination of the surface prior to cell culture did not impair the adherence of the cells. **Conclusion:** we demonstrated that PLA- $\epsilon$ -CL coated with PHMB has good biocompatible properties with antimicrobial activity thus revealing the polymer to be a suitable material for the development of medical devices that are able to prevent bacterial contaminations and infections.

Received 7th January 2025,  
Accepted 6th March 2025

DOI: 10.1039/d5ma00013k

rsc.li/materials-advances

## 1. Introduction

Produced by renewable resources, biodegradable polymers with their competitive mechanical properties, thermal stability and biocompatibility are important alternatives to synthetic materials for use in medical devices, especially in regard to sustainability.<sup>1,2</sup> Polylactide (PLA) is one of the main biodegradable polymers used in biomedical research and applications such as surgical plates,

suture yards, suture anchors, pins, wires, arrows, darts, wedges, washes, buttons and screws.<sup>3–6</sup> Since PLA is bioabsorbable, its use is however limited to applications requiring removal or degradation of the device. However, bacteria can also adhere to biodegradable materials resulting in biofilm formation with the development of an extracellular matrix, protecting the bacteria from the host's immune system and antibiotics.<sup>7</sup> Once infected, the medical device has to be removed and further antibiotic therapy may follow.<sup>8</sup>

Infected medical devices may be responsible for nosocomial infections *i.e.* such as lower respiratory tract infections, leading to patient discomfort, prolonged hospitalization, increased medication administration, and even increased morbidity.<sup>9,10</sup> Mechanical ventilation is a major risk since bacteria can be dispersed to the lower respiratory tract. Antimicrobially coated endotracheal suction catheters may be one measure to reduce the risk for ventilator-associated pneumonia.

To prevent bacterial contamination, various coating strategies of material surfaces, among other things for the modification of

<sup>a</sup> Institute of Hygiene and Environmental Medicine, Ferdinand-Sauerbruch-Str., University Medicine, D-17475 Greifswald, Germany.

E-mail: paula.zwicker@med.uni-greifswald.de; Tel: +49 (0)3834/86-4830

<sup>b</sup> Leibniz Institute for Plasma Science and Technology (INP e.V.), D-17489 Greifswald, Germany<sup>c</sup> VSB - Technical University of Ostrava, Ostrava-Poruba, Czech Republic<sup>d</sup> INM – Leibniz-Institut für Neue Materialien gGmbH, D-66123 Saarbrücken, Germany<sup>†</sup> Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d5ma00013k><sup>‡</sup> All these authors contributed equally to this work.

polymers,<sup>11</sup> have been developed.<sup>9,10,12–15</sup> In general, two types of antimicrobial coated surfaces can be distinguished: (I) active materials that can attack microorganisms at the cell wall, metabolism, or genome by releasing antibiotics or by irreversibly immobilized biocidal ingredients killing microorganisms upon contact, and (II) passive materials that prevent microbial attachment by a modified surface structure.<sup>16,17</sup> Active materials mainly comprise devices provided with quaternary ammonium compounds, antibiotics, antimicrobial peptides and antiseptics,<sup>18–21</sup> and passive devices comprise, for example, superhydrophobic surfaces.<sup>22,23</sup>

In previous works, we already demonstrated the efficacy of poly(hexamethylene biguanide) [PHMB],<sup>24–26</sup> a cationic polymer with a broad spectrum of activity against Gram-positive and Gram-negative bacteria, fungi, yeasts, and some viruses,<sup>27,28</sup> as a possible biocompatible agent for coating implant surfaces. Furthermore, due to its high biocompatibility,<sup>29</sup> it is a possible antiseptic agent for surface modification of medicinal materials supported by its polycationic structure and its primary amino end group that enables immobilization on polymer surfaces. The aim of the present study is the immobilization of PHMB on a polylactide- $\epsilon$ -caprolactone polymer as a potential antimicrobial material for proposed clinical use, for example, as suction catheters. Therefore, the cell compatibility of the raw material as well as of the material with immobilized PHMB and the antibacterial properties were tested *in vitro*.

The surface properties were analyzed by Fourier transform infrared spectroscopy (ATR-FTIR). The antibacterial performance of the samples was tested against the biofilm producing Gram-positive *S. epidermidis*. Finally, the *in vitro* cytocompatibility of the samples was studied using mouse fibroblast L929 cells and human buccal mucosa TR146 cells.

## 2. Materials and methods

### 2.1. Characterization of the polymer material and coating

**2.1.1. Test materials, polymer test sample extracts and PHMB.** Polylactide- $\epsilon$ -caprolactone 703S test specimens (PLA- $\epsilon$ -CL; 10 × 10 × 2 mm), provided by the INM – Leibniz Institute for New Materials (Saarbrücken, Germany) and sterilized *via*  $\beta$ -radiation (Herotron E-Beam Service GmbH, Bitterfeld-Wolfen, Germany) were used. The random co-polymer is merged in a 70:30 ratio (PLA:CL).

The preparation of the PLA- $\epsilon$ -CL test sample extracts for cytotoxicity testing of the raw material was carried out under aseptic conditions in a ratio of 2 g polymer per mL cell culture medium for 24 h at 37 °C and 5% CO<sub>2</sub> under humid conditions as given in DIN EN ISO 10993-5.

A polyhexanide solution (Fagron, Germany, Lot 15C20-B06-309679) containing 20% (w/v) PHMB in water and its dilutions were used.

**2.1.2. PHMB coating procedure.** The two-step procedure A and the direct procedure B were applied to define the optimal coating procedure for immobilization of PHMB onto the PLA test sample surface (Fig. 1).

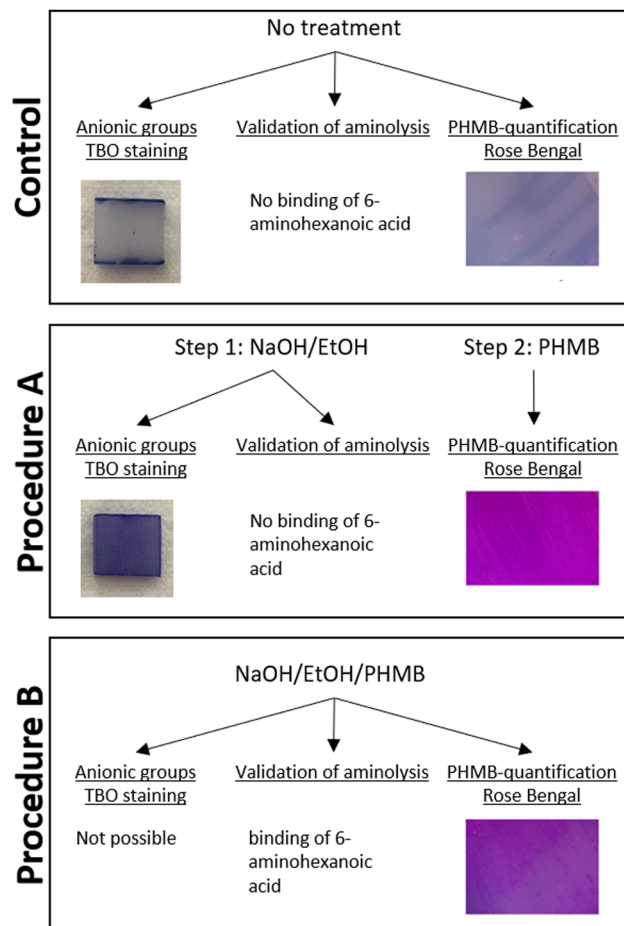


Fig. 1 Schematic overview of the treatment procedures for PLA- $\epsilon$ -CL test samples. The treatment of the control samples is shown in the upper panel, the two-step procedure A in the middle panel, and the direct treatment process B in the lower panel. Anionic surface groups were stained with toluidine blue O (TBO), validation of aminolysis was done with the surrogate substance 6-aminohehexanoic acid, and PHMB-coating was proved by using Rose Bengal (M. Hornschuh *et al.*, *Acta Biomater.*, 2020, **106**, 376–386).

**Procedure [A].** PLA- $\epsilon$ -CL disks were placed in sterile 20 mL glass vials containing 2.0 mL of solution A (0.25 M sodium hydroxide [NaOH] and 30% ethanol [EtOH], both from Carl Roth, Karlsruhe, Germany). After an incubation period of 6 h at 60 °C, the specimens were transferred in a 24-well microplate (Sarstedt, Nümbrecht, Germany) and rinsed three times with 1.5 mL of sterile deionized water under aseptic conditions. After purification of the plastic surface, incubation was carried out in 6% PHMB solution in water for 48 h.

**Procedure [B].** Immobilization of PHMB was performed in 20 mL glass vials containing 2.0 mL of solution B (0.25 M NaOH, 30% EtOH, and 6% PHMB) over a period of 6 h at 60 °C. Afterwards, the specimens were rinsed three times with 1.5 mL of deionized water and dried under aseptic conditions.

**2.1.3. Anionic functional group staining.** Anionic functional groups present on the polymer surface after hydrolysis (procedure A) were detected by incubating the test specimens



with the toluidine blue O reagent (TBO, pH10, Merck KGaA, Darmstadt, Germany).<sup>30,31</sup> After hydrolysis in NaOH/EtOH, the specimens were placed in a 24-well microplate and incubated with 1 mL of TBO (0.5 mM) solution for 5 h at room temperature while shaking at 200 rpm. After incubation, the test specimens were rinsed three times with 2.0 mL of NaOH (1 mM) solution until no more dye could be removed. For qualitative evaluation, the stained disks were evaluated by stereomicroscopy (Nikon SMZ 18).

**2.1.4. Rose Bengal method.** Adsorbed PHMB on the specimens' surface was quantified *via* Rose Bengal staining (ROS) as described earlier.<sup>32</sup> Test specimens were placed in a 24-well microplate and covered with 2.5 mL of Rose Bengal solution (0.0173 mM with 0.4% sodium acetate, both from Merck KGaA, Darmstadt, Germany). The microplate was covered with adhesive foil and incubated for 24 h at room temperature protected from light. Then, the test specimens were removed and rinsed once with 1.5 mL of deionized water while shaking at 400 rpm. The purplish red colored specimens were qualitatively assessed *via* stereomicroscopy. Quantitatively, the residual dye concentration in the supernatant after staining was determined spectrophotometrically at its absorption maximum at 549 nm (PowerWave XS, BioTek Instruments GmbH, Vermont, USA). Potentially released combinations of PHMB with Rose Bengal were measured at 576 nm; the absorption maximum of the complex was built up by PHMB and Rose Bengal. For the calculation of the amount of dye consumption, a calibration curve at 549 nm was applied.<sup>32</sup> The content of bound PHMB was investigated also by using a calibration curve.

**2.1.5. Binding of 6-aminohexanoic acid to the specimen surface.** The primary amino end groups of both PHMB and 6-aminohexanoic acid are suited for the aminolysis of ester groups present on the surface of PLA creating amide-links. For the validation of aminolysis, the surrogate substance 6-aminohexanoic acid was applied.

Similar to procedure B, test specimens were placed in 500  $\mu$ L of a mixture of 30% (v/v) ethanol and 0.25 mM NaOH with the addition of 1 mg mL<sup>-1</sup> 6-aminohexanoic acid (Carl Roth, Karlsruhe, Germany). After incubation for 6 h at 60 °C, specimens were removed and remaining primary amino groups in the supernatant were quantified with 2,4,6-trinitrobenzene sulfonic acid (TNBS) using a calibration curve of 6-aminohexanoic acid.<sup>33</sup> An amount of 100  $\mu$ L of supernatant was transferred to a 24-well microplate and 5  $\mu$ L of TNBS (Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) assay reagent was added. The assay reagent was prepared by mixing 700  $\mu$ L of TNBS stock solution (50 mg mL<sup>-1</sup>) with 300  $\mu$ L of water, followed by the addition of 95.4 mg of disodium tetraborate decahydrate (Sigma-Aldrich/Merck KGaA, Darmstadt, Germany).<sup>33</sup> After incubation for 10 min at room temperature protected from light, 900  $\mu$ L of a monobasic potassium phosphate solution (0.2 M, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) containing potassium sulfite (4 mM, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) supplemented immediately before the experiment, was added to the reaction mixture. Absorbance was measured at 416 nm.

**2.1.6. ATR-FTIR.** Samples have been characterized using ATR-FTIR spectroscopy (attenuated total reflection Fourier-transform infra-red spectroscopy) with the spectrum one spectrometer (PerkinElmer, USA) and related software Spectrum v5.3.1 and OriginPro 2022b at the Leibniz Institute for Plasma Science and Technology (Greifswald, Germany). The method was employed to evaluate the chemical functional groups of untreated PLA- $\epsilon$ -CL and PHMB-PLA- $\epsilon$ -CL samples after procedure A (PLA- $\epsilon$ -CL-A) and after procedure B (PLA- $\epsilon$ -CL-B). The spectra were collected in the range of 700 to 4000 cm<sup>-1</sup> using a diamond ATR crystal, with a resolution of 4 cm<sup>-1</sup> and an accumulation number of 11.

## 2.2. Biocompatibility tests

**2.2.1. Cell culture.** Mouse fibroblast L929 cells (ZVB/FLI, Greifswald-Insel Riems, Germany) were cultured in Minimum Essential Medium with Earle's salts (MEM, PAN-Biotech, Aidenbach, Germany), supplemented with 5% fetal bovine serum (FBS, Life Technologies, Karlsbad, CA, USA) and 2 mM L-glutamine (PAN-Biotech, Aidenbach, Germany), in a humidified atmosphere of 5% CO<sub>2</sub> and 95% RH at 37 °C. Human buccal mucosa TR146 cells (ECACC, Wiltshire, UK) were cultured in Dulbecco's modified Eagle medium/Ham's F-12, 1:1 mix (DMEM/F12, c.c. pro, Oberdorla, Germany), supplemented with 5% FBS and 2 mM L-glutamine, in a humidified atmosphere. The cells were harvested at 70–80% confluence using trypsin 0.05%/EDTA 0.02% (PAN-Biotech, Aidenbach, Germany). After centrifugation, the cell pellet was resuspended in the respective medium and adjusted to the necessary cell density.

**2.2.2. Cytotoxicity testing (DIN EN ISO 10993-5)**<sup>34</sup>. For cytotoxicity testing, the L929 cell density in the suspension was adjusted to  $1 \times 10^5$  cells per mL in cell culture medium. Subsequently, 100  $\mu$ L of this suspension were transferred to each cavity of a 96-well plate (Sarstedt, Nümbrecht, Germany) and cultured for 24 h under a humidified atmosphere at 37 °C. After decanting the supernatant, 100  $\mu$ L of polymer test sample extract of the raw material (pristine PLA- $\epsilon$ -CL) and its serial dilutions were added per cavity with six technical replicates each. Cells were cultured for further 24 h under cell culture conditions. The cell viability was analyzed by using the thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) assay and neutral red (NR, Honeywell International Inc. Fluka, Mosbach, Germany) uptake assay. Therefore, the test sample extract was decanted and the cells were washed with 100  $\mu$ L of phosphate buffered saline with Ca<sup>2+</sup> and Mg<sup>2+</sup> (wPBS). After washing, 100  $\mu$ L of MTT medium (0.5 mg mL<sup>-1</sup>) or neutral red solution (0.01%) in cell culture medium were added per well following incubation for 3 h. Afterwards, cells were washed once with wPBS and the dye was solubilized by the addition of 100  $\mu$ L of 2-propanol/HCl (0.04 M HCl) for the MTT assay or 150  $\mu$ L of EtOH/HAc (50% EtOH, 1% HAc) for the NR assay. Absorption was measured at 570 nm (MTT) or 540 nm (NR).

**2.2.3. 3D oral mucosa model.** The assessment of the irritation *in vitro* was performed on a reconstructed 3D oral mucosa model prepared as described before.<sup>35</sup> In short, cells with a



density of  $1 \times 10^6$  cells per mL were seeded in cell culture inserts (Millicell, 12 mm diameter, Merck-Millipore, Massachusetts, USA) and placed in 6-well cell culture plates. After three days in submerged culture, the cells on the membrane of the inserts were dried for 10 min under aseptic conditions and an air-lift culture of these cells followed with only basolateral medium supply for a total of 12 days.

To ensure that the multilayered oral mucosa models have grown correctly, measurement of the transepithelial electric resistance (TEER, EVOM World precision instruments) was used. After washing the cells in the inserts with 500  $\mu$ L of wPBS, they were placed in 24 well cell culture plates filled with 600  $\mu$ L of wPBS. Then, 400  $\mu$ L of wPBS were added to the inserts and the TEER was measured. Following the successful growth of multilayered oral mucosa models, 50  $\mu$ L of PLA- $\epsilon$ -CL test sample extract were applied. The test sample extracts were used both undiluted and 50% diluted in DMEM/F12. As the positive control, 1% Triton™ X-100 (Sigma-Aldrich/Merck KGaA, Darmstadt, Germany) was used. The inserts were placed in 1.5 mL of fresh medium and after incubation under cell culture conditions for 24 h, the surrounding medium was collected and stored at  $-20^\circ\text{C}$  for cytokine measurements. Again, 1.5 mL of fresh medium were added per well and the test sample extracts were removed from the mucosa tissue. After further incubation for 18 h without test and/or control solutions, the media were repeatedly collected for the quantitative determination of cytokines and stored at  $-20^\circ\text{C}$ . ELISA-kits (PeproTech, Hamburg, Germany) were used for the quantification of interleukin (IL)-1 $\alpha$ , IL-6 and TNF $\alpha$  in the supernatants following the instructions of the manufacturer.

The TEER was measured again after the removal of the test sample extracts to detect possible damage to the diffusion barrier. Evidence of the resulting viability of the epithelial cells was obtained after incubating the 3D models with MTT solution for 4 h by adding 500  $\mu$ L of MTT medium to a 24-well cell culture plate. Elution of the dye was performed in 2 mL of EtOH/HCl (0.04 M HCl) for 18 h at  $4^\circ\text{C}$ .

### 2.3. Cell adherence test

The cell adherence of human buccal mucosa cells (TR146) was tested on pristine PLA- $\epsilon$ -CL specimens and specimens with immobilized PHMB (PLA- $\epsilon$ -CL-A/B-). The cells were harvested as described before and adjusted to a final concentration of  $2 \times 10^6$  cells per mL in cell culture medium. Of this suspension, 50  $\mu$ L were applied to the specimens' surfaces and incubated for 6 h under cell culture conditions. Thereafter, the specimens were transferred to 1 mL of cell culture medium to remove non-adherent cells from the surface. The viability of adherent cells was proofed by an XTT-based assay (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; AppliChem, Darmstadt, Germany). For that, 500  $\mu$ L of XTT-reagent (containing 1 mg mL $^{-1}$  XTT and 0.025 mM phenazine methosulfate (*N*-methylphenazonium methyl sulfate), AppliChem, Darmstadt, Germany) was added per well already containing 1 mL of fresh cell culture medium. After 3 h of incubation at  $37^\circ\text{C}$ , the absorption of the XTT-medium was measured at 450 and 650 nm spectrophotometrically.

Non-adherent cells in the supernatant were detected by the use of an ATP assay (Promega, Wisconsin, USA). The assay was performed as described by the manufacturer. Briefly, 100  $\mu$ L of cell suspension was mixed with 100  $\mu$ L of BacTiter-Glo™ reagent (Promega, Wisconsin, USA). Subsequently, luminescence was determined in triplicates in a TriStar LB 941 (Berthold Technologies, Bad Wildbad, Germany) using white 96-well microplates (Berthold Technologies, Bad Wildbad, Germany). Microscopic evaluation was performed after fixation and actin staining of the adhered cells using Alexa Fluor™ 488 Phalloidin (Thermo Fisher Scientific, Waltham, MA, USA) with a confocal laser scanning microscope (LSM510 Exciter, Carl-Zeiss, Oberkochen, Germany) as previously described.<sup>24</sup>

### 2.4. Antibacterial testing

**2.4.1. Bacterial culture.** Cryo-stored *S. epidermidis* (ATCC 35984) was plated onto casein-soy-peptone-agar (CSA, Carl Roth, Karlsruhe, Germany) and incubated for 24 h at  $37^\circ\text{C}$ . One day before the experiment, a second subculture was plated on CSA and incubated for 24 h at  $37^\circ\text{C}$ . The bacteria were harvested with 1.5 mL of wPBS, centrifuged (13 000 rpm, 3 min) and rinsed with 1 mL of 1% Triton™ X-100. Two further centrifugation and washing steps with wPBS followed. Last centrifugation was done with 5500 rpm for 3 min. Afterwards, the bacterial pellet was resuspended in 1 mL of co-culture-medium (DMEM/F12, 10% tryptone-soy-bouillon (TSB), 5% FBS, and 2 mM L-glutamine) and vortexed for 10 s.<sup>25</sup>

**2.4.2. Bacterial reduction assay.** Test specimens (without and with immobilized PHMB) were arranged into a Petri dish (Sarstedt, Nümbrecht, Germany) and inoculated with 50  $\mu$ L of *S. epidermidis* suspension containing about  $1\text{--}5 \times 10^3$  colony-forming units (cfu) per mL. The suspension was evenly spread on the surface of the specimens using an inoculation loop. The Petri dish was placed in the incubator at  $37^\circ\text{C}$  for 6 h using a humidified chamber to prevent dehydration. For quantification, adherent bacteria were resuspended by shaking the test specimens in 1 mL of inactivator solution (30 g of Tween 80 [AppliChem GmbH, Darmstadt, Germany], 30 g of saponin, 1 g of L-cysteine [both from Sigma-Aldrich/Merck KGaA, Darmstadt, Germany], 1 g of L-histidine [Carl Roth GmbH + Co. KG, Karlsruhe, Germany], 1000 mL A. dest.) on sterile glass beads ( $42.85\text{--}3.45 \pm 0.3$  mm diameter; Carl Roth, Karlsruhe, Germany) on an orbital shaker (400 rpm) for 2 min. In the next step, the antibacterial activity of PHMB had to be inhibited. For that, the bacteria were incubated in inactivator solution for five minutes. Afterwards, serial dilutions were prepared in inactivator solution and 0.1 mL of each dilution was plated in duplicate on CSA plates. The cfu of the test microorganisms were counted after 48 h of incubation at  $37^\circ\text{C}$ . The log<sub>10</sub> (lg) microbicidal reduction for each contact time was calculated according to the formula:

$$\lg \text{ microbial reduction} = \lg_{\text{nc}} - \lg_{\text{nd}}$$

where nc is the number of cfu of viable cells on the control surface and nd is the number of cfu of viable cells after contact with the PHMB-immobilized surface. All antibacterial efficacy tests were conducted at least in triplicate.



**2.4.3. Contaminated cell culture assay.** For contamination of the test specimens with *S. epidermidis*, the bacterial suspension was adjusted to  $1-5 \times 10^5$  cfu per mL in co-culture medium. The test specimens were placed in sterile Petri dishes and 50  $\mu$ L of the bacterial suspension were applied followed by incubation at 37 °C for 6 h in a humidified atmosphere. Afterwards, the supernatant was removed and 50  $\mu$ L of a TR146 cell suspension in DMEM/F12 with a density of  $2 \times 10^6$  cells per mL were spread on the test specimen following further 6 h of incubation under cell culture conditions. Next to the PLA- $\epsilon$ -CL-A/B test specimen, non-treated uncontaminated PLA- $\epsilon$ -CL discs were used as a reference. Afterwards, the test specimens were transferred to a 24-well plate containing 1 mL of fresh cell culture medium in each well. Non-adhering cells were determined by the ATP assay as already described. PLA- $\epsilon$ -CL specimens with adherent TR146 cells were transferred to a new 24-well plate and actin filaments were stained with Alexa Fluor® 488 conjugated phalloidin as described previously<sup>24</sup> and qualitatively evaluated with a confocal laser scanning microscope (LSM510 Exciter, Carl-Zeiss, Oberkochen, Germany).

### 2.5. Statistical analysis

For testing statistical significance, GraphPad Prism 9.4.1 was used. One-way ANOVA tests were followed by Dunnett's multiple comparison.

## 3. Results

### 3.1. Characterization of the test specimens and PHMB immobilization

PHMB was immobilized on PLA- $\epsilon$ -CL specimens in two different ways; procedures A and B (Fig. 1). PLA- $\epsilon$ -CL-A specimens were tested for anionic groups on their surface *via* TBO staining. They showed a uniform blue staining of the specimen surface indicating the presence of anionic groups (Fig. 2 and 3) in contrast to control specimens.

To determine the amount of adsorbed PHMB to the specimens' surface, Rose Bengal staining was used.<sup>25</sup> Both PLA- $\epsilon$ -CL-A and PLA- $\epsilon$ -CL-B showed coloration after Rose Bengal

treatment (Fig. 2). Procedure B allowed the immobilization of higher amounts of PHMB, thus darker coloring of the test specimen occurred. Control surfaces only show negligible staining. The amount of Rose Bengal in the supernatant was spectrophotometrically measured and adsorbed dye molecules were calculated in relation to the initial solution. Referring to this, PLA- $\epsilon$ -CL-A adsorbed about  $7.6 \pm 3.3 \mu\text{g cm}^{-2}$  ( $n = 3$ ) of PHMB, whereas PLA- $\epsilon$ -CL-B specimens adsorbed  $23.65 \pm 1.5 \mu\text{g cm}^{-2}$  ( $n = 3$ ) of PHMB (Table 4). The non-treated surface did not enable PHMB immobilization.

The binding of PHMB to the PLA- $\epsilon$ -CL surface using procedure B was proved by the use of 6-aminohexanoic acid as a surrogate substance (primary amino group) for PHMB (Fig. 3). The amount of 6-aminohexanoic acid in the supernatants was quantified with the TNBS assay. Comparing the concentration of 6-aminohexanoic acid in the solution before and after the treatment of the specimens, on average  $248.9 \pm 43.8 \mu\text{g cm}^{-2}$  of 6-aminohexanoic acid was immobilized ( $n = 4$ ). Using procedure A, no 6-aminohexanoic acid was adsorbed by the surface.

The IR spectroscopy results (Fig. 4) revealed a characteristic fingerprint region. All samples exhibit fundamental absorption bands specific to PLA- $\epsilon$ -CL. Particularly, bands at  $1755 \text{ cm}^{-1}$  (C=O stretching),  $1453 \text{ cm}^{-1}$  (C-H scissoring),  $1356 \text{ cm}^{-1}$  (C-H rocking),  $869 \text{ cm}^{-1}$  (C-H bending),  $753 \text{ cm}^{-1}$  (C-H wagging), and an interval of several caprolactone stretching modes ( $1208-1041 \text{ cm}^{-1}$ , C-O-C) were observed. The sample treated with procedure A showed a high similarity to the pristine PLA- $\epsilon$ -CL material, confirming the finding that procedure A leads to minimal coverage of PLA- $\epsilon$ -CL by PHMB. On the other hand, procedure B generates a sample with dramatic changes in the vibrations of the caprolactone structures. The intensity of bands within  $1208-1041 \text{ cm}^{-1}$  changes and shifts to higher wavenumbers indicating new bonding states in the polymer network. Additionally, there is an increased band at  $1755 \text{ cm}^{-1}$ , indicating strengthened C=O bonds in the PLA- $\epsilon$ -CL-B sample. These results serve as indirect but significant indicators that PHMB has been deposited on PLA- $\epsilon$ -CL. Note that the direct observation of the NH absorption bands was not successful with this method. Also, the NH vibrations were not clearly detectable due to their lower concentration and intensity compared to the vibrational modes of the basic PLA- $\epsilon$ -CL material.

### 3.2. Biocompatibility of PLA- $\epsilon$ -CL specimens

To determine possible cytotoxic properties of the pristine material PLA- $\epsilon$ -CL, MTT and NR uptake tests with L929 mouse fibroblast cells were performed according to DIN EN ISO 10993-5 using extracts of the polymer material. Both tests revealed that the polymer test sample extract has no cytotoxic effect on L929 cell monolayers (Table 1). Compared with the control, a reduction of cell viability to  $87.5 \pm 1.8\%$  in the MTT assay was shown when the cells were treated with the undiluted polymer test sample extract (Table 1). In the NR uptake assay, cell viability was about  $105.0 \pm 8.9\%$  (Table 1). Viability of cells treated with the diluted polymer test sample extract was equal to the viability of cells treated with the undiluted test sample extract.

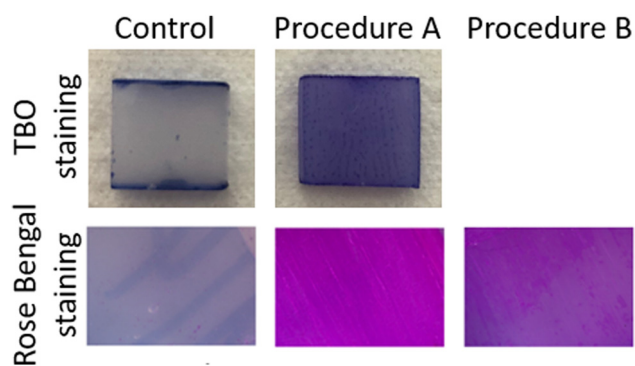


Fig. 2 Representative image of staining anionic groups after the hydrolysis of test specimens in NaOH/EtOH (top row) and of staining specimens after procedure A and B with Rose Bengal for the quantification of immobilized PHMB (bottom row).



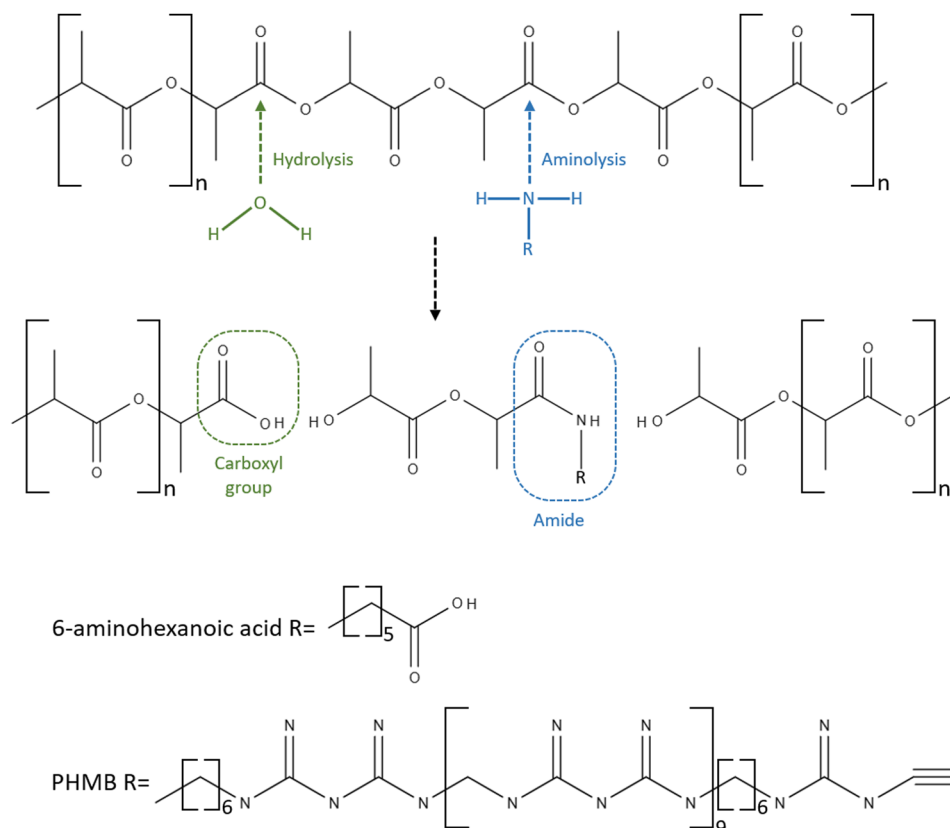


Fig. 3 Reaction of PHMB with the PLA- $\epsilon$ -CL surface when using procedure A or B. Using procedure A, carboxylic groups after hydrolysis enable PHMB immobilization, whereas using procedure B, PHMB is tethered via an amide group to the PLA- $\epsilon$ -CL surface after aminolysis.

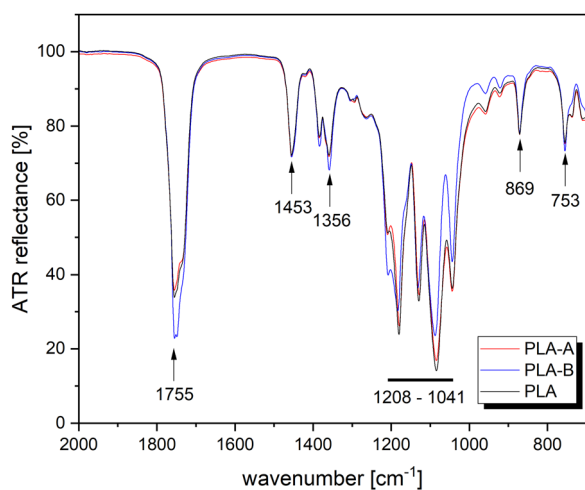


Fig. 4 Characteristic ATR-FTIR spectra in the fingerprint region: reference sample PLA- $\epsilon$ -CL (PLA) and PLA- $\epsilon$ -CL coated with PHMB using procedures A and B (PLA-A and PLA-B, respectively).

TR146 cells cultured directly on the surface of pristine PLA- $\epsilon$ -CL specimens exhibited an adhesion rate of about  $90.4 \pm 1.3$  (Table 2 left). Comparable amounts of cells adhered on surfaces with immobilized PHMB (Table 2). The microscopic evaluation revealed a dense cell structure, evenly spread over the whole area (Fig. 5).

Table 1 Viability of L929 cells after treatment with the test sample extract of pristine PLA- $\epsilon$ -CL for 24 h under cell culture conditions measured by MTT and neutral red (NR) uptake assays. As positive controls, SDS in different concentrations ( $50$ ,  $75$ ,  $100$ , and  $125 \mu\text{g mL}^{-1}$ ) was used. Viability was calculated in percent (%)  $\pm$  SD relative to untreated L929 cells,  $n = 7$

| Test solution                 | Viability [%] using MTT assay | Viability [%] using NR assay |
|-------------------------------|-------------------------------|------------------------------|
| Positive control              |                               |                              |
| $50 \mu\text{g mL}^{-1}$ SDS  | $105.8 \pm 10.8$              | $92.6 \pm 3.0$               |
| $75 \mu\text{g mL}^{-1}$ SDS  | $82.7 \pm 7.3$                | $35.7 \pm 17.3$              |
| $100 \mu\text{g mL}^{-1}$ SDS | $4.9 \pm 1.3$                 | $5.2 \pm 2.5$                |
| $125 \mu\text{g mL}^{-1}$ SDS | $0.8 \pm 0.4$                 | $4.7 \pm 5.2$                |
| Polymer test sample extract   |                               |                              |
| 100%                          | $87.5 \pm 1.8$                | $105.0 \pm 8.9$              |
| 90%                           | $82.1 \pm 1.8$                | $104.7 \pm 7.3$              |
| 80%                           | $82.1 \pm 1.8$                | $101.9 \pm 6.8$              |
| 70%                           | $84 \pm 2.0$                  | $102.7 \pm 2.3$              |
| 60%                           | $84.7 \pm 2.1$                | $101.3 \pm 3.1$              |

For testing properties regarding the cytocompatibility of the polymer test sample extracts in a more realistic condition, TR146 cells were cultured in a 3D mucosa model.<sup>35</sup> Before treatment with the polymer test sample extract, the TEER of all 3D models was measured (Fig. 6A), which resulted in values of about  $0.14 \pm 0.01 \text{ k}\Omega$ . After treatment with the undiluted polymer extract and incubation for 18 h, the mucosa model displayed a TEER of  $0.29 \pm 0.06 \text{ k}\Omega$  (Fig. 6B). The TEER value of



**Table 2** Summarized results for PHMB-immobilization, antibacterial efficacy and TR146 cell adherence after the cell adherence test and the contaminated cell culture assay on specimens without and with PHMB immobilization using procedure A and B. The amount of adhered TR146 on the surface of treated and untreated PLA- $\epsilon$ -CL specimens was measured by ATP and is expressed in percent  $\pm$  SD; *N* is the number of independent experiments

| Specimen                      | Cell adherence [%]                 |   |
|-------------------------------|------------------------------------|---|
|                               | Cytotoxicity assay<br><i>N</i> = 4 | Contaminated cell culture assay<br><i>N</i> = 4 |
| PLA- $\epsilon$ -CL = control | 90.4 $\pm$ 1.3                     | 97.5 $\pm$ 1.9                                  |
| PLA- $\epsilon$ -CL-A-        | 91.7 $\pm$ 4.9                     | 92.8 $\pm$ 8.0                                  |
| PLA- $\epsilon$ -CL-B-        | 95.8 $\pm$ 7.1                     | 94.0 $\pm$ 10.7                                 |

the negative control was about (NC; cell culture medium)  $0.27 \pm 0.05$  k $\Omega$ . The polymer test sample extract treated cells also show a slightly higher cell viability of  $127.7 \pm 12.7\%$  compared to the NC (100%). After further incubation for 24 h without the test sample extracts, both, for the control models and the treated 3D cell models, the TEER values increased to  $0.52 \pm 0.19$  k $\Omega$  and  $0.55 \pm 0.16$  k $\Omega$ , respectively (Fig. 6A). A strong decrease in the TEER values was expected due to treatment with the positive control 1% Triton<sup>TM</sup> X-100 for 30 min and 120 min (Fig. 6C).

Quantitative determination of pro-inflammatory mediators secreted by the 3D oral mucosa model was carried out by ELISA. Compared to the negative control, the concentration of IL-1 $\alpha$ , IL-6 and TNF $\alpha$  released into the culture medium was significantly increased 24 h after contact to the positive control (1% Triton<sup>TM</sup> X-100 for 30 min). In contrast, the PLA- $\epsilon$ -CL test sample extract did not evoke changes in cytokine secretion except for IL-1 $\alpha$  that was slightly but statistically significantly increased

(Table 3). TNF $\alpha$  secretion was slightly but not statistically significantly decreased.

### 3.3. Antimicrobial testing of PHMB coated PLA- $\epsilon$ -CL

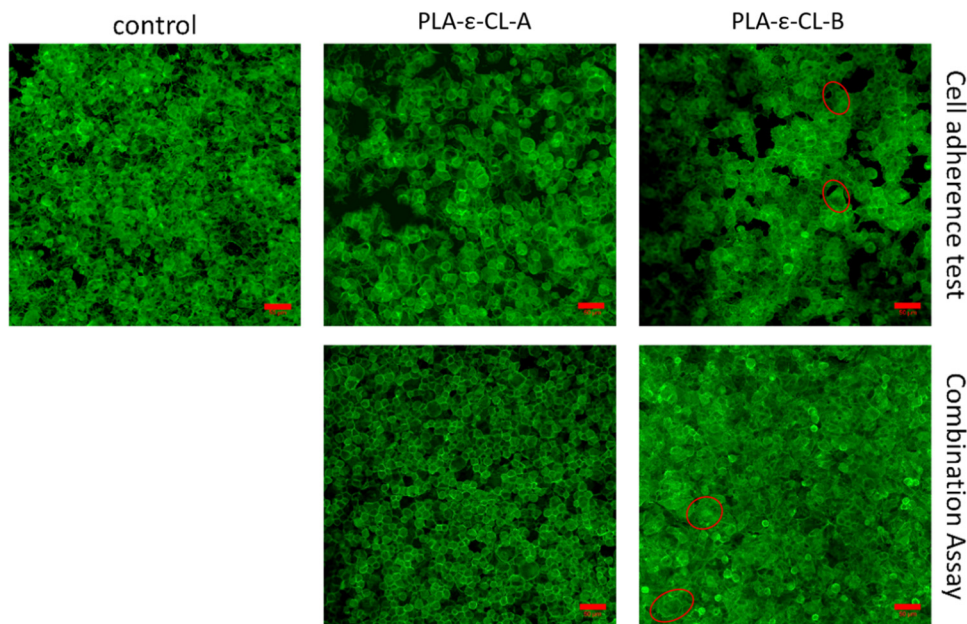
After 6 h of incubation, a statistically significant lg-reduction of  $4.3 \pm 0.4$  ( $p < 0.0001$ ,  $n = 6$ , PLA- $\epsilon$ -CL-A) or rather  $4.4 \pm 0.5$  ( $p < 0.0001$ ,  $n = 8$ , PLA- $\epsilon$ -CL-B) of *S. epidermidis* on PLA- $\epsilon$ -CL specimens with adsorbed PHMB was determined (Table 4). At the same time, on control specimens (PLA- $\epsilon$ -CL) without immobilized PHMB, growth of  $4.1 \pm 0.6$  lg ( $n = 21$ ) of bacteria was detected.

The combined assay of bacterial culture and cell culture was used to determine the cell proliferation after the bacterial contamination of the PLA- $\epsilon$ -CL-A/B- surfaces. After 6 h of incubation with *S. epidermidis*, TR146 cells were added to the specimens and adherence was tested after further cultivation for 6 h. On control surfaces without bacterial contamination,  $97.5 \pm 1.9\%$  of the TR146 cell adhered on the surface. On pre-contaminated surfaces with immobilized PHMB,  $92.8 \pm 8.0\%$  (PLA- $\epsilon$ -CL-A) and  $94.1 \pm 10.7\%$  (PLA- $\epsilon$ -CL-B) of the cells adhered (Table 2). The microscopic evaluation of the actin filament staining displayed no obvious phenotypic deviation in the number of cells on the surface between pre-contaminated PHMB-immobilized and non-treated surfaces (Fig. 5). On PLA- $\epsilon$ -CL-A, TR146 cells are rather spherical in morphology, whereas on the coated surfaces B and on the control surfaces, more stretched and flatten cells were present (Fig. 5).

## 4. Discussion

### 4.1. Immobilization of PHMB

There are various polymer surface modification techniques for the covalent or non-covalent attachment of bioactive



**Fig. 5** Representative images of microscopic evaluation of TR146 cells on PLA- $\epsilon$ -CL specimens (control) and PLA- $\epsilon$ -CL-A/B specimens with immobilized PHMB without and after previous bacterial contamination, using actin filament staining. Scale bar 50  $\mu$ m.



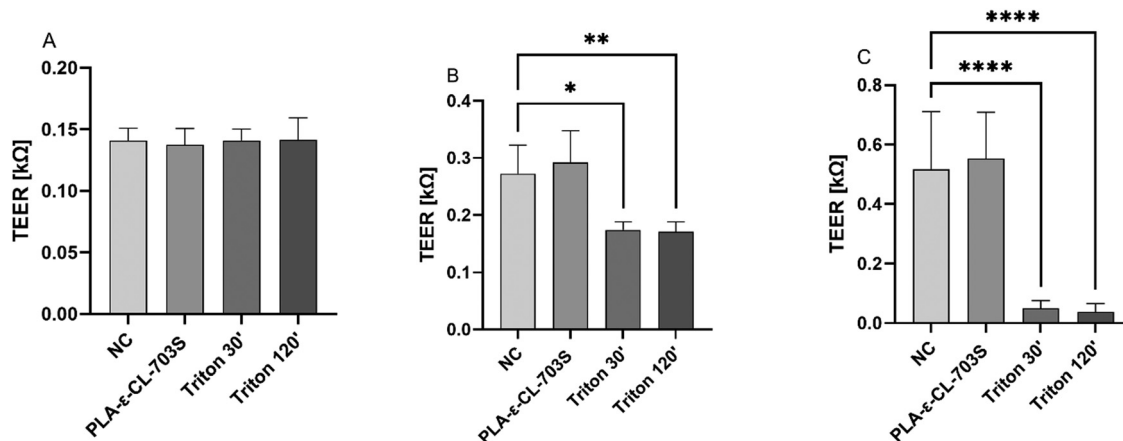


Fig. 6 TEER values (A) before the treatment of the cells with the PLA- $\epsilon$ -CL test sample extract or positive controls, (B) 18 h after treatment, and (C) 48 h after treatment. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\*\* $p < 0.0001$ .

**Table 3** Concentration of secreted cytokines in the supernatants from 3D-mucosa models treated with polymer test sample extracts for 24 h or 1% Triton X-100 for 30 min or 120 min; mean  $\pm$  SD;  $n = 3$ , \* $p < 0.05$ , \*\*\* $p < 0.001$

| Test sample               | Cell viability [%] | IL-1 $\alpha$ [pg ml $^{-1}$ ] | IL-6 [pg ml $^{-1}$ ] | TNF $\alpha$ [pg ml $^{-1}$ ] |
|---------------------------|--------------------|--------------------------------|-----------------------|-------------------------------|
| Negative control          | 100                | 29.7 $\pm$ 36.1                | 27.4 $\pm$ 23.7       | 74.8 $\pm$ 93.2               |
| PLA- $\epsilon$ -CL       | 127.7 $\pm$ 12.7   | 65.7 $\pm$ 49.0*               | 26.4 $\pm$ 0.2        | 51.9 $\pm$ 4.1                |
| 1% Triton X-100 (30 min)  | 32.3 $\pm$ 4.2     | 597.6 $\pm$ 124.9***           | 418.4 $\pm$ 134.2***  | 1469.4 $\pm$ 558.1***         |
| 1% Triton X-100 (120 min) | 17.8 $\pm$ 3.8     | 3087.13 $\pm$ 337.9***         | 2833.7 $\pm$ 341.1*** | 4295.0 $\pm$ 543.6***         |

**Table 4** Comparison of test specimens' treatment and results using procedure A and B for PHMB immobilization

|  | Without treatment          | Procedure A                              | Procedure B                              |
|--|----------------------------|--|--|
| Immobilized PHMB [ $\mu$ g per specimen]       | —                          | 7.6 $\pm$ 3.3 ( $n = 3$ )                | 23.65 $\pm$ 1.5 ( $n = 3$ )              |
| Bacterial regrowth                             | 4.1 $\pm$ 0.6 ( $n = 21$ ) | —  | —  |
| Microbial reduction <i>S. epidermidis</i> [lg] | —                          | 4.3 $\pm$ 0.4 ( $p < 0.0001$ , $n = 6$ ) | 4.4 $\pm$ 0.5 ( $p < 0.0001$ , $n = 8$ ) |

compounds<sup>36,37</sup> as binding antimicrobial agents to medical devices to avoid infections. An example is the binding of hexanediamine to PLA by previous incorporation of maleic acid<sup>38</sup> or the immobilization of polysaccharides on the PLA surface to immobilize glucosamine and chondroitin as antibacterial agents.<sup>39</sup> Sampath *et al.* compared the efficacy of antibiotics and antiseptics that were bound to catheters, figuring out that the antiseptic coating was more effective against resistant staphylococcus strains than antibiotic treatment.<sup>40</sup>

Furthermore it is already shown that PHMB is able to adsorb to various metals.<sup>26,41</sup> PHMB can also be anchored to (chemically modified) cellulose.<sup>42,43</sup> Wang *et al.*<sup>44</sup> as well as Wang and Kan<sup>42</sup> immobilized PHMB by physical adsorption on cellulose without or with the use of a binder (polyurethane derivative).<sup>45</sup>

Llorens *et al.* loaded PLA nanofibers with PHMB during electrospinning. The resulting 3D degradable scaffolds containing 0.1–0.25 wt% PHMB were antimicrobially active against *E. coli* CECT 101 and *M. luteus* CECT 245 by releasing the active agent. Biocompatible characteristics for the adhesion and proliferation of epithelial MDCK and fibroblastic MRC-5 cells<sup>46</sup> were revealed. In former studies, we already demonstrated the antimicrobial efficiency of PHMB immobilized on titanium

surfaces against *Staphylococcus* strains and *Pseudomonas aeruginosa*.<sup>24</sup>

Based on these data, we intended to immobilize the anti-septic agent PHMB onto the PLA- $\epsilon$ -CL surface with the assumption that using procedure B, the primary amino group of PHMB is tethered *via* an amide group to the PLA- $\epsilon$ -CL surface after aminolysis. The use of the primary amino end group of PHMB was already shown by other groups as Zhi *et al.* who have shown that the terminal primary amino group of PHMB is reactive with the epoxy group of allyl glycidyl ether.<sup>47</sup> Additionally, an allyloxy polyethylene glycol substituted PHMB oligomer was synthesized due to a reaction between tosyl-allyloxy polyethylene glycol and PHMB. During this process, the terminal primary amino group of PHMB substituted the tosyl group. Both allyl-substituted PHMB oligomers were used in plasma UV-assisted surface-initiated bottlebrush-like polymerization to coat a silicone rubber surface, which exhibited reusable antimicrobial and antibiofilm properties towards *E. coli* ATCC 8739, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231 and demonstrating no cytotoxicity applying human aorta smooth muscle cells CC-2571.<sup>47</sup> Chindera *et al.* have also shown that the amino end-group



is usable for modifications. They used the end-group for conjugation with FITC.<sup>48</sup>

Furthermore, the possibility to immobilize primary amino groups containing substances onto the PLA surface after aminolysis was shown by several research groups,<sup>49–52</sup> thus confirming the possibility of tethering of PHMB *via* amide groups onto the PLA- $\epsilon$ -CL surface.

Thus, we hypothesize that procedure B enables a direct immobilization of PHMB *via* the aminolysis of the PLA surface under alkaline conditions. However, it cannot be excluded that additional amounts of PHMB are adsorbed onto free carboxylic groups as a result of hydrolysis which is supported by the presence of significantly greater amounts of PHMB after the application of procedure B compared with procedure A. In the present investigation, we show that PHMB can be immobilized to a polylactide polymer by two different wet chemical processes while maintaining its antimicrobial efficacy and simultaneously being biocompatible to cells. Procedure A produces anionic groups resulting from the alkaline hydrolysis of ester groups on the polymer surface as adapted from Yang *et al.*<sup>53</sup> enabling the adsorption of the polycationic agent PHMB. Using procedure B in addition to the adsorption of PHMB to carboxylic groups, aminolysis by primary amino-groups takes place on the polymer surface<sup>49</sup> enabling the tethering of PHMB *via* amide groups. This was supported by using 6-aminohexanoic acid as a surrogate substance instead of PHMB in procedure B. In the same manner, as 6-aminohexanoic acid was shown to be immobilized on the PLA- $\epsilon$ -CL surface, the immobilization of PHMB might be possible because free primary amino end groups are available. Even if the amount of absorbed 6-aminohexanoic acid is much higher than that of absorbed PHMB, which might result from steric hindrance because of PHMB's molecular weight, the method might explain the molecular processes. The presence of terminal primary amino-groups in PHMB molecules was shown by Creppy *et al.* who revealed an occurrence of 16.5%.<sup>54</sup> In procedure A, only anionic carboxylic groups are present, which enable no binding of 6-amino hexanoic acid.

#### 4.2. Cytocompatibility and antimicrobial activity

The cytocompatibility of PHMB-coated PLA- $\epsilon$ -CL specimens was shown using L929 cells whose viability was not reduced >30% in comparison to the untreated control, thus revealing no cytotoxicity according to EN ISO 10993-5. Moreover, other studies confirm that PLA- $\epsilon$ -CL co-polymers are not cytotoxic.<sup>55,56</sup> We therefore assumed that the co-polymer PLA- $\epsilon$ -CL represents a reliable basis for the intended use.

To use a more realistic model, 3D mucosa models were applied for further testing as the coated PLA- $\epsilon$ -CL is intended to be used in suction catheters. We used the TEER value to assess the effect of the polymer test sample extract on the integrity of the tissue. When treating the 3D models of oral buccal mucosa with the undiluted polymer test sample extract, the 3D mucosa model displayed a more loosely cell bond compared to cells treated with cell culture medium. However, since the TEER value is rising after treatment, we hypothesize a recovery of the tissue marked by increasing barrier resistance.<sup>57</sup>

However, it has to be mentioned that too high amounts of PHMB can have negative effects on cell viability.<sup>24</sup>

The quantification of cytokine production shows no abnormality between PHMB-immobilized and untreated samples with the exception of interleukin 1 $\alpha$  (IL-1 $\alpha$ ) secretion. IL-1 $\alpha$  can be produced by macrophages, endothelia cells and fibroblasts and influences the skin barrier function and diverse immune reactions. In addition, IL-1 $\alpha$  is able to stimulate the production of dermis components like collagen by fibroblasts.<sup>58</sup> The increase of IL-1 $\alpha$  secretion after treatment with the PLA- $\epsilon$ -CL polymer test sample extract for 24 h may indicate a stimulation of the stabilization of the barrier function. The absence of TNF $\alpha$  secretion furthermore emphasizes that no negative effect in cell proliferation or cell differentiation occurred and inflammatory effects may be excluded. However, it has to be mentioned that due to little biological replicates, the results only reveal a trend and are not statistically secured. This is increased by the high standard deviations that might occur from single experiments producing outliers.

For testing the antimicrobial efficiency of the PHMB coating, a relevant Gram-positive microorganism (*S. epidermidis*) was used. *S. epidermidis* is growing in the endogenous skin flora frequently causing foreign body infections. It is known that *S. epidermidis* strains are able to bind on polymer surfaces and form biofilms by multiplication and slime formation.<sup>59,60</sup> Compared with *S. aureus*, *S. epidermidis* prefers to attach to polymer surfaces rather than metallic surfaces<sup>61</sup> building a matrix of proteins as fibrinogen and collagen,<sup>62,63</sup> which cover foreign bodies in the host. Foreign body-associated infections often have a chronic course because the bacterial cells present in the depth of biofilms are largely protected against the effects of antibiotics and the immune system.<sup>59,64</sup> As a consequence, the removal of the infected foreign material is necessary.<sup>54,65</sup> We demonstrated that PLA- $\epsilon$ -CL with immobilized PHMB is antimicrobially effective and even after contamination with *S. epidermidis*, cell compatibility is not impaired.

## 5. Conclusion

In this study, a co-polymer consisting of PLA and polycaprolactone in a ratio of 70:30 was proved to be non-cytotoxic towards cells in monolayers and 3D tissue models. Furthermore, PHMB was successfully immobilized on the surface without inhibiting the antimicrobial efficiency of the antiseptic agent. The applied procedure B that uses an immobilization of PHMB in one step seems to be the most optimal one for an effective antimicrobial coating of the PLA polymer surface combined with non-cytotoxic properties. Consequentially, PLA- $\epsilon$ -CL may be a suitable basic material to produce medical devices such as suction catheters equipped with PHMB to prevent foreign body infections.

## Author contributions

Conceptualization: GM; methodology: GM; validation: MH, PZ, and TS; formal analysis: MH; investigation: MH, TS, JS, and PZ;



resources: CBW and MJ; writing – original draft: MH and PZ; writing – review & editing: GM, AK, JS, CBW, and MJ; supervision: GM.

## Data availability

The datasets analyzed during the current study are available in the ESI† of this article and from the corresponding author on request.

## Conflicts of interest

The authors declare no potential conflicts of interest.

## Acknowledgements

This work was supported by the Ministry of Economics, Employment, and Health of Mecklenburg-Vorpommern in Germany (grant no. TBI-V-1-059-VBW-020). We thank Herotron E-Beam Service GmbH, Bitterfeld-Wolfen, Germany for the sterilization of the polylactide-ε-caprolactone specimens. JS acknowledges support of the European Union under the REFRESH—Research Excellence for Region Sustainability and High-tech Industries project number CZ.10.03.01/00/22\_003/0000048.

## References

- 1 K. Škrlová, K. Malachová, A. Muñoz-Bonilla, D. Měřinská, Z. Rybková, M. Fernández-García and D. Plachá, Biocompatible Polymer Materials with Antimicrobial Properties for Preparation of Stents, *Nanomaterials*, 2019, **9**, 1548, DOI: [10.3390/nano9111548](https://doi.org/10.3390/nano9111548).
- 2 F. Carrasco, P. Pagès, J. Gámez-Pérez, O. O. Santana and M. L. MasPOCH, Processing of poly(lactic acid): Characterization of chemical structure, thermal stability and mechanical properties, *Polym. Degrad. Stab.*, 2010, **95**, 116–125, DOI: [10.1016/j.polymdegradstab.2009.11.045](https://doi.org/10.1016/j.polymdegradstab.2009.11.045).
- 3 S. Vacaras, M. Baciut, O. Lucaci, C. Dinu, G. Baciut and L. Crisan, *et al.*, Understanding the basis of medical use of poly-lactide-based resorbable polymers and composites – a review of the clinical and metabolic impact, *Drug Metab. Rev.*, 2019, **51**, 570–588, DOI: [10.1080/03602532.2019.1642911](https://doi.org/10.1080/03602532.2019.1642911).
- 4 D. Garlotta, A Literature Review of Poly(Lactic Acid), *J. Polym. Environ.*, 2001, **9**, 63–84, DOI: [10.1023/A:1020200822435](https://doi.org/10.1023/A:1020200822435).
- 5 L. Xiao, B. Wang, G. Yang and M. Gauthier, Poly(Lactic Acid)-Based Biomaterials: Synthesis, Modification and Applications, in *Biomedical Science, Engineering and Technology*, ed. D. N. Ghista, Erscheinungsort nicht ermittelbar, IntechOpen, 2012, DOI: [10.5772/23927](https://doi.org/10.5772/23927).
- 6 L. S. Nair and C. T. Laurencin, Biodegradable polymers as biomaterials, *Prog. Polym. Sci.*, 2007, **32**, 762–798, DOI: [10.1016/j.progpolymsci.2007.05.017](https://doi.org/10.1016/j.progpolymsci.2007.05.017).
- 7 C. R. Arciola, New concepts and new weapons in implant infections, *Int. J. Artif. Organs*, 2009, **32**, 533–536, DOI: [10.1177/039139880903200901](https://doi.org/10.1177/039139880903200901).
- 8 J. W. Costerton, Biofilm theory can guide the treatment of device-related orthopaedic infections, *Clin. Orthop. Relat. Res.*, 2005, 7–11, DOI: [10.1097/00003086-200508000-00003](https://doi.org/10.1097/00003086-200508000-00003).
- 9 M. Lehocý, P. F. F. Amaral, M. A. Z. Coelho, P. Šťáhel, A. M. Barros-Timmons and J. A. P. Coutinho, Attachment/detachment of *Saccharomyces cerevisiae* on plasma deposited organosilicon thin films, *Czech. J. Phys.*, 2006, **56**, B1256–B1262, DOI: [10.1007/s10582-006-0359-0](https://doi.org/10.1007/s10582-006-0359-0).
- 10 M. Lehocý, P. Šťáhel, M. Koutný, J. Čech, J. Institoris and A. Mráček, Adhesion of *Rhodococcus* sp. S3E2 and *Rhodococcus* sp. S3E3 to plasma prepared Teflon-like and organosilicon surfaces, *J. Mater. Process. Technol.*, 2009, **209**, 2871–2875, DOI: [10.1016/j.jmatprotec.2008.06.042](https://doi.org/10.1016/j.jmatprotec.2008.06.042).
- 11 H. Qiu, Z. Si, Y. Luo, P. Feng, X. Wu and W. Hou, *et al.*, The Mechanisms and the Applications of Antibacterial Polymers in Surface Modification on Medical Devices, *Front. Bioeng. Biotechnol.*, 2020, **8**, 910, DOI: [10.3389/fbioe.2020.00910](https://doi.org/10.3389/fbioe.2020.00910).
- 12 A. Asadinezhad, M. Lehocý, P. Sáha and M. Mozetič, Recent progress in surface modification of polyvinyl chloride, *Materials*, 2012, **5**, 2937–2959, DOI: [10.3390/ma5122937](https://doi.org/10.3390/ma5122937).
- 13 F. Bílek, K. Sulovská, M. Lehocý, P. Sáha, P. Humpolíček, M. Mozetič and I. Junkar, Preparation of active antibacterial LDPE surface through multistep physicochemical approach II: graft type effect on antibacterial properties, *Colloids Surf., B*, 2013, **102**, 842–848, DOI: [10.1016/j.colsurfb.2012.08.026](https://doi.org/10.1016/j.colsurfb.2012.08.026).
- 14 R. D. Kale, V. G. Gorade, N. Madye, B. Chaudhary, P. S. Bangde and P. P. Dandekar, Preparation and characterization of biocomposite packaging film from poly(lactic acid) and acylated microcrystalline cellulose using rice bran oil, *Int. J. Biol. Macromol.*, 2018, **118**, 1090–1102, DOI: [10.1016/j.ijbiomac.2018.06.076](https://doi.org/10.1016/j.ijbiomac.2018.06.076).
- 15 H. Luo, X.-Q. Yin, P.-F. Tan, Z.-P. Gu, Z.-M. Liu and L. Tan, Polymeric antibacterial materials: design, platforms and applications, *J. Mater. Chem. B*, 2021, **9**, 2802–2815, DOI: [10.1039/d1tb00109d](https://doi.org/10.1039/d1tb00109d).
- 16 A. R. Statz, J. P. Park, N. P. Chongsiriwatana, A. E. Barron and P. B. Messersmith, Surface-immobilised antimicrobial peptoids, *Biofouling*, 2008, **24**, 439–448, DOI: [10.1080/08927010802331829](https://doi.org/10.1080/08927010802331829).
- 17 I. Banerjee, R. C. Pangule and R. S. Kane, Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms, *Adv. Mater.*, 2011, **23**, 690–718, DOI: [10.1002/adma.201001215](https://doi.org/10.1002/adma.201001215).
- 18 J.-B. D. Green, T. Fulghum and M. A. Nordhaus, Review of immobilized antimicrobial agents and methods for testing, *Biointerphases*, 2011, **6**, CL2–CL43, DOI: [10.1116/1.3645195](https://doi.org/10.1116/1.3645195).
- 19 L. Ferreira and A. Zumbuehl, Non-leaching surfaces capable of killing microorganisms on contact, *J. Mater. Chem.*, 2009, **19**, 7796, DOI: [10.1039/b905668h](https://doi.org/10.1039/b905668h).
- 20 J. Yi, Y. Zhang, W. Lin, B. Niu and Q. Chen, Effect of poly-hexamethylene biguanide functionalized silver nanoparticles



- on the growth of *Staphylococcus aureus*, *FEMS Microbiol. Lett.*, 2019, **366**, DOI: [10.1093/femsle/fnz036](https://doi.org/10.1093/femsle/fnz036).
- 21 S. Ramasamy, S. Muthusamy, S. Nagarajan, A. V. Nath, J. S. Savarimuthu, J. Jayaprakash and R. M. Gurunadhan, Fabrication of collagen with polyhexamethylene biguanide: A potential scaffold for infected wounds, *J. Biomed. Mater. Res., Part B*, 2022, **110**, 535–546, DOI: [10.1002/jbm.b.34933](https://doi.org/10.1002/jbm.b.34933).
  - 22 K. Chae, W. Y. Jang, K. Park, J. Lee, H. Kim and K. Lee, *et al.*, Antibacterial infection and immune-evasive coating for orthopedic implants, *Sci. Adv.*, 2020, **6**, eabb0025, DOI: [10.1126/sciadv.abb0025](https://doi.org/10.1126/sciadv.abb0025).
  - 23 P. Tang, W. Zhang, Y. Wang, B. Zhang, H. Wang, C. Lin and L. Zhang, Effect of Superhydrophobic Surface of Titanium on *Staphylococcus aureus* Adhesion, *J. Nanomater.*, 2011, **2011**, 1–8, DOI: [10.1155/2011/178921](https://doi.org/10.1155/2011/178921).
  - 24 M. Hornschuh, P. Zwicker, T. Schmidt, B. Finke, A. Kramer and G. Müller, Poly(hexamethylene biguanide), adsorbed onto Ti-Al-V alloys, kills slime-producing *Staphylococci* and *Pseudomonas aeruginosa* without inhibiting SaOs-2 cell differentiation, *J. Biomed. Mater. Res., Part B*, 2020, **108**, 1801–1813, DOI: [10.1002/jbm.b.34522](https://doi.org/10.1002/jbm.b.34522).
  - 25 M. Hornschuh, P. Zwicker, T. Schmidt, A. Kramer and G. Müller, In vitro evaluation of contact-active antibacterial efficacy of Ti-Al-V alloys coated with the antimicrobial agent PHMB, *Acta Biomater.*, 2020, **106**, 376–386, DOI: [10.1016/j.actbio.2020.02.016](https://doi.org/10.1016/j.actbio.2020.02.016).
  - 26 G. Müller, H. Benkhail, R. Matthes, B. Finke, W. Friedrichs and N. Geist, *et al.*, Poly(hexamethylene biguanide) adsorption on hydrogen peroxide treated Ti-Al-V alloys and effects on wettability, antimicrobial efficacy, and cytotoxicity, *Biomaterials*, 2014, **35**, 5261–5277, DOI: [10.1016/j.biomaterials.2014.03.033](https://doi.org/10.1016/j.biomaterials.2014.03.033).
  - 27 A. M. Carmona-Ribeiro and L. D. de Melo Carrasco, Cationic antimicrobial polymers and their assemblies, *Int. J. Mol. Sci.*, 2013, **14**, 9906–9946, DOI: [10.3390/ijms14059906](https://doi.org/10.3390/ijms14059906).
  - 28 W.-Y. Wang, H.-W. Hu, J.-C. Chiou, K.-F. Yung and C.-W. Kan, Poly(hexamethylene biguanide) hydrochloride (PHMB)-based materials: synthesis, modification, properties, determination, and application, *Polym. Chem.*, 2023, **14**, 5226–5252, DOI: [10.1039/D3PY01148H](https://doi.org/10.1039/D3PY01148H).
  - 29 G. Müller and A. Kramer, Biocompatibility index of anti-septic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity, *J. Antimicrob. Chemother.*, 2008, **61**, 1281–1287, DOI: [10.1093/jac/dkn125](https://doi.org/10.1093/jac/dkn125).
  - 30 A. Ben Fradj, R. Iafi, L. Gzara, A. H. Hamzaoui and A. Hafiane, Spectrophotometric study of the interaction of toluidine blue with poly(ammonium acrylate), *J. Mol. Liq.*, 2014, **194**, 110–114, DOI: [10.1016/j.molliq.2014.01.008](https://doi.org/10.1016/j.molliq.2014.01.008).
  - 31 L. D'Ilario and A. Martinelli, Toluidine blue: aggregation properties and structural aspects, *Modell. Simul. Mater. Sci. Eng.*, 2006, **14**, 581–595, DOI: [10.1088/0965-0393/14/4/003](https://doi.org/10.1088/0965-0393/14/4/003).
  - 32 P. Zwicker, N. Geist, E. Göbler, M. Kulke, T. Schmidt and M. Hornschuh, *et al.*, Improved Adsorption of the Antimicrobial Agent Poly (Hexamethylene) Biguanide on Ti-Al-V Alloys by NaOH Treatment and Impact of Mass Coverage and Contamination on Cytocompatibility, *Coatings*, 2021, **11**, 1118, DOI: [10.3390/coatings11091118](https://doi.org/10.3390/coatings11091118).
  - 33 A. K. Hazra, S. P. Chock and R. W. Albers, Protein determination with trinitrobenzene sulfonate: A method relatively independent of amino acid composition, *Anal. Biochem.*, 1984, **137**, 437–443, DOI: [10.1016/0003-2697\(84\)90110-6](https://doi.org/10.1016/0003-2697(84)90110-6).
  - 34 European Committee for Standardization. Biologische Beurteilung von Medizinprodukten Teil 5: Prüfungen auf In-vitro-Zytotoxizität 2009.
  - 35 P. Zwicker, M. Zumpe, A. Kramer and G. Müller, A 3D Model of Human Buccal Mucosa for Compatibility Testing of Mouth Rinsing Solutions, *Pharmaceutics*, 2023, **15**, 721, DOI: [10.3390/pharmaceutics15030721](https://doi.org/10.3390/pharmaceutics15030721).
  - 36 J. M. Goddard and J. H. Hotchkiss, Polymer surface modification for the attachment of bioactive compounds, *Prog. Polym. Sci.*, 2007, **32**, 698–725, DOI: [10.1016/j.progpolymsci.2007.04.002](https://doi.org/10.1016/j.progpolymsci.2007.04.002).
  - 37 P. S. Ferreira, S. M. Ribeiro, R. Pontes and J. Nunes, Production methods and applications of bioactive polylactic acid: a review, *Environ. Chem. Lett.*, 2024, **22**, 1831–1859, DOI: [10.1007/s10311-024-01729-z](https://doi.org/10.1007/s10311-024-01729-z).
  - 38 J. Pan, Y. Wang, S. Qin, B. Zhang and Y. Luo, Grafting reaction of poly(D,L)lactic acid with maleic anhydride and hexanediamine to introduce more reactive groups in its bulk, *J. Biomed. Mater. Res., Part B*, 2005, **74**, 476–480, DOI: [10.1002/jbm.b.30208](https://doi.org/10.1002/jbm.b.30208).
  - 39 I. Karakurt, K. Ozaltin, D. Vesela, M. Lehoccky, P. Humpolíček and M. Mozetič, Antibacterial Activity and Cytotoxicity of Immobilized Glucosamine/Chondroitin Sulfate on Polylactic Acid Films, *Polymers*, 2019, **11**, 1186, DOI: [10.3390/polym11071186](https://doi.org/10.3390/polym11071186).
  - 40 L. A. Sampath, S. M. Tambe and S. M. Modak, In vitro and in vivo efficacy of catheters impregnated with antiseptics or antibiotics: evaluation of the risk of bacterial resistance to the antimicrobials in the catheters, *Infect. Control Hosp. Epidemiol.*, 2001, **22**, 640–646, DOI: [10.1086/501836](https://doi.org/10.1086/501836).
  - 41 J. Zhao, S. Jin, A. H. S. Delgado, Z. Chen, J. P. Matinlinna and J. K.-H. Tsoi, Self-Assembled PHMB Titanium Coating Enables Anti-Fusobacterium nucleatum Strategy, *Coatings*, 2021, **11**, 1190, DOI: [10.3390/coatings11101190](https://doi.org/10.3390/coatings11101190).
  - 42 C. Guo, J. Zhang, X. Feng, Z. Du, Y. Jiang and Y. Shi, *et al.*, Polyhexamethylene biguanide chemically modified cotton with desirable hemostatic, inflammation-reducing, intrinsic antibacterial property for infected wound healing, *Chin. Chem. Lett.*, 2022, **33**, 2975–2981, DOI: [10.1016/j.ccllet.2021.12.086](https://doi.org/10.1016/j.ccllet.2021.12.086).
  - 43 R. S. Blackburn, A. Harvey, L. L. Kettle, J. D. Payne and S. J. Russell, Sorption of poly(hexamethylenebiguanide) on cellulose: mechanism of binding and molecular recognition, *Langmuir*, 2006, **22**, 5636–5644, DOI: [10.1021/la053002b](https://doi.org/10.1021/la053002b).
  - 44 W.-Y. Wang, J.-C. Chiou, W.-X. Chen, J.-L. Yu and C.-W. Kan, Biosafety evaluation and quantitative determination of poly(hexamethylene biguanide) (PHMB) coated on cellulosic fabrics by Kubelka–Munk equation, *Cellulose*, 2021, **28**, 6651–6661, DOI: [10.1007/s10570-021-03939-y](https://doi.org/10.1007/s10570-021-03939-y).



- 45 W.-Y. Wang and C.-W. Kan, An eco-friendly dyeing method: bromophenol blue (BPB) applied for dyeing cotton fabrics coated with cationic finishing agents, *Cellulose*, 2020, **27**, 9045–9059, DOI: [10.1007/s10570-020-03375-4](https://doi.org/10.1007/s10570-020-03375-4).
- 46 E. Llorens, S. Calderón, L. J. Del Valle and J. Puiggalí, Polybiguanide (PHMB) loaded in PLA scaffolds displaying high hydrophobic, biocompatibility and antibacterial properties, *Mater. Sci. Eng., C*, 2015, **50**, 74–84, DOI: [10.1016/j.msec.2015.01.100](https://doi.org/10.1016/j.msec.2015.01.100).
- 47 Z. Zhi, Y. Su, Y. Xi, L. Tian, M. Xu and Q. Wang, *et al.*, Dual-Functional Polyethylene Glycol-*b*-polyhexanide Surface Coating with in Vitro and in Vivo Antimicrobial and Anti-fouling Activities, *ACS Appl. Mater. Interfaces*, 2017, **9**, 10383–10397, DOI: [10.1021/acsami.6b12979](https://doi.org/10.1021/acsami.6b12979).
- 48 K. Chindera, M. Mahato, A. K. Sharma, H. Horsley, K. Kloc-Muniak and N. F. Kamaruzzaman, *et al.*, The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes, *Sci. Rep.*, 2016, **6**, 23121, DOI: [10.1038/srep23121](https://doi.org/10.1038/srep23121).
- 49 T. I. Croll, A. J. O'Connor, G. W. Stevens and J. J. Cooper-White, Controllable Surface Modification of Poly(lactic-co-glycolic acid) (PLGA) by Hydrolysis or Aminolysis I: Physical, Chemical, and Theoretical Aspects, *Biomacromolecules*, 2004, **5**, 463–473, DOI: [10.1021/bm0343040](https://doi.org/10.1021/bm0343040).
- 50 T. Haddad, S. Noel, B. Liberelle, R. El Ayoubi, A. Ajji and G. D. Crescenzo, Fabrication and surface modification of poly lactic acid (PLA) scaffolds with epidermal growth factor for neural tissue engineering, *Biomatter*, 2016, **6**, e1231276, DOI: [10.1080/21592535.2016.1231276](https://doi.org/10.1080/21592535.2016.1231276).
- 51 T. Yamaoka, Y. Takebe and Y. Kimura, Surface modification of poly (L-lactic acid) film with bioactive materials by a novel direct alkaline treatment process, *Kobunshi Ronbunshu*, 1998, **55**, 328–333.
- 52 Y. Zhu, C. Gao, X. Liu and J. Shen, Surface Modification of Polycaprolactone Membrane *via* Aminolysis and Biomacromolecule Immobilization for Promoting Cytocompatibility of Human Endothelial Cells, *Biomacromolecules*, 2002, **3**, 1312–1319, DOI: [10.1021/bm020074y](https://doi.org/10.1021/bm020074y).
- 53 J. Yang, Y. Wan, C. Tu, Q. Cai, J. Bei and S. Wang, Enhancing the cell affinity of macroporous poly(L-lactide) cell scaffold by a convenient surface modification method, *Polym. Int.*, 2003, **52**, 1892–1899, DOI: [10.1002/pi.1272](https://doi.org/10.1002/pi.1272).
- 54 E. E. Creppy, A. Diallo, S. Moukha, C. Eklou-Gadegbeku and D. Cros, Study of epigenetic properties of Poly(HexaMethylene Biguanide) hydrochloride (PHMB), *Int. J. Environ. Res. Public Health*, 2014, **11**, 8069–8092, DOI: [10.3390/ijerph110808069](https://doi.org/10.3390/ijerph110808069).
- 55 H. Jeong, J. Rho, J.-Y. Shin, D. Y. Lee, T. Hwang and K. J. Kim, Mechanical properties and cytotoxicity of PLA/PCL films, *Biomed. Eng. Lett.*, 2018, **8**, 267–272, DOI: [10.1007/s13534-018-0065-4](https://doi.org/10.1007/s13534-018-0065-4).
- 56 P. F. M. Finotti, L. C. Costa, T. S. O. Capote, R. M. Scarel-Caminaga and M. A. Chinelatto, Immiscible poly(lactic acid)/poly( $\epsilon$ -caprolactone) for temporary implants: Compatibility and cytotoxicity, *J. Mech. Behav. Biomed. Mater.*, 2017, **68**, 155–162, DOI: [10.1016/j.jmbbm.2017.01.050](https://doi.org/10.1016/j.jmbbm.2017.01.050).
- 57 B. B. Karakocak, S. Keshavan, G. Gunasingam, S. Angeloni, A. Auderset, A. Petri-Fink and B. Rothen-Rutishauser, Rethinking of TEER measurement reporting for epithelial cells grown on permeable inserts, *Eur. J. Pharm. Sci.*, 2023, **188**, 106511, DOI: [10.1016/j.ejps.2023.106511](https://doi.org/10.1016/j.ejps.2023.106511).
- 58 A. E. Postlethwaite, R. Raghov, G. P. Stricklin, H. Poppleton, J. M. Seyer and A. H. Kang, Modulation of fibroblast functions by interleukin 1: increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1 alpha and beta, *J. Cell Biol.*, 1988, **106**, 311–318, DOI: [10.1083/jcb.106.2.311](https://doi.org/10.1083/jcb.106.2.311).
- 59 M. Otto, *Staphylococcus epidermidis*—the ‘accidental’ pathogen, *Nat. Rev. Microbiol.*, 2009, **7**, 555–567, DOI: [10.1038/nrmicro2182](https://doi.org/10.1038/nrmicro2182).
- 60 P. D. Fey and M. E. Olson, Current concepts in biofilm formation of *Staphylococcus epidermidis*, *Future Microbiol.*, 2010, **5**, 917–933, DOI: [10.2217/fmb.10.56](https://doi.org/10.2217/fmb.10.56).
- 61 E. Barth, Q. M. Myrvik, W. Wagner and A. G. Gristina, In vitro and in vivo comparative colonization of *Staphylococcus aureus* and *Staphylococcus epidermidis* on orthopaedic implant materials, *Biomaterials*, 1989, **10**, 325–328, DOI: [10.1016/0142-9612\(89\)90073-2](https://doi.org/10.1016/0142-9612(89)90073-2).
- 62 H. Rohde, C. Burdelski, K. Bartscht, M. Hussain, F. Buck and M. A. Horstkotte, *et al.*, Induction of *Staphylococcus epidermidis* biofilm formation *via* proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases, *Mol. Microbiol.*, 2005, **55**, 1883–1895, DOI: [10.1111/j.1365-2958.2005.04515.x](https://doi.org/10.1111/j.1365-2958.2005.04515.x).
- 63 D. G. Conrady, C. C. Brescia, K. Horii, A. A. Weiss, D. J. Hassett and A. B. Herr, A zinc-dependent adhesion module is responsible for intercellular adhesion in staphylococcal biofilms, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 19456–19461, DOI: [10.1073/pnas.0807717105](https://doi.org/10.1073/pnas.0807717105).
- 64 M. Otto, Molecular basis of *Staphylococcus epidermidis* infections, *Semin. Immunopathol.*, 2012, **34**, 201–214, DOI: [10.1007/s00281-011-0296-2](https://doi.org/10.1007/s00281-011-0296-2).
- 65 M. Sabaté Brescó, L. G. Harris, K. Thompson, B. Stanic, M. Morgenstern and L. O'Mahony, *et al.*, Pathogenic Mechanisms and Host Interactions in *Staphylococcus epidermidis* Device-Related Infection, *Front. Microbiol.*, 2017, **8**, 1401, DOI: [10.3389/fmicb.2017.01401](https://doi.org/10.3389/fmicb.2017.01401).

