

## Cytocompatibility of coated titanium surfaces impregnated with an antiseptic to staphylococci and fibroblasts

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**INTRODUCTION:** *Staphylococcus aureus* and *Staphylococcus epidermidis* are both commonly associated with open fractures and external fixators. These bacteria account for 3-40% of reported cases<sup>1,2</sup>, can lead to osteomyelitis and septicaemia, and with the rise in antibiotic resistant bacteria are an important issue<sup>3</sup>. Once adhered, *S. aureus* and *S. epidermidis* form biofilms which can be difficult to clinically treat because the bacteria are protected from phagocytosis and antibiotics<sup>4</sup>, hence the need to prevent initial bacterial adhesion. This study describes the cytocompatibility of different coated titanium surfaces with/without chlorhexidine diacetate (CHA) to *S. aureus*, *S. epidermidis*, and hTERT fibroblasts.

**MATERIALS AND METHODS:** To visualise *S. aureus* and *S. epidermidis* adherence on different surfaces (Poly-D,L-lactide (PDLLA), politerefate (PTF), anodic plasma chemical deposited calcium-phosphate (CaP/APC), polyurethane (PU), and polyvinylpyrrolidone (PVP)), bacteria were cultured on the different surfaces in brain heart infusion broth (BHI) at 37°C for 2h, 24h, 48h and 96h, then fixed for visualisation with an scanning electron microscope (SEM). To quantify the amount of bacteria on the surfaces, adherent bacteria were detached by sonication in Tween 80, then stained with a live/dead assay, and then counted with a Partec PAS flow cytometer. The amount of bacteria in the media was also counted using the same procedure. To determine the cytocompatibility of the surfaces to hTERT fibroblasts, cells were cultured on the surfaces in DMEM with 10% FCS at 37°C for 48h, then fixed for the SEM and the amount of spreading analysed. The cumulative release kinetics of CHA from the coated surfaces was analysed, and the adhesive strength of the coating mechanically tested.

**RESULTS:** On the surfaces without CHA, both staphylococcal strains and spread fibroblasts were observed, but on the CHA impregnated surfaces few bacteria (Fig. 1) and no intact fibroblasts were seen (Fig. 2). Flow cytometry found fewer bacteria in the media and on the surfaces containing CHA in comparison to the surfaces without CHA (Fig. 3). The release kinetics varied from slow to burst release: PDLLA > PTF > PU > CaP/APC = PVP.

With the exception of PU and PVP, the other three coatings passed the mechanical testing.

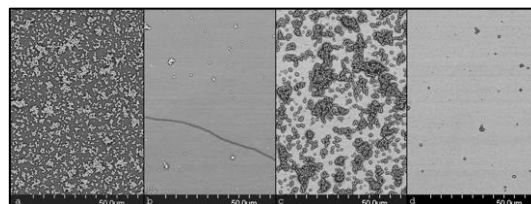


Fig. 1. SEM images of *S. aureus* (a-b) and *S. epidermidis* (c-d) on PU without CHA (a & c) and with CHA (b & d) after culturing for 48h.

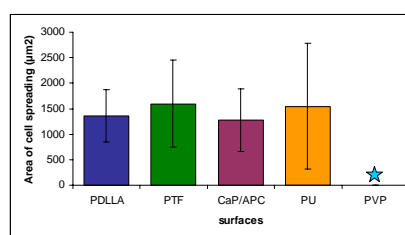


Fig. 2. Average spreading of hTERT fibroblasts on the different surfaces without CHA.. Star = little or no cell spreading so was not analysed.

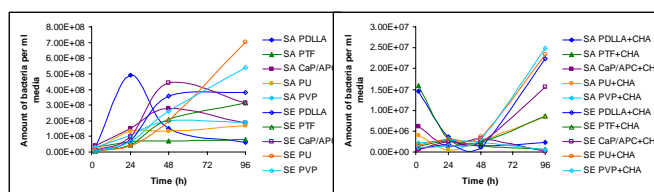


Fig. 3. Flow cytometer analysis of the media after culturing *S. aureus* (SA) and *S. epidermidis* (SE) for 2h, 24h, 48h and 96h on a) different surfaces without CHA, and b) with CHA.

**DISCUSSION & CONCLUSIONS:** This study showed that PDLLA and Politerefate (PTF) have potential as coatings for drug delivery, since they were cytocompatible to hTERT fibroblasts, eluted CHA effectively, and passed mechanical testing. However, since CHA was toxic to hTERT fibroblasts *in vitro*, the cytotoxicity of CHA needs further evaluation before it can be tested *in vivo*.

**REFERENCES:** <sup>1</sup>Lee-Smith J, Santy J, Davis P, Jester R, Kneale J (2001) J Orthop Nursing 5:37-42; <sup>2</sup>Khatod M, Botte MJ, Hoyt DB, Meyer RS, Smith JM, Akeson WH (2003) J Trauma 55:949-954; <sup>3</sup>Lowy FD (1998) New Eng J Med 339: 520-532; <sup>4</sup>Hoyle BD, Costerton JW (1991) Prog. Drug Res. 37:91-105.

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