# A bone infection organ model for biomaterials research of human origin

Theodor Kuehling[1], Pia Schilling[1], Hermann O. Mayr[1], Annerose Serr[2], Marc Bohner[3], Michael Seidenstuecker[1]
[1]Medical Center Albert-Ludwigs-University, Freiburg, Germany, [2]Institute of Microbiology and Hygiene, Medical Center University Freiburg, Germany,
[3]Robert Mathys Foundation, Switzerland
michael.seidenstuecker@uniklinik-freiburg.de

INTRODUCTION: Although over the years procedures have become safer, the American College of Surgeons National Surgery Quality Improvement Program reported that 30 day post arthroplasty complications occurred in 4.2% of performed Total hip- and 5.55% of performed total knee arthroplasties [1], with periprosthetic joint infection (PJI) being one the most frequent complications [2]. Infection rates during the first two years after primary joint replacement vary depending on the joint location – under 1% in hip and shoulder prothesis, less than 2% in knee prothesis and under 9% in elbow prosthesis [3]. This leads to higher revision rates, as PJI are accounted for 14.8% of hip revision surgeries and are the most common cause for knee revision surgeries being responsible for 25.2% of knee revisions. The main aim of this study was to achieve a reproducible, solitary S. aureus infection within a human organ model, which was subsequently to be eliminated by inserting a hydrogel- and clindamycin-loaded β-TCP composite. We wanted to prove our hypothesis of an at least four-week lasting germ count reduction using a β-TCP composite as a drug release system, leading to antibiotic concentrations exceeding the minimum inhibitory concentration over the whole observation time. So far, to the best of our knowledge, no such investigations of ceramics with interconnected pores have been made, with a recent focus on PMMA carriers for drug delivery [4, 5]. Our approach, however, allows a drug release not only from the implants surface, but its whole body, due to interconnected pores, loaded with hydrogels and antibiotics. By developing this human bone infection organ model we could directly investigate the β-TCP drug release in a setting close to in vivo conditions.

METHODS: The production of the β-TCP ceramics used was performed according to our specifications by the RMS Foundation. A bone organ model previously described by our group [6] was used to develop this infection model. Human tibia plateaus (positive ethical vote of the ethics committee of the University of Freiburg (420/19)), collected during total knee replacement surgery, were used as a source of bone material. Bone cylinders with a diameter of 15 mm were then milled out of the tibial plateaus, the height was shortened to 6 mm, and a 7 mm hole was drilled in the center for later placement of β-TCP. The loading of the composites was carried out by means of a flow chamber [7] with ADA gelatin gel containing 50 mg/ml clindamycin. Samples were infected with S. *aureus ATCC29213* and treated with differently loaded β-TCP composites (alginate +/- clindamycin, ADA-gelatin +/- clindamycin, unloaded).. The infection was observed for 28 days, quantifying bacteria in the medium and the osseus material on day 1, 7, 14, 21 and 28. All samples were histologically processed for bone vitality evaluation. Bone infection could be consistently performed within the organ model.

RESULTS SECTION: A reproducible infection of the bone samples was possible using the described method with bacterial counts being detected in the infection medium as well as in the sonicated bone sample after a 24-hour lasting bacterial exposition. Mean germ counts 24 hours after the infection were 8.29 ± 0.32 log10 (CFU/ml, n=5) in the sonicated bone tissue and 8.99 ± 0.15 log10 (CFU/ml, n=33) in the infection medium. Once the hydrogel-loaded β-TCP composites were placed inside the bone samples bacterial counts started to differ, depending on the antibiotic loading of the composite. Exact counts (log10 CFU/ml) are listed in Table 1.

DISCUSSION: The current findings of the workgroup support this effect of local drug release with a strong initial drop in both groups treated with clindamycin. There are two possible explanations to the long-term reduction of bacteria (1) an initial burst release combined with a continuous release of clindamycin out of β-TCP; (2) accumulation of clindamycin in the bone tissue itself [6], followed by a germ count reduction originating there. The finally measured bacterial elimination can be, most likely, traced back to a combination of both effects.

SIGNIFICANCE/CLINICAL RELEVANCE: The common treatment of bone infections is debridement and systemic administration of antibiotics. In some cases, antibiotic-containing carriers are already used, but these must be removed again. Our work is intended to show another treatment option. The scaffold we have developed, made of a calcium phosphate ceramic and a hydrogel as the active substance carrier, can, in addition to releasing the active substance, also assume a load-bearing function of the bone and is biodegradable. In addition, the model we developed can also be used for the analysis and treatment of bone infections other than those of the musculoskeletal system. More importantly, it can also serve as a substitute for previously used animal experiments.

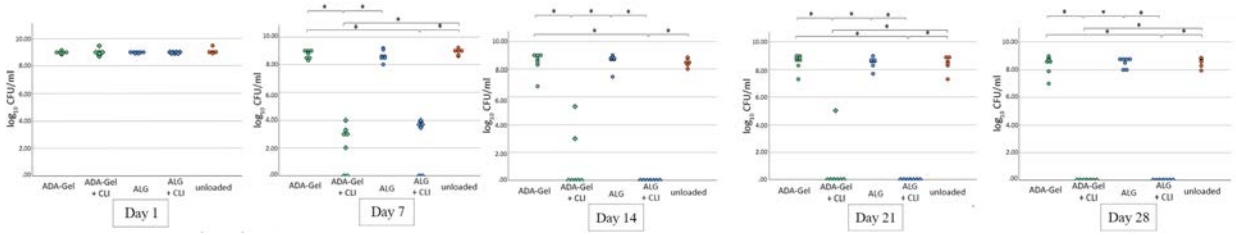


Fig. 1: *Bacterial counts in the infection organ model. Infection course in the bone-surrounding medium over a 4-week period treated with differently loaded β-TCP composites; ADA-Gel (green dots); ADA-Gel + CLI (green squares); ALG (blue dots); ALG + CLI (blue squares); unloaded (orange dots) Statistical analysis: Kolmogorov-Smirnov-Test for testing of normal distribution, Wilcoxon-Test for mean value comparison.*

Tab. 1: *Bacterial counts (log10 CFU/ml)*

| **Bacterial counts ($log_{10}$ CFU/ml)** | | | | | |
|---|---|---|---|---|---|
| β-TCP composite | infection control medium | day 7 | day 14 | day 21 | day 28 |
| ADA-Gel (n=7) | 8.99 ± 0.15 (n=33) | 8.70 ± 0.28 | 8.42 ± 0.77 | 8.55 ± 0.60 | 8.40 ± 0.71 |
| ADA-Gel + CLI (n=7) | | 2.19 ± 1.60 | 1.19 ± 2.13 | 0.71 ± 1.89 | 0.00 ± 0.00 |
| ALG (n=8) | | 8.63 ± 0.40 | 8.61 ± 0.51 | 8.51 ± 0.41 | 8.52 ± 0.37 |
| ALG + CLI (n=7) | | 2.68 ± 1.84 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| unloaded (n=6) | | 8.90 ± 0.25 | 8.52 ± 0.33 | 8.44 ± 0.66 | 8.55 ± 0.41 |

REFERENCES: [1] Belmont et al. J. Bone jt. Surg. Ser A96; 2014; 20-26. [2] Lambertz et al. Dtsch. Arztebl. Int. 114; 2017. [3] Trampuz et al. Curr. Infect. Dis. Rep. 10; 2008; 394-403. [4] Oungeun et al ACS Omega 4; 2019; 14860-14867. [5] Bettencourt et al. J. Microencapsul. 29; 2012; 353-367. [6] Thabit et al. Int. J. Infect. Dis 81; 2019; 128-136. [6] Zankovic et al. Materials (Basel). 14; 2021. [7] Seidenstuecker et al.; J Func biomater; 2015;6: 1085-98