Dual release of Daptomycin and BMP-2 from a composite of microporous β-TCP ceramic and ADA gelatin for bone regeneration

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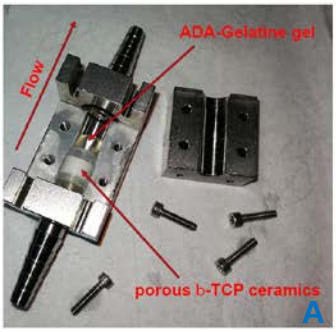
INTRODUCTION: Osteomyelitis is an inflammation of the bone that usually affects bone (osteitis), bone marrow (osteomyelitis), and periosteum (periostitis). The surrounding soft tissue may also be affected. It is caused by infection with various microorganisms and ultimately leads to destruction of the bone. A special form of the exogenous form is implant-associated osteo-myelitis. It is characterized by a biofilm of colonizing bacteria in which the bacteria multiply less and are more resistant to antibiotics than outside the biofilm. Based on this, targeted systemic or local antibiotic therapies are administered. Antibiotic-containing carrier systems are one option that offers the advantage of releasing active ingredients over a longer period of time. In vitro sustained drug release from a carrier system consisting of microporous β-TCP ceramic and alginate has been reported in the literature [1-4]. Alginate dialdehyde (ADA) gelatin gel showed both better mechanical properties when loaded into a β-TCP ceramic and higher biodegradability than pure alginate. The possibility of dual drug release has not yet been investigated in this system, which could improve patient outcomes by combining anti-infective treatment, bone growth promoting therapy, and better stability in bone due to the β-TCP ceramic.

METHODS: The most promising ADA gelatin gel for drug release from microcapsules was used to fill microporous β-TCP ceramics under directed flow in a dedicated loading chamber [1]. Dual release of daptomacin and BMP-2 was measured on days 1, 2, 3, 6, 9, 14, 21, and 28 by high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). After release, the microbial efficacy of the daptomacin was verified and the biocompatibility of the composite was tested in cell cultures using the Live Dead Staining Kit, WST-I and LDH assay. In addition, the mechanical stiffness of the composite was compared with a blank ceramic after 28-day incubation in SBF and 60-day incubation in TRIS buffer pH 5.0 and pH 7.4.

RESULTS SECTION: Daptomycin and the model compound FITC protein A (n=30) were released from the composite over 28 days. The daptomycin release was 57 ± 4 %. The DAP release above the minimum inhibitory concentration (MIC) by day 9 and burst release of 68.5 ± 6.3% were observed in the loaded ceramics. BMP-2 was released from the loaded ceramics at low concentrations over 28 days. The loaded ceramics sustained ~10% more load on average. Loading with gels (alginate as well as ADA gelatin) after 60d in TRIS buffer (EN ISO 10993-14:2009) resulted in a reduction of the maximum tolerated load at pH 7.4 as well as at pH 5.0 to 66 and 38% of the initial value of 1000N, respectively.

DISCUSSION: The release of active substances from β-TCP and hydrogel have already been extensively studied. Directional flow loading is a special procedure in which the ceramic could act as a stabilizer in the bone and, as a biodegradable system, enables a single-stage surgical procedure. Whether ADA-gelatin gel is suitable for this procedure as a more biodegradable alternative to pure alginate or whether a dual release is possible in this composite has not yet been investigated. The change in the mechanical strength of the composite in which a gel such as ADA gelatine is present in the degradation test in TRIS buffer (pH 7.4 and 5.0) has also only been investigated by us to date.

SIGNIFICANCE/CLINICAL RELEVANCE: Osteomyelitis is an inflammation of the bone that can usually also affect the surrounding soft tissue. The typical therapy for a bone infection consists of surgical removal of the site of infection and subsequent determination of the pathogen. Based on this, targeted systemic or local antibiotic therapies are administered. In contrast, local antibiotic applications can achieve greater efficacy at the site of infection and reduce the systemic side effects of therapy. However, systems based on PMMA chains that have been used up to now, must be removed again. An implantable biodegradable system would have many advantages for the patient, in particular due to the one-time surgery required.



Release period [d]	Daptomycin-Release [µg/ml]	BMP-2 Release [ng/ml]
1	1232.16 ± 130.74	22.73 ± 44.10
2	289.04 ± 39.56	59.80 ± 90.0
3	89.34 ± 12.18	101.86 ± 92.04
6	11.74 ± 4.90	669.00 ± 148.40
9	0.13 ± 0.28	208.96 ± 43.20
14	0 ± 0	71.54 ± 47.63
21	0 ± 0	148.31 ± 100.86
28	0 ± 0	1.75 ± 6.14
Recovery [%]:	89.42 ± 2.00	2.63 ± 9.15

B

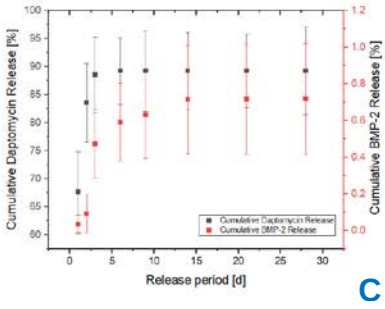


Fig. 1: Flow chamber with β-TCP and ADA-gel (A); released DAP and BMP-2 concentrations (B); cumulative release curves for DAP and BMP-2 (C)

REFERENCES: [1] Seidenstuecker et al.; J Func biomater; 2015;6: 1085-98 [2] Kissling et al.; BMC Biotechnol; 2016;16;44 [3] Seidenstuecker et al. Acta Biomater; 2017; 51:433-46 ; [4] Ritschl et al.; J Mater Sci Mater Med; 2023; 34:39.