

Bone response and mechanical strength of rabbit femoral defects filled with injectable CaP cements containing TGF- β 1 loaded gelatin microparticles

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Abstract

This study focused at the potential of transforming growth factor β 1 (TGF- β 1) loaded gelatin microparticles to enhance the bone response and mechanical strength of rabbit femoral defects filled with injectable calcium phosphate (CaP)/gelatin microparticle composites. Therefore, TGF- β 1 loaded composites and non-loaded controls were injected in circular defects as created in the femoral condyles of rabbits and were left in place for 4, 8 and 12 weeks. The specimens were evaluated mechanically (push-out test), and morphologically (scanning electron microscopy (SEM), histology, and histomorphometry). The results showed a gradual increase in mechanical strength with increasing implantation periods. Histological and histomorphometrical evaluation showed similar results for both composite formulations regarding histological aspect, new bone formation and bone/implant contact. However, TGF- β 1 loading of the composites demonstrated a significant effect on composite degradation after twelve weeks of implantation. The results of this study showed that CaP/gelatin composites show excellent osteogenic properties and a rapid increase in mechanical strength. The addition of TGF- β 1 significantly enhances the bone remodeling process.

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1. Introduction

Calcium phosphate (CaP) ceramics are widely used as bone substitutes in reconstructive orthopedic and oral surgery because of their beneficial effects on bone healing. These ceramics can be applied as granules or prefabricated porous blocks, but they can also be formulated as an injectable CaP paste that can be shaped according to the required dimensions [1–5]. These so-called CaP cements are highly compatible with soft and hard tissues after setting in situ [6]. In view of tissue reconstruction, CaP cements are supposed to be subject of biological degradation and concomitant replacement by bone tissue. However, the

degradation of CaP cements is known to be slow [7], which is likely due to the limited extent of porosity. CaP cements contain only an intrinsic nanoporosity that allows transport of nutrients and waste through the material, but the dimensions of this nanoporosity are insufficient to obtain tissue ingrowth [6]. As a consequence, attempts have been made to increase the porosity of the material to allow tissue ingrowth and to accelerate degradation [8,9]. For the additional creation of microporosity, different methods have already been applied; the most commonly used method at our department is the incorporation of high molecular weight poly(lactic-co-glycolic acid) (HMW-PLGA) microparticles to generate CaP/PLGA composites [9–12]. A limitation regarding the use of PLGA for microparticle production is the relative slow degradation of this polymer (6–12 weeks) [11]. This slow degradation

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still prevents cells to penetrate into the implants early after *in vivo* application, which is the probable cause of delayed bone formation in the composites [9,12–14].

Therefore, a method was developed to prepare composites in which microparticles of gelatin were incorporated into CaP cement [15,16]. Gelatin microparticles are biodegradable, biocompatible, and non-toxic and can be crosslinked with glutaraldehyde in order to increase thermal and mechanical stability of the microparticles under physiological conditions [17]. Furthermore, the crosslinking agent concentration and reaction period can be varied, allowing the creation of microparticles with different degradation properties [17–19].

Inherent to the creation of porosity is the loss of mechanical strength of the CaP cement material. This makes the composites less suitable for use under load-bearing conditions. In an ideal situation, the cement would retain its mechanical strength, while being resorbed and replaced by newly formed bone. Previous research already showed that the decreased mechanical properties are partly compensated by the ingrowth of bone in the cement porosity [12].

To further stimulate bone formation and cement resorption, microparticles can be used for the delivery of an appropriate growth factor. Among the candidate growth factors for such an application is transforming growth factor $\beta 1$ (TGF- $\beta 1$), which plays a significant role in wound healing [20–22] by enhancing the repair of injured tissue like skin and bone [23]. TGF- $\beta 1$ acts on osteoblasts, chondrocytes, and cells of the osteoclastic lineage [24] and has been reported to stimulate osteogenesis at orthotopic sites [25–27].

In view of the above mentioned, this study investigated the potential of TGF- $\beta 1$ loaded gelatin microparticles to enhance the bone response and mechanical strength of rabbit femoral defects filled with injectable CaP/gelatin microparticle composites.

2. Materials and methods

2.1. Calcium phosphate (CaP) cement

CaP cement (Calcibon[®]; Merck Biomaterial GmbH, Darmstadt, Germany) was used for the preparation of the implants. The chemical composition of this cement is 61% α tri-calcium phosphate (α -TCP), 26% CaHPO₄, 10% CaCO₃ and 3% precipitated hydroxyapatite (PHA). Before usage, the cement powder was sterilized by gamma radiation with 25 kGy (Isotron B.V., Ede, The Netherlands).

2.2. Gelatin microparticles

Gelatin microparticles were prepared as described by Holland et al. [28]. Briefly, a gelatin solution was prepared by dissolving negatively charged acidic gelatin (type B; pI = 4.7–5.2; Sigma, St. Louis, MO, USA) in deionized water at 37 °C for 1 h. Then, this solution was drop wise added to 125 ml chilled olive oil (Acros Organics, Geel, Belgium) while stirring at 500 rpm. After 30 min of stirring, 50 ml chilled acetone (4 °C; HPLC grade, Labscan Ltd., Dublin, Ireland) together with 500 μ l 25 wt% glutaraldehyde (Merck, Darmstadt, Germany) was added to the

emulsion. After an additional 30 min of stirring, the microparticles were collected by filtration and washed with chilled acetone (4 °C). Finally, the microparticles were frozen and lyophilized.

Microparticle size distribution was assessed by morphometrical analysis using a light microscope (Leica Microsystems AG, Wetzlar, Germany) and computer-based image analysis techniques (Leica[®] Qwin Pro-image analysis system, Wetzlar, Germany).

2.3. TGF- $\beta 1$ microparticle loading

For the TGF- $\beta 1$ -loaded composites, 600 mg gelatin microparticles were swollen in 3.6 ml 4 mM HCl/BSA [0.1%] solution containing 10 μ g TGF- $\beta 1$ (R&D Systems Minneapolis, MI, USA), vortexed and lyophilized again. This solution volume is below the microparticles' theoretical swelling volume in order to allow complete growth factor adsorption. In this way, the positively charged TGF- $\beta 1$ formed a polyionic complexation with the negatively charged gelatin microparticles [29]. Eventually, 250 ng TGF- $\beta 1$ was present within each implant.

2.4. Injectable CaP/gelatin composites

Composites containing 5% (w/w) gelatin microparticles were generated as follows. Gelatin microparticles (30 mg), either or not loaded with TGF- $\beta 1$, were pre-swollen in 180 μ l water and mixed for 15 s in a 2 ml syringe (Sherwood medical monoject) with closed tip using a mixing apparatus (Silamat, Vivadent, Schaan, Liechtenstein) to obtain an even distribution of the water content in the microparticles. CaP cement powder (570 mg) was added afterwards in the 2 ml syringe. The final composite was created by mixing 210 μ l of Na₂HPO₄ (1 wt%; filter sterilized) with 600 mg of this CaP/gelatin combination for 15 s using the mixing apparatus. After mixing, the cement was immediately injected into the defect.

Final composite formulations in this study were:

1. CaP/gelatin (5% w/w gelatin microparticles/CaP cement composite).
2. CaP/gelatin+TGF- $\beta 1$ (5% w/w TGF- $\beta 1$ -loaded gelatin microparticles/CaP cement composite; 250 ng TGF- $\beta 1$ per composite).

Furthermore, additional samples were prepared with the goal to determine the total porosity of the composites by correlating the weight of CaP samples with the weight of the CaP/gelatin samples after placement in a furnace at 650 °C for 2 h (to remove the gelatin microparticles) [11].

2.5. Surgery

Thirty-six female New Zealand White rabbits (4–5 months) with a weight of approximately 3.5 kg were used as experimental animals. The animal experiment was approved by the Animal Ethical Committee of the Radboud University Nijmegen Medical Center and national guidelines for the care and use of laboratory animals were observed. Each rabbit received only one sample of both CaP/gelatin composite formulations. The composites were inserted in trabecular bone defects as created in the left medial and right medial distal femur [30]. In this way, 72 composites were injected in 36 rabbits.

Surgery was performed under general inhalation anesthesia. Anesthesia was induced by an intravenous injection of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone) and atropine, and maintained by a mixture of nitrous oxide, isoflurane and oxygen through a constant volume ventilator. To reduce the peri-operative infection risk, the rabbits received antibiotic prophylaxis (Baytril 2.5% (enrofloxacin), 5–10 mg/kg).

During anesthesia, the rabbits were immobilized on their back and the surgical areas were shaved and disinfected with povidone-iodine. A longitudinal incision was made down to the periosteum. Subsequently, a midline incision was created in the periosteum. The periosteum was undermined and lifted off the distal femora. The defects in the femora were drilled from the medial direction with an increasing burr diameter (from 2.0 to 4.0 mm) to obtain cylindrical defects with a depth of 6 mm and a diameter of 4 mm. Following injection of the composites, the

material was left to set for approximately 10 min. Subsequently, the periosteum and soft tissue were closed using resorbable vicryl 4–0 sutures.

The rabbits were sacrificed in groups of 12 at 4, 8 and 12 weeks ($n = 12$ per formulation at each time period) post-surgery by an overdose of Nembutals (pentobarbital).

2.6. Mechanical testing (i.e. push-out test)

To determine the mechanical strength of the rabbit femoral defects filled with CaP/gelatin composites, half of the retrieved specimens ($n = 6$ for each composite formulation at each time point) were used. Evaluation consisted of a push-out test [31,32], using a mechanical testing bench (MTS 858 Mini Bionix II, Gouda, The Netherlands). The retrieved specimens with their surrounding tissue were transported to the laboratory on ice. After arrival, the distal femur was grinded at both sides to obtain flat surfaces, with the composite implant perpendicular to these flat surfaces to make the push-out test possible. Subsequently, each specimen was fixated on a support jig with a hole of 4.4 mm (10% larger than the implant diameter of 4.0 mm) to minimize the effect of the test condition on the push-out results [31,32]. This support jig enabled the application of a vertical force using a 3.85 mm diameter lever (at a constant displacement speed of 0.5 mm/min) on the CaP/gelatin specimens including the new bone formation. When the peak force was reached, the test was immediately stopped to ensure minimal displacement of the samples. The mechanical strength of the defects filled with composites was calculated by following formula:

$$\text{shear strength (MPa)} = \frac{\text{Push-out force(N)}}{\pi * \text{implant diameter (mm)} * \text{femoral thickness (mm)}}$$

2.7. Scanning electron microscopy (SEM)

After mechanical testing, the specimens were fixated in 10% formalin solution, dehydrated in a graded series of ethanol, and embedded in methylmethacrylate. After polishing, the specimens were sputter-coated with gold, and examined with SEM (Jeol 6310 SEM, Boston, MA, USA) to determine the fracture plane as occurred during the push-out testing (e.g. in the cement, at the interface bone–cement, in the surrounding bone). SEM was performed at the Microscopic Imaging Center (MIC) of the Nijmegen Center for Molecular Life Sciences (NCMLS), the Netherlands.

2.8. Histological and histomorphometrical evaluation

The other half of the retrieved implants with their surrounding tissue (six specimens of each experimental group at each time point) were prepared for histological evaluation. The samples were fixated in 4% formalin solution (pH = 7.4), dehydrated in a graded series of ethanol, and embedded in methylmethacrylate. Following polymerization, three 10 μm thick, longitudinal sections were prepared per specimen through the center of the composites, using a sawing microtome technique [33]. The sections were stained with methylene blue and basic fuchsin and investigated using a light microscope. For histomorphometrical analysis, all sections per specimen were evaluated using computer based image analysis software (Leica® Qwin Pro-image analysis system, Wetzlar,

Germany). The quantitative evaluation of newly formed bone was done by determining a region of interest (ROI), which was set using a circle of 4.0 mm in diameter with the composite positioned in the center. Within this ROI, new bone formation was distinguished from composite through structure and color differences. New bone formation was expressed in mm^2 , using standardization of the two-dimensional area of analysis.

Bone/implant contact evaluation was defined by the percentage of composite perimeter at which direct bone to composite contact, without an intervening soft tissue layer, was present. Degradation of the CaP/gelatin composites was determined by calculating the remaining surface area of the composites through structure and color differences. The remaining composite was expressed in mm^2 , using standardization of the two-dimensional area of analysis.

2.9. Statistical analysis

Statistical analysis was performed with GraphPad® InStat 3.05 software (GraphPad Software Inc., San Diego, CA, USA) using one-way analysis of variance (ANOVA) with a Tukey multiple comparison post-test. Significance was set at $p < 0.05$.

3. Results

3.1. Composite characterization

The distribution of the unswollen gelatin microparticles varied between 1 and 49 μm with an average size of $8.4 \pm 7.6 \mu\text{m}$, while the water-swollen microparticles varied between 1 and 66 μm with an average size of $20.7 \pm 14.6 \mu\text{m}$. The porosity of the composite formulations after setting was $45.0 \pm 1.3\%$ (Table 1).

3.2. General observations of animals

All 36 rabbits in this experiment remained in good health and did not show any wound complications after surgery. The original defects were completely filled with the injectable composites without the presence of entrapped air bubbles. At retrieval, no visual signs of inflammatory or adverse tissue reactions were observed.

3.3. Mechanical testing

The results of the push-out test (Fig. 1) showed a gradual increase in mechanical strength with increasing implantation periods. After 4 weeks of implantation, the push-out value of the CaP/gelatin composites including new bone formation was $3.7 \pm 1.7 \text{ MPa}$, which increased to $6.6 \pm 1.6 \text{ MPa}$ at 8 weeks, and finally measured $10.0 \pm 2.9 \text{ MPa}$ after 12 weeks of implantation. The CaP/gelatin + TGF- β 1 composites

Table 1
Implant porosity, gelatin microparticle sizes and TGF- β 1 loading

	CaP/gelatin	CaP/gelatin + TGF- β 1
Macroporosity	$45.0 \pm 1.3\%$	
Microparticles size (before swelling)	$8.4 \pm 7.6 \mu\text{m}$ (1–49 μm)	
Microparticles size (after swelling)	$20.7 \pm 14.6 \mu\text{m}$ (1–66 μm)	
TGF- β 1 loading	–	250 ng

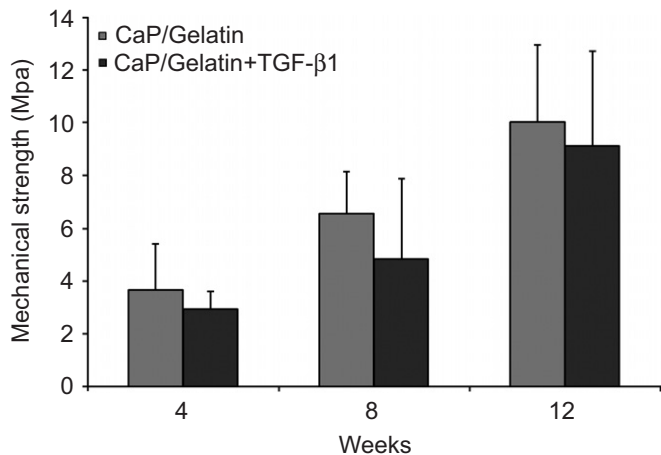


Fig. 1. Push-out test results (in MPa) of CaP/gelatin composites with or without TGF- β 1 after 4, 8 and 12 weeks of implantation. Bars represent means \pm SD ($n = 6$).

including new bone formation showed a mechanical strength value of 2.9 ± 0.7 MPa after 4 weeks of implantation, which increased to 4.9 ± 3.0 MPa at 8 weeks, and finally measured 9.1 ± 3.6 MPa after 12 weeks of implantation. At individual implantation periods, no significant differences of mechanical strength values were present between CaP/gelatin and CaP/gelatin + TGF- β 1 composite formulations.

3.4. Scanning electron microscopy (SEM)

SEM examination (Fig. 2) of the fracture plane of the specimens subjected to the mechanical test showed similar results for CaP/gelatin and CaP/gelatin + TGF- β 1 composites. After 4 weeks of implantation, samples showed a fracture plane at the bone–cement interface (Fig. 2A), while the 8-week samples showed a fracture, which was found in the CaP/gelatin composites, as well as in the newly formed bone (Fig. 2B). Finally, the 12 week samples demonstrated fractures only in the newly formed bone (Fig. 2C).

3.5. Histological and histomorphometrical evaluation

Histological evaluation showed similar results for CaP/gelatin and CaP/gelatin + TGF- β 1 composites (Fig. 3). Within first 4 weeks after implantation, limited bone formation occurred at the implant periphery, whereas after 8 weeks of implantation peripheral microparticles were degraded and replaced by newly formed bone. Finally, after 12 weeks of implantation more bone ingrowth was observed deeper into the composites, but no tissue formation was present in the central part of the composites. Furthermore, degradation of the composites was observed, indicated by the loss of integrity of the implant and by the decline in composite diameter and concomitant replacement by newly formed bone with increasing implantation time. Also, this newly formed bone around the composites appeared to be denser than the surrounding trabecular

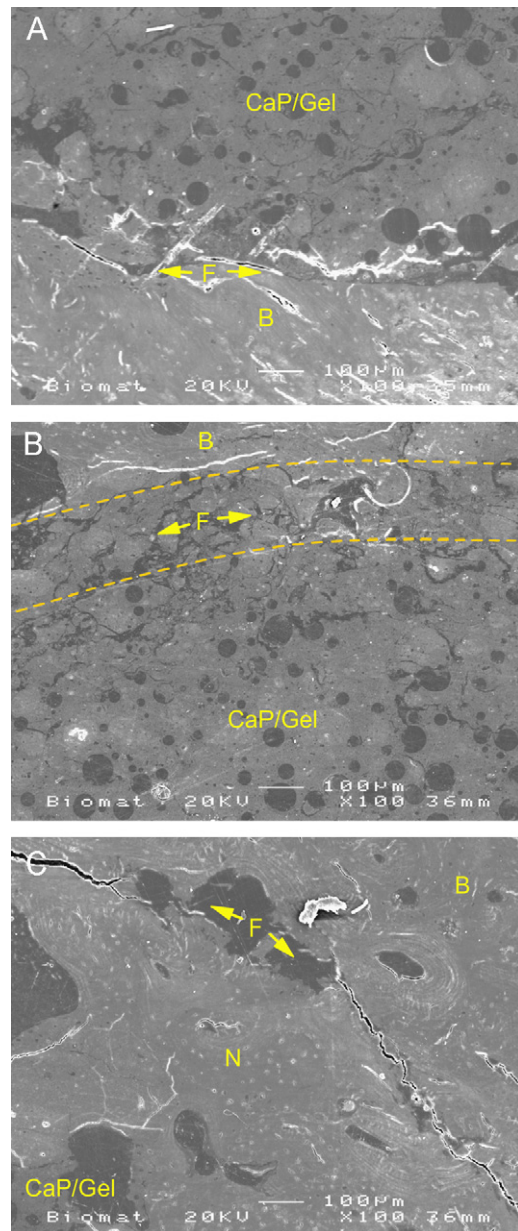


Fig. 2. Scanning electron microscopy (SEM) of CaP/gelatin composites with or without TGF- β 1 after 4 (A), 8 (B) and 12 (C) weeks of implantation. CaP/Gel = calcium phosphate cement with incorporated gelatin microparticles; B = trabecular bone; F = fracture (plane); N = newly formed bone.

bone. In three samples (out of 36), an intervening fibrous tissue layer was observed between composite and surrounding bone. One was found after 8 weeks of implantation in the CaP/gelatin group, while the other two were found in the CaP/gelatin + TGF- β 1 at 4 and 8 weeks of implantation. None of the composites were associated with the presence of multi-nucleated giant cells.

Histomorphometrical evaluation showed no significant differences between formulations containing CaP/gelatin or CaP/gelatin + TGF- β 1 (Fig. 4). Bone ingrowth into the CaP/gelatin composites after 4 weeks of implantation was

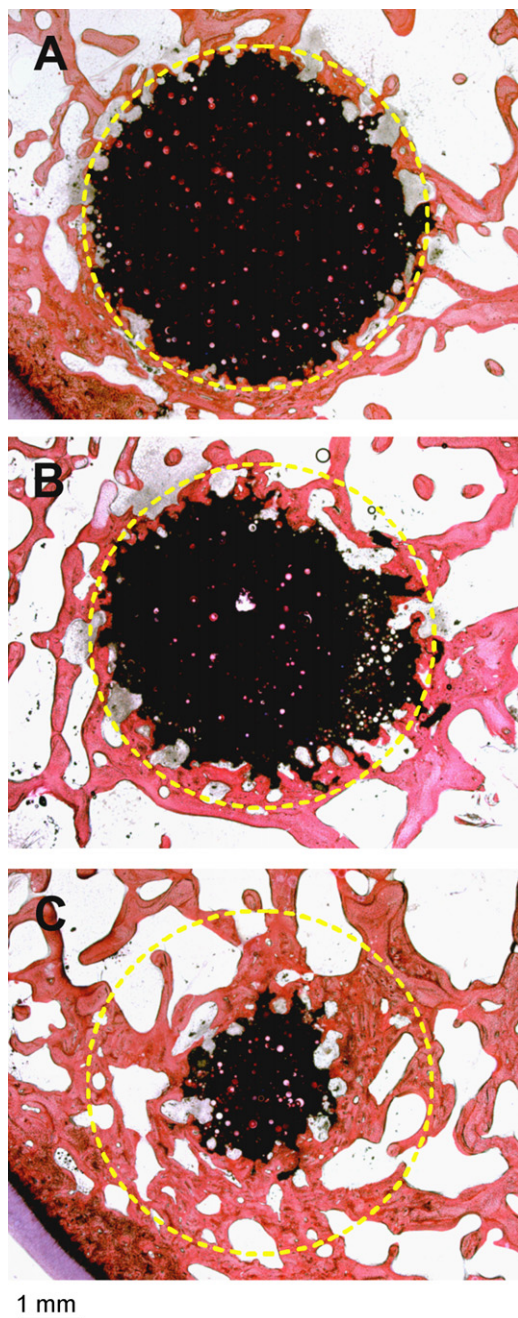


Fig. 3. Histological sections of CaP/gelatin + TGF- β 1 composites after 4 (A), 8 (B), and 12 (C) weeks of implantation (dashed circle marks the original defect).

$0.6 \pm 0.5 \text{ mm}^2$, which increased to $1.8 \pm 0.4 \text{ mm}^2$ at 8 weeks, and finally measured $2.2 \pm 1.0 \text{ mm}^2$ after 12 weeks of implantation. CaP/gelatin + TGF- β 1 composites showed bone ingrowth of $1.0 \pm 0.7 \text{ mm}^2$ after 4 weeks of implantation, which increased to $1.7 \pm 0.9 \text{ mm}^2$ at 8 weeks, and finally measured $3.8 \pm 2.0 \text{ mm}^2$ after 12 weeks of implantation.

Bone/implant contact of the CaP/gelatin composites (Fig. 5) after 4 weeks of implantation was $61.0 \pm 11.4\%$, which after 8 weeks measured $64.8 \pm 15.1\%$, and finally was $64.2 \pm 15.8\%$ after 12 weeks of implantation. CaP/

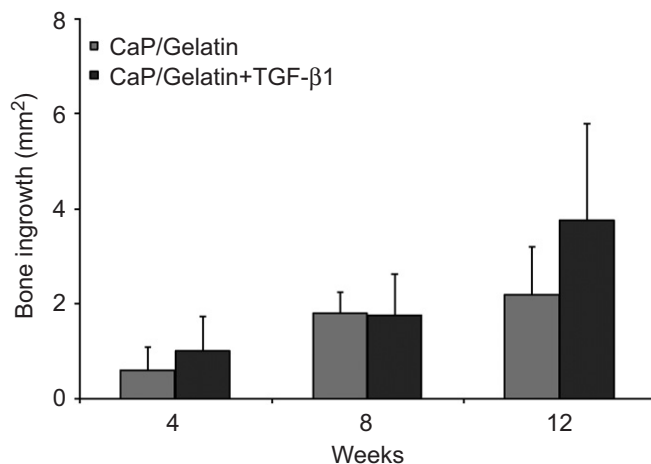


Fig. 4. Bone ingrowth (in mm^2) into the CaP/gelatin composites with or without TGF- β 1 after 4, 8 and 12 weeks of implantation. Bars represent means \pm SD ($n = 6$).

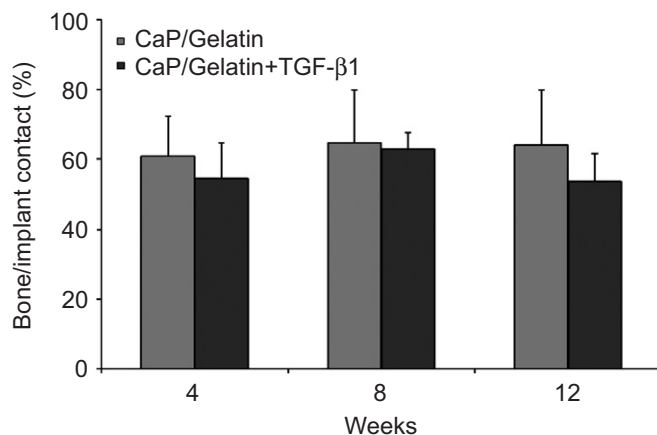


Fig. 5. Bone/implant contact (in %) of the CaP/gelatin composites with or without TGF- β 1 after 4, 8 and 12 weeks of implantation. Bars represent means \pm SD ($n = 6$).

gelatin + TGF- β 1 composites showed a bone/implant contact of $54.6 \pm 10.2\%$ after 4 weeks of implantation, which after 8 weeks measured $62.9 \pm 4.8\%$, and finally was $53.7 \pm 8.0\%$ after 12 weeks of implantation.

The remaining surface area of the CaP/gelatin composites (Fig. 6) after 4 weeks of implantation was $11.2 \pm 1.0 \text{ mm}^2$, which decreased to $9.2 \pm 1.0 \text{ mm}^2$, and finally measured $7.7 \pm 1.7 \text{ mm}^2$ after 12 weeks of implantation. CaP/gelatin + TGF- β 1 composites showed a remaining surface area of $10.4 \pm 1.0 \text{ mm}^2$ after 4 weeks of implantation, which decreased to $8.4 \pm 2.0 \text{ mm}^2$ at 8 weeks, and finally measured $3.8 \pm 2.3 \text{ mm}^2$ after 12 weeks of implantation. The difference between CaP/gelatin composites and CaP/gelatin + TGF- β 1 was significant after 12 weeks of implantation.

4. Discussion

In this study, the bone response and mechanical strength of a rabbit femoral defect filled with an injectable CaP

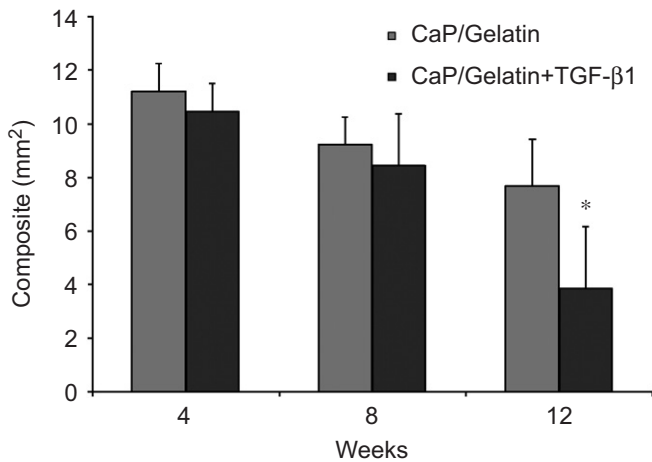


Fig. 6. Remaining surface area (mm²) of the CaP/gelatin composites with or without TGF-β1 after 4, 8 and 12 weeks of implantation. Bars represent means ± SD ($n = 6$). (* $p < 0.05$.)

cement containing gelatin microparticles, either or not loaded with TGF-β1, was examined. The loading of gelatin microparticles with TGF-β1 was enrolled as a parameter to evaluate potential effects of this growth factor on bone formation and cement resorption.

The porosity of both composite formulations (CaP/gelatin with or without TGF-β1) was $45.0 \pm 1.3\%$, which should be high enough to obtain an interconnective network after microparticle degradation [33]. Nonetheless, no fibrous tissue or bone formation was observed in the central part of the implants. This might be due to the delayed degradation of gelatin microparticles in the central part of the composites, or the relatively low mass ratio of microparticles (5 wt%) in the composite formulations. The lack of interconnectivity might further be explained by the formation of apatite crystals on gelatin, which has been reported previously [15,34]. This phenomenon might cause filling of the created pores with precipitate, and hence hamper the formation of an interconnective network. This problem can be solved partly by increasing the mass ratio, but we know also that setting problems occur if the mass ratio is above a certain threshold [15], which makes such composite formulations unfavorable for clinical usage.

In this study, implants were grinded to obtain a flat surface for the push-out test. Still, parameters like composite thickness, composite diameter, angularity of the bone defect and positioning of the lever can influence the results of the push-out test [31]. Nevertheless, the push-out results represent an approximation of the actual mechanical strength. Remarkably, the results of the push-out test showed similar or even higher values compared to the compression strength of 4–10 MPa of trabecular bone [35–37]. This observation is in accordance with Ikenaga et al. [37], who also found massive new bone formation around degraded CaP cement, which was mainly composed of β-TCP. This new bone formation resulted in an increased bone density directly around the degraded

implant, and hence higher push-out values of the composites compared to trabecular bone. Furthermore, the applied push-out test utilizes a similar lever for all samples. In view of composite degradation and replacement by newly formed bone at later implantation periods, the used method is not testing the actual interface of the composite with the bone tissue of these samples, but rather the denser bone structure directly around the degraded composite. The increase in mechanical strength with implantation time was further confirmed by SEM examination, which evidently supports the suggestion that the composite appears to be suitable for load-bearing purposes.

Histological evaluation showed that bone ingrowth started at the defect edges and proceeded into the micropores, which were created after degradation of the gelatin microparticles. This was also observed in other *in vivo* experiments with CaP cement based composites [12,13,38]. This might be due to the already excellent osteogenic properties of the CaP cement. Kroese-Deutman et al. [39] reported that porous CaP cement itself was sufficient to bridge a critical-sized cranial defect in rabbits, without the presence of any growth factors. On the other hand, the cement material in their study did not show a clear sign of degradation despite the complete filling of the implant porosity with bone tissue. This is in contrast to the current study where the TGF-β1 loaded specimens were significantly more degraded than the non-loaded specimens, which suggests that the released TGF-β1 evoked an enhanced bone remodeling resulting in increased cement degradation.

Further, it has to be noticed that we decided to employ a dose of 250 ng TGF-β1 (3.3 ng per mm³ implant) per injectable composite. Since the optimal TGF-β1 concentration is unknown, the amount of included TGF-β1 was based on previous research [40]. Amounts of 0.5 ng TGF-β1 per mm³ implant have been reported to have an effect on bone formation in a rat cranial defect [41]. Also, Vehof et al. [42] and Beck et al. [43] both described the incorporation of up to 25 ng TGF-β1 per mm³ implant to have an effect on bone formation in a rabbit cranial defect.

Considering the release kinetics of TGF-β1 from the gelatin/CaP composite, previous research by Habraken et al. [44] showed that the release of TGF-β1 from gelatin microparticles incorporated in CaP cement was limited to 14%, and that the release of adsorbed TGF-β1 on CaP cement alone was limited to 33% after 6 weeks of incubation. From literature, it is further known that CaP cements have a strong binding affinity for proteins [45,46]. Most likely, after degradation of the gelatin microparticles, the released TGF-β1 was bound to the CaP cement, which after cement degradation was released again. Therefore, the released TGF-β1 is still able to stimulate the bone response after longer implantation periods. Besides, it is proposed that even in a biomaterial-bound state, a growth factor can retain its biological activity [47,48].

Although almost all implants showed good bone ingrowth, three samples showed an intervening fibrous

tissue layer between composite and surrounding bone. This could be explained by an initially suboptimal bone/implant contact, which can result in fibrous tissue formation [12]. The added water inside the gelatin microparticles and the presence of body fluids can contribute to a delayed setting time [15,49]. This hampers setting of the composite on the defects periphery, and can lead to composite material being flushed away at the bone defect wall. Although bleeding of the bone defects was tried to be minimized by packing the defects with gauzes before injecting the composites, the presence of limited amounts of blood at the defect site could not be completely prevented.

The bone/implant contact measurements showed a contact of approximately 60% during all implantation periods. Ooms et al. [7] reported that a CaP cement without microparticles resulted in a bone/implant contact of 80–90%. By adding microparticles in the CaP cement the surface area of the CaP cement is increased after degradation of the microparticles. Therefore, new bone formation has to fill up the created pores, which might explain the differences in bone/implant contact with dense CaP cement.

5. Conclusions

In conclusion, this study demonstrated that after injection and setting of a CaP/gelatin composite, the mechanical strength of the composite including the new bone formation, is sufficient after 12 weeks of implantation for load-bearing purposes. Further, degradation of the composites was observed, indicated by the loss of integrity of the composites, and by the decline in composite diameter and concomitant replacement by newly formed bone with increasing implantation time. The addition of TGF- β 1 to the gelatin microparticles in the composite formulation did not result in higher mechanical strength values or improvement of the bone response, but significantly increased composite degradation after 12 weeks of implantation.

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