In vitro growth factor release from injectable calcium phosphate cements containing gelatin microspheres

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Abstract: To improve the *in vivo* resorption of an injectable calcium phosphate cement (CPC) for bone tissue engineering purposes, in previous experiments macroporosity was introduced by the *in situ* degradation of incorporated gelatin microspheres. Gelatin microspheres are also suitable carriers for osteoinductive drugs/growth factors, where release occurs concomitantly with degradation of the hydrogel. Introduction of these microspheres into CPC can alter the release pattern of the cement, which usually shows a marginal release of incorporated drugs. The goal of this study was to determine the *in vitro* release characteristics of gelatin microsphere CPC. For this, recombinant human TGF-β1, bFGF, and BMP-2 were labeled with ^{125}I and loaded onto gelatin type A (porcine, pI = 7.0–9.0)/ type B (bovine, pI = 4.5–5.0) microspheres for a short (instant) and longer (prolonged) time before mixing them with the cement. Radioactivity of the resulting 5 or 10 wt % gelatin microsphere CPC composites was monitored for 6 weeks when subjected to proteolytic medium. Drug-loaded CPC was used as control. Results showed that release pattern/efficiency of gelatin microsphere CPCs and CPC controls was highly dependent on the type of growth factor but unaffected by the amount of growth factor. With gelatin microsphere CPC, release was also dependent on the type of gelatin, total volume of incorporated microspheres, and loading method. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 91A: 614–622, 2009

Key words: gelatin microspheres; calcium phosphate cement; growth factors; release; radioactive label

INTRODUCTION

Calcium phosphate cements (CPCs) are commonly used as bone-filling materials in the field of dentistry, orthopedic, and postoperative surgery. These cements have proven be biocompatible and osteoconductive, and because of the *in-situ* setting abilities, a perfect fit with the site defect can be accomplished.[1,2] One disadvantage is the high density and slow degradability of the material, which makes it less applicable for tissue engineering purposes.[3] Therefore, in earlier studies by our group microspheres made of poly(lactic-*co*-glycolic acid) (PLGA)[4–8] and gelatin[9,10] were added to an apatite cement. These microsphere CPCs exhibited good injectability characteristics and setting properties. During *in vitro/in vivo* degradation of the spheres, porosity increased concomitantly, yielding a structure of spherical pores and a decrease in mechanical strength. PLGA microspheres produced an acidic environment due to the hydrolysis of the ester groups.[8] On the other hand, gelatin microspheres degraded gradually by proteolysis and no additional decrease in pH was observed.[9] Drug release studies with microsphere CPC were also performed as the addition of osteoinductive drugs can improve bone remodeling at the implant site.[11,12] The drug was incorporated or adsorbed onto the microspheres to give a release pattern that differs from the usual diffusion dependent release from CPCs.[13,14] *In vitro/ in vivo* release data of bone morphogenic protein-2 (BMP-2) from PLGA microsphere CPC overall showed a pattern that was characterized by a small burst release, followed by a period of slow, sustained release.[4,5] Furthermore, by changing the molecular weight of the polymer release was tailored within certain ranges as low molecular weight PLGA

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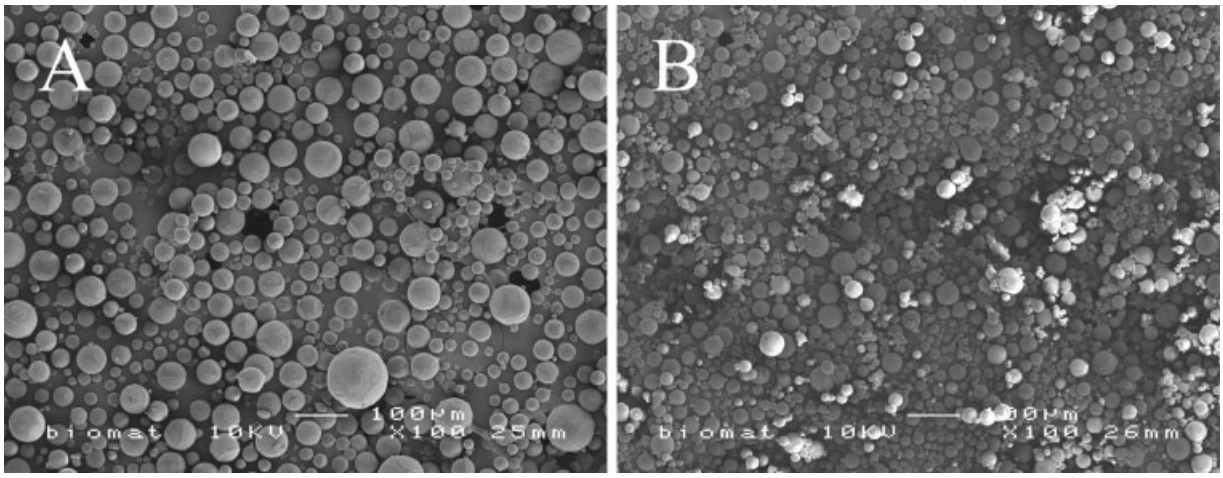

Figure 1. Morphology microspheres by scanning electron microscopy; (A) GELA microspheres, (B) GELB microspheres (original magnification 100×).

microsphere CPC showed a significant higher release after 4 weeks *in vivo*.[5] In an *in vitro* release study using gelatin microsphere CPC,[10] microspheres were loaded with bovine serum albumin (BSA) and release from the microsphere CPC was monitored for 9 weeks. Two loading methods were applied; BSA was loaded onto the microspheres directly before mixing (instant loading) or 24 h before mixing (prolonged loading) to establish a strong electrostatic bond between the basic gelatin (type A, pI = 7.0–9.0) and the acidic BSA (pI = 5.0).[15] Results showed a release pattern without initial burst and a delayed release after 3 days that decreased in time. Release up to three weeks was significantly higher with the instant loaded scaffolds, whereas the prolonged loaded scaffolds exhibited a more sustained release. Structure analysis by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) showed peaks of intact BSA after 3, 7, and 17 days. Gelatin microsphere CPC therefore seems to be a suitable scaffold for drug delivery; however, growth factors like BMP-2, transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) can show a completely different release pattern than BSA.[1] In addition, there exist two types of gelatin; type A (pI = 7.0–9.0) and type B (pI = 4.5–5.0) that because of their opposite isoelectric point exhibit different electrostatic interactions with various drugs/proteins.[15]

The goal of this study was to determine the release characteristics of gelatin type A or type B microsphere CPC by loading the scaffolds with various types of growth factor (TGF-β, bFGF, and BMP-2) and applying two loading mechanisms. Also, different amounts of growth factor were loaded onto the gelatin microspheres to investigate whether release from these composites is concentration dependent. Release was measured by labeling the growth factors with ^{125}I after which radioactivity of the loaded composites and release medium (phosphate buffered saline, PBS) was monitored for a total of 6 weeks.

MATERIALS AND METHODS

Materials

Cell culture tested gelatin type A (GELA) (pI = 7.0–9.0, bloom = 300) and gelatin type B (GELB) (pI = 4.7–5.2, bloom = 225) (Sigma-Aldrich, St. Louis, USA) were used for the fabrication of the microspheres. The CPC used is commercially available under the product name Calcibon® (Biomet Merck, Darmstadt, Germany) and consists of 61% α-tricalcium phosphate (α-TCP), 26% Ca_2HPO_4, 10% $CaCO_3$ and 3% precipitated hydroxyapatite (pHA). Collagenase 1A (Sigma) was used as gelatin degrading enzyme. Recombinant human transforming growth factor β1 (TGF-β1, pI = 9.5), basic fibroblast growth factor (bFGF, pI = 9.6), and bone morphogenic protein-2 (BMP-2, pI = 8.5) (R&D systems Europe Ltd., Abingdon, UK) were the growth factors used in the release experiment.

Preparation of gelatin microspheres

Totally, 2.5 g of GELA or GELB was dissolved in 25 mL ddH_2O at an elevated temperature (30 min at 60°C). The resulting clear solution was added slowly (10-mL pipette) to a 250-mL three-necked round bottom flask containing 125 mL olive oil while stirring at 500 rpm (Teflon upper stirrer). During stirring, the round bottom flask was put in an ice bath. After 30 min, 50 mL of chilled acetone (4°C) and glutaraldehyde (0.5 mL = 6.25 m*M*) was added slowly. The solution was stirred for another hour. Microspheres were collected by filtration (D3, Schott Duran, Mainz, Germany) and washed several times with acetone (~1 L) to remove residual olive oil. Following this, micro-

TABLE I
Average Size Gelatin Microspheres (n = 200)

	Average Size (μm)	
	Dry	Swollen
GELA microspheres	15.5 ± 13.8	37.4 ± 31.1
GELB microspheres	8.4 ± 7.6	20.7 ± 14.6

spheres were stored in a drying chamber until further use. Morphology of the microspheres was visualized by scanning electron microscopy (SEM) (JEOL 6400-LINK AN 10000 at 10 kV) and is shown in Figure 1. Average size of the microspheres is given in Table I and was determined using digital image software (Leica Qwin®, Leica Microsystems AG, Wetzlar, Germany).

Radioiodination of growth factors

TGF-β1, bFGF and BMP-2 were labeled with ^{125}I according to the iodogen method.[16] Briefly, in a 500-μL eppendorf tube coated with 50-μg iodogen 10 μL of 0.5*M* phosphate buffer saline (PBS) was added and adjusted to 100 μL with 50 m*M* PBS. Growth factor (8 μg) and 10–15 MBq ^{125}I (Perkin-Elmer, Boston, MA) was added and incubated at room temperature for 10 min. After that, 100 μL of saturated Tyrosine solution in PBS was added and labeling efficiency of the reaction was 36%, 13%, and 43% for TGF-β1, bFGF, and BMP-2, respectively. To remove the nonbound I^{125} activity, the reaction mixture was eluted with 0.1% BSA in PBS on a prerinsed disposable Sephadex G25M column (PD-10; Pharmacia, Uppsala, Sweden). The radiochemical purity of the ^{125}I-labeled growth factors was determined by instant thin layer chromatography (ITLC) on Gelman ITLC-SG strips (Gelman Laboratories, Ann Arbor, MI) with 0.1*M* citrate, pH 5.0 as a mobile phase. The radiochemical purity of the growth factors is presented in Table II.

TABLE II
Radiochemical Purity ^{125}I-Labeled Growth Factors

	TGF-β1	bFGF	BMP-2
Radiochemical purity	95.6%	83.9%	97.8%

Preparation of gelatin microsphere/CPC composites

For the release study, 10 wt % gelatin type A microsphere CPCs (GELA CPCs) and 5 wt % gelatin type B microsphere CPCs (GELB CPCs) were prepared as described in previous studies.[9,10] The weight % of microspheres inside the cement corresponds to the maximum amount of microspheres that can be incorporated to still obtain a composite with manageable handling properties.[10] TGF-β1, bFGF, and BMP-2 was loaded onto the composites by two loading methods where growth factor was mixed with the cement directly after loading them to the microspheres (instant loading), or following a period of swelling before cement was added (prolonged loading). Briefly, growth factor loaded 5/10 wt % gelatin microsphere CPCs were made by adding a growth factor solution in PBS (300/560 μL) to 50/100 mg of dry gelatin microspheres inside a BSA coated 2-mL syringe. With instant loading, after 15 s of vigorous stirring using a Silamat® (Vivadent, Schaan, LIE) mixing apparatus, 950/900 mg CPC was added and the constituents were stirred again for 15 s or until an equal distribution of spheres inside the cement was obtained. With the prolonged loaded composites, the gelatin microspheres were allowed to swell for 24 h at 4°C before they were mixed with the cement. Following both procedures, 280 μL of 1% Na_2HPO_4 was added and after 15 s of stirring, the resulting paste was injected into Teflon moulds (cylindrical, d = 4.5 mm, h = 9.0 mm). Samples were hardened at room temperature overnight. SEM-micrographs of the gelatin microsphere CPCs are given in Figure 2. Growth factor-loaded samples without microspheres (CPC) were used as control and prepared by adding a growth factor solution to preset cement samples (adsorbed). Calculated amount of growth factor per sample is 50 ng. Furthermore, drug entrapment efficiency was

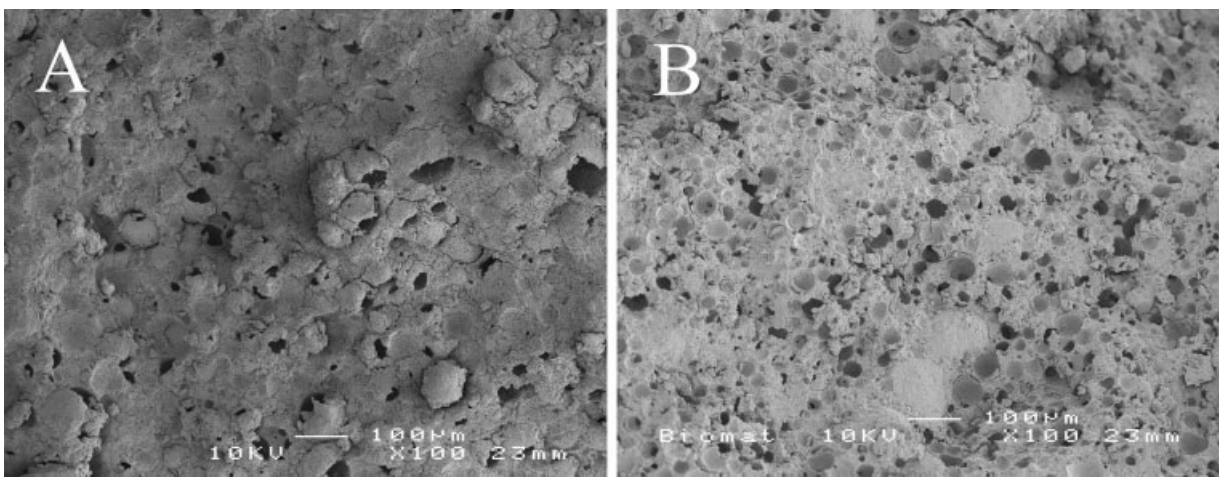

Figure 2. Morphology gelatin microsphere CPCs; (A) 10% GELA CPC, (B) 5% GELB CPC (original magnification 100×).

TABLE III
Vol % of Microspheres Inside Gelatin Microsphere CPC

Formulation	Volume % of Microspheres
5% GELA CPC	48.7 ± 1.8%
10% GELA CPC	57.5 ± 1.4%
5% GELB CPC	48.4 ± 1.8%

determined by measuring the γ-irradiation of the preset samples after which it was divided by the γ-irradiation of the corresponding calculated amount of labeled growth factor.

With BMP-2 some supplementary studies were performed. To investigate whether growth factor release is concentration dependent, different amounts of BMP-2 were incorporated into the cement. Next to 50 ng of ^{125}I-labbeled BMP-2 per scaffold, samples were loaded with 500 ng and 5 μg of BMP-2 applying hot/cold ratios of 1/10 and 1/100. Furthermore to investigate the effect on drug release of the total amount/volume of incorporated microspheres, with BMP-2 also a 5 wt % GELA CPC was formulated. Volume % of gelatin microspheres inside the 5 and 10 wt % GELA CPC[10] and 5 wt % GELB CPC is presented in Table III. Finally, in addition to the adsorbed CPC controls, CPC samples were prepared where BMP-2 was dissolved into the liquid hardener (1% Na_2HPO_4) during cement preparation (incorporated). In Table IV a scheme of the different parameters used during the experiment is presented.

Release study

Samples were placed in 3 mL PBS containing 373 ng/mL collagenase 1A[17] inside 10 mL polypropylene tubes. Tubes were put in a water bath containing a rotating plate (70 rpm) at 37°C. Medium was renewed every 3–4 days. At the same time, γ-irradiation of the sample/medium was measured for a total of 6 weeks. Samples were taken and measured in triplicate ($n = 3$).

Statistical analysis

Data were presented as mean ± standard deviation. Significant differences were determined using analysis of variance (ANOVA). Results were considered significant if $p < 0.05$. Calculations were performed using GraphPad Instat®.

TABLE IV
Parameters Used During the Release Experiment

Formulation	Type of Loading	Growth Factor Loaded (ng)				
				BMP-2		
		TGF-β1	bFGF	1×	10×	100×
10% GELA CPC	Instant	50	50	50	500	5000
	Prolonged	50	50	50	500	5000
5% GEL B CPC	Instant	50	50	50	500	5000
	Prolonged	50	50	50	500	5000
5% GELA CPC	Instant	–	–	50	–	–
	Prolonged	–	–	50	–	–
CPC (control)	Adsorbed	50	50	50	500	5000
	Incorporated	–	–	50	–	–

RESULTS

The drug entrapment efficiencies of the samples are given in Table V. For all types of gelatin microsphere CPC, drug entrapment efficiencies were around 70–75%. Differences in entrapment efficiency between instant and prolonged loaded scaffolds were observed, though no clear trend was observed. CPC controls where the BSA was adsorbed exhibited higher entrapment efficiencies (82–94%) than the gelatin microsphere CPC, though the incorporated CPC showed an efficiency of only 51%.

The release pattern for the CPC is given in Figure 3. Overall, CPC where the drug was adsorbed onto the surface exhibited a release pattern consisting of an initial burst, followed by a sustained release that was slowly diminishing in time. The release pattern of both BMP-2 and bFGF was similar, whereas the TGF-β1 showed a significant lower initial burst and sustained release. In contrast to the BMP-2 adsorbed CPC, release from the BMP-2 incorporated cement

TABLE V
Entrapment Efficiency Gelatin Microsphere CPC and CPC as Percentage of Calculated Amount of Growth Factor (1× = 50 ng, 10× = 500 ng, 100× = 5 μg)

			BMP-2		
	TGF-β1	bFGF	1×	10×	100×
CPC (adsorbed)	81.7 ± 3.4	82.3 ± 3.1	93.7 ± 4.2	90.7 ± 4.7	88.5 ± 2.9
10% GelA CPC instant	74.8 ± 1.8	75.7 ± 2.6	77.7 ± 2.3	78.8 ± 1.5	75.7 ± 1.9
10% GelA CPC prolonged	66.8 ± 1.7	76.1 ± 0.5	71.0 ± 2.0	72.1 ± 1.3	68.3 ± 1.6
5% GelB CPC instant	72.4 ± 2.8	69.4 ± 0.4	77.8 ± 2.4	81.0 ± 4.3	73.1 ± 4.4
5% GelB CPC prolonged	68.5 ± 0.2	68.8 ± 1.6	71.4 ± 0.9	71.1 ± 1.0	70.0 ± 1.3
CPC (incorporated)	–	–	50.9 ± 2.3	–	–
5% GelA CPC instant	–	–	69.8 ± 1.5	–	–
5% GelA CPC prolonged	–	–	75.8 ± 1.8	–	–

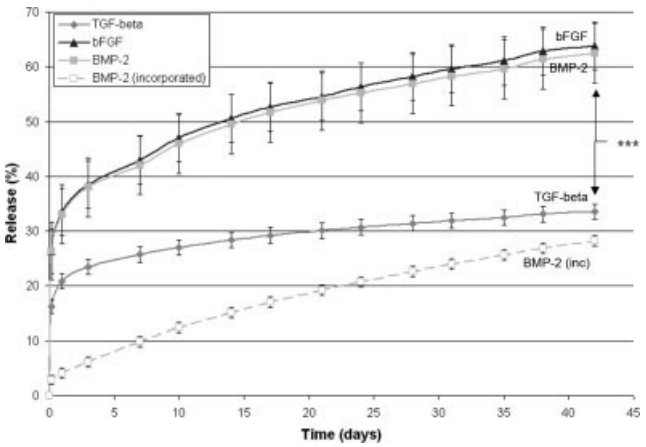

Figure 3. Growth factor release from CPC, *** = $p < 0.001$.

did not show an initial burst. Furthermore, sustained release was comparable to the BMP-2 adsorbed cement though release efficiency (Table VI), due to the initial burst, was significantly lower ($p < 0.001$).

Similar to the CPC samples, both 10% GELA CPCs (Fig. 4) and 5% GELB CPCs (Fig. 5) exhibited a pattern consisting of a (small) initial burst followed by sustained release of the growth factor. Also here, release was dependent on the type of growth factor, where bFGF exhibited a higher burst release than BMP-2 or TGF-β1. Sustained release and release efficiency (Table VI) was in the following order; bFGF > BMP-2 ≫ TGF-β1. The 10% GELA CPC also showed a significant higher sustained release from the prolonged loaded scaffolds when compared to the instant loaded scaffolds for all growth factors. With TGF-β1 the prolonged loaded scaffolds experienced a 33% higher release after 6 weeks, whereas with bFGF and BMP-2 this was 8% and 13%. The 5% GELB CPC did not show such a trend, as with bFGF the instant loaded scaffolds showed a significant higher release ($p < 0.01$), and differences between instant and prolonged loaded scaffolds with BMP-2 and TGF-β1 were small.

In Figure 6 the BMP-2 release from all formulations is depicted. It was observed that sustained release from the 5% GELA CPC was significantly lower ($p < 0.001$) than the 10% GELA CPC, 5% GELB CPC and CPC controls. Furthermore, sustained release from the incorporated CPC was identical to the 5% GELB CPC and instant loaded 10% GELA CPC, though slower than the prolonged loaded 10% GELA CPC.

Figure 7 shows the absolute release from the BMP-2 loaded composites. The release patterns at the different BMP-2 concentrations were identical for all formulations and exhibited respectively a 10/100 fold increase in burst or sustained release and absolute release after 6 weeks (Table VI) when implants were loaded with a 10/100-fold higher amount of BMP-2.

DISCUSSION

In this study, growth factor release from injectable CPCs with incorporated gelatin microspheres was investigated. For this purpose, TGF-β1, bFGF, or BMP-2 was added to the gelatin microspheres before they were mixed with the cement using two loading methods. Furthermore, scaffolds were prepared using either type A or type B gelatin microspheres. In addition, for BMP-2 the effect of growth factor concentration and amount of incorporated microspheres on the release pattern was investigated. Samples without gelatin microspheres (CPC) were used as control where the growth factor was adsorbed onto the scaffolds or added to the liquid hardener (BMP-2).

Results showed that the drug entrapment efficiency of the CPC controls was dependent on the loading method as the incorporated CPC sample

TABLE VI
Release Efficiency After 6 Weeks of Gelatin Microsphere CPC and CPC as Percentage of Actual Loaded protein (1× = 50 ng, 10× = 500 ng, 100× = 5 μg)

			BMP-2		
	TGF-β1	bFGF	1×	10×	100×
CPC (adsorbed)	33.5 ± 1.4	63.9 ± 4.4	62.5 ± 5.4	56.2 ± 2.8	57.4 ± 2.3
10% GelA CPC instant	13.3 ± 0.3	50.5 ± 1.2	28.8 ± 0.3	30.5 ± 1.0	29.2 ± 0.5
10% GelA CPC prolonged	17.8 ± 0.5	54.3 ± 1.4	32.5 ± 1.4	32.0 ± 0.8	34.2 ± 1.4
5% GelB CPC instant	13.8 ± 0.2	53.7 ± 0.4	27.1 ± 0.3	28.0 ± 1.0	28.3 ± 0.6
5% GelB CPC prolonged	14.4 ± 0.4	50.7 ± 1.0	28.1 ± 0.4	28.0 ± 0.6	28.3 ± 0.2
CPC (incorporated)	–	–	28.3 ± 1.0	–	–
5% GelA CPC instant	–	–	20.7 ± 0.3	–	–
5% GelA CPC prolonged	–	–	18.6 ± 0.3	–	–

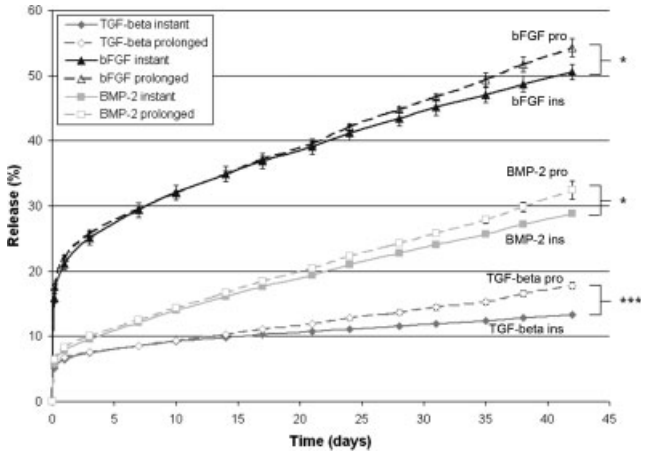

Figure 4. Growth factor release from 10% GELA CPC, * = $p < 0.05$, *** = $p < 0.001$.

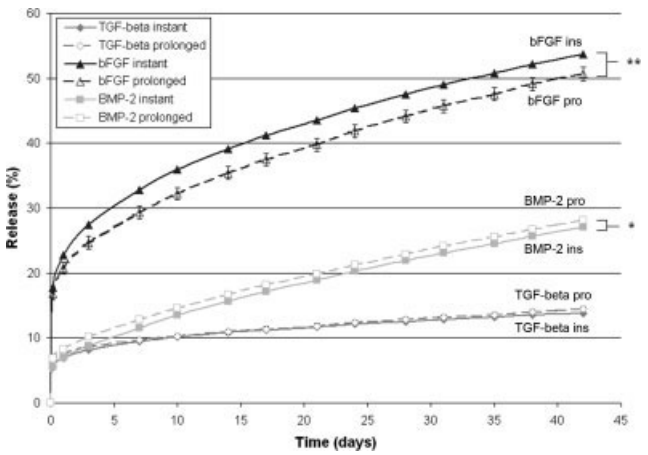

Figure 5. Growth factor release from 5% GELB CPC, * = $p < 0.05$, ** = $p < 0.01$.

clearly exhibited a significant lower efficiency than the adsorbed CPC samples. For the incorporated CPC sample, losses occurred when drug adsorbed to the syringe during mixing or when drug was absorbed to the Teflon mould during cement hardening. Entrapment efficiency of the gelatin microsphere composites was intermediate. This indicates that the (electrostatic) interaction between the drug and microspheres was strong enough to retain drug inside the spheres during mixing; however, part of the growth factor will be dispersed into the surrounding cement.[15]

With most formulations an initial burst release of the growth factor was observed. With the growth factor-adsorbed CPC most of the drug was located at the outside of the sample, which explains the high burst release.[13,14] The CPC with incorporated BMP-2 did not show a burst release as the growth factor in this sample was dispersed more evenly through the cement, which is known for its protein-binding capacity.[13,14,18] Occasionally, with the gelatin microsphere CPCs a substantial burst release was obtained. Although the BMP-2 and TGF-β1 loaded composites showed a burst release of 5–7%, with bFGF a burst release of 16–18% was observed. This burst release is probably due to the suboptimal radiochemical purity of I^{125} labeled bFGF, which was 84%. The radioactive preparation used in this experiment therefore contained 16% free Iodine-125 that is expected to exhibit a fast release from the scaffolds. Regarding this radiochemical purity, burst release from gelatin microsphere CPC was low. As gelatin scaffolds/microspheres often show a high burst release,[15,17,19] the low burst observed here is a result of protein binding to the cement after release from the microspheres.

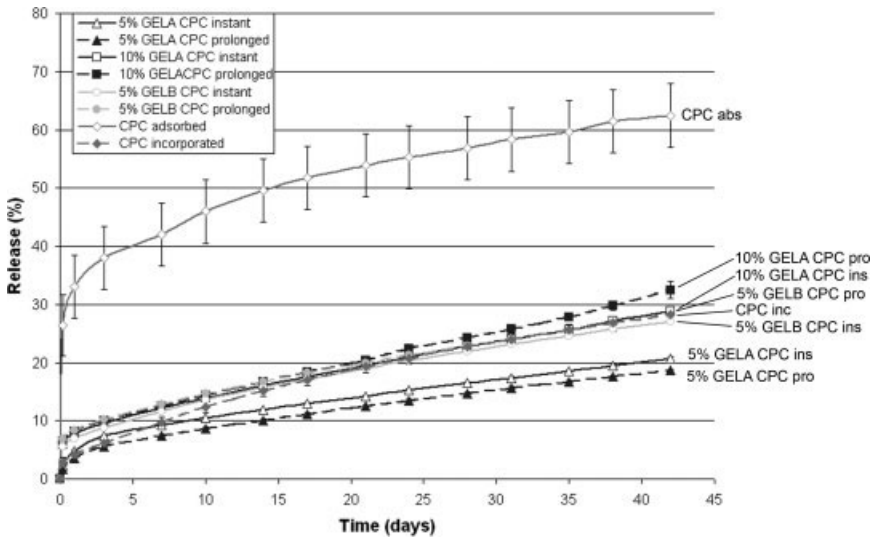

Figure 6. BMP-2 release from gelatin microsphere CPC and CPC.

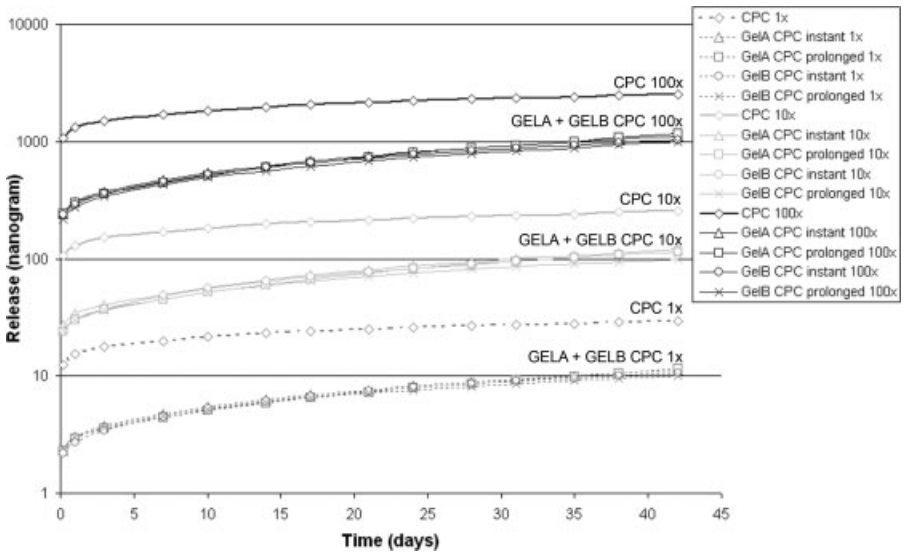

Figure 7. Cumulative BMP-2 release from 10% GELA CPC, 5% GELB CPC and CPC with different amount of incorporated BMP-2 (1× = 50 ng, 10× = 500 ng, 100× = 5 μg).

Following the burst release, with each growth factor a sustained release was observed. With the CPC controls, this sustained release is probably due to diffusion of drug through the cement[20,21] as the CPC does not show signs of degradation or dissolution[8–10]. Figure 8 shows that BMP-2 release from the incorporated CPC is proportional to the square root of time, which is typical for a diffusion dependent release. Prolonged loaded 10% GELA CPC, however, shows a more linear sustained release. With the gelatin microsphere CPCs drug release can also be the result of the proteolytic degradation of microspheres. Supposedly degradation of gelatin microspheres inside the CPC is a gradual process from the outside of the implant to the inner part,[10] though in these samples also an initial burst of free gelatin chains was observed. According to literature,[15] electrostatically bonded growth factor releases concomitantly with release of gelatin degradation products. The observation that the release from the 10% GELA CPC is more rapid than from the 5% GELA CPC indicates that a similar mechanism indeed occurs. Furthermore as the used collagenase specifically degrades collagen or gelatin,[22] no proteolytic degradation of the growth factor is expected.

In most cases the magnitude of the sustained release from the gelatin microsphere CPC was comparable to the CPC samples, whereas it was significantly lower with 5% GELA CPC. This can be due to the fact that proteolytic gelatin degradation does not occur in a high extent for the 5% GELA CPC and most of the growth factor is retained in the gelatin microspheres. In previous degradation studies[9,10] it was concluded that due to the low interconnectivity of the microspheres inside a 5% gelatin microsphere CPC, the enzyme did not penetrate the composite as it experienced physical interactions with the surrounding cement. The release that is observed from the 5% GELA CPC is mostly due to diffusion from the cement, which is higher with the CPC controls. Figure 6 also shows that BMP-2 release from the 5% GELB CPC is significantly higher than release from the 5% GELA CPC, whereas the vol % of microspheres inside the composite is similar (Table III). This can be explained by a better distribution of the GELB microspheres into the cement, which are smaller in size than the GELA microspheres, leading to a higher interconnectivity and improved microsphere degradation. Also, the lower isoelectric point of gelatin type B can have caused the higher sustained release from the GELB CPC, as the used

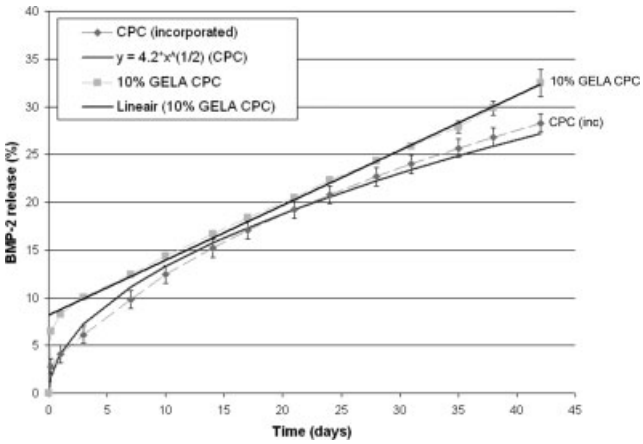

Figure 8. BMP-2 release curves from BMP-2 incorporated CPC and 10% GELA CPC with additional trend lines.

growth factors all have an isoelectric point of 8.5–9.5. The electrostatic interactions with the acidic GELB microspheres (pI = 4.5–5.0) will be stronger than with the GELA microspheres (pI = 7.0–9.0), leading to smaller losses to the surrounding cement during composite preparation. However, next to electrostatic interactions, physical entanglements also play a role in drug loading. Similar to the GELA CPC, an increase in the percent of microspheres inside the GELB CPC is supposed to improve drug release from these composites. However, like food-grade gelatin microspheres in a previous study,[9] with GELB microspheres the addition of more than 5 wt % of microspheres renders a composite with insufficient setting properties.

Apart from the implant material, the release test showed large differences in the sustained release of the various growth factors, that is showing a fast release of bFGF and a much slower release of TGF-β1. In agreement with other studies with cement or gelatin as scaffold material,[15,19,23] release in this study was highly dependent on the type of growth factor. Intrinsic and physical parameters like the isoelectric point,[15,19] hydration,[24] and size of the drug play an important role in drug release; however, these parameters do not always have the same effect on the release pattern/efficiency. Charge distribution and chain conformation of the drug are markedly influenced by environmental conditions like buffer capacity (pH) and consistency of the medium.[15,24] Also the state of the cement surface changes when these environmental conditions alter, resulting in a more positively/negatively charged substrate.[25] It is therefore not always straightforward to predict release from these CPCs, despite the similarities between the drugs.

In the previous BSA release study[10] it was shown that the loading method also can be used to tailor release from the 10% GELA CPCs. In accordance to this study, current results indeed showed that absorption of the growth factor for a longer period onto the microspheres (prolonged loading), led to a higher sustained release with these composites. The explanation for this phenomenon is a higher release from the gelatin microspheres during proteolytic degradation as result of stronger electrostatic interactions between the gelatin and growth factor, and less losses to the surrounding cement. GELA CPC (5%) and GELB CPC (5%) do not, or to a lesser extent, show this feature.As discussed earlier, proteolytic degradation did not occur in a high extent for the 5% GELA CPC and therefore it is not surprising that with this formulation the prolonged loaded samples did not show a higher sustained release than the instant loaded samples. Furthermore, as electrostatic interactions between the gelatin and growth factors are expected to be stronger with GELB microspheres, these interactions already could be optimal using instant loading.

Another feature that was observed in the BSA release study[10] was a substantial higher release from the instant loaded scaffolds within the first weeks due to weaker electrostatic interactions between the drug and gelatin. In our study, in most cases growth factor release up to 3 weeks was similar for both instant and prolonged loaded samples. With bFGF loaded 5% GELB CPC there was a 10% higher release from the instant loaded scaffolds within the first weeks, though with BSA a 100% higher release was obtained. Comparison of both studies, the type of drug (BSA/growth factor), substrate medium (demineralized water/PBS), and drug concentration (1.5 mg/50–5000 ng) used during the release experiment all could have influenced drug release. The use of demineralized water in the BSA release study also resulted in a strong decrease of the pH. An increase in drug concentration can influence release properties as protein-binding sites are saturable.[26] Increasing BMP-2 loading from 50 ng to 5 μg in this study did not lead to a different release pattern or efficiency, but a proportional increase in absolute release. Within this range, sustained release from the gelatin microsphere CPCs and CPC controls therefore can be tuned to a certain therapeutic amount per week.

CONCLUSION

Gelatin microsphere/CPC composites can be applied for the sustained release of growth factors. The composites showed release efficiencies of 14–55% and release patterns consisting of a small initial burst followed by a sustained release up to 6 weeks. CPC controls showed a higher burst release as well as release efficiency when drug was adsorbed at the outside of the sample, but a comparable pattern and release efficiency when drug was incorporated during cement preparation. Sustained release from the gelatin microsphere CPCs was highly dependent on the type of growth factor and was also dependent on the total volume of microspheres and type of gelatin. Also the loading mechanism was important, as instant loaded 10% GELA CPC showed a significant higher sustained release than prolonged loaded composites. Furthermore, release patterns/efficiencies from the gelatin microsphere composites and CPC controls did not differ significantly when BMP-2 concentration was increased with a factor of 10–100.

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