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Incorporation of polymeric microparticles into collagen-hydroxyapatite scaffolds for the delivery of a pro-osteogenic peptide for bone tissue engineering

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Collagen-hydroxyapatite scaffolds are outstanding materials for bone tissue engineering as they are biocompatible, bioresorbable, osteoconductive, and osteoinductive. The objective of the present work was to assess the potential of increasing their regenerative capacity by functionalising the scaffolds for therapeutic delivery. This was achieved by the utilization of polymeric drug carriers. With this purpose, alginate, chitosan, gelatine, and poly(lactic-co-glycolic acid) (PLGA) microparticles eluting PTHrP 107-111, an osteogenic pentapeptide, were fabricated and tested by incorporating them into the scaffolds. Among them, PLGA microparticles show the most promising characteristics for use as drug delivery devices. Following the incorporation of the microparticles, the scaffolds maintained their interconnected porous structure and the mechanical properties of the materials were not adversely affected. In addition, the microparticles released all their PTHrP 107-111 cargo. Most importantly, the delivered peptide proved to be bioactive and promoted enhanced osteogenesis as assessed by alkaline phosphatase production and osteocalcin and osteopontin gene expression when pre-osteoblastic cells were seeded on the scaffolds. While the focus was on bone repair, the release system described in this study can be used for the delivery of therapeutics for healing and regeneration of a variety of tissue types depending on the type of collagen scaffold chosen. © 2014 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License. [http://dx.doi.org/10.1063/1.4902833]

The role of scaffolds for bone tissue engineering has evolved from being a supportive matrix for cell proliferation and differentiation to also acting as a reservoir for the controlled release of active molecules that enhance tissue regeneration.1 In this sense, significant efforts have been made with ceramics, synthetic polymers, and hydrogels for developing complex devices for the sequential and responsive release of growth factors and other therapeutics.2-4 We have recently developed, in our labs, three dimensional porous collagen-hydroxyapatite (CHA) scaffolds with immense potential for bone repair as they are biodegradable, biocompatible, osteoconductive, and osteoinductive.5,6 However, enhancing their regenerative capacity through the incorporation and release of therapeutics presents challenges given the chemically labile nature of proteins. Consequently, the utilization of many post-fabrication procedures for functionalising matrices to achieve controlled drug-delivery may compromise the integrity of the scaffold.

One way to incorporate drugs into the CHA scaffold would be to load the therapeutics into polymeric carriers that could be subsequently integrated into the protein network during its fabrication,
TABLE I. Fabrication conditions for the PTHrP 107-111 loaded polymeric microparticles by spray-drying using a Buchi mini spray dryer B-290.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent</th>
<th>Inlet temperature (°C)</th>
<th>Aspiration (%)</th>
<th>Pump (%)</th>
<th>Nozzle cleaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Water</td>
<td>140</td>
<td>80</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Chitosan</td>
<td>1% acetic acid</td>
<td>160</td>
<td>80</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1.6% glutaraldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatine</td>
<td>0.1% mannitol</td>
<td>160</td>
<td>80</td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
</table>

an approach that has been successfully used in other collagen-based materials.\cite{7,8} These carriers would ensure the stability of the drugs during the fabrication process and would also allow their release after implantation. In addition, they would act as a reservoir, protecting the therapeutic molecules from degradation \textit{in vivo}. With this in mind, the present work evaluated the feasibility of incorporating four different polymeric microparticles into CHA scaffolds and determined the effects on the scaffold’s characteristics. Alginate, chitosan, gelatine, and poly(lactic-co-glycolic acid) (PLGA) were chosen for the fabrication of the microparticles, as these biocompatible polymers have been widely used with drug delivery purposes.\cite{9-12} As a proof of concept, prior to incorporation into the scaffolds, the microparticles were loaded with the fragment 107-111 from the parathyroid hormone (PTHrP 107-111), a pentapeptide with pro-osteogenic activity.\cite{13} The release of the peptide from the microparticles/scaffold composites was analysed, and the anabolic effect of the released PTHrP 107-111 on pre-osteoblastic cells was tested \textit{in vitro}.

Alginate, chitosan, gelatine, and PLGA were chosen as polymers for the encapsulation of the model molecule, the pro-osteogenic peptide PTHrP 107-111.

A spray drying process, detailed elsewhere,\cite{14} was used for the fabrication of alginate, gelatine, and chitosan microparticles. Briefly, in this fabrication procedure, a polymeric feed solution is atomized and forced to pass through a chamber at high temperature where the solvent from the droplets is evaporated. As a result, particulate powder of the polymer is collected. Drug loaded materials can be produced by this method by adding the therapeutic molecule, PTHrP 107-111 in the present work, to the polymeric solution.

Table I summarizes the conditions used for the fabrication of the different microparticles with a Buchi Mini Spray Dryer B-290. In all cases, the ratio of PTHrP 107-111:polymer was 25.2 mg:100 g. Alginate microparticles were crosslinked after fabrication; with this purpose, the microparticles were suspended in 10 ml acetonitrile and then poured into 1.2% (w/v) calcium chloride while stirring for 10 min. Afterwards, they were vacuum filtered through a 0.45 µm nylon membrane, washed twice with 10 ml distilled water, and freeze-dried at −56 °C overnight.

PLGA microparticles were fabricated by a water-in-oil-in-water emulsion (W/O/W) method. Briefly, 0.5 ml of an aqueous solution of 9.45 mg of PTHrP 107-111 was added to 5 ml of dichloromethane where 375 mg of PLGA had been previously solved. A primary emulsion was generated by ultrasonication of this mixture. This W/O emulsion was added dropwise while sonicing to 30 ml of an aqueous solution containing 1 g of polyvinyl alcohol (PVA) and 1.13 g of NaCl. The resulting W/O/W emulsion was dispersed into 70 ml of the same aqueous solution. This emulsion was stirred for 4 h at room temperature and the resulting microparticles were collected by centrifugation (5000 rpm, 10 min). The particles were washed three times with 1.13% (w/v) NaCl and freeze-dried at −56 °C overnight.

Morphological characterization was carried out by Scanning Electron Microscopy (SEM) using a Zeiss Supra Variable Pressure Field Emission Scanning Electron Microscope. For SEM observations, samples were mounted on copper studs with carbon glue and then sputtered with gold.

In order to assess the release kinetics of PTHrP 107-111 from the microparticles, 20 mg of each composition were immersed in 2 ml phosphate buffered saline (PBS) at 37 °C. At given timepoints, the medium was removed after centrifugation and replaced by fresh pre-warmed PBS. Media were analysed by high performance liquid chromatography (HPLC) following a protocol detailed by Manzano \textit{et al}.\cite{15}
The fabrication of the CHA scaffolds was done following a procedure previously published and patented by our group. Briefly, 1.8 g of micro-fibrillar bovine tendon collagen was added to 320 ml of 0.5 M acetic acid solution. This was blended at 15 000 rpm for 90 min using an overhead blender in a reaction vessel which was kept at 4 °C using a circulation system to prevent denaturation of the collagen due to the heat generated during the process. Aliquots of 10 ml of a solution of 3.6 g of hydroxyapatite particles, with a mean diameter of 5 µm (Plasma Biotal Limited, North Derbyshire, UK), in 40 ml of 0.5 M acetic acid solution were added every 60 min, and the resulting slurry was blended for 60 additional minutes. Then, the slurry was degassed by vacuum in order to remove air bubbles that could produce uncontrolled porosity in the final scaffold. A suspension of 100 mg of the microparticles (fabricated using the techniques described above) in 1 ml distilled water was gently mixed with 9 ml of this CHA slurry, and the mixture was pipetted into a stainless steel tray prior to lyophilization at −10 °C using a constant cooling freeze-drying protocol. Depending on the microparticle type, scaffolds are denoted PLGA-CHA, alginate-CHA, chitosan-CHA, and gelatine-CHA. A similar procedure was carried out adding 1 ml of distilled water to the slurry in order to produce a CHA scaffold, used as blank control, which was denoted blank-CHA. After freeze-drying, both sides of the scaffolds were exposed to short wavelength (254 nm) UV-light (at 2300 µW/cm²) for 15 min in order to crosslink them.

The porosity of the scaffolds with and without microparticles was calculated by the following equation:

\[
\text{% porosity} = 100 \times \left[ 1 - \left( \frac{\rho_{\text{actual}}}{\rho_{\text{theoretical}}} \right) \right].
\]

The actual density (\(\rho_{\text{actual}}\)) of the scaffolds was calculated by dividing the actual mass of the scaffolds to calculated volume of the scaffolds. The theoretical density (\(\rho_{\text{theoretical}}\)) was estimated using the values of collagen, PLGA, chitosan, gelatine, and/or alginate taken from the literature.

In order to check the influence of the addition of microparticles into the scaffold over its mechanical properties, wet compression testing was performed using a uniaxial tensile testing machine (Z050, Zwick/Roell, Ulm, Germany). Disks of scaffolds (n = 9) with a diameter of 6.5 mm were pre-hydrated prior to transfer to the testing rig which contained distilled water. Uniaxial compression testing was performed up to a maximum strain of 10%. The modulus was calculated from the slope of the stress-strain curve over the range of 2%–5% strain. Compressive testing results were analysed using an in-house designed Microsoft Excel macro.

SEM characterization of the surface and the cross-section of the different composite scaffolds were performed using Zeiss Supra Variable Pressure Field Emission Scanning Electron Microscope. Samples were mounted on copper studs and were sputtered with gold prior to observation.

In order to assess the PTHrP 107-111 release kinetics from the composite scaffolds, 6.5 mm diameter disks were soaked in 2 ml PBS at 37 °C. At given timepoints, the medium was replaced with fresh pre-warmed PBS and analysed by HPLC.

MC3T3-E1 murine pre-osteoblasts were used to study the effects of the peptide on osteoblast differentiation in vitro since they exhibit similar properties as osteoprogenitor cell lines including high alkaline phosphatase (ALP) activity. These cultures form calcified bone tissue in a process closely resembling intramembranous ossification. MC3T3-E1 cells were cultured in α-MEM supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 2% penicillin/streptomycin. Media were changed every 3 days and cells were removed from the flasks using a trypsin–EDTA (ethylenediaaminetetraacetic acid) solution 0.25%. Cells (3 × 10^5, quantified with an haemocytometer) were seeded on the surface of 6.5 mm diameter CHA scaffolds and CHA-PLGA scaffolds (1.5 × 10^5 cells on each side) and then incubated in 2 ml of pro-osteogenic medium (α-MEM supplemented with 10% FBS, 50 µg/ml ascorbic acid, 10 mM β-glycerol-phosphate, 1% penicillin/streptomycin, and 0.25 mg/ml amphotericin B) under standard conditions (37 °C, 5% CO₂).

The osteogenic potential of MC3T3-E1 cells on the scaffolds was assessed and compared to blank CHA scaffolds by quantifying the activity of ALP, a widely exploited early biochemical marker for osteogenic activity 3 and 7 days post-seeding. At the endpoint of the study, constructs containing MC3T3-E1 cells were washed in PBS, lysed according to the manufacturer’s protocol in lysis buffer (SensoLyte pNPP Alkaline Phosphatase Assay Kit), and incubated at 4 °C. The supernatant was collected for quantification of ALP. This method utilizes p-nitrophenylphosphate...
(pNPP) that is hydrolyzed by ALP to produce a yellow product. The amount of colored product is proportional to the amount of enzyme in the reaction mixture.

In order to determine the expression of different osteogenic genes as indicators of differentiation into an osteoblastic phenotype, ribonucleic acid (RNA) was extracted from MC3T3-E1 cells after 14 days of culture by using Qiazol Lysis Reagent (Qiagen) and RNeasy Mini Kit (Qiagen). RNA was quantified using a Nanodrop 2000 Micro-Volume UV–Vis Spectrophotometer for nucleic acid and protein quantitation (Thermo Scientific). RNA extraction was followed by complementary DNA reverse transcription by using QuantiTect Reverse Transcription Kit (Qiagen). The samples were prepared for polymerase chain reaction (PCR) analysis by using SYBR Green PCR kit (Qiagen). Real time polymerase chain reaction (RT–PCR) was performed on 10 ng RNA, and reactions were run on a Real–Time PCR System (Eppendorf Mastercycler ep Realplex 4S_Version1). Gene expression of osteopontin (OPN, Qiagen, QT00157724), osteocalcin (OCN, Qiagen, QT00259406), and 18S (Qiagen, QT01036875) as an endogenous control were investigated. Results were analyzed by using 2-∆∆CT Method.21 Fold change is defined as the relative expression compared to the expression of each gene from cells seeded on blank CHA scaffolds and cultured at 37 °C.

The data are represented as means ± standard error of the mean. Statistics were carried out using GraphPad Prism software using a general linear model ANOVA with Bonferroni posttest analysis performed for multiple comparisons. All experiments were performed with a sample size of 3 per treatment group unless otherwise stated. Statistical significance was taken at p < 0.05 unless otherwise stated.

SEM observations, shown in Figure 1, confirm that irrespective of the fabrication procedure and the composition, all microparticles were in the 1–10 µm size range, in concordance with results obtained by dynamic light scattering measurements (data not shown). The micrographs also demonstrate that PLGA, chitosan, and alginate microparticles had a spherical shape with a smooth surface, while gelatine based materials had a wrinkled texture, a phenomenon that has been described before for this type of products.22

As shown in Figure 2, diverse peptide release profiles were obtained from the different microparticles in the in vitro studies. On the one hand, PLGA and alginate particles released all their contents in the first 10 h of the study while, on the other hand, the delivery from chitosan and gelatine microparticles was sustained until 48 h. There was no further release of peptide from that timepoint to the end of the study (14 days) in any of the studied polymers (data not shown).

Having determined that polymeric microparticles effectively eluted PTHrP 107-111 with the experimental procedures detailed above, they were then incorporated into the CHA slurry, prior to its lyophilisation. SEM micrographs of the final constructs, displayed in Figure 3, demonstrate

FIG. 1. SEM micrograph of alginate (a), chitosan (b), PLGA (c), and gelatine (d) PTHrP 107-111 loaded microparticles. Scale bar represents 10 µm. In contrast with the rest of polymers, gelatine microparticles have a wrinkled texture.
FIG. 2. PTHrP 107-111 release kinetics from the different polymeric microparticles. The percentage of drug released is calculated from the amount of peptide added during the fabrication of the microparticles. PLGA and alginate release the peptide content in the first 10 h of the study, while gelatine and chitosan display a sustained delivery.

FIG. 3. SEM micrographs of collagen-hydroxyapatite scaffolds incorporating the different polymeric microparticles. Images from top to bottom were taken at 6000, 1000, and 100× magnification, respectively. Scale bar from top to bottom represents 10, 50, and 500 μm, respectively. With the exception of gelatine, microparticles can be distinguished in the walls of the microparticle/collagen scaffolds.

that the microparticles were homogeneously distributed through the CHA scaffolds walls, with the exception of gelatine microparticles which could not be distinguished in the sample. In addition, the micrographs suggest that the porosity and pore interconnectivity were maintained in all scaffolds following microparticle incorporation. These observations are corroborated by the results from porosity quantification (Figure 4(a)), as all the scaffolds had porosity values over 96%.

The incorporation of the microparticles into the collagen network did not have a negative effect on the mechanical properties of the CHA scaffolds, as shown in Figure 4(b). Indeed, the compressive modulus of chitosan-CHA scaffold was significantly higher than the blank-CHA control.

When the PTHrP 107-111 release profiles were analysed, gelatine-CHA degraded in the PBS before the first sample was obtained (2 h) and no PTHrP 107-111 was detected in the media where the chitosan-CHA scaffolds were releasing, at any of the timepoints. Thus, only the release of PTHrP 107-111 from alginate-CHA and PLGA-CHA scaffolds, displayed in Figure 5, could be studied. The percentage of drug release shown in Figure 5 has been calculated based on the data presented in Figure 2, by assuming that the release from the microparticles after 14 days represents the total drug incorporated into these microparticles. The drug delivery profiles from these scaffolds are similar to those determined from the corresponding microparticles alone, with the majority of
FIG. 4. (a) Porosity and (b) mechanical properties of collagen-hydroxyapatite scaffolds incorporating alginate, chitosan, gelatine, or PLGA microparticles. ***p < 0.001 denotes significance compared with all other groups.

FIG. 5. Release profiles of PTHrP 107-111 from PLGA and alginate microparticles incorporated into collagen-hydroxyapatite scaffolds. The percentage of the drug released is calculated from the total amount of peptide incorporated with the microparticles.

The drug being released during the first 10 h. Interestingly, only around 60% of the incorporated drug was delivered from the alginate-CHA samples after 14 days of study (data not shown).

Taking these results into consideration, the PLGA-CHA scaffolds, which displayed the best properties in terms of peptide delivery efficiency, were selected for further in vitro studies where bioactivity assays were performed with preosteoblastic cells, results of which are detailed in the following section.

The osteogenic differentiation potential of MC3T3-E1 cells in direct contact with a peptide eluting PLGA-CHA scaffold and a blank-CHA scaffold was assessed and compared. ALP cellular
FIG. 6. (a) MC3T3 ALP activity following 3 and 7 days culture on PTHrP 107-111 eluting and blank collagen hydroxyapatite scaffolds. *p < 0.001 denotes significance compared with the blank collagen hydroxyapatite scaffolds. (b) Osteocalcin and osteopontin relative expression of MC3T3 after 14 days of culture on PTHrP 107-111 eluting and blank collagen hydroxyapatite scaffolds. *p < 0.001 and **p < 0.01 denote significance compared with the blank CHA scaffolds.

activity, an early marker for osteoblastic differentiation, was examined after culturing the cells on the scaffolds for 3 and 7 days (Figure 6(a)). A significant increase in ALP activity was observed from cells grown on peptide eluting scaffolds compared to cells grown on blank CHA controls at 3 days.

Figure 6(b) shows the osteogenic gene expression results from cells cultured on the scaffolds for 14 days. These results corroborate the pro-osteogenic effect implied by ALP analysis, as evidenced by significant increases in expression of the osteogenic markers OCN and OPN in cells cultured on peptide eluting PLGA-CHA scaffolds.

The aim of the present study was to assess the feasibility of incorporating different therapeutic-loaded polymeric microparticles into a CHA scaffold and assess their potential to enhance osteogenesis. This experimental approach may overcome the inherent difficulties of loading drugs in these bioactive materials, as they are fabricated using acidic solvents which can harm labile products such as proteins or peptides. With this purpose in mind, the microparticles were mixed with the CHA slurry during an intermediate fabrication step, prior to the final freeze-drying process. In order to determine if a bioactive molecule could be loaded and released from these microparticle-scaffold constructs, the pro-osteogenic pentapeptide PTHrP 107-111 was preloaded into the microparticles. This work demonstrates that, from the evaluated polymers, CHA scaffolds incorporating PLGA microparticles show the most promising characteristics for use as drug delivery devices. Following the incorporation of the PLGA microparticles, the scaffold retained its interconnected porous structure and the mechanical properties of the materials were not adversely affected. In addition, the microparticles released all their PTHrP 107-111 cargo from the scaffolds unlike the other compositions. Most importantly, the delivered peptide proved to be bioactive and promoted enhanced osteogenesis as assessed by alkaline phosphatase production and osteocalcin and osteopontin gene expression when pre-osteoblastic cells were seeded on the scaffolds.

The fabrication of the PTHrP 107-111 loaded microparticles was carried out by a double emulsion technique (with PLGA) and a spray-drying process (with alginate, chitosan, and gelatine) which yielded materials with the expected micrometric size and spherical shape.9–12 Regarding the PTHrP 107-111 delivery, PLGA and alginate microparticles released their cargo after only 10 h of immersion in PBS, while gelatine and chitosan delivered the peptide in a more controlled manner.
fashion, which suggests a stronger chemical interaction between the pentapeptide and these specific polymers.

The incorporation of PLGA, alginate, and chitosan microparticles to the CHA slurry and its subsequent freeze-drying yielded composite scaffolds with microparticles homogeneously distributed throughout the scaffold walls. However, gelatine microparticles were not distinguishable under SEM examination, which may indicate that the gelatine dissolved as a result of the acidic pH of the slurry. In addition, gelatine-CHA scaffolds degraded very quickly in PBS during the drug release studies confirming that these microparticles are not suitable for inclusion into the collagen matrix using the procedure described in this study. Encouragingly, the addition of the microparticles did not have a negative effect on the mechanical properties of the CHA scaffolds. Indeed, the incorporation of chitosan augmented significantly the compressive modulus of the scaffolds. The increased stiffness, which may be beneficial for osteoblasts attachment, differentiation, and proliferation, may be attributed to the presence of residual glutaraldehyde, which was used for crosslinking the microparticles. Glutaraldehyde is a well-known crosslinking agent of protein-based scaffolds, and its existence in the CHA slurry would also explain that the PTHrP 107-111 was not released from the chitosan-CHA scaffolds, as the peptide was probably also being crosslinked to the collagen matrix. Importantly, the cytotoxicity of glutaraldehyde has been well documented and thus the existence of its residues in the scaffold should be minimized.

PTHRP 107-111 release was detected from alginate-CHA and PLGA-CHA composites only. The delivery kinetics from these scaffolds in vitro followed the same trend as the release from the corresponding microparticles alone, i.e., quick release in 10 h. However, while PLGA microparticles released all their cargo from the scaffolds, assuming that the release from the microparticles after 14 days represents the total drug incorporated into these microparticles, only a fraction of around 60% was delivered from alginate-CHA samples. This is probably due to the degradation of the peptide accumulated in the outer layers of the microparticles when they were immersed in the acidic CHA slurry before lyophilization; the aggregation of encapsulated molecules on the surface of spray-dried microparticles is an expected consequence of this fabrication process.

Thus, PLGA microparticles-CHA scaffolds were selected for carrying out in vitro studies in order to check the bioactivity of the released PTHrP 107-111.

The analysis of the effect of the peptide on osteoblasts grown in direct contact with the PLGA-CHA scaffolds confirmed its pro-anabolic activity as the released PTHrP 107-111 led to a significant increase in ALP activity, which is indicative of an enhancement of cell differentiation towards a pro-osteogenic lineage. Further confirmation of the anabolic effect of released peptide was obtained from the augmented expression of OPN and OCN genes, markers of distinct stages of osteoblast maturation. The significant increase in the levels of these two genes in cells grown on PLGA-CHA scaffolds is indicative of differentiation and mineralization, corroborating the previous ALP results. This pro-osteogenic effect can be attributed to the release of the peptide from the scaffolds, as it was not observed in a parallel study carried out with non-eluting PLGA-CHA constructs (data not shown). Thus, in vitro results confirm that PTHrP 107-111 was bioactive after loading into the microparticles and the subsequent incorporation into the CHA scaffold.

Taken together, these results demonstrate the development of a fabrication procedure which allows microparticles to be incorporated in CHA scaffolds for the local delivery of therapeutics. The present study shows that, for releasing a small molecule such as PTHrP 107-111, PLGA-CHA is the optimal combination. However, promising results were also obtained for alginate-CHA, demonstrating their potential for the targeted release of drugs. Chitosan-CHA scaffolds could also be used if a non-toxic alternative crosslinking method was employed during the fabrication of the microparticles. The slow release from these microparticles, which may have been affected by glutaraldehyde, could be attractive for the delivery of certain therapeutics and may be tuned depending on the cross-linking agent utilised. It must be noted that, in order to avoid the potential toxicity of a high, burst release, the release kinetics from these constructs can be tuned by modifying and functionalizing the microparticles prior to their incorporation into the CHA scaffold, an approach that is being studied currently in our laboratories. Also, by varying the composition of the collagen-based scaffold, this strategy can be applied to deliver therapeutics to a wide range of target tissues.
The fabrication of collagen-hydroxyapatite scaffolds incorporating polymeric microparticles for the localized delivery of therapeutics was investigated. The pentapeptide PTHrP 107-111 was tested as model molecule for the delivery studies. Scaffolds incorporating PLGA microparticles displayed porosity and mechanical properties similar to those of collagen-hydroxyapatite scaffolds along with the highest ratios of peptide loading and release. In addition, PTHrP 107-111 released from these composite scaffolds showed pro-osteogenic effects on bone cells, demonstrating the potential of these systems for bone tissue regeneration. However, by tuning the release kinetics, the active molecule, and the composition of the collagen scaffold, these platforms have the potential to be applied for a wide range of applications in addition to bone.

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