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# Delivery of osteoinductive growth factors from degradable PEG hydrogels influences osteoblast differentiation and mineralization

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**Abstract**

Degradable poly(ethylene glycol) (PEG) hydrogels with varying mass loss profiles were investigated to assess their applicability as delivery vehicles for osteoinductive growth factors in bone tissue engineering. Protein release is readily controlled by changes in both the structure (i.e., macromer concentration) and chemistry (i.e., number of degradable units) of the starting macromer. In vitro studies indicate an increase in total protein levels, alkaline phosphatase, and mineralization by osteoblasts cultured in the presence of osteoinductive growth factors compared to cells cultured with standard media. When growth factors are delivered from a 25 wt% hydrogel, a significant increase in both alkaline phosphatase and mineralization was seen after 3 weeks of culture over growth factor delivery in a bolus fashion. Additionally, gene expression levels of both osteocalcin and type I collagen were higher at early timepoints when growth factors were released from hydrogels. These results indicate that growth factors remain active after photoencapsulation, that the sustained delivery of growth factors alters various markers of osteoblastic differentiation, and that these networks could be useful as delivery vehicles for growth factors in bone tissue engineering. Finally, ectopic bone formation was present in subcutaneous rat tissue after implantation of hydrogel networks loaded with osteoinductive growth factors.

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*Keywords:* Hydrogels; Photopolymerization; Osteoinductive growth factors; Bone tissue engineering; Growth factor delivery

## 1. Introduction

A clinical need exists for alternative treatment options for regeneration of bone tissue in large defects in long bones or the craniofacial region, spinal fusions, and even periodontal applications, all of which can be damaged by either trauma or disease. Although considered to be the best current treatment option, there are many limitations with bone grafting procedures, including the introduction of a second surgical site to obtain the graft, the possibility for graft rejection by the patient, and the

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potential for limited integration between the graft material and native tissue [1]. Additionally, invasive metallic implants, often used in conjunction with bone grafts, cause stress shielding at the injured site and must either be removed with a second surgery or remain as a permanent foreign body. To address these issues, tissue engineering is emerging as a field with significant potential to regenerate musculoskeletal tissues such as cartilage, tendon, and bone [2,3].

In general, bone tissue engineering involves the development of an osteoconductive material [4,5], the delivery of osteoprogenitor cells [6,7], the delivery of osteoinductive agents [8,9], or a combination of the above treatments. The delivery of osteoinductive agents, such as bone morphogenetic proteins (BMPs), has significantly enhanced the regeneration of bone tissue in animal models [10]. Advances in recombinant protein production and protein isolation directly from animal tissue now allow for the production of large quantities of therapeutic molecules that are beneficial for bone tissue engineering.

The current question that researchers are posing is: how do we deliver these molecules to the appropriate site and maximize their therapeutic potential? Specifically, degradable polymeric biomaterials are proving useful in answering this question. Some of the delivery vehicles that have been investigated for the delivery of osteoinductive factors include collagen networks [11], gelatin [12], poly(lactic acid) (PLA) [13,14], and polyanhydrides [15]. Synthetic hydrogel networks provide specific advantages for growth factor delivery due to their high water content, general biocompatibility, controlled degradation and, consequently, controlled drug delivery.

One specific class of synthetic hydrogels that may be a useful carrier for osteoinductive growth factors are formed from multifunctional PLA-b-PEG-b-PLA macromers [16], which consist of a PEG core that gives the hydrogel its hydrophilicity, PLA linkages that give the hydrogel degradability, and acrylate groups that allow for formation of a crosslinked network upon exposure to light and the addition of an appropriate photoinitiator. Although originally developed for prevention of postoperative adhesions [17], these networks have been investigated for controlled drug delivery [18,19] and for the encapsulation of cells for tissue engineering [20].

One additional benefit to these photopolymerizable hydrogels is the potential for non-invasive implantation through transdermal photopolymerization [21]. In this process, a liquid macromer solution incorporating either cells or growth factors is injected subcutaneously while the surface of the skin is exposed to either ultraviolet or visible light. This technique was used previously for the delivery of photoencapsulated chondrocytes for cartilage regeneration [22] and for post-surgical tissue adhesion [23], but is easily adapted for other tissue engineering and drug delivery applications.

The overall objective of this study is to illustrate the feasibility of crosslinked PEG hydrogel networks for sustained and localized release of active growth factors for bone regeneration. Specifically, we aim to answer the following fundamental questions related to the photoencapsulation of osteoinductive growth factors in hydrogel materials: (1) To what extent can the delivery of large molecules be controlled with these degradable PEG hydrogels? (2) Do the growth factors remain active after photoencapsulation in hydrogel networks? (3) Will osteoinductive growth factors released from hydrogel networks promote mineralized tissue formation in both in vitro and in vivo systems? and (4) Will sustained delivery (i.e., from hydrogels) of osteoinductive growth factors be more beneficial in stimulating osteoblast function than an initial dose of growth factors.

## 2. Materials and methods

### *2.1. Macromer synthesis and network formation*

Multifunctional PLA-b-PEG-b-PLA macromers were synthesized as described in detail elsewhere [16]. Briefly, lactic acid units were added to PEG-4600 cores through a ring opening polymerization of lactide (Polysciences) on the exposed hydroxy end groups. This molecule was functionalized with acrylate groups through the addition of acryloyl chloride in the presence of triethylamine. After precipitation in diethyl ether and purification, the macromer (shown in Fig. 1) was dissolved in deionized water, mixed with the desired protein, and photopolymerized by exposure to ultraviolet light (~4 $mW/cm^2$) for 10 min and the addition of 0.05 wt% of the photoinitiatior 2-hydroxy-1-[4-(hydroxy-

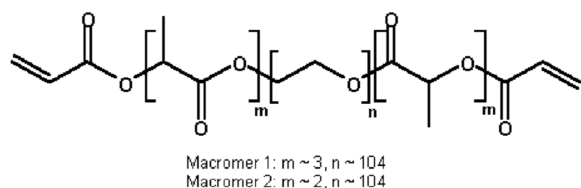


Fig. 1. Chemical structure of diacrylated PLA-b-PEG-b-PLA macromer, where $n$ is the number of ethylene glycol repeat units and $m$ is the number of lactic acid repeat units.

ethoxy)phenyl]-2-methyl-1-propanone (I2959, Ciba Geigy). This specific initiating system was determined to be cytocompatible when polymerized in the presence of cells by Bryant et al. [24]. All chemicals were obtained from Aldrich and used as received unless noted otherwise.

### *2.2. Release studies*

For solute release experiments, 0.04 wt% bovine serum albumin (BSA, Sigma) was added to the macromer solution before photopolymerization. This concentration is accurately measured upon release and is below the solubility limit for BSA, which serves as a model protein to investigate the release behavior from these hydrogel networks. The influence of both changes in macromer concentration (10, 25, and 50 wt%) and changes in the macromer chemistry (i.e., number of lactic acid units) on drug release kinetics was investigated. Hydrogel disks (1 cm diameter, 1 mm thick) were degraded in phosphate buffered saline (PBS, pH=7.4) at 37 °C. At desired times, the buffer solution was sampled and protein release was quantified with a total protein assay (Bio-Rad). A differential color change in the assay reagent was quantified by measuring the absorbance at 595 nm (Perkin Elmer, Lambda 40) and related to known protein standards.

### *2.3. In vitro osteoblast studies*

Primary rat calvarial osteoblasts were harvested and cultured as described previously [25]. After three passages, osteoblasts were trypsinized from culture plates and seeded at a density of $5\times10^4$ cells/cm$^2$ in 12-well transwell plates (Costar). Growth factors (2 μg per hydrogel, donated by Sulzer Biologics, Wheatridge, CO, USA) were photoencapsulated in hydrogels (10 and 25 wt% of Macromer 1) with a volume of 40 μl (5 mm diameter, 2 mm thick). The growth factors used in this study were isolated directly from bovine tissue and include a mixture of proteins such as bone morphogenetic proteins, transforming growth factor-β, and fibroblast growth factor [26]. Media was changed every 48 h throughout the study. Hydrogels were suspended above monolayers of osteoblasts after 24 h of attachment (designated as day 0). Cells exposed to a bolus of growth factors (2 μg per well) for 48 h to mimic a dose delivery of growth factors, as well as no growth factors, were used as control systems.

At day 0 (before exposure to growth factors), and after 1, 2, and 3 weeks of culture, the cells were rinsed twice with PBS, and lysed by exposure to a 1% Triton X-100 (Electron Microscopy Sciences) solution for 15 min, collected with cell scrapers, and sonicated (W-380, Heat Systems-Ultrasonic) for 5 min. Cell lysates were frozen at −80 °C until assaying. The total DNA in the cell lysate samples was quantified with a PicoGreen dsDNA Quantitation Reagent (Molecular Probes) using standard manufacturer's protocols. The fluorescence (excitation ~485 nm, emission ~535 nm, Perkin Elmer Wallac Victor2) was measured for all samples in 96-well plates, and DNA was quantified using calf thymus DNA for standards. Total protein in the cell lysate solutions was measured using the BioRad total protein assay described above.

Alkaline phosphatase (ALP) activity was determined using a commercially available kit (Sigma, Kit 245). Briefly, 20 μl of the lysate solution were incubated with *p*-nitrophenyl phosphate at room temperature for 5 min. The absorbance was measured at 405 nm (Perkin Elmer Lambda 40) in 1-min increments, and the slope of the absorbance versus time plot was used to calculate the ALP activity. The ALP activity is reported as a value normalized to the total DNA in each sample. After 3 weeks of culture, wells were rinsed twice with PBS, and osteoblast layers were fixed in a 2.5% glutaraldehyde solution for 15 min. Mineral deposits were stained with a 1.5% silver nitrate solution and exposure to ultraviolet light for 30 min. After rinsing thoroughly with deionized water, the total mineralized area was

quantified using NIH Image Software for a minimum of five images from three samples of each treatment.

At desired timepoints (day 0, 1, 2, and 3 weeks), osteoblast cultures were washed with PBS, and total RNA was isolated using a guanidinium thiocyanate/phenol reagent (TRI reagent, Sigma) and standard manufacturer's protocols. After allowing the RNA pellet to dry, it was resuspended in DEPC-treated water, and the RNA quantity and purity were analyzed via spectrophotometry at 260 and 280 nm. RT-PCR was performed using the SuperScript™ oligo (dT) system (Invitrogen). A 100-ng total RNA sample was used for single strand cDNA synthesis. The reverse transcription reaction was incubated at 42 °C for 50 min and terminated at 70 °C for 15 min. PCR was conducted using 200 nmole of primers specific for osteocalcin (OCN, sense: 5′-CAGCCCCCTACCCAGAT-3′, anti-sense: 5′-TGTGCCGTCCATACTTTC-3′), α1(I) procollagen (COL I, sense: 5′-TCTCCACTCTTCTAGTTCCT-3′; anti-sense: 5′-TTGGGTCATTTCCACATGC-3′) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, sense: 5′-ACCACAGTCCATGCCAT-CAC-3′, anti-sense: 5′-TCCACCACCCTGTTGCT-GTA-3′). PCR thermal cycling temperatures were performed in an Eppendorf Mastercycler Personal at 94 °C for 2 min, then 35 cycles of 94 °C for 75 s, 60 °C for 75 s and 72 °C for 90 s followed by final extension at 72 °C for 5 min. PCR products were analyzed on a 1.5% agarose gel with 0.5 μg/ml ethidium bromide. Gel images were captured using a Kodak DC290 camera with bundled software and quantified using NIH Image software. Quantified products were standardized to a standard housekeeping gene, GAPDH.

### 2.4. In vivo subcutaneous implantation

Hydrogels were prepared under the conditions described above with 35 μg of osteoinductive growth factors encapsulated within each hydrogel. Macromer solutions (10, 25, and 50 wt% of Macromer 1) were polymerized in a cylindrical mold with a height and diameter of approximately 0.25 and 1 cm, respectively. Four hydrogel constructs were placed in subcutaneous pockets in each rat (Long–Evans rats, ~150 g), and the construct (if the hydrogel was not completely degraded) and surrounding tissue were explanted from the animals after 21 days. Histological sections were prepared under standard techniques and stained for mineral deposits using a Von Kossa/hematoxylin and eosin stain.

### 2.5. Statistical analysis

Statistical analysis was performed using a Student's *t*-test with a minimum confidence level of 0.05 for statistical significance. All experiments were performed in triplicate and values are reported as the mean and standard deviation of the mean.

## 3. Results and discussion

### 3.1. Network structure and release behavior

Degradable hydrogels were fabricated by the photoinitiated polymerization of multifunctional PLA-b-PEG-b-PLA macromers (shown in Fig. 1) to investigate the feasibility of osteoinductive growth factor release from hydrogels for bone regeneration. Network degradation is readily controlled by changes in the molecular weight of the PEG core, the type of degradable linkage (e.g., lactic acid versus caprolactone), the number of degradable linkages, and the weight fraction of macromer used to fabricate the hydrogel. The release of a model protein, BSA, was used to investigate the influence of these last two parameters on the kinetics of protein release from these degradable hydrogels. Although this is not directly correlated to the release of growth factors of different sizes, these studies show the controllable nature of large molecule release from these hydrogel networks.

The delivery of BSA from hydrogels fabricated with macromer concentrations of 10, 25, and 50 wt% are shown in Fig. 2a. Changes in macromer concentration gave dramatically different release profiles with protein being totally released in ~8 days for the 10-wt% hydrogel, ~28 days for the 25-wt% hydrogel, and up to ~64 days for the 50-wt% hydrogel. The macroscopic release of proteins from these networks is directly affected by diffusion of protein through the hydrogel and consequently, the mesh size of the network. In general, an increase in macromer concentration will decrease the overall

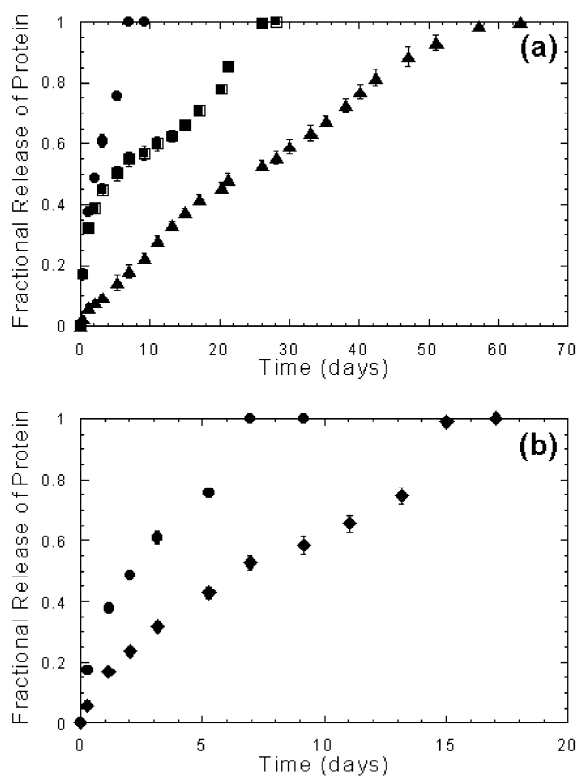


Fig. 2. Release profiles of BSA delivered from hydrogel networks fabricated from (a) 10 (●), 25 (■), and 50 (▲) wt% macromer 1 and (b) 10 wt% of macromer 1 (●) and 10 wt% of macromer 2 (♦).

mesh size of the networks through changes in network swelling due to changes in network structural properties. For instance, an increase in the amount of solvent during network formation will cause an increase in cyclization with the probability of both vinyl groups on one macromer molecule reacting into the same kinetic chain. Likewise, an increase in solvent concentration will increase the length of kinetic chains during polymerization since the diffusion of polymerizing radicals will not become limited until later times during polymerization [27]. All of these changes play a part in the release of protein from hydrogel networks.

For both the 10- and 25-wt% hydrogel, a burst of protein release is seen within the first 48 h of degradation. Potentially, the mesh size of the network is above a critical size for BSA diffusion and, thus, protein diffusion is not as readily controlled by the degradation properties of the hydrogel. For the 50-wt% hydrogel, protein release is relatively linear throughout the experiment. The lack of a burst of protein at short degradation times indicates that protein release is highly dependent on the degradation of the polymer network. In this case, protein release is inhibited until polymer chains are cleaved by hydrolysis and released from the networks to produce a mesh size that enables diffusion of protein molecules from the network.

In Fig. 2b, protein release kinetics are shown for different macromer chemistries. In both cases, the macromer was polymerized in a 10-wt% solution, but the number of lactic acid repeat units on the macromer was changed. For the lower number of lactic acid units (Macromer 2), the polymer takes ~17 days for complete protein release, whereas all of the protein is released with ~8 days when the number of lactic acid repeat units was increased (Macromer 1) from ~2 to ~3. For a PEG molecule to be released from the network, an ester linkage must be degraded on each side of the molecule. When the number of lactic acid units is increased, the number of hydrolytically degradable ester linkages increases, and the probability that a crosslink cleaves, which increases the diffusivity of the entrapped molecules, increases. The overall acrylation efficiency of Macromer 1 was lower than that of Macromer 2, which can also influence the network structure and, consequently, protein release. These results show that polymer degradation and, consequently, protein delivery can be tailored for a variety of applications through various changes in the polymer chemistry and network structure. Since these results were obtained for BSA (~66 kDa), they only provide an approximation for the release of other proteins such as transforming growth factor-β1 (~25 kDa) and bone morphogenetic protein-2 (~26 kDa), which have different molecular weights and conformations and will have different release kinetics.

### 3.2. In vitro release in osteoblast cultures

To determine whether sustained delivery is beneficial for the delivery of osteoinductive factors, the control of protein release via network structural changes, which was demonstrated in the previous BSA release studies, was utilized. In this study,

osteoblasts were cultured without growth factors present, with growth factors, but only for the initial 48 h of culture to mimic a burst release (i.e., injection), and from hydrogels fabricated from 10 wt% (BSA release in ~8 days) and 25 wt% (BSA release in ~28 days) of Macromer 1. Since the media was changed throughout the experiment to provide proper nutrients to the cultured osteoblasts, the concentration of growth factors increased over each 48-h period and then dropped to zero when the media was replenished.

Several biochemical properties related to these experiments were measured and are summarized in Table 1. An increase in DNA (i.e., number of osteoblasts) was noted between day 0 and 3 weeks, but there was no significant difference in DNA between the various treatments. Since the osteoblasts were seeded at a near confluent density, the increase in DNA was expected as osteoblasts form 3-dimensional nodules in culture, but the results indicate that the growth factors do not have a stimulatory effect on osteoblast proliferation. In contrast to the DNA results, a significant increase in total protein ($P<0.05$) was seen between day 0 and 3 weeks, but an increase was also seen between cells cultured without growth factors and with growth factors under the various delivery conditions. However, there was no significant change in protein levels between the different release conditions. This similarity in protein levels shows both: (i) that the growth factors used in this study influence the formation of extracellular matrix production in the osteoblasts since the DNA levels did not change and (ii) that the growth factors released from the hydrogel networks survived the photoencapsulation process since the protein levels were higher than in control samples.

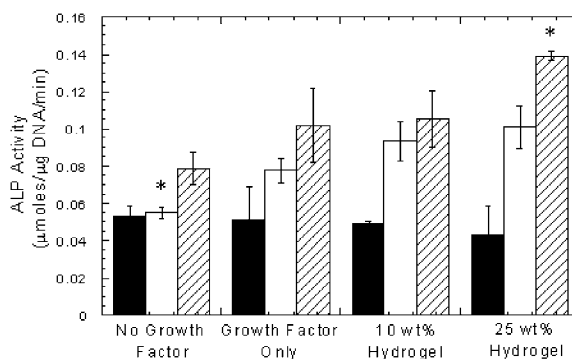


Fig. 3. ALP activity of primary rat calvarial osteoblasts cultured under a variety of release conditions for 1 week (black), 2 weeks (white), and 3 weeks (striped). *Statistical difference ($P<0.05$) from all other treatments at the same timepoint.

The levels of ALP activity, a common marker of osteoblastic differentiation, are shown in Fig. 3. After 1 week of culture, there was little difference seen between all of the treatments. After 2 weeks, there was a statistical difference between the cells treated with growth factors and the control cells, with enhanced ALP activity seen with the addition of the osteoinductive growth factors. After 3 weeks, there was a statistical difference in ALP activity for cells treated with growth factors released from the 25-wt% hydrogels and all of the other samples. For

Table 1
Biochemical properties of rat calvarial osteoblasts after 3 weeks of in vitro culture under various growth factor release conditions

| Culture time | Growth factor delivery | DNA (μg) | Total protein (mg) | % Mineralization |
|---|---|---|---|---|
| Day 0 | Before growth factor delivery | 4.02±0.30* | 0.39±0.06* | Not applicable |
| 3 weeks | No growth factors | 4.85±0.01 | 0.70±0.14 | 33.1±2.4* |
| 3 weeks | Bolus of growth factors for first 48 h | 5.08±0.42 | 0.91±0.12 | 46.1±4.8 |
| 3 weeks | Growth factors delivered from 10 wt% hydrogel | 4.97±0.50 | 0.89±0.11 | 46.2±4.4 |
| 3 weeks | Growth factors delivered from 25 wt% hydrogel | 4.70±0.22 | 0.88±0.15 | 50.1±4.2 |

*Indicates a statistical ($P<0.05$) difference from all other samples.

example, the ALP activity was 0.139±0.003 μmoles/μg DNA/min when growth factors were released from a 25-wt% hydrogel, but only 0.079±0.009 μmoles/μg DNA/min without any growth factors present during 3 weeks of culture. These results indicate that a change in the release behavior of the osteoinductive growth factors can have a substantial effect on osteoblast function. Specifically, the controlled and sustained release of these growth factors encapsulated in photocrosslinked hydrogels shows an increase in ALP activity in rat calvarial osteoblasts over a dose release at initial culture times.

Mineral deposition by the cultured osteoblasts after 3 weeks under these treatments was quantified and is outlined in Table 1. There was a significant increase in mineralization between the cells not exposed to growth factors and those exposed to the various growth factor release profiles. There was also an increase when the growth factors were released from the 25-wt% hydrogel over the 10-wt% gel and the dose delivery. Light micrographs of the stained mineral areas for the control with no growth factors (a) and the growth factors released from the 25-wt% hydrogel (b) are shown in Fig. 4. The intensity difference indicates changes in the mineralization between the two conditions.

Gene expression profiles for the cultured osteoblasts are shown in Fig. 5 and quantified in Table 2. Although a large amount of variability exists when the expression profiles are quantified and normalized to levels of GAPDH, general trends are evident. For instance, an increase in osteocalcin expression was seen after 1 week of culture when growth factors were delivered from the hydrogel networks. However, after 2 and 3 weeks of culture, levels of osteocalcin expression between the different culture conditions were equivalent. In general, osteocalcin is present in mature osteoblasts and is known to have a role in regulating mineralization [28]. Similar trends in expression were observed for type I collagen, with an increase in expression after 1 week of culture when growth factors were delivered from the hydrogel networks, whereas little variation in expression levels was seen after 2 and 3 weeks of culture. Since type I collagen is the primary non-mineral component of bone tissue, the expression of type I collagen is essential in bone remodeling.

In general, the differentiation process of osteoblasts begins with an increase in cell density, continues with an increase in various protein levels, including ALP, and continues with mineralization of a matrix secreted by the osteoblasts [29]. In vitro cultures of rat calvarial osteoblasts in the presence of osteoinductive growth factors follow this differentiation process with enhancement of various differentiation markers (i.e., ALP) and total mineralization when the growth factors are delivered from degrad-

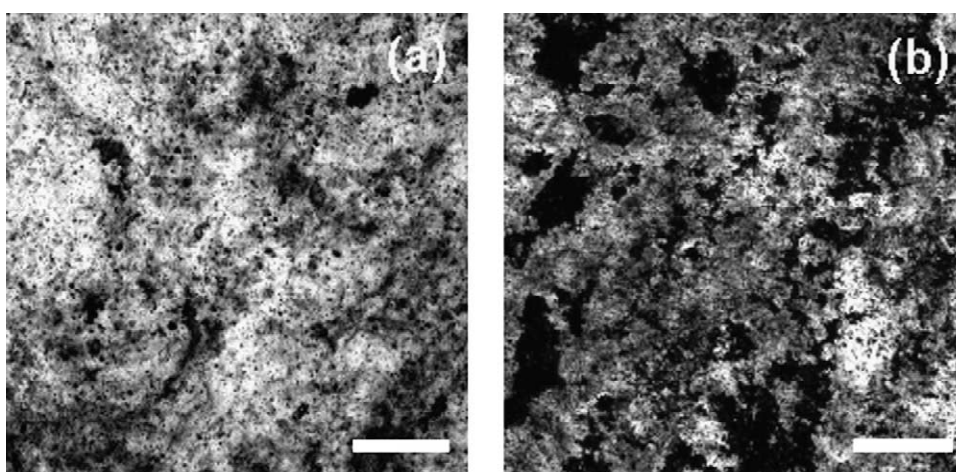


Fig. 4. Light micrographs of Von Kossa staining of primary rat calvarial osteoblasts cultured (a) without growth factors present and (b) with growth factors released from a 25-wt% hydrogel (bar=100 μm).

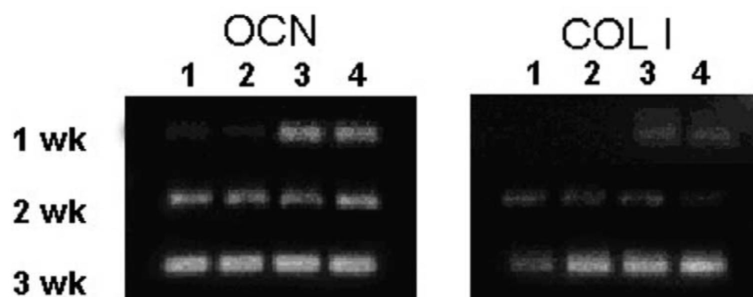


Fig. 5. Ethidium bromide stained agarose gels of osteocalcin (OCN) and type I collagen (COL I) gene expression for rat calvarial osteoblasts cultured under various growth factor release conditions after 1, 2, and 3 weeks. Lanes are as follow: (1) no growth factors, (2) growth factor only, (3) growth factors delivered from a 10-wt% hydrogel, and (4) growth factors delivered from a 25-wt% hydrogel.

able PEG hydrogels. Gene expression studies indicate that growth factor release from hydrogel networks may accelerate this differentiation process with an increase in both osteocalcin and type I collagen expression at early timepoints.

### *3.3. In vivo subcutaneous implantation*

Histological sections stained with a Von Kossa/hematoxylin and eosin stain to illustrate both cell morphology and mineralized tissue are shown in Fig. 6. When no growth factors are released from the hydrogels, only a small capsule of tissue surrounds the implant, indicating a generally good biocompatible response to the material. For growth factor release experiments, the amount of osteoinductive growth factors (35 μg) remained constant, but the hydrogel composition was altered (10, 25, and 50 wt% of Macromer 1) to give a variety of release profiles. With all implants releasing growth factors, mineral deposits (indicated by black staining of histological sections) were present in the surrounding tissues. This occurrence of ectopic bone tissue further supports that at least a fraction of the photoencapsulated growth factors remained active after polymerization.

Although observed only qualitatively, the amount of mineralization was similar for the 10- and 25-wt% hydrogels, but decreased substantially with the 50-wt% hydrogel. This phenomenon may be explained by two factors. First, the increase in macromer concentration dramatically decreases the rate of growth factor release from the networks. Due to the high vascularity of the subcutaneous tissue, the amount of growth factor surrounding the implant could have been very low compared to the amount necessary to induce bone tissue formation. Additionally, the higher macromer concentration would increase the local radical concentration during polymerization and increase the possibility of radicals

Table 2
Osteocalcin (OCN) and type I collagen (COL I) gene expression normalized to levels of GAPDH of primary rat calvarial osteoblasts cultured under various growth factor release conditions for 1, 2, and 3 weeks

| Growth factor delivery | 1 week | | 2 week | | 3 week | |
|---|---|---|---|---|---|---|
| | OCN | COL I | OCN | COL I | OCN | COL I |
| No growth factors | 0.12±0.07 | 0.03±0.02 | 0.09±0.01 | 0.04±0.003 | 0.21±0.13 | 0.05±0.04 |
| Bolus of growth factors for first 48 h | 0.13±0.12 | 0.09±0.13 | 0.11±0.02 | 0.04±0.01 | 0.17±0.11 | 0.09±0.10 |
| Growth factors delivered from 10 wt% hydrogel | 0.22±0.08 | 0.13±0.06 | 0.11±0.01 | 0.04±0.01 | 0.20±0.13 | 0.12±0.09 |
| Growth factors delivered from 25 wt% hydrogel | 0.19±0.09 | 0.15±0.09 | 0.10±0.01 | 0.04±0.01 | 0.16±0.11 | 0.13±0.08 |

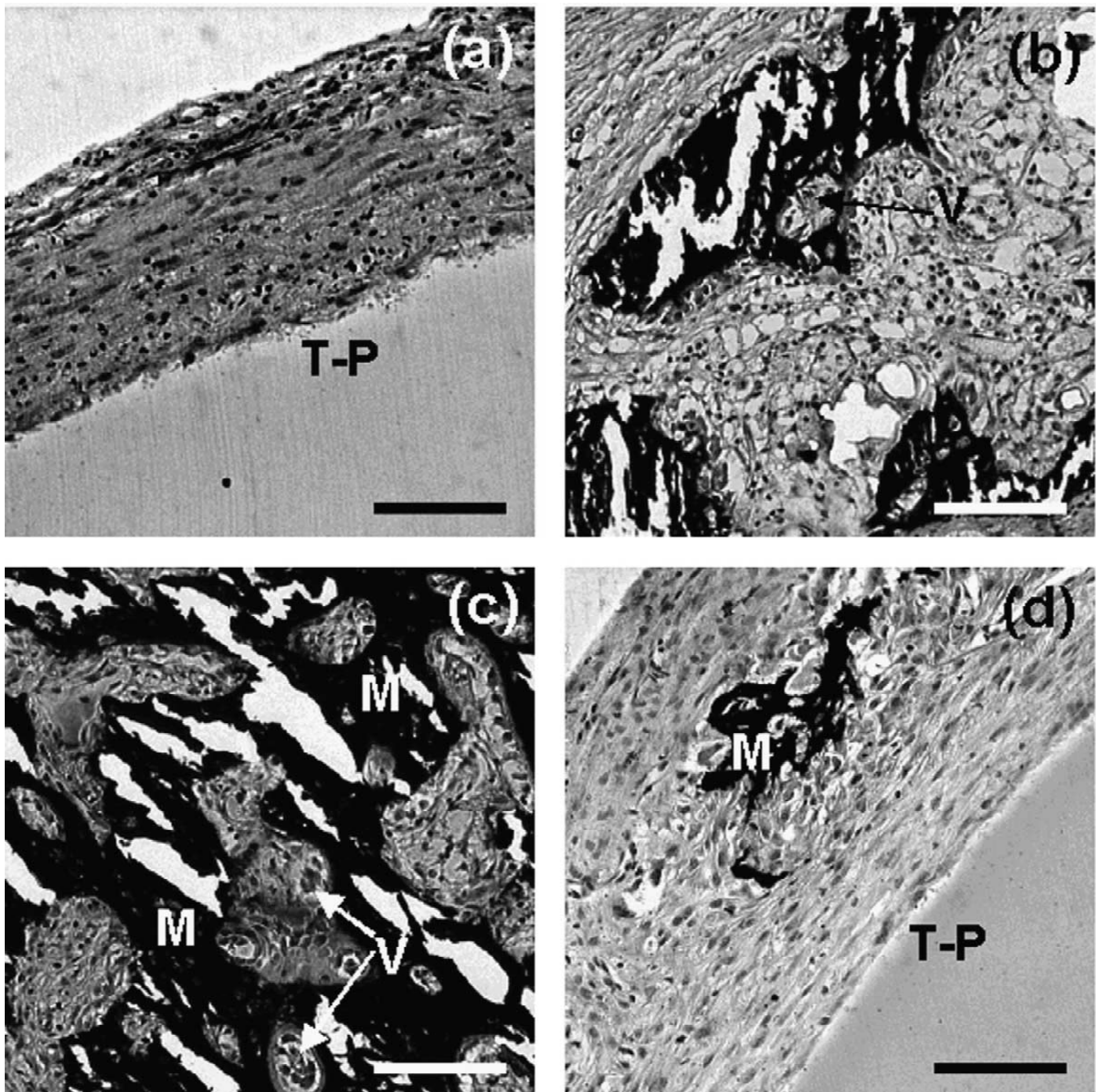


Fig. 6. Von kossa/hematoxylin and eosin stained histological sections of tissue surrounding (a) 25 wt% hydrogel, no growth factors; (b) 10 wt% hydrogel, 35 μg growth factors; (c) 25 wt% hydrogel, 35 μg growth factors; and (d) 50 wt% hydrogel, 35 μg growth factors 3 weeks after implantation. T–P=tissue/polymer interface, M=mineralized tissue, and V=vascular structures (bar=100 μm).

interacting and denaturing the encapsulated growth factors. With the 25-wt% samples, the tissue formed resembles mature bone tissue with cells encapsulated in mineralized tissue and vascular structures present throughout. In general, these results indicate that the delivery of osteoinductive growth factors from degradable hydrogel networks will induce bone formation and have potential to accelerate healing in the field of bone tissue engineering.

## 4. Conclusions

Osteoinductive growth factors were successfully

photoencapsulated in degradable PEG hydrogels and released in both in vitro and in vivo environments to promote mineralized tissue formation. The delivery method of these growth factors influenced the differentiation process of primary rat calvarial osteoblasts. Specifically, enhanced gene expression of both osteocalcin and type I collagen was seen after 1 week in culture, and significantly higher levels of both mineralization and alkaline phosphatase were noted when growth factors were delivered from a 25-wt% hydrogel. Growth factors released from hydrogel implants in subcutaneous tissue also produced ectopic mineralized tissue. Overall, these results indicate that osteoinductive growth factors remain active after photoencapsulation in hydrogel networks and that the sustained delivery of growth factors may be beneficial in bone tissue regeneration.

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