Photopolymerization for filling porous ceramic matrix: Improvement of mechanical properties and drug delivering behaviour.

Running title: Hydrogels filling ceramic scaffolds by photopolimerization

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ABSTRACT

The present work presents the photopolymerisation of composite scaffolds where a prefabricated ceramic scaffold was impregnated with Poly(ethylene) glycol dimethacrylate (PEGDMA) macromer solution. The PEGDMA solution penetrates the porosity of ceramic scaffold where it cures in situ thereby enhancing their mechanical properties. The mechanical properties of the obtained composite approximate the mechanical properties of cancellous bone. The Young´s Modulus of the composite developed is 106±5 MPa. PEGDMA is a polymer that shows excellent percentages of gel fraction (96%), which preserves when ceramic scaffold is introduced, and swelling (134%). Furthermore, thanks to their swelling properties, the composite is able to control the release vancomycin and dexamethasone. The efficacy of in situ delivery of vancomycin is demonstrated by the inhibition of bacteria colony proliferation. Dexamethasone released is recorded for up to ten days using UV Vis spectroscopy. These excellent results make photopolymerization and PEGDMA strong candidates for the manufacture of different loaded bone void fillers.

INTRODUCTION

An ideal material for use in bone tissue engineering applications should be biocompatible, biodegradable, bioactive and osteoinductive [1]. Apart from these biological properties, the choice of scaffold also depends on a variety of factors, including porosity, resorption rate, and mechanical strength [2]. Porosity is a very important factor in bone tissue engineering as it plays a vital role in tissue formation and function [3] as an interconnected porosity allows cell colonization and vascularization [4]. However, increased porosity decreases the mechanical properties of the scaffold [5]. Hence, scaffolds should be tailored to offer optimal strength [6] as an inability to contribute to load bearing can lead to non-unions [7] whereas excessive mechanical properties can lead to stress shielding [8]. Hence in an ideal situation the bone tissue engineering construct would offer similar biomechanical strength to the native bone [6].

Ceramic materials have been investigated for these applications for decades [9]. These bioceramics may be bioresorbable such as tricalcium phosphate (TCP) or bioactive such as hydroxyapatite (HA), bioactive glasses and glass-ceramics [10,11,12,13,14]. In addition calcium silicates, such as wollastonite (W), are characterized by their excellent osteoinductive properties and rapid bioresorption characteristics [15,16]. However, brittleness is an intrinsic drawback of ceramic scaffolds [17]. One way to improve mechanical properties is reinforce the ceramic structure by addition of polymers or hydrogels [1,18].

There are different ways to process polymeric-ceramic composites. These include but are not limited to extrusion[19], freezing-thawing [20,21] 3D printing [22] or photo-

polymerization[3,5,23,24].Photo-polymerization is of particular interest as it is used to obtain complex structures in a cost-effective manner in comparison to other techniques [23]. For example, powders of inorganic phase can be dispersed in the monomer solution and scaffolds have been obtained by emulsion and following photopolymerization. [25] In other hand, this method allows the easy filling of a porous structure or scaffold with a liquid monomer which can be polymerized in situ through an Ultra violet-Visible (UV-Vis) photo-initiated reaction initiated by a photo-initiator molecule. Once the polymerization reaction is ended the ceramic structure is completely embedded in a polymer matrix.

Several acrylate based monomers can be used for these applications, however it is desirable to utilize maromonomers as hydrogels synthesised using these materials have been reported to exert a minimal toxicological response and can be modified to be either bio-inert or biocompatible [26,27]. Examples of photo-polymerisable macromers include Poly(ethylene) glycol (PEG) methacrylate and polyvinyl alcohol (PVA) derivatives [26,28]. PEG is an FDA approved component [26]. Modified PEG based hydrogels are an attractive material due to tunable mechanical, and biological properties such as biocompatibility and lack of toxic influence on surrounding tissue [28,30]. Poly(ethylene) glycol dimethacrylate (PEGDMA) is an excellent candidate for photo-polymerization due to their mechanical properties and the water solubility of its macromolecular monomer precursor. However, PEGDMA is not easily dissolved once it polymerizes. Nevertheless, due to its excellent swelling properties it may be phagocyted as is reported with polyvinylpyrrolidinone hydrogels crosslinked with PEGDMA[31]. Additionally, PEGDMA hydrogels have shown potential in the controlled release of antimicrobials [5,23]. This is an important factor in orthopaedics as infections remain to be one of the main challenges in the orthopedic field and is one of the most common reasons of implants failure and revision surgery [32]. To avoid infections two main strategies are employed: sterilization prior to implantation and treatment with antibiotics. Regarding sterilization, UV-Vis radiation is commonly used to sterilize [33].

Hence, the current study was designed to assess the mechanical properties of porous ceramic scaffolds, which were infused with PEGDMA prior to in situ photo polymerization. These systems loaded with antimicrobial agents to determine their efficacy at killing bacteria and with dexamethasone, a corticosteroid which can minimize implantation-associated inflammation [34] enhances the osteogenic differentiation of progenitor cells [35] which is highly desirable for successful bone regeneration to take place.

EXPERIMENTAL

Materials

The fabrication process of the composite can be divided in two main steps. The first step involved the preparation of the ceramic scaffold, which the second step involved the infusion and in situ of the polymer phase of the scaffold.

Ceramic scaffolds were prepared from a mixture of 80% Wollastonite (W) (M400, NYAD®) and 20 % β-Tricalcium phosphate (β-TCP) powders obtained by precipitation. β-TCP powders were synthesized by the neutralization reaction of Hydroxyapatite (HA) (analytical grade, LabSynth) with the required amount of Orthophosphoric acid (85% analytical grade, Panreac) to obtain a Ca / P ratio of 1.50. The suspension was stirred for 30 min and subsequently dried at 65 °C for 24 hours. The dried powders were treated at 1000 °C for 15 hours. Both powders, W ($CaSiO_3$) and β-TCP (Ca_3PO_4), were individually milled by attrition in isopropyl alcohol with alumina balls (1-2 mm of diameter size) for 4h. Ground particles were subsequently dried with circulating air at 65 °C for 24h and ground with Agatha mortar and pestle.

Ceramic powders will be processed to obtain scaffolds (sponges). An aqueous suspension of W-β-TCP powders was prepared with 56wt% of powders in distilled water. To maintain the powders in suspension 0.3 wt% of dispersant (Dolapix PCN, Zschimmer-Schwarz) and 1 wt% of PVA binder (Optapix PAF 35, Zschimmer-Schwarz) were added. Scaffolds are prepared by the foam replication method [36]. Pieces of 60 pores per inch (ppi) polyurethane foams were impregnated with the suspension by dipping to obtain the replicas. These pieces were dried at 60 °C for 30 min, followed by sintering. The sintering schedule starts with a slow heating rate, 2 °C/min up to 600 °C. A slow heating rate is used to avoid the explosive exit of CO_2 as a result of the thermal decomposition of polyurethane. Subsequently, the heating rate was increased to 5 °C/min until the second dwell at 1350 °C for 2 hours. At this temperature sintering take place as well as the transformation of W to pseudo (ps)-W [37]. At this temperature α-TCP is the stable phase, hence to promote the transformation to β-TCP was promoted through cooling the ceramic composition to 1000 °C at a rate of 2 °C/min. Cooling rate must be slow to promote the phase transformation, whereas at a faster cooling rate the α-phase remains stable [10].

To prepare the PEGDMA based macromonomer solution, 45 wt% of PEGDMA (m.w. 600, Polysciences Inc), 8 wt% of Acrylic Acid (AA), as a crosslinking comonomer, and 0.1 wt% photoinitiator 4-(2-hydroxyethoxy)phenyl-(2-hydroxy-2-propyl)ketone (Irgacure 2959, Ciba Specialty Chemicals) was prepared in aqueous solution. The solution was placed in a 50ml beaker, mixed using a magnetic stirrer for 1 h, and finally sonicated for 30 min until a homogenous mixture was achieved[5]. The PEGDMA based solution was divided into aliquots, which were

placed in different recesses of a silicon mould. Ceramic scaffolds were immersed into the PEGDMA solution. Subsequently, UV curing was achieved following 60 minutes in a UV curing system (Dr. Gröbel UV-Electronik GmbH). The irradiation chamber utilized was a controlled radiation source with 20 UV tubes that provide a spectral range of between 315 and 400 nm at an average intensity of 10–13.5 mW=cm^2.

Once the scaffolds and composites are obtained a phase composition was realised by X-Ray Diffraction (XRD). Moreover, the incorporation of ceramic scaffolds in the polymer matrix was demonstrated by Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Physical properties such as swelling, gel fraction and compression strength were studied for the composites and compared with the measurements obtained for the polymer matrix without ceramic scaffolds embed. In case of compression strength, these measurements were also compared with the ceramic scaffold without polymer matrix.

Biodegradability, bioactivity and cytotoxicity of the composites were studied by in vitro asssays. Moreover, in vitro assays were also used to evaluate the composites as a drug delivery system able to release antibiotics as Vancomycin and other drugs such as Dexamethasone

The different techniques, assays and parametres used in the characterizations and study of different properties are described below.

X-Ray Diffraction

Phase composition of ceramic scaffolds and composites were studied by XRD. For the analysis scaffolds were milled using an Agate mortar and pestle. Diffraction pattern were obtained using a Bruker Multiload D8 diffractometer (Germany). Data was collected between 10° and 60° (2θ) in 0.05° steps, counting for 1s per step and using CuKα radiation. The X-ray tube was operated at 40kV at 40mA. The Eva-version 6.0.0.1 Diffract plus software was used to evaluate the diffraction patterns.

Attenuated total reflectance Fourier-transform infrared spectroscopy

ATR-FTIR was carried out using a Perkin Elmer (Spectrum One) spectrometer fitted with a universal ATR sampling accessory. All data was recorded in the spectral range of 4000 – 520 cm^{-1}.

Apparent Total Porosity

The percentage of apparent total porosity was calculated by the geometric volume (V_g) and the theoretical density (δ_t) and the weight (w) of the samples using equation 1:

$$\% \text{ Porosity} = [(w / \delta t)/V] \, 100 \qquad (1)$$

Swelling

After photopolymerization, samples were placed in a buffer solution (pH 7.4) to determine their water up take. Samples were removed after 24 hours and their weigh was measured. The equilibrium water contains (EWC) was calculated using equation 2:

$$EWC\ (\%) = (Ws - Wd)/Wd*100 \quad (2)$$

Where Wd and Ws are the weights of the composites after photopolymerisation, dried and in the swollen state respectively. Results are expressed as mean (± SD) (n=5) [23].

Gel fraction

The gel fraction was calculated from the weight of dried samples before and after swelling and according with equation 3:

$$\%G = (Wd / Wds) *100 \quad (3)$$

Where Wd represented the weight of the dried composites before swelling and Wds represented the composite weight after swelling for 24 hours [23].

Compression measurements

Compression tests were performed on the Lloyd Lr10K screw-driven mechanical testing machine fitted with a 2.5KN load cell with a bespoke 30mm diameter testing head. Prior to testing, the composites were allowed equilibrate at room temperature for 72h in buffer solution pH 7.4. Unconfined compression tests were carried out at a speed of 0.5mm/min and samples were strained to 60% (n=5).

Biodegradation and bioactivity tests

Biodegradation and bioactivity studies were carried out in simulated body fluid (SBF) pH = 7.4, prepared according with the international standard ISO/FDIS 23317 which is based on Kokubo assays [38]. Samples were incubated in SBF for 24 hours and 1 week. After incubation, samples were dried at 37 °C for 24h. It was observed that during drying, the polymer phase of the composite cracked which enabled easy imaging/analysis of the ceramic scaffold. Before and after incubation in SBF, scaffold surfaces were analyzed by Raman spectroscopy using a Reinshaw inVia Raman Microscope (infrared laser 785 cm^{-1} with 10% power). The spectra were acquired in the range from 250 to 1200 cm^{-1} recording 5 times for 10 s of each accumulation. The samples were visually assessed following sputter coating with the aid of a Hitachi TM-1000 scanning electron microscopy (SEM) with a backscattered electrons (BSE) detector and magnification between 20 and 10000X.

Cytotoxicity

Scaffold cytotoxicity was evaluated by elution assay. MC3T3-E1 cells were seeded at 10,000 cells per well in 96 well microtitre plates and incubated in a humidified atmosphere of 5% CO_2: 95% air at 37°C for 24 h. Cultures were exposed to pre-warmed test media containing 100-0% extracts control and sample hydrogels for a further 24 h at 37°C. 100 µl fresh culture media supplemented with 0.5 mg ml^{-1} MTT was added to each well and plates were incubated for a further 4 h at 37°C. The MTT media was aspirated off and DMSO was added to each well. Plates were shaken for 15 s and incubated at room temperature for 10 min prior to recording optical densities at 540 nm (victor). Cell viabilities were calculated as a percentage of untreated control cells ± the standard error of the mean (SEM) using equation 4:

% Viability = Absorbance (540nm) treated cells / Absorvance (540 nm) untreated cells (4)

Drug loading

Active pharmaceutical ingredients (API) Dexamethasone and Vancomycin were loaded into the PEGDMA phase of the composite at a loading of 10 and 4 mg/hydrogel sample prior to UV curing respectively. Subsequently, the PEGDMA solution was infused into the ceramic composite and cured as described previously.

Drug release

Drug release studies were performed using Dexamethasone. Tests were carried out in triplicate on specimens with a surface area of 340 mm^2 approximately. Samples were placed into 5mL of SBF at 37 °C. At predetermined time intervals of: 1, 2, 3 and 10 days, SBF was removed from the samples and replaced with 5ml of fresh SBF. Drug release was determined by performing UV-Vis spectroscopy on the collected media. The UV-Vis Spectrometer used was a PerkinElmer Lambda 950. Concentrations were calculated based on the maximum absorbance of Dexamethasone at 240 nm with reference to a calibration preformed with known concentrations.

Antibacterial activity

Antimicrobial inhibition testing were performed *in vitro* on the growing cultures of *Staphylococcus aureus* ATCC 8325-4 and RN4220 and *Staphylococcus epidermidis* ATCC 1457 and CSF41498 distributed on Mueller-Hinton agar plates. The British Society for Antimicrobial Chemotherapy (BSAC) guidelines, Version 10.2, which outlines the disc diffusion method for antimicrobial susceptibility testing were used and adapted where needed for this experiment (BSAC 2011). In this experiment, composites with Vancomycin entrapped within the sample were assessed. The controls were blank hydrogel based composites without drug. Each drug-loaded disc and control disc was placed dry on the agar surface; specimens were gently pressed

into the agar to ensure intimate contact with the agar, and incubated at 37 °C for 20 h. After incubation, the inhibition zones on the plates were assessed as efficacy of the released vancomycin. The zone of inhibition was calculated using the formula shown in equation 5:

$$W = (T–D) \quad (5)$$

Where: W represents the inhibition zone; T was the total diameter of the composite and the clear zone around the composite and D was the diameter of the specimen (5mm diameter) [23]. Tests were conducted in two replicate tests containing four samples per replicate.

RESULTS AND DISCUSSION

The dimensions of the ceramic scaffold prepared in this work were 5mm in diameter and 1-2 mm in length. The interconnected porosity can be observed in Fig. 1A. The phase composition of ceramic scaffolds studied by XRD (Fig. 1B) is consisting with a majority phase psW and a minority phase β-TCP as could be expected after the thermal treatment. It means that initial W was successfully converted to psW by heating at 1350 °C and α-TCP to β-TCP transformation was ensured by slow cooling rate. [39,40]

Ceramic scaffolds were impregnated with PEGDMA based macromonomer solution and a composite structure was formed following photopolymerization, where PEGDMA was observed to fill the porous structure of the ceramic scaffold (Fig. 1C).

FTIR spectra shown in Figure 3 reveals the characteristic bands of PEGDMA: at 1712 and 1638 cm^{-1}, C=C bending, at 1457 cm^{-1}, CCH_2 bending, at 1349 cm^{-1}, CH vibrations of CH_3 groups and at 1083 cm^{-1}, O-R stretching. [23] When ceramic scaffold structure is immersed in the polymeric matrix the corresponding inorganic signals appear. Bands at 682 and 902 cm^{-1} are assigned to SiO_2 groups of wollastonite. Signals at 938, 968, 1022 and 1107 cm^{-1} are attributed to $(PO_4)_3^2$ groups from TCP. [20]

Swelling

Swelling and gel fraction of composites were measured and compared with PEGDMA without inorganic phase to observe the possible changes introduced with the ceramic scaffold. From swelling analysis, it was found that the percentage swelling of the composite was 30% lower than that of the PEGDMA polymer without scaffold (Fig. 3A). These results are attributed to the inability of the ceramic phase of the composite to swell. Moreover, the weight percentage of ceramic phase in the composite is approximately 30%, which closely matches the difference in swelling behaviour of both specimens. This indicates that the polymer phase maintains its swelling capability while embedded in the ceramic scaffold.

Gel fraction

Gel fraction results show similar percentages for the composite and the polymer matrix without inorganic phase, 93 and 95% respectively (Fig. 3B). These values indicate a high curing efficiency of the PEGDMA matrix which was not decreased when the matrix was cured in situ inside the ceramic scaffold. Similar results were found by Killion et al [3] who used a similar system with powdered bioactive glass particles and reported gel fraction values of between 92 and 97%. It should be noted that the ceramic material used in this work was opaque which would prevent the transmittance of UV light. However, this ceramic material has been processed as scaffolds, specifically ceramic pieces have been obtained as sponges. These sponges are characterized by an interconnected porous network. The porosity of these scaffolds have an apparent total volume of 50±2 %. The macroporosity is shown in Figure 1a and it is in the range between 500 µm to 1mm .Hence, these results indicate that UV light is able to arrive to the photoinitiator molecules placed within the porous network and once free radicals are generated a highly cross-linked structure is formed in into the interconnected porosity of the scaffold.

Compression measurements

The Young´s modulus and the stress at limit have been studied to evaluate the mechanical properties of the composites (Fig. 4). As could be expected, Young´s modulus and the stress at limit values of the composite are between the corresponding values for the single components (PEGDMA and the ceramic scaffold). The Young´s Modulus of ceramic scaffold decreased when combined with PEGDMA. This reduction in Young's modulus means that the composite undergoes elastic deformation at lower loads compared with the scaffold without PEGDMA (Fig. 4A). This resulted in the composite having a Young´s Modulus equal to 106±5 MPa, which is within the range of that of natural bone. It has a modulus of 50-500Mpa for cancellous bone [41] which is the most common source of autologous bone grafts. Furthermore, this value is quiet similar to the estimated in 142 MPa from apparent density by regression models, which include cancellous bone from vertebrae, proximal tibia, and proximal femur. [42]

In case of the stress limit measurements (Fig. 4B), the ceramic scaffold possesses the highest values. The scaffold is able to bear higher stress forces without deformation compared to PEGDMA or the composite. However, it is well-known that ceramic materials are characterized by their brittle nature [17] which can lead to catastrophic failure of the implant without deformation. Conversely, PEGDMA is mechanically weak and poorly able to contribute to load bearing. In this work it was found that the composite experienced an interesting synergistic effect where the polymer provides to the scaffold a plastic deformation prior to failure, whereas the ceramic phase increases the value of stress at the limit. This synergistic effect is a result of the polymer matrix transferring loads to the ceramic surface.

Biodegradation and bioactivity

Other important properties in an implant, from the biological point of view, are the biodegradability and the bioactivity. It is well-known that PEGDMA alone does not contribute to favourably to this bioactivity [3], however, its high swelling ratios should promote biodegradation through phagocytosis[30,31]. Additionally, the ceramic scaffold is based on psW and β-TCP, both are soluble in aqueous solutions which further promotes scaffold degradation. Degradation of psW delivers SiO_3^{2-} ions to the which promote osteoinduction[43]. The degradation products of β-TCP are Ca^{2+} and PO_4^{3-}. When the ion concentration surpasses the solubility product, an hydroxyapatite (HA) layer precipitate onto the surface, thereby promoting bone-bonding to the scaffold. [4]

After incubation on SBF during one and seven dayscomposites were removed and dried. The dried polymer matrix was easily removed and the ceramic surface analysed. The Raman spectra obtained from the surface of the ceramic portion of the scaffold show signals due to the vibration modes of SiO_3^{2-} , from psW [44], and PO_4^{3-}, from β-TCP [42] (Fig. 5A). After 1 day of incubation, the intensity of signals due to the vibration modes of SiO_3^{2-} decreased. This was due to the rapid dissolution of psW. The dissolution was observed in photomicrographs recorded by SEM imaging as increased pitting and surface irregularities in the ceramic surface(Fig. 5B) when it is compared with the sample prior to incubation (Fig. 5C). This rapid dissolution of the ceramic phase enhances the bioresorption process by increasing the scaffold surface area which encourages cell colonization and new bone formation [45] while producing osteoinductive ingredients [46]. SEM results also indicate that a HA layer had not precipitated onto the surface of the scaffold. This is likely a result of the short incubation period (1 day) and the rapidly eroding surface. HA deposition was also not evident from Raman spectroscopy as the vibrational modes of PO_4^{3-} groups of HA (961 cm^{-1}) and β-TCP (950 cm^{-1} and 970 cm^{-1}) overlap [47]. Hence, it is difficult to observe small quantities of HA precipitated by Raman. Nevertheless, the Raman spectra obtained for samples incubated for 7 days, a new signal appeared around 850 cm^{-1} in the Raman spectra which is associated with impurities of CO_3^{2-} and or HPO_4^{2-} in some substituted HA [48]

Cytotoxicity

Materials used for implants have to be biocompatible and their degradation products must not be cytotoxic. For this reason, cell viability was studied by direct contact and elution assays with MC3T3 cells. It is well-known that calcium phosphates and silicates are biocompatible and the ions product of their dissolution has not cytotoxic As these degradation products are Ca^{2+}, PO_4^{3-} and SiO_3^{2-} ions, which possess beneficial properties promoting bone bonding and osteoinduction [2].

According with the elution assay percentages of viability in both cases, PEGDMA and PEGDMA/Ceramic composite, were approximately 90% compared to positive controls (Fig. 6). Hence, it can be concluded that the degradation products of PEGDMA and PEGDMA/Ceramic composite are non-toxic under the conditions tested. Similarly, Lin-Gibson et al [49] stated that hydrogels containing PEGDMA have been shown to be biocompatible and that the unreacted dimethacrylates have a relatively low cytotoxicity.

Drug release and antibacterial activity

The PEGDMA matrix was examined for its potential use as vehicle for the delivery of active pharmaceuticals. In this work, dexamethasone a corticosteroid which can minimize implantation-associat ed inflammation [34] and enhances the osteogenic differentiation of progenitor cells [35] and Vancomycin an antimicrobial agent which is used for methicillin-resistant Staphylococcus aureus (MRSA) [50] were selected due to their potential uses in the treatment of orthopaedic indications.

The efficacy of the composite to deliver Vancomycin was evaluated with different bacteria populations: Staphylococcus Aureus and Epidermidis. Vancomycin loaded composite scaffold generated a zone of inhibition of 37 ± 2mm and 41 ± 2mm for scaffolds incubated with Staphylococcus Aureus and Staphylococcus Epidermidis for 24 hours respectively.

In case of Dexametazone, its delivery from the PEGDMA matrix, as well as its delivering rate and mechanism were studied by UV-Vis spectroscopy. PEGDMA and composite samples, which contain Dexametazone, delivered active pharmaceutical with a non-linear behaviour with reference to time (Figure 7 A). As can be seen in figure 7 b, in the PEGDMA it is possible to observe a linear ratio between the dexametazone delivered and the square root of the time in full range of time. It is in agreement with a diffusion-controlled mechanism. It occurs thanks to the excellent swelling behaviour of the PEGDMA phase. In the case of the composite, this behaviour takes place during the first 72 hours of extraction. After 3 days 80% of entrapped Dexametazone was delivered by the composite while thereafter the polymer material released its payload more slowly. A possible explanation for this could be related to the ceramic portion of the scaffold. Drug diffusion through its interconnected microporous structure is quicker than those through the polymer matrix. It means that during the first 72 hours, most of the delivered molecules followed the interconnected microporous pathway of the ceramic structure. However, after 72 hours most of the molecules followed the PEGDMA matrix pathway.

CONCLUSIONS

Ceramic scaffold has been developed sponges for bone regeneration applications with the aim to mimic extracellular matrix of bone tissue. However, ceramics are naturally brittle, which is

attributed to the high strength ionic bonds. Moreover, strength dramatically decreases with porosity increasing.

Photopolymerization is a possible and smart solution to easy fill the porosity of ceramic scaffolds with a cost-effective method resulting in enhanced mechanical properties. At the same time, this polymer matrix can be charged with drugs or molecules, such as antibiotics or growth factors, to avoid infections or promote osteoinduction.

Results presented in the current study show that filling of a porous ceramic scaffold with PEGDMA results in a composite, which has excellent gel fraction percentages despite the tortuosity inside of the ceramic scaffold. It has been demonstrated that PEGDMA is able to fill the porosity getting the same gel fractions when ceramic scaffold is embedded. Moreover, mechanical properties approached those of natural bone.

Regarding the biological properties, biodegradation of the ceramic in SBF has been observed by Raman spectroscopy and SEM. The biodegradation products are Ca^{2+}, PO_4^{3-} and SiO_3^{2-} ions that possess beneficial properties promoting bone bonding and osteoinduction. While newly precipitated HA was not observed at days one or seven by Raman or SEM, a new band in Raman spectra appear at 850 cm^{-1} after seven days which could be assigned to the presence of CO_3^{2-} and/or HPO_4^{2-} ions in partially substituted HA.

Finally, the composites were evaluated as vehicle for in situ drug delivering. Vancomycin and Dexamethasone were successfully delivered. In case of Dexamethasone, the delivering process was over 10 days and it occurs by a diffusion mechanism.

All these properties and characteristics suggest the processing of PEGDMA by photopolymerization is an excellent method to fill the porosity of porous scaffold to enhance their mechanical properties while also acting as a delivery vehicle for active pharmaceutical ingredients. These properties combined, indicate that in situ photopolymerization of PEGDMA within the structure of a porous scaffold has potential for the treatment of loaded bone voids.

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FIGURE LEGENDS

Fig. 1 Characterization of ceramic scaffolds and composites A) morphology of ceramic scaffold observed by SEM micrograph B) micrographs from the fracture of the composite and C) phase composition of ceramic scaffold studied by XRD.

Fig. 2 ATR-FTIR of PEGDMA and composite, where --- indicate the characteristic bands of PEGDMA and * and o indicate the characteristic bands of PO_4^{3-} and SiO_3^{2-} respectively.

Fig. 3 Measurements of A) Swelling and B) Gel fraction of polymeric ceramic composites compared with polymer matrix. Error bars show P=0.05 according with student test.

Fig. 4 Mechanical properties of composites compared with the corresponding and individual ceramic or polymeric phases. A) Young modulus and B) Stress limit.

Fig. 5 Biodegradability and bioactivity assays. A) Raman spectra of the scaffold surface from composites incubated for one and seven days, compared with a non-incubated sample, where * are vibrations modes of SiO_3^{2-} and ● correspond to vibrations modes of PO_4^{3-} B) SEM photomicrographs of scaffold surface incubated 1 day and C) scaffold surface which has not been incubated on SBF.

Fig. 6 Viability study by elution assay with MC3T3 cells.

Fig. 7 Drug delivery assay with Dexametazone in SBF. A) Concentrations of Dexametazone delivered calculated from absorbance measurements in UV-Vis spectroscopy and B) vs the $t^{1/2}$.

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