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Obtaining new composite biomaterials by means of mineralization of methacrylate hydrogels using the reaction–diffusion method

Yousof Ramadan [a], M. Isabel González-Sánchez [b], Karl Hawkins [c], Jorge Rubio-Retama [a], Edelmira Valero [b], Stefano Perni [d,f], Polina Prokopovich [d,e,f], Enrique López-Cabarcos [a,*]

[a] Department of Physical Chemistry II, Faculty of Pharmacy, Complutense University of Madrid, 28040 Madrid, Spain
[b] Department of Physical Chemistry, School of Industrial Engineering, Castilla-La Mancha University, 02071 Albacete, Spain
[c] Centre of Nanohealth, Institute of Life Sciences, College of Medicine, Swansea University, Singleton Park, Swansea SA2 8PP, Wales, UK
[d] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff CF103NB, UK
[e] Institute of Medical Engineering and Medical Physics, School of Engineering, Cardiff University, Cardiff, UK
[f] Department of Biological Engineering, MA Institute of Technology, Cambridge, USA

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ABSTRACT

The present paper describes the synthesis and characterization of a new polymeric biomaterial mineralized with calcium phosphate using the reaction–diffusion method. The scaffold of this biomaterial was a hydrogel constituted by biocompatible polyethylene glycol methyl ether methacrylate (PEGMEM) and 2-(dimethylamino)ethyl methacrylate (DMAEM), which were cross-linked with N-N'-methylenebisacrylamide (BIS). The cross-linking content of the hydrogels was varied from 0.25% to 15% (w/w). The gels were used as matrix where two reactants (Na_2HPO_4 and $CaCl_2$) diffused from both ends of the gel and upon encountering produced calcium phosphate crystals that precipitated within the polymer matrix forming bands. The shape of the crystals was tuned by modifying the matrix porosity in such a way that when the polymer matrix was slightly reticulated the diffusion reaction produced round calcium phosphate microcrystals, whilst when the polymer matrix was highly reticulated the reaction yielded flat calcium phosphate crystals. Selected area electron diffraction performed on the nanocrystals that constitute the microcrystals showed that they were formed by Brushite ($CaHPO_4.2H_2O$). This new composite material could be useful in medical and dentistry applications such as bone regeneration, bone repair or tissue engineering.

1. Introduction

The study of the precipitate formation within a gel after the encounter of two reaction fronts that diffuse from opposite sides enables to relate scientific fields that are normally separated such as gel structure, progress of reaction fronts, crystal growth in gels, nanoparticles synthesis, simulation of nonlinear phenomena, etc. This is an old research subject and during most of the early period, the main interest was held by the phenomenon of Liesegang rings; however, more recently a new approach was taken in the context of order formation out of chaos and simulation of the process [1–5]. For several decades mineralization of an organic matrix has been an important topic in bone-tissue engineering [6]. There are numerous reports about mineralization, for example, through immersion in simulated body fluid [7] or an alternate soaking process [8] and a natural diffusion process through a biopolymer membrane [9]. However, these methods form 2D mineralised surfaces on the biomaterial, which is not useful in bone repair and osseoregeneration processes. As 3D mineralization was observed in the hydrogels, very recently they started been used as a good model to study this process, in consequence that many mineralization processes take place in gelling environments [10–12]. Most of the research in this field was performed using physical gels such as gelatin, agarose, silica, starch, polyvinyl alcohol) hydrogels [2,13,14] whilst only few studies were conducted with chemical gels [15].

The integration of micro- or/and nano- size particles in synthetic hydrogels can be a way to create new biomaterials. One difficulty lies in getting a homogeneous distribution of the inorganic particles across the organic matrix and in controlling their composition and crystal size. Hydrogels have been associated with some biomineralization systems [10] such as: tooth enamel in mammals [16], nacreous layer in mollusk shells [17] and trout otoliths [18]; therefore, hydrogels could provide the matrix to grow inorganic calcium phosphate particles. Organic matrixes made of cross-linked hydrogels based on methacrylates have been proven non cytotoxic, non-immunoreactive and their porosity could be controlled by the amount of cross-linker used in

* Corresponding author.
E-mail address: cabarcos@farm.ucm.es (E. López-Cabarcos).

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their synthesis [19]. Furthermore, lately, hydrogels based on methacrylates have been used as tissue expanders before vertical ridge augmentation to facilitate the wound closure and to prevent the exposure of the bone grafts to the oral cavity demonstrating the possible applications of these materials in dentistry [20].

It has been previously shown that a new biomaterial could developed combining the cohesion provided by the gel with the bone regeneration properties of calcium phosphate particles [21–23]. In this work, we have prepared a new copolymer hydrogel composed of two well known and widely used methacrylate polymers [24–28] containing calcium phosphate nanoparticles. The particle formation within the gel was achieved through a reaction diffusion process:

$$Na_2HPO4 + CaCl_2 + 2H_2O \rightarrow \quad CaHPO_4 \cdot 2H_2O + 2ClNa$$

Electrolyte solutions of sodium hydrogen phosphate and calcium chloride were allowed to diffuse from opposite sides of the gel; the low solubility of calcium phosphate resulted in the precipitation of nanoparticles when saturation was reached. The properties of the inorganic nanoparticles formed within the gel, as well as the physical properties of the gels, were studied in this work. The growth of calcium phosphate particles in methacrylate hydrogels constituted, a new biomaterial which could have potential benefits in dentistry and orthopaedic tissue regeneration.

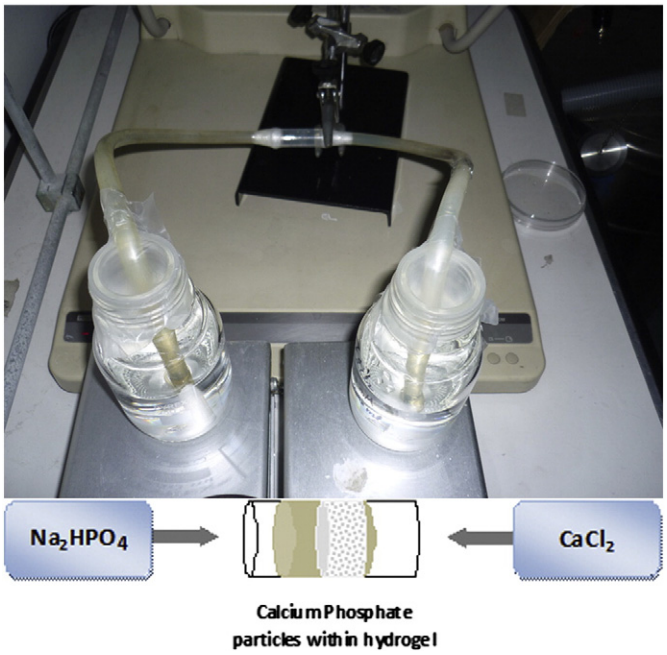

Fig. 1. Schematic representation of the diffusion of the electrolyte solutions from opposite ends within a gel (left side) and the corresponding set up of the experiment (right side).

2. Materials and methods

2.1. Chemicals

The monomers polyethylene glycol methyl ether methacrylate (PEGMEM), and 2-dimethylamino ethyl methacrylate (DMAEM), and the cross linker N,N′-methylenebis(acrylamide) (BIS) were purchased from Sigma Aldrich. The initiator ammonium persulfate (APS) was purchased from Fluka. $Na_2HPO_4 \cdot 2 \cdot H_2O$ (DHP) and $CaCl_2$ were purchased from Panreac.

2.2. Synthesis of the gel

The gels were prepared by adding 2 ml of PEGMEM (0.0065 mol) and 1 ml of DMAEM (0.0065 mol) to 5 ml deionised water at room temperature. Subsequently, appropriate amounts of BIS and APS were added under stirring, finally the mixture was allowed to polymerize for 25 minutes. During the polymerization the temperature was recorded every 30 seconds. After preparation, the gels were dialyzed against milliQ water at room temperature for two weeks to remove the unreacted monomers and oligomers [29].

Two groups of gels were prepared as described above: i) initiator content was varied between 1 mg/mL and 7 mg/mL and no crosslinker was used in the preparation of the gels; ii) crosslinker concentration varied between 0.25% and 15% (w/w) whereas the concentration of initiator was kept at 1 mg/mL.

2.3. Reaction diffusion experiments

A piece of the swelled gel was inserted in the middle of a PTF tube (1.5 cm in diameter and 7 cm in length) and connected to both sides with silicone tubes of 54 cm length and 8 mm diameter, that connected both sides with two bottles of 200 ml that served as reservoirs for Ca (solution of 20 mM) and phosphate (solution of 20 mM) [5,30]. The formation and evolution of the calcium phosphate precipitate was followed every day using a Canon RE-650 video recorder. The pictures obtained were analyzed using Image J to measure the lengths of the gel, the width of the calcium phosphate precipitate formed in the gel and the position of the centre of the band; for the latter purpose the border of the gel from which the solution of $CaCl_2$ diffuses was assumed as origin for the position measurements. The experimental setup and a scheme of the experiment are shown in Fig. 1.

2.4. Gel Characterization Methods

The chemical composition of the gel was studied using FTIR (Thermo Nicolet IR200) covering the wave number range between 500 and 3500 cm^{-1} [31]. Bruker Avance 250 MHz ^1H NMR was used to investigate the ratio of both monomers used in the synthesis in the final gel [32] The decomposition temperature and water content of the gel were measured with DSC Mettler 820 equipped with a cooler operated by liquid Nitrogen; the samples were heated at a rate of 5 °C/min [33]. The characterization of the calcium phosphate precipitate formed on the surface of the gel by reaction diffusion method was carried out by scanning electron microscope (SEM) JSM 6335 F (JEOL) operated at 10 and 20 kV. In addition, transmission electron microscope (TEM) JEM 1010 (JEOL) working at 100 kV was used to image the calcium phosphate particles as well as selected area electron diffraction patterns. The samples examined by TEM were prepared in a similar way as biological samples; the gel was embedded in resin for 48 to 72 hours at 60 °C, then a microtome was used to cut thin slices (100 nm thick) that were ready for use.

2.5. Gel swelling behaviour

The gels were immersed completely in deionised water and they were weighted daily [29]. The hydration of the gel was obtained according to Eq. (1).

$$H = \frac{\left(m_f - m_i\right)}{m_i} \tag{1}$$

where m_i and m_f are the weights of the gel before and after swelling respectively.

The effect of NaCl solutions with different concentration in swelling process was also investigated. For these experiments pieces of a fully swollen gel (2.5% cross-linking) were placed in NaCl solutions with