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Silver nanoparticle based antibacterial methacrylate hydrogels potential for bone graft applications

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ABSTRACT

Infections are frequent and very undesired occurrences after orthopedic procedures; furthermore, the growing concern caused by the rise in antibiotic resistance is progressively dwindling the efficacy of such drugs. Artificial bone graft materials could solve some of the problems associated with the gold standard use of natural bone graft such as limited bone material, pain at the donor site and rejections if donor tissue is used. We have previously described new acrylate base nanocomposite hydrogels as bone graft materials. In the present paper, we describe the integration of silver nanoparticles in the polymeric mineralized biomaterial to provide non-antibiotic antibacterial activity against *Staphylococcus epidermidis* and Methicillin-resistant *Staphylococcus aureus*. Two different crosslinking degrees were tested and the silver nanoparticles were integrated into the composite matrix by means of three different methods: entrapment in the polymeric hydrogel before the mineralization; diffusion during the process of calcium phosphate crystallization and adsorption post-mineralization. The latter being generally the most effective method of encapsulation; however, the adsorption of silver nanoparticles inside the pores of the biomaterial led to a decreasing antibacterial activity for adsorption time longer than 2 days.

1. Introduction

Hydrogels are suitable for a variety of applications in the pharmaceutical and medical industry. They have been used in ophthalmology, for drug delivery, orthopedics and medical devices [1–3]. Among their characteristics are: soft and rubbery consistence, excellent biocompatibility and high permeability to oxygen, nutrients and other water soluble metabolites. All these characteristics make them particularly attractive as scaffolds in tissue engineering applications [4]. Very recently hydrogels have been used as a model to study biomineralization since these processes take place in gelling environments [5]. Biomineralization of an organic matrix has been an important topic in bone–tissue engineering [6–9] as a possible process to prepare hybrid biomaterials for orthopedic tissue engineering where three-dimensional biomimetic mineralization is highly desired [10].

Hydrogels based on methacrylates are biocompatible, non-toxic, and non-immuno-reactive and their porosity is easily controllable [11]. After the pioneering work in the 1960s [12], methacrylate hydrogels have been applied in drug delivery systems, tissue regeneration, contact lenses and synthetic membranes for biosensors [13–18]. Very recently hydrogels based on methacrylates have been also used as tissue expanders in dentistry clinics [19].

We lately synthesized a copolymer hydrogel using as monomers PEG methyl ether methacrylate (PEGMEM) and (dimethylamino)ethyl methacrylate (DEM) [20]. Using Na_2HPO_4 and $CaCl_2$, we were able to produce brushite microparticles in situ using the gel network as a microreactor for microparticle formation by means of a reaction–diffusion process (Fig. 1). In this process, phosphate and calcium ions migrate from the opposite sides of the gel and when they encounter the insoluble calcium phosphate particles form and precipitate. This composite hydrogels could be used in medical and dentistry applications as artificial bone graft material because of the osseconductive properties provided by the calcium phosphate microparticles homogeneously distributed in the gel matrix.

Infections associated with medical implants are becoming increasingly common and result in significant morbidity and, in some cases,

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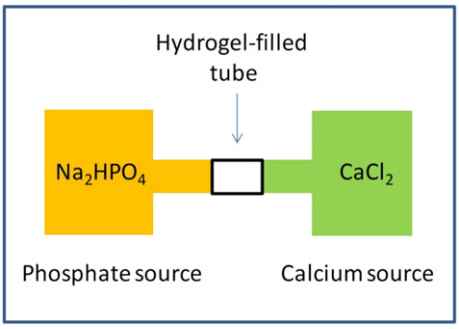

Fig. 1. Representation of the reaction–diffusion process to produce calcium phosphate nanoparticles inside the hydrogel matrix.

mortality [21]. Very frequently biofilms, bacteria that attach to surfaces and aggregate in a hydrated polymeric matrix, are associated to infections because of their high resistance to antibacterial drugs [22]. Thus the synthesis of hydrogels with bactericidal properties has special interest. Some authors have been able to mix bactericidal agents into hydrogels [23,24]. Another promising approach is the addition of metal nanoparticles as alternative to antibiotics because of the increasing bacteria population exhibiting resistance to these drugs consequently reducing their applicability [25,26]. Silver nanoparticles have been shown effective to combat bacteria, viruses and eukaryotic micro-organisms [27,28]. Besides, silver nanoparticles are also reported to possess anti-inflammatory [29] and anti-angiogenic activity [30], which makes these nanoparticles suited for medical purposes. Various synthetic routes have been developed to prepare silver nanoparticles and the conditions are responsible for the size, shape and surface charge of the resulting nanomaterials [31–33].

In this work, we examined ways to encapsulate silver nanoparticles in methacrylate hydrogels containing calcium phosphate to obtain a new multifunctional biomaterial that could be applied as a synthetic bone draft since it combines homogeneous 3D-biomineralization and antibacterial properties.

2. Materials and methods

2.1. Chemicals

Polyethylene glycol methyl ether methacrylate with average Mn = 300 (PEGMEM), 2-dimethylamino ethyl methacrylate (DEM), N,N′-methylenebis(acrylamide) (BIS), silver nitrate and citric acid were purchased from Sigma Aldrich. The initiator ammonium persulfate (APS) was purchased from Fluka (Spain). $Na_2HPO_4 \cdot 12H_2O$ and $CaCl_2$ were purchased from Panreac quimica SAU (Spain). All chemicals were reagent grade and used as received.

2.2. Synthesis of the silver nanoparticles

Silver colloids were prepared by the reduction of $AgNO_3$ with citrate at near-boiling temperature. 125 ml of silver nitrate solution 1 mM was heated and as soon as boiling commenced, 5 ml of 1% sodium citrate solution were added. Heating was continued until a color change from uncolored to pale yellow was evident. Then the solution was removed from the heating element and stirred until it had cooled down to room temperature.

2.3. Silver nanoparticle characterisation

UV–vis spectra (350 – 600 nm, 1 nm resolution) of the conjugated nanoparticles dispersed in PBS (1 mg/ml) were recorded in 1 cm quartz cells with a U-3000 Hitachi, UV–vis spectrometer. For transmission electron microscopy (TEM) characterization a 4 μl droplet of nanoparticle suspension was placed on a plain carbon-coated copper TEM grid and allowed to evaporate in air under ambient laboratory conditions for several hours. Bright field TEM images were obtained using a TEM (Philips CM12, FEI Ltd, UK) operating at 80 kV fitted with an X-ray microanalysis detector (EM-400 Detecting Unit, EDAX UK) utilizing EDAX's Genesis software. Typical magnification of the images was × 100,000. Images were recorded using a SIS MegaView III digital camera (SIS Analytics, Germany) and analyzed with the software ImageJ.

2.4. Synthesis of the gel

The gels were prepared by adding 2 ml PEGMM (0.0065 mol) and 1 ml of DEM (0.0065 mol) to 5 ml of deionized water at room temperature. Subsequently, appropriate amounts of BIS and 1 mg/ml APS were added under stirring and finally the mixture was allowed to gel. It generally took around 25 min for a firm gel to form. After obtaining the gels, they were dialyzed in ultrapure water at room temperature for two weeks to remove the excess monomers and unwanted reaction products.

To obtain the hydrogel with silver nanoparticles entrapped, 5 ml of silver nanoparticle suspension at two different concentrations, 1 mM and 0.5 mM in water, was used instead of deionized water. The rest of the process was exactly the same.

2.5. Reaction diffusion experiments

A piece of the swelled gel was inserted in the middle of a plastic tube (diameter: 1 cm; length: 7 cm) connected to both sides with silicone tubes that ended each one in a bottle (500 ml) containing an aqueous solution of either $CaCl_2$ (20 mM) or Na_2HPO_4 (20 mM), thus insuring a reservoir that guaranteed the continuous diffusion of the reactants through the gel. The reaction proceeded at room temperature for one week.

One of the methods of integration silver nanoparticles used in this work consisted in diluting the phosphate with the silver nanoparticle suspension (1 and 0.5 mM) and operating as described above.

2.6. Adsorption of silver nanoparticles in the hydrogels

Mineralized hydrogels were immersed in silver nanoparticle suspensions (5 ml) at two concentrations: as obtained from the synthesis (1 mM) and diluted 1:1 in distilled water (0.5 mM). The gels were stored at room temperature for up to 7 days. Every day a set of samples was removed from the silver nanoparticle suspension and rinsed in sterile PBS prior to further testing.

2.7. Silver content determination

Gel pieces of known weight were placed in glass bottles containing 5 ml of aqua regia (HCl:HNO_3 3:1); the bottles were sealed and stored at room temperature until the gels were completely dissolved. The silver ion content in the dissolved acid solution was determined by inductively coupled plasma–mass spectroscopy (ICP–MS) analysis (Optima 2100DV OES; Perkin Elmer, Waltham, MA, USA) against the Primar 28 element standard.

2.8. Bacterial species

The bacteria used were Methicillin-resistant *Staphylococcus aureus* (MRSA) (NCTC12493) and *Staphylococcus epidermidis* (RP62a). Bacteria were maintained by sub-culturing on Brain Heart Infusion (BHI) agar (Oxoid, Basingstoke, UK) and storing plates at 4 °C for no more than a week. For experimental purposes, bacteria were grown aerobically in 10 ml BHI broth (Oxoid, Basingstoke, UK) statically at 37 °C for 24 h.