Materials Science and Engineering C 59 (2016) 818–827

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Strontium-rich injectable hybrid system for bone regeneration

Nuno Neves [a,b,c,*], Bruno B. Campos [d], Isabel F. Almeida [e], Paulo C. Costa [e], Abel Trigo Cabral [c], Mário A. Barbosa [a,b,f], Cristina C. Ribeiro [a,b,g]

[a] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
[b] INEB — Instituto de Engenharia Biomédica, Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal
[c] FMUP — Faculdade de Medicina da Universidade do Porto, Departamento de Cirurgia, Serviço de Ortopedia, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
[d] FCUP — Faculdade de Ciências da Universidade do Porto, Centro de Investigação em Química, Departamento de Química e Bioquímica, Rua do Campo Alegre 1021/1055, 4169-007 Porto, Portugal
[e] FFUP — Faculdade de Farmácia da Universidade do Porto, Laboratório de Tecnologia Farmacêutica, Departamento de Ciências do Medicamento, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[f] ICBAS — Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[g] ISEP — Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 10 September 2015
Accepted 12 October 2015
Available online 23 October 2015

Keywords:
Biomaterials
Injectable bone substitute
Strontium
Alginate
Hydroxyapatite

ABSTRACT

Current challenges in the development of scaffolds for bone regeneration include the engineering of materials that can withstand normal dynamic physiological mechanical stresses exerted on the bone and provide a matrix capable of supporting cell migration and tissue ingrowth. The objective of the present work was to develop and characterize a hybrid polymer–ceramic injectable system that consists of an alginate matrix crosslinked *in situ* in the presence of strontium (Sr), incorporating a ceramic reinforcement in the form of Sr-rich microspheres. The incorporation of Sr in the microspheres and in the vehicle relies on the growing evidence that Sr has beneficial effects in bone remodeling and in the treatment of osteopenic disorders and osteoporosis. Sr-rich porous hydroxyapatite microspheres with a uniform size and a mean diameter of 555 μm were prepared, and their compression strength and friability tested. A 3.5% (*w*/*v*) ultrapure sodium alginate solution was used as the vehicle and its *in situ* gelation was promoted by the addition of calcium (Ca) or Sr carbonate and Glucone-δ-lactone. Gelation times varied with temperature and crosslinking agent, being slower for Sr than for Ca, but adequate for injection in both cases. Injectability was evaluated using a device employed in vertebroplasty surgical procedures, coupled to a texture analyzer in compression mode. Compositions with 35% w of microspheres presented the best compromise between injectability and compression strength of the system, the force required to extrude it being lower than 100 N. Micro CT analysis revealed a homogeneous distribution of the microspheres inside the vehicle, and a mean inter-microspheres space of 220 μm. DMA results showed that elastic behavior of the hybrid is dominant over the viscous one and that the higher storage modulus was obtained for the 3.5%Alg–35%Sr-HAp-Sr formulation.

1. Introduction

Osteoporosis is a systemic disease that affects a significant part of the aging population in western countries. It results in progressive loss of mineralization and consequent changes in bone architecture leading to increased susceptibility to fractures. Because of the raise in expectancy of life, osteoporosis has become a serious public health issue that will probably be worsened in the near future. The treatment of bone defects in osteoporotic fractures remains a significant challenge. The use of calcium phosphate ceramics in bone regeneration, either alone or in combination with a polymeric phase, is now a common practice, since these materials provide good biological responses, based on their osteoconductive properties, and adequate mechanical properties [1–5]. The development of injectable materials for filling bone defects allows for the use of minimally invasive techniques. Most injectable ceramic materials consist of micro or nanoparticles suspended in appropriate vehicles [6–11]. Spherical particles are more suitable for implantation than non-homogeneous granules, since they conform better to irregular implant sites and present more predictable flowing properties during injection [12–15]. The space between particles is critical for the success of a scaffold for bone regeneration since blood vessels and cells should be able to invade the inter-particle network to promote bone formation throughout the filled defect. It is generally accepted that a size of 100–200 μm in diameter is suitable for bone in-growth [16].

The addition of inductive factors or osteoprogenitor cells is a current strategy to improve osteogenesis in osteoporosis [17–20]. Sr is a trace element that plays a dual role in bone metabolism, stimulating bone

* Corresponding author at: INEB — Instituto de Engenharia Biomédica, Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal.
E-mail address: nsmneves@gmail.com (N. Neves).

http://dx.doi.org/10.1016/j.msec.2015.10.038
0928-4931/

formation and inhibiting bone resorption [21–26]. *In vitro* pre-clinical studies indicate that Sr decreases bone resorption by directly inhibiting the differentiation, resorbing activity and apoptosis of osteoclasts [21, 25–27]. It was also observed that it enhanced the replication of preosteoblastic cells and osteogenic differentiation, reduced osteoblasts apoptosis and, secondarily, promoted bone matrix synthesis [21,24,26, 28,29]. At least three mechanisms are involved in the opposite effects of Sr: activation of the calcium-sensing receptor (CaSR), nuclear factor of activated Tc (NFATc)/Wnt signaling, and modulation of osteoprotegerin (OPG) and receptor activator of nuclear factor κ-B ligand (RANKL) [21]. The available *in vivo* data are consistent with the *in vitro* studies showing the beneficial effect of Sr in increasing bone architecture and bone strength in both intact and osteoporotic animal models [22, 30–35]. Sr has been used in clinical practice against osteoporosis as oral Sr ranelate, and has shown effectiveness in the prevention of both vertebral and non-vertebral osteoporotic fractures [21,36,37]. Incorporating Sr in calcium phosphate cements may be a strategy to achieve high Sr concentrations not in a systemic but in a local environment, using the osteoanabolic and anti-osteoclastic activities for enhancement of new bone formation [38–40].

Our group has developed an injectable system based on hydroxyapatite microspheres and an alginate-based vehicle with gel-forming ability [41]. We have extensively studied various types of microspheres, namely of calcium alginate [42], hybrid calcium phosphate/alginate [43] and calcium phosphates [44,45].

In the present work we propose an injectable hybrid system that consists of a polymeric matrix crosslinked *in situ* with Sr, reinforced with Sr-rich calcium phosphate microspheres, to be used for bone regeneration. The reasoning behind this strategy is that our hybrid system will provide both a scaffold capable of supporting cell migration and tissue ingrowth and incorporate two Sr release kinetics, in order to guarantee an effective bone remodeling since the early stages of implantation.

2. Materials and methods

2.1. Microspheres preparation and characterization

2.1.1. Microspheres preparation

HAp-microspheres and Sr-HAp microspheres were prepared using the droplet extrusion method combined with ionotropic gelation in the presence of Ca^{2+} and Sr^{2+} respectively. A homogeneous paste, obtained by dispersing the ceramic powder (HAp, Plasma Biotal) in a 3% (w/v) sodium alginate solution, was extruded drop-wise into a $CaCl_2$ or $SrCl_2$ crosslinking solution with a concentration of 0.1 M, where spherical-shaped particles were instantaneously formed due to the rapid establishment of Ca or Sr mediated junctions between polyguluronate chains on the polymer backbone.

Different ceramic-to-polymer solution ratios were tested, the 0.2 w/w being the one that resulted in more reproducible results. The size of the microspheres was controlled by regulating the extrusion flow rate using a syringe pump, and by applying a coaxial air stream (Encapsulation unit Var J1, Nisco Engineering AG). At completion of the gelation period the microspheres were recovered and rinsed in water. Finally, they were dried and sintered at 1100 °C and 1200 °C (Eurotherm 2408 MLE, Termolab).

2.1.2. Microspheres characterization

HAp and Sr-HAp microspheres were characterized in terms of diameter (laser beam diffraction — Coulter LS, Beckman Coulter), porosity (mercury porosimetry — AutoPore IV, Micromeritics) and specific surface area (gas adsorption according to the Brunauer, Emmel and Teller-BET method — ASAP 2000, Micromeritics). Morphological and physic-chemical characterization of the microspheres was carried out using scanning electron microscopy (SEM–EDS), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The SEM/EDS exam was performed using a high resolution (Schottky) Environmental Scanning Electron Microscope with X-ray Microanalysis and Electron Backscattered Diffraction analysis: Quanta 400 FEG ESEM/EDAX Genesis X4M. Samples were coated with an Au/Pd thin film, by sputtering, using the SPI Module Sputter Coater equipment. For FTIR and XRD analysis, microspheres were reduced to powder and analyzed respectively as KBr pellets using a spectrophotometer (Spectrum 2000, PerkinElmer) and as powder particles using a diffractometer (Philips X-Pert). Zeta potential of hydroxyapatite microspheres was determined from streaming potential measurements with a commercial electrokinetic analyzer (EKA, Anton Paar GmbH) using a special cylindrical cell with a powder insert for granular or powder samples. The electrolyte used was 1 mM KCl and the pH was slowly changed from 3 to 10. Mechanical characterization of the microspheres (friability and rupture force) was performed using a friability tester and a texture analyzer. Friability tests were implemented according to a procedure described in the European Pharmacopeia (7th edition) with minor modifications. Briefly, 2 g of microspheres were loaded into a drum (F1, Sotax AG,) operating at 25 rpm. The fall height was 150 mm and the same microspheres were used for three cycles of 4 min each. After each cycle, the desegregated powder was blown out and the microspheres were collected and weighted again [41]. Friability was reported as percentage of total weight lost. Microsphere rupture force (hardness) was evaluated in a texture analyzer (TA-XT2i, Stable Micro Systems). The load was applied vertically to individual microspheres, using a load cell of 5 kg and a cylindrical metallic probe with a diameter of 2 mm at a displacement rate of 0.1 mm/s. The rupture force was determined from the maximum force reached (breaking point). In each experiment, 10 microspheres were assayed and the mean value from at least three experiments was calculated.

2.2. Vehicle characterization

2.2.1. Alginate molecular weight determination

An ultra-pure sodium alginate (NovaMatrix, FMC Biopolymers) with >60% content of guluronic *vs*. mannuronic acid units was used in the experiments. The molecular weight of the alginate was calculated by gel permeation chromatography/size exclusion chromatography (GPC/SEC). The analysis was performed at room temperature (circa 23 °C), using a modular system, composed by an isocratic pump, a vacuum degasser and an autosampler module (GPC Max, Viscotek), a viscometer/right angle laser light scattering (RALLS) and a dual detector (T60, Viscotek), and a refractive index detector (K-5002, Knaeur), operating at the same wavelength as the RALLS detector (670 nm). Separations were performed in a set of PL aquagel-OH mixed columns. The mobile phase consisted of 0.1 M $NaNO_3$ with 0.02% *w*/*v* NaN_3 and the flow-rate was maintained at 1.0 mL/min. Samples were dissolved in the mobile phase at 1 mg/mL, filtered, and 100 μL of sample were injected by the autosampler on an automatic injection valve equipped with a 200 μL loop. All modules were controlled and sample data analyzed by the OmniSEC Triple Detection/Light Scattering GPC/SECModular GPCMax software. Samples were analyzed in quadruplicate.

2.2.2. Gel formation

Internal gelation of the Na alginate solution was promoted using a method previously described by Kuo and Ma [46] with minor modifications, described elsewhere [41]. Briefly, a Ca or Sr salt with limited solubility, in this case $CaCO_3$ or $SrCO_3$, was mixed with the alginate solution and used as a source of Ca or Sr ions respectively. The release of Ca or Sr into the solution was promoted by the generation of an acidic pH with Glucone-δ-lactone (GDL, Sigma), a slowly dissociating acid, which was also incorporated in the solution. Once released, Ca or Sr ions can participate in the interchain ionic binding between carboxyl groups (COO^-) of guluronic acids blocks in the polymer chain, giving rise to a crosslinked gel. The $CaCO_3$/GDL or $SrCO_3$/GDL ratio was set at 0.5 to yield a neutral pH value. All components were pre-equilibrated