Materials Science and Engineering C 33 (2013) 4203–4212

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Hydrogel/bioactive glass composites for bone regeneration applications: Synthesis and characterisation

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ARTICLE INFO

Article history:
Received 18 October 2012
Received in revised form 4 March 2013
Accepted 10 June 2013
Available online 24 June 2013

Keywords:
Hydrogels
Bioactive glasses
Mechanical properties
Biomineralisation

ABSTRACT

Due to the deficiencies of current commercially available biological bone grafts, alternative bone graft substitutes have come to the forefront of tissue engineering in recent times. The main challenge for scientists in manufacturing bone graft substitutes is to obtain a scaffold that has sufficient mechanical strength and bioactive properties to promote formation of new tissue. The ability to synthesise hydrogel based composite scaffolds using photopolymerisation has been demonstrated in this study. The prepared hydrogel based composites were characterised using techniques including Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), Energy-dispersive X-ray spectrometry (EDX), rheological studies and compression testing. In addition, gel fraction, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), porosity and swelling studies of the composites were carried out. It was found that these novel hydrogel bioglass composite formulations did not display the inherent brittleness that is typically associated with bioactive glass based bone graft materials and exhibited enhanced biomechanical properties compared to the polyethylene glycol hydrogel scaffolds along. Together, the combination of enhanced mechanical properties and the deposition of apatite on the surface of these hydrogel based composites make them an ideal candidate as bone graft substitutes in cancellous bone defects or low load bearing applications.

1. Introduction

Bone is a complex and highly specialised form of connective tissue with exceptional mechanical and biological properties [1]. Bone behaves as a dynamic tissue as it has the distinctive capability to regenerate and remodel through the action of osteoblasts, osteoclasts and osteocytes. However, in the case of critical size defects, the defect becomes too large for the aforementioned cells to repair the damaged tissue [2]. Bone defects remain a major problem in orthopaedic surgery where defects may arise from trauma [3], tumour resection [4–6] and osteomyelitis [7]. The treatments of choice for these types of defects are bone grafting procedures. Currently, the most common type is biological grafts and they include autografts, allografts and xenografts. However, each biological graft has its own limitations: autografts for example require an extra surgery and increase the risk of morbidity and may involve blood loss, sepsis and pain [8,9]. On the other hand, allografts (both freeze dried and fresh frozen) as well as xenografts carry histocompatibility antigens different from the host and therefore, increase risk of rejection [10]. They are also expensive and require stringent handling protocols.

Nevertheless, it has been estimated that 2.2 million bone grafting procedures are performed worldwide each year to stimulate bone healing [11]. The market for European bone grafts and bone cements was worth $692.1 million in 2009 and is expected to almost double to $1248.0 million by 2016 [12]. Due to the anticipated increase in the market size and the current issues with biological grafts, synthetic bone graft substitutes are expected to play a vital role in bone regeneration.

Hydrogels are 3-D networks formed from hydrophilic polymers which are crosslinked to form insoluble polymer matrices [13]. Hydrogels have excellent biological properties due to their ability to mimic extracellular matrix [14]. Their aqueous environment allows transportation of substances such as nutrients and by-products from cell metabolism. Their properties are reliant on type of crosslinking and crosslink density [15]. One particular material that has been comprehensively studied for tissue engineering applications is polyethylene glycol hydrogels (PEGs) [16–18]. PEG is biocompatible and has the ability to form in situ [19,20], however, PEG hydrogels generally lack the mechanical properties to replicate in vivo conditions for load bearing systems such as encountered in bone

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http://dx.doi.org/10.1016/j.msec.2013.06.013

regeneration applications [21]. This lack of mechanical strength frequently requires the utilisation of metal stabilisation implants to supplement the bones strength when patients ambulate. This additional surgery adds to the patient's morbidity and adds extra expense to an already costly procedure. These short-comings have limited their application to soft tissue repair [22–24]. Additionally, PEG hydrogels alone display poor mineralisation properties upon implantation, therefore restricting the ability of these hydrogels to promote formation of new bone through osteoconduction and osteoinduction [25].

Bioactive glasses were first discovered by Hench et al. in 1969 [26]. Bioactive glasses have the ability to form chemical bonds (i.e. apatite) on the surface layer of the scaffold to promote growth of new bone [27]. The advantage of bioactive glasses over other bioceramics, i.e. hydroxyapatite, is that they exhibit much faster formation of apatite on the surface of the scaffolds in physiological fluid and in vivo. Studies have also shown that bioactive glasses can improve cellular adhesion, increase osteoblast proliferation and differentiation [28]. Phosphate based glasses however have drawbacks in terms of particle migration, settling and handleability. Also, they have poor standalone flexibility and fatigue strength and are brittle in nature and therefore, are not suitable or reliable as load bearing bone graft substitutes [29].

As described above, both hydrogels and bioceramics show a number of deficiencies in terms of the properties required as bone graft materials. To overcome these limitations, a new generation of polymer and bioceramic composites have been developed [30–34]. The mechanical properties of these composites can be modified to produce a synthetic bone graft substitute that exhibits toughness and plasticity from the polymeric phase and the compressive strength and mineralisation properties typical of the bioceramics. From the literature it was found that a variety of methods of loading bioceramics in polymeric systems have been developed for bone regeneration to date. These include twin-screw extrusion [35], injection moulding [30], gas forming and particulate leaching [36]. To our knowledge, no previous work has investigated photopolymerised hydrogels with such bioactive glass particles incorporated in order to promote compressive strength and bioactive properties of the hydrogel based composites. In this study, the effect of bioglass loading, polymer molecular weight and concentration was evaluated with the goal of assessing the feasibility of using photopolymerisable hydrogel based composites for bone regeneration applications.

Recently it has been noted that in the field of tissue engineering scientists should seek to develop new materials that can establish key interactions with the biological host to trigger regenerative and reparative processes [37], it is in this regard that Sr and Zn doped bioglasses have emerged as materials of particular interest, and there is increasing evidence that these ions may control bone formation in both healthy individuals and those with metabolically compromised tissues [38]. Both Sr and Zn have significant therapeutic potential, in the case of the former enhanced bone remodelling it has been clinically demonstrated and linked to the duality of roles that Sr can play in bone remodelling (enhancing osteoblastic proliferation and decreasing osteoclastic turnover) [39]. In respect of Zn, this agent provides for enhanced antibacterial efficacy and is linked with improved bone quality. It has been shown that Sr/Zn based silicate glasses perform with appropriate responses in metabolically compromised tissue [40], and in this regard they continue to be of significant interest in bone tissue engineering.

2. Materials and methods

2.1. Materials

The macromolecular monomers, poly(ethylene glycol) dimethacrylate (*Mw*) 600–1000 were obtained from PolySciences Inc. The photoinitiator utilised was 4-(2-hydroxyethoxy)phenyl-(2-hydroxy-2-propyl)ketone (Irgacure® 2959) supplied by Ciba Specialty Chemicals. All materials were used as received.

2.2. Bioglass synthesis

One glass was synthesised; $0.2SrO/0.2Na_2O/0.1CaO/0.1ZnO/0.4SiO_2$ (mol. fraction). Appropriate amounts of analytical grade calcium carbonate, strontium carbonate, zinc oxide and silicon dioxide (Sigma Aldrich, Canada), were weighed out in a plastic tub and homogeneously mixed in a NalgeneR plastic container (Sigma Aldrich, Canada) for 1 h. Each batch of powder was placed in platinum crucibles (50 mL), then fired (1480 °C, 1 h) using a Bench-Top High Temperature Muffle Furnace (EQ-KSL, MTI Corporation, USA) and shock quenched into distilled water. The resulting glass frit was dried in an oven (120 °C, 1 day), pulverised in an agate planetary mill (Pulverisette 7; Laval Labs Inc., Canada) and sieved to retrieve particulates of <45 μm. Glass powders were subsequently stored in dry desiccators for subsequent evaluation. The glass composition in this study is the result of optimisation of bone graft performance from preceding studies [41,42].

2.3. Composite hydrogel formulation

Highly crosslinked hydrogels have been prepared previously through photopolymerisation [21,43]. Hydrogels were photopolymerised using a UV curing system (Dr. Gröbel UV-Electronik GmbH). The irradiation chamber utilised 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. The prepolymerised mixtures were prepared by combining desired amounts of macromolecular monomer (PEGDMA) with a specified amount of distilled water, bioactive glass powder and 0.1 wt.% photoinitiator. The batches were placed in a 50 mL beaker, mixed using a magnetic stirrer for 1 h, and finally sonicated for 30 min until a homogenous mixture was achieved. The solutions were pipetted into silicone moulds and photopolymerisation was carried out for 10 min, after which time gelation had occurred. The compositions of the control and hydrogel based composites are listed in Table 1.

2.4. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed to evaluate the effective weight percentages of bioglass in the composite scaffold. Tests were conducted using a Perkin Elmer TGA 7 Thermogravimetric Analyzer, coupled with a Perkin Elmer Thermal Analysis Controller TAC7/DX under nitrogen atmosphere. The tests were run from 30 °C to 600 °C, at a heating rate of 10 °C/min.

2.5. X-ray diffraction

Phase analysis was conducted using X-ray diffraction (XRD) to detect phase composition and crystallinity of bioactive glass powder and composite materials. Tests were carried out at room temperature

Table 1
Formulated composition of control and composites prior to photopolymerisation.

Composite code	PEGDMA 1000 (wt.%)	PEGDMA 600 (wt.%)	Bioglass loading (wt.%)	Distilled water (wt.%)
A0	75		0	25
A5	75		5	25
A20	75		20	25
B0	50		0	50
B5	50		5	50
B20	50		20	50
C0		75	0	25
C5		75	5	25
C20		75	20	25