Materials Science and Engineering C 77 (2017) 513–520

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Design and characterization of biodegradable macroporous hybrid inorganic-organic polymer for orthopedic applications

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ARTICLE INFO

Article history:
Received 16 July 2016
Received in revised form 30 December 2016
Accepted 20 March 2017
Available online 21 March 2017

Keywords:
Hybrid
Biodegradable
Macroporous
Inorganic-organic polymer

ABSTRACT

We have engineered hybrid polymer products based on a hybrid inorganic-organic comacromer consisting of hydroxyapatite (HA), carboxyl terminated polypropylene fumarate (CTPPF), PEG300 and ascorbic acid (AA) as a bone graft material. The integration and the spatial distribution of HA in the polymer backbone were facilitated by silanisation and 1-ethyl-3-(−3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) coupling technique. These comacromers and crosslinked polymer products were characterized by Fourier transform infrared spectroscopy (FTIR), Nuclear magnetic resonance (NMR), Scanning electron microscopy (SEM) and Raman mapping techniques. SEM and EDAX analysis substantiate high *invitro* bioactivity of the polymer products. SEM studies depict a distinct macroporous structure with pore size of 50 to 300 μm. These crosslinked hybrid products demonstrated no significant difference in compressive moduli after 4 weeks immersion in SBF. In particular, the compressive moduli were found to be comparable with that of trabecular bone. We suggest that the formation of an apatite layer on the surface of the composites deter initial degradation leading to better mechanical stability. As expected, the polymer products displayed negligible degradation in SBF during the first 4 weeks which increased to a maximum of 25% by the end of the 8 weeks time period. In addition these crosslinked products which are hydrophilic exhibit favorable albumin adsorption, cell viability, HOS cell adhesion and exemplary compatibility. Cumulatively, the results deduced in the present study suggest that these hybrid products have potential as a bone graft material.

1. Introduction

Bioceramics such as HA, calcium deficient hydroxyapatite (CDHA) and tri calcium phosphate (TCP) are being directly utilized for implant coatings and clinical treatment of bone defects [1–3]. In particular, hydroxyapatite (HA) manifests commendable properties including biocompatibility, osteoconductivity and bioactivity [4]. This bioactivity promotes the formation of a hydroxyl carbonate layer on its surface, leading to a chemical bond, and supports osseointegration which augments implant efficiency. However, they are limited by an inherent brittleness that makes it especially susceptible to fracture [5]. This mechanical incompetence has been addressed by researchers by developing ceramic/polymer composites [6,7]. These designed composites exhibit properties such as bioactivity and osteoconductivity of ceramics alongside formability and flexibility of polymers. For example, HA mechanically mixed with polymers like poly(glycolic acid) and poly(lactic acid) enhance mechanical properties of these composites, that play a considerable role in bone tissue engineering [8,9]. The bioactivity of these engineered composites also depends upon the shape, size and concentration of filler employed. In addition the use of fillers in the nano regime enhances interfacial bonding leading to augmented mechanical properties [10].

Since bone is primarily a collagen/nano HA mineral composite [11], attempts have been made to disperse HA in polymer and copolymer matrices to mimic the native structure of bone. Sharifi et al. [12] investigated a biodegradable nanocomposite based on poly(hexamethylene carbonate fumarate) and HA. Qui et al. [13] reported a composite of poly(1,8-octanediol-citrate) and HA, which elicited no inflammatory response *in vivo*. Gyawali et al. [14] developed a citric acid based composite for cell delivery and orthopedic applications. Cui et al. [15] prepared a HA/collagen/PLA composite and evaluated it's *in vivo* potential in a rabbit segmental defect model. More recently, Jayabalan et al. [16,17] has prepared and evaluated poly (propylene fumarate-*co*-ethylene glycol) and covalently bonded HA-poly (propylene fumarate-*co*-citrate-co-PEG) for application as scaffolds for critical bone defects.

Many critical requirements need to be addressed for the development of a successful bone grafting biodegradable composite. These include adequate load bearing capability with biomechanical stability, optimal porosity to permit bone in growth and vascularisation, optimal biocompatibility and osteointegrative properties [18–20]. In addition surface properties of the composites influence a series of events such

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http://dx.doi.org/10.1016/j.msec.2017.03.171
0928-4931/

as protein adsorption, proliferation of cells and ultimately bone tissue deposition. Therefore numerous reports list ongoing efforts to modify surfaces of implant materials for enhanced tissue response [21–27]. Favorable integration of a bone graft with tissue also reckons on surface as well as bulk characteristics which augment bone mineralization, required biomaterial stability and osteointegration. It is often difficult to achieve these characteristics in biodegradable polymer filler based composites. In this work we have attempted to address these drawbacks and have explored the design of hybrid inorganic-organic comacromer based on carboxyl terminated polypropylene fumarate (CTPPF), PEG300, ascorbic acid (AA) and HA as a bone graft material for successful bone regeneration.

2. Materials and methods

2.1. Materials

PEG 300, 1,2 propylene glycol, maleic anhydride, AA, aminopropyltriethoxysilane (APTS), EDC, N-vinyl pyrrolidone (NVP), dibenzoyl peroxide, *N*, *N*-dimethylaniline, morpholine and sodium acetate were procured from Merck Chemicals USA. Disodium hydrogen phosphate, calcium chloride, trisodium citrate (TSC), bovine serum albumin and fibrinogen employed in protein adsorption studies were obtained from Sigma Chemicals respectively. All chemicals listed were utilized as available unless otherwise specified.

2.2. Synthesis of poly (PEG-co-propylene fumarate-co-ascorbate)

CTPPF was initially synthesized by following previously established protocols [28]. Briefly Maleic anhydride and 1,2-propanediol were mixed and refluxed at 148 °C under nitrogen atmosphere, followed by vacuum condensation at 185–190 °C for 15 min. Sodium acetate and morpholine were added to catalyze the polymerization and isomerisation reaction. The reaction product obtained was subsequently purified by dissolving in acetone and washing with 25% aqueous methanol. The CTPPF resin was then reprecipitated in ether, filtered, and dried using a rotary evaporator.

The synthesis of poly (PEG -*co*-propylene fumarate-co-ascorbate) [coded as PFA] resin mainly involved a one pot poly condensation reaction. AA, PEG 300 and CTPPF at a molar ratio of 1:3:5 were melted and refluxed at 160 °C for an hour under nitrogen atmosphere. The mixture was then vacuum condensed at 180–190 °C for 20 min to remove water to obtain a yellow colored solution. This solution was then purified by dissolving in acetone and reprecipitating in ether to obtain the purified comacromer resin. The molecular weight of the PFA resin was determined using a Waters HPLC system with Styragel-HR-5E/4E/2/0.5 columns in series with the mobile phase tetrahydrofuran.

2.3. Preparation of HA nanoparticles

The needle shaped HA nanoparticles were synthesized by a facile co-precipitation technique. Calcium chloride (1.84 g) and tri sodium citrate (0.9 g) was dissolved in 120 mL of distilled water. The solution was stirred for 15 min before 100 mL disodium hydrogen phosphate (2.23 g) solution was added drop wise from a burette. The reaction was allowed to proceed under stirring for 16 h. The resulting suspension obtained was washed with distilled water, centrifuged, and lyophilized. The nanoparticles obtained were collected and stored for further use.

2.4. Synthesis of covalently bonded HA- poly (PEG-co-propylene fumarate-co-ascorbate)

HA nanoparticles in suspension were subsequently treated with APTS and coupled to AA employing EDC chemistry by following previously established protocols [29]. Briefly, 50 mg of HA nanoparticles was dispersed in 50 mL of ethanol. 3 mL of APTS and 2 mL of water was subsequently added followed by stirring for 24 h. The HA-APTS nanoparticles obtained were then coupled with AA using EDC chemistry. 25 mg of AA was dissolved in water. EDC (15 mg) was then added into the solution and reaction allowed to stir overnight to activate their COOH groups. Subsequently 50 mg of HA-APTS nanoparticles was added to this solution. The final product was lyophilized to obtain covalently bonded HA-AA. The HA content in the final covalently bonded HA-AA was varied to prepare two different batches, containing 15 wt% and 20 wt% with respect to the total weight of the AA. The covalently bonded HA-A-A containing 15 wt% of HA and 20 wt% of HA with respect to the total weight of the AA has been abbreviated as 15A and 20A respectively.

Covalently bonded HA- poly (PEG-*co*-propylene fumarate-co-ascorbate) was then prepared as hybrid comacromers using covalently bonded HA-AA, PEG 300 and CTPPF at a molar ratio of 1:3:5 and refluxed at 160 °C for an hour under nitrogen atmosphere. The mixture was then vacuum condensed at 180 °C for 20 min to remove water to obtain a yellow colored solution. The hybrid comacromers prepared with 15A and 20A have been coded as 15PFA and 20PFA respectively.

2.5. Preparation of crosslinked polymer products

The comacromer PFA and hybrid comacromers 15PFA and 20PFA were then crosslinked with N-vinyl pyrrolidone (NVP) to obtain crosslinked polymer products. The comacromers and NVP were mixed at a mass ratio of 1:0.5. The crosslinking was triggered by adding 2% *w*/w dibenzoyl peroxide and 0.2% *w*/w *N*, *N*-dimethylaniline as initiator and activator respectively. The mixture was then stirred and transferred to mold and cured at ambient condition. The setting temperature and time were measured accurately for all the prepared polymer products. The crosslinked comacromer PFA has been coded as NPFA and the crosslinked hybrid comacromers prepared with 15PFA and 20PFA have been coded as, 15NPFA and 20NPFA respectively.

2.6. Physiochemical characterization, mechanical evaluation and monitoring of degradation profile of crosslinked polymer products

The functional moieties present in the comacromer PFA, hybrid comacromers15PFA and 20PFA resins and cross linked polymer products 15NPFA and 20NPFA were systematically assessed in a fourier transform infrared (FTIR) spectrometer (Jasco, FT/IR-4200, USA). The proton NMR and C13 NMR spectra of the PFA resin were acquired using a NMR spectrometer (AV 400, Bruker, India). The distribution and integration of HA in the hybrid comacromer was assessed by Raman spectral imaging (532 nm laser line, 40 mW laser power) using Raman spectroscopy (Witec-Alpha 300, USA). The contact angle of the polymer products NPFA, 15NPFA and 20NPFA were measured using Wilhelmy method (KSV Sigma 701 tensiometer). SEM imaging of the crosslinked polymer products was carried out using Environmental scanning electron microscopy (ESEM, FEI, Quanta 200, USA). The density of the polymer products was measured by geometrical weight/volume evaluation and the volume porosity was consequently calculated. The open porosity was evaluated by Hg-porosimetry (Quantachrome. Auto scan-92 porosimetry, USA).

Compressive testing were performed for $n = 5$ samples using a material testing system using the universal automated mechanical test analyzer (Instron, 3345 Bioplus, India) at room temperature with a 5 KN load cell at a crosshead speed of 5 mm/min. Cylindrical specimens measuring 6 mm in diameter and 12 mm in height were compressed along their longitudinal axis until failure. The compressive stress, load and Young modulus of the crosslinked polymer products were determined using Instron's proprietary Bluehill 3 software. Similarly the change in compressive stress, load and Young modulus after aging in simulated body fluid (SBF) solution for four weeks has also been evaluated. Shore-D hardness of these crosslinked polymer products was determined using a durometer using cylindrical samples having 6 mm diameter.