Materials Science & Engineering C xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

Introducing an attractive method for total biomimetic creation of a synthetic biodegradable bioactive bone scaffold based on statistical experimental design

Sara Shahbazi[a], Ali Zamanian[a,*], Mohammad Pazouki[b], Yaser Jafari[c]

[a] Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center, Karaj, Alborz, Iran
[b] Department of Energy, Materials and Energy Research Center, Karaj, Alborz, Iran
[c] Department of Analytical Chemistry, Faculty of Chemistry, University of Kashan, Kashan, Iran

ARTICLE INFO

Keywords:
Total biomimetic
Biodegradable
Bioactive
Bone scaffold
Experimental design

ABSTRACT

A new total biomimetic technique based on both the water uptake and degradation processes is introduced in this study to provide an interesting procedure to fabricate a bioactive and biodegradable synthetic scaffold, which has a good mechanical and structural properties. The optimization of effective parameters to scaffold fabrication was done by response surface methodology/central composite design (CCD). With this method, a synthetic scaffold was fabricated which has a uniform and open-interconnected porous structure with the largest pore size of 100–200 μm. The obtained compressive ultimate strength of ~35 MPa and compression modulus of 58 MPa are similar to some of the trabecular bone. The pore morphology, size, and distribution of the scaffold were characterized using a scanning electron microscope and mercury porosimeter. Fourier transform infrared spectroscopy, EDAX and X-ray diffraction analyses were used to determine the chemical composition, Ca/P element ratio of mineralized microparticles, and the crystal structure of the scaffolds, respectively. The optimum biodegradable synthetic scaffold based on its raw materials of polypropylene fumarate, hydroxyethyl methacrylate and nano bioactive glass (PPF/HEMA/nanoBG) as 70/30 wt/wt%, 20 wt%, and 1.5 wt/wt% (PHB.732/1.5) with desired porosity, pore size, and geometry were created by 4 weeks immersion in SBF. This scaffold showed considerable biocompatibility in the ranging from 86 to 101% for the indirect and direct contact tests and good osteoblast cell attachment when studied with the bone-like cells.

1. Introduction

The design of a successful bone porous scaffold with a high similarity to the natural bone, it needs to understand the bone biology and structure. Natural bone is an open cell composite material containing the extracellular matrix (ECM) proteins, osteogenic cells (i.e., osteoblasts and osteocytes), growth factors, mineralized calcium in the form of hydroxyapatite, and a vascular system. Calcium reservoir in the bone is mostly (85%) in the hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) crystal form. In addition to, the self-assembled collagens (97% collagen type I) form 90% of the total weight of ECM proteins. The bone strength and stiffness is due to mineral crystals and assembled collagen fibers being usually oriented in the particular direction; In addition, to provide stiffness, the hydroxyapatite crystals also develop osteoconductivity properties. The collagen fibers enhance fracture toughness and form integrated structure to achieve a high porosity and a large surface area which is necessary for cell viability and proliferation [1].

Engineering bones typically require an artificial highly porous scaffold that can support the promoting cell behaviors (such as cell attachment, spreading, migration and proliferation) and new bone tissue formation in three dimensions (3D). An ideal scaffold should be biocompatible, biodegradable, osteoconductive and possess proper mechanical and physical properties [2].

The various materials including metals, ceramics, polymers (synthetic and natural) and their combinations have been applied to the fabrication of bone scaffolds. Although metals are a good choice for medical implants, they are non-degradable in a biological environment, and concern about their corrosion always exist [3]. Also, applications of ceramics in tissue engineering are much limited to the difficulty of their processability, particularly with porous structure fabrication. Nevertheless, ceramic materials are attractive for bone tissue engineering applications due to their excellent mechanical properties and osteoconductivity creation using some of them such as three calcium phosphates (TCPs) [4]. In contrast, polymers possess the high flexibility to

* Corresponding author.
E-mail address: a-zamanian@merc.ac.ir (A. Zamanian).

https://doi.org/10.1016/j.msec.2017.12.033
Received 19 May 2017; Received in revised form 14 October 2017; Accepted 28 December 2017

scaffold fabrication due to their various composition and structure which can be modified to the specific requires. Biodegradability of the polymer is an attractive property that can be controlled through molecular design of polymers. Therefore, polymeric materials have been widely used and studied for bone tissue engineering applications. However, they are involved with low mechanical properties and issues relating to biocompatibility [5].

Recently, unsaturated, biodegradable, and biocompatible polyesters have been applied as promising materials for bone tissue engineering. One of the best material is poly (propylene fumarate) (PPF), a linear, unsaturated polyester that can be in-situ cross-linked and degraded over time. Ester linkage property and existence of double bonds in the main chain of PPF is particular it's- advantages which allow the polymer to be hydrolyzed in the water media. Also, this double bonds can be formed a self-crosslinked network or created a network with other unsaturated reagents [6–8]. The body can absorb propylene glycol (PG) and fumaric acid (FA), both compounds produced by hydrolysis producer, without showing cytotoxicity. Therefore, PPF has a potential to the in-situ fabrication of a three-dimensional scaffold. Using PPF in tissue engineering applications has been extensively investigated [9–12].

Unsaturated monomers, such as methyl methacrylate (MMA), N-vinylpyrrolidone (NVP), and diethyl fumarate (DEF), have often added to PPF resins to promote physical and mechanical properties, accelerate the crosslinking reaction and control PPF network degradation process [13–16]. The 2-hydroxyethyl methacrylate monomer (HEMA) is an unsaturated (containing C═C group) and sufficiently soluble monomer in water that can usually be crosslinked during bulk-redox polymerization frequently using azobisisobutyronitrile (AIBN) or the pair of benzoyl peroxide (BPO) with a substituted amine [17]. Poly (hydroxyethyl methacrylate) (pHEMA) is a widely used and researched polymer. Every repeated unit of pHEMA has one of a hydroxyl group (−OH) which makes it highly hydrophilic and biocompatible material for applying in different biomedical devices like, soft contact lenses, artificial skin, drug delivery systems, bone substitute, and scaffolds for tissue engineering [18–23]. It is reported that pHEMA is a biocompatible but not biodegradable material. However, incorporating HEMA monomers with other agents contain ester linkages, such as ethylene glycol dimethacrylate, usually can provide degradation partially. Several attempts have been made to use pHEMA in combination with hydrolytically biodegradable materials such as poly (ε-caprolactone) and L-cystine. It is conferred that the pending species of the HEMA-biodegradable oligomers have good biocompatibility which can be excreted urinary by the kidneys [24–28]. Thus, HEMA unsaturated monomers can be applied as a biocompatible and hydrophilic agent for crosslinking the unsaturated polyesters such as PPF in the bone applications [12].

Due to the cell-scaffold and protein-scaffold interactions done on the pore surfaces of the scaffold, attempts to grow nanoparticles of bone-like apatite on pre-fabricated porous polymeric scaffolds in a simulated body fluid (SBF), have frequently been done. In this method, the wall surfaces of internal pores have been efficiently modified with bone-like apatite with any significant change in the bulk structure and scaffold properties [29–32]. The created biomimetic apatite in an SBF is partially carbonated HA (HCA). The HCA crystals are more similar to the natural bone apatite (calcium deficient Ca/P ~ 1.5) than the stoichiometric HA crystals (Ca/P ~ 1.67). The HCA should degrade faster than the stoichiometric HA crystals and therefore can be a better component for the scaffold fabrication and new bone tissue formation [33]. To further mimic a macroporous scaffold, the combination of nano-fibrous organic component (such as collagen, alginate) with the HCA has been mostly investigated. The soaking, such scaffolds in an SBF at a reasonable time results in creating a uniform and dense layer of nano-HCA which covered the wall surfaces of internal pores [34–36].

Bioactive glasses (BGs) are the biocompatible bioceramics that make a strong interfacial bonding with the bone. Because the good biodegradability, bioactivity, and osteoconductivity [37–39], BGs have been used in the clinical assays as the bone repair materials for more than ten years [40,41]. BGs bioactivity is due to HCA formation on their surfaces. The rate of tissue bonding depends on the rate of HCA formation, which refers to the interactions between the bioimplant and the surrounding physiological fluids [42]. Recent several studies showed that increasing specific surface area of BGs will significantly accelerate the HCA mineralization procedure since the ion exchange occurs at the surface of bioactive glasses [39]. It has been shown that the biomaterials in nano-scale could enhance the interaction between biomaterials and cells [43,44]. The stiff bioactive nanocomposites can be produced through the incorporation of nanoBG particles with biodegradable polymers. The most commonly combined polymers with nanoBGs are polylactide (PLA), polyglycolic (PGA) and their copolymers (PLGA) (PLGA), which use for clinical applications [45].

Recently, chemometrics methods and experimental designs used to select the optimum parameters in chemical synthesis formulations. Due to the simultaneous evaluation of effective parameters on properties of the synthetic material, this approach is more accurate, shorter and cheaper route than the classical optimization. Chemometrics methods are usually done using an analytical software at the initial (designs a statistical matrix) and subsequently followed by experimental assays (finding the responses to optimization parameters).

Both HEMA water absorbability and PPF hydrolytic degradability in an aqueous solution (such as SBF) at the reasonable soaking time results in creating a porous structure. Controlling the factors affecting the construction properties can lead to form an optimum porous structure without using any scaffold producing techniques such as salt leaching, electrospinning, 3D printing, etc. [46]. Also, incorporation of nanoBG particles with the PPF/HEMA polymer matrix not only provides proper mechanical properties but also can cause to apatite nucleation on the wall surfaces of internally created pores through immersion of PPF/HEMA/nanoBG composites in SBF [12]. Therefore, soaking such composites in SBF at the reasonable time could result in produce a synthetic biodegradable scaffold with interconnected open-pores which their wall surfaces are significantly covered with HCA. In this way, not only the coating of the pores surfaces will be formed through immersion PPF/HEMA/nanoBG in SBF but also the internal interconnected pores of the scaffold created. Therefore, the hydroxyapatite type and porosity structure (round interconnected pores) of the created scaffolds will be more similar to natural bone structure.

Recently, S. Prem Victor et al. have been tried to develop a porous material for successful bone regeneration. In this work, a hydrophilic biocompatible polymer (polyethylene glycol) and an attractive copolymer (propylene fumarate-*co*-ascorbate) as the degradable polyester with wide applications in bone tissue engineering have been used to matrix preparation. Also, hydroxyapatite has incorporated into the copolymer matrix for enhancing mineralization and osseointegration. However, no experimental design work for evaluating simultaneously effective parameters on porosity creation has been done [47].

The novelty of this paper is the fabrication of a bioactive and biodegradable synthetic bone scaffold based on PPF/HEMA/nanoBG through a total biomimetic technique. In this approach, the HEMA water absorbability and PPF hydrolytic degradability in SBF solution at 4 weeks soaking time have been used to create porosity of the scaffold. Also, nanoBG has been added to the PPF/HEMA matrix for HCA formation on the wall surfaces of internal pores in the final scaffold. To fabricate a utilizable scaffold, it is necessary to optimize the effective factors correctly for creating the desired properties. The relationship between functional parameters of the raw material composition including PPF/HEMA ratio, the amount of nanoBG and initiator (BPO + DMA)/PPF + HEMA ratio has been done by response surface methodology/central composite design (CCD). The main objective of CCD is simultaneously optimization levels of the variables to get the best response for scaffold creation in SBF solution with the good porosity and bioactivity. Finally, the best-fabricated scaffold has been characterized with additional tests to evaluate its properties as bone