Materials and Design 109 (2016) 434–442

Contents lists available at ScienceDirect

Materials and Design

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

Osteogenic differentiation of mesenchymal stem cells on a poly (octanediol citrate)/bioglass composite scaffold *in vitro*

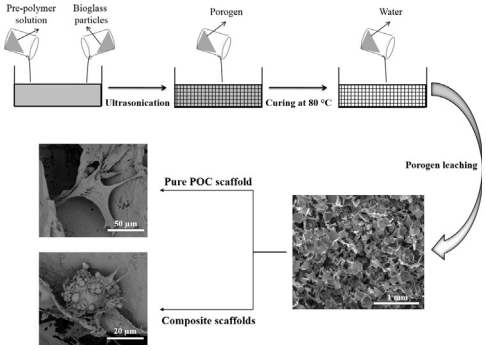
Ehsan Zeimaran [a], Saktiswaren Mohan [b], Sara Pourshahrestani [a], Belinda Pingguan-Murphy [a], Nahrizul Adib Kadri [a,*], Malliga Raman Murali [b], Hanumantha Rao Balaji Raghavendran [b], Khairunnisa Hasikin [a], Tunku Kamarul [b], Mark R. Towler [a,c,**]

[a] Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Tissue Engineering Group (TEG), National Orthopaedic Centre of Excellence in Research and Learning (NOCERAL), Department of Orthopaedic Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[c] Department of Mechanical & Industrial Engineering, Ryerson University, Toronto M5B 2K3, ON, Canada

HIGHLIGHTS

- The responses of mesenchymal stem cells to a poly (octanediol citrate)-bioglass composite scaffold were evaluated *in vitro*.
- Mesenchymal stem cells appeared to flatten on the neat scaffold while maintaining rounded shape on the composite scaffolds.
- Composite scaffolds showed enhanced osteogenic differentiation compared to the unfilled poly (octanediol citrate) scaffold.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 14 July 2016
Accepted 18 July 2016
Available online 20 July 2016

Keywords:
Composite scaffold
Bioactive glass
Human bone marrow mesenchymal stem cells
Osteogenic differentiation
Bone tissue engineering

ABSTRACT

This study investigated the effect of composite scaffolds composed of poly (octanediol citrate) (POC) and a bioactive glass (composition, 48%SiO_2-12%CaO-32%ZnO-8%Ga_2O_3) on the growth and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). All the scaffolds, regardless of the amount of bioglass incorporation, were able to support the growth of hBMSCs and guide their osteogenic differentiation without osteogenic media stimulation. The expression of bone-associated genes (runt-related transcription factor 2, type I collagen, bone morphogenetic protein 2, osteonectin and osteocalcin) was significantly increased by a culture time of up to 2 weeks, particularly for the composite scaffold loaded with 10% bioactive glass. The composite scaffolds significantly stimulated alkaline phosphatase (ALP) activity compared to the pure POC scaffold. Cellular mineralization of the secreted extracellular matrix illustrated a higher calcium level on the composites than on the pure POC and increased with culture time. These results suggest that composite scaffolds of POC and a bioactive glass can provide favourable conditions for osteogenic differentiation of hBMSCs and can potentially be used to induce bone healing and regeneration.

* Corresponding author.
** Correspondence to: M.R. Towler, Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail addresses: nahrizuladib@um.edu.my (N.A. Kadri), mtowler@Ryerson.ca (M.R. Towler).

http://dx.doi.org/10.1016/j.matdes.2016.07.096
0264-1275/

1. Introduction

Bone is one of the most commonly transplanted tissues of the body with over 2 million grafting procedures annually worldwide [1]. However, skeletal defects caused by tumors or traumatic bone loss present the need for complex treatment strategies [2], often requiring the use of autografts, allografts, or metallic and ceramic implants, each of which has its own disadvantages such as donor site morbidity, disease transmission, and mismatch of mechanical properties with the native bone [3]. Recently, tissue engineering has emerged as an alternative approach to create *de novo* tissue by growing cells on 3D scaffolding [4]. An ideal bone graft substitute or tissue scaffold should provide the necessary support for the cells to attach, proliferate and facilitate ingrowth [5]. In parallel with tissue formation, an ideal scaffold should degrade and create open space for new bone formation until regeneration is achieved. Accordingly, biodegradable scaffolds have the potential to reduce the number of surgeries, since there is no need for an additional operation to remove the implant.

A crucial factor identified in the failure of many tissue-engineered constructs is inadequate tissue regeneration around the biomaterial immediately after implantation [6]. Since the interaction of cells with biomaterials is a fundamental parameter in the evaluation of a scaffold, a number of recent contributions to the literature have focused on the design and development of biomaterial structures that facilitate favourable interactions and augment tissue regeneration. *In vivo* bone formation involves osteogenic reparative cells originating from mesenchymal stem cells (MSCs) in bone marrow, the presence of a regeneration template, and the provision of regulatory signals [7]. Human mesenchymal stem cells (hMSCs) isolated from bone marrow serve as an ideal cell source for a wide variety of cell-therapy concepts due to their self-renewal ability, multilineage differentiation potential and immunomodulatory properties [8]. They are also capable of secretion of biomolecules such as cytokines, chemokines, growth factors, and extra cellular matrix (ECM) molecules, in a paracrine or even autocrine manner, which influence the surrounding environment to promote angiogenesis, reduce inflammation, and enhance tissue repair [9]. For the application of hMSCs to bone regeneration, a better understanding of the interactions occurring between hMSCs and biological scaffold material is essential because the interaction at the cell-biomaterial interface plays a major role in the bonding of implant materials to bone.

The choice of scaffold is a critical factor in the development of competent tissues with the desired characteristics [10]. Synthetic biomaterials are now being designed with a combination of both resorbable and bioactive characteristics to stimulate regeneration of living tissue. So far, there is no single biomaterial that is able to satisfy all the requirements for an ideal bone graft driving tissue engineers to create 3D scaffolds made up bioceramic and polymeric materials to facilitate normal bone growth.

Poly (octanediol citrate) (POC) has been investigated as such a scaffold due to its biomimetic viscoelastic properties, linear degradation profile and non-toxic degradation products. The mechanical properties and biodegradation rate of POC can be controlled by altering curing conditions (time and temperature) and the initial monomer molar ratio to mimic the pliancy of certain soft tissues such as blood vessels, urinary bladder smooth muscle and myocardium [11]. POC appears to have good compatibility with a number of cells, including articular chondrocytes, endothelial cells, myoblasts and osteoblasts, without requiring any additional treatment [12–15]. However, poor mechanical properties have retarded its use in load bearing applications driving attempts to manipulate the physicochemical properties of POC by synthesis and fabrication of co-polymers and composites suitable for hard tissue engineering [16–18]. POCs low stiffness makes it a suitable material for accepting a large amount of fillers without the detrimental effect of stress shielding [19]. Several studies have been conducted to develop composites of POC with ceramics such as hydroxyapatite (HA), calcium silicate and bioactive glass in order to improve both mechanical properties and the osteoinductivity [17,19,20]. It has been shown that tissue ingrowth into POC-HA is increased compared to ingrowth into poly (L-lactic acid), which highlights an additional benefit of these novel biomaterials. HA exhibits a low dissolution rate, whereas bioactive glasses are more soluble and their degradation products are released into the surrounding environment, potentially inducing bioactivity, depending upon what constitutes those degradation products [21].

Bioactive silicate glasses have achieved great success in clinical applications due to their well-known osteoconductive and osteoinductive properties [21]. For example, ionic dissolution products of bioglasses can up-regulate the expression of genes of osteoblastic cells, which in turn control osteogenesis and promote bone formation [22]. Bioglasses can bond to both hard and soft tissues through rapid formation of hydroxyl carbonate-apatite on the glass surface upon implantation, which promotes cell migration and differentiation. Bioactive glasses can be doped with therapeutic elements to modulate the desired tissue responses. For instance, the release of magnesium or potassium ions can increase bioactivity, silver, zinc (Zn) or gallium (Ga) ions can impart antibacterial properties, and strontium ions can enhance bone cell responses [23]. Although there have been many studies of the interaction of composite scaffolds with MSCs, to our knowledge there have been no studies of stem cell response to composites of POC and a bioactive glass.

Recently, we have shown that a POC scaffold loaded by a bioactive glass containing Zn^{2+} and Ga^{3+} can stimulate collagen type I and III secretion in human osteoblast-like cells as well as imparting effective antibacterial activity against *E. coli* and *S. aureus* bacteria [16,24]. In the present study, a range of composite scaffolds comprising POC/bioglass were cultured with hBMSC to observe the possible influence of the bioglass phase on *in vitro* osteogenic differentiation potential.

2. Materials and methods

2.1. Fabrication and characterization of composite scaffold

Synthesis and characterization of POC pre-polymer and fabrication of POC-bioactive glass ($0.48SiO_2$-0.12CaO-0.32ZnO-$0.08Ga_2O_3$ molar fraction) composite scaffolds was carried out as previously reported [16]. In brief, POC pre-polymer was synthesized successfully by the polycondensation reaction of high purity citric acid and 1,8-octanediol (1:1 mole ratio), then dissolved in 1,4-dioxane and mixed with various amount of bioactive glass (≤45 μm) to obtain composites with 10, 20 and 30 wt% bioactive glass, named hereafter POC-10%BG, POC-20%BG and POC-30%BG respectively. After that the solutions were sonicated for 30 mins followed by the addition of 90 wt% of salt porogen (200–300 μm). The resulting materials were post-polymerized at 80 °C for 7 days. Salt in the resulting composites was washed for 4 days in distilled water and subsequently the porous scaffolds were freeze-dried. Disk-shaped scaffolds (6 mm diameter × 3 mm thickness) were punched out for the subsequent experiments. The scaffold morphology was analyzed by a field emission scanning electron microscopy (FESEM: Quanta™ 250 FEG—FEI, USA), at 20 kV. The scaffolds were gold-coated using a 150 rotary-pumped sputter coater (Quorum Technologies).

2.2. Cell culture and seeding

Using standard laboratory protocols, hBMSCs were isolated (ethical approval for human bone marrow collection was obtained from the medical ethics committee of University of Malaya Medical Centre; MECID.NO: 201412-865). The cells were cultured in a medium (Invitrogen, Carlsbad, CA, USA) supplemented with 15% fetal bovine serum (FBS; Invitrogen), 100 U/ml penicillin (Sigma-Aldrich, USA), and 100 mg/ml streptomycin (Sigma-Aldrich) in tissue culture flasks at 37 °C under a humidified atmosphere of 5% CO_2·When the cells