Acta Biomaterialia 62 (2017) 352–361

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Sol gel-derived hydroxyapatite films over porous calcium polyphosphate substrates for improved tissue engineering of osteochondral-like constructs

Whitaik David Lee [a], Rahul Gawri [a,f], Robert M. Pilliar [a,b], William L. Stanford [a,c,d,e], Rita A. Kandel [a,f,*]

[a] Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College St., Toronto, Ontario M5S 3G9, Canada
[b] Faculty of Dentistry, University of Toronto, 124 Edward St., Toronto, Ontario M5G 1G6, Canada
[c] Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, 501 Smyth Road, Box 511., Ottawa, Ontario K1H 8L6, Canada
[d] Department of Cellular & Molecular Medicine, University of Ottawa, 501 Smyth Road, Box 511., Ottawa, Ontario K1H 8L6, Canada
[e] Department of Biochemistry, Microbiology and Immunology, University of Ottawa, 501 Smyth Road, Box 511., Ottawa, Ontario K1H 8L6, Canada
[f] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario M5G 1X5, Canada

ARTICLE INFO

Article history:
Received 24 April 2017
Received in revised form 10 August 2017
Accepted 12 August 2017
Available online 14 August 2017

Keywords:
Calcium polyphosphate
Scaffold
Sol-gel coating
Inorganic polyphosphate
Calcification of cartilage

ABSTRACT

Integration of *in vitro*-formed cartilage on a suitable substrate to form tissue-engineered implants for osteochondral defect repair is a considerable challenge. In healthy cartilage, a zone of calcified cartilage (ZCC) acts as an intermediary for mechanical force transfer from soft to hard tissue, as well as an effective interlocking structure to better resist interfacial shear forces. We have developed biphasic constructs that consist of scaffold-free cartilage tissue grown *in vitro* on, and interdigitated with, porous calcium polyphosphate (CPP) substrates. However, as CPP degrades, it releases inorganic polyphosphates (polyP) that can inhibit local mineralization, thereby preventing the formation of a ZCC at the interface. Thus, we hypothesize that coating CPP substrate with a layer of hydroxyapatite (HA) might prevent or limit this polyP release. To investigate this we tested both inorganic or organic sol-gel processing methods, as a barrier coating on CPP substrate to inhibit polyP release. Both types of coating supported the formation of ZCC in direct contact with the substrate, however the ZCC appeared more continuous in the tissue formed on the organic HA sol gel coated CPP. Tissues formed on coated substrates accumulated comparable quantities of extracellular matrix and mineral, but tissues formed on organic sol-gel (OSG)-coated substrates accumulated less polyP than tissues formed on inorganic sol-gel (ISG)-coated substrates. Constructs formed with OSG-coated CPP substrates had greater interfacial shear strength than those formed with ISG-coated and non-coated substrates. These results suggest that the OSG coating method can modify the location and distribution of ZCC and can be used to improve the mechanical integrity of tissue-engineered constructs formed on porous CPP substrates.

Statement of Significance

Articular cartilage interfaces with bone through a zone of calcified cartilage. This study describes a method to generate an "osteochondral-like" implant that mimics this organization using isolated deep zone cartilage cells and a sol-gel hydroxyapatite coated bone substitute material composed of calcium polyphosphate (CPP). Developing a layer of calcified cartilage at the interface should contribute to enhancing the success of this "osteochondral-like" construct following implantation to repair cartilage defects.

1. Introduction

In a joint, articular cartilage covers the articulating surfaces of the bones and facilitates smooth and painless joint movements. Joint injury or disease can damage articular cartilage, leading to pain and limited mobility. Cartilage has little, if any, intrinsic

* Corresponding author at: Dept. of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada.
E-mail address: rita.kandel@sinaihealthsystem.ca (R.A. Kandel).

http://dx.doi.org/10.1016/j.actbio.2017.08.016
1742-7061/

capacity for regeneration; therefore, tissue engineering has been widely viewed as a promising approach to replace damaged tissues [1]. Since joint loading puts articular cartilage under mechanical stress, strong integration of tissue-engineered cartilage to its underlying substrate is critical for a successful repair.

In healthy cartilage, a zone of calcified cartilage (ZCC) exists at the articular cartilage-subchondral bone interface. This ZCC serves as a transition region for force transmission between soft tissue (cartilage) and hard tissue (subchondral bone), which laterally dissipates forces [2]. ZCC also enhances fixation between two tissues by mechanical interdigitation at the interface region, thereby providing improved resistance to applied compressive and shearing forces [3]. We have developed biphasic constructs that consist of scaffold-free cartilage tissue formed on and integrated to a porous bone substitute material, calcium polyphosphate (CPP) [4,5]. The porous CPP substrate serves as a replacement for subchondral bone, which often also shows abnormalities in an osteoarthritic joint [6,7] or may have been damaged in symptomatic joint injuries [8,9]. The substrate is also a means for fixation of the tissue-engineered cartilage and native bone, which would be achieved by bone ingrowth into the porous CPP in the subchondral region and interdigitation of cartilage and the porous CPP substrate at the interface. Incorporating a ZCC at this interface would enhance its resistance to shearing forces. However, CPP is biodegradable, resulting in the release of inorganic polyphosphates (polyP) as a degradation by-product [10]. PolyP is a known inhibitor of apatite crystal growth, and biphasic constructs grown under mineralizing conditions form a ZCC in close proximity to, but separated from, the CPP substrate by a distinct non-mineralized layer [11]. Thus, a method to limit the polyP release must be devised to achieve a functional ZCC which is able to transmit these shearing forces [2].

Sol-gel thin film processing is a non-line-of-sight method, suitable for coating complex surfaces such as those presented by porous CPP. Calcium phosphate sol can be synthesized from either organic or inorganic precursors to deposit a crack-free, thin calcium hydroxyapatite coating [12]. In a previous study, porous CPP substrates were coated with hydroxyapatite using the inorganic sol-gel (ISG) method, in which the coating was intended to serve as a barrier to inhibit polyP release [13]. This approach generated constructs with a ZCC forming in close proximity to the substrate. However, mechanical testing did not demonstrate significantly increased interfacial shear strength of the samples compared to constructs with a ZCC formed on CPP substrates without the hydroxyapatite barrier coating. A comparative study of coating films formed using the ISG method and the organic sol-gel (OSG) method on porous-surfaced titanium implants showed that the OSG-derived coating appeared less nanoporous than the ISG-derived coating [12]. Therefore, we hypothesized that hydroxyapatite barrier coating formed using the OSG method on porous CPP substrates would inhibit the polyP release more effectively and further increase the interfacial shear strength of the construct due to the localization of the ZCC.

In this study, we directly compared the formation of hydroxyapatite (HA) barrier coatings by either OSG or ISG thin film coatings applied to porous CPP substrates in order to identify which would create biphasic constructs with an enhanced capacity for withstanding shear forces at the cartilage-CPP interface.

2. Materials and methods

2.1. Sol-gel processing of porous CPP disks

Two different calcium phosphate sol formulations were prepared for forming thin film coating by dip-coating of porous CPP disks as previously described [12]. To prepare an organic sol, triethyl phosphite (Sigma-Aldrich, Oakville, Ontario, Canada) was first hydrolyzed in excess ddH_2O for 24 h. Calcium nitrate tetrahydrate (Sigma-Aldrich) was dissolved in ethanol and added to hydrolyzed triethyl phosphite solution at a calcium-phosphate ratio of 1:1.67. The sol was sealed, aged at 40 °C for 4 days, then diluted to 67% with ethanol and further aged at room temperature for 2 days. The pH of the resulting sol was about 2. To prepare an inorganic sol, aqueous solutions of ammonium dihydrogen phosphate (Sigma-Aldrich) and calcium nitrate were mixed in the presence of ammonium hydroxide (Sigma-Aldrich) at the same calcium-phosphate ratio of 1:1.67. The sol was sealed with a porous membrane to allow for ventilation and aged at room temperature for 7 days. After removing the supernatant, the sol was diluted to 75% with distilled water. The pH of the resulting sol was about 10.

Porous CPP disks of 4 mm diameter and 2 mm height were prepared by gravity sintering 75–150 μm CPP particles as previously described [10,14]. The fabricated disks had an approximate volume porosity of 30% with interconnecting pores in the size range of 100–250 μm as previously described [10]. Disks were pre-incubated in phosphate-buffered saline for a week. Buffer was changed every other day. Each layer of film was formed by dip coating porous CPP disks, followed by annealing for 15 min at 210 °C. With the organic sol, disks were sequentially coated twice using a withdrawal speed of 20 cm/min [12] while for the inorganic sol, disks were sequentially coated eight times using a withdrawal speed of 30 cm/min [13]. After the final dip coating, disks were annealed at 500 °C for 20 min (organic sol) or 60 min (inorganic sol) and gradually cooled to room temperature. The coated disks were placed in Tygon tubing to create a well-like barrier to prevent cell leakage during seeding. Subsequently these tubed CPP disks were sterilized with γ-irradiation (2.5 MRad). The fabrication process and the experimental design used in this study is outlined in Fig. 1.

2.2. Characterization of HA coating on CPP disks

Sol-gel-formed coatings on porous CPP disks were characterized as previously described [13]. The outer and fracture surfaces of non-coated and coated CPP substrates were sputter-coated with gold (Desk II, Denton Vacuum, Moorestown, NJ, USA) to make them electrically conducting for viewing by scanning electron microscopy (XL30, FEI, Portland, OR, USA). The surface coverage and microstructure of films were examined using secondary electron imaging. Some coated porous CPP disks were purposely fractured in order to form exposed fracture surfaces across the coatings and micrographs of cross-sectional fractured regions were used to estimate the thickness of the coatings. The crystallographic form of the coatings was determined by electron diffraction (Tecnai 20, FEI) of portions of the thin coating (along with some inadvertently detached CPP particles) scraped from the coated disks. To survey the distribution of coating throughout the porous disks, coated disks were embedded in methyl methacrylate (Osteo-Bed bone embedding kit, Polysciences Inc., Warrington, PA), bisected and imaged using backscattered electron imaging (BSEI). Contrast and brightness were adjusted to show the coating and CPP at different signal intensities. To characterize the composition of the coatings, coated CPP disks were sonicated in 0.5 N hydrochloric acid for 20 min to selectively dissolve the coating. The dissolution products were neutralized with sodium hydroxide solution before analysis. Calcium content was determined using the *o*-cresolphthalein complexone assay and measuring absorbance at 570 nm [15]. Phosphate content was determined using the heteropoly blue assay and absorbance measured at 620 nm [15]. Non-coated CPP disks were used as negative control.