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Silver doped resorbable tricalcium phosphate scaffolds for bone graft applications

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ABSTRACT

Bone graft procedures, in particular maxillofacial repair, account for half of the orthopedic procedures done in the US each year. Infection is a major issue in surgery, and should be of primary concern when engineering biomaterials. Silver is of renewed importance today, as it has the ability to potentiate antibiotics against resistant bacterial strains. In order to reduce long term infection risks, it is necessary for the scaffold to maintain a silver ion release for the length of the healing process. In this study, silver doped porous β-tricalcium phosphate (β-TCP) scaffolds were engineered using liquid porogen based method with the goal of meeting these requirements. Silver was added to the β-TCP at three different dopant levels: 0.5 wt% Ag_2O, 1 wt% Ag_2O and 2 wt% Ag_2O. Immersion in pH 5 acetate buffer over a 60 day period resulted in a total cumulative ion release between 32 and 54 μM for dense control scaffolds, and between 80 and 90 μM for porous scaffolds. Porosity increased the dissolution rate of the scaffolds by a factor of 2. Human osteoblast cell lines were grown on the scaffolds to measure cytotoxicity and cell proliferation. Porosity increased osteoconduction by doubling the cell growth, and there was no significant cytotoxic effect even for the 2 wt% Ag_2O, as cells were observed on all the samples. Our results showed that silver can be released over a long period without compromising the biocompatibility of the scaffolds.

1. Introduction

The skeleton plays a crucial role in the anatomy of all vertebrates as it is the load bearing structure that the rest of the body is built around. As a result of this, some of the most crippling ailments are afflictions of the musculoskeletal system, which are bone related diseases and traumas. Although very rarely life threatening today, musculoskeletal diseases including accident related trauma and arthritis, are a heavy burden on society. 50% of adults over the age of 18 are affected by them only in the USA [1]. Musculoskeletal (MSK) conditions was found to affect more than 1.7 billion people worldwide according to a study conducted in 2012 [2]. The same study also found MSK conditions as the second greatest cause of disability. This burden is only set to increase, driven up by factors such as an ageing population, more active lifestyles, and increased life expectancy. Data from the center for disease control (CDC) shows that in the US in 2010, there were over 5 million surgical procedures on the musculoskeletal system [3]. Out of these 5 million procedures, the vast majority treated patients over the age of 30. Over 1 million procedures were total joint replacements, and a further 2 million were bone grafts. On top of these grafts and implants, countless screws, plates, and pins are used to stabilize fractures and breaks.

Luckily for us, bone is one of only two biological materials along with the liver that can repair itself when damaged. This does not however eliminate the need for bone grafts and implants, both permanent and temporary. Autografts, where the recipient and donor is the same person, are considered the gold standard for bone repair, however they are limited by donor site availability and morbidity, and can only be used for small defect repair [4]. Allografts, bone grafts from a different individual, and exografts, where the donor is from another species, are limited by recipient autoimmune response as well as disease transmission. The development of synthetic engineered biomaterials has therefore been crucial in satisfying the demands of what has become a $2.5 billion industry [5]. Advances in biomaterials research have resulted in improvements in performance, durability, and cost of synthetic implants. Bone grafts used in maxillofacial repair constitute half of the orthopedic procedures done in the US each year. These grafts are usually made from synthesized calcium phosphate (CaP) bioceramics, which have the same mineral composition of bone.

This study focuses on tricalcium phosphate (TCP) bioceramics, which have the advantage of being bioresorbable meaning they will naturally dissolve within the body over time [6]. Because the chemistry is similar to that of natural bone [7–8], cells will adhere and proliferate easily on the material. This material property is called osteoconductivity. Osteoconductivity is also increased by the structure of the material, as open pores can provide a three dimensional structure for the bone to regrow as the scaffold dissolves [9–11]. The advantage of bioresorbable

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osteoconductive bioceramics is that once the patient is healthy there is no need for removal surgery. However their poor mechanical properties preclude them from being used in load bearing applications. The two most critical material properties for orthopedic grafts are osteoconductivity and osteoinductivity, which describe the material's ability to further promote cell differentiation, proliferation, and formation of bone structures [7]. Two things determine osteoconductivity, both the morphology of the material, in particular porosity, as well as its chemical and bioactive properties. Osteoinductivity is provided by locally delivered growth factors and hormones.

With all these advances persists the oldest, most common, and costliest problem that orthopedic surgeons have to contend with: post-surgery periprosthetic infection [12–15]. The presence of a foreign body reduces the necessary number of bacteria to cause infection by a factor of a thousand [5]. The resulting infections are difficult to diagnose, and end up leading to extra surgeries, and further complications with the patients' health. With the development of nanotechnology and nanoscale processing techniques, attempts have been made to combat infection in various ways, including nanotube surface coatings for drug delivery, encapsulated antibiotics, and other localized delivery methods [7,16–17]. Long term infection risks complicate the issue further, as biofilms develop quickly and are very hard to eliminate. These surviving bacteria colonies greatly increase the risk of secondary infection, which can be generalized and life threatening [18]. This long term risk creates the need for a graft material which can retain antibacterial properties over a significant time period. Effective local antibiotic treatment became possible with the advent of nanotechnology, however controlled spatial and temporal delivery, in particular of antibiotics, remains one of the principal challenges for tissue engineers [19]. In addition to well-known antibacterial properties [20–22] silver has proven to be a potentiator for antibiotics, to the point of resensitizing resistant bacteria strains in recent *in vivo* studies [23]. Additionally, silver can easily be used as an elemental dopant [20,24–26].

The aim of this study was to create a viable porous bone graft that will supply a local concentration of silver ions for the lifetime of the implant, thus diminishing the long term infection risk that exists for patients today. The effects of silver on material processing, physical properties, *in vitro* ion release through dissolution, and cytotoxicity of TCP scaffolds were examined. The role of porosity on the *in vitro* dissolution kinetics and osteoconduction of resorbable β-TCP scaffolds was also investigated.

2. Materials and methods

2.1. Silver doped bone graft fabrication

The TCP used in this study was prepared according to the Eq. (i) from precursors calcium phosphate and calcium carbonate (High purity from sigma Aldrich).

$$CaCO_3 + 2CaHPO_4 \rightarrow Ca_3(PO_4)_2 + CO_2 + H_2O \quad \text{(i)}$$

The precursors are mixed in stoichiometric amounts, ball milled then the mixture was heat treated at 1050 °C for 24 h. The resulting TCP is then grinded and doped with the appropriate mass of silver oxide (sigma Aldrich 99.9% purity). The doping levels chosen are 0.5 wt%, 1 wt% and 2 wt% Ag_2O, keeping a batch of pure TCP as a control. These doping levels were chosen after careful analysis of previous research [27]. Previous work done on compact samples with silver indicated that the ideal loading level to avoid cytotoxicity and yet still observe antibacterial effects is between 2 and 4 wt% Ag_2O. Since this study is interested in porous samples, which have an increased surface area and a higher dissolution rate, the optimal loading was kept below 2 wt%. The TCP powders were then ball milled in ethanol with zirconia milling media for 6 h in order to obtain a uniform mixing. For ball milling, the powder to ethanol ratio is 2 g for 3 mL, and the mass ratio of powder to milling media is 1:5. The batches of powder are then processed into all the different samples required to complete the study.

Two main types of samples, porous and compact, were made. The compact samples were prepared by uniaxial pressing. For mechanical testing, compact samples with 7.5 mm diameter and 10 mm height were made. The compact samples were further processed in a cold isostatic press (CIP). The samples are vacuum sealed into a double layer of latex gloves, then put in liquid for 5 min at 50 psi. Since the porosity of bone is in the range 30% to 75%, two porosities were chosen, one at each end. In order to engineer porosity into the TCP samples, an emulsion method was used. Kolliphor EL, a commercial product based on castor oil, was used as an emulsifying agent to stabilize porogen droplet size. The emulsifier was added to paraffin oil and TCP powder, and the whole mixture was then stirred for less than 45 s with a stirring bar. The porosity of the samples is dictated by the volume fraction of powder to porogen in the initial mixture. To obtain highly porous samples (75% porosity) 20 g of TCP were mixed with 20 g of paraffin oil, to which 13.4 mL of emulsifier were added. The homogeneous slurry obtained is a mixture of powder and dispersed porogen droplets, with the emulsifier stabilizing the porogen. The slurry was poured into 1 in. tall cylindrical molds with a diameter of 3/4″. These molds were put into an oven at 80 °C for several days to dry and harden before removing them from the mold for sintering. The slurry was found to be stable for pure TCP samples. However, once the dopant was added, the stability of the slurry was greatly reduced. The slurry was very difficult to obtain, and the oil droplets had a tendency to separate from the slurry.

The samples were sintered in a high temperature furnace (Zircar). The sintering procedure established by Perera et al. [28], which has been optimized by this research group is to sinter at 1250 °C for 2 h. The high temperature sintering is preceded by an hour dwell time at 300 °C and 600 °C to dry and burn out additives before sintering. The heating rate up to 600 °C was 1 °C per minute for the porous samples and 3 °C per minute for the dense samples. From 600 °C to 1250 °C the heating rate for all samples was 1 °C per minute.

2.2. Characterization: phase, density and porosity

Phase analysis of the sintered scaffolds was carried out using a diffractometer (PW 3040/00 Xpert MPD, Philips, Netherlands), with K_α radiation and a Ni filter. The intensity was measured over the range 20° to 60° in steps of 0.05° and 0.5 s. Phase percentage was determined by calculating the relative intensity of the three principal peaks for each phase using the Eq. (ii).

$$\text{Relative intensity} = \frac{\text{intensity of peak of phase analyzed}}{\text{intensity of all the peaks of all the phases}} \quad \text{(ii)}$$

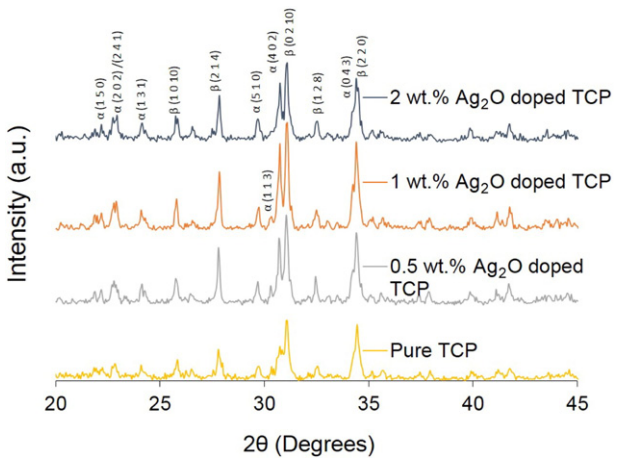

Fig. 1. XRD spectra of pure and silver doped TCP scaffolds sintered at 1250 °C for 2 h.