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Extracellular matrix formation and mineralization on a phosphate-free porous bioactive glass scaffold using primary human osteoblast (HOB) cells

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Abstract

Sol–gel derived bioactive glasses of the 70S30C (70 mol% SiO_2, 30 mol% CaO) composition have been foamed to produce 3D bioactive scaffolds with hierarchical interconnected pore morphologies similar to trabecular bone. The aim of this study was to investigate primary human osteoblast response to porous bioactive glass scaffolds. The scaffolds supported osteoblast growth and induced differentiation, within the 3-week culture period, as depicted by enhanced ALPase enzymatic activity, without the addition of supplementary factors such as ascorbic acid, β-glycerophosphate and dexamethasone. This is the first time this has been observed on a bioactive glass that does not contain phosphate. Deposition of extracellular matrix was also confirmed by enhanced production of the extracellular matrix protein collagen type I. SEM showed indications of mineralized bone nodule formation without the addition of growth factors. The 70S30C bioactive glass scaffolds therefore fulfil many of the criteria for an ideal scaffold for bone tissue engineering applications.

Keywords: Osteoblast; Bioactive glass; Porous scaffold; Ion release; Mineralization

1. Introduction

An artificial scaffold is required that can be used in tissue engineering applications to restore diseased or damaged bone to its natural state and function. One strategy for bone tissue engineering involves the harvesting of stem cells or osteoblasts from the patient, which are then cultured on a scaffold *in vitro*, where conditions are optimized to begin the first stages of (immature) bone formation. The tissue/scaffold composite (tissue engineered construct) can then be implanted in the defect of the patient, where the bone should regenerate at the rate at which the scaffold resorbs [1,2]. The body should then remodel the immature bone into mature bone creating a structure that is dictated by the loading at the defect site.

An ideal scaffold for bone tissue engineering applications should fulfil several criteria. First, the scaffold should be biocompatible (not toxic) and act as a three-dimensional (3D) template for *in vitro* and *in vivo* bone growth. It therefore must consist of an interconnected macroporous network with a modal interconnected pore diameter of at least 100 μm to allow cell migration, bone ingrowth and eventually vascularization [3,4]. The scaffold material should be one that promotes cell adhesion and activity and ideally stimulates osteogenesis at the genetic level [5] so that a tissue engineered construct can be grown *in vitro*, ready for implantation. This construct should have mechanical properties matching that of the host bone. The scaffold should bond to the host bone, creating a stable interface and the scaffold should then resorb at the

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same rate as the bone is regenerated, with non-toxic degradation products. The processing technique for the scaffolds should also be one that can produce irregular shapes to match that of the bone defect, that allows sterilization of the scaffold and has the potential to be scaled up to allow commercial production to the required international standards for clinical use.

Bioactive glass was chosen as a scaffold material because bioactive glasses bond to bone and stimulate bone growth (osteoinduction) [5]. Melt-derived bioactive glass of the original Bioglass® composition (46.1% SiO_2, 24.4% NaO, 26.9% CaO and 2.6% P_2O_5, in mol%) has been used clinically in a powder form since the mid 1980s, as a regenerative bone filler, under the product names Perioglas and Novabone. (Novabone Corporation, Alachua Florida). The bone bonding ability of the glasses has been attributed to their ability to form a surface layer of hydroxycarbonate apatite (HCA) [6] and osteoinduction is thought to be due to the ionic dissolution products released from the glasses, which stimulate osteogenic cells at the genetic level. Critical amounts of silicon and calcium ions have been found to up-regulate seven families of genes found in osteoblasts [7].

This has been supported by work by Reffitt et al. [8] who showed enhanced differentiation of osteoblastic cell lines when exposed to soluble silica (orthosilicic acid) and showed that type I collagen synthesis increased in all treated cells at orthosilicic acid concentrations of 10 and 20 μM. Type I collagen mRNA was also shown to increase significantly in primary osteoblast cells seeded with orthosilicic acid concentrations from 5 to 50 μM [9].

Sol–gel derived bioactive glasses tend to have more simple compositions than melt-derived bioactive glasses, such as Bioglass®, and exhibit enhanced bioactivity and resorbability, due to a mesoporous texture (pore diameters in the range 2–50 nm) that is inherent to the sol–gel process and increases the specific surface area of the glass [10].

Sol–gel derived bioactive glasses have been foamed to produce macroporous 3D resorbable scaffolds for tissue engineering applications [11]. A hierarchical structure was obtained, with macropores in excess of 500 μm connected by interconnects with diameters in excess of 100 μm, and a mesoporous texture (pore diameters of 10–20 nm). Many processing variables can be used to control the hierarchical structure, such as the glass composition, surfactant and gelling agent concentration and the processing temperature [12–14].

Previously, primary adult trabecular bone derived human osteoblasts were cultured on porous scaffolds of 58S composition (60 mol% SiO_2, 36 mol% CaO, 4 mol% P_2O_5) [15]. The study found that mineralized bone nodule formation occurred on the scaffolds within 10 days of culture. However, it was unclear as to whether phosphate was required in the glass composition. Previous work on the materials properties of the foam scaffolds found that the phosphate free 70S30C (70 mol% SiO_2, 30 mol% CaO) composition was more susceptible to the foaming process and was bioactive in simulated body fluid (SBF) [12]. In fact, scaffolds with compressive strengths of 2.4 MPa were produced while maintaining interconnected pore diameters in excess of 100 μm [14], therefore it would be beneficial if phosphate free glasses stimulate similar cell response as the phosphate containing scaffolds. In this study human adult trabecular bone derived cells were also utilized. These cells are well established and extensively characterized sources of primary osteoblasts (enriched in osteoblasts and their immediate precursors) [16] that appear to have multipotential mesenchymal progenitor cell characteristics [17], making them an attractive cell source for bone tissue engineering applications. The addition of osteogenic supplements such as glucocorticoids, ascorbic acid and β-glycerophosphate are extensively used by researchers for bone tissue engineering experiments *in vitro* and have been shown to induce matrix mineralization in a number of bone cell cultures. β-glycerophosphate is commonly used as an exogenous phosphate source for cultured osteoblasts to synthesize mineralized material. Ascorbic acid promotes synthesis and accumulation of type I collagen in chick, mouse, rat and human osteoblasts [18,19] and it is required for the expression of alkaline phosphatase and osteocalcin and the development of a mature osteoblast phenotype *in vitro* [20]. Dexamethasone, a glucocorticoid, is routinely used for *in vitro* experiments to induce proliferation, osteoblast maturation and extracellular matrix mineralization, however its use is controversial and does not reflect physiological conditions [21,22]. The main aim of this work was to examine the initial response of human osteoblasts and to phosphate-free bioactive glass scaffolds to investigate their potential to fulfil the criteria of an ideal scaffold for bone tissue engineering. A second aim was to establish whether the addition of commonly used osteogenic factors is necessary for osteogenesis on these scaffolds.

2. Methods

2.1. Foam synthesis

Colloidal solutions (sols) of the 70S30C composition (70 mol% SiO_2, 30 mol% CaO) were prepared by mixing (in order); deionized water, 2 N nitric acid, tetraethyl orthosilicate (TEOS) and calcium nitrate tetrahydrate (all Sigma, Gillingham, UK) [23]. The molar ratio of water to TEOS (R ratio) was 12:1. Porous scaffolds were produced by foaming 50 ml aliquots of sol by vigorous agitation with the addition of 0.35 ml Teepol (Thames Mead Ltd., London) and 1.5 ml 5% (v/v) HF (a gelation catalyst). Teepol is a detergent containing a low concentration mixture of anionic (15%) and nonionic surfactants (5%). As the gelling point was approached the foamed solution was cast into cylindrical polymethyl propylene moulds [11]. Samples were aged (60 °C), dried (130 °C) and thermally stabilized (600 °C) according to established procedures [24].

2.2. Material characterization

Foam scaffolds were produced to a bulk density of 0.25 g/cm^3 (measured geometrically), which, from previous work corresponds to a modal interconnected pore diameter of 120 μm and a total porosity of 91% [14]. The modal interconnected pore diameter for 3 typical scaffolds from the batch used was measured using mercury intrusion porosimetry (Quantachrome Poremaster 33, Quantachrome Corp. Boynton Beach,