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ScienceDirect
Acta Biomaterialia 3 (2007) 563–572
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Effect of ternary phosphate-based glass compositions on osteoblast and osteoblast-like proliferation, differentiation and death *in vitro*

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Received 2 June 2006; received in revised form 17 November 2006; accepted 19 November 2006

Abstract

There is currently a need to expand the range of graft materials available to orthopaedic surgeons. This study investigated the effect of ternary phosphate-based glass (PBG) compositions on the behaviour of osteoblast and osteoblast-like cells. PBGs of the formula (in mol.%) P_2O_5(50)–CaO(50−*X*)–Na_2O(*X*), where *X* is either 2, 4, 6, 8 or 10, were produced and their influence on the proliferation, differentiation and death *in vitro* of adult human bone marrow stromal cells (hBMSCs) and human fetal osteoblast 1.19 (HFOB 1.19) cells were assessed. Tissue culture plastic (TCP) and hydroxyapatite (HA) were used as controls. Exposure to PBGs in culture inhibited cell adhesion and proliferation and increased cell death in both cell types studied. There was no significant difference in percentage cell death between the PBGs, which was significantly greater than the controls. However, compared with other PBGs, a greater number of cells were found on the 48 mol.% CaO which may have been due to either increased adherence or proliferation, or both. This composition was capable of supporting osteogenic proliferation and early differentiation, and supports the notion that chemical modification of the glass could lead to a more biologically compatible substrate with the potential to support osteogenic grafting. Realisation of this potential should lead to the development of novel grafting strategies for the treatment of problematic bone defects.

Keywords: Phosphate-based glass; Osteoblasts; Bone; Graft

1. Introduction

Bone deficit as a result of trauma often requires bone grafting. Various sources are available to surgeons – autologous grafts, allogenic grafts as well as synthetic grafts such as hydroxyapatite (HA). Because autologous and allogenic graft materials have limitations in terms of availability and risk of infection, there is a need to explore novel synthetic graft materials [1]. Grafting should ideally close osseous deficits by integrating successfully into the surrounding tissue without disruption and stimulate the bone repair process. The graft material ideally should possess osteogenic, osteoinductive, osteoconductive and resorptive properties [2].

At present, commonly used materials such as calcium phosphate ceramics are suitable only for small deficits [1]. Other materials, such as Bioglass® [1,3,4], use silicon tetraoxide (SiO_4) as the network former and produce a scaffold for growth that has been found to exhibit beneficial characteristics *in vivo*. However, the SiO_2 component is rarely absorbed, it inhibits the rate of resorption, and the long-term reaction locally and systemically is not yet fully understood, raising concerns over its long-term use *in vivo* [3,5].

As an alternative degradable grafting material for hard tissue engineering, phosphate-based glasses (PBGs) [3] have been developed for a number of years [4,6,7]. The network

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1742-7061/$ - see front matter
doi:10.1016/j.actbio.2006.11.008

former of PBGs is a phosphate (PO_4^{3-}) which is highly degradable and the glass can be engineered to include mineral elements, such as Ca^{2+}, Na^+, Mg^{2+}, Sr^{2+} and K^+. The introduction of these elements, known as network modifiers, has been shown to alter the phosphate network and consequently affect the degradation properties. The degradation rates can therefore be controlled by selectively varying the amounts of these constituents incorporated into the glass [8]. Ternary based PBGs contain varying levels of P_2O_5–CaO–Na_2O, but contain no silicon. Several attempts have been made to harmonise the degradation behaviour with an end application [9]. In this ternary system, substituting Na_2O with CaO while maintaining P_2O_5 constant yielded glass systems that were less degradable, since Ca^{2+} ions have much stronger field strengths than Na^+ and a chelating structure could be formed with ionic bonding between two adjacent (PO_4^{3-}) tetrahedrons to strengthen the P–O–P bond [10]. Altering the proportion of CaO in the PBGs modifies their rate of dissolution and resorption rate from a few hours to weeks [4].

It has been postulated that the constituent atoms of these PBGs are similar to the inorganic component of bone and hence aid its successful incorporation into surrounding tissues [4,7]. Preliminary studies have provided evidence that their chemistry could be manipulated during manufacture and enhanced to provide a useful adjuvant material. As PBGs are soluble, they aid apatite formation by releasing ions into the host tissue – a calcium phosphate layer forms that is then transformed into an apatite layer, ensuring a stable attachment for the implant to adhere to and integrate into the natural phase of bone. Studies have reported that the composition and subsequent solubility of the glass profoundly affects cell attachment and proliferation. It has been shown that glasses based on the above ternary system with a CaO content lower than 40 mol.% exhibited fewer adherent cells compared with glasses containing higher amounts of CaO (>40 mol.%). This in turn has led to better biocompatibility; for example, an enhanced bone cell growth together with an up-regulation of antigen expression has been observed in cells incubated with extracts from low solubility glasses [7].

It has been suggested [7] that PBGs might provide a candidate substrate for a synthetic orthopaedic graft material. Previous studies of these glasses have used MG63 and HOS-TE85 human cell lines in addition to bone-derived cells [7,11]. MG63 and HOS-TE85 cells have osteoblast-like characteristics and are often used as experimental models for investigating aspects of osteoblast function. However, their proliferation and alkaline phosphatase expression are not representative of bone cell behaviour and they are therefore not ideally suited to studying these aspects of osteoblast function [12].

This study used adult human bone marrow derived cells (hBMSCs), which are heterogeneous in nature [13] but known to contain cells of the osteogenic lineage at different stages of osteogenic differentiation [14]. It is these marrow cells that are predominantly recruited *in vivo* to effect a successful fracture repair [15,16] and would also therefore interact with the PBG in a graft situation. For this reason, hBMSCs provide an optimal model system to investigate cell–material interactions for orthopaedic use. The human fetal osteoblast 1.19 (HFOB 1.19) cell line was also used [17]. HFOB 1.19 cells are conditionally immortilized with a gene coding a temperature-sensitive mutant (tsA58) of the SV40 large T antigen. Cells cultured at the permissive temperature of 33.5 °C undergo rapid cell proliferation. At the restrictive temperature of 39.5 °C proliferation ceases and differentiation occurs. The HFOB cell line provides a homogeneous, rapidly proliferating model system to study human osteoblast proliferation and differentiation [17].

A range of PBGs were compared with hydroxyapatite and tissue culture plastic controls *in vitro* to assess their effect on the proliferation and differentiation of osteoblast cells (hBMSCs) and osteoblast-like cells (HFOBs). The solubility of the PBGs, its products, and the effect on media pH was also assessed.

2. Materials and methods

2.1. Manufacture of PBGs

Glass rods were prepared at compositions shown in Table 1. This was carried out by thoroughly mixing three starting reagents (NaH_2PO_4, P_2O_5 and $CaCO_3$) in a platinum crucible. The crucible was placed in a carbolite furnace preheated at 700 °C. Heating at 700 °C ensures that excess gas produced (CO_2 and water vapour) due to the chemical reaction is expelled. After 10–15 min the temperature of the furnace was increased to 1100 °C for 1 h, at which temperature the glass forms a melt. The crucible was then removed from the furnace and the glass melt quickly poured into a graphite split mould consisting of two 12 mm diameter cylindrical holes that had been preheated in a casting furnace at 420 °C. The graphite mould was instantly placed back into the casting furnace and heated for 1 h at 420 °C in order to eliminate any residual stress. The glass was then furnace cooled to room temperature. This enabled the glass to go through a process of rapid quenching and annealing to form a solid amorphous glass rod of very high viscosity. Once the graphite mould reached room temperature the rods were removed and then cut at a cross section to discs of 2 mm thickness using a Testbourne diamond saw cutter.

Table 1
Glass codes and composition in mol.%

Glass code	CaO	Na_2O	P_2O_5
C40	40	10	50
C42	42	8	50
C44	44	6	50
C46	46	4	50
C48	48	2	50

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