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A novel *ex vivo* culture system for studying bone repair☆

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Summary Repair of large bony defects still remains a challenge for surgeons. Hydroxyapatite (HA) is well known for its biocompatibility and osseoconduction properties in the osseous environment. In this study the biofunctionality of a newly developed scaffold comprising of collagen and HA, with variable macropores was examined. The biological response was evaluated using primary human osteoblast cells (HOBs). Cell infiltration, proliferation and differentiation were assessed. The results showed that HOBs were able to migrate from the collagen into the HA pores with greater cell migration and infiltration observed in those scaffolds with larger pores. Furthermore, it was shown that Alkaline Phosphatase, a differentiation marker for HOBs was enhanced as the average macropore size increased. This *in vitro* model provides a more relevant method of testing the biofunctionality and migration ability of cells at a trauma site following implantation in bone and cartilage.

Introduction

Tissue/organ repair has been the ultimate goal of surgery from ancient times to the present. Repair traditionally has taken two forms: (i) tissue grafting and organ transplantation; and (ii) alloplastic or synthetic material replacement. Both approaches, however, have known limitations.[4,7,22,32] Grafting requires second surgical sites with associated morbidity and is restricted by limited amounts of material, especially for organ replacement. The development of autologous, vital, vascularized, bone flaps has provided a means of circumventing many of these problems, but requires highly complex surgical techniques and generates donor site morbidity.[21] Synthetic materials often integrate poorly with host tissue and fail over time due to wear and fatigue or adverse body response.

Tissue engineering represents the future of musculoskeletal repair; hence suitable *in vitro* models are needed to study the mechanisms of cell prolif-

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eration and differentiation. An ideal bone graft substitute comprises regulatory osseoinductive factors to induce bone regeneration, an osseoconductive scaffold or matrix to provide physical support and direction for repair, and also osteogenic cells, which have the ability to differentiate and regenerate bone.[14]

With increasing advances in biomimetic materials for tissue engineering, there is greater scope for the application of materials into mainstream reconstructive surgery. Experimental modelling of bone regeneration and the factors influencing bone matrix secretion is hampered by a lack of *in vitro* models. Whilst *in vivo* experimentation has yielded considerable information on the processes taking place, the ability to investigate the influence of specific factors *ex vivo* offers significant advantages.

Porous materials have been used as bone implants for many years. The internal architectural attributes, including pore size, shape and interconnectivity play an important role in the *in vivo* and mechanical performance of the implants. They control the degree of bone regeneration,[12,29,31,35,42,49] path of bone regeneration[33] and determine the mechanical properties of the implants.[10,23]

Scaffolds for tissue engineering have been created using a wide variety of techniques and materials. Based on the structural similarity between natural and synthetic apatite, porous hydroxyapatite (HA) has proved to be a very useful material for orthopaedic, maxillofacial and dental applications. Most applications of HA are aimed at good implant fixation to bone, osseoconduction into the pores, and eventual resorption of the implant and replacement by bone.[9] HA has found use as a surface coating for joint arthroplasty, and its most striking effects have been its ability to enhance bone growth across a gap around an implant in both stable and unstable mechanical conditions and to even convert motion-induced fibrous membrane into a bony anchorage.[43,44]

HA has been shown to promote and support the apposition/deposition or integration of bone, when placed within a bony defect *in vivo*. HA mimics the normal pattern of osteoid production and mineralisation to form bone.[1] HA also provides structural support for the defect during healing and bonds with the surrounding host bone.[52] The non-mechanical bond with bone is of significant strength,[28] and although the mechanical strength of porous HA is low, it can be significantly increased as bone grows into it.[24] The formation of such an intimate bond between the tissues surrounding the implant and the implant material obligates cellular adherence. Osteoblast cells fail to proliferate, grow and/or differentiate if they are denied a substrate on which they can adhere.[46] This anchorage dependence is a prerequisite for bone formation *in vitro*[51] and *in vivo*.[47] Therefore, osseoconduction is closely associated with the phenomenological behaviour of cells (that is, the anchorage, attachment, adhesion and spreading) and the process of bone formation requires a surface that allows/favours this cell behaviour.

The physical structure of HA is a key factor that will determine the success of the implant *in vivo*. In 1972, Hulbert et al.[27] showed that when porous discs of a near inert ceramic were implanted in muscle they exhibited thinner fibrous encapsulation with faster healing in the surrounding tissues when compared to dense discs of the same composition. It is now known that provision of a suitable macroporous structure is important in order to obtain good implant integration through rapid vascularisation, bone ingrowth, and where the implant is resorbable, possibly remodelling.[48]

In vivo studies have investigated the effect of macropore size on bone formation.[5,8,19,23,31,35] In these studies it was generally claimed that a minimum pore size of 100 μm was necessary for bone ingrowth into the porous implants. Flatley et al.[17] reported that a pore size of 500 μm was the optimal, as did Kuhne et al.[31] but was only compared with a pore size of 200 μm. There is a general consensus that an average pore size for HA to maximise osseoconduction is between 100 μm and 500 μm.[8] Many authors have concluded that this reflects a dependence of bone ingrowth on pore size.[25,26,30,50] It is worth noting that these studies used porous HA with random pore geometry and therefore have a wider range of pore sizes.

The average size of macropores in porous HA is not considered to be the only parameter affecting the ingrowth of bone. Other parameters include the degree and size of the connectivities within the sponge like structure. Blind-ending pores will prevent the ingrowth of bone, whereas large interconnecting channels will allow migration of cells and growth factors. It has been claimed that the size and frequency of interconnections is of more importance to osseoconduction rather than the size of the pores themselves.[16,31] However, as connectivity generally increases with increasing pore size, separating the contributions of each parameter is difficult.

Knowledge of events taking place at the tissue-biomaterial interface will assist in the design of improved orthopaedic/dental biomaterials able to induce specific and desirable responses from surrounding cells/tissues, optimise the function of osteoblasts, and consequently enhance long-term bone-implant bonding.[11] Hence in this study an *ex*