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Osteogenic priming potential of bovine hydroxyapatite sintered at different temperatures for tissue engineering applications

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ABSTRACT

Bovine hydroxyapatite, a widely used scaffold in tissue engineering, is sintered at varied temperatures (600 °C, 700 °C, 750 °C, 800 °C, 900 °C and 1000 °C) and was evaluated for its osseo-integration potential with human bone marrow derived mesenchymal stromal cells. The cell attachment, distribution, morphology, and osteogenic marker assays were analysed. SEM analysis on day 11 revealed the porous structure and well-defined equiaxed HAp grain morphology and its affinity for cell attachment. While confocal microscopy showed viable cells on all samples, the proliferation assay indicated that cells in monolayer is significantly higher on day 11 and 15 than the scaffold ($p < 0.05$). Significant differences were observed only between certain groups (750:600 and 750:1000) at day 11, however, no difference was observed between the groups after day 15. Osteogenic marker assays revealed that the bovine scaffold sintered at 750 °C exhibited superior osteoinductivity than others. These findings indicate that the bovine HAp sintered at 750 °C may act as a potential biomaterial for possible tissue engineering applications.

1. Introduction

Development of biomaterials for the repair/replacement of hard tissue has earned much interest in the past few years. Although, the conventional method of using autogenous (host) or allogenic (donor) bone was most preferred for the treatment of bone defects, disadvantages including, the limited quantity and the risk of transmission of infection (e.g. HIV, Hepatitis, etc.) [1,2] have led researchers to pursuit for a possible alternative. Owing to its physical and chemical similarities to inorganic mineral component, synthetic HAP with chemical formula of Ca_{10} $(PO4)_6(OH)_2$ was the most frequently studied. However, due to the absence of other trace elements (e.g., Na, Mg, and Al), it was not able to mimic apatite similar to natural source such as fish and bovine bones [3], which is surplus; easily obtainable with lower cost than synthetic HAp.

Though, the conversion of natural spongy (cancellous) bone extracted from animals offers the potential of developing into a natural porous HAP structure with excellent biocompatibility, bioactivity and osteoconduction properties, their poor mechanical properties restrict its use in weight bearing areas. Mechanical properties of the scaffolds depend on the porosity and morphological structure of the materials. Matching mechanical properties and interconnected porous structure similar to the bone structure and property are highly desirable for different TE applications [4,5]. The sintering behavior of hydroxyapatite (HAp), their mechanical properties and its microstructure were found to be dependent on the thermal history during the fabrication process. Although studies have exhibited the effects of sintered bone at different temperature, individually [6], controversy regarding the onset of chemical and structural changes and the effects of such structural modification for optimal osseo-integration are still elusive. Therefore, the present study aims to evaluate the response of human bone marrow derived mesenchymal stromal cells (hBM-MSCs) and its osseo-integration potential on bovine HAp sintered at varying temperatures (600 °C–1000 °C) in vitro.

2. Materials and methods

HAp Sample Preparation and processing: HAp was obtained from the femur bone of a bovine (aged between 2 and 3 years

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