HUMAN MESENCHYMAL STEM CELL ADHESION, PROLIFERATION AND DIFFERENTIATION ON FULLY DENSE APATITE-WOLLASTONITE GLASS AND GLASS-CERAMIC

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Introduction

An understanding of the effect on cell adhesion and differentiation of a material's properties, in combination with biological factors, can be utilised in tissue regeneration applications. To this direction, fully dense Apatite-Wollastonite (A-W) Glass (G) and Glass-Ceramic (GC) substrates were prepared in order to examine the effect of surface chemistry on human mesenchymal stem cell (MSC) adhesion and differentiation towards an osteogenic lineage.

Materials and Methods

Substrates: Fully dense A-W G and GC substrates were prepared. The glass was melted using reagent grade SiO_2, $CaCO_3$, P_2O_5, MgO and CaF_2 at 1450 °C and cast into water to produce an amorphous frit. Differential Thermal Analysis (DTA) was used to confirm the A-W composition. The frit was dried, ground and then remelted and cast into a preheated graphite mould. The resulting glass rod was cut into discs and some of the samples heat treated to prepare the GC. A-W G and GC samples were subsequently ground with a P2500 SiC paper and polished, and the surface topography of the materials characterised using Confocal Laser Scanning Microscopy (CLSM) and Scanning Electron Microscopy (SEM). The chemistry of the samples and their homogeneity were examined using Energy-Dispersive X-ray Spectroscopy (EDX). The crystalline phases formed in the free surface of the GC were identified by X-ray Diffraction (XRD). Water contact angle (WCA) measurements were used to examine the surface energy of the substrates.

Cells: Primary human MSCs were extracted from knees obtained from routine knee replacement surgery (Harrogate and York Hospitals, following ethical approval). Cells were cultured in MSC medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (p/s) (basal). To promote osteogenic differentiation, MSCs, after adhesion to the substrates were cultured either in basal or osteogenic media; basal supplemented with 50 μg/ml L-ascorbic acid phosphate, 5 mM β-glycerophosphate and 10 nM dexamethazone. A-W G and GC substrates were seeded with $5x10^4$ cells/ml and examined after 0, 7, 14 and 21 in culture. Cell adhesion and proliferation was examined by SEM and CLSM, after F-actin and DAPI staining. The osteoconductivity and osteoinductivity of the substrates was evaluated by measuring the alkaline phosphatase (ALP) activity in basal and osteogenic media and normalizing it to DNA, using the fluorescent nucleic acid stain picogreen.

Results and Discussion

DTA analysis showed two exothermic events, with peak maxima at 888 °C and between 940-1000 °C indicating that crystallisation occurred with two crystal phases generated; XRD revealed that the starting material (G) was indeed completely amorphous and confirmed that the phases that had been crystallised in the case of the GC were apatite and wollastonite. EDX mapping of both A-W G and GC suggested that both phases were homogenously distributed, with the Ca/(Si+P) ratios being 2 for the G and 1.65 for the GC. Macro-roughness measurements showed that both G and GC were smooth after grinding and polishing, and the average surface roughness was 2±0.2 μm. The WCA on G was 48±5°, while that for the GC <5°.

Cell adhesion studies showed that both the A-W G and the GC substrates facilitated MSC adhesion, spreading and proliferation, with the GC presenting a higher number of adherent cells. The determination of the ALP activity of the hMSCs cultured in basal and osteogenic media on A-W G and GC and on tissue culture plastic (TCP) showed that both G and GC presented much higher activity in comparison to the TCP, in both basal and osteogenic media. The activity though was higher for the GC in comparison to the G, for all the tested time points, possibly due to its superior hydrophilicity. These results indicate that the A-W GC is a good osteoconductive and osteoinductive material that can be used in tissue engineering towards bone regeneration.

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