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Chemotactic Behaviour of Human Mesenchymal Stem Cells in Response to Silicate Substituted Hydroxyapatite

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Abstract : Silicate-substituted hydroxyapatite (SiHA) has been shown to enhance bone regeneration in vivo compared with phase pure stoichiometric hydroxyapatite. Evidence suggests that substrate chemistry dependent formation of a permissive protein layer on the surface of synthetic bone graft substitute materials is key for bioactivity and cell attachment. However, little information is available on whether the substrate chemistry may affect cell migration and recruitment. The aim of this study is to investigate whether or not human Mesenchymal Stem Cells (hMSCs) exhibit a chemotactic response to SiHA porous granules and if it can be linked to either the ion exchange or protein sequestering and enrichment on the surface of the material. 150mg of SiHA granules with 80% total porosity and 20% strut porosity were incubated in 1ml of either Serum Free Media (SFM) or 10% Serum Containing Media (SCM) under static cell culture conditions (37°C, 5% CO2) in absence of cells. Protein sequestering and exchange of calcium, phosphate and silicate ions were analysed at 0.5, 1, 2, 4, 8, 16 and 24 hours with n=12 per time point. Migration of hMSCs in the presence of 150mg of SiHA granules was assessed over 24 hours using a modified transwell migration system in either SFM or SCM (n=6) with 30% serum containing media acting as a positive control. At 24 hours protein sequestering and ionic exchange were analysed, and the number of cells was quantified using a high throughput confocal microscope (IN Cell Analyser 6000). In acellular condition, both calcium and phosphate ion concentrations in media showed a decrease at 24 hours which was greater in SFM than in SCM. This suggests possible formation and precipitation of a bone like apatite on the surface of SiHA. Reduction in this activity observed in SCM indicates that the presence of serum proteins is interfering with the ion exchange at the material and media interface. Adsorbed protein levels showed fluctuation over time followed by sharp decrease at 24 hours, suggesting a possible protein rearrangement on the surface of the material. The ion analysis performed on SFM and SCM after 24-hour incubation with cells in the presence of granules showed a greater reduction in phosphate concentration in both SFM and SCM compared to phosphate levels in acellular condition. Silicate concentration in SCM increased from 1.6mM (absence of cells) to 5.1mM (presence of cells). This indicates that the cells are promoting the uptake of phosphate and release of silicate ions. No significant change was seen in levels of adsorbed proteins in the presence and absence of cells. Further analysis is required to determine whether the species of these proteins change over time. The analysis of cell migration after 24-hour incubation showed more cells migrating towards the granules, 12.7% in SFM and 8.3% in SCM, than in positive control, 4.5% in SFM and 3.6% in SCM respectively. These results suggest that SiHA has a chemotactic activity independent of serum proteins. A property which has not previously been demonstrated for a synthetic bone graft material.

Keywords : cell migration, hMSCs, SiHA, transwell migration system
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