Zinc and Magnesium doped Calcium Phosphate Nanomatrix for Bone Tissue Engineering

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Statement of Purpose: The calcium phosphate based ceramics has been utilized for treating bone defects as it is similar to natural bone and is highly biocompatible. A porous matrix (ZnCaMgP) developed from calcium phosphate containing specific percentages of magnesium and zinc exhibited excellent attachment and proliferation of osteoblast cells (1). This demonstrated its superior surface properties that are favorable for cell growth, cell matrix interaction and tissue formation. Calcium phosphates found in natural bone is in the form of nanometer-sized needle-like crystals of approximately 5-20 nm width by 60 nm length, with a poorly crystallized non-stoichiometric apatite phase containing other trace ions, which gives strength to the bone. Mimicking this nano structure will be beneficial in improved bone ingrowths. Therefore, an attempt has been made to develop a nanomatrix with the same combination of calcium phosphate with magnesium and zinc. Growth of osteoblast like cells onto this matrix was evaluated for the possible application of this matrix towards bone tissue engineering.

Methods: Calcium chloride, zinc chloride, magnesium chloride were from Sigma Chemical Co., USA. Di sodium hydrogen phosphate and other chemicals were from Merck India Ltd. Mumbai. Calcium phosphate was precipitated from the solution containing a mixture of calcium chloride, zinc chloride and magnesium chloride in the presence of simulated body fluid (1.5*SBF) and chitin nanofibres. Precipitation was initiated by slow addition of di sodium hydrogen phosphate into this mixture by stirring and 200 RPM. Final reaction mixture was kept for 24 hours without stirring and filtered. This gel like material was freeze dried to obtain porous nanomatrix. Osteoblast like cells MG 63 were cultured onto this matrix. The cell growth was studied using scanning electron microscope (SEM) and confocal microscope. The distribution of zinc, magnesium and calcium phosphate was evaluated using confocal raman microscope.

Results: The particle size of the precipitate evaluated using dynamic light scattering has shown that the particles are in the range of 20-80 nm. This was confirmed by transmission electron microscopy. The porous nanomatrix was evaluated using confocal Raman microscope. Maping was done after evaluating individual raman spectra of calcium, zinc and magnesium phosphates. The spectral map as shown in figure 1 indicates that components are uniformly distributed across the nanomatrix except magnesium phosphate which is indicated in blue. The SEM micrograph of the nanomatrix is shown in figure 2(a). It is a porous structure with 100 to 200 micron pores which seems to be optimal for bone ingrowth because macroporosity (pores >50 µm) is thought to contribute to osteogenesis by facilitating cell and ion transport (2). Figure 2(b) exhibits the growth of osteoblast like cells on to the nanomatrix. It exhibits the growth and proliferation of osteoblast like cells into the pores of the nanmatrix in 48 hours of culture. The cell growth and spreading was confirmed by evaluating using confocal microscope as shown in figure 3.

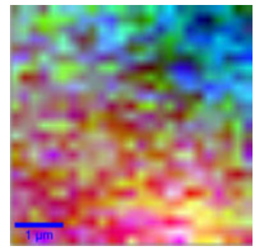

Figure 1. Raman spectral mapping of the nanomatrix. Red showing calcium phosphate, green showing zinc phosphate and blue showing magnesium phosphate.

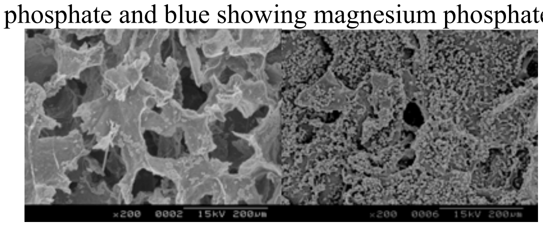

Figure 2. SEM micrograph of the (a) ZnCaMgP nanomatrix and (b) osteoblast like cells grown onto it.

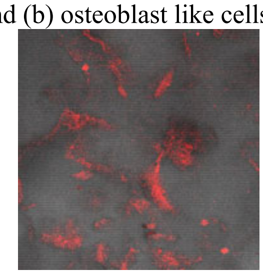

Figure 3. Confocal micrograph of osteoblast like cells grown onto the nanomatrix (actin stained).

Conclusions: The nanomatrix developed by mimicking the natural mineral formation, incorporating zinc and magnesium ions, exhibited excellent growth of osteoblast cells. For developing an ideal nanophase bone graft material, factors that are capable of triggering osteogenesis, i.e. osteoinductive growth factors like bone morphogenic proteins, also need to be incorporated into the nano-phase matrix.

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References:

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