Cytocompatibility and Phase Assemblage Study of heat treated potassium magnesium phosphate–silicate ceramics

Bikramjit Basu*, Ravi Kumar*, Sushma Kalmodia*, Shekhar Nath*, Dileep Singh**

*Laboratory for Advanced Ceramics, Department Materials and Metallurgical Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, UP, India

** Nuclear Engineering Division, Argonne National Laboratory, Argonne, IL, USA

Statement of Purpose: This article will report the study on a new generation bioactive ceramic, based on $MgKPO_4$ (Magnesium Potassium Phosphate, abbreviated as MKP) for biomedical applications. A series of heat treatment experiments on the slip cast silica (SiO_2) containing MKP ceramics were carried out at 900, 1,000 and 1,100^{o}C for 4 h in air. The density of the slip cast ceramic increased to 2.5 gm/cm^3 upon heat treatment at 900^{o}C. However, no significant change in density was measured upon heat treatment to higher temperature of 1,000 and 1,100^{o}C. On the basis of XRD results, the presence of $K_2MgSi_5O_{12}$ and dehydrated $MgKPO_4$ were confirmed and complementary information was also obtained using FT-IR and Raman spectroscopy. In order to confirm the in-vitro cytocompatibility property, the cell culture tests were carried out on selected samples and the results revealed good cell adhesion and spreading of L929 mouse fibroblast cells. MTT assay analysis with L929 cells confirmed non-cytotoxic behavior of MKP containing ceramics and the results were comparable with sintered HAp ceramics. It is expected that the newly developed MKP based materials could be a good substitute for hydroxyapatite (HAp or HA) based bioceramics.

Methods: The MKP ceramic was synthesized by reacting desired amounts (as per reaction 1) of calcined magnesium oxide (MgO) powder with potassium phosphate (KH_2PO_4) in aqueous conditions.

$$MgO + KH_2PO_4 + 5H_2O = MgKPO_4 \cdot 6H_2O \quad (1)$$

Gibbs free energy change for the reaction described by reaction 1 is -969.8 kJ/mol at 25^{o}C, which indicates the feasibility of spontaneous formation of MKP. In the present study, up to 50 wt% of silica (commercially available, size <50 μm) filter was added in the dry powder mix. After the step described by reaction 1, heat treatment of the as slip cast ceramic pellets were carried out at 900, 1,000 and 1,100^{o}C for 4 h in conventional pressureless sintering furnace.

Results: XRD spectra of MKP-1 (without heat treatment) shows the presence of MgO and $MgKPO_4 \cdot 6H_2O$ phases. It was found that MgO phase completely disappears on sintering above temperature 900^{o}C. The presence of potassium magnesium silicate ($K_2MgSi_5O_{12}$) phase was detected in all the sintered samples. The major peaks, detected for the sintered samples within the XRD detection limit were found to be SiO_2, $MgKPO_4$ and $K_2MgSi_5O_{12}$. Complementary information on phases was obtained through FTIR and Raman Spectra. The results of FTIR and Raman Spectra corroborated well with the XRD results. Figure 1 shows the SEM evidence of the adhesion of fibroblast L929 cells on MKP-4 (1100^{o}C, 4hr) and pure HAp after 3 days of culture.

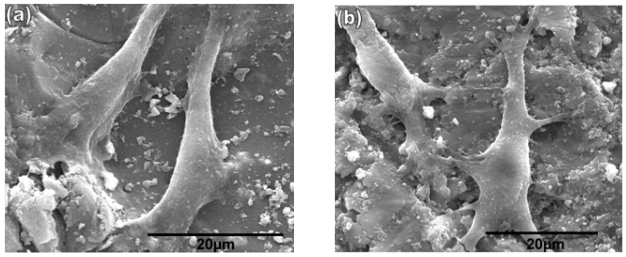

Figure1. SEM images, revealing the adhesion of L929 fibroblast cells on (a) MKP-4 (1,100^{o}C, 4 h) (b) pure HAp

It is clear from Fig. 1a that mouse fibroblast L929 cells can adhere and proliferate on the MKP ceramics (MKP-4). Cell proliferation, extra cellular matrix (ECM) formation and cell–cell contacts were the major observations in cell adhesion experiments. The SEM image (Fig. 1a) clearly shows cytoplasmic extension/cellular bridge formation on the surface of investigated ceramic. MTT results showed that the numbers of metabolically active cells in case of MKP ceramics are either equal or more than pure HAp. All the test samples i.e. pure HAp, and MKP-1 (as-cast), MKP-2 (900^{o}C, 4hr), MKP-3 (1000^{o}C, 4hr) and MKP-4 (1100^{o}C, 4hr) possess comparable cell proliferation.

Conclusions:

[1] XRD analysis confirms the presence of crystalline $K_2MgSi_5O_{12}$ and $MgKPO_4$ phases in the heat treated Complimentary information of the phosphate and silicate phases was obtained using FT-IR and Raman spectroscopy.

[2] The cell viability study using MTT analysis of the MKP samples, heat treated for 4 h, clearly indicates that the newly investigated phosphate ceramics are as non-cytotoxic as sintered HAp.

[3] The results of the cell culture experiments establish good cytocompatibility properties of MKP ceramics. The spreading/adhesion/proliferation characteristics of L929 cells on MKP containing ceramics are clearly comparable with that of pure HAp and control specimens.

[4] Based on the present results, it can be concluded that the slip casting and heat treatment combination can lead to the development of low density biocompatible non-cytotoxic phosphate–silicate ceramics.