

Supplementary Information

Mesoporous 45S5 bioactive glass: Synthesis, *in vitro* dissolution and biomineralization behaviour

Anil Kumar¹, Sevi Murugavel^{1*}, Anusha Aditya² and Aldo R. Boccaccini³

¹*Department of Physics and Astrophysics, University of Delhi, Delhi-110007*

²*CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi--110025*

³*Institute of Biomaterials, University of Erlangen-Nuermberg, 91058 Erlangen, Germany*

Cell culture studies

In order to evaluate the initial cell attachment and proliferation of the cells, elution test (ISO 10993-5) was carried out at different stages of the mesoporous bioglass at concentration of 10, 20, 50, and 100 µg/ml were incubated with U2OS cells 24 h and subjected to MTT assay. In MTT assay, the viability of cells grown in the presence of different concentrations of bioglass was assessed by a calorimetric MTT assay and it measures the reduction of the tetrazolium component into formazan crystals by viable cells reflecting the level of cell metabolism. For the assay, cells were then seeded onto 96 well culture plates with a density of 104 cells per well and they were incubated under standard culturing conditions. In order to carry out the assay, initially 100 mL of 10% MTT reagent was added and the plate was covered with foil leaving some free space to allow air inside and incubated for 2 h at 37 °C under cell culture conditions. After 2 h the plate was taken out and then 100 µL of dimethyl sulfoxide (DMSO) was added by removing the cell culture medium and incubated for 20 min at cell culture conditions to dissolve formazan crystals. After the incubation, the plate was taken out and read with 570 nm and 630 nm wavelengths using TECAN multimode plate reader. An obtained absorbance for each of the wells was averaged and the cell numbers determined by comparison with a typical calibration curve prepared using the data obtained from the wells containing known number of cells. The cells cultured

in DMEM medium were used as positive control. All the experiments were run with five samples and the data was illustrated as mean standard deviation. The statistical difference was analyzed using Students GraphPad and a P value of < 0.05 was obtained. A noticeable increase of cell proliferation was observed with increase of culture time, which could be considered good cytocompatibility of 45S5 glass due to superior textural property.