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Silk Fibroin-Alginate Based Beads for Human Mesenchymal Stem Cell Differentiation in 3D

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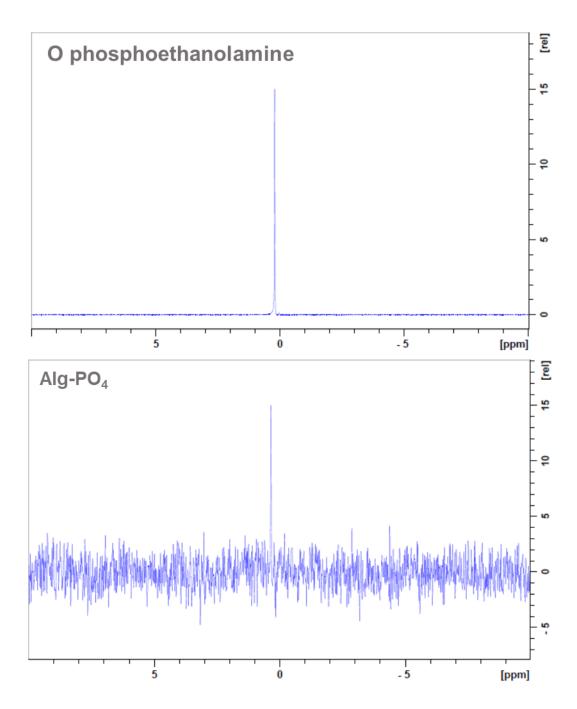


Figure S1. 31 P-NMR spectra of o-phosphoethanolamine and alginate conjugated with o-phosphoethanolamine (Alg-PO₄) in D₂O.

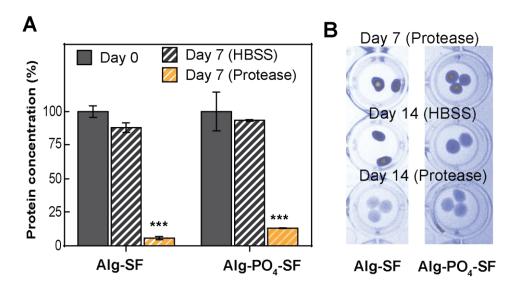


Figure S2. Degradation study of beads (A) Concentration of silk fibroin protein determined by BCA assay showing degradation of silk fibroin in presence of protease. (B) Beads stained with Coomassie Brilliant Blue R-250, the blue color in gels confirms presence of the silk fibroin protein. The image was captured and then pseudo coloured using UVP's IT² gel documentation system. (Two way ANOVA, *** p<0.001)

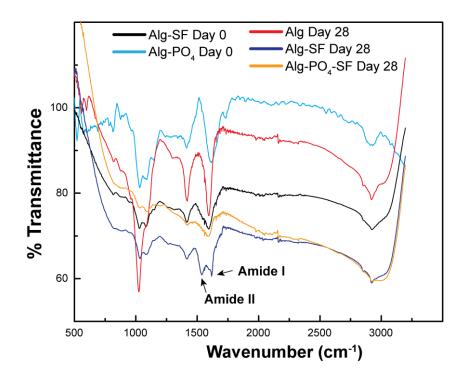


Figure S3. Attenuated total reflection-fourier transform infrared (ATR-FTIR) spectroscopy confirming presence of β sheet formation in beads containing silk fibroin after 28 days of incubation in HBSS.

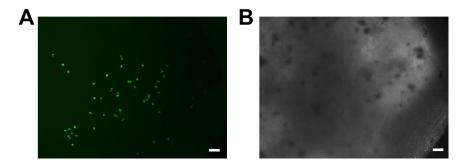


Figure S4. Live dead staining of alginate-silk fibroin bead treated with ethanol and then seeded with MG-63 cells after 14 days of incubation showing (A) fluorescence microscopy image (B) bright field image. Green: viable cells. Scale bar: $100 \, \mu m$.

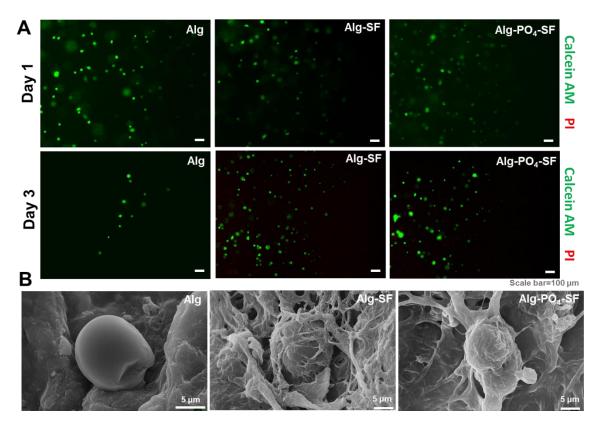


Figure S5. (A) Fluorescence microscopy images of live dead staining of hMSCs seeded beads after 24 h and 72 h incubation (merged image: red, green). (B) Scanning electron micrograph of lyophilized beads, 7 days after hMSCs seeding.

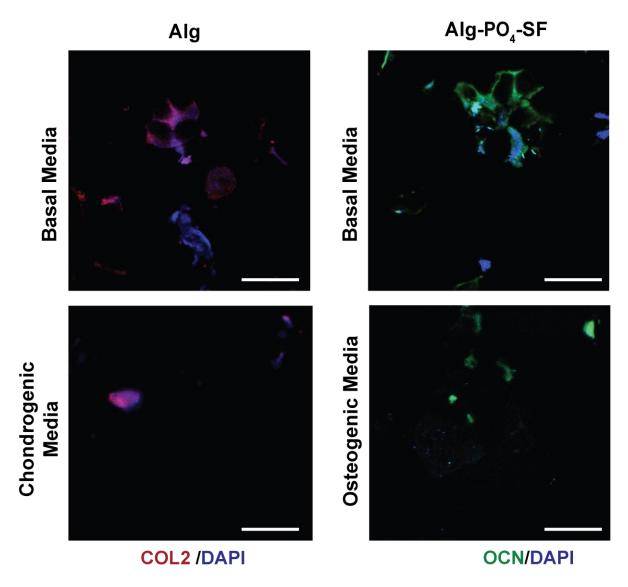


Figure S6. Confocal microscopy images of hMSCs in gels after 28 days of incubation in basal media or differentiation media. The hMSCs were stained with antibodies against collagen II (COL2) and osteocalcin (OCN). Blue: DAPI, Red: Alexa Fluor 594, Green: Alexa Fluor 488. Objectives used for imaging: 20x, 60x. Scale bar:50 μm