Microsphere scaffolds provide a unique three-dimensional micro-environment for the cells cultured on their surfaces through their curvature. Many studies have shown previously that the topographical cues like curvature have an effect on the differentiation abilities of the stem cells. Similarly, studies have also shown that mechanical cues like matrix stiffness and various other biomolecular signals can play a crucial role in determining the stem cell fate. In this study, we designed a three-dimensional composite scaffold by encapsulating gelatin microspheres in collagen hydrogels, in order to present a combination of topographical, mechanical, and biomolecular signals to human adipose derived stem cells (ADSCs) and studied their osteogenic differentiation abilities. Topographical cues are provided in the form of microsphere curvature by culturing ADSCs on gelatin microspheres to form cell-microsphere aggregates. Further, varying amounts of these cell-microsphere aggregates are then encapsulated into collagen hydrogels to fabricate microsphere-hydrogel composite scaffolds with varying mechanical properties. The mechanical properties of such scaffolds were studied using rheometry. ADSCs encapsulated in such composite scaffolds with varying mechanical properties were induced towards osteogenic lineage and further characterized using gene expression studies of osteogenic marker genes and by measuring the alkaline phosphatase activity. We found that encapsulating increased amounts of microspheres in collagen hydrogels increases the storage modulus of the gels and favors the osteogenic differentiation of ADSCs. To further accentuate the osteogenic differentiation of ADSCs, we then provided the biomolecular cues by encapsulating basic fibroblast growth factor (bFGF) into the scaffolds and releasing it in a controlled manner. Gene expression of osteogenic marker genes and alkaline phosphatase activity of ADSCs upon differentiation in the bFGF encapsulated scaffolds seem to be enhanced compared to the scaffolds with bFGF supplementation directly in the media. Overall, this study shows that osteogenic differentiation of ADSCs can be enhanced by culturing them in microsphere–hydrogel composite scaffolds which can subsequently be used as effective injectable delivery vehicles for ADSCs as well as various biomolecules.

Acknowledgement: The authors would like to acknowledge funding from the National University of Singapore.