Tissue regeneration using autologous adult human mesenchymal stem cells (hMSCs) has become an attractive approach due to the reduced probability of transplant rejection. The classic differentiation approach involves a combination of mechanical and chemical stimulation. Use of a batch bioreactor flowing media containing differentiation growth factors while the hMSCs remain adhered to a rigid culture surface is widely accepted. However, this system of differentiation requires a significant amount of time. When in a clinical setting and quickly trying to treat and regenerate tissue for patients, time is considered an antagonist. The ability to differentiate hMSCs without a bioreactor or specific differentiation media would substantially contribute to the creation of more efficient biocompatible scaffolds that incorporate the differentiated hMSCs of the patient. This work examined the differentiation of hMSCs via shear stimulation through biocompatible plastic tubing in a suspended flow.

The in vitro investigation of mechanically and chemically stimulated hMSCs in a suspension was performed by: (1) Determining the length of plastic tubing which induced a differentiation response of the hMSCs. (2) Examining intracellular receptors using fluorescent markers to resolve differentiation toward the adipogenic lineage. (3) Incubating the hMSCs with fluorescently tagged antibodies corresponding to differentiation along the adipogenic and chondrogenic lineages. (4) Analyzing the genetic fold changes related to the shear stimulation of hMSCs.

At a flow rate equivalent to physiological shear stress, 15 dyn/cm$^2$, undifferentiated hMSCs were exposed to an environment of shear through syringes connected to biocompatible tubing with varying lengths. The resulting fluid stimulation, which took place for approximately 20 minutes, was capable of accelerating the differentiation of hMSCs toward the adipogenic and chondrogenic lineages. Differentiation toward adipocytes was confirmed through the observations of accumulated lipid triglycerides and the increased fold change for adipogenic markers such as LPL1, CFL1, and SSP1. The increased concentration of Type 2 Collagen on the surface of shear stimulated hMSCs with the upregulation of MAPK1, SOX9, and FARP1, demonstrated the capabilities to induce sustained differentiation into the chondrogenic lineage. This work of shear stimulating hMSCs, in combination with chemical stimuli, illustrates ameliorated differentiation of hMSCs toward the adipogenic and chondrogenic lineages.

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