"Bone Marrow Microenvironment Culturing System for Mesenchymal Stem Cells"

Taylor Marohl, Maddie Meier, Veronica Porubsky, and Michelle Tong

BME Design Excellence Award Executive Summary – Team 11

Degenerative musculoskeletal diseases such as Osteoarthritis (OA) affect the majority of the aging adult population. OA is characterized by a breakdown of cartilage that results in extreme pain, stiffness, and swelling of the joints. Current therapies, such as pharmacological approaches and physical therapy, only influence surface-level symptoms and do not target the underlying disease mechanism. To address the shortcomings of existing OA therapies, our client Professor Wan-Ju Li, aims to develop a regenerative medicine approach using human mesenchymal stem cells (hMSCs). hMSCs are self-renewing cells capable of differentiating into osteocytes, adipocytes, and chondrocytes. Clinical applications often extract hMSCs from bone marrow, as this is considered the most accessible and enriched source. While the bone marrow microenvironment is characterized by a low stiffness extracellular matrix, low oxygen tension, and a variety of secreted factors, *in vitro* cell culture often consists of a stiff polystyrene surface, a high oxygen tension of 21%, and selected factors included in cell culture media. In an attempt to maintain hMSCs in the quiescent state of that found in natural bone marrow, Professor Li has requested a culturing system that recapitulates the substrate stiffness and oxygen tension of bone marrow. To this end, we aimed to develop a RGD-conjugated PEGDA hydrogel and a cell culture chamber with user-set oxygen tension.

In order to mimic the bone marrow extracellular matrix with a hydrogel, we first conducted a comprehensive literature search to determine the stiffness of bone marrow, which can range from 0.1 – 0.2 kPa. We also obtained bovine and porcine femoral bone samples in order to achieve a better understanding of the composition and appearance of bone marrow. We chose to focus on polyethylene glycol diacrylate (PEGDA) as a biomaterial due to its inertness and biocompatibility, as well as ease of use and manipulation. Since PEGDA does not contain cell adhesive sequences, we aimed to include an Arg-Gly-Asp (RGD) sequence. Initial testing with 700 Da PEGDA yielded gels of higher stiffness (1 - 7 kPa) than that of bone marrow. We altered our molecule to 2000 Da PEGDA in order to achieve a lower-stiffness gel; these hydrogels are still in the production stage. After production and stiffness characterization, the gels will be evaluated for their ability to maintain hMSC quiescence by a 7 day cell culture experiment. The resulting cell population will be evaluated for quiescence cell markers through FACS and senescence markers p16 and p21 through RT-PCR.

In order to mimic the low oxygen tension found in bone marrow, we aimed to produce a cell culture chamber that could be set by the user to any oxygen tension. Since hypoxia chambers are available commercially, we focused on producing a chamber that was inexpensive and easy to assemble. This system allows the maintenance of consistent gas concentrations within the chamber, allowing for cell culture at low oxygen tension while maintaining appropriate conditions for the cells. The effects of low oxygen tension on hMSC quiescence will be evaluated using the same cell culture experiment described above for the hydrogel.

At the final stage, the hypoxia chamber and PEGDA gels will be combined to examine the effects of low oxygen tension and low gel stiffness on hMSC quiescence, demonstrating that the bone marrow microenvironment can be recreated *in vitro* for potential use in regenerative medicine.