

Establishing an allogeneic mesenchymal stem cell bank for regenerative medicine research at the UF Veterinary Teaching Hospital

Mesenchymal stem cells (MSCs) are multipotent stem cells present in virtually all adult tissues that participate in homeostasis and repair by the secretion of paracrine factors and differentiation in tissue-specific cells. MSCs can be isolated with high yield and maintain their proliferative capacity and genetic stability for several passages *in vitro*. They are classified as low or non-immunogenic as they express low levels of MHC I and lack MHC II and other co-stimulatory molecules. They do not cause T-cell activation and consequent rejection when allogeneically or xenogeneically transplanted, and due to their limited plasticity, they do not form tumors *in vivo*. MSCs have been tested for multiple therapeutic applications in regenerative medicine, including neurodegenerative disorders, renal failure, diabetes, and skin wound healing. Their ability to promote cell growth, angiogenesis, and immunomodulation has been widely described and supported by basic, pre-clinical, and clinical studies. The goal of this study is to establish a canine and feline MSC bank for future regenerative medicine applications at the UF Veterinary Teaching Hospital. The student will participate in the following aims of this study:

Aim 1: Isolate, expand, and cryopreserve canine and feline adipose-derived mesenchymal stem cells

MSCs will be isolated following our established protocols, from fragments of adipose tissue discarded during elective surgeries (spaying) of healthy donors at UF Veterinary Teaching Hospital. Isolated cells will be cultured and passaged for expansion *in vitro* and cryopreserved in liquid nitrogen for downstream applications.

Aim 2: Validate cell identity through phenotypic characterization and trilineage differentiation

MSC stemness validation will be performed by immunostaining for CD90+, CD73+, CD105+, CD34-, CD45-, and differentiation to osteo-, chondro-, and adipogenesis following our established protocols.

This project is significant because it will help to establish a UF veterinary stem cell bank that will be available for collaborative research across the college. The student will learn a broad range of laboratory techniques including primary cell culture, flow cytometry, immunocytochemistry, and qPCR. Additionally, the student will be instructed about stem cell biology and regenerative medicine, as well as the regulations required for cellular therapies in veterinary medicine.

This project is part of a broader study aiming to investigate the MSCs' therapeutic potential in a series of diseases and conditions, using *in vitro*, rodent models, and clinical trials. These conditions include sepsis, chronic skin wounds, and chronic kidney disease, and will be tested in collaboration with clinical faculty (Dr. Ronald Goncalves). Other possibilities, such as non-healing bone fractures, diabetes, and spinal cord injuries may be also studied in future collaborations.