***Specific Aims***

As modern medicine has eliminated many causes of early life mortality, human life expectancy has increased rapidly. Because of this, most people now live long enough to experience disability resulting from age-related degenerative disease. By the year 2030, it is expected that the percent of people over the age of 65 in the US population will be roughly 20% (Powell, 2010). Currently, the demographic over the age of 65 suffers from degenerative diseases at alarmingly high rates: 50% suffer from osteoarthritis, 50% suffer from xerostomia, 10% suffer from Alzheimer’s disease, 25% suffer from type II diabetes, and many more suffer from a myriad of other age-related degenerative diseases including, but not limited to, Parkinson’s disease, multiple sclerosis, macular degeneration, sarcopenia, etc. Together, these result in varying degrees of disability among elderly populations and represent a substantial and growing public health concern.

Implicated in the pathogenesis of all of the diseases mentioned, and perhaps all age-related degenerative disease, is inflammation. Due to their ability to modulate inflammation locally and systemically via paracrine effects by secreted cytokines, human mesenchymal stem cells (MSCs) represent a promising treatment for a variety of age-related diseases. However, there remain significant hurdles to realizing their full therapeutic potential. Due to availability, immunological concerns, and risk of disease transmission from allograft tissue, it is preferable to use autologous cells. However, the quality and quantity of mesenchymal stem cells declines with age limiting their therapeutic potential. In fact, in preliminary work, we have demonstrated that elderly MSC populations possess a substantial number of senescent cells that secrete a number of proinflammatory cytokines termed the senescence-associated secretory phenotype (SASP) (ref?). Hence, elderly MSCs may promote tissue degeneration rather than aid regeneration. In order for stem cell-based therapies to realize their full potential, it may be essential that a strategy be developed for reliably rescuing the regenerative capacity of human mesenchymal stem cells from elderly donors.

Our group has recently licensed a technology from University of Texas Health San Antonio, for the isolation and expansion of a sub-population of “youthful” bone-marrow derived mesenchymal stem cells from elderly donors [REF]. The approach utilizes fluorescence activated cell sorting (FACS) to isolate a sub-population, characterized by high expression of SSEA-4 and small cell size. This sub-population represents a small proportion of the overall population (5-10%) in donors over the age of 65, but following isolation from what appears to be a negative in situ micro environment these cells are demonstrated to be phenotypically and functionally similar to BM-MSCs from a young donor. Upon isolation, these cells are expanded using our group’s core technology, a biomimetic microenvironment elaborated by stem cells (Bone Marrow- High Performance Microenvironment or BM-HPME®). Expansion on BM-HPME® facilitates the rapid proliferation of the sub-population without the loss of stemness resulting from replicative senescence or spontaneous differentiation that one often observes using traditional two-dimensional cell culture strategies. Using this two-pronged strategy, large quantities of high quality cells may be obtained from otherwise compromised stem cell populations.

While promising, no data yet has indicated that these cells are capable of therapeutic effect in humans or animals. Some of the most striking data from earlier work done on this cell population reveals that while elderly MSC populations express the SASP the small, SSEA-4+ sub-population does not. This suggests that elderly MSCs may contribute to age-related degeneration and inflammation, by their paracrine effects, whereas the small, SSEA-4+ sub-population once isolated and placed in a more conducive micro environment may have a more typical MSC cytokine profile (anti-inflammatory/immunomodulatory). Based on this data, we propose a feasibility test to determine whether this sub-population could hold promise for the treatment of chronic systemic inflammation and other resulting diseases of aging. In order to determine feasibility, we intend to test the hypothesis that the “youthful” sub-population possesses a cytokine profile that is immunomodulatory and anti-inflammatory. In order to test this hypothesis, we propose the following aims:

**Specific Aim 1:** To compare immunomodulatory capability of young, elderly and small(+) MSCs expanded on either TCP or BM-HPME® in a lymphocyte proliferation assay.

**Specific Aim 2:** To characterize the cytokine profile of small(+) MSCs relative to young and elderly MSCs.

The proposed study will allow our group to determine the feasibility of using our innovative approach to address a pressing global health issue. This line of work has the potential to drastically alter the treatment of an array of degenerative illnesses, and builds on our company’s core competencies. The primary inventor on the relevant patent is the PI on this grant, the inventor of HPME® is a scientific advisor, and we have existing collaborations with UT Health San Antonio, BioBridge Global, and the US Army Institute for Surgical Research that would be useful in translating any promising results to preclinical models and then scaling for commercial applications. The expertise, infrastructure, and intellectual property position of our company and our collaborators make us uniquely well-suited to pursue this line of questioning and to commercialize the resulting products/services.