Isolation and Characterization of Unique Cell Populations from Human Anterior Cruciate Ligament for Use in Tissue Engineering

Natalie L. Leong, MD1, Michael G. Yeranosian2, David R. McAllister, MD1, Denis Evseenko, MD, PhD1, Frank A. Petrigliano, MD1.

1University of California, Los Angeles, Los Angeles, CA, USA, 2Rutgers New Jersey Medical School, Newark, NJ, USA.

Disclosures:

Introduction: Rupture of the anterior cruciate ligament (ACL) is one of most common ligament injuries of the knee1, with over 200,000 patients diagnosed with ACL disruptions annually2. Due to the ACL’s inherent inability to heal, as many as 175,000 cases of diagnosed ACL injuries require surgery each year in the United States2, with an estimated cost of one billion dollars annually3. Due to various limitations in current grafts and advances in biology and materials science, there is a growing interest in tissue engineering of an ACL replacement. A challenge in ACL tissue engineering is finding an appropriate cell source that survives in vivo transplantation and can contribute to the regeneration of the ACL in vivo. The most logical choice is adult ACL fibroblasts, but these cells alone are usually insufficient in that they have low proliferation and synthetic capacity. Recent progress in stem cell biology has shown that mesenchymal stem cells (MSCs) not only function as a cell source during tissue regeneration, but also provide trophic support to parenchymal cell types. We hypothesize that native MSCs that reside within adult ACL will promote survival, proliferation, and function of structural ligament-forming fibroblasts (LFF) during ACL regeneration. Isolation and ex vivo expansion of purified mesenchymal populations naturally present in ligament tissue, with subsequent co-implantation of these cells on biodegradable scaffolds with isolated structural ligmental fibroblasts represent a novel, previously unexplored approach for ligament restoration. In this study, we identify the presence of different MSC populations in the ACL using immunohistochemistry. Additionally, we isolate these different populations using FACS and examine their differentiation potential in vitro.

Methods: After obtaining appropriate IRB approval from our institution, ruptured human ACL tissue was obtained from 5 adult patients undergoing primary ACL reconstruction surgery. The samples were embedded in paraffin and prepared into 5-micron thick sections, which were immunohistochemically stained with a number of mesenchymal and stem/progenitor cell markers including CD146, CD44, and CD34. Through immunohistochemistry, three unique populations of cells were identified. Next, human ACLs were enzymatically digested with collagenase I and DNAse and sorted by FACS. The resulting 3 cell populations were plated in tissue culture flasks in medium supplemented with platelet derived factor and fibroblast growth factor-2. Cell proliferation was assayed using BrdU assay, and differentiation potential was assessed by inducing bone, cartilage, and fat using commercially available differentiation medium.

Results: Immunohistochemistry showed there are two distinct MSC populations and one fibroblast population within the ACL. Cells that were positive for CD146 and CD44 and negative for CD34 were observed in the perivascular region. These cells were pericytes, or perivascular stem cells, a type of MSC (Figure 1a) which were classified as ligament perivascular cells (LPC). Another mesenchymal population was mapped in the loose interstitial sheaths located between structural collagen fibers of the ligament body. These cells were not structurally associated with blood vessels and were positive for CD34 and CD44, but not CD146. These cells were an adventitial mesenchymal population which were classified as ligament interstitial cells (LIC). Lastly, ligament-forming fibroblasts (LFF) were observed within highly aligned dense collagen fibers. These cells were highly positive for CD44 and showed no expression of CD34 or CD146. Flowcytometric analysis also demonstrated these three main cell subsets highly. The three populations were isolated and defined as follows: 1) LFF: CD146negCD34negCD44+, 2) ligamental perivascular cells (LPC): CD146negCD34 negCD44+, and 3) ligamental interstitial cells (LIC): CD146negCD34negCD44+ (Figure 1b).
All cell populations demonstrated significant
potential for ex vivo expansion. Based on the cell proliferation assay, the LIC population was the most proliferative, followed by the LPCs. Proliferation capacity of LFF cells, based on the average doubling time, was almost 5 fold lower than for the LIC population. Both the LPC and LIC populations were able to differentiate into bone, cartilage, and adipose-like phenotypes with the addition of the appropriate differentiation media (data not shown).

**Discussion:** These two mesenchymal-like cell populations (LPC and LIC) have never been previously isolated and described in ligamental tissues and may represent a specialized pool of progenitors. All three cell populations isolated showed significant potential for ex vivo expansion, which is important in culturing sufficient cells for use in tissue engineering applications. Both LIC and LPC were capable of making bone, cartilage and fat in vitro, indicating that both of these cell types are multipotent, as would be expected of mesenchymal stem cell populations. All together, these studies demonstrate the existence of two mesenchymal cell-like, highly expandable populations in the ACL tissue and offer a strategy for the FACS-based isolation of these populations. Future work will focus on defining the therapeutic potential of these cells when co-cultured at in vitro and in vivo.

**Significance:** This study identified two novel populations of ligament-derived mesenchymal stem cell-like cells for use in ligament tissue engineering. Potentially, these cell populations, which are native to the ACL, could provide more robust support of ligament-forming fibroblasts in terms of facilitating cell survival, proliferation, and matrix elaboration, allowing for an enhanced tissue-engineered replacement for the ACL.

**Acknowledgments:** Supported in part by the H H Lee Research Program.


ORS 2014 Annual Meeting
Poster No: 1376