Study of the Stem Cells from Goat Bone Marrow Stroma Placed on a Biological Graft for Artificial Ligament

Qiuying Liu[1], Yifei Wang[1], Xinlei Peng[1] Yongliang Lin[2], Bin Xu[2], Xiaoming Guo[2], Guofeng Xu[2], and Bruce Z Gao[3]

[1]Guangzhou Jinan Biomedicine Research & Develop Center, Jinan University, Guangzhou, China

[2]Grandhope Biotech Co., Ltd., Guangzhou Science Park, Guangzhou, China

[3]Clemson University, Clemson, SC

Statement of Purpose: The anterior cruciate ligament (ACL) is a strong, rounded band of tissue that extends laterally from the head of the tibia to the intercondyloid notch of the femur to prevent the tibia from slipping backward. It is the most commonly injured ligament in the human knee. When a torn ligament cannot be repaired by the body itself or through a surgical intervention, it must be replaced. Most replacements come from connective tissues in the patient's own body. However, autograft reconstruction of the ligament often results in additional complications at the graft donor site; to overcome this problem, artificial ligaments have been developed. One approach to reconstructing the ligament artificially is the use of a treated animal tendon and ligament as a scaffold to grow the required ligament either in vitro or in vivo. Grandhope Biotech has developed an animal tissue treatment technique involving epoxy crosslinking fixation, diversified minimization of antigen, special tissue tanning, and surface activity modification that has been used as the base for the development of a unique biological ligament graft. Transplantation of this graft into a goat ACL injury model has demonstrated regeneration of functional ligament. In this report we present our study results on the in vitro cultivation of stem cells from goat bone marrow stroma on the biological graft. This study is the first step towards future exploration of the effect of seeded patient's stem cells on the regeneration rate of the ligament.

Methods: The preparation procedure of the scaffold from fresh porcine tendon included bioburden, impurity elimination, pretreatments, epoxy crosslinking fixation, protein molecule modification to raise the mechanical strength, diversified antigen eliminations, surface activity modification, and terminal sterilization. The tissue was harvested and treated with two pieces of bone connected at the two ends for the convenience of transplantation (Fig.1). The stem cells were harvested from goat bone marrow and purified by using the adhesive assay. The differentiation potential of the purified stromal stem cells was verified by inducing the cells to differentiate into adipocytes (Fig. 2). After three passages, the stem cells were seeded onto the graft placed inside a T75 flask. The cell numbers were counted by trypsinizing cells on the scaffold, and cell development was analyzed by using conventional histological methods. MTT assay was employed to assess the effect of bFGF on the development of the stem cells on the scaffold.

Results: The histological analysis is shown in Fig. 3. The number of healthy cells attached to the graft was counted as $1.5\times10^5/cm^2$ after one week of stem cell seeding and $2.2\times10^5/cm^2$ after two weeks. The stem cells were attached on the surface of the graft, composed of dense, aligned collagens. Addition of bFGF in the tissue culture flask was proved to promote the growth of the stem cells attached to the graft.

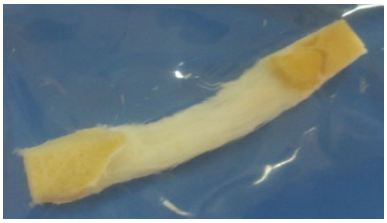

Figure 1. Porcine ligament graft

Figure 2. Adipocytes induced from purified bone marrow stromal cells

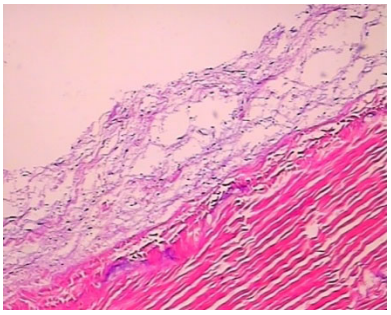

Figure 3. Histological image of bone marrow stromal stem cells on ligament graft (X40): on the surface of the tightly aligned collagen, stem cells were attached and grown normally two weeks after cell seeding

Conclusions: The unique tissue-treatment technique is able to effectively eliminate the heterogenic collagens on the porcine tendons and ligaments that would result in immune responses. Purified bone marrow stromal cells with certain differential capacity can be grown on the scaffold normally. These preliminary results have laid the foundation for the next phase of study to explore the possibility of placing stem cells from the patient on the graft before transplanting it into the patient's knee.

References:

1. Wang K, Zhu L, Cai D, Zeng C, Lu H, Xu G, Guo X, Lin X, Chen S. Microsurgery. 2008;28:44-53.