Biologically Enhanced Healing of the Human Rotator Cuff: 8-month Postoperative Histological Evaluation

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Abstract: Given the high percentage of persistent rotator cuff defects, investigators have begun exploring techniques that use biologic adjuvants to recreate a biomechanically equivalent layer of connective tissue. To evaluate the efficacy of a mesenchymal stem cell, platelet-rich plasma, and dermal allograft construct, a histological comparison of native rotator cuff tissue and biologically enhanced rotator cuff tissue was performed. The evaluation indicated that this treatment modality in conjunction with an adjusted rehabilitation protocol may successfully recreate a transition zone and restore a synovial lining similar to native tissue.

Successful repair of the rotator cuff requires tendon-to-bone healing. Despite reported improvements in techniques and instrumentation,1-3 retear rates are as high as 94%.1-8 Following rotator cuff repair, the fibrocartilaginous transition zone becomes infiltrated with scar tissue.9-15 Without restoration of physiologic tissue, the ability of the tendon to transmit force from muscle to bone is obstructed. The structural integrity of the tendon is weakened, increasing susceptibility to repetitive tears. Consequently, investigators have begun exploring techniques that use biologic adjuvants to restore a native fibrocartilaginous transition zone.16-20

Mesenchymal stem cells (MSCs) are nonhematopoietic, multipotent cells capable of differentiating into tissue-forming cell lineages.21-23 Given their potential to differentiate into tenocytes and chondrocytes, several studies have investigated the ability of MSCs to facilitate tendon healing in animal models.24-28 Despite evidence that MSCs are incapable of single-handedly mediating tendon-to-bone healing,28 recent research has indicated that the additional application of growth factors is necessary to optimize the microenvironment and the healing capacity of MSCs.29

In a rabbit patellar tendon model, Zhang and Wang30 demonstrated that a platelet-rich plasma (PRP) releasate application can stimulate tendon stem cell differentiation into tenocytes and increased collagen production. Although these findings are encouraging, few studies have investigated whether these results can be extrapolated to humans.31

In an initial report, a MSC, PRP, and dermal allograft construct was used to repair a repetitively failed human rotator cuff.31 Because the optimization of biologic augmentation was dependent on the timing of mechanical stimulation, the rehabilitation protocol was designed in accordance with the temporal parameters of healing.32 Magnetic resonance imaging (MRI) confirmed the integrity of the cuff at 3, 6, and 9 months. By the 9-month clinical evaluation, the patient had regained normal use of the shoulder. Although promising, further investigation of this treatment modality to recreate a biomechanically equivalent layer of connective tissue is warranted.

The purpose of the current study was to further evaluate the efficacy of a MSC, PRP, and dermal allograft construct coupled with alterations in the rehabilitation protocol to restore native rotator cuff tissue. A histological comparison of native rotator cuff tissue and...
biologically enhanced rotator cuff tissue was performed. It was hypothesized that the biologically augmented rotator cuff tissue would feature histological similarities to normal rotator cuff tissue.

Informed written consent was obtained from the patient to publish text and images for scientific and educational purposes. Her confidentiality and privacy were ensured and maintained. Data were abstracted into a coded and secure database. Potential conflicts do not exist.

Case Report

In August 2011, a 52-year-old woman presented to the outpatient clinic reporting pain in her left shoulder. Magnetic resonance images confirmed a full-thickness retear with approximately 2.7 cm of the supraspinatus tendon retracted (Figure 1A). Given her history of recurrent tears, the patient elected to undergo rotator cuff repair using a MSC, PRP, and dermal allograft construct. In September 2011, the procedure was performed. The surgical procedure and rehabilitation protocol adhered to previously described techniques.

At the initial follow-up 10 days postoperatively, the patient reported minimal pain and no longer required pain medication. The surgical incision was clean, dry, and intact. Upper compartments were not swollen. Palpation of the right shoulder demonstrated no tenderness.

Two months postoperatively, the patient was in a motor vehicle accident. Pain in the left and right shoulders returned. Magnetic resonance images confirmed the integrity of the left rotator cuff (Figure 1B) but indicated a full-thickness retear of the right rotator cuff with approximately 3.5 cm of the supraspinatus tendon retracted (Figure 1C). The patient elected to undergo a rotator cuff repair of the right native shoulder using a MSC, PRP, and dermal allograft construct.

The orthopedic surgeon (J.K.H.) spent considerable time discussing the risks associated with biopsying a previously repaired rotator cuff. After multiple discussions, the patient agreed to undergo biopsies of both shoulders at the time of right shoulder rotator cuff repair. She reasoned that the biopsies have the potential to extend the existing scientific knowledge of this technology and may eventually serve as a treatment option for other patients with repetitively failed rotator cuff repairs.

In May 2012, eight months after rotator cuff repair, the orthopedic surgeon biopsied a fragment of tissue from the left (augmented) and right (native) rotator cuffs. The biopsy of the augmented rotator cuff was obtained from the central aspect of the supraspinatus tendon at the musculotendinous junction. The biopsy from the normal rotator cuff was obtained from an area that was distinct from the site of injury.

The biopsies were fixed in 10% buffered formalin. Each measured approximately 1 cm at the greatest dimension. The tissue was serially sectioned and submitted for histologic evaluation. The pathology was reviewed by the Chief of Bone and Soft Tissue Pathology in the Lehigh Valley Health Network in Allentown, Pennsylvania (G.A.S.). Following routine processing, the tissue was embedded in paraffin. Five-micron-thick sections were stained with hematoxylin-eosin. Additional sections were stained with Verhoeff-van Gieson elastin stain for examination by routine light microscopy.

Results

Intraoperative Findings

Incorporation of the graft was evident intraoperatively. The graft appeared contiguous with the surrounding tissue (Figure 2A). After the biopsy was obtained, a rich capillary ingrowth was visualized (Figure 2B).

Histological Assessment

The native biopsy revealed a typical thin, dense band of collagen consistent with fibrotendinous tissue, and the
superficial surface exhibited adipose and neurovascular tissue (Figure 3A). The deep surface showed a thin synovial lining with abundant capillaries (Figure 3B).

The augmented biopsy showed a dense band of collagen and elastin, approximately 4 times as thick as the native side, consistent with the dermal collagen graft (Figure 3C). The graft, which was devitalized and acellular at the time of placement, demonstrated a diffuse population of viable fibroblasts and small chondrocyte-like cells elaborating a delicate myxoid matrix, resembling normal fibrocartilage (Figure 3D). The surface juxtaposed to the joint now showed a synovial-type lining rich in capillaries, formed on the subdermal surface of the augmented tissue.

Because the allograft was devitalized and contained no living cells prior to implantation, these findings suggest that the collagen–elastin scaffolding of the allograft had become secondarily populated by chondrocytes and fibroblasts. However, it is not possible to determine whether the chondrocytes, fibroblasts, and synovium differentiated from donor MSCs or migrated from the patient’s adjacent native tissue. In either case, the biologically augmented construct was fully incorporated and underwent tissue remodeling. Evidence existed of collagen fiber reorganization and fibrocartilage formation that resembled normal rotator cuff tissue.

The subdermal surface of the augmented biopsy was interspersed with vascular and neural components. Similar to the normal rotator cuff, the subdermal surface now showed a synovial lining.

Although the concomitant delivery of MSCs, PRP, and a dermal allograft during rotator cuff repair followed by subsequent adjustments in the rehabilitation protocol appear to accelerate the healing cascade and aid in the repair of repetitively failed full-thickness rotator cuff tears, the current case report evaluated the histological composition of the fibrocartilaginous transition zone 8 months after rotator cuff repair using a previously reported repair technique. The acellular dermal allograft served as a delivery vehicle that introduced the MSCs and PRP to the local cellular environment in close proximity. Presumably, when activated, the platelets released growth factors that provided the necessary cellular and molecular signals to differentiate the MSCs. This was evidenced by collagen fiber reorganization and fibrocartilage formation that was similar to native tissue. Working together, the growth factors and MSCs effectively remodeled and incorporated the acellular dermis. The presence of neural and vascular tissues also indicated that the correct cellular and molecular signals were activated to induce angiogenesis and neural infiltration.

Had an additional sample of donor MSCs been available or had the sex of the donor and recipient been different, molecular studies, such as polymerase chain reaction or sex chromosome centromeric fluorescence in situ hybridization, could have been used on the paraffin-embedded tissue to confirm the persistent presence of donor MSCs in the graft, further elucidating the physiology underlying this treatment modality.

Collectively, the results of the current histological evaluation...
tion suggests incorporation and microanatomic remodeling of the graft into more physiologic rotator cuff tissue. These findings demonstrate the effectiveness of biological enhancement and alterations in the rehabilitation protocol to induce vascularization, promote neural infiltration, and repopulate damaged tissue with chondrocytes and fibroblasts in the human rotator cuff.

This combination of biological adjuvants and adjustments in the rehabilitation protocol appear capable of recreating a fibrocartilaginous transition zone similar to native tissue. Future studies capable of differentiating donor MSCs from host cells are warranted to further understand the physiologic process of healing in the presence of a MSC, PRP, and dermal allograft construct.

REFERENCES