# Rotator Cuff Repair with Purified Exosome Product (PEP) in a Canine Model.

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INTRODUCTION:
Rotator cuff consist of four muscles with tendons, surrounding the shoulder joint capsule circumferentially and stabilize the joint biomechanically. The most common cause of shoulder pain and dysfunction in all age groups is rotator cuff tears, which affect over 35% of the population over 50. More than 4.5 million patients visit orthopedic surgeons and approximately 300,000 outpatient surgical repairs are performed at a cost of $26,000-50,000 per procedure with a return to work between 7-11 months. Surgical repair is a common treatment, but recurrent tears occur in 20 to 90% of the patients, especially in those with large or massive tears. Recently, biomaterial or mechanical augmentations has been used to accelerate tissue healing and increase mechanical strength of the repaired rotator cuff and to improve the functional outcomes following repair. Research on this topic has been energized in recent decades including cell-based therapy, tissue engineering, bioactive growth factors, etc. In our lab, a new technique to improve the repair has been developed using a biological method. Recently, Mayo Clinic Center for Regenerative Medicine developed and manufactured a GMP grade purified exosome product (PEP) for clinical translation. With the strong support of our promising preliminary data. Exosomes transfer biological signals from the donor cell to the target cell and are critically involved in both physiological and pathological processes. Compared with other biological strategies such as plasma, PRP and stem cells, exosomes are immune-privileged and exhibit low species differences in structure and elements. A clinical translation, preclinical large animal model is needed, to show whether PEP can be effective when the repair and healing is scaled up with clinically used surgical repair techniques. The aim of this study is to define the role of the PEP on rotator cuff healing, enthesis regeneration and functional restoration using a canine rotator cuff tear model.

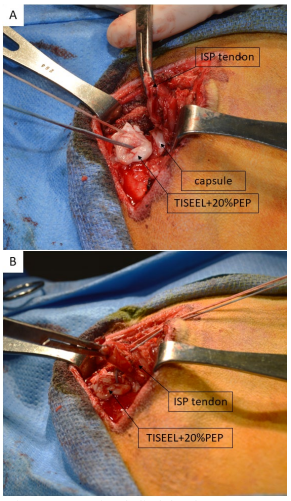

Fig 1. Surgical field of TISSEEL+PEP group. A, TISSEEL with 20%PEP put on the footprint and penetrated by #2 FiberWire sutures; B, TISSEEL with 20%PEP going covered with ISP tendon.

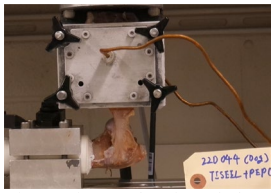
Fig 2. Dissected series of the humerus, ISP tendon and muscle on a servohydraulic testing machine to measure maximum tensile failure load and stiffness.

METHODS: A total of 36 shoulders from 18 female and male canine about 1 year of age and weighing around 30kg were used. The left or right shoulder was selected randomly with the infraspinatus (ISP) tendon being exposed using a deltopectoral approach under general anesthesia. Then, the ISP tendon was detached at its insertion using a sharp knife, along with the capsule beneath, to expose the glenohumeral joint to simulate an intrasynovial tendon injury condition in a clinical scenario. A decortication of the footprint was performed using chisels. and a double-row repair was performed with 4.5 mm Corkscrew FT suture anchors (Arthrex, Naples, FL) loaded with #2 FiberWire (Arthrex). The medial-row anchors were placed along the articular margin. with the lateral-row anchors placed 10 mm apart from medial-row anchors. Sutures were passed through the ISP tendon and tied with a mattress configuration. Canines were allocated to 3 groups (each, n=6): normal repair (control) group, fibrin glue added repair (TISSEEL repair) group and fibrin glue and PEP added repair (TISSEEL+PEP) group. In TISSEEL group, 4ml of commercially provided fibrin glue (TISEEL, Baxter, Chicago, IL) was put between the detached ISP tendon and the decorticated footprint. In TISSEEL+PEP group, 4ml of TISSEEL which contained 20% PEP was put in the same way as in the TISSEEL group (Fig 1). A high radial neurectomy was performed with the radial nerve being sharply transected above the triceps brachii to denervate the triceps muscle thus preventing elbow extension and thereby preventing weight bearing postoperatively to protect repaired ISP tendon. The canines were euthanized 6 weeks after the surgery. Immediately after the euthanasia, time-0 control group, whose procedure was completely same the surgery above, was performed on the shoulder not chosen for the surgery done on live animals to evaluate the pure strength of the suture-anchor repairment without tissue healing. The humerus, ISP tendon and muscle was dissected in series with the width and thickness of the ISP tendon being measured. The dissected specimens were tested on a servohydraulic testing machine (858 MiniBionix II; MTS Systems Corp, Eden Prairie, MN; Fig.2) to measure their maximum tensile failure load and stiffness. The tested specimens were fixed, decalcified and sliced into series sections for histological evaluation by H&E staining to determine the failure mode. Additionally, ultrasound shear wave elastography (SWE) of ISP muscle on the surgical side was measured every two weeks using an ultrasound system (SuperSonic® MACH™ 30, Hologic, Marlborough, MA) to evaluate the recovery of the ISP muscle. All procedures were approved by IACUC.

RESULTS: Average values of maximum tensile failure load (N) were 205.56±67.16(N) in time-0 control group, 446.19±12.49(N) in control group, 430.66±136.67(N) in TISSEEL group and 677.55±66.13(N) in TISSEEL+PEP group, respectively; the value of time-0 control group was significantly lower than those of the other groups and the value of TISSEEL+PEP was significantly higher than those of the other groups. Average values of Stiffness (N/mm) were 27.29±3.98(N/mm) in time-0 control group, 52.94±3.36(N/mm) in control group, 55.24±20.58(N/mm) in TISSEEL group and 95.63±1.08(N/mm) in TISSEEL+PEP group, respectively; the value of time-0 control group was significantly lower than those of the other groups and the value of TISSEEL+PEP was significantly higher than those of the other groups. The average tendon width was 14.5±0.5(mm) in control group, 15.5±0.5(mm) in TISSEEL group and 17.25±0.25(mm) in TISSEEL+PEP group. The average tendon thickness was 5.25±0.25(mm) in control group, 5.85±0.35(mm) in TISSEEL group and 7.25±0.25(mm) in TISSEEL+PEP group. Histological staining revealed that one specimen in the TISSEEL group raptured in the muscle and tendon junction were all specimens raptured in the tendon bone junction. Average values of SWE (kPa) in control group were 26.68±3.56kPa at 2$^{nd}$ week, 35.80±5.67 at 4$^{th}$, 27.80±4.56 at 6$^{th}$, respectively; in TISSEEL group 26.68±4.45kPa at 2$^{nd}$ week, 32.80±5.26 at 4$^{th}$, 24.55±4.26 at 6$^{th}$, respectively; in TISSEEL+PEP group 21.82±3.22kPa at 2$^{nd}$ week, 23.30±2.08 at 4$^{th}$, 21.11±3.99 at 6$^{th}$, respectively; comparing the values at 4$^{th}$ week, the value in TISSEEL+PEP group was significantly lower than those in other groups.

DISCUSSION: Mechanical test showed that both the maximum tensile failure load and stiffness of the TISSEEL+PEP group were significantly higher than other groups and that those of the time-0 control group were significantly lower than other groups. The main strength of the repaired ISP tendon reconstruction seems to be healing of the surrounding tissue and not the suture anchor itself. Additionally, the width and thickness of the ISP tendon in TISSEEL+PEP group was higher than the other groups. Weekly SWE measurements showed that TISSEEL+PEP could suppress postoperative increasing of ISP muscle stiffness. PEP seemed to help the tendon healing after repair.

SIGNIFICANCE/CLINICAL RELEVANCE: TISSEEL+20% PEP improve both the maximum tensile failure load by 1.57 times and the stiffness 1.82 times against normal ISP tendon repair. PEP seems to be a good candidate for an additive to accelerate tissue healing and increase the mechanical strength of the repaired rotator cuff.