Effect of purified exosome products on healing in a rat rotator cuff injury model

Zhao Gongyin, M.D.[1,2], Tsukamoto Ichiro, M.D.[1], Yousefi Farbod, M.D.[1], Wan Rou, M.D.[1], Zhu Weihong, M.D[1,3], Reisdorf Ramona L.[1], Brosig, Maggie A.[1], Zhao Chunfeng, M.D.[1]
[1]Mayo Clinic, Rochester, MN, [2]Changzhou Medical Center of Nanjing Medical University, Changzhou, Jiangsu, China, [3]The Second XiangYa Hospital, Central South University, Changsha, Hunan, China.
Zhao.gongyin@mayo.edu

Disclosures: Nothing to disclose

INTRODUCTION:

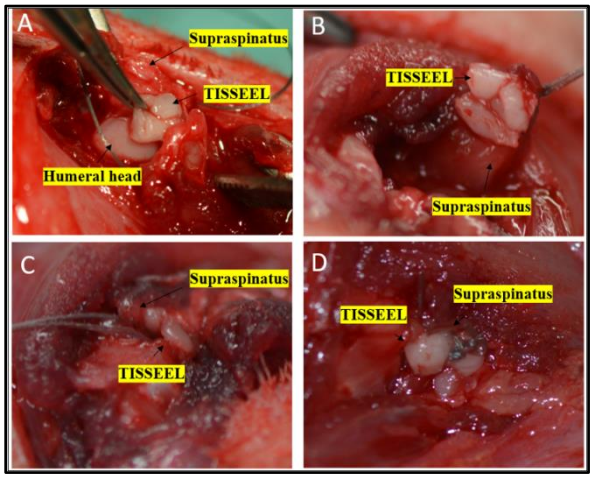

***Fig 1**. Specific surgical procedures: **A)** The supraspinatus tendon, is severed at the footprint; **B)** The TISSEEL containing PEP is sutured to the supraspinatus tendon, **C, D)** The severed supraspinatus tendon is sutured to the rotator cuff. severing site*

METHODS: Under Institutional Animal Care and Use Committee (IACUC) approved guidelines, a total of 120 SD retired breeder rats with equal numbers of both sexes and ages from 18-24 months old will be used. 120 rats will be divided into 12 groups based on three treatment variables (repair alone, repair treated with TISSEEL alone, and repair treated with PEP-TISSEEL) and four-time points of survival (1, 3, 6, and 12 weeks after surgery). Under general anesthesia, the supraspinatus tendon is exposed and then transected at the insertion site of the greater tuberosity. (**Fig.1A**) The TISSEEL with or without PEP is then secured to the supraspinatus tendon with a 5-0 Ethibond suture. (**Fig.1B**). A 0.5-mm hole is drilled transversely along the proximal humerus in an anteroposterior direction, and the other end of the suture is passed through the 0.5-mm hole. The suture is sutured to the tendon at the insertion point of the tendon on the greater tuberosity (**Fig.1C, D**). The rats in 1 and 3-week survival groups (n=6) will be used only for biological analysis (RT-PCR and Histology) since functional analyses (healing strength and gait) are not relevant within such short-term follow-up. The rats in 6 and 12-week follow-ups (n=14) will be analyzed in both function and biology.

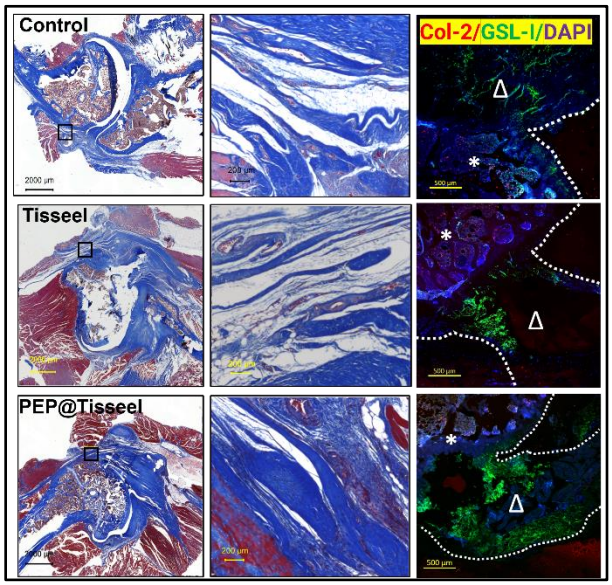

***Fig 3**. Masson staining and IF of rotator cuff injuries in rats in the 12-week group. "*" represents the humeral head and the "Δ" represents the supraspinatus tendon.*

The progressive deterioration of the tendon structure precipitates a notable decline in the intrinsic capacity to effectuate restitution at the tendon-to-bone juncture, concomitant with the inability to reinstate the indigenous transitional tissue, known as endosteum, at this interface. The conundrum of enhancing the clinical outcomes associated with reparative interventions targeting the rotator cuff has loomed as a substantive and unresolved quandary. Exosomes, as fundamental extracellular vesicles, have emerged as pivotal agents in orchestrating intricate intercellular communication, mediating signal transduction, and modulating pivotal cellular biological processes. The exosomes present in the Purified Exosome Product (PEP), meticulously cultured within the precincts of the Advanced Product Incubator (API) at the Mayo Clinic Center for Regenerative Medicine, utilizing archives of expired blood constituents, exhibit a conspicuous potential for fostering regeneration across diverse tissue substrates, encompassing wound, nerve, muscle, and tendon tissues. To further investigate the therapeutic effects of PEP on rotator cuff injuries, our evaluation of Purified Exosome Product (PEP) within a rat model has illuminated its potential as a therapeutic modality targeting the rotator cuff and has loomed as a substantive and unresolved quandary.

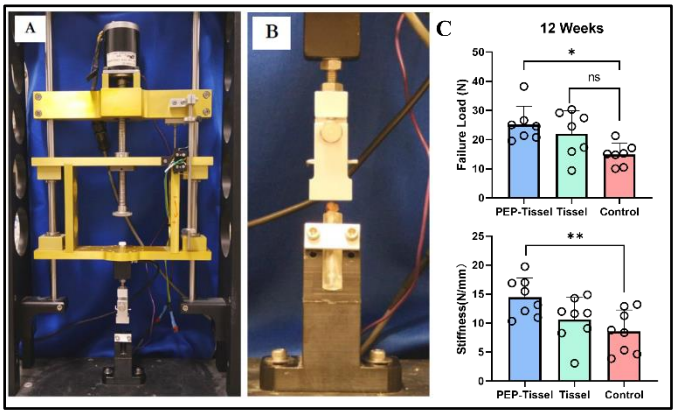

***Fig 2**. **A)** The Mini-MTS system used for this study with fixtures used for rat supraspinatus testing mounted. **B)** Mounted test sample. **C)**Failure Load and Stiffness.*

RESULTS: The failure load of the PEP@TISSEEL group was significantly higher than that of the Control group, and the stiffness of the PEP@TISSEEL group was significantly higher than that of the Control group. (P=0.0248, P=0.0134) (**Fig.2C**) Masson can be seen that the collagen content of the PEP@Tisseel group is significantly higher than that of the Control and Tisseel groups compared to the Control group and more cell proliferation can be found. Cheap fluorescence suggests that the GSL-1 protein content in the PEP@Tieessl group is significantly higher than the other two groups. This protein mainly represents vascularization and tissue regeneration, and the content of type 2 collagen is also significantly higher than the other two groups. (**Fig.3**) Digi gait suggests that the oscillation amplitude of the PEP@Tisseel group was significantly lower than the control and Tisseel groups. (P=0.0004, P=0.0006), while the stride amplitude was significantly higher than the Tisseel group. (P=0.0298).

DISCUSSION: Previous research revealed polyethylene glycol's wound healing and tendon-bone regeneration potential. Our latest study demonstrates Tisseel-based PEP's role in accelerating supraspinatus tendon revascularization and collagen formation, thus enhancing rotator cuff injury healing and symptom relief. This likely involves dampening inflammation and boosting relevant protein expression, necessitating further mechanistic exploration.

SIGNIFICANCE: If our goal is successfully achieved, we would have developed a novel cell-free biotherapeutics that is clinically translational for rotator cuff repair, since our PEP is GMP grade, and the PEP carrier (TISSEEL) is an FDA approved biological produce.

REFERENCES: 1. Ren, Y., et al., Effects of purified exosome product on rotator cuff tendon-bone healing in vitro and in vivo. Biomaterials, 2021. 276: p. 121019.