Use of Biomimetic Polymer Adhesive to Regenerate Achilles Enthesis Injuries in Rats

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DISCLOSURES: V. Pastukh: None. S. Hattori: None. D. Maloney: None. J. Zhang: None. JH-C. Wang: None.

INTRODUCTION: Injury may occur to an enthesis after acute trauma or to a degenerated tendon after normal activity or minor trauma [1]. The primary challenge in regenerating a tendon to the bone lies in the mechanical intricacies of joining a flexible material (tendon) with a rigid material (bone) [2]. Post-injury, enthesis regeneration does not take place following surgical repair. Instead, fibrovascular tissue, characterized by reduced biomechanical properties, develops at the repair site, impeding the seamless integration between the tendon and bone [3]. Regenerating the enthesis after injury poses significant challenges in orthopaedic medicine. This study aimed to improve enthesis tissue healing, reparation and regeneration using a newly developed biomimetic polymer adhesive.

METHODS: Synthesized in our lab, the polymer LDI-Gly-DOPA-Cys (lysine diisocyanate, glycerol, phenylalanine, and cysteine) exhibits non-toxic, biodegradable, and bio-adhesive properties. A foam that forms during polymerization after ingredients are mixed serves as a scaffold for enthesis tissue remodeling. We used a rat Achilles enthesis repair model to assess healing and regeneration after polymer implantation in repair site on 3 rats/group of eight-week-old SD female rats for control and treatment groups. Enthesis repair following enthesis surgical transection was done by transossal fixation of Achilles tendon to calcaneus (**Fig. 1**). This study was approved by IACUC.

RESULTS: In the treatment group, polymer was applied in the enthesis repair site before suturing to calcaneus. New tissue formation in enthesis repair site was histologically analyzed in 4 weeks post-surgery (**Fig. 2**). Histological images showed the formation of organized gradient transition from bone to tendon through mineralized and demineralized fibrocartilage tissue (**Fig. 2D-F**), compared to formation of disorganized connective tissue in control group (**Fig. 2A-C**). Increase of glycosaminoglycans and fibrocartilage organized tissue expression in enthesis repair site was found in treatment group (**Fig. 2J-L**) compared to formation of disorganized fibrocartilage and connective tissue in control group (**Fig. 2 G-I**). Similar changes demonstrated new enthesis tissue formation in the treatment group (**Fig. 2P-R**), whereas formation of disorganized fibrocartilage and connective tissue were observed in enthesis repair site in control group (**Fig. 2M-O**).

DISCUSSION: We have developed a novel biomimetic amino acids-based urethane polymer as a tissue/bone adhesive. This polymer adhesive can form strong bonding with collagen and Ca2+ in bone tissues and can be conjugated with bio-agents. In this study, we demonstrated that the biomimetic properties of the polymer stimulate the regeneration of specific enthesis tissue at the repair site. The use of this polymer adhesive in conjunction with sutures can facilitate the gradual transfer of physical loading from muscle to bone. In contrast, when sutures are used alone, they tend to result in the formation of disorganized connective and fibrocartilage tissue, which lacks the necessary mechanical strength. This deficiency can lead to repetitive trauma, pain, and long-term disability. Our ongoing research is focused on further characterizing the biological, biochemical, and biomechanical properties of the newly formed enthesis in our rat enthesis injury model.

SIGNIFICANCE: Our biomimetic polymer adhesive may be used as a new tissue engineering approach to regenerate injured enthesis effectively.

ACKNOWLEDGEMENTS: This work was supported in part by a DOD Award (W81XWH-22-9-0016-014) and Pittsburgh Foundation Grants (AD2022-130408; AD2023-134256).

REFERENCES: 1) Derwin, K.A., et al., J Bone Joint Surg Am, 2018. 100(16): p. e109. 2) Lu, H.H. and S. Thomopoulos, Annu Rev Biomed Eng, 2013. 15: p. 201-26. 3) Chen, W., et al., Biomaterials, 2021. 271: p. 120714.

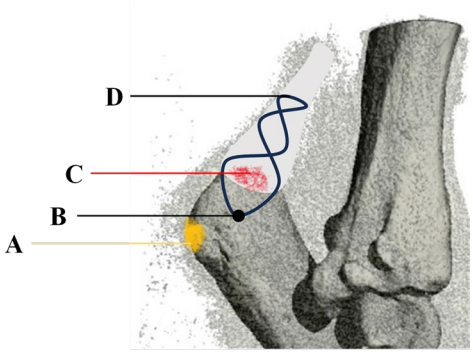

Fig. 1 Achilles enthesis repair model in rats.
Original Achilles tendon enthesis (**A**); Calcaneus drilling point (**B**); Enthesis repair site (**C**); Achilles – calcaneus transossal suture (**D**).

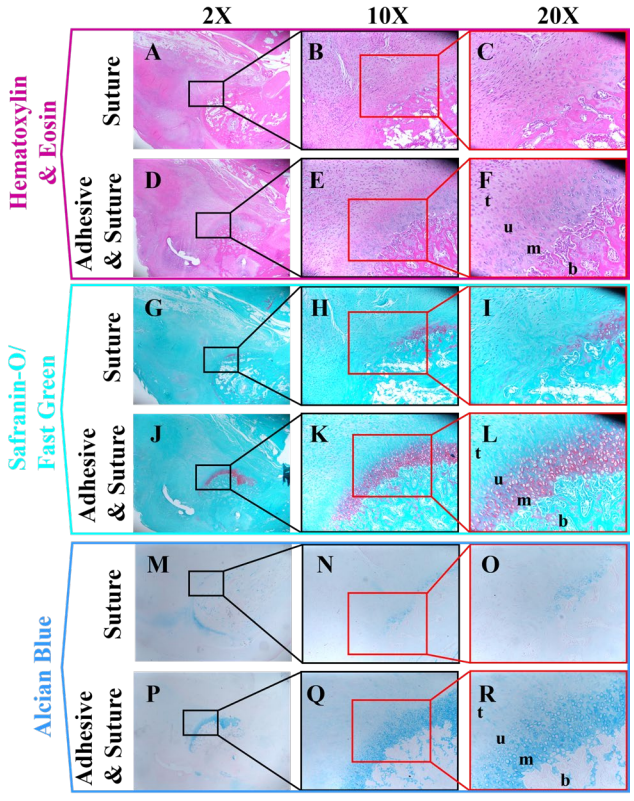

Fig. 2 Application of the biomimetic polymer leads to enthesis tissue formation. Rat ankle joint images show the enthesis repair site and surrounding tissues for both control (Suture: **A, B, C, G, H, I, M, N,** and **O**) and treatment (Adhesive + Suture: **D, E, F, J, K, L, P, Q, and R**).Compared to the control group, the treatment group shows the regeneration of the tendon-to-bone interface, characterized by the extensive formation of fibrocartilage zone, as revealed by H&E, and two other staining methods. **t:** tendon, **u:** unmineralized fibrocartilage, **m:** mineralized fibrocartilage, and **b:** bone.