Advanced Bioadhesives for Regenerative Healing of Dense Connective Tissues

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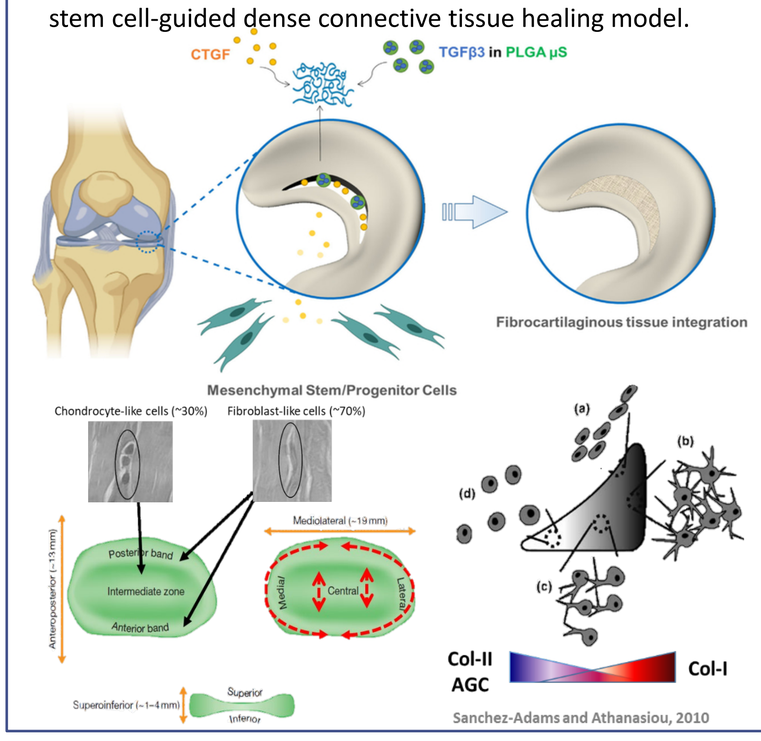
97th Annual Greater New York Dental Meeting
November 26th, 2021 – December 1, 2021

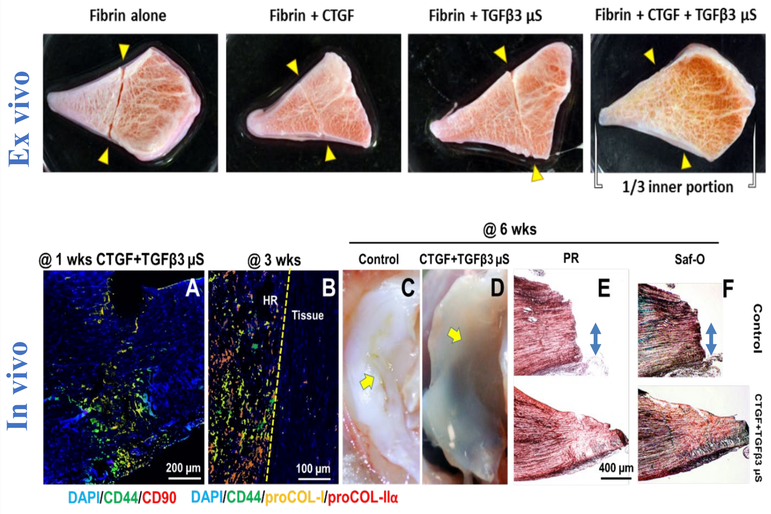
INTRODUCTION

- Various bioadhesives have been synthesized to facilitate stem cell-induced healing of fibrocartilaginous tissues such as temporomandibular joint (TMJ) disc and knee meniscus.
- Outstanding challenges include poor wet-adhesion and mechanical strength of bioadhesives.
- To address these weaknesses, we have synthesized several bioadhesives and applied chemical modifications, including double-crosslinked gelatin, fibrin-based with genipin crosslinking, or heparin conjugation.
- We have evaluated these bioadhesives in our well-established stem cell-guided dense connective tissue healing model.



BACKGROUND

Avascular Fibrocartilage Healing via Endogenous syMSCs

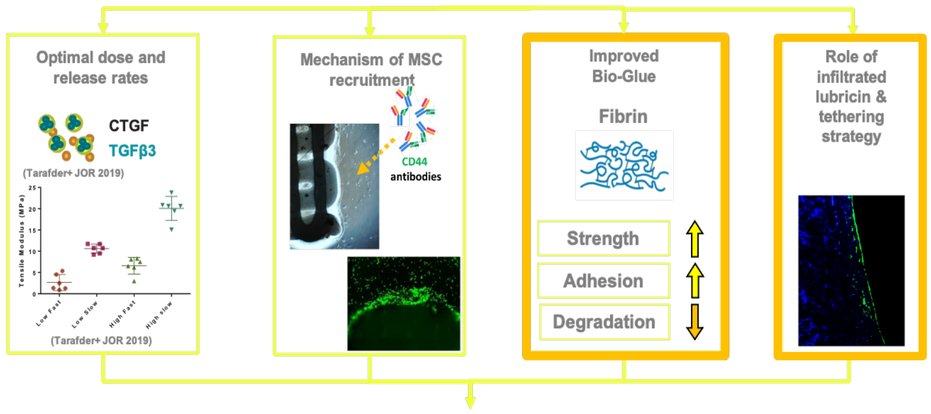


- Knee meniscus and TMJ discs possess near identical histologic composition with similar tissue healing challenges with respect to damage to the avascular inner third.
- The meniscus is characterized by a multiphase biochemical composition and complex structure: The outer third possesses a dense, vascularized fibrous matrix. The middle third possesses a mixed population of chondrocytes and fibroblasts.
- Our laboratory has demonstrated in vivo & ex vivo regeneration of inner avascular third regions with stepwise CTGF and microsphere-encapsulated TGFβ3 delivery.
- Still, the hydrogel used for growth factor delivery remains mechanically inadequate.

METHODS & RESULTS

Overview of Objectives

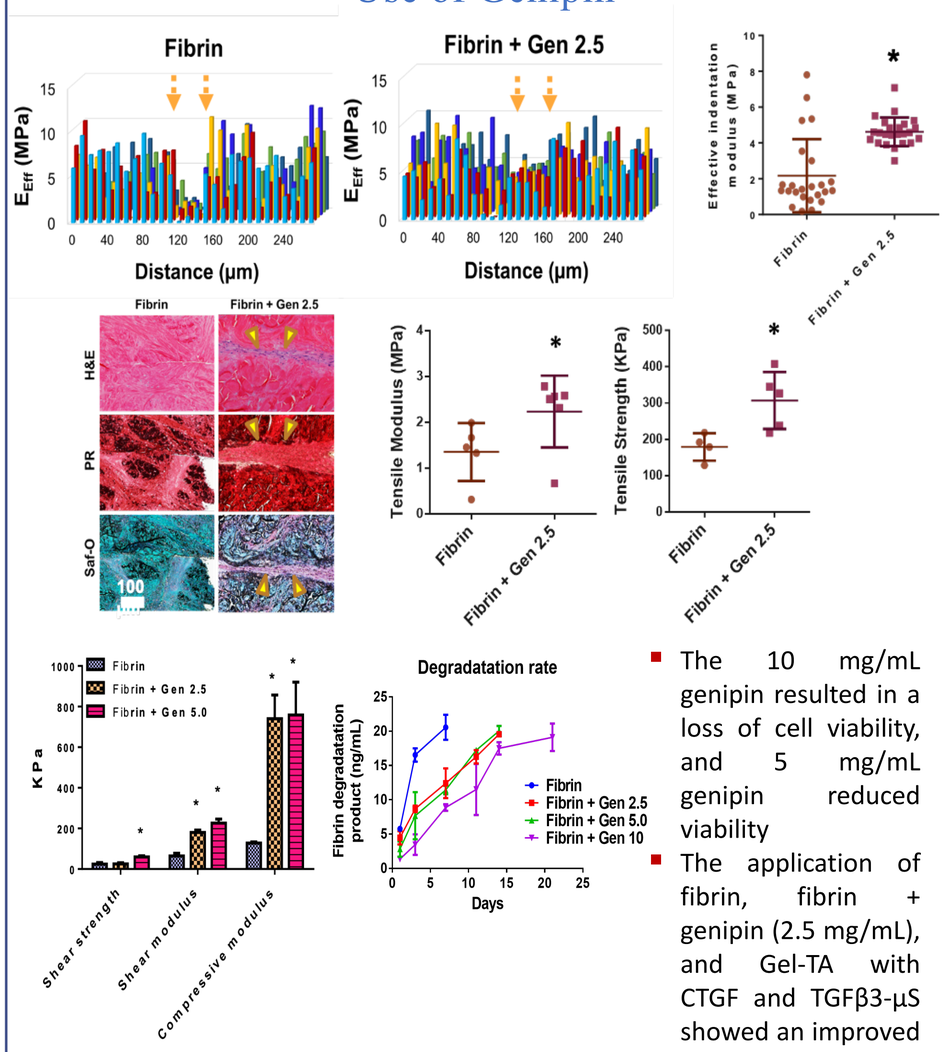
Toward regenerative engineering of avascular meniscus healing



Pre-clinical studies

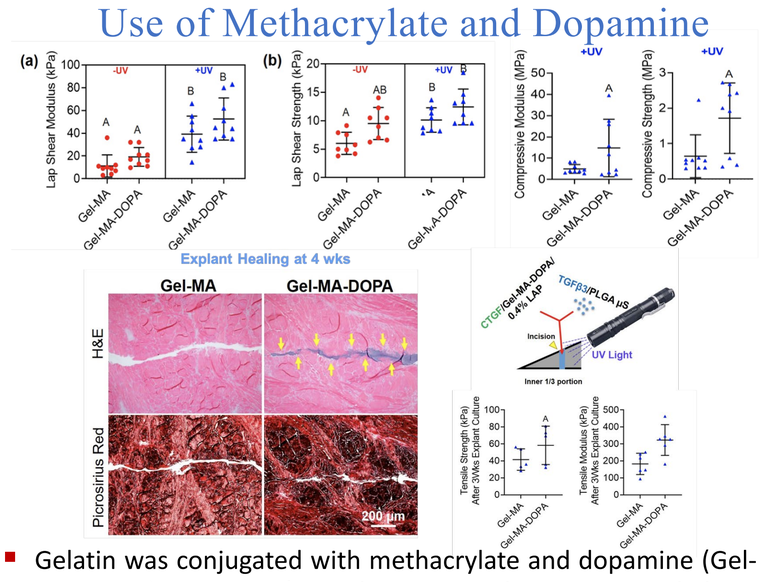
- Per our established protocol, growth factors were delivered to induced avascular meniscus tears via newly synthesized hydrogels. These included genipin-crosslinked fibrin, heparin was conjugated gelatins through further covalent bonding, and methacrylate/dopamine bonded gelatins in the presence of crosslinkers.
- The synthesized bioadhesives were first tested under lap shear, compression and tensile stress. *In vitro* degradation tests were performed.
- Then, mechanical efficacy of each bioadhesive was tested after 4-5 weeks of tissue healing.
- Reintegration of tissue at damaged sites was measured in meniscus explant healing model with controlled delivery of bioactive cues.

Use of Genipin



- All tested hydrogels showed adequate CTGF release and MSCs recruitment.
- Overall, the addition of genipin to fibrin significantly improved mechanical properties of each hydrogel in a dose-dependent manner.
- The 10 mg/mL genipin resulted in a loss of cell viability, and 5 mg/mL genipin reduced viability
- The application of fibrin, fibrin + genipin (2.5 mg/mL), and Gel-TA with CTGF and TGFβ3-μS showed an improved healing of meniscus tears, with the best outcome achieved by fibrin+2.5 mg/mL genipin.

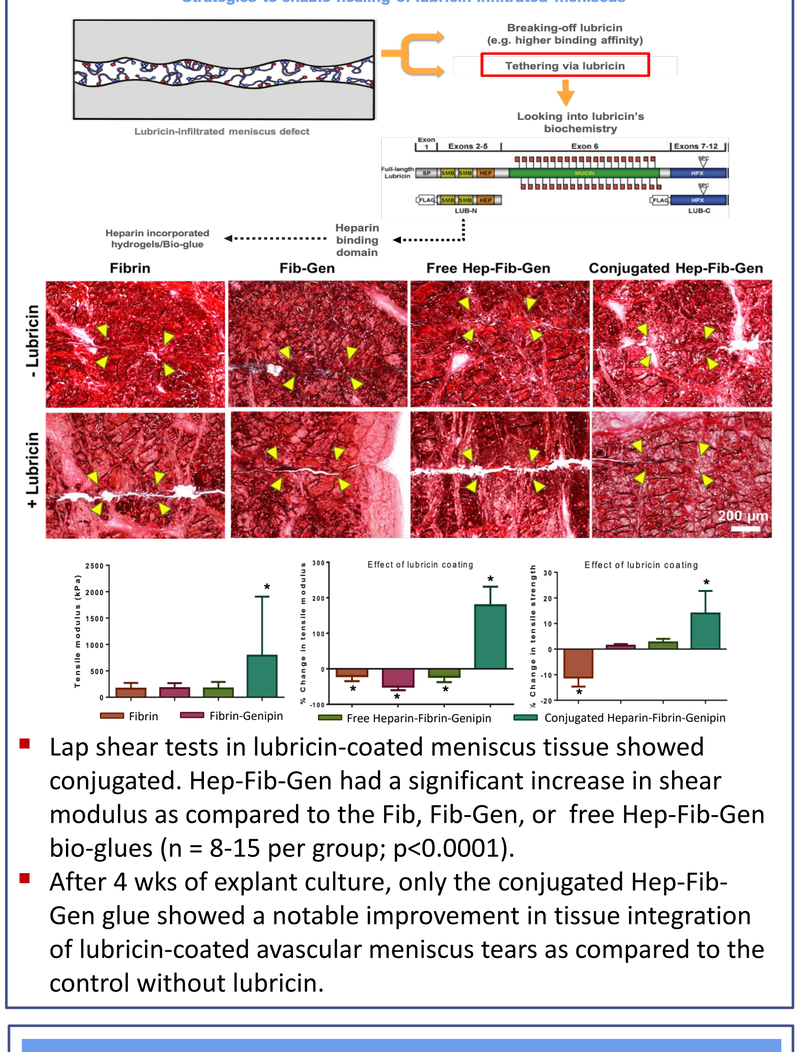
Use of Methacrylate and Dopamine



- Gelatin was conjugated with methacrylate and dopamine (Gel-MA-DOPA) using EDC/NHS chemistry and various cross-linkers. Gel-MA-DOPA with Fe3+ or Fibrin showed successful instant gelation.
- UV- exposed gels exhibited significantly higher lap shear moduli and strength (except Gel- MA). The Gel-MA-DOPA exhibited significantly higher compressive modulus & strength.
- Overall, Gel-MA-DOPA displayed superior mechanical properties. Explants with growth factor-loaded Gel-MA-DOPA showed significantly higher tensile moduli (322.78±101.37 kPa) and strength (58.31±22.61kPa) vs Gel-MA alone.
- Application of Gel-MA-DOPA significantly improved healing of avascular menisci regions via increased integration of fibrocartilaginous tissues.

Use of Heparin for Lubricin Tethering

Strategies to enable healing of lubricin-infiltrated meniscus



- Lap shear tests in lubricin-coated meniscus tissue showed conjugated. Hep-Fib-Gen had a significant increase in shear modulus as compared to the Fib, Fib-Gen, or free Hep-Fib-Gen bio-glues (n = 8-15 per group; p<0.0001).
- After 4 wks of explant culture, only the conjugated Hep-Fib-Gen glue showed a notable improvement in tissue integration of lubricin-coated avascular meniscus tears as compared to the control without lubricin.

DISCUSSION

- Genipin cross-linking significantly increased compression and lap shear properties of fibrin-based hydrogels in a dose-dependent manner.
- Heparin conjugation improved the tensile modulus of hydrogels, but high concentrations resulted in brittle glue at despite lubricin sequestration.
- Gel-MA-DOPA had mechanical properties superior to that of gels with methacrylate (Gel-MA), but the overall bonding strength of Gel-MA-DOPA was weaker than genipin/heparin enhanced fibrin-based bioadhesives.
- In explant healing, each modification with genipin, heparin, and double-crosslinked methacrylate/ dopamine, each significantly enhanced functional properties of healed tissues.

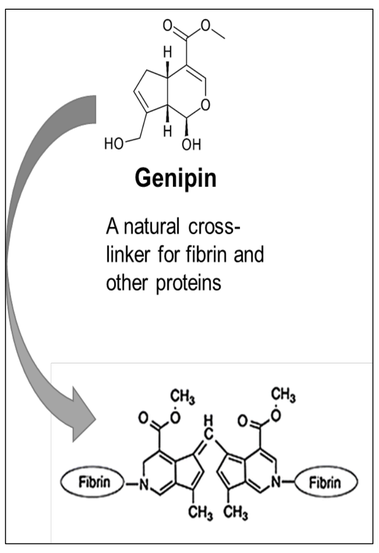
CONCLUSION

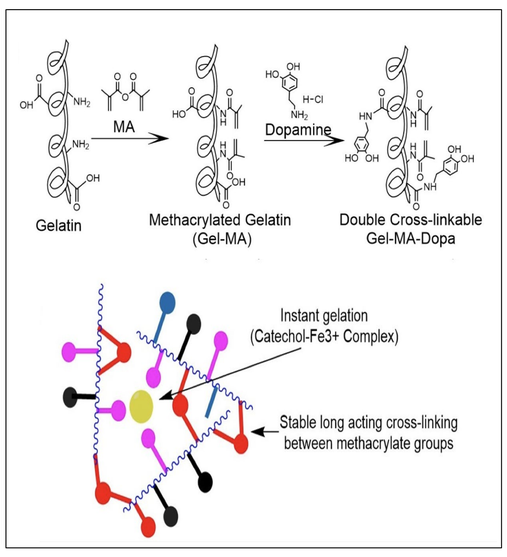
- Bioadhesives augmented with additional moieties possessing crosslinking potential to induce greater adhesion generally demonstrated a significant increase in mechanical properties as compared to those without.
- Each approach showed advantages and disadvantages, suggesting further improvement through cross-modification among the tested moieties.
- In conclusion, our study may serve as a vital foundation towards development of efficient bioadhesives for stem cell-guided regenerative healing in dense connective tissues like avascular meniscus and TMJ discs.

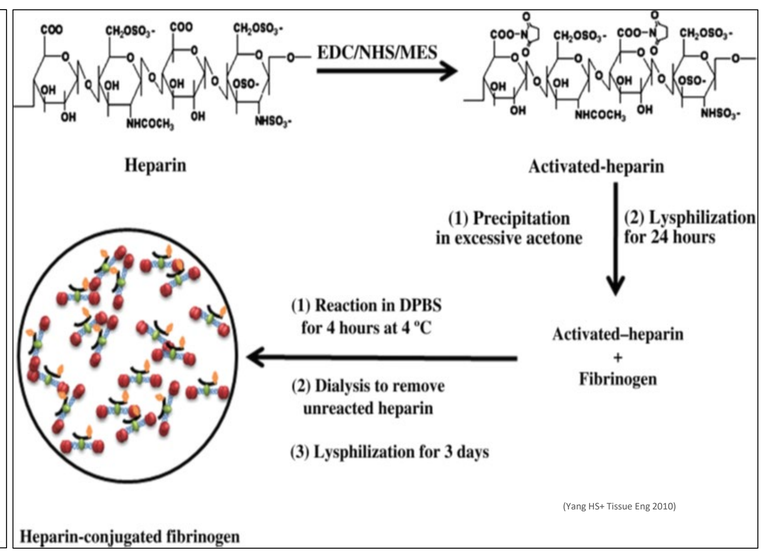
SUMMARY OF FINDINGS

- In explant healing, the modifications with genipin, heparin, and double-crosslinking methacrylate and dopamine each significantly enhanced the functional properties of healed tissues.

	Fibrin-Genipin 2.5 (Fib Gen 2.5)	Gelatin-Methacrylate-Dopamine (Gel-MA-DOPA)	Heparin-conjugated fibrinogen (Hep-Fib-Gen)
PROs	• Improved Adhesion	• Safer physiologically • Faster degradation expected	• Tethers lubricin • Further improved adhesion
CONs	• Slow degradation (toxicity)	• Weak adhesion overall	• Brittle properties







ACKNOWLEDGEMENTS

- Research reported in this publication was supported by the National Institute of Dental & Craniofacial Research of the National Institutes of Health under Award Number 1R01DE029321-01A1. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.