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Dual-Crosslinked Gelatin to Facilitate Stem Cell-Guided Fibrocartilage Healing

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INTRODUCTION

- Various bio-glues have been tested to facilitate stem cell-based healing of temporomandibular joint (TMJ) disc and meniscus fibrocartilaginous tissues .
- Outstanding challenges in bio-glues include poor wet-bonding adhesion and mechanical strength.
- To overcome these weaknesses, we developed a dual-crosslinked gelatin with methacrylate and dopamine and tested its efficacy in our stem cell-guided fibrocartilage healing model (**Fig.1**).

Stem cell-guided regeneration, regionally variant vascularity

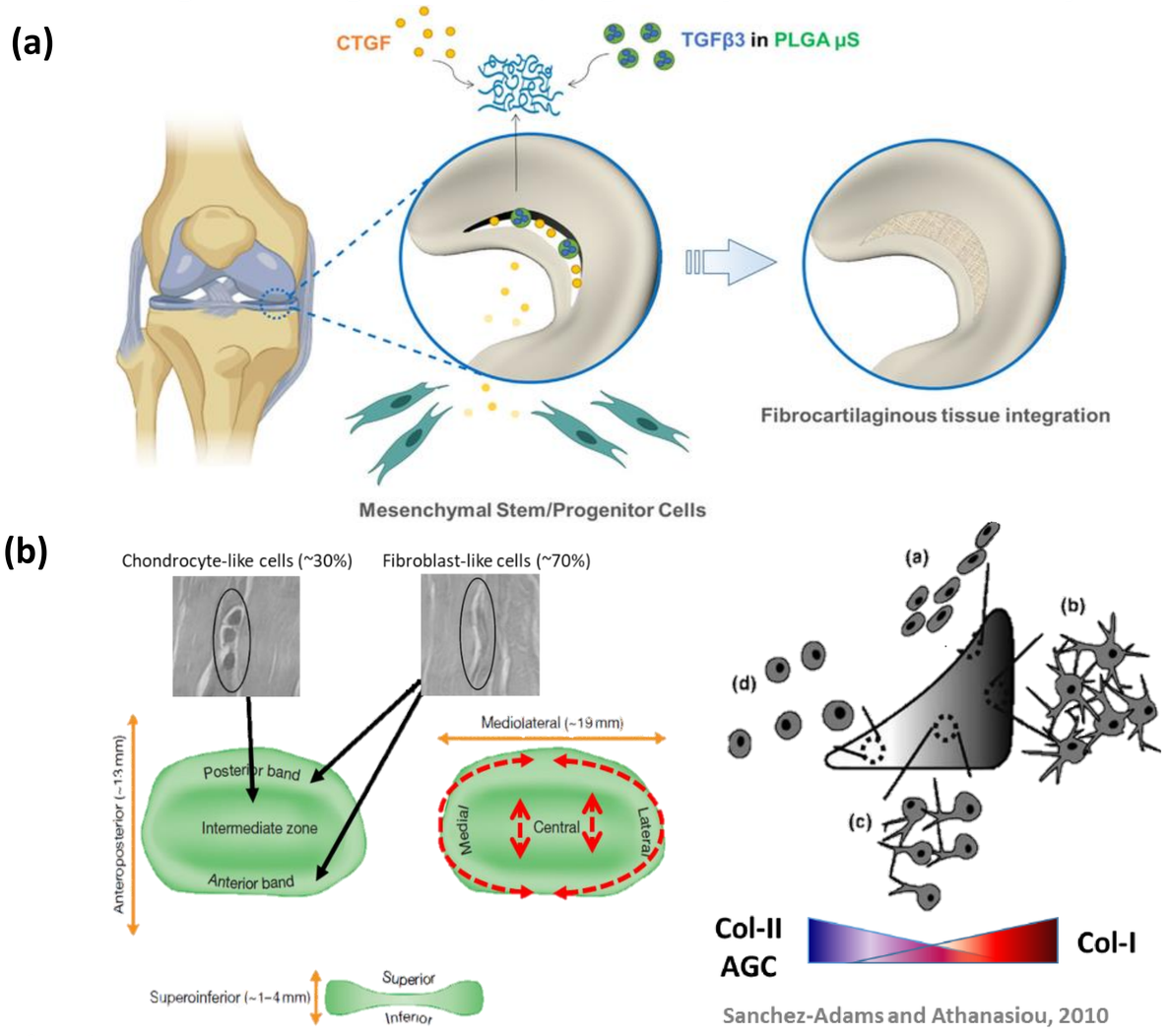
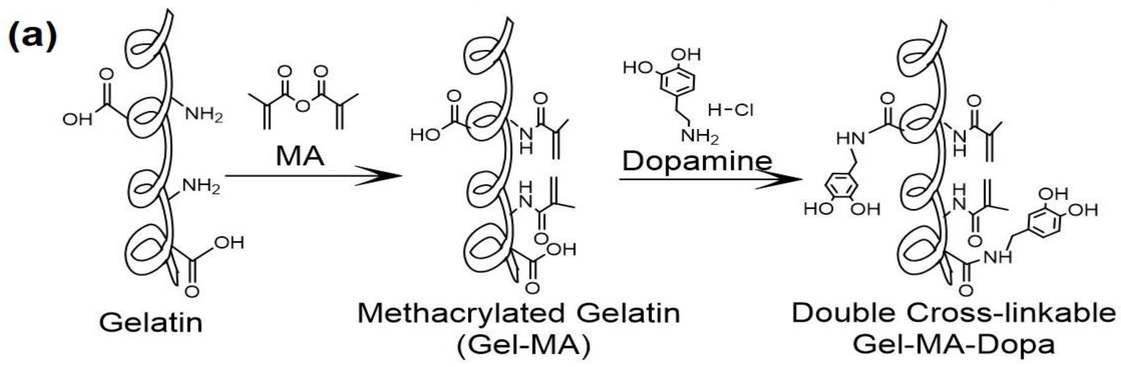


Fig. 1. Inducing seamless healing of avascular meniscus tears by via step-wise recruitment of synovial MSCs (a) to avascular region. Fibrocartilage discs have regionally variant cell/matrix phenotypes and vascularity (b) leaving them prone to poorly healing tears or perforations in the inner avascular third.

MATERIALS & METHODS

- Methacrylated (Gel-MA) and further dopamine-conjugated (Gel-MA-DOPA) gelatin were synthesized per established protocols (**Fig.2a**)

Synthesis of a double-conjugated, double-cross-linkable bioglue



- Fibrin, Fe^{3+}, UV/visible light photoinitiator concentrations tested to optimize adhesion & mechanical stability by lap shear & compression tests (**Fig.2a&b).**
- Our synthesized bio-glues were loaded with CTGF (100-ng/ml) and TGF-β3 encapsulated PLGA microspheres (10-mg), applied for healing of an incision made at the avascular inner third of bovine meniscus, cultured with human bone marrow mesenchymal stem cells (hBM-MSCs) in fibrochondrogenic culture, and harvest for analysis and testing at 3-wk (**Fig.2b**).

Lap-shear test, in vitro explant culture

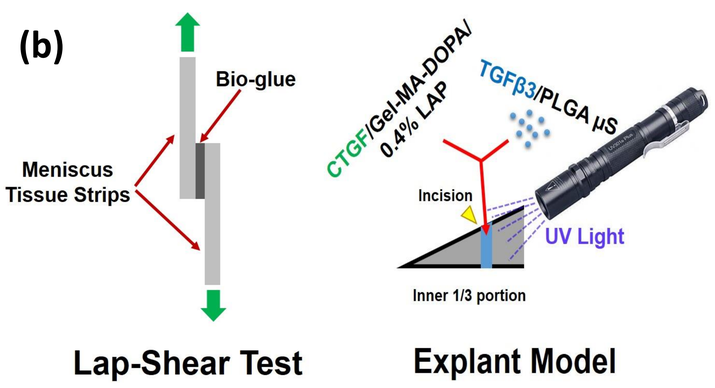


Fig. 2. Schematic representation of double cross-linkable Gel-MA-DOPA bio-glue synthesis (a), lap shear testing + explant culture (b).

RESULTS

Mechanical testing: Lap shear, compression, explant tensile

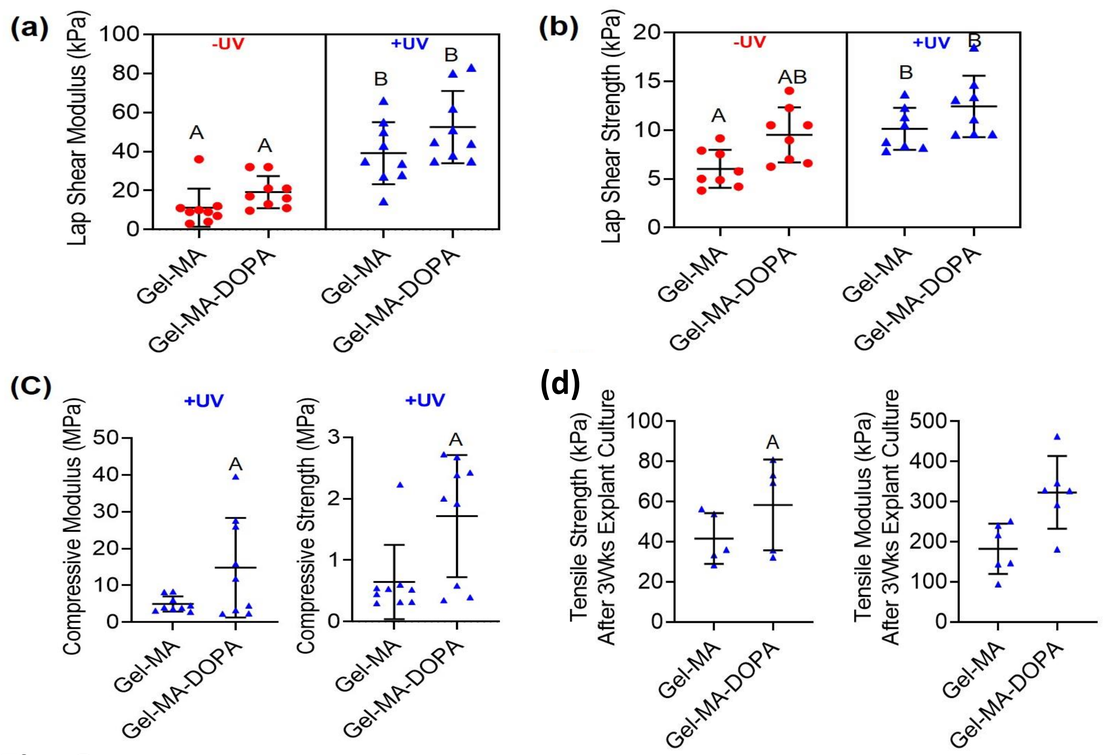


Fig. 3. Lap shear (a &b) and compression (c) tests for Gel-MA and Gel-MA-DOPA (n = 8-10 per group: *:p<0.05). Tensile properties after 4 weeks explant culture with Gel-MA and Gel-MA-DOPA (n=5-6 per group: p<0.05). No statistically significant difference between two groups sharing same letter.

- Gel-MA-DOPA with Fe^{3+} or Fibrin showed successful instant gelation.
- UV-exposed gels exhibited significantly higher lap shear moduli and strength than gels not exposed to UV (except Gel-MA) **(Fig.3a&b).**
- The Gel-MA-DOPA exhibited significantly higher compressive modulus and strength than Gel-MA (**Fig.3c**).
- Overall, Gel-MA-DOPA displayed superior mechanical properties to Gel-MA.

Avascular meniscus tissue regeneration in explant cultures

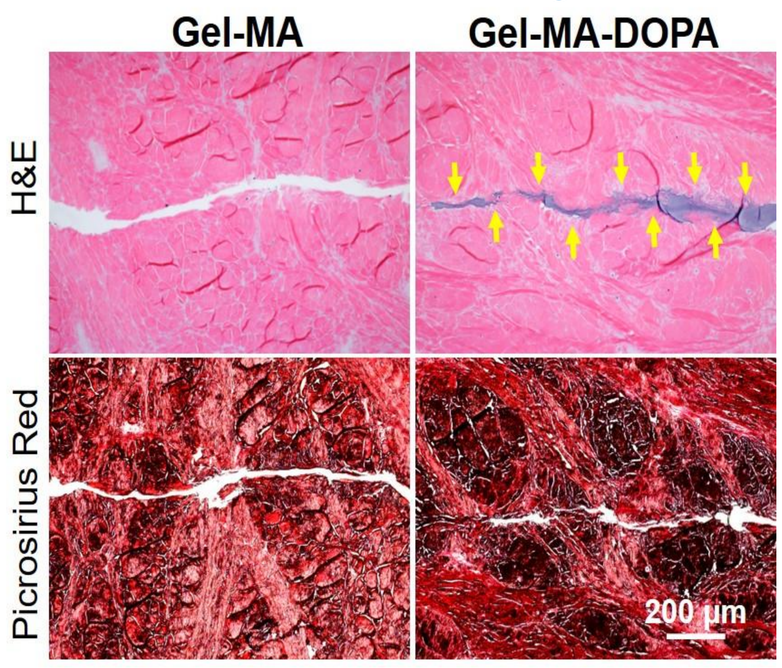


Fig. 4. Healing of avascular meniscus tears by Gel-MA and Gel-MA-DOPA after 4 wks. Gel-MA-DOPA exhibited improved tissue integration most likely through its ability to form bonds with the native tissue.

- Explants cultured with hBM-MSCs received growth factor-loaded Gel-MA-DOPA which showed significantly higher tensile moduli (322.78 ± 101.37 kPa) and strength (58.31 ± 22.61kPa) than those with Gel-MA (**Fig.2d**).
- Application of Gel-MA-DOPA significantly improved healing of avascular menisci regions through increased integration of fibrocartilaginous tissues (**Fig.4**).

DISCUSSION & CONCLUSION

- Gel-MA-DOPA demonstrated instant gelation via reversible chelation making our bioglue easy-to-use.
- Gel-MA-DOPA exhibited enhanced tissue integration most likely via irreversible covalent bonds with tissue upon catechol to quinone oxidation.
- Follow-up studies will investigate gluing mechanisms, refinement processes to improve gel mechanics, and long-term outcomes.
- In conclusion, the dual-crosslinked bio-glue conjugated with dopamine and methacrylate, Gel-MA-DOPA, may serve as a novel, efficient tissue adhesive vehicle for stem cell-guided healing of fibrocartilaginous tissues including meniscus and TMJ discs.

ACKNOWLEDGEMENT

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