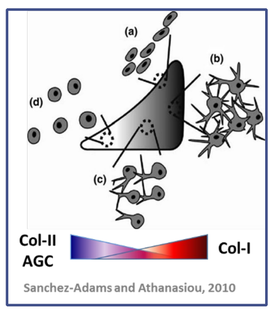
Hydrophilic Polydopamine (hPDA) Incorporated Fibrin Bio-Adhesive For Improved Healing Of Fibrocartilaginous Tissues

Maya Jeremias, Aminah Sarowar, Solaiman Tarafder, Chang H Lee

Columbia University College of Dental Medicine

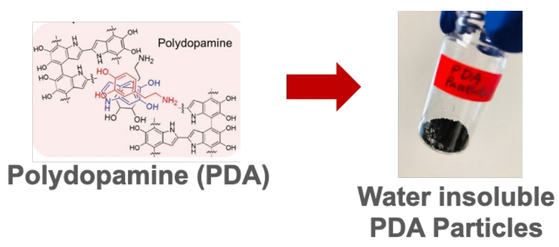
INTRODUCTION

- The temporomandibular disc and meniscus are complex fibrocartilaginous tissues with regionally variant cell/matrix phenotypes and vascularity
- Tears or perforation to these tissues hardly heal, and with current limited treatment, they frequently progress to joint deterioration and osteoarthritis.
- There is no robust available regenerative therapy for the TMJ or meniscus.
- Polydopamines (PDA) are structurally similar to marine mussel secreted adhesion proteins with strong wet adhesion to almost any material surfaces, and its addition to an already established fibrin-based bioactive glue could serve as a novel, bio-adhesive to promote integrated fibrocartilaginous tissue repair.
- However, PDA becomes insoluble in solution, which is why its adhesive power has not been explored in many biomedical and tissue engineering applications.

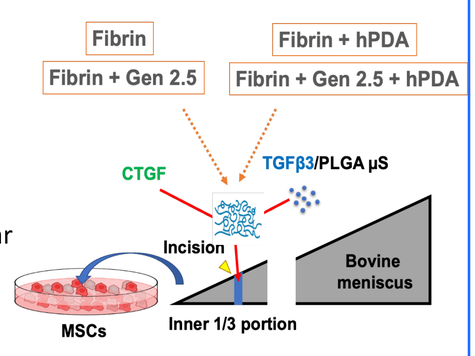


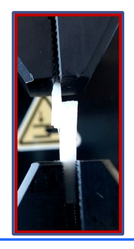
OBJECTIVES

1. Synthesize soluble Polydopamine (hPDA) from insoluble PDA particles and film



2. Assess hPDA's cytotoxicity, mechanical properties (lap shear testing), degradation rate and capacity to heal in our explant model (tensile properties and indentation modulus).





METHODS & MATERIAL

- **hPDA Synthesis:** hPDA was extracted from water-insoluble polydopamine synthesized from dopamine (2.5 mg/mL) dissolved in TRIS/HCL buffer (pH=8.5).
- **Live-dead assay**: Tested cytotoxicity of hPDA in 2D and 3D cell cultures using hBMSCs. *In vitro* degradation testing prepared Fib with and without hPDA labeled with Alexa Fluor® 488 dye. FibGen gel (2.5 mg/ml genipin) and FibGenhPDA (2.5 mg/ml genipin and 6 mg/mL hPDA) were prepared for comparison.
- **Lap-shear testing**: Isolated inner-third zone menisci from bovine knee joints and applied 20 μl of bio-glue (Fib, FibGen, FibhPDA and FibGenhPDA) between tissue strips followed by displacement using CellScale UniVert uniaxial mechanical testing (CellScale Biomaterials Testing, Waterloo, Canada).
- **In Situ Regeneration**: Bio-glues were applied to our meniscus explant healing model through controlled delivery of bioactive cues. After 6 wks, all the harvested explants were analyzed for healing of avascular tears using histology, biochemical assays, and multi-scale mechanical tests.

RESULTS

Live-Dead Assay

2D Testing

Figure 1a

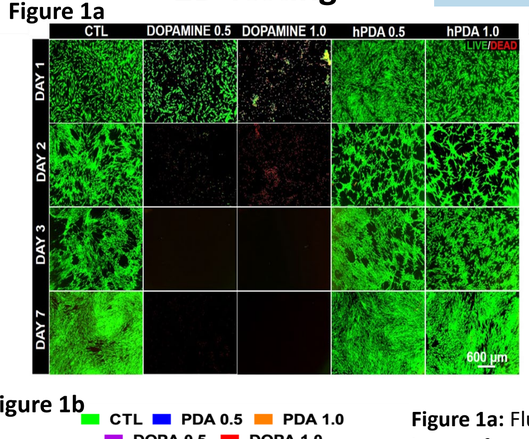


Figure 1b

Figure 1a: Fluorescent images from Live-Dead assay showing live (green) and dead (red) cells with control, Dopamine, and hPDA. **Figure 1b:** Percent live cells in control, dopamine, and PDA for 7 days.

3D Testing

Figure 2a

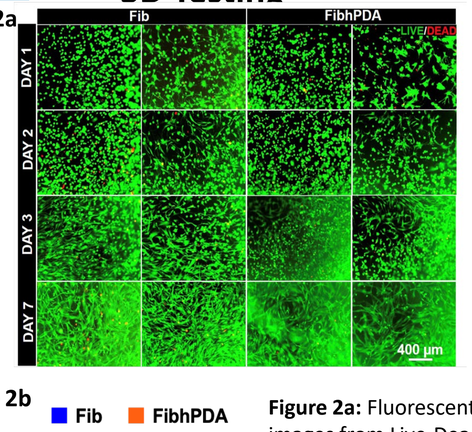


Figure 2b

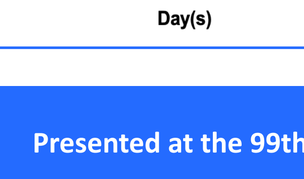


Figure 2a: Fluorescent images from Live-Dead assay showing live (green) and dead (red) cells in Fib and FibhPDA gels
Figure 2b: Percent live cells in Fib and FibhPDA gels for 7 days.

RESULTS

Synthesis of hPDA

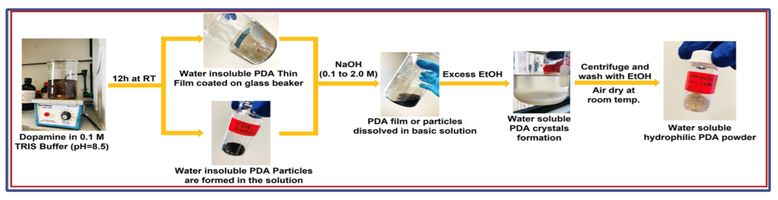


Figure 5a: Synthesis route for water-soluble PDA

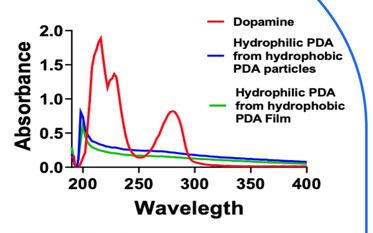


Figure 5b: UV absorbance for dopamine and hPDA.

Degradation Assay

Figure 3

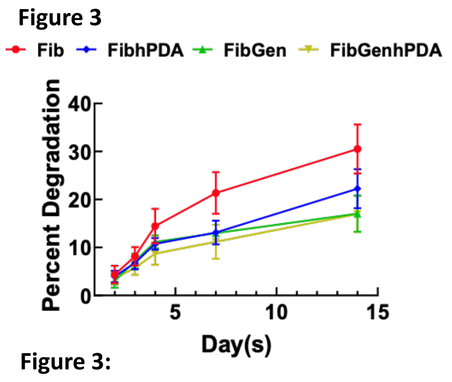


Figure 3: Percent degradation with Alexa Fluor® 488 dye for Fib, FibhPDA, FibGen, and FibGenhPDA gels for 14 days.

Lap-Shear Testing

Figure 4a

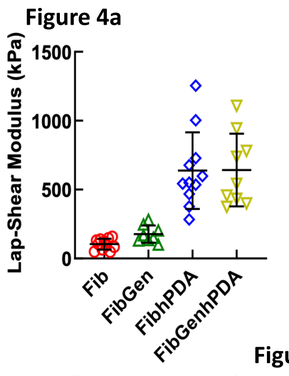


Figure 4b

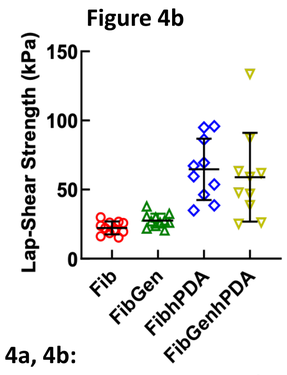


Figure 4a, 4b: Lap-Shear modulus (n=8-11 per group; $p<0.0001$) and lap-Shear strength (n=10-11 per group, $p<0.001$) Groups not sharing same letter are statistically significant.

Avascular meniscus healing @ 6 wks

Figure 6

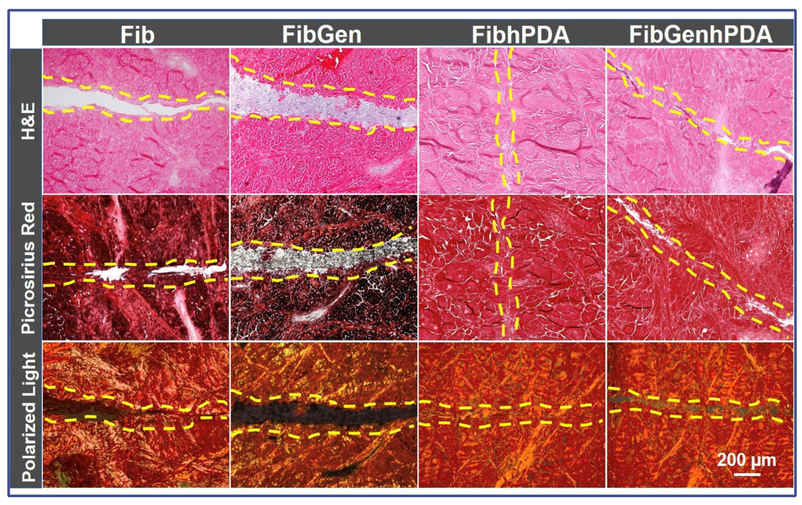


Figure 6: H&E, Picorious Red, and Polarized Light images for healing of avascular meniscus tears by Fib, FibGen, FibGenhPDA and FibhPDA after 6 wks, showing improved tissue integration and healing by hPDA.

Indentation Modulus @ 6 wks

Figure 7

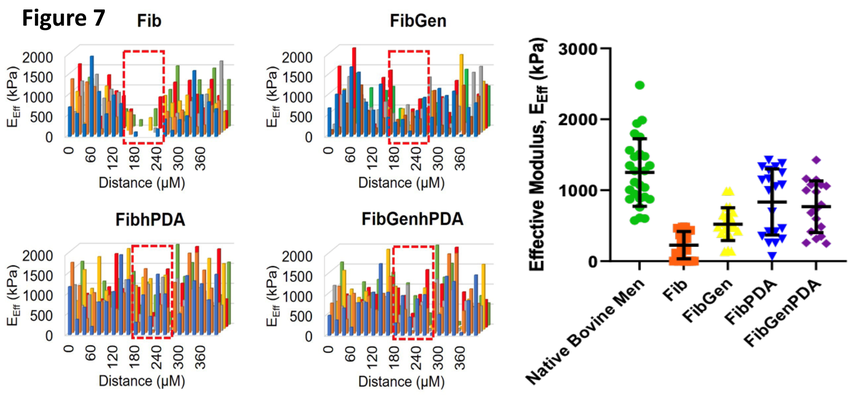


Figure 7: Effective Indentation modulus of Fib, FibGen, FibhPDA, and FibGenhPDA compared to native bovine meniscus. Groups not sharing same letter are statistically significant.

Tensile Properties @ 6 wks

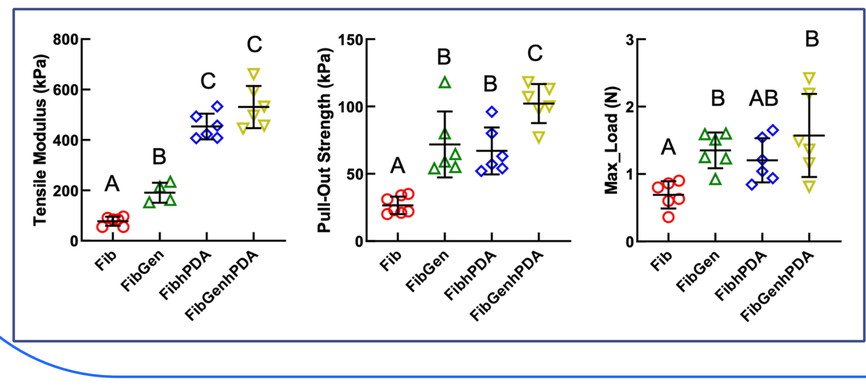


Figure 8: Tensile Modulus, Pull-Out Strength and Max Load for Fib, FibGen, FibhPDA, and FibGenhPDA.

CONCLUSION

- Polydopamine (PDA) has structural similarity to marine mussel secreted adhesion proteins with strong wet adhesion to various substrates. The high adhesiveness of PDA is attributed to the active catechol and primary amine groups on PDA that facilitate excellent wet adhesion to almost all material surfaces.
- Due to its insolubility in aqueous solutions, the adhesive power of PDA could not be explored in many biomedical and tissue engineering applications. Here, we developed a method to synthesize hPDA from insoluble PDA film and particles and showed that hPDA has great potential in bio-adhesive development for fibrocartilaginous tissue repair and healing.
- Incorporation of hPDA in fibrin gel resulted in improved adhesion of the gel to the meniscus tissue surface, likely through the interactions of many functional groups (i.e., catechol, amine, hydroxyl groups) present in hPDA.
- hPDA incorporated Fib may serve as a novel, efficient tissue adhesive for avascular meniscus and TMJ disc tear healing given its excellent biocompatibility and tissue adhesion.

ACKNOWLEDGMENTS

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