Designing a Novel Gelatin Fiber-Based HA (GelHA) Plug to Treat Perianal Fistulas in Crohn's Patient

MAO RESEARCH GROUP

JOHNS HOPKINS WHITING SCHOOL of ENGINEERING

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Background

Crohn's disease (CD) is an immune mediated inflammatory bowel disease impacting 780,000 patients in the US. CD is an illness that produces swelling in the digestive tract tissue and can result in chronic wounds and soft tissue loss.[1] Current therapies available are geared towards alleviating the symptoms of patients (Diarrhea, fever, fatigue, abdominal pain). Perianal fistulas (PAF) appears in 30-40% of CD patients, a condition where an abnormal tunnel connects the anal canal to the skin of the buttocks.[2] This is a chronic wound where natural soft tissue repair is extremely rare.

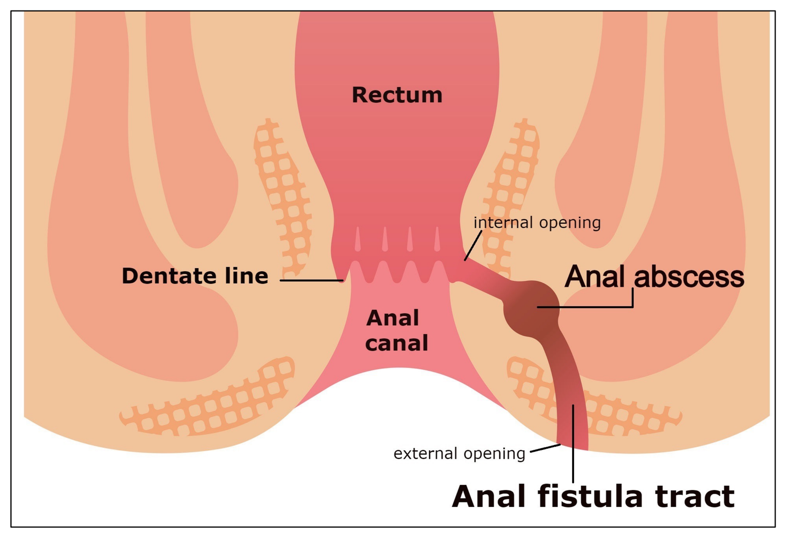


Figure 1. Schematic of anal fistula tract

Common symptoms of CD-PAF:

- Feculent drainage
- Perianal pain
- Redness, soreness, itching
- Reduced quality of life.

Study Objectives

Aim 1: Develop a biodegradable Gelatin-hyaluronic acid (GelHA) composite (nanofiber hydrogel composite) NHC plug to withstand mechanical conditions of perianal tracts.

Aim 2: Evaluate the ability of the GelHA composite plug co-delivered with adipose-derived stems cells (ADSCs) to promote remodeling of PAF through *in vitro* cell culture studies.

Methods

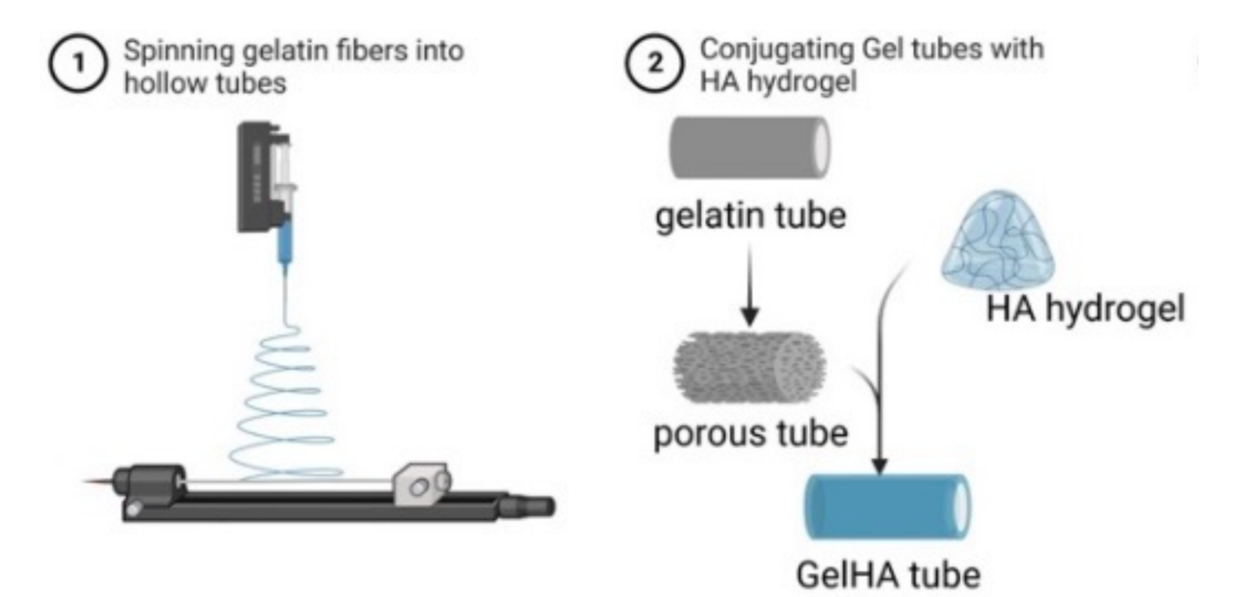


Figure 2. Schematic of (1) electrospinning process of gelatin B tube and (2) covalent crosslinking of HA hydrogel with gelatin B tube.

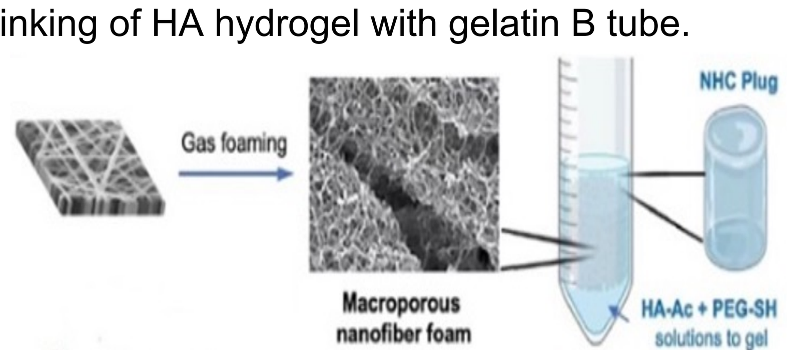
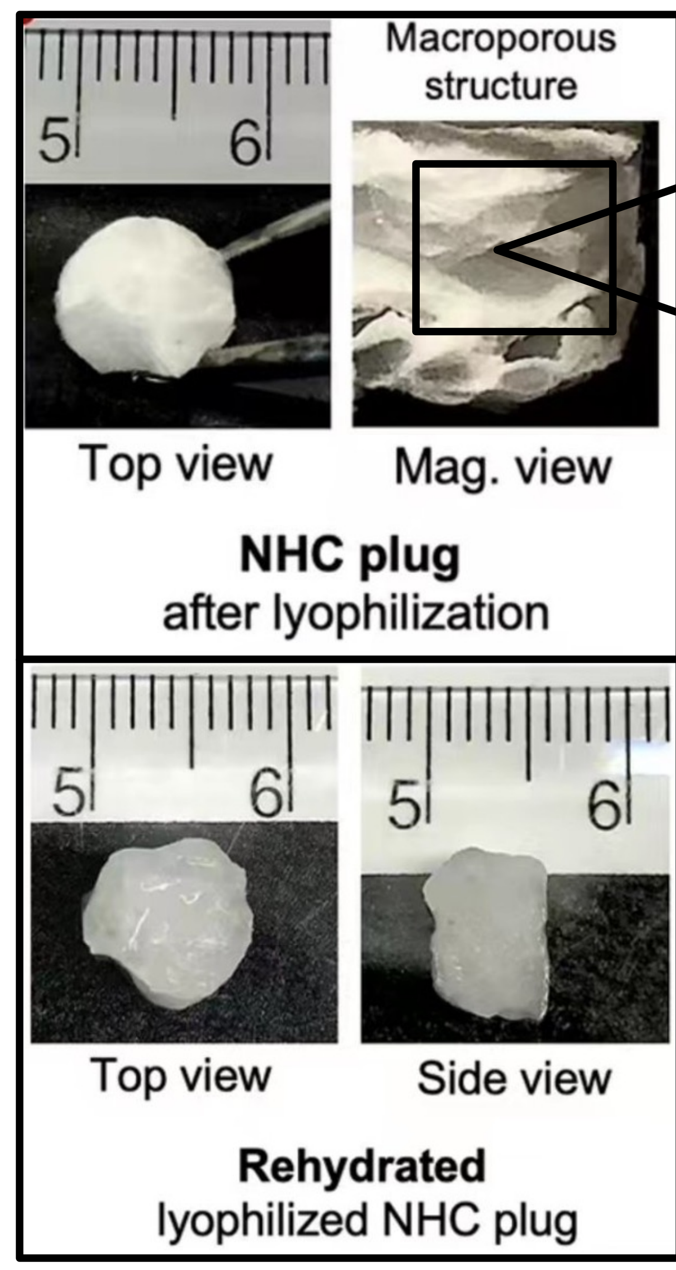


Figure 3. Gas foaming of gelatin B by $NaBH_4$ and the resulting foam structure.

Results

Aim 1. NHC Plug Synthesis and Testing



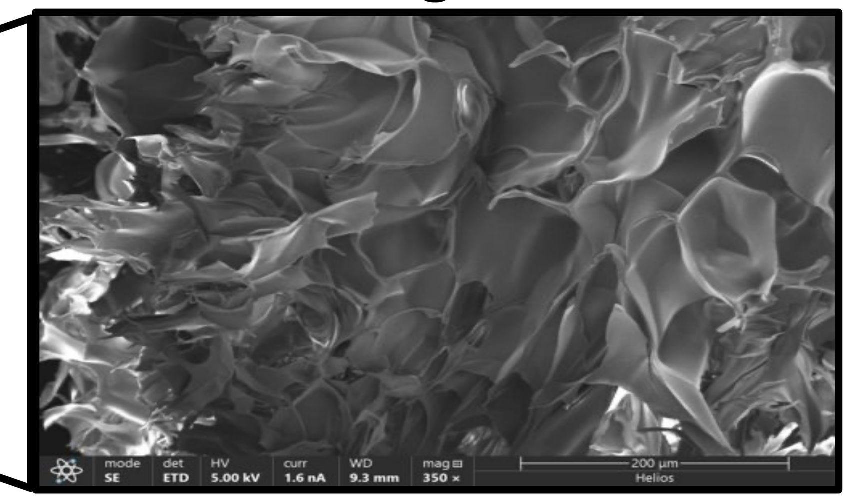


Figure 4. SEM imaging of GelHA plug highlighting open pores inside the scaffold.

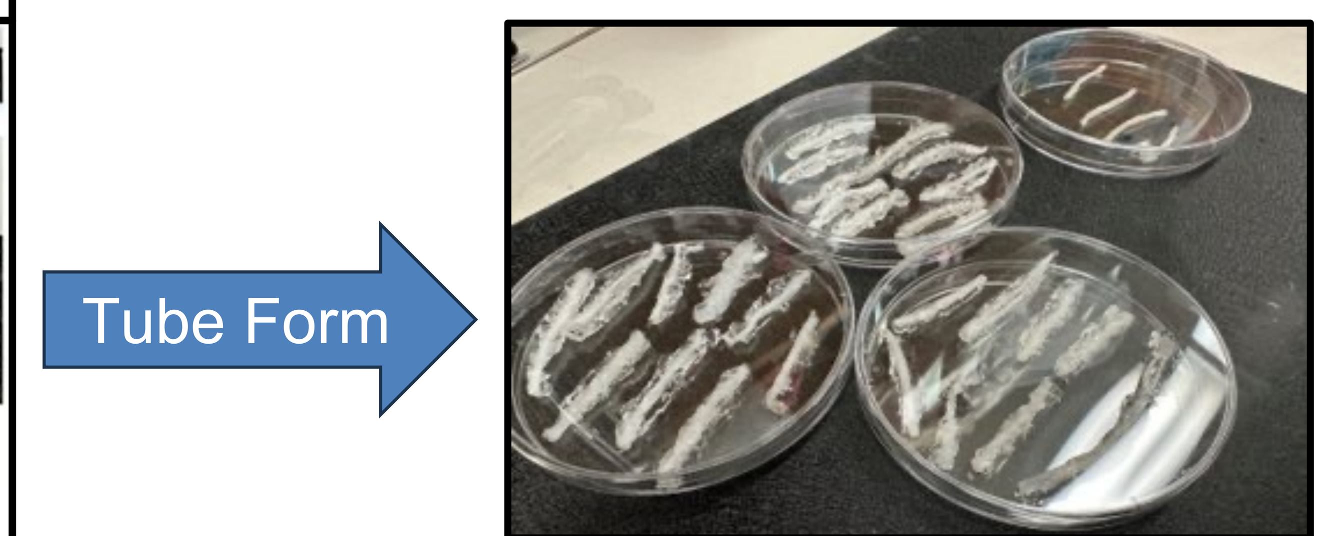


Figure 5. Rehydrated NHC plug as a tube.

- ~ **3-fold increase** in elastic modulus of NHC plug shows inclusion of gelatin B improved shape retention when compared to the pure HA gel.
- ~ **32-fold increase** in storage modulus of NHC plug highlights the plug's enhanced energy storage capabilities.

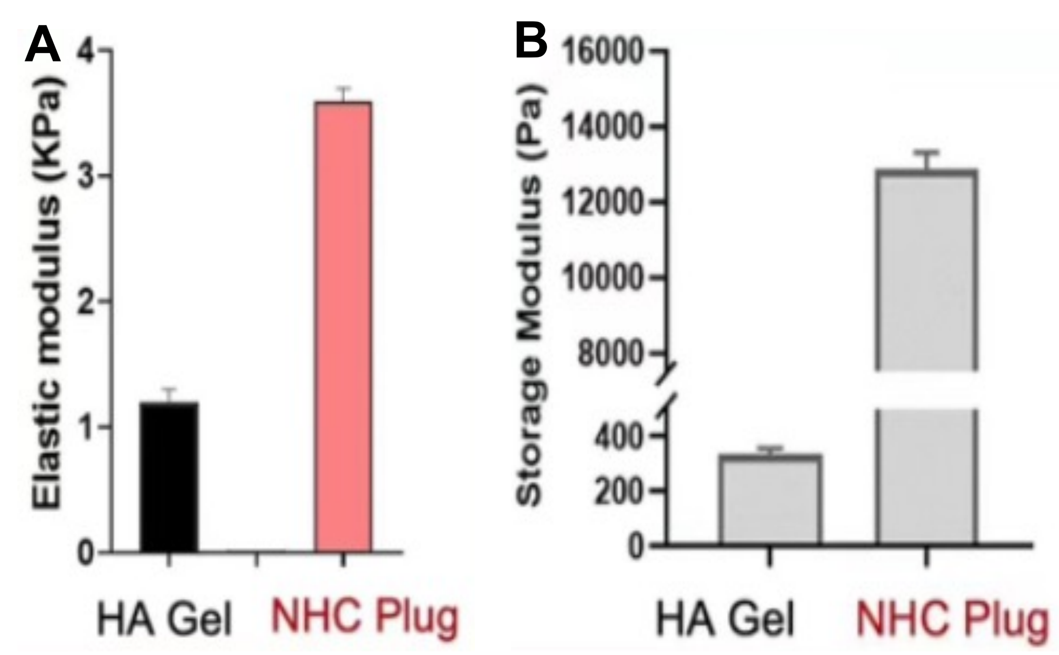


Figure 6. Comparison of (A) elastic modulus and (B) storage modulus for HA only gel and NHC plug.

Aim 2. Assessment of Biocompatibility

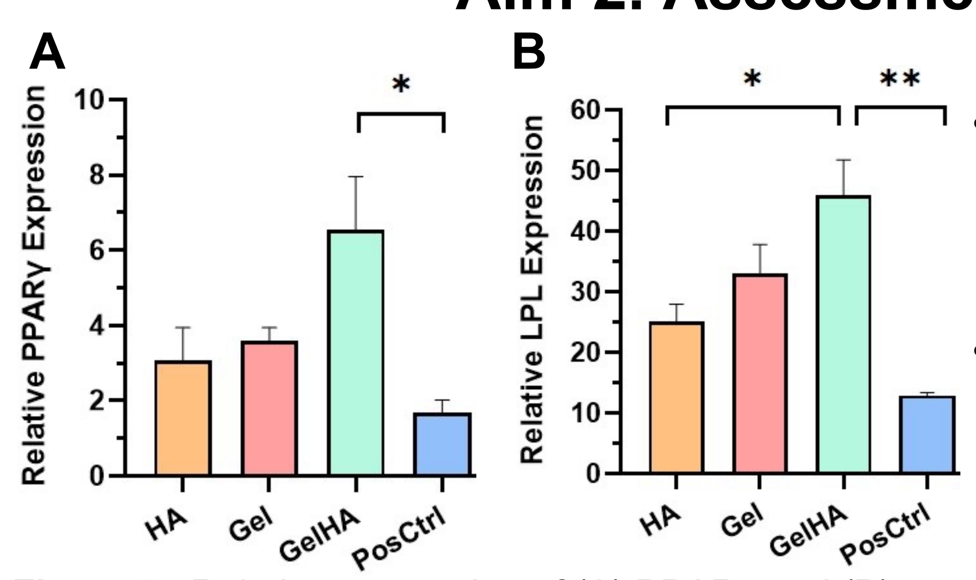


Figure 7. Relative expression of (A) PPARγ and (B) LPL in HA, Gel, GelHA, and positive control.

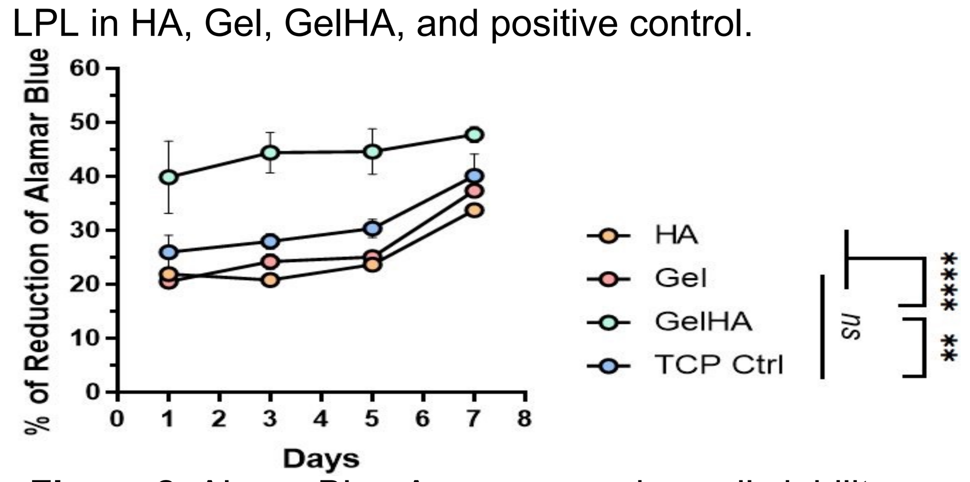


Figure 2. AlamarBlue Assay assessing cell viability.

- PPARγ encodes nuclear receptor necessary for adipocyte differentiation[3]; **GelHA scaffold** allowed for **highest expression** of PPARγ.
- LPL one of the first genes induced during adipogenesis[4]; heightened expression in GelHA scaffold indicates **increased differentiation** of preadipocytes into adipocytes.
- Increased reduction of AlamarBlue within GelHA scaffold indicates significant improvement in **cellular proliferation of ADSCs and cell viability.**
- AlamarBlue reduction signals for an **increase in molecular oxygen permeation** and the cells' ability to utilize oxygen effectively.

Conclusion

- GelHA scaffold allows for enhanced elasticity and shape retention, as well as a high water swelling ratio, desirable features when designing a plug aimed to seal perianal fistulas and preventing abscess formation.
- Mechanical testing highlights the potential of the GelHA plug in conforming to the irregular contours of the fistula tract that may be encountered.
- SEM imaging and AlamarBlue assay confirm the presence of a highly porous microstructure inside the GelHA that enhances cell viability and directs ADSCs adipogenic differentiation.
- Upregulation of PPARγ and LPL in ADSCs delivered within GelHA plug, confirming microenvironment within 3D scaffold conducive to cellular proliferation and the formation of adipose tissue, vital for soft tissue repair.

Future Directions

- Evaluation of different gelatin B and HA crosslinking methods to enhance crosslinking efficiency.
- Investigating additional nanofibers that may allow for enhanced mechanical properties and biocompatibility.
- In vivo subcutaneous implantations to assess adverse immune responses and monocyte infiltration/polarization.
- H&E and IHF imaging to assess host cell infiltration and micro-vessel network formation.
- Evaluation of soft tissue restoration of GelHA plug co-delivered with ADSCs in-vivo in a rat fistula model.

References

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