Engineering Antibacterial and Regenerative Hydrogel for Minimally Invasive Treatment of Fistula

Reihaneh Haghniaz*, Fatemeh Zehtabi, Fahimeh Zarei, Bahareh Mirmashhouri, Hossein Montazerian, Ankit Gangrade, Natan Roberto de Barros, Ali Khademhosseini*

Terasaki Institute for Biomedical Innovation, Woodland Hills, Los Angeles, CA 91367, USA.

Aim: Perianal fistula is an abnormal connection between the anal canal and perianal skin, often resulting from infections. Despite advances in surgical techniques, no successful treatment exists. Patients endure repeated interventions with a postoperative recurrence rate of ~50% and fecal incontinence risk of up to 40%. This results in poor quality of life with increasing healthcare costs of > $80,000 per patient. This study aims to revolutionize the treatment of fistula *via* a minimally invasive platform that employs an injectable antibacterial and regenerative bioadhesive hydrogel, comprising thiolated-gelatin (GS), polydopamine nanoparticles (PDA) and poly (ethylene glycol) maleimide (PEM, as a crosslinker) to occlude fistula tracts. **Methods:** The GS prepolymers (20% w/v) were prepared in ultrapure water at 37°C. PDA was synthesized by oxidative polymerization of dopamine hydrochloride. PDA (0-10% w/v in water) was mixed with PEM (40% w/v in PBS) at a ratio of 1:1. Hydrogels were prepared by co-injecting equal volumes of GS with PDA-PEM mixtures using a dual-barrel syringe (**Fig. 1A**).
Results: Crosslinking between maleimide and thiol groups, facilitated by PDA, resulted in gel formation within a few seconds (**Fig. 1B**). PDA-containing hydrogels demonstrated superior mechanical strength with a storage modulus of ~100-fold greater than PDA-free hydrogels (**Fig. 1C**). Human dermal fibroblast cells maintained >90% viability after 5 days of incubation with hydrogels (**Fig. 1D**). PDA imparted the hydrogels with antibacterial activities of >70% against *E. coli,* compared to Ciprofloxacin (**Fig. 1E**). PDA-enriched hydrogels accelerated wound healing two times more than untreated controls, *in vitro* (**Fig. 1F**). Additionally, PDA inclusion enhanced adhesive strength by 400% compared to commercial adhesive (Tisseel), allowing hydrogels to withstand pressures >300 mmHg at a flow rate of 120 ml/min using *ex vivo* pig belly fistula model (**Fig. 1G**).

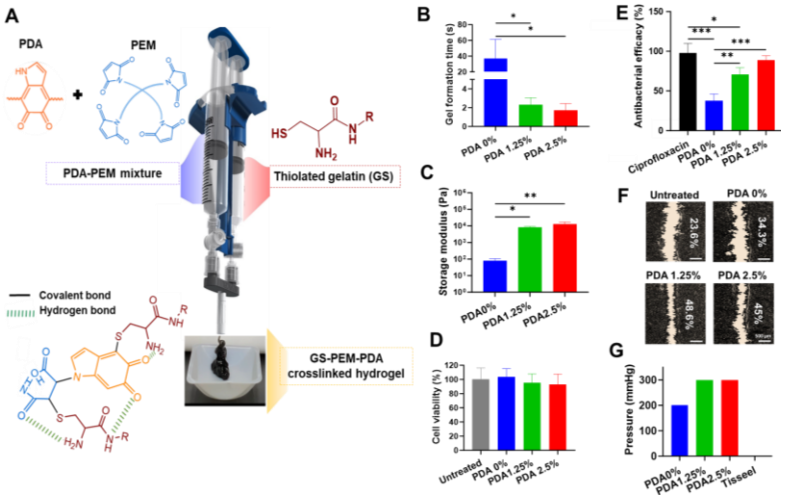

Fig. 1. A). Schematic illustration of the GS-PEM-PDA hydrogel formation. B) Sol-gel transition time. C) Storage moduli (*G'*), 15 min after gel formation. D) Cell viability 5 days after incubation of hydrogels with human dermal fibroblasts (HDF) vs. untreated control. E) Antibacterial efficacy against *E.coli.* F) Percent wound healing measured by scratch assay, 12 h after incubation of hydrogels with HDF cells. G) Results of pressures obtained from embolization test using *ex vivo* fistula model.

Conclusions: The hydrogels exhibit remarkable mechanical properties and tissue adhesion, effectively promoting wound healing and reducing bacterial infection. Based on these promising results the hydrogels will be further tested for their fistula closure efficacy, *in vivo* (ongoing study). These hydrogels not only aim to treat fistula but also hold the potential for healing other infected body cavities, *e.g.*, gumline cavities.

Acknowledgments: The authors acknowledge funding from Rousselot Company, Belgium.