A Nanofiber-Hydrogel Composite to Treat Fistula in Crohn's Disease in a Porcine Model

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Statement of Purpose: Fistulas are abnormal connections that can develop between the intestinal tract and the skin [1, 2]. Perianal fistulizing Crohn's disease is associated with significant pain, purulent or feculent drainage, frank fecal incontinence, and a high risk of super-imposed infections. At present, there are no reliable, minimally invasive treatments for fistulas, which remain a major unmet need in the care of Crohn's patients as biologic therapy, and surgical treatment have all shown limited success [3, 4]. Preliminary studies on stem cells are promising, but still only report a 50% rate of success [4]. The objective of this study is to develop a therapeutic strategy integrating adipose-derived stem cells (ADSCs) and an injectable nanofiber-hydrogel composite (NHC) as filler graft, and to test its efficiency in treating the perianal fistula track in a swine model.

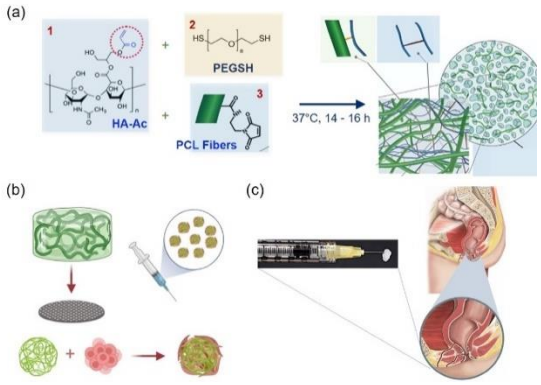

Figure 1. (a) Synthesis scheme of the nanofiber-hydrogel composite (NHC). (b) Generation of NHC microbeads and culturing ADSCs on NHC microparticles. (c) Illustration of a procedure to inject ADSC-NHC microparticles into perianal fistula track through a 27-gauge needle.

Methods: The NHC is composed of acrylated hyaluronic acid (HA-Ac) crosslinked by dithiol poly (ethylene glycol) (PEG-SH) and surface-functionalized poly (ε-caprolactone) (PCL) nanofibers. After crosslinking, NHC was processed into microparticles with an average size of 200 to 250 μm and cultured with ADSC spheroids with a similar size in an incubator for 24 h. To optimize the stiffness of NHC for cells growth and proliferation, we tuned HA concentration from 5 to 15 mg/mL. ADSC-coated NHC microparticles were injected subcutaneously in porcine model to assess cell infiltration and ADSC retention in the NHC matrix. Tissue samples were harvested at post-operative days (PODs) 14 and 42, and processed for cryosection and evaluation via immunohistochemistry staining for vascular ingrowth, vessel density, and neo-tissue formation.

Results: The storage modulus of the NHC was modulated from 104.3±11.9 Pa to 438.0±30.3 Pa, by increasing HA concentration from 5 to 15 mg/mL. Results from the subcutaneous injection demonstrated that the soft NHC (HA-5) induced blood vessel formation more favorably inside the implants (Figs. 2b and 2c). No significant difference in blood vessel density was found among the groups at POD 14, although more vessels were observed in the 104-Pa NHC at POD 42.

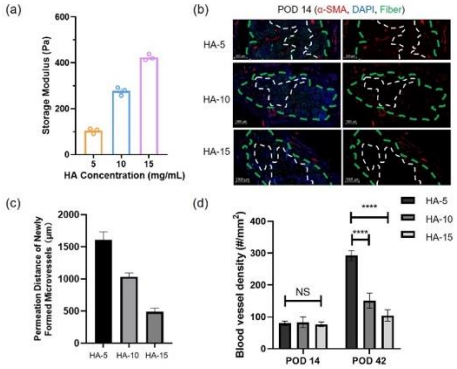

Figure 2. (a) Storage modulus of NHC prepared at different HA concentrations. (b) Confocal images showing new blood vessel formation inside the NHC at POD14, the green and white dash lines circle out the area where the vessels were formed. (c) Quantitative results showing the permeation distance of blood vessel formation inside the NHC. (d) Quantitative results presenting the blood vessel density inside the NHC at POD 14 and 42, ****p<0.0001.

Conclusions: The combination of NHC microparticles with ADSCs displayed a higher degree of micro-vessel formation inside the matrix in a stiffness-dependent approach. The NHC has the potential as an efficacious cell carrier for fistula treatment as a conformal composite scaffold filling fistula track. Its ability to promote angiogenesis and host tissue integration can facilitate effective cell delivery and retention, vascular infiltration, neo-tissue formation, while limiting scar tissue formation during the fistula healing process.

References

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