Self-healable and sustained release zwitterionic cryogels for wound healing

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Statement of Purpose: Scar formation, or fibrosis, is a fundamental complication of an extremely wide variety of conditions and disease processes that results in significant healthcare expenditure and morbidity.[1,2] Here, we report a method to prepare self-healable zwitterionic cryogels for sustained release of therapeutic miRNA-loaded cerium oxide nanoparticles for wound healing. Recently, we showed that crosslinked poly(SBMA) zwitterionic cryogels have good protein loading and release properties.[3] However, these gels are highly crosslinked, resulting in mechanically tough and brittle gels, which may not be suitable for wound dressing applications. An ideal wound dressing material should be flexible and self-healable to resist crack formation due to mechanical stress generated during wound healing.[4] Here, we developed a method to prepare zwitterionic cryogels in the absence of any crosslinkers. The prepared cryogels were flexible, injectable, and self-healable, with sustained nanoparticle release. The promise of the developed materials in wound healing applications was demonstrated in a mouse model.

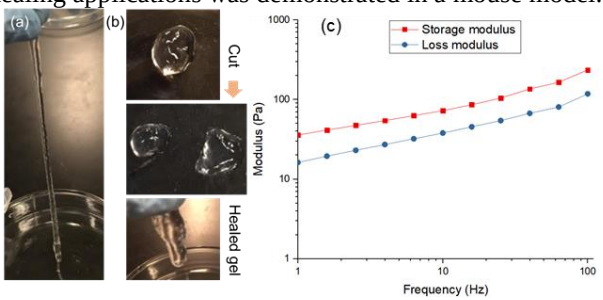

Figure 1. Cryogels prepared using SBMA and HEMA monomers. (a) Photograph showing an elongated gel. (b) Photographs showing self-healing ability of the gel. (c) Storage and loss moduli of the cryogel.

Methods: Zwitterionic gels were prepared using [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA) as the zwitterionic monomer and 2-hydroxyethyl methacrylate (HEMA) as the comonomer by dissolving in water and polymerization initiated with ammonium persulfate (APS) and N,N,N',N'-Tetramethylethylene diamine (TEMED). Polymerization of the hydrogel solutions was performed at -20 ºC for 24 h to form cryogels. The storage and loss moduli of the gels were determined via compressive dynamic mechanical analysis and released fluorescein-labeled cerium oxide nanoparticles (FITC-CNP) was quantified using a microplate reader. 12-week-old homozygous diabetic (Db/Db) mice were used for wound healing studies. 8-mm wounds were created on each mouse and empty or CNP-miR146a-loaded zwitterionic gels were administered one time. The wound surface area was measured every other day until full closure. Animals were euthanized 4 weeks after wound closure for biomechanical testing to measure the maximum load, extension, and tensile strain.

Results: The flexibility and self-healing ability of the cryogels were demonstrated (Fig. 1a,b). Rheology showed the viscoelastic nature of the prepared cryogels (Fig. 1c). The controlled nanoparticle release from the cryogels was demonstrated with FITC-CNP (Fig. 2). Cryogels had a sustained release profile for more than 2 months.

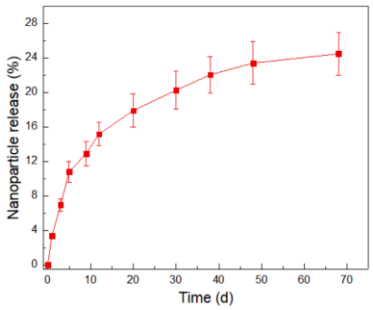

Figure 2. Nanoparticle release from copolymer cryogels prepared using SBMA and HEMA monomers.

Having desired sustained release properties and mechanical properties we tested them for their ability to promote wound healing in diabetic mice. Db/Db mouse wounds treated with CNP-miR146a-loaded cryogel demonstrated more rapid wound closure compared to the control gels: 14 d vs. 20 d ($p=0.002$; Fig. 3), and the repair tissue from these treated wounds had improved maximum load, modulus and tensile strength (data not shown).

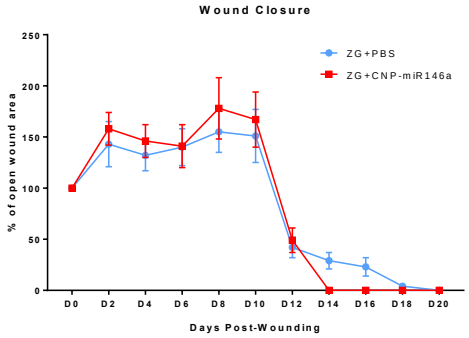

Figure 3. Wound healing response in diabetic mice to zwitterionic gels loaded with CNP-miR146a.

Conclusions: In conclusion, zwitterionic cryogels with, sustained release profiles, self-healing ability and good mechanical properties were developed for wound healing applications. Diabetic mouse wounds treated with the miRNA-CNP-laden zwitterionic cryogels demonstrated improved time to complete wound healing, strength, elasticity, and resistance to stress after healing.

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References: 1) Kossi JA. *World J Surg* **2004**; 28: 666-670. 2) Williams F. *Burns* **1998**; 24(4): 329-335. 3) Sener G., Krebs MD. *RSC Adv.* **2016**; 6:29608-29611. 4) Boateng JS. *J. Pharma. Sci.* **2008**; 97:2892-2923.