Engineering Hyaluronic Acid Hydrogels with Design of Experiments for Controlled Protein Delivery

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Statement of Purpose: Severe musculoskeletal injuries that fail to heal can be treated with the delivery of recombinant proteins from biomaterial scaffolds.[1,2] Bone morphogenic protein-2 (BMP-2) is used to treat traumatic bone injuries. However, current biomaterials provide poor control over BMP-2 delivery, leading to rapid protein release away from the site of injury and adverse effects such as abnormal mineralization and inflammation.[3] There is a need for rationally designed delivery vehicles that can better control local protein delivery.[1] Our goal was to develop a hydrogel delivery vehicle using hyaluronic acid (HA), which is a naturally occurring biopolymer.[2] Interactions between multiple hydrogel physicochemical properties were explored using design of experiments (DOE) to engineer a vehicle for controlled protein delivery.[4] DOE uses critical setpoints (high, medium, low) to screen ranges of independent variables and find significant interactions between independent variables (factors) on outcomes (responses). This method allows minimization of the number of experiments required and predicts the optimal formulation to achieve the desired hydrogel responses (swelling = 0%, compression modulus > 20kPa, gelation time = 2 min). I investigated polymer concentration and crosslinker ratio (low=-1, medium=0, high=1) as factors and evaluated their effects on swelling and compression modulus as responses.

Methods: A hydrazone click reaction between hydrazide and aldehyde functional groups was used to form an HA hydrogel crosslinked with dynamic covalent bonds. To investigate the interactions between protein release and gel properties, I tested hydrogels fabricated with ranges of total polymer concentrations (1-2.5 w/v %), and crosslinker ratios (0.8:1-5.4:1) of carbohydrazide-HA to oxidized-HA. *MODDE pro (Umetrics)* DOE software was used to model the interactions between variables in hydrogel fabrication.

Results: Hydrogel formulations with high polymer content ($CH_{2.25}Ox_{2.25}$ 2.25 w/v%) combined with high crosslinker ratio (5.4 CH:1 Ox) demonstrated the highest compression modulus at 40 kPa **(Fig. 1)**. High CH to Ox ratio provided a greater range of tunability for optimizing compression modulus compared to polymer concentration. BMP-2 (10 ng) was mixed into hydrogels containing varying w/v% of carbohydrazide-HA and oxidized-HA, and BMP-2 release was monitored over 48 hours **(Fig. 2A)**. Preliminary results demonstrated that all hydrogel formulations provided sustained BMP-2 release, and no burst release of the protein was observed **(Fig. 2B)**. DOE was then used to optimize interactions between hydrogel swelling, gelation time and protein release. The HA-hydrogel predicted to be minimally swelling using DOE Magic Formula "MF" (CH 2.22% and Ox 1.82%) demonstrated a negligible change in mass **(Fig. 2C)**, indicating successful optimization. Future work will confirm that the MF yields sustained release of BMP-2 over 7 days. The effects of additional hydrogel properties, such as compression modulus, on BMP-2 release will also be evaluated. Using DOE to tune multiple, interacting factors in a complex system at once will facilitate controlled BMP-2 release from HA hydrogels.

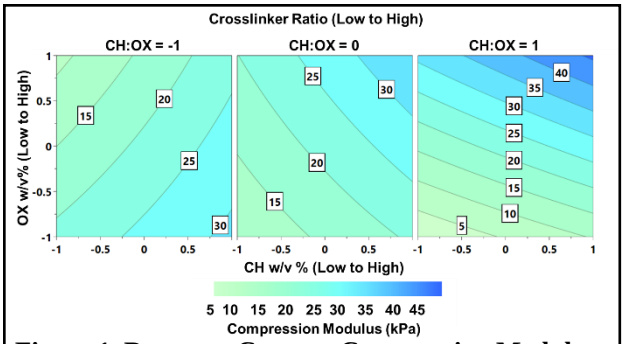

Figure 1. Response Contour Compression Modulus. Interaction between polymer concentration and cross-linker ratio.

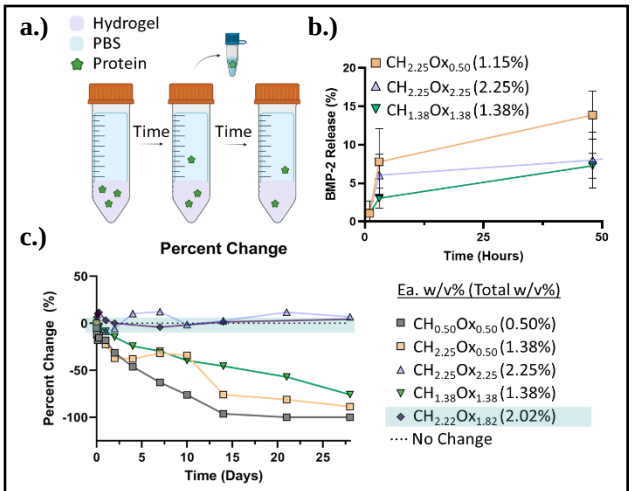

Figure 2. Modified Hydrogel Optimization. (a) Release schema. (b) BMP-2 release combinations. (c) Swelling over time with modified HA combinations.

References:

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4.) Lam, J., et al. *Adv Healthc Mater* **2015**, *4* (4), 534.