Hemostatic Wound Bandage for Blood Clotting Deficiencies

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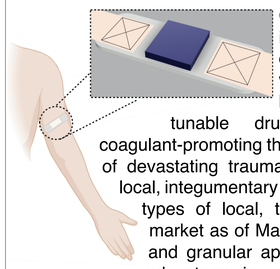
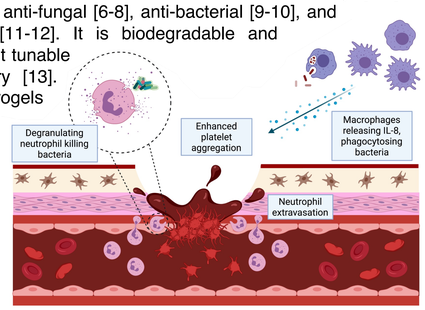
Introduction and Motivation

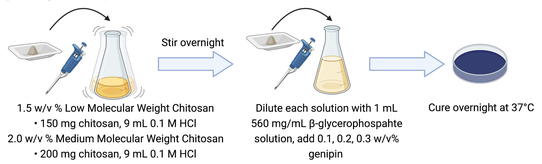


Current hemostatic technologies for individuals with blood clotting disorders do not mimic the natural wound-healing environment, often lead to complications, and do not use tunable drug delivery systems. Systemic coagulant-promoting therapeutics are ideal during scenarios of devastating trauma; however, most instances require local, integumentary system treatment. There are different types of local, topical hemostatic products on the market as of March 2020: gauze, sponges, sealants, and granular applications [1]. Chitosan has several advantages in wound healing: increasing macrophage recruitment [2], stimulating interleukin-8 release [3], and initiating a coagulation response outside of the traditional pathway [4-5]. Chitosan also is anti-fungal [6-8], anti-bacterial [9-10], and an antioxidant [11-12]. It is biodegradable and porous, making it tunable for drug delivery [13]. Chitosan hydrogels recapitulate the natural, moist environment of the body, accelerate angiogenesis, and offer protection from pathogens [14].

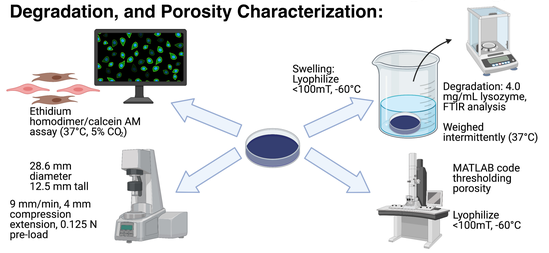


Methods

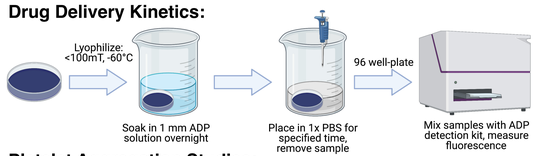
Hydrogel Preparation:



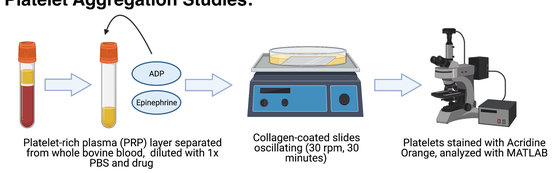
HUVEC, HDF Cell Viability, Compression Testing, Swelling, Degradation, and Porosity Characterization:



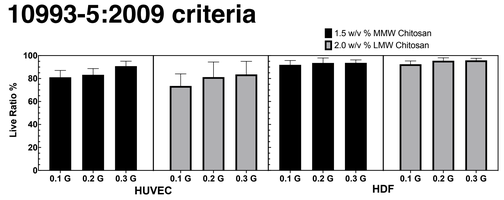
Drug Delivery Kinetics:

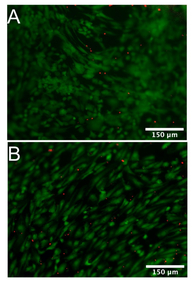


Platelet Aggregation Studies:



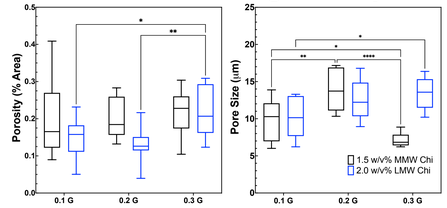
Testing for *in vitro* cell viability meets ISO 10993-5:2009 criteria

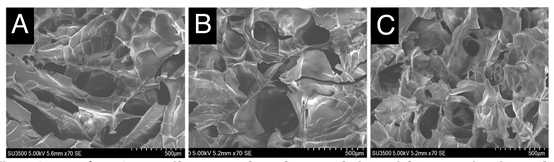




Average cell viability is above 70% for almost all hydrogels. Two physiologically relevant cells in wound healing, dermal fibroblasts (HDFs) and endothelial cells (HUVECs) were analyzed. Live cells (TRITC- green) and the nuclei of dead cells (FITC- red) were shown using a calcein-AM/ethidium homodimer live/dead assay. Image **A** is HDFs on a 1.5 w/v % low molecular weight chitosan, 0.1 w/v % genipin hydrogel, while **B** displays HUVECs on the same gel formulation.

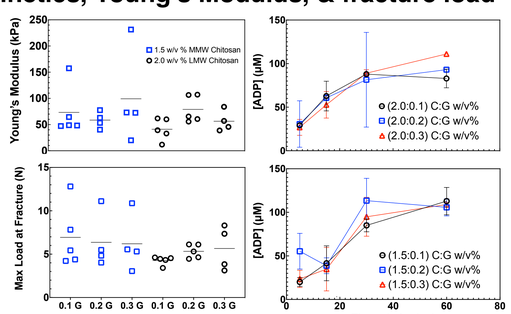
Hydrogels are in the macropore size range, which is ideal for fast drug delivery





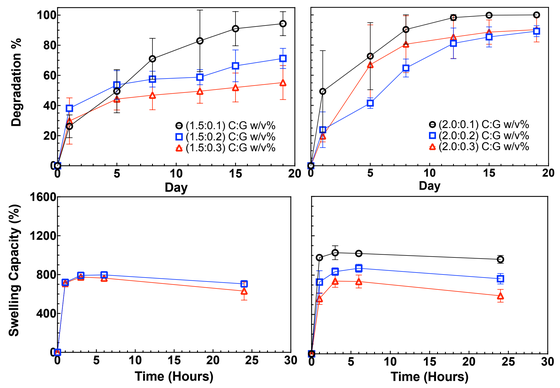
The 1.5 w/v % medium molecular weight chitosan hydrogels have a statistically significant difference in the mean pore size. There is a statistically significant difference in the % area of pores in the low molecular weight chitosan samples. Images **A-C**, 2.0 w/v % medium molecular weight chitosan, 0.1-0.3 w/v % genipin respectively.

Hydrogels display similar drug delivery kinetics, Young's Modulus, & fracture load



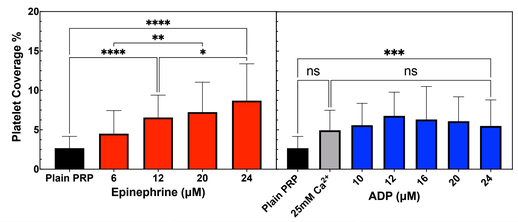
Diffusion of 1 mM ADP from the hydrogel matrices displays a first-order drug delivery curve. The maximum load the hydrogels could withstand was about 5 N despite differences in formulation, in addition to a Young's Modulus around 100 kPa.

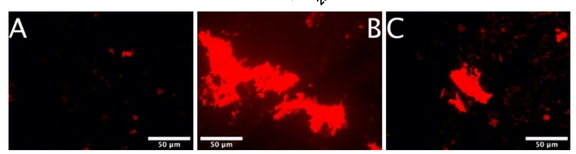
With less genipin, hydrogels degrade at a faster rate, exhibit higher swelling capacity



Within 21 days, most 0.1 w/v % genipin hydrogel samples decayed completely in 4 mg/mL lysozyme solution. The maximum swelling capacity (2.0 w/v % chitosan) between the 0.3 w/v % genipin samples and the 0.1 w/v % genipin samples is significantly different. Samples were shaken at 30 rpm continuously for degradation studies (37°C).

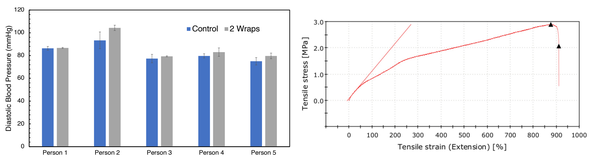
Statistically significant platelet aggregation for both ADP and epinephrine





24 µM epinephrine is observed to significantly increase platelet aggregation against all ADP concentrations (except 12 µM). Calcium ions at 25 mM are not significantly different as compared to the negative control. Images **A-C**, represent plain platelet-rich plasma (negative control), 24 µM epinephrine, and 12 µM ADP respectively.

Final design doesn't vasoconstrict arm, can withstand high tensile strain while wrapping



The TPU filament bandage demonstrates an ability to withstand 190.81 N before it slips during experimentation. The diastolic pressure data illustrates an increase in application pressure by measuring the differences in blood pressure between the control (no wrapping) and two wraps of the final bandage design. The difference in diastolic pressures is not statistically significant. The bandage is not restricting blood flow to the distal portion of the arm.

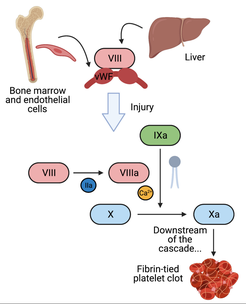
Conclusions

- ***Almost all hydrogel formulation*** *are shown to be biocompatible and* ***exhibit low cytotoxicity***
- *Hydrogels all show* ***macro-porous*** *structure,* ***increasing drug delivery*** *kinetics*
- *Lower genipin hydrogels* ***degrade faster*** *in physiologically-relevant conditions, and* ***swell more*** *for pooled blood applications*
- *Both epinephrine and ADP display* ***significant platelet aggregation*** *as opposed to no drug,* ***at the expense of higher epinephrine doses***
- *The final device design* ***does not constrict blood flow****, and is* ***able to withstand high strain*** *while wrapping.*

Future Directions

Transitioning from the characterization of the hydrogel and the overall bandage backing, the next steps are to test the product *in vivo*.

These *in vivo* studies will take place in mice with a coagulation factor VIII (F8) gene knockout. These genes are inherited from an X-linked autosomal recessive pass-down from a previous generation and are what cause Hemophilia A. The efficacy will be determined by the time to clot, platelet aggregation, and tests of increased coagulation cascade signaling. Hemophilia B, von Willebrand disease, amongst others may also be evaluated.



Literature Cited

[1] Peng, et al. *Mil. Med. Res.*, **7**, 1-18 (2020). [2] Ueno, et al. *Adv. Drug Deliv. Rev.*, **52**, 105-15 (2001). [4] Minami, et al. *Carbohyd. Poly.*, **36**, 151-5 (1998). [5] Khan, et al. *Int. J. of Biol. Macromol.*, **124**, 138-47 (2019). [6] Shih, et al. *Microbiol.*, **10**, 1-14 (2019). [7] Ing, et al. *Int. J. of Miomat.*, **2012**, 1-9 (2012). [8] Alburquenque, et al. *Med. Mycol.*, **48**, 1018-23 (2010). [9] Atay, et al. *Functional Chitosan*, **2020**, 457-89 (2010). [10] Raafat, et al. *Microb. Biotech.*, **2**, 186-201 (2009). [11] Ngo, et al. *Food Nutr. Res.*, **73**, 15-31 (2014). [12] Tring, et al. *Int. J. of Carbohyd. Chem.*, **2015**, 1-6 (2015). [13] Ahmadi, et al. *Res. Pharm. Sci.*, **10**, 1-16 (2015). [14] Hu, et al. *Biomat.*, **8**, 2084-2101 (2020).

Acknowledgements

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