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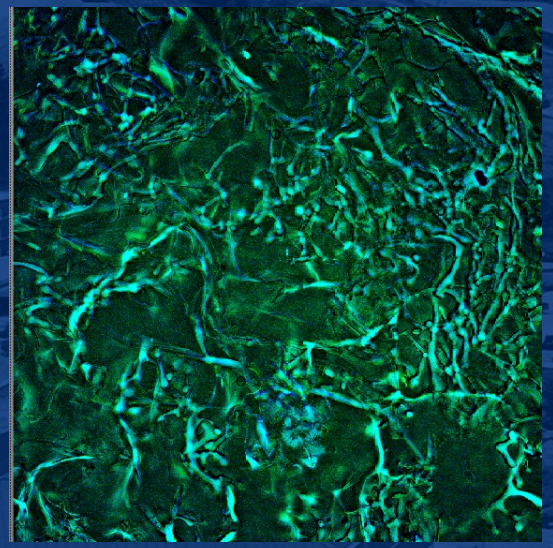

TREATING DIABETIC FIBROBLASTS THROUGH TUNABLE THERAPEUTIC HYALURONONAN-BINDING SILK FIBROIN HYDROGELS

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Nanoscience and Biomedical Engineering Department
South Dakota School of Mines

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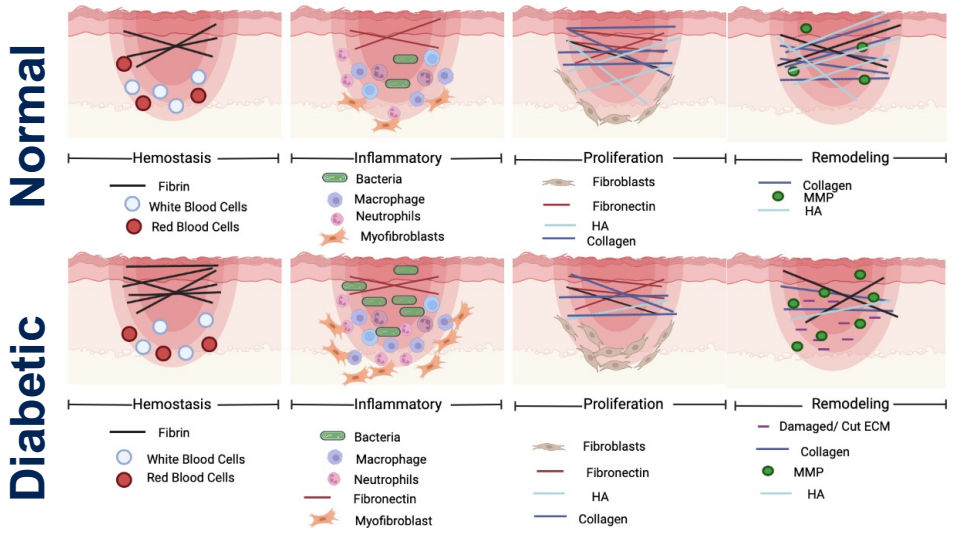
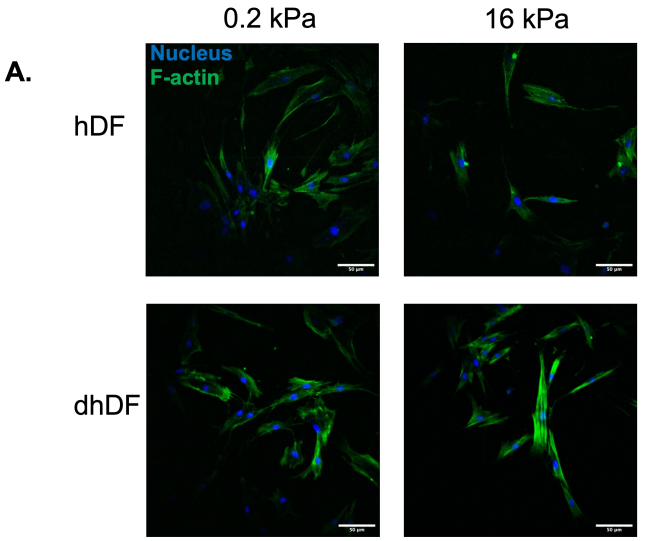
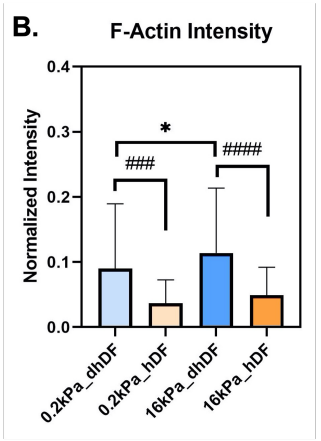
WOUND HEALING







- More bacteria
- Longer inflammation stage
- Less collagen and HA
- More damaged ECM
- Stiffer wound environment

- Softer surfaces result in less stress fibers and more healthy fibroblast like behavior

(Huffer, A et al, 2024)

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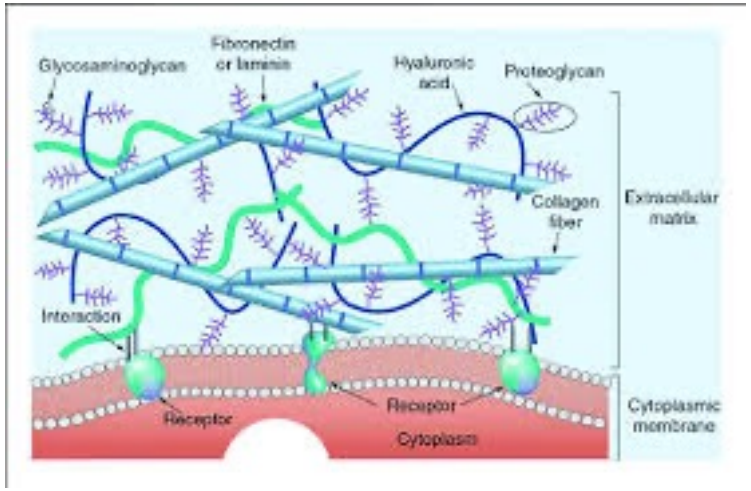
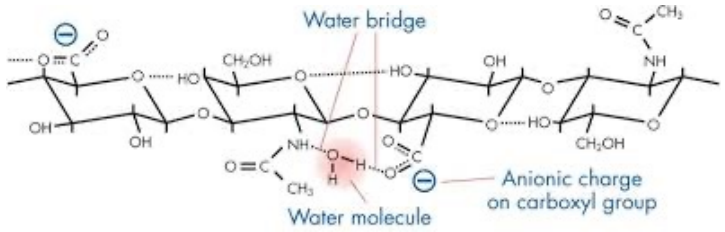
ECM

Importance of HA in the skin

- A single HA monomer consists of a disaccharide of D-Glucoronate-1,3—N-acetylglycosaminoglycan at each repeating unit
- Cells recognize HA through different binding receptors (CD44)
- In the ECM HA attracts water which leads to increased hydration and regulates the stiffness of the extracellular space
- High molecular weight HA (>900 kDa) has anti-inflammatory effects
- Low molecular weight HA (<120 kDa) is pro-inflammatory effects
- Fibroblasts produce the majority of the skin's HA
- Swelling of HA in at the ECM prevents crowding of other ECM components, regulates collagen organization, controls neovascularization and immune function.





https://hcs-pharma.com/the-extracellular-matrix-a-focus-on-glycosaminoglycans-and-hyaluronan/

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ECM

Role of HA in Wound Healing

- In hemostasis stage
  - HA in serum regulates platelet aggregation and fibrin mesh formation.
- In inflammatory stage
  - HA interacts with both innate and adaptive immune cells.
- As previously discussed, there is less HA present in diabetic wounds

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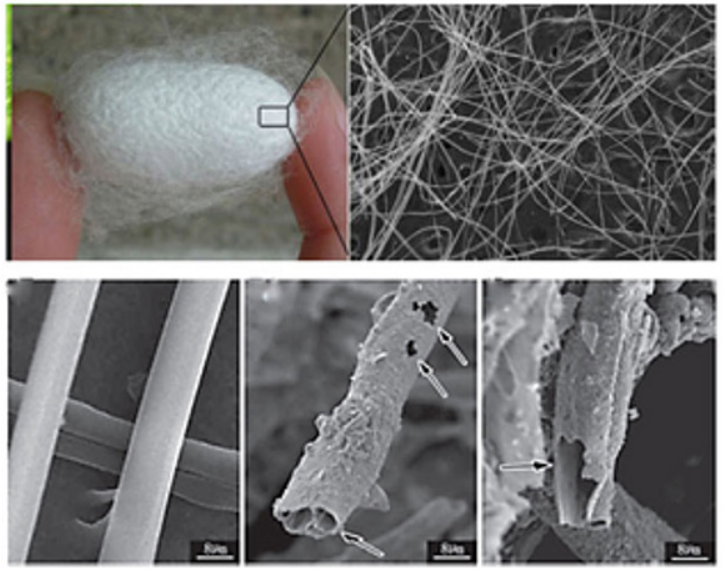
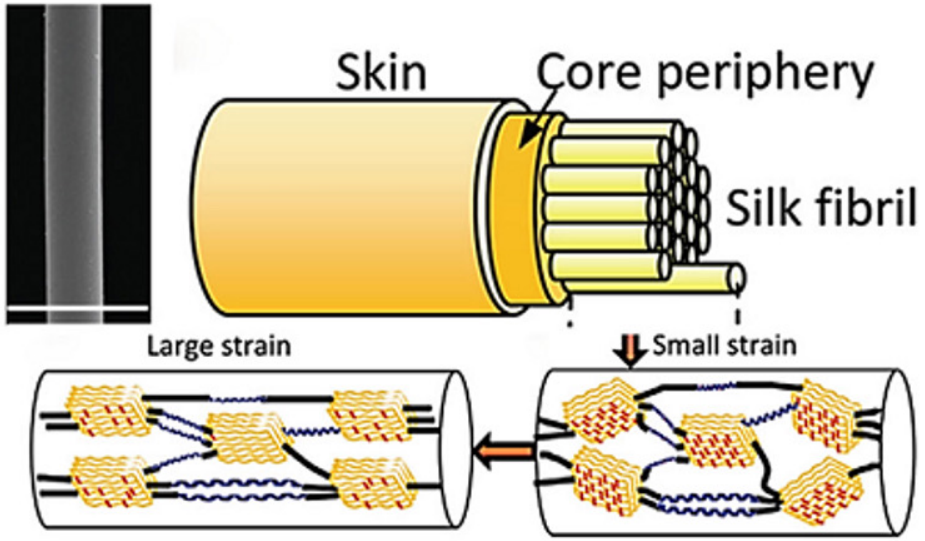
SILK AS A BIOMATERIAL





- Highly Biocompatible
- Great for Wound healing and tissue engineering
- Tunable
- Missing the biological components of the ECM

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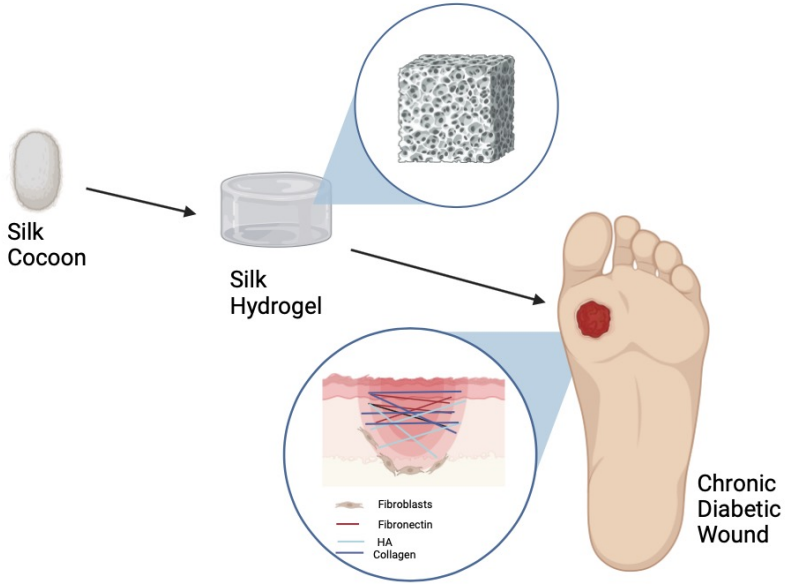
CREATE A TUNABLE SF HYDROGEL THAT ATTRACTS THE ENDOGENOUS HA TO TREAT DIABETIC FIBROBLASTS

We hypothesize that by attracting endogenous HA to a widely used silk fibroin biomaterial system can have a therapeutic effect on diabetic fibroblasts.

- Creating a tunable silk hydrogel
- Functionalizing the hydrogels with a novel HA attracting peptide tailored towards silk fibroin materials



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GOALS OF THE STUDY

Design a Tunable Biomaterial that also contains the Biological Components

- ❑Make a SF hydrogel with homogenous porosity
- ❑Tune the stiffness of the hydrogels
- ❑Characterize the mechanical properties
- ❑Ensure cytocompatibility of the hydrogels
- ❑Functionalize hydrogels with HABP

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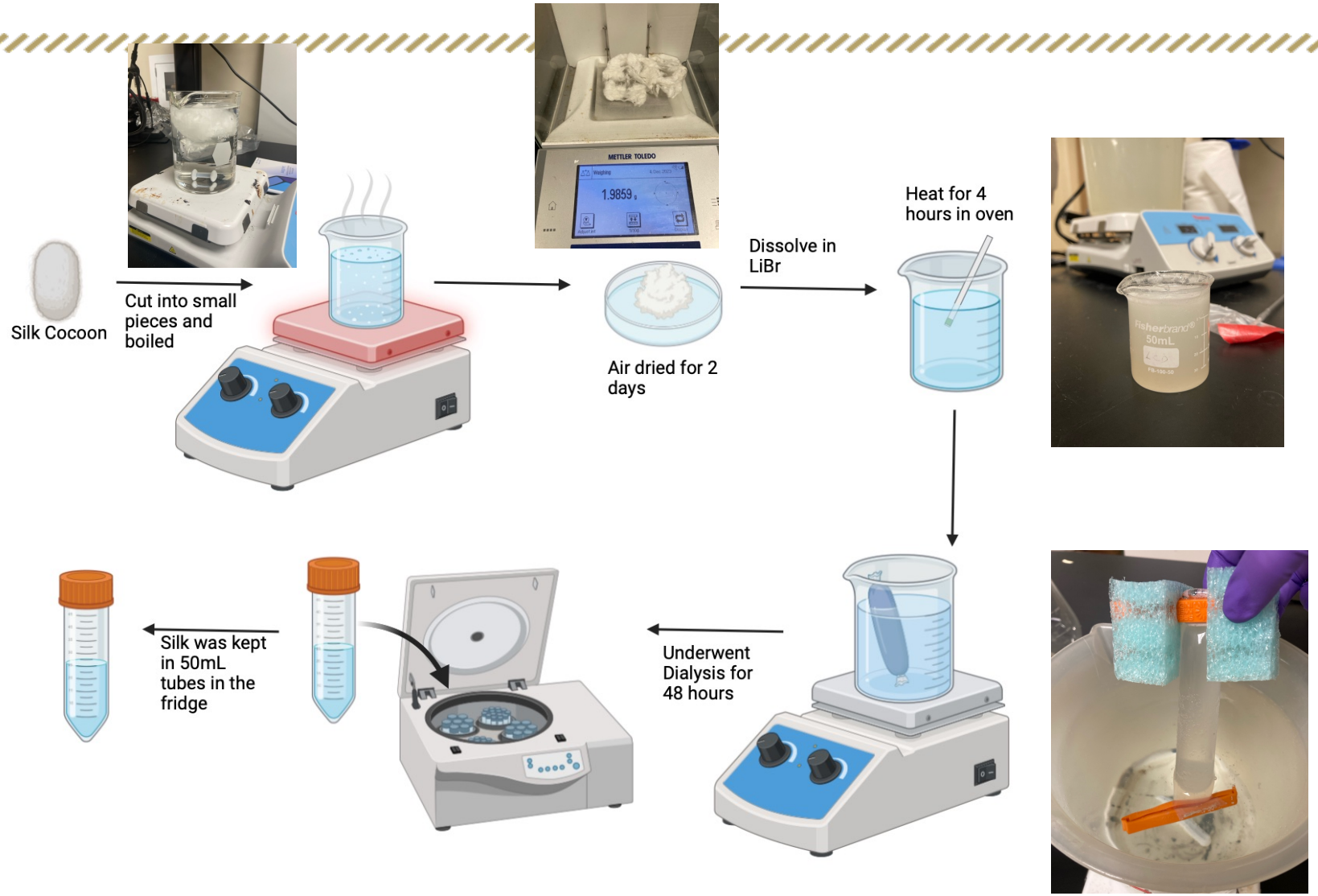
REGENERATION OF SILK FIBROIN FROM SILKWORM COCOONS



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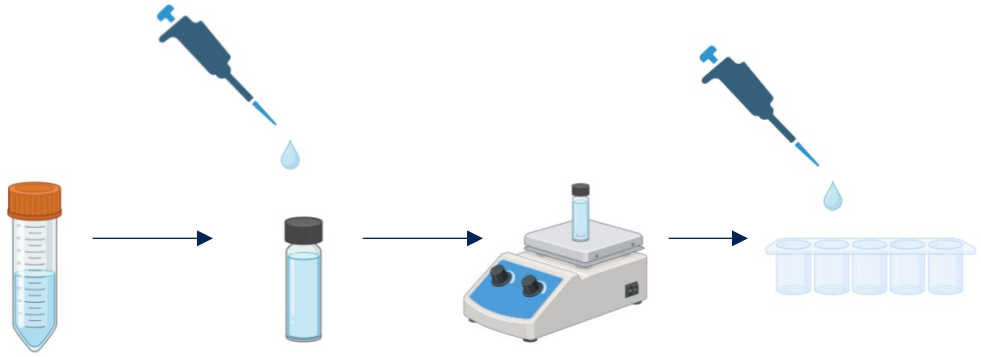
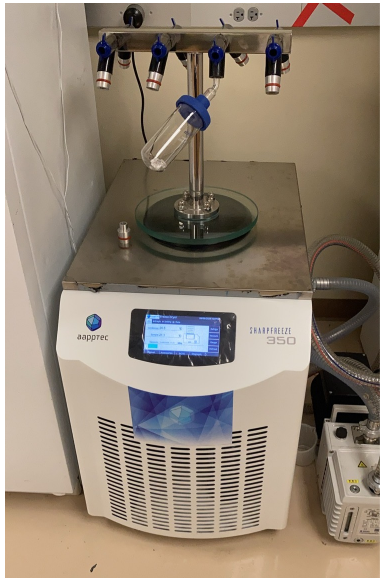
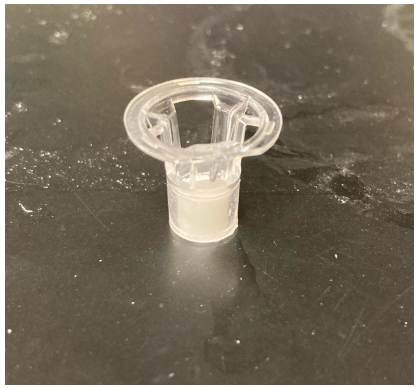
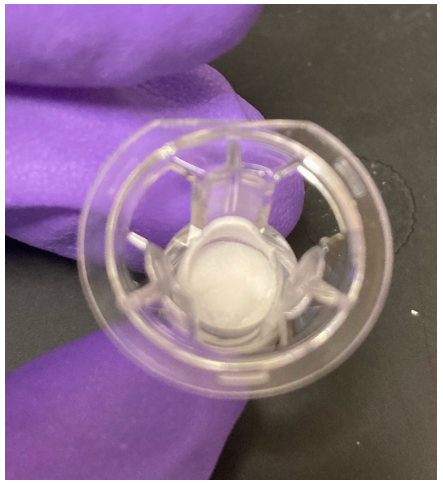
CREATING THE SF HYDROGELS







- This lyophilization process creates highly porous hydrogels
- Due to the hydrophobicity of the silk the water molecules stay together during freezing.
- When in the lyophilizer those molecules create micro pores.



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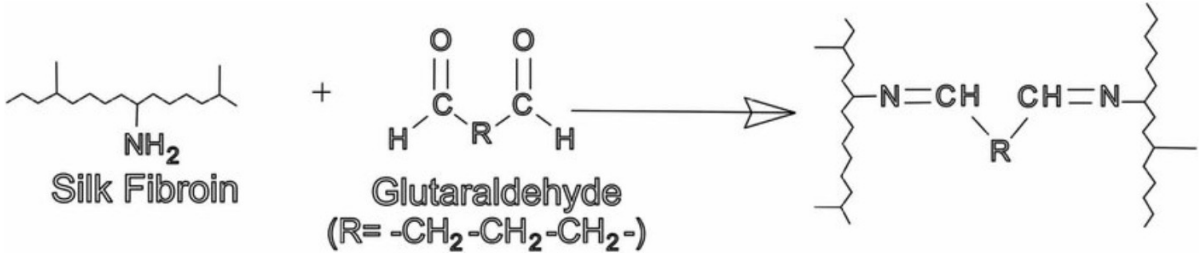
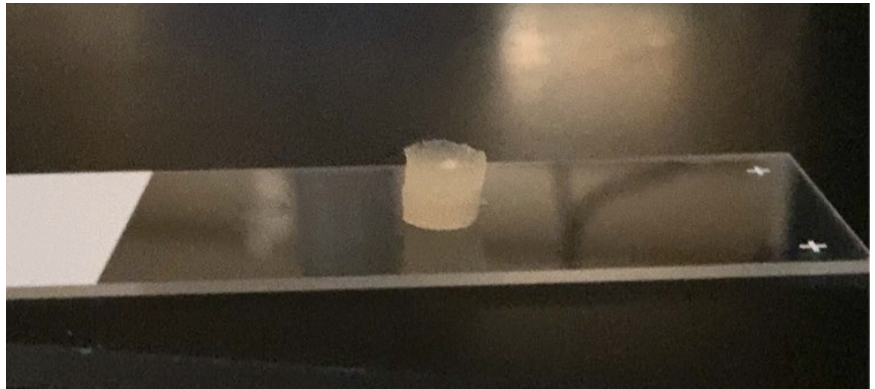
GLUTARALDEHYDE CROSSLINKING

- The amine group of the N-terminus of the peptide and surface lysine of the carrier protein are targeted in this method
- Glutaraldehyde vapor is used because of the low toxicity levels compared to the liquid form





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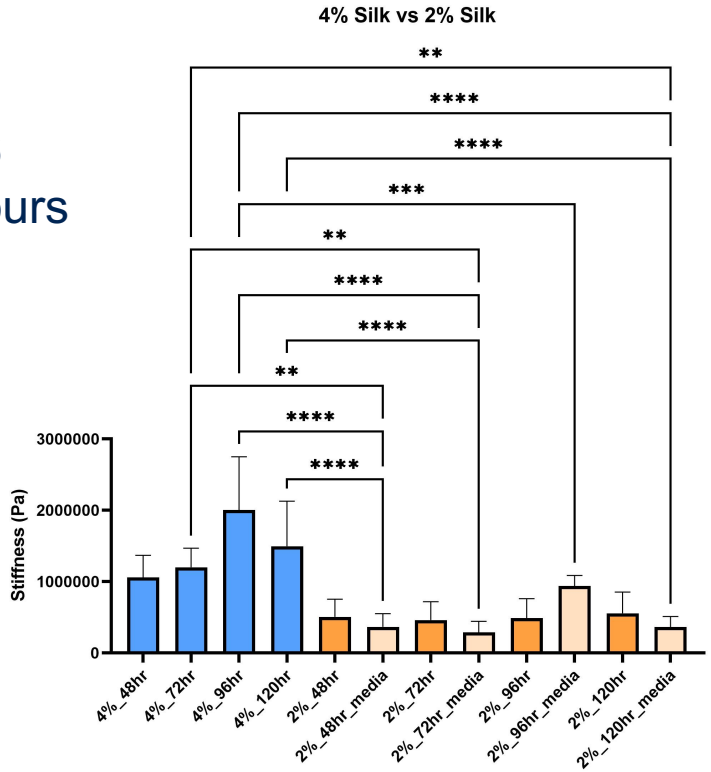
RHEOMETER

- Use the Rheometer-DHR3 running the DMA-compression protocol
- All crosslinked samples were measured

★ 96 hours



4% Silk

4% Silk w/ Media

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SWELLING RATIO OF HYDROGELS

$$\frac{wet\ weight - dry\ weight}{dry\ weight} = swell\ ratio$$

Sample	Insert weight (g)	Insert + Silk (g)	After lyophilization (g)	Swelling Ratio
1	0.5390	0.7388	0.5468	24.6153846
2	0.5414	0.0.7361	0.5488	25.3108108
3	0.5388	0.7304	0.5460	25.6111111
4	0.5417	0.7343	0.5482	28.6307692

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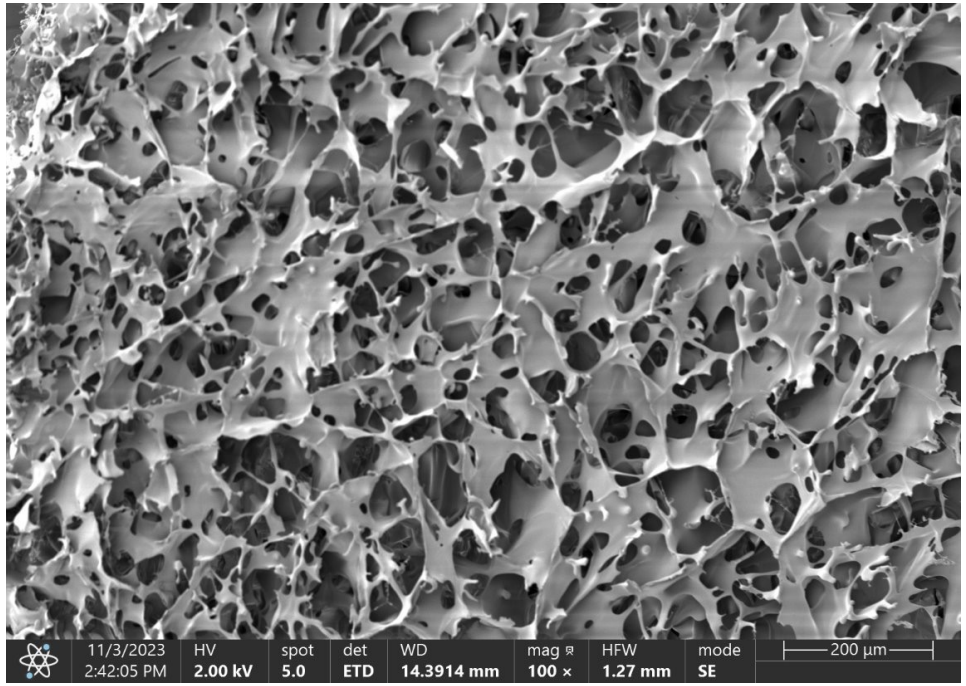
PORE SIZE

- Measured the pore size for 300 pores over 5 SEM (Scanning Electron Microscope) images
- Took the average and the standard deviation
- Pore size was found to be 62.056 ± 31.985 µm



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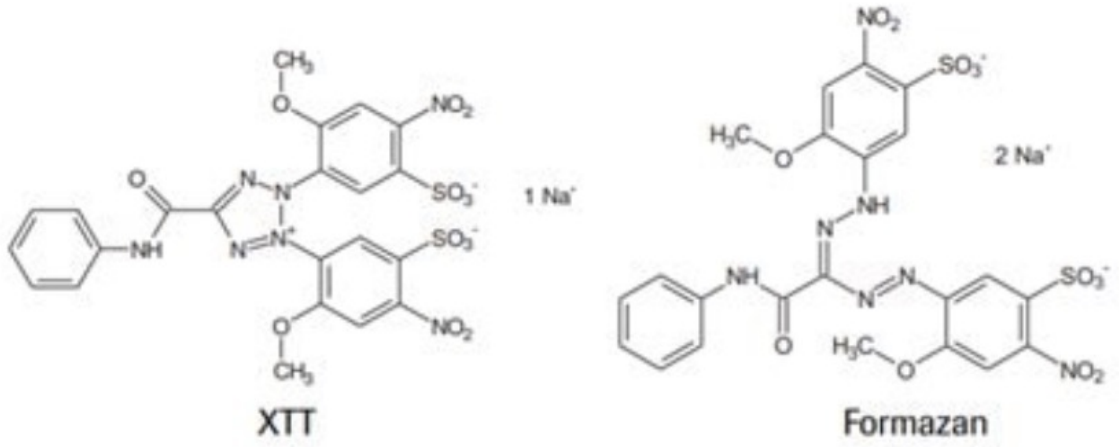
METABOLIC ACTIVITY

- XTT Assay is based on cleavage of yellow tetrazolium salt (XTT) to form an orange formazan dye by metabolically active cells
- An increase in the number of living cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample
  - Therefore, more metabolic activity means more orange formazan formed



XTT Formazan

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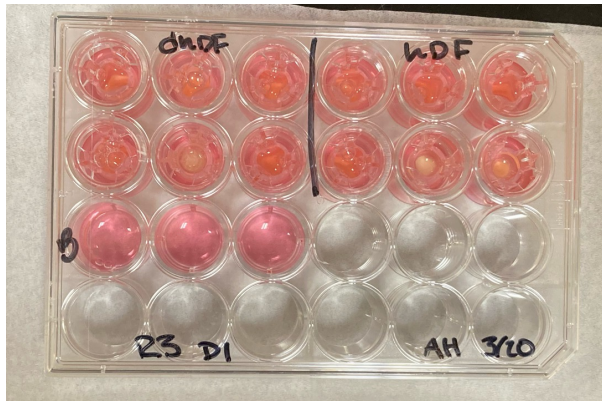
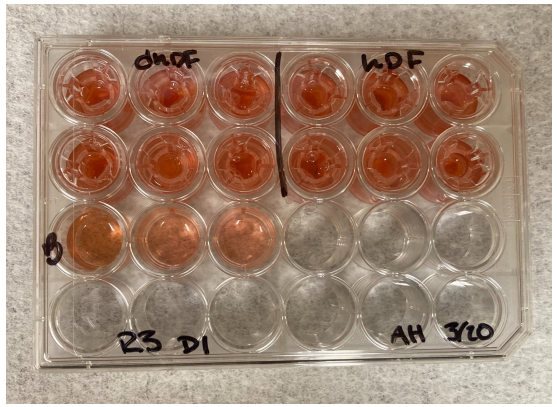
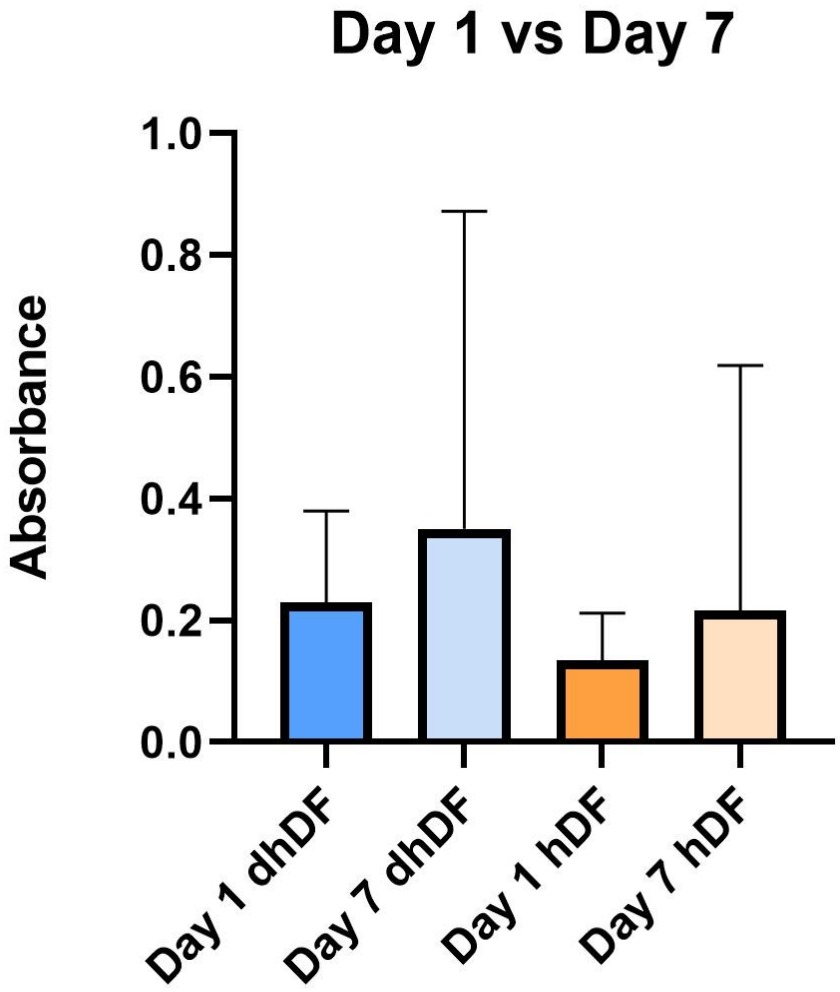
METABOLIC ACTIVITY IN SILK HYDROGELS

- Cell Types: dhDF and hDF
- Seeding Density: 50,000 cells/ scaffold
- Seed On: 4% SF Hydrogels crosslinked for 24 hours
- Incubation time for XTT: 6 hours
- Running 3 repeats on Days 1 and 7







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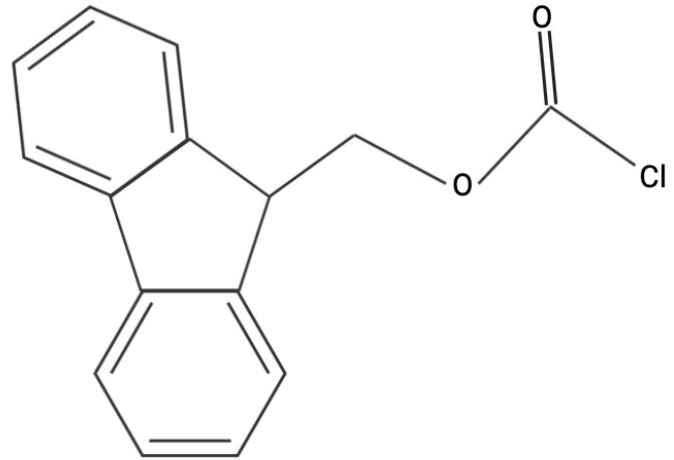
NEXT STEP – PEPTIDE SYNTHESIS

- Peptide synthesis is the method where you chemically link amino acids together in a desired sequence
- We use Fmoc Solid Phase Peptide Synthesis
  - This means there is a resin used as the building block for the peptide change and each time an amino acid is added Fmoc is used to cap it to prevent unwanted bonding or aggregation.



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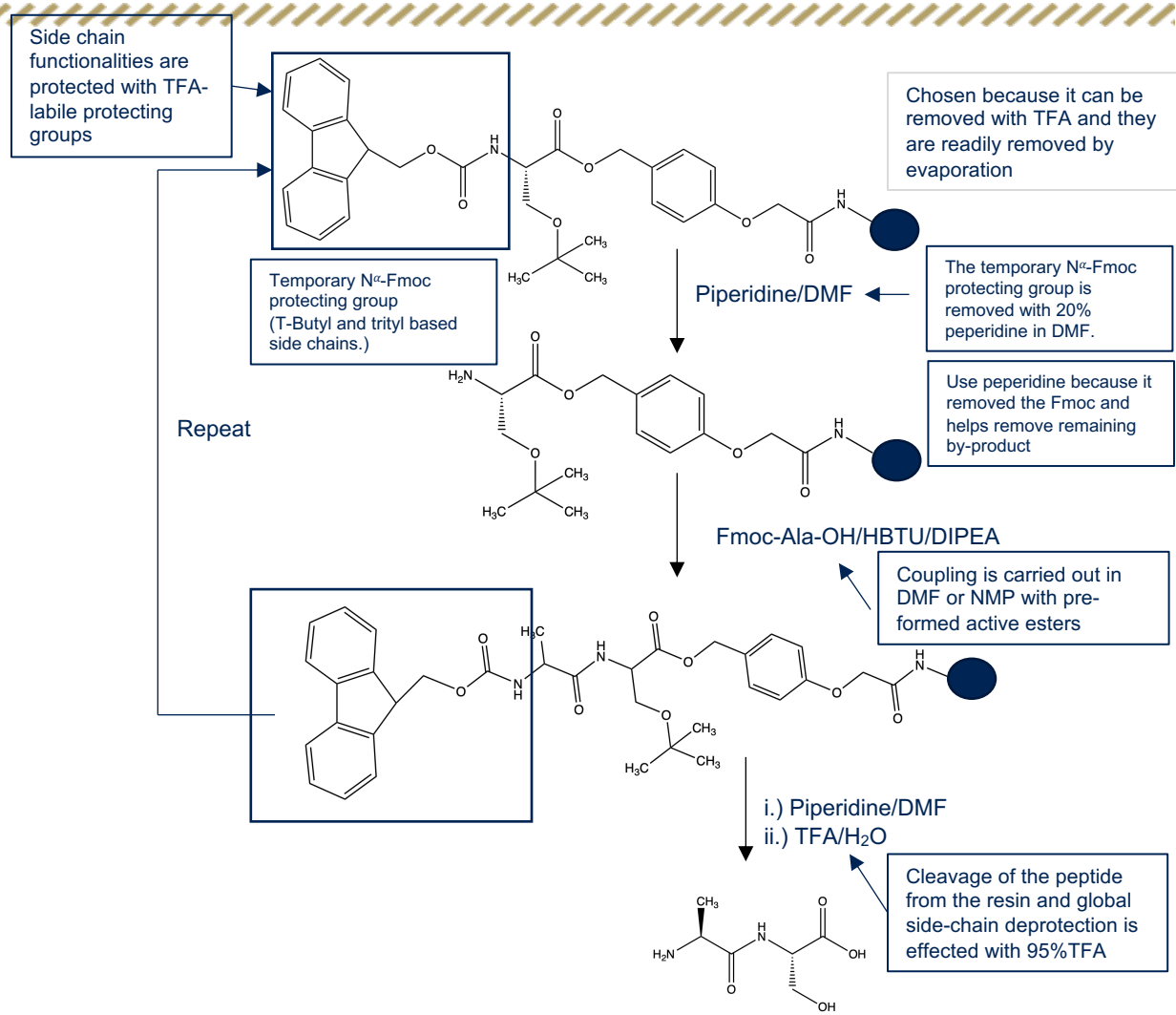
NEXT STEP – PEPTIDE SYNTHESIS

- The Fmoc/tBu method is based on an orthogonal protecting group strategy, using base-labile N-Fmoc group for protection of the α-amino group and acid-labile side chain protecting groups and resin linkage agent.
- Temporary and permanent protection are affected by different chemical mechanisms.
- The side-chain protecting groups and linkage agents can be removed under milder conditions



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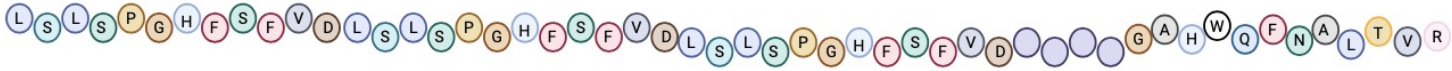
NEXT STEP – PEPTIDE SYNTHESIS

- We are creating a novel peptide sequence
- Once created the peptide is
  - Cleaved from the resin
  - Frozen and lyophilized
  - Ran through the NMR to ensure the proper peptide has formed
- The peptide will then be functionalized to the silk hydrogels
- Then cells will be seeded, and further testing will be done to observer the dhDF and hDF and their reactions to the surfaces.



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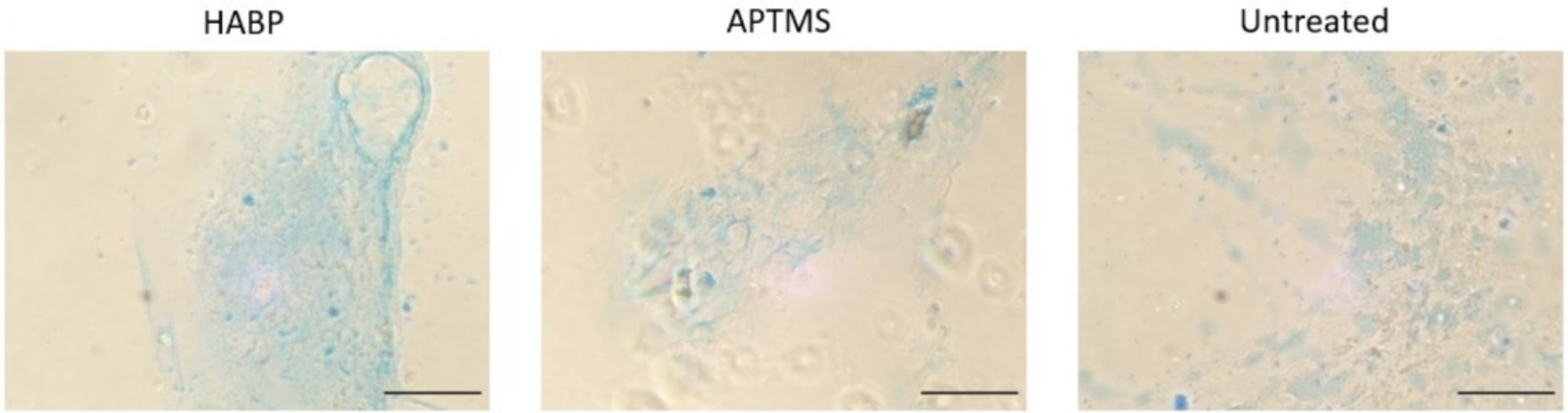
PRELIMINARY DATA FOR HABP



- Preliminary Studies conducted by Beth Blake et al (MSc student, Ozdemir Lab) indicate that the HABP attracts HA to the surface more than untreated surfaces
- Cytotoxicity assays (Alive/Dead and Presto Blue) also indicate that the cells are not negatively affected by the HABP

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CONCLUSION

- We aimed to create a tunable silk hydrogel with the ability to attract endogenous HA.
- The silk hydrogels
  - Have homogenous pores
  - An optimal crosslinking time of 96 hours
  - No cytotoxic effect on the fibroblasts
- Current experiments show no therapeutic effect of just silk on diabetic fibroblast metabolic activity.
- Next step is to synthesize the novel peptide and functionalize it to the silk hydrogels

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ACKNOWLEDGEMENT

- Dr Travis Walker and Laura Brunmaier
  - Walker Lab in the Chemical and Biological Engineering Department at South Dakota School of Mines and Technology for the use of the Rheometer and aid characterizing the stiffness
- Dr. Steve Smith
- Head of Nanoscience and Biomedical Engineering Department at South Dakota School of Mines and Technology for access to the microscopes.

- This research was funded by the South Dakota School of Mines and Technology Startup Fund


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THANK YOU
Questions?

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