A Biomaterials Approach to Kidney Engineering: Structural and Chemical Modification of Silk Fibroin Scaffolds for *in vitro* Tubulogenesis

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Introduction

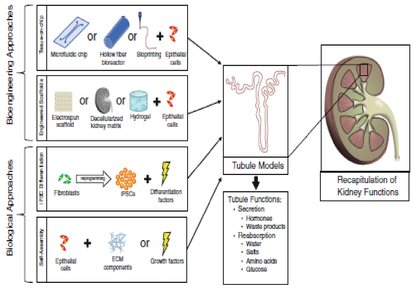
Addressing a global public health concern:

Chronic kidney disease (CKD), an umbrella term for various disorders which affect the structure and function of the kidney, impacts about 14% of the general population.[1] Of patients within the United States, over 700,000 experience kidney failure.[2] At this stage of CKD, where patients have an expected lifespan of 3-5 years,[3] the primary treatment options consist of hemodialysis and organ transplant. Kidney transplantation is an unsustainable solution, as there are fewer available donors and an 11-13% yearly increase in desired operations.[4]

Similarly, while hemodialysis is considered the gold standard for medical treatment of CKD, there are numerous drawbacks to this process. Dialysis requires multiple treatment sessions each week, and results in the undesired removal of necessary bodily fluids and electrolytes. Furthermore, many patients do not have access to hemodialysis care, as spending for the procedure is around $91,000 each year per patient.[2] With end-stage renal disease becoming a major health problem, it is critical to explore further solutions to overcome the shortcomings of the current treatment standard.

Tissue engineering- a promising solution:

Tissue engineering involves the use of a biomaterial as a temporary scaffold for supported cell growth and differentiation. This is achieved through the integration of both physical and chemical cues, promoting an appropriate microenvironment to simulate the native extracellular matrix (ECM).

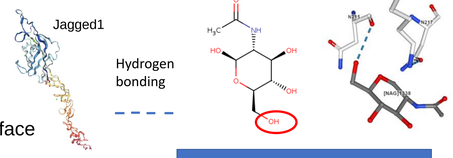


Overview of various approaches to kidney tubule engineering[5].

Objective: To incite synthetic tubule formation via physical and chemical cues within silk fibroin (SF) scaffolds

→Approach 1: Structural
- Desired properties: high porosity, tubular pore structures, allow for proper gas and nutrient exchange

→Approach 2: Chemical
- Ligand of choice: Jagged1 (Jag1)
- Notch signaling: proximal tubule fate determination for stem cells
- Jag1 only functional when bound to surface

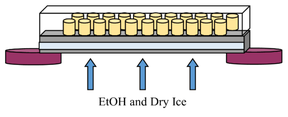


Example of Native Binding Ligand to Jagged1

Experimental

A) Scaffold Development

Diagram for dir



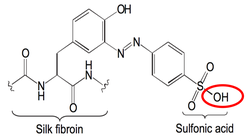
Directional freezing approach:
- load PDMS-treated well plate with SF solution
- cover with insulated metal plate
- metal plate in EtOH and dry ice (45min), lyophilize (2 days), autoclave for β-sheet formation

B) Chemical Modification

Motive: to introduce sulfonic acid (SO_2-OH) groups to SF backbone through electrophilic aromatic substitution of tyrosine

Reagents for 1 molar equivalent of diazonium salt

0.2 mmol of 4-sulfanilic acid + 0.8mmol p-toluene sulfonic acid in 2mL DI H_20 → Cooled on ice → 23.45µL sodium nitrite → Vortexed→ Cooled→ Reacted with Silk Scaffolds (6%) – 30 minutes

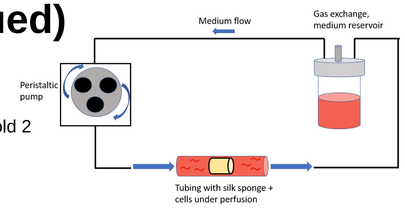


Experimental (Continued)

C) Cell-based Studies

- Renal Proximal Epithelial Cells (RPTECs)
- Culture *in vitro*, seed into sponges, culture scaffold 2 weeks→ testing

Conditions:
Perfused vs. Static Medium
Random vs. Directional Sponge Pores



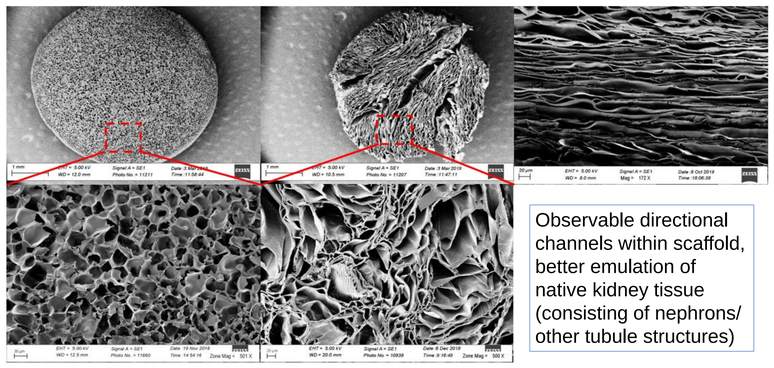
Schematic for perfusion flow testing of RPTEC sponges.

Results

Materials Characterization

Scanning Electron Microscopy (SEM): Used to take images at the micron scale and exhibit surface morphology

← Transverse Cross Section → Longitudinal Cross Section

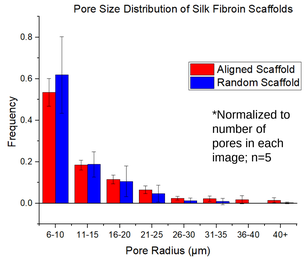


Observable directional channels within scaffold, better emulation of native kidney tissue (consisting of nephrons/ other tubule structures)

Structural and Mechanical Analysis

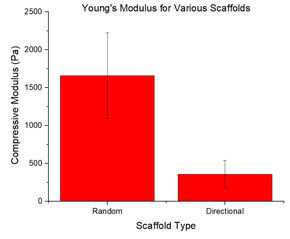
	Random Sponge	Directional Sponge
Porosity	18.9 ± 6.1 %	38.8 ± 6.2 %

Pore Size Distribution of Silk Fibroin Scaffolds



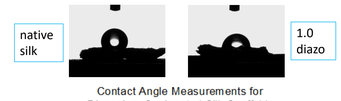
Directional sponges ~2x porous, but significantly weaker mechanical properties compared to the random sponges.

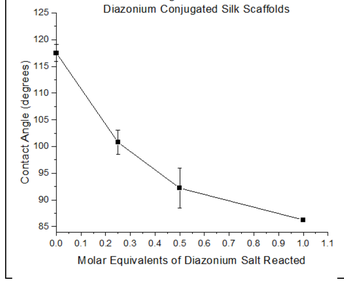
Dynamic Mechanical Analysis (DMA)



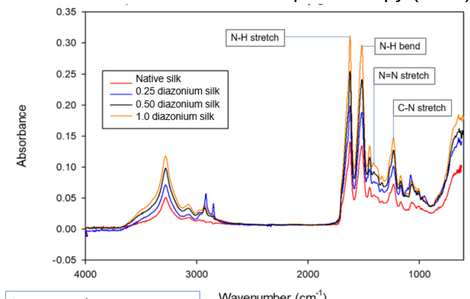
Chemical Modification

Contact Angle Goniometry





Fourier Transform Infrared Spectroscopy (FTIR)



Increase in hydrophilicity and FTIR absorbance with increasing diazonium conjugation of silk

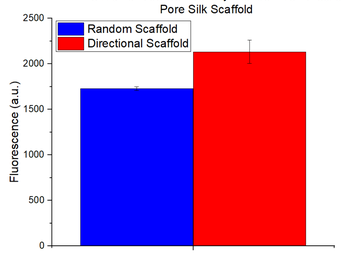
Increasing conjugation

Results (Continued)

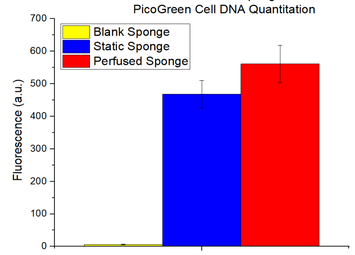
Preliminary studies confirm that directional sponges allow for higher cell density due to higher surface area and ability for greater mass transport of nutrients.

Cell Studies:

RPTEC AlamarBlue Cell Density: Random vs. Directional Pore Silk Scaffold



RPTEC Directional Sponges: PicoGreen Cell DNA Quantitation



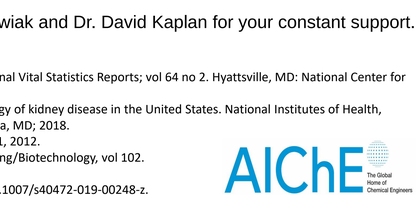
Increased Tubulogenesis with Directional Scaffolds + Perfusion:

PCR:

List of PCR markers and gene's role in tubulogenesis

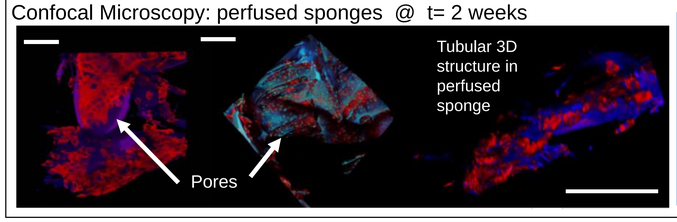
Genetic Marker	Function
Tight Junction Protein 1 (TJP1)	Encodes protein possibly for signal transduction at cell-cell junctions. Tight junctions prevent leakage of solutes during transport within paracellular pathways.
Aquaporin 1 (AQP1)	Encodes for protein of common water channel within membrane of proximal tubules and lower limb of Loop of Henle. Also serves as non-selective cation channel.
Solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1)	Encodes for protein which facilitates transport of glucose across membranes.
P-glycoprotein 1/ ATP-binding cassette sub-family B member 1 (ABCB1)	Encodes for protein present in proximal tubule which pumps xenobiotic compounds out of cell.
Cubilin (CBN)	Encodes for protein receptor for intrinsic factor-vitamin B_{12} complexes.
Multidrug and toxin extrusion protein 1/ Solute carrier family 47 member 1 (SLC47A1)	Encodes for protein within the multidrug and toxin extrusion protein (MATE) family, which excretes toxic electrolytes through urine.
Sodium–hydrogen antiporter 3/ solute carrier family 9 member 3 (SLC9A3)	Encodes for a sodium-hydrogen antiporter protein on proximal tubules; responsible for the balance of Na+.
Sodium glucose cotransporter 2/ solute carrier family 5 member 2 (SLC5A2)	Encodes for sodium-dependent glucose transport protein.

Relative Gene Expression Levels: Perfused vs. Static RPTEC Sponges



PCR indicates an upregulation of genes pertaining to proximal tubule development and functionality when exposed to flow, improving tubulogenesis.

Confocal Microscopy: perfused sponges @ t= 2 weeks



Imaging confirms tubule-structure formation; confluent lining of pore, cytosolic matter exchange (TJP1). Scale bar= 50µm

Conclusions

- Altering the freezing procedure of SF solution allowed for the formation of unidirectional channels within the scaffold, providing greater surface area and simulates native tubule structures within nephron.
- Diazonium coupling of tyrosine residue allows for controlled incorporation of hydroxyl groups for introduction of Jag1 to scaffold. Next steps → Jag1 bind/release study and culture with iPSCs.
- Directional pores allow for improved cell seeding, proliferation, and recapitulation of independent proximal tubule structures.

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