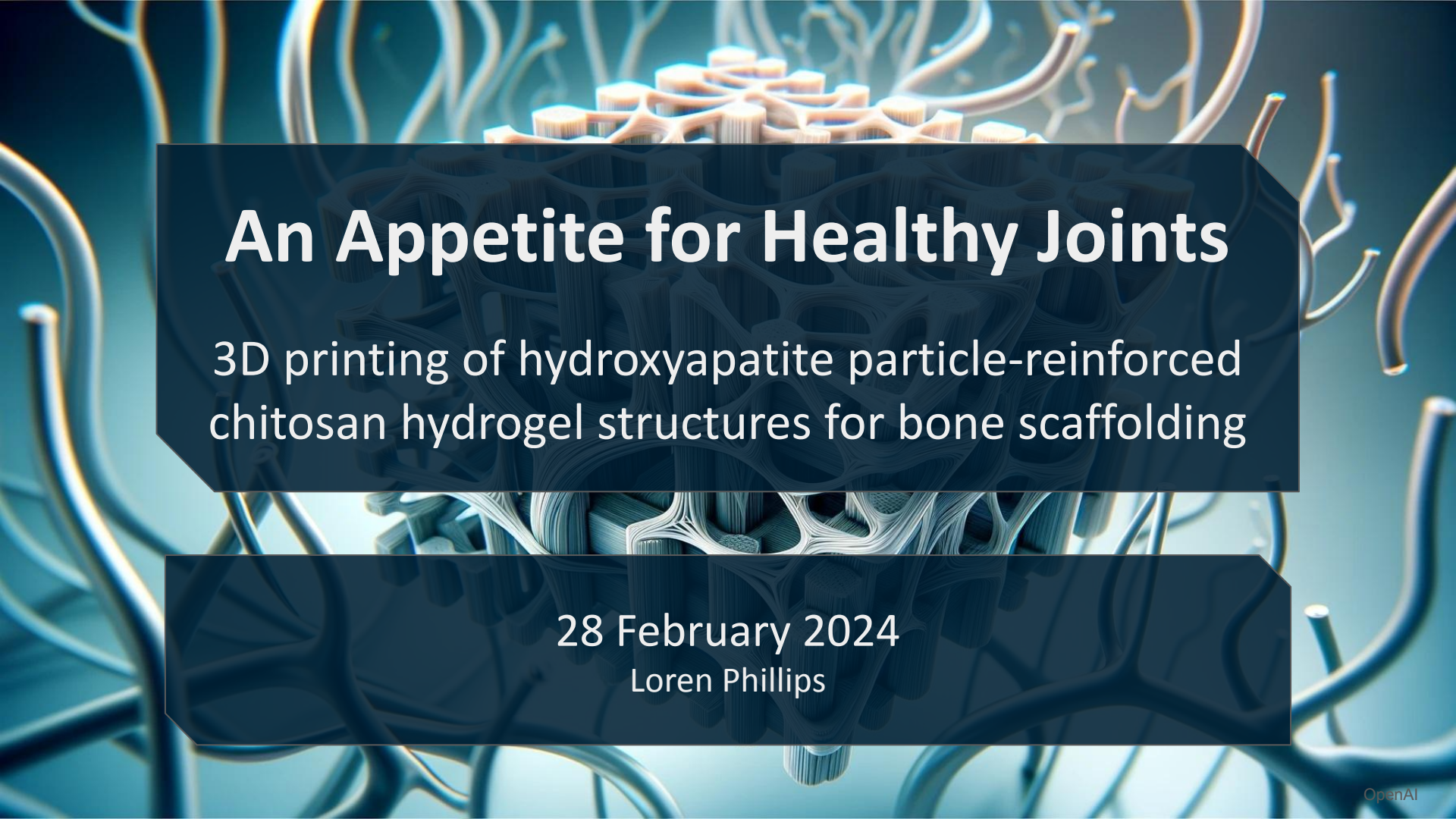


An Appetite for Healthy Joints

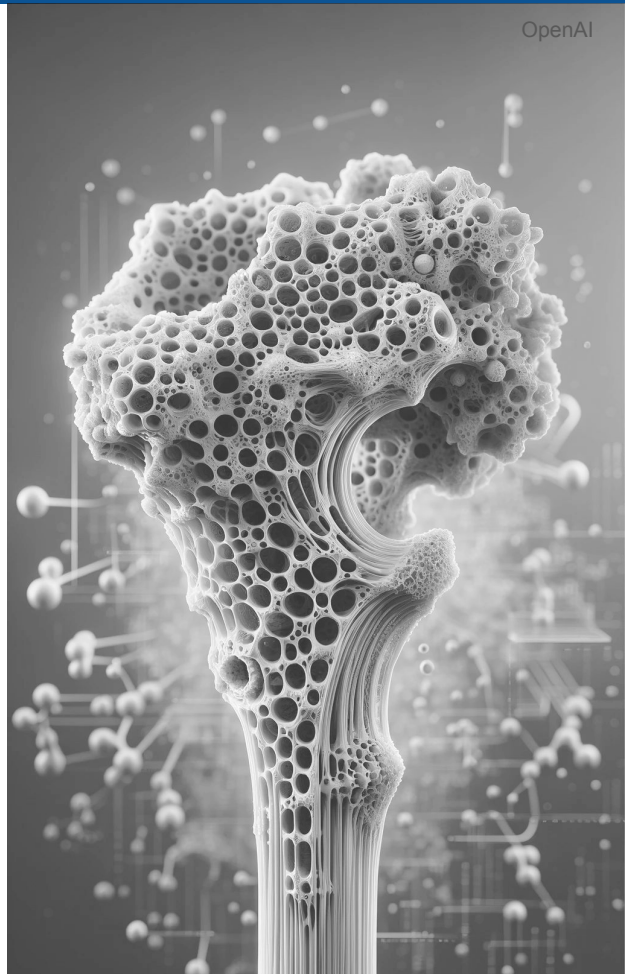
3D printing of hydroxyapatite particle-reinforced chitosan hydrogel structures for bone scaffolding

28 February 2024

Loren Phillips

Agenda

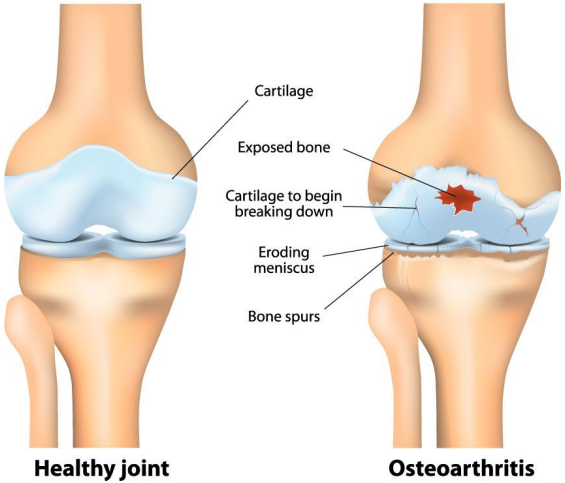
- ❑ **Motivation & Rationale** — 3m
- ❑ **Background and Previous Work** — 3m
- ❑ **Research Overview** — 2m
  - ❑ Specific Aim 1 — 3m
  - ❑ Specific Aim 2 — 3m
  - ❑ Specific Aim 3 — 3m
- ❑ **Anticipated Impact and Deliverables** — 3m
- ❑ **Questions** — 5m



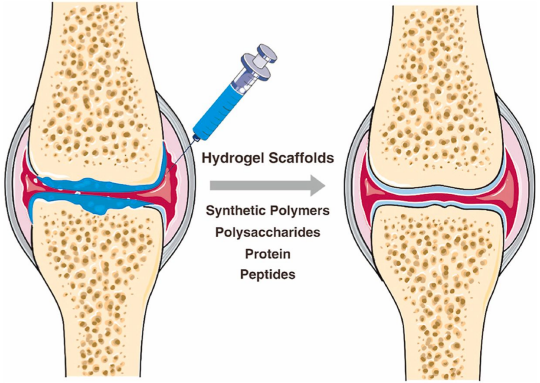
RESEARCH MOTIVATION

Societal Problem: Articular Cartilage (AC) damage.

- Specialized connective tissue of joints [2]
  - Softens/smooths surface for articulation
- Cartilage lesions have limited potency for inherent self-healing.
- Cause of significant pain, swelling limited range of motion [9]
- Common Transplantation clinical techniques struggle due to the donor deficiency and disease transfer.
- Clinical surgeries are also limited to lesion thickness.
- Over 32.5 million US adults have osteoarthritis. [10]



Sadringham Orthopedic Specialists



Wei, Wei et al. "Advanced hydrogels for the repair of cartilage defects and regeneration."

Engineering Challenge:

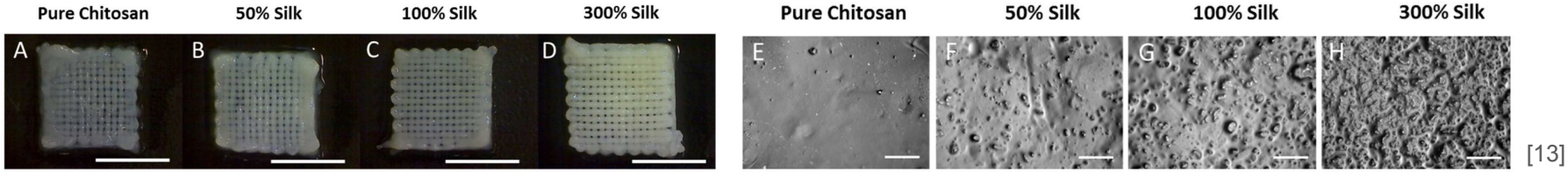
- Opportunity to use hydrogel scaffolds, many challenges…
- Critical to select suitable biomaterials.
- 3D printed hydrogel scaffolds suffer from low printing accuracy and poor mech. properties due to soft nature.
- Also suffer from a tendency to shrink.

PREVIOUS WORK: SILK-REINFORCED CHITOSAN

Chitosan material has received considerable attention as a scaffold material for the mentioned application.

But, low stiffness and compressive strength of chitosan makes it unstable and prone to collapse! [5,6,7]

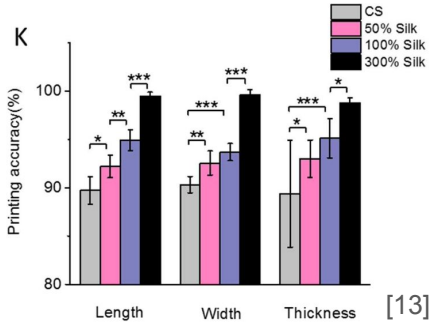
Shrinkage is also common - the biggest problem for maintaining shape fidelity after printing. [6]



[13]

Compared with pure chitosan scaffolds, the addition of silk particles resulted in up to a 5-fold increase in compressive modulus, significantly better printing accuracy, and improved scaffold stability [13].

Particle loading also enabled tuning of the surface roughness of the scaffolds. Printed composite hydrogel scaffolds showed no cytotoxicity and supported adherence and growth of human fibroblast cells [13].



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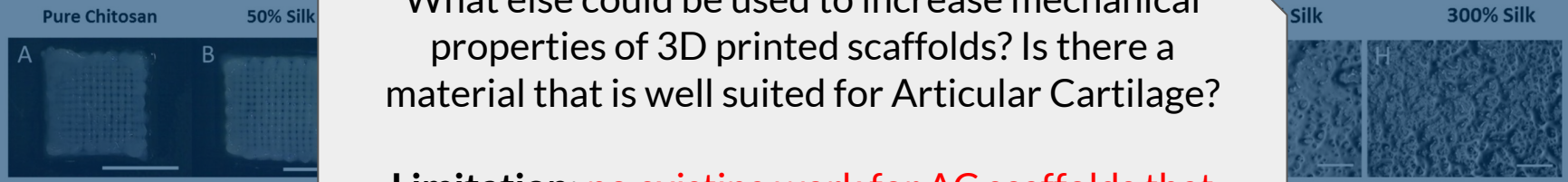

What else could be used to increase mechanical properties of 3D printed scaffolds? Is there a material that is well suited for Articular Cartilage?

Limitation: no existing work for AC scaffolds that employs nanoparticles to support scaffold.

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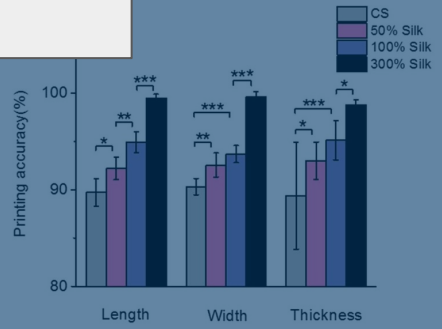


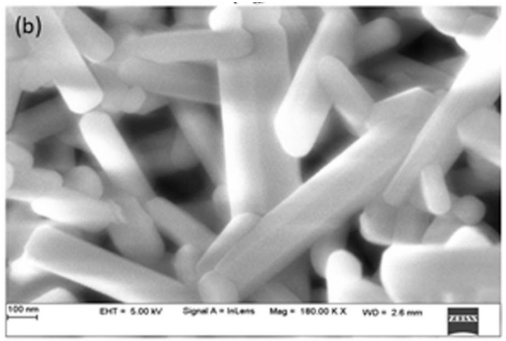
PREVIOUS WORK: HYDROGEL WITH HYDROXYAPATITE NANORODS (HAnr) [1]

Fabrication of HANr reinforced chitosan nanocomposite hydrogel for tissue-engineered articular cartilages.

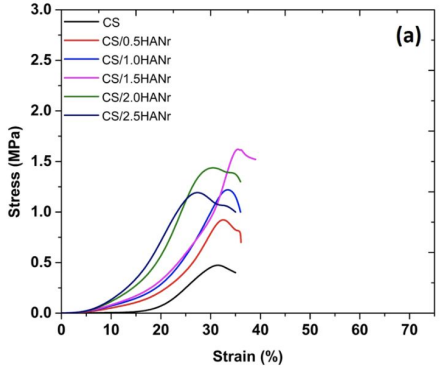
Characterized these physically, chemically, mechanically and biologically with different concentrations.

Prepared the hydroxyapatite nanorod from cuttlefish bone through a mechanochemical method.

The compressive mechanical properties of the hydrogels are summarized below.



SEM of synthesized hydroxyapatite nanorod [1]



Unconfined compression stress-strain curve of composite hydrogel [1]

Pure chitosan hydrogel shows the least compression strength with a significant deformation.

However, composites show bimodal trend: compression strength increases for 1.5 wt% of HANr and as HANr concentration increases above 1.5 wt%, compressive strength decreases due to the agglomeration.

PROJECT OVERVIEW

Research Hypothesis: Optimizing the incorporation of hydroxyapatite nanorods into chitosan scaffolds will enhance printing accuracy, mechanical strength, and biological compatibility, addressing the engineering challenge of creating suitable biomaterials for cartilage regeneration with superior mechanobiological properties.

Through rigorous testing for mechanical robustness, stability, and cytotoxicity, this approach aims to provide a means to surpass limitations of current cartilage repair techniques, proving it may offer a viable alternative to traditional clinical methods.

Specific Aim 1

Accurately print with hydroxyapatite + chitosan bioink

[image]

Specific Aim 2

Characterize the mechanical properties of the printed scaffolds

[image]

Specific Aim 3

Assess the biocompatibility of the scaffolds

[image]

SPECIFIC AIMS

Specific Aim 1 → Printing

Specific Aim 2 → Mechanical Characterization

Specific Aim 3 → Assess Biocompatibility

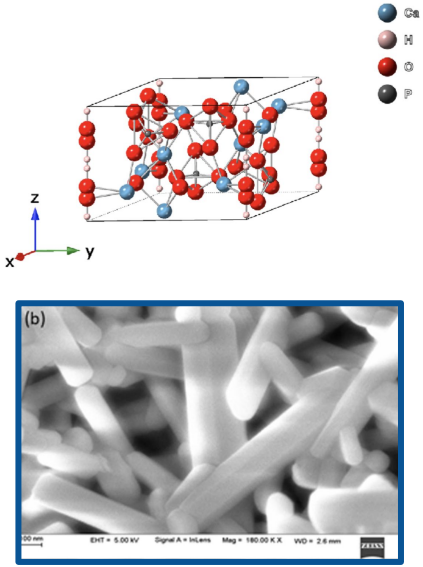
Specific Aim 1 encompasses three main goals: **Create** the Bioink, **Print** with the ink, and perform basic **Characterization** on the print.

Ink Creation - Nanosystems Integration

Nanorods Growth: Cuttlefish bones (CB) washed, powdered, and sieved. Then they are dissolved in demineralized water, followed by the addition of orthophosphoric acid (H3PO4) in order to maintain a proper stoichiometric molar ratio of Ca/P. After long period of stirring the slurry id dried and calcined in a muffle furnace.

CS/HANr Bioink: Add different amounts (0.5, 1.0, 1.5, 2.0, and 2.5 wt%) of HANr particles to chitosan solution. We will also print 0% wt iterations as **control.**

Creation of 'paste' that can be centrifuged (bubbles). Check with FTIR. [15]



Top: Unit cell of hydroxyapatite [image]. Bottom: SEM of synthesized hydroxyapatite nanorod [1]

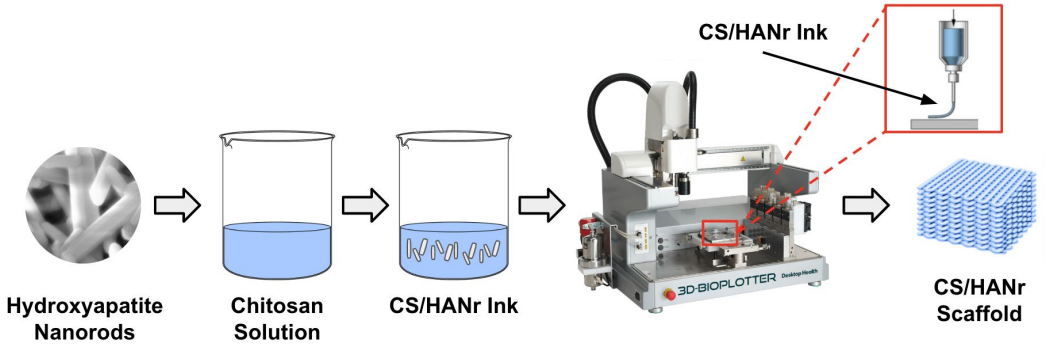
SPECIFIC AIMS

Specific Aim 1	Specific Aim 2	Specific Aim 3
Printing →	Mechanical Characterization →	Assess Biocompatibility

Scaffold Printing Process

The prepared CS/HANr inks will be transferred into injection cartridges and mounted on a 3D Bioplotter. A lattice CAD model file will be used to guide layer- by-layer extrusion. 0 and 90° angle steps will be taken between each layer (for surface, dimension and mechanical characterization). We will tune the following:

- Nozzle Diameter
- Extrusion Pressure
- Printing Speeds



Each layer will be solidified after printing by manually pipetting coagulant solution. Another dense membrane structure will also be prepared for stability tests.

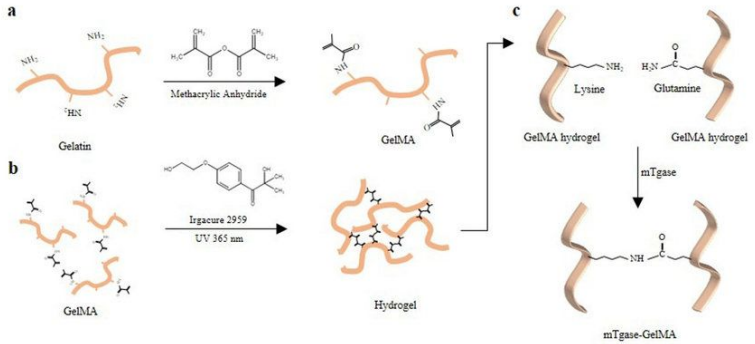
SPECIFIC AIMS

Specific Aim 1	Specific Aim 2	Specific Aim 3
Printing →	Mechanical Characterization →	Assess Biocompatibility

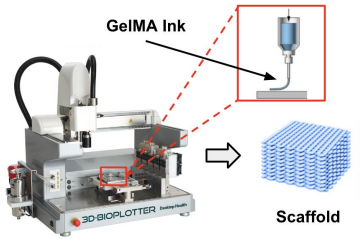
Scaffold Printing Control

In addition to printing without nanorods, we will use a well-documented bioink to ensure printer works well and understand the limits of printing accuracy…

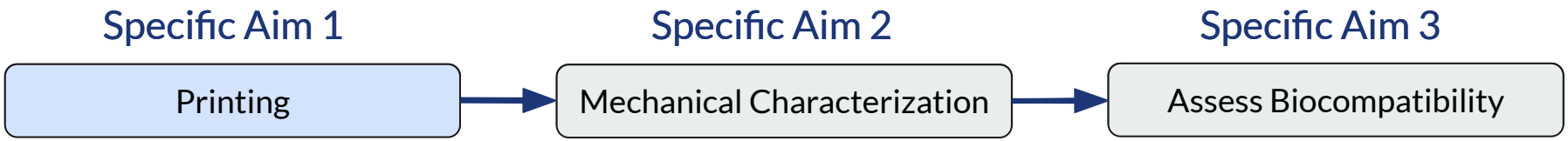
- Bioink based on methacrylated derivatives such as GelMA (gelatin methacryloyl)
- GelMA quickly photocrosslinks yielding stable structures with high fidelity to the original design.
- This crosslinking helps maintain the shape and dimensions of the printed structure, thus minimizing shrinkage.



Up: Photocrosslinking of GelMA [image]. Right: Printing schematic



SPECIFIC AIMS



Print Characterization → Accuracy, Roughness

The dimensions of the printed scaffolds can be measured using a Vernier caliper and compared to the CAD model to determine **printing accuracy [12]** using

$$\text{Printing accuarcy } (\%) = \left[1 - \frac{|D_i - D|}{D}\right] \times 100$$

Roughness caused by particle loading will be demonstrated with laser profilometry. The addition of HANr particles should theoretically increase the surface roughness of the printed scaffolds.

Particle loading could be an effective way to tune the surface roughness from submicron to micrometre range, which is an important for the biological performance of the scaffolds [13].

SPECIFIC AIMS

Specific Aim 1	Specific Aim 2	Specific Aim 3
Printing →	Mechanical Characterization →	Assess Biocompatibility

Compression tests: measure the compressive properties of the printed structure. Compression tests performed using mechanical testing machine with a load cell

Scaffolds can be tested using a wet compression bath. Compressive strength will be tested using a linear strain rate from 0–50% of the initial length at a determined extension rate

To approximate biological conditions, tests will be conducted at determined temperature (~40C) in PBS (pH 7.4) solution. [2,3]

[image]

SPECIFIC AIMS

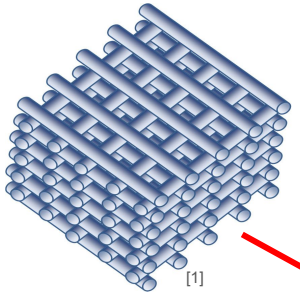
Specific Aim 1	Specific Aim 2	Specific Aim 3
Printing →	Mechanical Characterization →	Assess Biocompatibility

Scaffolds will be tested using a wet compression bath (right)

As a **control**, a commercial tissue engineering scaffold with proven performance and similar porosity to chitosan-HANr scaffolds will be tested.

This will validate that the test setup is capable of measuring high-strength materials accurately.

Our negative **control** will be the pure chitosan without nanorod reinforcement, to demonstrate the sensitivity of the test to detect differences in compressive strengths, and ensure that any increases in strength are due to the HANr reinforcement.


[1]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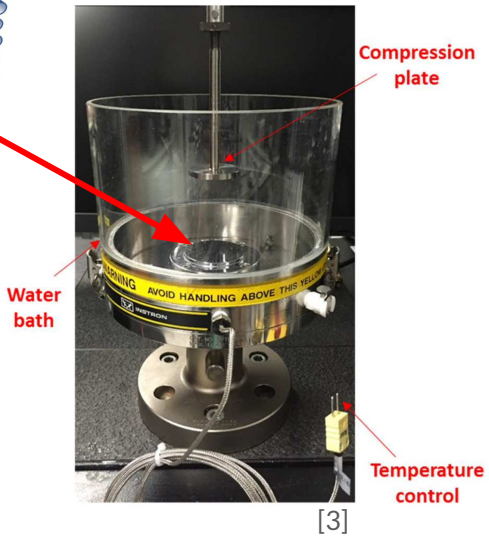


[3]

SPECIFIC AIMS

Specific Aim 1	→	Specific Aim 2	→	Specific Aim 3
Printing		Mechanical Characterization		Assess Biocompatibility

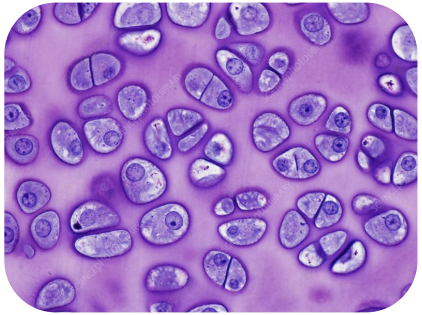
Cytotoxicity: 3-day test duration with sterilized samples (ISO 10993)

- Immersion in ethanol → repeat for multiple rinses.
- Wash with phosphate buffered saline (PBS) and equilibrate in cell culture medium for 72 hours
- Extract any chemical leachates released by the scaffolds.

Articular chondrocytes seeded into standard well plates. [13,14]

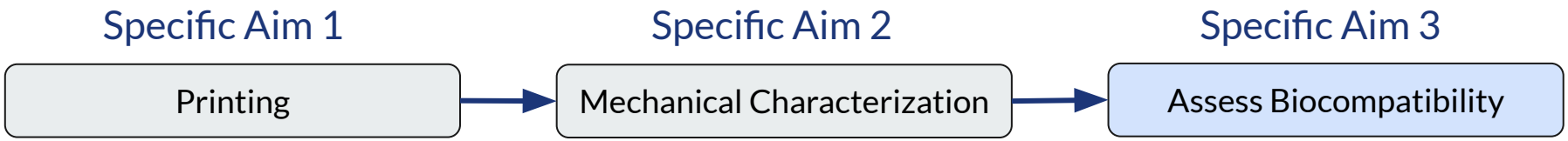
MTS Assay: Remove culture mediums, add color-development solution to wells, incubate, and measure absorbance using a microplate reader. [13]

Negative Control: Dimethyl sulfoxide (DMSO) at 5% v/v in medium
Positive Control: Cell culture medium



Chondrocytes (up) are highly specialized, metabolically active cells that play a unique role in the development, maintenance, and repair of the ECM. [image]

SPECIFIC AIMS



Scaffold Stability: Printed scaffolds incubated in Dulbecco's Modified Eagle's Medium (DMEM) media.

- Scaffolds removed and weighed at different times during 2 weeks to determine scaffold state.
- Repeat above stability study with cells added to scaffold
  - Determine if the cells contributed to degradation

Control: Dulbecco's Modified Eagle's Medium (DMEM) media
Control: Scaffold with no nanorods (chitosan). Synthetic Commercial Scaffold.

Cell Proliferation: Articular chondrocytes seeded on the scaffolds [13,14]

- Rinsed with PBS at various days post cell seeding and transfer to new wells.
  - Removes non adherent cells
- MTS assay to test proliferation

Control: Tissue Culture Plastic (TCP) - material with high level of biological affinity
Control: Hydrogen Peroxide (H_2O_2)- cytotoxic agent known to reduce cell viability

PROJECT SUMMARY

Specific Aim 1

Accurately print with hydroxyapatite + chitosan bioink

[image]

Specific Aim 2

Characterize the mechanical properties of the printed scaffolds

[image]

Specific Aim 3

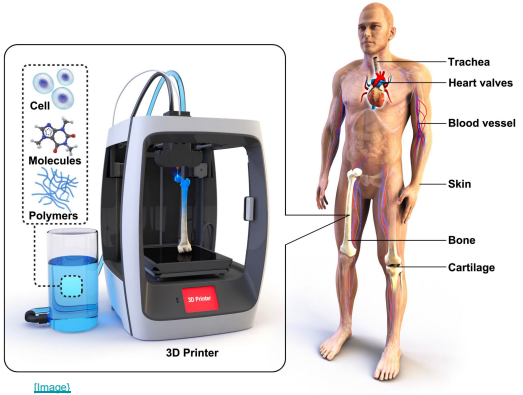
Assess the biocompatibility of the scaffolds

[image]

IMPACT AND DELIVERABLES

Deliverables:

1. Optimized 3D Printing Protocol and Ink Characterization
   - Size of nanorods and scaffold structure sizes
   - Percentage of nanorods in the ink
   - Range of printing pressures for optimal accuracy
2. Mechanical Characterization Report
   - Analysis of the structural and mechanical properties
   - Compression test results, stress/strain curve data
3. Biocompatibility Data
   - Cytotoxicity assays and long-term stability tests
   - Confirm the safety and effectiveness of the scaffolds for clinical use
4. Prototype of Hydroxyapatite Nanorods in Chitosan Scaffolds
   - Prototype scaffolds that can be used for preclinical studies



[Image]

Future Impact:

1. Clinical Impact
   - More reliable, efficient, and less invasive cartilage repair treatment
2. Technological Advancement
   - Advancements in 3D printing techniques for biomedical applications
3. Economic Impact
   - Potential reduction in healthcare costs

[Image]

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THANK YOU
Questions?
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