IIB Project Notes

3D Printing of Multi-Material Hydrogels

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① Project Overview

1.1. Points of Contact

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1.2. Project Goals

Post-surgical cavities resulting from the resection of soft tissues damaged by trauma, can lead to internal tissue adhesions. A potential strategy to reduce the complications associated, is through filling large resection cavities with a hydrogel construct exhibiting tissue-mimic physical properties. An ideal hydrogel construct should be injectable to comply with surgical procedures, forming a complex shape fitting into the cavity post injection, with good tissue interface. We aim to synthesise remotely deformable biocompatible soft materials, in which an electromagnet can be used to guide the shape of a soft material. This soft material can be bonded to a hydrogel, which can be used in a 3D bioprinter as cell-loaded bioink.

Michaelmas goal: to model and optimise the properties of a magnetic fibre in a hydrogel, and synthesise the fibres.

Lent goal: to load the fibres into the hydrogel and test printing feasibility.

1.3. Project Assessment

Michaelmas Week 5: Mid-term review. Presentation with department.

② Literature Review and Background

2.1. Background

Regenerative medicine is an emerging field in biomedical science, involving the *in vivo* formation of personalised tissues (e.g. skin, bone, organ tissue). In order to successfully integrate with the host tissue, implanted tissues must contain:

- Matrix / scaffold: polymeric 3D structure mimicking the extracellular matrix (ECM).
- Signalling molecules: various biomolecules e.g. growth factors, Yamanaka factors
- Cells: live cells, depending on the type of tissue to be regenerated e.g. stem cells.

An alternative cell-free approach to tissue engineering involves removing cells from the formulation, and instead using 'homing' of host cells into the new scaffold, promoted by the biomolecules.

Scaffold: typically made of synthetic resorbable aliphatic polyesters (e.g. PCL, PTMC). It must have a porous interconnected structure (to allow nutrient flow), be biodegradable/bioresorbable at a rate matching the cell proliferation rate, have suitable surface chemistry to permit cell attachment and proliferation, have matching mechanical properties and be easily processed into various shapes.

The scaffold geometry must have very high surface area. For this purpose, geometries using triply periodic minimal implicit surfaces (e.g. Schwarz "P" unit cell) or space-filling curves can be used. Scaffolds can also be made by decellularising tissue *ex vivo*, leaving a natural matrix.

Bioprinting: automated and controllable deposition of matrix-cell-biomolecule bioinks.

Bioink contains live cells in a hydrogel-biomolecule formulation. The bioink may contain the fully-formed scaffold, or its precursors (controlled induced cross-linking). Bioprinting can be done by various methods, adapted from and inspired by the approaches in conventional 3D printing.

- Extrusion: continuous deposition of a viscous bioink stream from a syringe.
- Inkjet: directed emission of bioink droplets from a piezoelectric nozzle.
- Projection: UV-initiated cross-linking of photosensitive hydrogels (**not** polymerisation like photolithography).

The rheological properties of the bioink (gelation kinetics, surface tension, viscoelasticity, shear-dependent and time-dependent viscosity) are important design parameters, depending on the printing method. The addition of cross-linkers into the bioink allows the solidification of the hydrogel in the scaffold once deposited. Depending on whether the hydrogel is UCST or LCST (orientation of water-polymer phase diagram), either the syringe or stage must be heated to form the gel before deposition. The cells can be supported on microcarriers suspended in the bioink, which are made of either natural (cellulose, gelatin, collagen) or synthetic (e.g. dextran, plastic, glass) hard materials. This arrangement permits high cell counts to be used without compromising viability, as well as improved nutrient exchange. Some types of cell e.g. chondrocytes can help to replace the scaffold with new ECM tissue if rigidity is desired. Functional materials (e.g. graphene, magnetic fibres, metal nanoparticles) can also be supplied in the bioink with careful control of suspension rheology and biocompatibility. Bioprinters can be designed to print from inputs given by parametric equations for the toolpath, G-code (low-level language in CAM), CAD models or images (by segmentation).

2.2. Experimental Techniques

Fabrication of Magnetic Fibres

(*Stretchable electromagnetic fibers for self-powered mechanical sensing*, Su *et al.*)

NdFeB magnetic powders, up to 70 wt%, were dispersed in the elastic Ecoflex scaffold to form a viscous liquid formulation. This mixture was wet-spinned through an ethanol bath into thin fibres (diameter ~ 2 mm) to fabricate stretchable (strain >50%) & magnetic (>40 mT) fibres. A conductive wire was wound around (2-3 turns per cm of fibre) the magnetic fibre like a magnetic core solenoid, allowing relative movements between magnetic and conductive fibres during one cycle of stretching/recovery.

When the fibres are stretched, only the magnetic part is extended while the wire remains motionless. This causes a negative lateral strain in the fibre (by Poisson's ratio), decreasing its cross-sectional area, decreasing the total flux linkage through the solenoid. By Lenz's law, an emf is developed when the fibre is stretched and restored, which can be detected with amplitude ~4 microvolts.

In our project, the conductive wire is not relevant, only the magnetic fibre component is required.

PEO as a Spinning Agent for Non-Spinnable Materials

(*PEO as spinnable polymer and spinning agent for non-spinnable materials*, Grothe *et al*.)

PEO has been shown to be a suitable polymer for use in electrospinning processes, in which materials are drawn out into thin strands. Not all materials are suitable, including our magnetic fibres, so PEO could be used (in addition to EcoFlex) to form the fibres instead. Alternatives include PEDOT:PSS (a semiconductive polymer) and polyurethane.

Review on Alginate-Gelatin Hydrogels for Bioprinting Applications

(*A Review on the Adaption of Alginate-Gelatin Hydrogels for 3D Cultures and Bioprinting - PMC*, Łabowska et al.)

③ Magnetic Fibre Composites (Michaelmas)

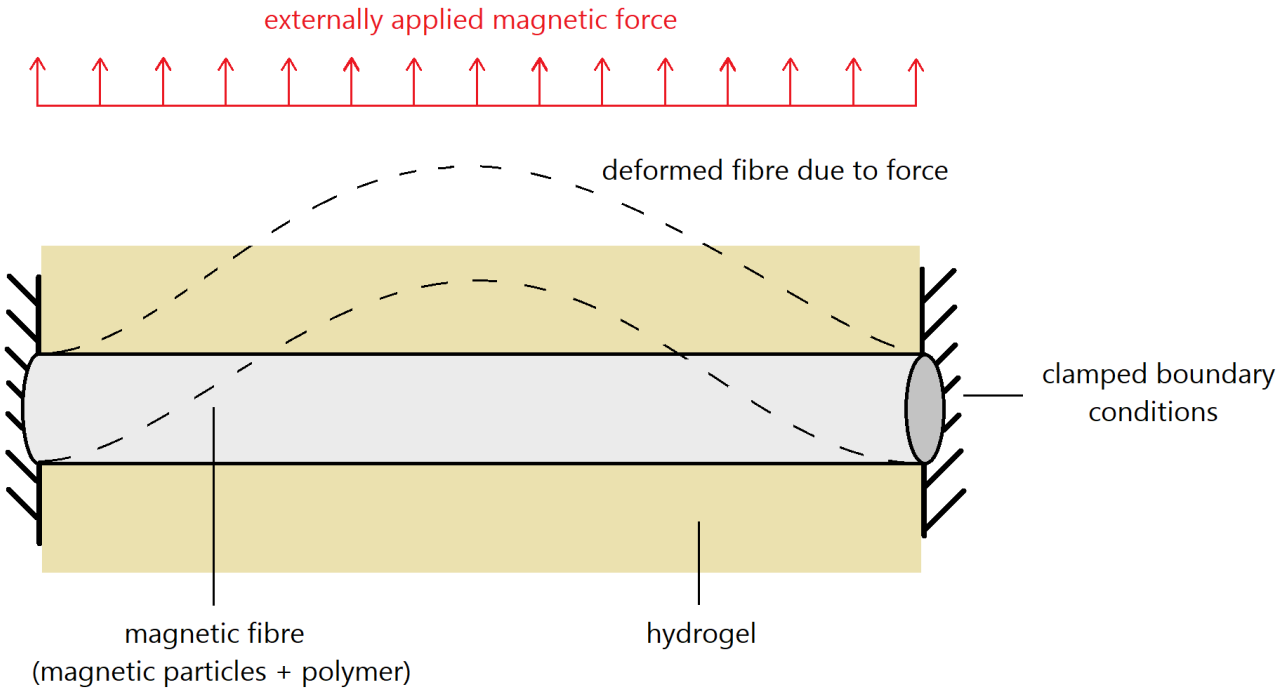
3.1. Theoretical Treatment

The magnetic fibre can be modelled as an elastic beam clamped at two ends. Due to the presence of powderised neodymium magnets ($Nd_2Fe_{14}B$) in the fibre, it exerts a permanent magnetic field around itself. The fibre is also enclosed in a hydrogel, which can be modelled as a continuous elastic material. The fibre is actuated (displaced) by an external magnetic field, provided by e.g. an electromagnet.

The following conditions must be met:

- The bending stresses in the fibre must not cause it to yield. A safer criterion is to ensure that the maximum stress is less than half of the yield stress, to provide protection against fatigue-induced failure.
- The stress in the hydrogel surface must not cause it to fail. Otherwise, the fibre will cut through it and destroy the material.

Diagram:



Assumptions:

- The system and forces are all symmetric about the vertical axis.
- Gravity (self-weight) is neglected.
- The materials are all linear elastic.
- Only steady-state behaviour is considered i.e. no time dependence, no viscosity.

Parameters:

- $2L$: length of fibre.
- A: cross-sectional area of fibre.
- E: Young's modulus of fibre.
- $B = EI$: flexural rigidity (modulus times second moment of inertia) of fibre.
- x: position coordinate along fibre length, so that the fibre runs from $-L < x < L$.
- y: position coordinate in transverse direction, measuring displacement.
- $\omega(x)$: force per unit length on the fibre at position x.
- F: net force exerted by magnetic field, so the reaction force at each clamp is $F/2$.

  By definition, $F = \int_{-L}^{L} \omega(x)\, dx = 2\int_{0}^{L} \omega(x)\, dx$.

Equilibrium Analysis

We can model the situation as a doubly-clamped (statically indeterminate) beam subject to a symmetric distributed load $\omega(x)$, superimposed with the resistive forces exerted by the hydrogel medium.

To restore static determinacy, consider the deformation of the beam under only the external load $\omega(x)$, with pinned supports. The variation of the internal bending moment $M_B(x)$ is

$$M_B(x) = \frac{x+L}{2} \times \left(\int_{-L}^{x} \omega(x)\, dx - F \right)$$

The angular rotation $\psi(x)$ satisfies $M_B(x) = EI\, \frac{d\psi}{dx}$, so the end rotation ψ_L is given by

$$\psi_L = \int_{0}^{\psi_L} d\psi = \frac{1}{EI} \int_{0}^{L} M_B(x)\, dx$$

By compatibility, the clamping moment M_C at each fixed support is therefore

$$\frac{2}{3}\psi_L = \frac{2ML}{3EI} \quad \Rightarrow \quad M_C = \frac{\psi_L EI}{L}$$

By superposition, the variation of bending moment $M(x)$ in indeterminate case is

$$M(x) = M_C + M_B(x)$$

Then $M(\pm L) = M_C$, and in general,

$$\psi(x) = \int_{0}^{\psi(x)} d\psi = \frac{1}{EI} \int_{-L}^{x} M(x)\, dx \quad \text{and} \quad y(x) = \int_{0}^{y(x)} dy = -\int_{-L}^{x} \psi(x)\, dx.$$

The midspan maximum deflection is $y(0)$.

The components of $\omega(x)$ can be separated into the magnetic force $m(x)$ and a contact force $\lambda(x)$ per unit length exerted due to the stress induced by the displacement of the hydrogel:

$$\omega(x) = m(x) + \lambda(x)$$

In principle, both components involve feedback with the displacement $y(x)$, since more displacement towards the externally applied magnet will increase the force applied to the fibre, as well as straining the hydrogel further, causing it to resist the movement more. These two effects are in opposing directions. We may need to consider whether one will dominate over the other, and/or whether the magnetic force can be considered independent of y.

A simple model for $\lambda(x)$ is a simple proportionality relation, $\lambda(x) = -ky(x)$, where k is a stiffness (like a spring constant). If $m(x) = m_0$ is also considered constant (i.e. y is small),

$$\omega(x) = m_0 - ky(x), k < \frac{m_0}{y(0)}$$

Applying the elastic law $M(x) = -EI\,y''(x)$ and differentiating twice, the equations modelling the deflection of the beam are

$$y''''(x) = \frac{1}{2EI}\Big((x + L)ky'(x) + 2ky(x) - 2m_0\Big)$$

$$y(-L) = 0,\ y'(-L) = 0,\ y''(-L) = \frac{-\psi_L}{L},\ y'''(-L) = \frac{-F}{2}$$

$$\psi_L = \frac{1}{EI}\int_0^L \left(\frac{x+L}{2} \times \left(\int_{-L}^{x} (m_0 - ky(x))\,dx - F\right)\right) dx,\ F = 2\int_0^L (m_0 - ky(x))\,dx$$

There is a circular dependency between the boundary conditions. This makes the system too complicated to solve, even numerically.

Therefore, we will set $k = 0$ i.e. the hydrogel does not impede the deflection at all. Now:

$$y''''(x) = \frac{-m_0}{EI} \Rightarrow M(x) = \frac{m_0}{6}\left(3x^2 - L^2\right),\ y(x) = \frac{m_0}{24EI}\left(2L^2x^2 - x^4 - L^4\right)$$

The maximum bending moment and maximum displacement are (with $I = \frac{\pi}{4}r^4$):

$$M_{max} = M(\pm L) = \frac{m_0L^2}{3},\quad y_{max} = |y(0)| = \frac{m_0L^4}{6\pi Er^4}$$

Material Selection

To prevent yield and fatigue, we require $\sigma_{max} = \frac{M_{max} r}{I} \leq \frac{\sigma_y}{2} \Rightarrow \frac{8 m_0 L^2}{3\pi r^3} < \sigma_y$.

For limiting performance, we want to maximise y_{max} subject to the constraint $\frac{8 m_0 L^2}{3\pi r^3} = \sigma_y$. This leads to a material selection performance index given by $\frac{\sigma_y^2 r^2}{m_0 E}$.

Assume that $m_0 = m_V r^2$ (i.e. constant magnetic force per unit volume). The performance index is then $\frac{\sigma_y^2}{m_V E}$. This appears as the materials below a line with gradient 2 on a log-log plot of E against σ_y, while having a small magnetic force per unit volume.

3.2. Experimental Procedures

Fibre Compositions

Trial 1:

- Solvent: 85% DMF + 15% acetone (by volume)
- Polymer: PEO, 30% wt
- Magnetic: iron(II, III) oxide, 30% wt

Trial 2:

- Solvent: 85% DMF + 15% acetone (by volume)
- Polymer: polystyrene, 30% wt
- Magnetic: iron(II, III) oxide, 30% wt

Trial 3:

- Solvent: 85% DMF + 15% acetone (by volume)
- Polymer: polyurethane, 20% wt
- Magnetic: iron(II, III) oxide, 40% wt

Trial 4:

- Solvent: 85% DMF + 15% acetone (v/v)
- Polymer: polystyrene, 10% wt
- Magnetic: iron(II, III) oxide, 40% wt

Trial 5:

- Solvent: 85% DMF + 15% acetone (v/v)
- Polymer: polystyrene, 20% wt
- Magnetic: iron(II, III) oxide, 40% wt

Mid-Term Review Notes

Could also try polyurethane (PU) in methyl ethyl ketone (MEK) solvent.
Plasma treatment - doesn't work when fibre is around hydrogel.
Preventing the fibres from decoupling.
Cutting the frame off with the fibre.
Investigate the use of optical sensor for displacement of the fibre.

Electromagnet

Circuit components needed: buy a kit of circuit parts

- Arduino + USB connector + solderless breadboard

Circuit components kit:

https://uk.rs-online.com/web/p/analogue-development-tools/8904008?searchId=274a4d12-5de5-4cfa-a9ff-78e78883fbb5&gb=s

http://www.robotsforfun.com/webpages/electromagnet.html

A Review on the Adaption of Alginate-Gelatin Hydrogels for 3D Cultures and Bioprinting
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7916803/

Review of soft magnetic fibres

https://onlinelibrary.wiley.com/doi/full/10.1002/adma.202212202

4D printing of patterned multi material magnetic hydrogel actuators

https://www.sciencedirect.com/science/article/pii/S2214860421006539

Steps for Making Magnetic Hydrogel Composite

Prepare Components

1. Collect small glass vial. Weigh it with the cap on and note down the dry mass.
2. Using a micropipette, add 850 µL of DMF and 150 µL of acetone.
3. Weight the mass of the solvent. Calculate the required masses of polystyrene and iron oxide.
4. Using a weighing boat, add the required masses of polystyrene and iron oxide.
5. Drop in a stir bar. Cap tightly, label and seal the top with parafilm.
6. Heat at 60 °C and stir gently for 2 hours.
7. Prepare the hydrogel composition using deionised water, adding gelatin and alginate.
8. Drop in a large stir bar. Heat-stir at 80 °C for 30 minutes.
9. Cross-link the hydrogel by freezing in the freezer until the fibres are ready.
10. Prepare the robot arm and spinner if using them.

Spin Fibres

1. Using an uncapped thin syringe, slowly draw up to 0.2 mL of the fibre solution.
2. Cap the syringe with a 25 gauge needle.
3. Get the frame and hold it in one hand. Ensure the length of the frame is extended.
4. Extrude some solution through the needle onto the glove until some fibres appear. Spin the fibres across the frame, aiming to get long and micron-thin fibres.
5. Once fibres are spun across the width of the frame, use a dropper pipette to place a small blob of silicone adhesive glue on the edges of the frame to secure the filaments.
6. Examine the frame on a petri dish under the light microscope at 20x magnification.
7. Using an uncapped thick syringe, slowly draw up some of the hydrogel and cap with a needle.
8. Extrude a small blobs of hydrogel into the fibres, covering both sides.
9. Test for magnetic responsiveness by moving the frame and using the magnet and/or electromagnet.

Work To Be Done

Dependent variables:

- Pure fibre properties:
    - Deflection at fixed magnetic fields
    - Mean diameter
    - Variance in diameter across 10 random sites
- Pure hydrogel properties
    -

Independent variables:

- Contraction strain (length of frame before spinning / length of frame after spinning)
    - 0%, 10%, 20%, 30%, 40%, 50%
- Solvent-polymer system
    - 85% DMF - 15% acetone / PS
- Solvent-polymer composition
    - 10%, 20%, 30%
- Solvent-Iron oxide composition
    - 10%, 20%, 30%
- Hydrogel composition
    - 6% gelatin / 1% alginate - 20 ml water + 1.2 g gelatin + 0.2 g alginate
    - 2% gelatin / 0.33% alginate

- 9 different fibre compositions to be made (solvent - polymer - iron)
    - 80/10/10, 70/20/10, 60/30/10
    - 70/10/20, 60/20/20, 50/30/20
    - 60/10/30, 50/20/30, 40/30/30
- 4 different hydrogel compositions to be made (water - gelatin - alginate)
    - 93/6/1, 97/2/1, 93.666/6/0.333, 97.666/2/0.333

To do today:

- Print 36 frames
- Setup the spinner and robot arms
- Code the robot arm
- Obtain 9 glass vials
- Prepare all the fibre solutions (with PS only)
- Spin all the fibres
- Prepare all the hydrogels

- To prepare each fibre set:
    - Measure the mass of the empty frame.
    - Start the spinner, with the slider at a fixed distance (10.0 mm).
    - Put the syringe in the robot arm, extrude and grab some fibre manually, place it on the frame and then start the robot arm program to spin across. Need to practise this and code the program.
    - Stop after a certain number of passes along the frame (TBC) and measure the mass of the spun frame, calculate fibre mass.
    - If there is time, repeat 3 times.
- To prepare each hydrogel:
    -

To do today (12/05)

- Collect the variable height sliders and see which one best fits the frame
- Prepare the printing file for 18 copies of this slider and frame and print
- Use the robot to extrude fibres into the frame, find the optimal rate of horizontal movement of the robot and practise the technique

To do 17/05

- Spin each of the 9 solutions into 9 frames
- Take lots of pictures of each one
- Prepare two hydrogel solutions
- Spin 9 more solutions if there is time
-