3D Printing of Multi-Material Hydrogels

An IIB Project Final Report by

Lorcan Nicholls (ln356@cam.ac.uk)
University of Cambridge, Girton College

26th May, 2024

Senior Supervisor: Prof. Yan Yan Shery Huang
Supervisor: Yaqi Sheng

Contents

① Technical Abstract

② Introduction

2.1. Background

Materials designed to mimic the adaptability and flexibility of biological tissues hold immense promise for a variety of highly interdisciplinary science and engineering applications. In the recent decade, rapid progress in the fields of 3D and 4D bioprinting and responsive materials has led to the convergent development of soft functional materials which have driven research into current and emerging technologies including mechanobiology research, organoids, tissue engineering, regenerative medicine, actuators for soft robotics and smart textiles for wearable technology.

One of the key challenges relevant to many of these applications is the high standard of biocompatibility required of the constituent materials. In the realm of materials science, hydrogels occupy a distinct niche as a class of substances characterised by a cross-linked polymer network with the ability to absorb and retain substantial quantities of water, providing them with the properties of mechanical softness, flexibility, and biocompatibility [1]. While some shape-memory hydrogel formulations can be designed to exhibit an intrinsic response to stimuli such as light, temperature and pH on their own as a result of the steric and electrostatic properties arising from their physico-chemical bonding, their scope is broadened further when combined with other responsive materials, such as semiconductors [2], ferromagnets [3] [4] [5], ferroelectrics [6] and even superconductors [7]. These heterogeneous biphasic functional composites possess tunable properties by means of varying the morphology of the dispersed phase within the hydrogel into arrangements such as nanoparticles [8], nano- and microfibres, thin films and amorphous multi-material networks.

The present project undertakes an exploration into the potential of incorporating hydrogels into three-dimensionally printed magnetic composites for actuator applications. It is proposed that this synergistic combination of materials has the potential to yield actuators with enhanced performance and expanded functionality, while being manufacturable to high dimensional tolerance without the need for specialised fabrication facilities. A range of possible materials was surveyed based on both existing literature and new experimentation, and various important properties of interest of the resulting composites are characterised and reported. The exploration of 3D printing techniques for fabricating magnetic hydrogel composites offers precise control over the material's structure, shape, and functionality, unlocking new possibilities for complex and customized actuator designs. Through this research, we aim to contribute to the advancement of soft actuator technology by developing 3D printable magnetic hydrogel composites, opening up exciting opportunities for applications in diverse fields.

In this introductory section, a summary of the main principles behind the two core components of the new composite - **magnetic fibres** and **hydrogels** - is presented, followed by a literature review of the main fabrication techniques in the laboratory and their applications across science and engineering, and finally the scope of the project throughout its development is discussed.

Hydrogels

Hydrogels composed of natural biopolymers are especially capable of mimicking the mechanical properties of biological tissues. These biopolymers are also polyelectrolytes, containing permanently ionised functional groups on their chains acting as hydrophilic binding sites for solvent water molecules, ions. If the polymer contains charges of both polarities, referred to as a polyampholyte, these sites can attract each other, permitting native physical cross-linking between chains, by ionic attractions, hydrogen bonding or physical entanglement. These polymers are highly water-soluble and incorporate water molecules into their 3D structures resulting in swelling and formation of a stable solid-like polymer network. For example, in gelatin, hydrogen bonding shifts the random conformation to a structured one in which single helices associate into triple helices, bringing about the sol-gel transformation [10]. Other natural polymers with similar physical crosslinking mechanisms include agarose, alginate, chitosan, collagen, heparin, hyaluronan, fibrin and matrigel, all of which are entirely natural in origin. Synthetic hydrogels have been developed to address concerns about the durability of these materials, which use man-made polymers lacking ionisation, instead enacting their phase transition by chemical crosslinking, such as by amidation, transesterification or photocatalytic bond formation by ultraviolet radiation, as exploited by their usage as bioinks in light projection bioprinting. The most common synthetic hydrogels are based on polyethylene glycol and its diacrylate (PEGDA), modified cellulose, methacrylated gelatin (GelMA) and proprietary poloxamers such as Pluronic F-127. These are widely used when control of the rate of natural degradation is necessary, such as in tissue engineering applications, where the rate of proliferation of the host cells must closely match the rate of *in vivo* biodegradation of the matrix while retaining its mechanical stability.

Natural hydrogels can be divided into two subclasses depending on the temperature dependence of their solubility. Those in which cooling from high temperature promotes crosslinking, forming solid gels at room temperature, are classed as being ‘lower critical solution temperature’ (LCST) hydrogels, and those with the opposite behaviour have an ‘upper critical solution temperature’ (UCST), as shown in Fig. 1(b). These phase transitions occur on account of thermodynamics, due to the free energy minimisation and polymer-solvent interactions as predicted by the Flory-Huggins solution theory of polymeric colloids, where the curves shown may represent either the binodal (stable coexistence) or spinodal (metastable) phase boundaries. Whether a particular polymer forms an LCST or UCST hydrogel depends on its chemical makeup, which are tunable for synthetic hydrogels, and has great importance for their use in bioprinting, where the nozzle and stage temperatures must be set appropriately for solidification on equilibration to ambient conditions.

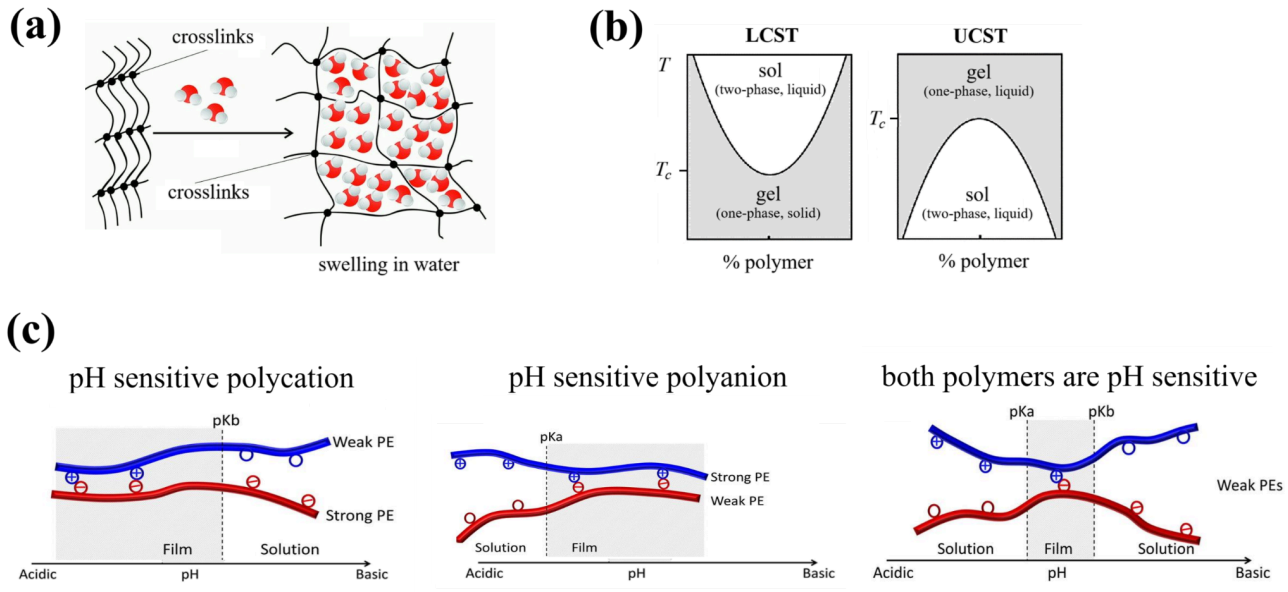

Fig. 1. Main principles of hydrogels. **(a)** Schematic of the swelling of crosslinked polymers due to addition of water. **(b)** The phase diagrams of LCST and UCST hydrogels, showing the sol-gel transition between the fields. **(c)** Intrinsically-responsive hydrogels showing sensitivity to pH, due to weak and/or strong ionisation of polyelectrolytes at different pH, resulting in differential swelling in solution.

Natural protein-based hydrogels can also have their solubility modified by addition of simple salts to the water solution. While the precise mechanism is not fully clear, it is generally observed that ‘kosmotropic’ ions - those that promote the structuring of water through its hydrogen bonding network - result in decreased polymer solubility, such as by citrate, phosphate and tartrate salts. Conversely, the ‘chaotropic’ ions promote disorder and tend to homogenise the phases, increasing solubility, such as by potassium, ammonium and guanidinium salts. These phenomena are widely exploited in various standard molecular biology assays where they result in ‘salting out’ or ‘salting in’ of target proteins respectively. The mechanical (as well as electrical transport) properties of the resulting natural hydrogel can be tuned in this way, where addition of kosmotropic anions during gel formation results in increased hydrogel stiffness due to promoted polymer agglomeration. pH-sensitive crosslinking results in intrinsically responsive hydrogels (Fig. 1(c)), a type of functionalised hydrogel, which has inspired numerous innovative new approaches to synthesise composite materials of hydrogels to respond to other stimuli such as light, electricity and magnetism. The latter is the primary focus of this project, where the hydrogel is used as a soft substrate which is actuated by a magnetic matrix interwoven with the hydrogel.

Magnetic Materials

Fibre Spinning

3D Printing

2.2. Literature Review

Survey of Current Hydrogel-Magnetic Composites

[9]

Printable Hydrogels

Comparison of Fibre Spinning Techniques

Applications of Magnetic Hydrogel Composites

2.3. Project Scope

Initial Goals and Context

Revised Project Goals

③ Theory and Experimental Design

3.1. Theoretical Treatment

3.2. Experimental Methodology

④ Materials and Methods

4.1. Apparatus

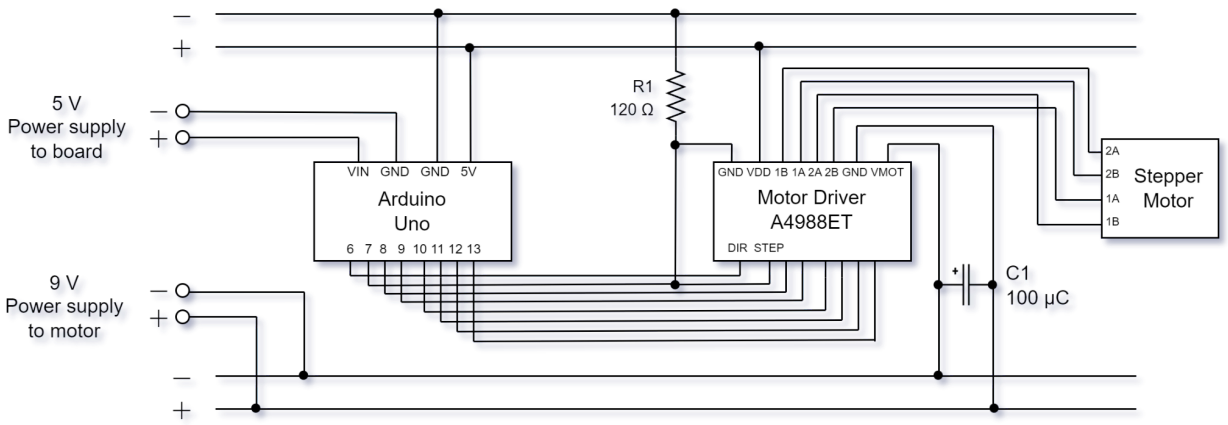

Fig. 1. Circuit schematic for the spinner, using an Arduino-controlled stepper motor.

4.2. Experimental Techniques

⑤ Results and Discussion

5.1. Analysis of Results

5.2. Discussion

⑥ Conclusions

6.1. Primary Findings

6.2. Future Work

⑦ References

[1] Utech, S., & Boccaccini, A. R. (2015). A review of hydrogel-based composites for biomedical applications: enhancement of hydrogel properties by addition of rigid inorganic fillers. *Journal of Materials Science*, *51*(1), 271–310. https://doi.org/10.1007/s10853-015-9382-5

[2] Liu, J., Garcia, J., Leahy, L. M., Song, R., Mullarkey, D., Fei, B., Dervan, A., Shvets, I. V., Stamenov, P., Wang, W., O'Brien, F. J., Coleman, J. N., & Nicolosi, V. (2023). 3D printing of multifunctional conductive polymer composite hydrogels. *Advanced Functional Materials*, *33*(37). https://doi.org/10.1002/adfm.202214196

[3] Brunsen, A., Utech, S., Maskos, M., Knoll, W., & Jonas, U. (2012). Magnetic composite thin films of FexOy nanoparticles and photocrosslinked Dextran Hydrogels. *Journal of Magnetism and Magnetic Materials*, *324*(8), 1488–1497. https://doi.org/10.1016/j.jmmm.2011.11.039

[4] Salahuddin, B., Aziz, S., Gao, S., Hossain, Md. S., Billah, M., Zhu, Z., & Amiralian, N. (2022). Magnetic hydrogel composite for wastewater treatment. *Polymers*, *14*(23), 5074. https://doi.org/10.3390/polym14235074

[5] Tang, S., Yan, Y., Lu, X., Wang, P., Xu, X., Hu, K., Yan, S., Guo, Z., Han, X., Zhang, F., & Gu, N. (2024). Nanocomposite magnetic hydrogel with dual anisotropic properties induces osteogenesis through the NOTCH-dependent pathways. *NPG Asia Materials*, *16*(1). https://doi.org/10.1038/s41427-024-00535-x

[6] Yang, L., Nandakumar, D. K., Miao, L., Suresh, L., Zhang, D., Xiong, T., Vaghasiya, J. V., Kwon, K. C., & Ching Tan, S. (2020). Energy harvesting from atmospheric humidity by a hydrogel-integrated ferroelectric-semiconductor system. *Joule*, *4*(1), 176–188. https://doi.org/10.1016/j.joule.2019.10.008

[7] Wang, Y., Zhang, W., Shan, Y., Yu, X., & Chen, K. (2023). Preparation of polyacrylamide/calcium alginate@$Ti_3C_2T_x$ composite hydrogels with high adhesive performance for flexible supercapacitor electrolytes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *666*, 131312. https://doi.org/10.1016/j.colsurfa.2023.131312

[8] Thoniyot, P., Tan, M. J., Karim, A. A., Young, D. J., & Loh, X. J. (2015). Nanoparticle–Hydrogel Composites: Concept, design, and applications of these promising, Multi‐Functional Materials. *Advanced Science*, *2*(1–2). https://doi.org/10.1002/advs.201400010

[9] Simińska-Stanny, J., Nizioł, M., Szymczyk-Ziółkowska, P., Brożyna, M., Junka, A., Shavandi, A., & Podstawczyk, D. (2022). 4D printing of patterned multimaterial magnetic hydrogel actuators. *Additive Manufacturing*, *49*, 102506. https://doi.org/10.1016/j.addma.2021.102506

[10] Kokol, V., Pottathara, Y. B., Mihelčič, M., & Perše, L. S. (2021). Rheological properties of gelatine hydrogels affected by flow- and horizontally-induced cooling rates during 3D cryo-printing. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *616*, 126356. https://doi.org/10.1016/j.colsurfa.2021.126356

⑧ Appendix

8.1. Appendix I: Symbols

Notation

l	end-to-end polymer chain length

Abbreviations

LCST	lower critical solution temperature
PBS	phosphate-buffered saline
UCST	upper critical solution temperature

8.2. Appendix II: Tabular Data

Table 1: properties of some natural and synthetic hydrogels.

Hydrogel	Origin	Temperature Dependence	Uses
Agarose	Natural	UCST	gel electrophoresis, affinity chromatography (Sepharose), immobilisation, cell encapsulation
Alginate	Natural	UCST	controlled drug release
Chitosan	Natural	UCST	
Gelatin	Natural	UCST	
Collagen	Natural	UCST	
Hyaluronan	Natural	LCST	
Fibrin	Natural	LCST	
Matrigel	Natural	LCST	
PEG diacrylate (PEGDA)	Synthetic	UCST	
Modified cellulose	Synthetic	both	
Methacrylated gelatin (GelMA)	Synthetic	both	
Pluronic F127	Synthetic	both	