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Porous PNIPAm hydrogels: Overcoming diffusion-governed hydrogel actuation

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Porous PNIPAm hydrogels: Overcoming diffusion-governed hydrogel actuation

Abstract

© 2019 Elsevier B.V. A custom centrifugal melt-spinning technique was used to prepare randomly arranged 3D fibre networks from commercially available shellac flakes. These fibre networks were implanted into thermally actuating poly(N-isopropylacrylamide)-alginate hydrogels and then removed chemically to yield an interconnected porous gel structure. Pore diameter was capable of being controlled through the fibre-spinning temperature, where it was shown that shellac fibres spun at a lower temperature yielded larger diameter pores in the resultant gels. These gels demonstrated a fast actuation, with a 77 % volume loss of a cylindrical sample in just 30 s when immersed in a 60 °C water bath. It was shown that the volume change mechanism overcame the well-known dimensional constraint, which has previously governed hydrogel swelling/de-swelling, through minimisation of the water diffusion distance from the gel to the porous network. With such rapidly actuating materials, potential applications for these hydrogels lie in thermally responsive valves and artificial muscles for soft robotics or microfluidics.

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Porous PNIPAm Hydrogels: Overcoming Diffusion-Governed Hydrogel Actuation

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Abstract:

A custom centrifugal melt-spinning technique was used to prepare randomly arranged 3D fibre networks from commercially available shellac flakes. These fibre networks were implanted into thermally actuating poly(N-isopropylacrylamide)-alginate hydrogels and then removed chemically to yield an interconnected porous gel structure. Pore diameter was capable of being controlled through the fibre-spinning temperature, where it was shown that shellac fibres spun at a lower temperature yielded larger diameter pores in the resultant gels. These gels demonstrated a fast actuation, with a 77% volume loss of a cylindrical sample in just 30 seconds when immersed in a 60°C water bath. It was shown that the volume change mechanism overcame the well-known dimensional constraint, which has previously governed hydrogel swelling/deswelling, through minimisation of the water diffusion distance from the gel to the porous network. With such rapidly actuating materials, potential applications for these hydrogels lie in thermally responsive valves and artificial muscles for soft robotics or microfluidics.

Introduction:

Channelled or porous hydrogels have shown promise for use in energy storage, energy harvesting, tissue vascularisation and artificial muscle development [1,2]. Since many hydrogels are intrinsically biocompatible, they have been widely investigated for various biomedical

applications [3–5]. More recently, responsive or smart hydrogels have been studied for uses in the field of soft robotics and microfluidics [6]; soft robotic actuators have been modelled through a gel volume change in response to an environmental variation in temperature, light intensity/wavelength, electric field or pH [7,8].

One of the challenges associated with hydrogel actuation is the diffusion speed of interstitial water from the bulk gel to the surface for successful expulsion. According to the swelling kinetics of hydrogel materials, it is well-known that the swelling/de-swelling time is directly proportional to the square of the smallest dimension [9]. Since the speed of gel volume change is governed by the removal of interstitial water from the hydrogel structure, research has focused on the preparation of porous hydrogels in an effort to reduce the diffusion distance and hence increase the actuation speed.

Several approaches to achieve macro-porous gels have been developed [1]. These include creating sacrificial particulate networks[10], 3D printing scaffolds with sacrificial networks [11], electrospinning [12], cryogelation [13], emulsion templating [14] and the preparation of hydrogel foams through bubble introduction [15,16]. However, for specific applications, precise control of channel dimensions, shape, orientation or density is required. For example, a microfluidic flow-cell may require a certain flow rate of material through channels that are aligned in a certain direction but distributed in a 3D array [17]. On the other hand, when designing biological scaffolds for the promotion of cell-growth, a randomised 3D array of channels within a certain size range is desirable [18]. Printing 3D scaffolds with sacrificial networks tends to yield highly ordered arrays of channels, but most commonly in a planar orientation and limited to larger fibre diameters [19]. Hydrogel foam structures often possess large, inconsistent pore sizes, many of which are closed, fully encapsulated within the gel and tend to have weakened mechanical characteristics; as such, they are often unsuitable for many of the aforementioned applications.

In order to address some of these issues, a previous study by Bellan et al. has shown that sacrificial shellac microfibrils can be used to prepare a porous hydrogel through chemical dissolution [20]. Similarly, the same research group has created a sacrificial PNIPAm fibre network to fabricate porous gelatine, capable of allowing media flow for optimised cell-growth [21]. Inspired by this approach, we have investigated the preparation of a hydrogel-based thermal actuator with reduced response time due to the presence of hollow channels.

Shellac is derived from the resinous secretions of the female lac bug insects found in trees in India and Thailand. Typical uses of shellac resin include wood varnish, nail polish and edible glazing agents. Lac resin is a natural polymer made up of hydroxy fatty acids, primarily a complex of

aleuritic acid (9,10,16-trihydroxyhexadecanoic acid) and hydroxy sesquiterpenic acids [22] . This acidic composition allows for facile dissolution using alcohols or aqueous bases, making it an excellent candidate for a sacrificial scaffold material.

Poly(N-isopropylacrylamide) (PNIPAm) is a thermo-responsive polymer, with transition temperature around 32°C [23]. Above this temperature, PNIPAm undergoes volume shrinkage. This is due to a polymer chain coil-to-globule transition, resulting in a local change of hydrophilicity to hydrophobicity, leading to ejection of intrinsic water from the hydrophobic areas of the hydrogel material. Previous studies have focused on the use of PNIPAm as a thermally actuating material for devices such as strain gauges, valves, sensors and artificial muscles [24,25]. For the purpose of preparing a more robust hydrogel, alginate is incorporated to form an ionic-covalent entanglement (ICE) network. Our previous research has demonstrated a strong, flexible actuating material, capable of rapid heat-responsive volume change based on this hydrogel formulation [26], with >50% volume reduction of a cylindrical sample after 1 minute at 60°C .

In the present study, incorporation of a randomised interconnected 3D porous network into strong PNIPAm-alginate hydrogels has been shown to elicit a faster thermal actuation response time. A sacrificial network of melt-spun shellac fibres was set in the host gel and subsequently chemically removed by washing with an alkaline solution, leaving hollow pores in the hydrogel. This essentially increased the surface area, and hence reduced in the distance over which water must travel in order to be expelled from the bulk material. Through this means of porous hydrogel preparation, the dimensionality constraint which previously governed the swelling and deswelling of gels has been overcome.

Experimental:

Materials: N,N'-Methylenebis(acrylamide) (MBAm), alginic acid sodium salt (alginate from brown algae), calcium chloride (CaCl₂) and ammonium persulfate (APS) were purchased from Sigma Aldrich (Australia). N,N,N',N'-Tetramethylethylenediamine (TEMED) and N-Isopropylacrylamide (NIPAm) were purchased from Novachem (Australia). Shellac flakes (Feast Watson Mastertouch) were purchased from Bunnings (Australia).

Fibre-Spinning: A commercially available candy floss machine (Cotton Candy Co.) was adapted to be used as a centrifugal melt-spinning instrument. The temperature of the device was controlled by varying the voltage, which was set to 125 V and allowed to reach 150°C at the centre of the spinneret. Spinneret diameter was 12.5 cm, speed: 3150 r.p.m. Temperature was monitored using a handheld digital laser thermometer. Shellac flakes were placed into the heated spinneret, where they melted, flowed through the spinneret holes and re-solidified upon cooling. Fibres

were collected manually from the floss drum (52 cm diameter) with the aid of a paper-covered stirring rod, held vertically, while moving around the edge of the floss drum; the function of the paper was to add a high-friction surface, onto which the fibres could gather.

Hydrogel Preparation: For a 20 mL hydrogel sample, 2 g NIPAm, 20 mg MBAm and 400 mg alginate were added to 20 mL distilled water (18.2 M Ω -cm) under rapid stirring (Stuart CB162 Hotplate Stirrer) until all solids were fully dissolved. The solution was cooled to 4°C before and after addition of 200 mg APS to prevent premature reaction activation. 80 μ L TEMED (4°C) was added prior to gel-casting as an accelerant.

Preparation of porous gel: Shellac floss (3D fibre mesh) was placed in a petri dish and spread out to fill the volume with minimal localised clumping (Figure 1b). Hydrogel-forming solutions were injected in between the floss network and allowed to cure for at least 1 hour at 4°C to avoid phase-separation between PNIPAm and alginate polymer chains (Figure 1c). Cylindrical (15 mm diameter) samples were cut out of the gel-shellac composite using a hole-punch and placed in a sodium hydroxide bath (NaOH, 0.5 M) for one to two days until complete dissolution of the shellac network was visible. Once shellac fibres were visibly removed, gels were re-swollen in water with several water-bath changes over the course of 24 hours. The gel samples were then soaked in a calcium chloride (CaCl₂, 0.5 M) bath for 24 hours to cross-link the alginate network, followed by further soaking in water to remove any free Ca²⁺ ions (Figure 1d).

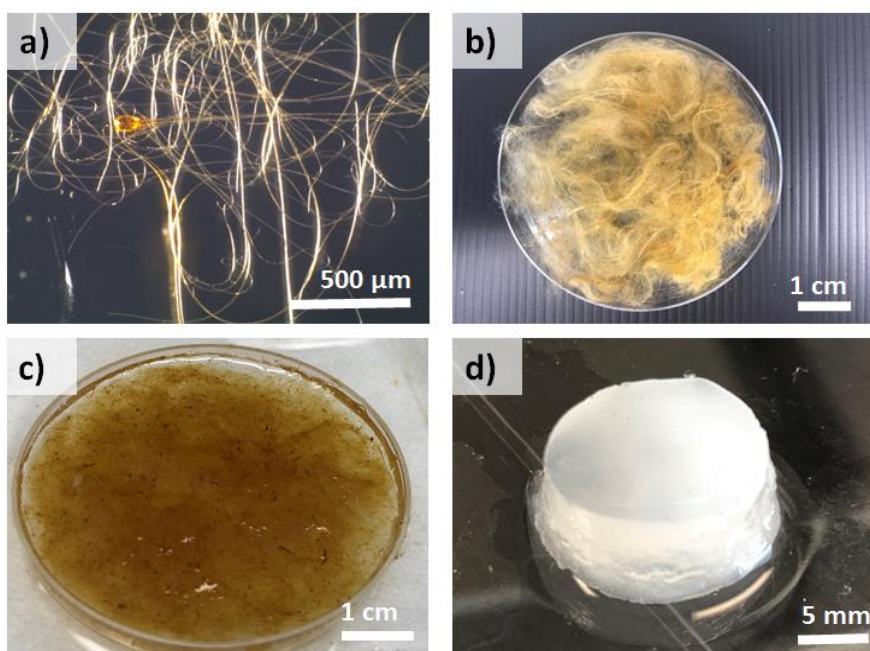


Figure 1. a) Microscopy image of some shellac fibres. b) Shellac fibres placed in a petri-dish, ready for gel-casting. c) PNIPAm-alginate hydrogel cast around shellac fibres. d) cylindrical PNIPAm-alginate hydrogel with fibres removed via NaOH soaking.

Characterisation: Rheological analysis of cylindrical hydrogels (roughly 15 mm diameter, 9 mm height) was carried out with an Anton Paar Physica MCR 301 Digital Rheometer (parallel plate tool; diameter, 15 mm). Dynamic modulus was measured as a function of shear strain (0.01 % - 10 %) to ascertain the parameters of the linear viscoelastic (LVE) region for each gel sample.

Mechanical compression testing of identical cylindrical samples was performed on a Shimadzu EZ-S Universal Tester at a compression rate of 2 mm/min, with a 50 N load-cell, until material failure.

Thermal actuation of cylindrical hydrogel samples was tracked using a laboratory balance (Mettler XS 64) and a Leica Z16 APO optical microscope (in tandem with Leica Application Suite Software). Mass measurements were recorded for up to 30 mins of actuation (volume loss) in a 150 mL, 60°C water bath. For each measured time period (0.5, 1, 2, 3, 4, 5, 10, 20 and 30 mins), the hydrogel sample was removed from the water bath, patted dry to remove any excess surface water, imaged and weighed, before being placed back into the water bath.

Scanning electron microscopy images of shellac fibres were obtained using a field emission SEM (FESEM JEOL JSM 7500-FA) operated at 5 kV and a spot size setting of 8 mm.

Results:

Shellac Floss Spinning:

Shellac fibres were prepared at various melt-temperatures between 150°C and 225°C and collected as described. There was a visible difference between bulk samples of fibres spun at the lower and higher temperatures. At 150°C, the samples contained shorter, thicker fibres which were coarse to touch. Using a melt temperature of 225°C, the fibres were much finer and the floss was softer to touch. Upon closer inspection with SEM, the differences in fibre diameter were confirmed (Figure 2). For example, the 150°C sample of shellac floss exhibited fibres of up to 100 µm diameter, whereas the 225°C sample displayed fibres with diameter less than 20 µm. This phenomenon can be attributed to the higher temperatures inducing a lower viscosity in the liquid shellac, resulting in thinner, longer fibres being drawn out during the centrifugal spinning process. Conversely, lower spinning temperatures result in high-viscosity melts, yielding shorter, thicker fibres.

The spherical beads of shellac which appear in all samples represent the termination of spun fibre arrays. The beads are pulled from the spinneret once the polymer melt has diminished to a size smaller than the spinneret hole and can no longer resist the centrifugal force applied by the extruded fibres. It was noted that the longer (and thinner) the fibre, the larger the beads tended to be; this can be attributed to greater centrifugal forces associated with the longer fibres.

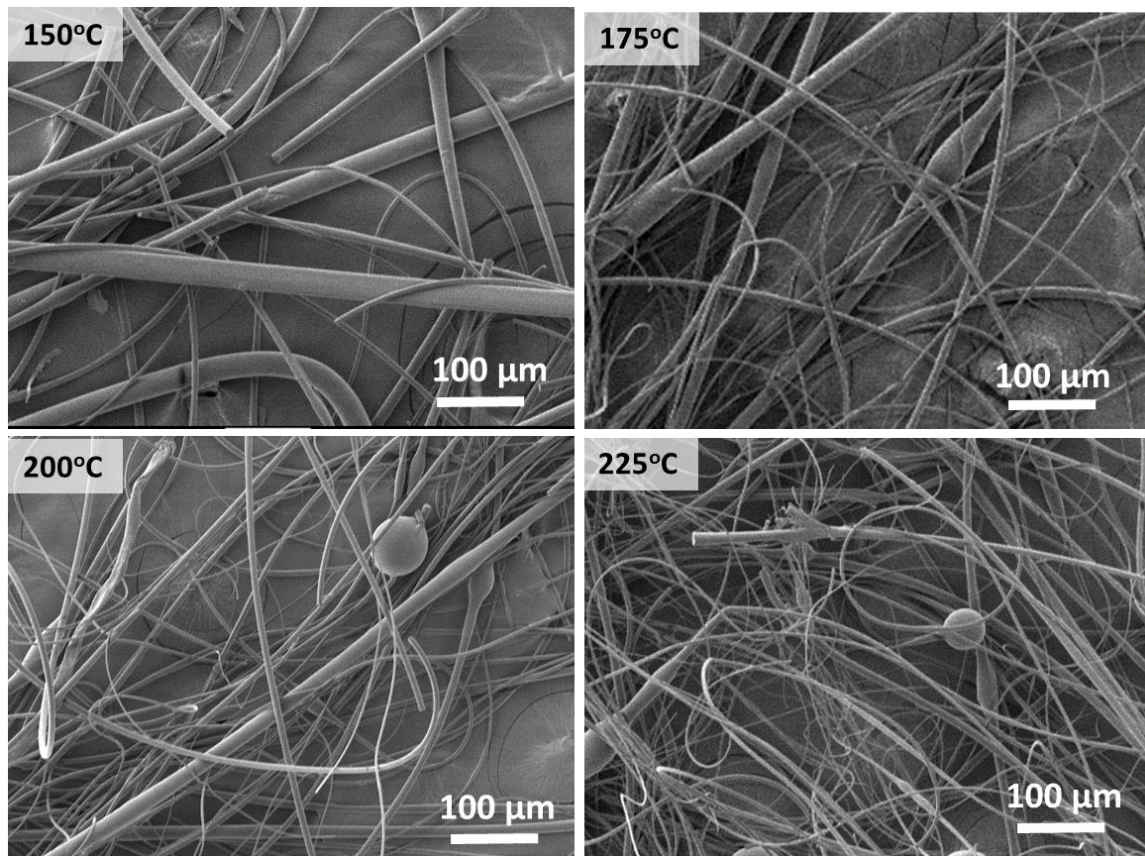


Figure 2. SEM images of shellac fibres spun at various temperatures between 150°C and 225°C

Porous Hydrogel Preparation:

Dual-network ionic-covalent entanglement (ICE) hydrogel-forming solutions were prepared from combinations of covalently cross-linked PNIPAm and ionically cross-linked alginate, as described in the Experimental section. Samples were formed with and without shellac. For comparison, gels were also prepared from PNIPAm without alginate. Again, these gels were made with and without shellac.

For the two samples containing shellac, 140 mg (0.7 wt% loading) shellac fibres, spun at 150°C, were placed in a petri dish (5.5 cm diameter) and spread out until evenly distributed through the whole volume. The cooled (4°C) hydrogel-forming solution (20 mL) was carefully pipetted into the petri dish around the shellac fibres, avoiding network collapse (clumping), air pockets and exposed fibre bundles at the surface. The hydrogel samples were allowed to thermally cure for at least 1 hour at 4°C and then cylindrical shapes were cut from the bulk sample in the petri dish. The four sample types were treated in one of two ways: left as prepared, or soaked in 0.5 M NaOH. A total of 8 different samples were prepared.

Rheological consistency of all gel samples were compared to the mechanical properties (Table 1). When focusing on elastic modulus, it can be seen that both types of gel (PNIPAm and PNIPAm-alginate) exhibited clear mechanical reinforcement with the inclusion of shellac fibres. The modulus of the neat PNIPAm-alginate gel increased from 50 ± 9 kPa to 79 ± 11 kPa with the addition of 0.7 wt% shellac. Similarly, as-prepared PNIPAm gels showed an almost 3-fold reinforcement due to the fibre network with neat modulus of 17 ± 2 kPa compared to 46 ± 10 kPa for the shellac-gel. This reinforcement can be explained by the presence of the entangled fibre network that can provide a physical scaffold for the hydrogel networks. The reinforcing trend was also observed through the comparison of the rheological storage modulus between gels with/without shellac fibres. It should also be noted that, in both cases for as-prepared gels, the water content was 1% lower when shellac fibres were present. This small effect is attributed to the fibre network restricting polymer displacement and hence, swelling in water.

As expected, dual-network PNIPAm-alginate hydrogels were consistently stiffer than their equivalent PNIPAm counterparts. This enhanced stiffness is due to inter-network entanglement, which has a synergistic reinforcement effect, as observed for previous studies on ICE hydrogels [27,28]. Swelling ratios for PNIPAm gels were also slightly higher due to the reduced network constraint offered by the alginate system. For all measured samples, soaking in NaOH resulted in a reduction of elastic modulus and increase in water content. There are two mechanisms contributing to this reduction: firstly, where alginate is present, there will be a partial ionic exchange between the divalent calcium and monovalent sodium ions, leading to a more loosely-bound polymer network. This accounts for the dramatic decrease in elastic modulus in samples 1 and 2, when compared to the as prepared PNIPAm-alginate. Secondly, NaOH has been shown to induce an increase in hydrophilicity in PNIPAm chains, accounting for the slight increase in measured water content and decrease in elastic modulus for the PNIPAm control sample. It is noted that the PNIPAm-alginate and PNIPAm samples with shellac removed via NaOH exhibited no difference between them, suggesting that there is no mechanical advantage in the inclusion of alginate

Table 1. Mechanical compression and rheology data for various gel-fibre combinations and treatments. Data represents the mean of 4 measured samples. Elastic modulus was calculated for 20-30% compressive strain, storage modulus was measured within the linear viscoelastic region at 0.01-1% shear strain.

Sample	Treatment	Elastic Modulus (kPa)	Storage Modulus (kPa)	Water Content (%)
1. PNIPAm-alginate + shellac	As prepared	79 ± 11	8.7 ± 1.2	93.2
	NaOH soak	17 ± 2	4.4 ± 0.7	94.6
2. PNIPAm-alginate	As prepared	50 ± 9	4.6 ± 0.3	94.2
	NaOH soak	21 ± 2	4.8 ± 0.3	95.1
3. PNIPAm + shellac	As prepared	46 ± 10	3.6 ± 0.4	94.0
	NaOH soak	16 ± 2	3.5 ± 0.2	95.1
4. PNIPAm	As prepared	17 ± 2	1.3 ± 0.1	95.1
	NaOH soak	12 ± 2	2.1 ± 0.5	95.4

To assess the thermal actuation of each gel type, the sample volume was tracked as a function of time in which the sample was immersed in a 60°C water bath (Figure 3). As-prepared gel samples were seen to have relatively poor initial actuation, and this was accentuated for samples containing shellac fibre networks (Figure 3a, samples 1 and 3). This could be attributed to either the fibre network physically constraining the gel and preventing actuation, or the fibre network reducing the pore-size, which would impede the removal of interstitial water and hence the actuation speed. Having undergone a treatment of immersion in NaOH to remove any shellac that may be present in the hydrogels, a dramatic improvement in actuation response time was observed for samples 1 and 3 (Figure 3b).

A volume loss of 85% was recorded for sample 1, PNIPAm-alginate with shellac fibres removed via NaOH treatment after one minute. This is considerably faster than previously studied PNIPAm-alginate hydrogels without channels which exhibited a volume loss of 60% after the same length of time [26]. The dramatic improvement in response time is due to the creation of a network of hollow pores in the absence of shellac, through which interstitial water can be readily ejected from the hydrogel sample. This gel composition and treatment type were selected as being optimum for all subsequent experiments.

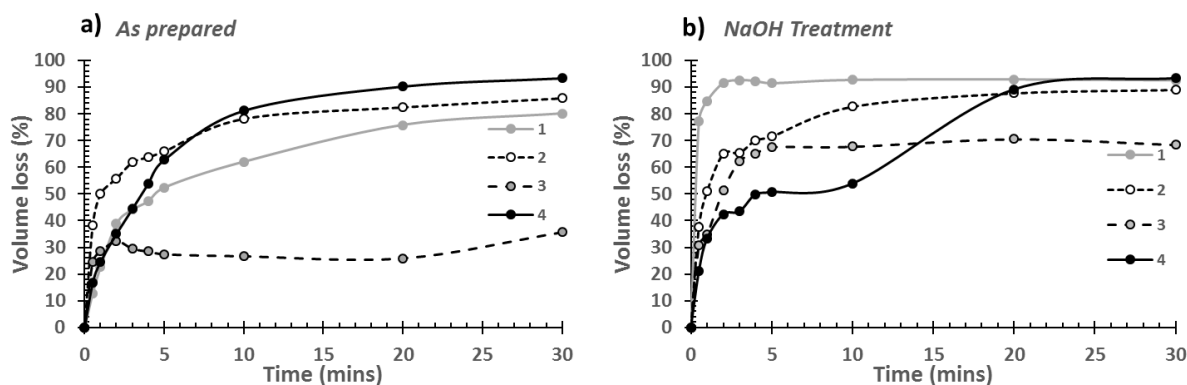


Figure 3. Thermal actuation of hydrogel samples. Data is represented as a volume reduction/loss over 30 minutes, when a cylindrical sample (diameter = 15mm, height = 9mm) is immersed in a 60°C water bath. a) Actuation of as-prepared hydrogels and b) actuation of hydrogels which have undergone a NaOH treatment. Sample 1: PNIPAm-alginate-shellac, sample 2: PNIPAm-alginate, sample 3: PNIPAm-shellac, sample 4: PNIPAm. Data series are representative of experiments performed in triplicate.

An important finding during these studies was that PNIPAm-alginate dual-network systems actuated much more quickly than PNIPAm hydrogels, validating its inclusion, despite previous findings of an insignificant mechanical reinforcement. Furthermore, a visible surface blistering effect was observed for the PNIPAm gels, meaning that water, which was being removed from the bulk of the gel, was becoming trapped beneath the outer layer (Figure 4a and b). Since this did not occur for PNIPAm-alginate samples, it is suggested that the presence of the alginate provides a level of surface porosity, allowing for the complete and unencumbered evacuation of the interstitial water and hence, the gel actuates more efficiently. The delayed increase in volume loss (water removal) of sample 4 (PNIPAm hydrogel) observed in Figure 3b is attributed to this effect.

A typical PNIPAm-alginate porous hydrogel was frozen in liquid nitrogen and cross-sectioned for SEM imaging (Figure 4c and d). Pores were observed to be randomly distributed throughout the bulk of the gel, as was expected. Furthermore, they were seen to be devoid of shellac and contain a coating of ice.

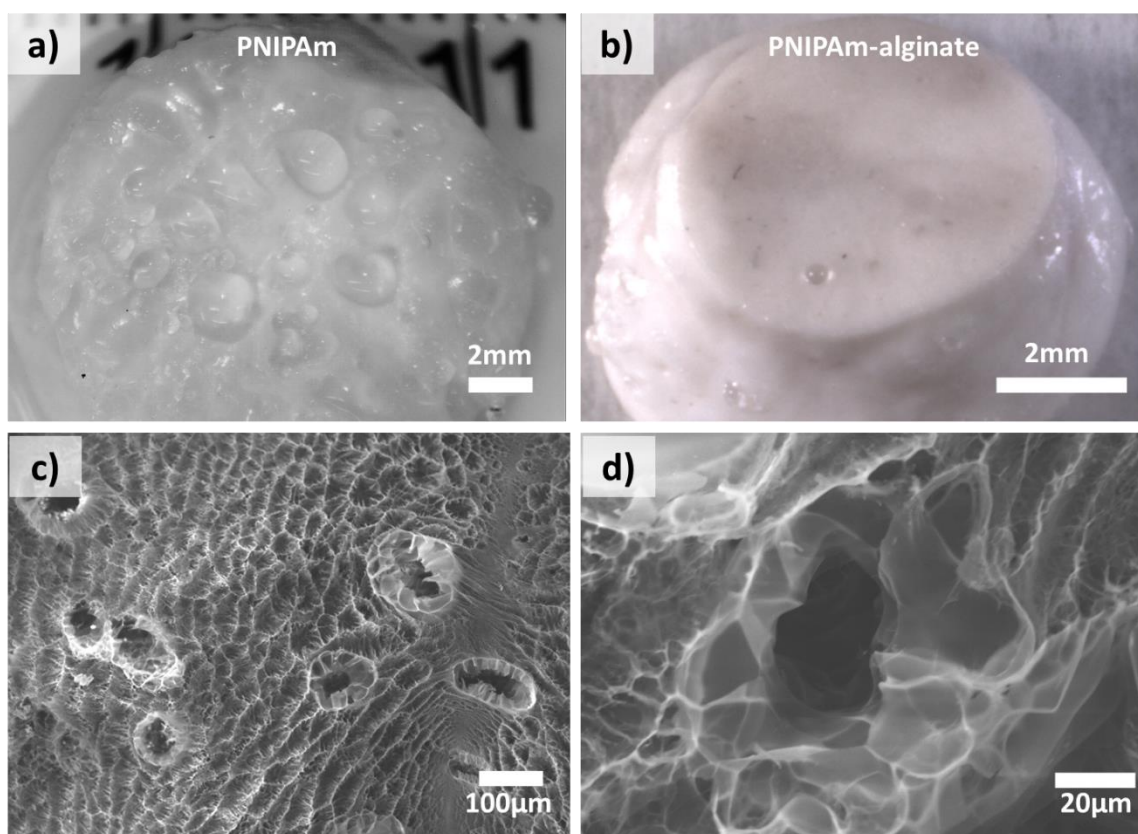


Figure 4. a) PNIPAm hydrogel after immersion in 60°C water bath for 5 mins, b) PNIPAm-alginate hydrogel after immersion in 60°C water bath for 5 mins. c) and d) SEM cross-sectional images of a PNIPAm-alginate hydrogel displaying hollow pores.

Since a variation in shellac fibre diameter was observed through SEM imaging (Figure 2), the effect of varying channel size was easily investigated. PNIPAm-alginate hydrogels (20 mL) were cast around shellac fibres from a range of spinning temperatures. The thermal actuation was assessed for fibre-spinning temperatures between 150°C and 225°C (Figure 5). By focusing on the initial 5 minutes of reaction, there is a clear trend between pore diameter and response time; the larger the diameter (lower spinning temperature), the faster the response. After 30 seconds of heating, the gels with pore networks resulting from fibres spun at 150°C and 225°C had volume losses of 77% and 19%, respectively, showing the considerable difference. This effect can allow for more precise control of the flow rate and actuation speed through channel size tailoring. Due to the desire to optimise response time for fast actuation, shellac fibres spun at 150°C will be used for subsequent experiments in this work.

While rheological shear testing of gels showed very little change in storage modulus between samples, mechanical compression analysis highlighted a distinct weakening effect (decrease in elastic modulus) for larger pore diameters (Table 2). This can be attributed to pore-collapse in

compression mode, which does not occur in shear. Intuitively, larger pore-sizes (150°C fibre spinning) induce a more significant collapse when compared to smaller pore sizes (225°C fibre spinning).

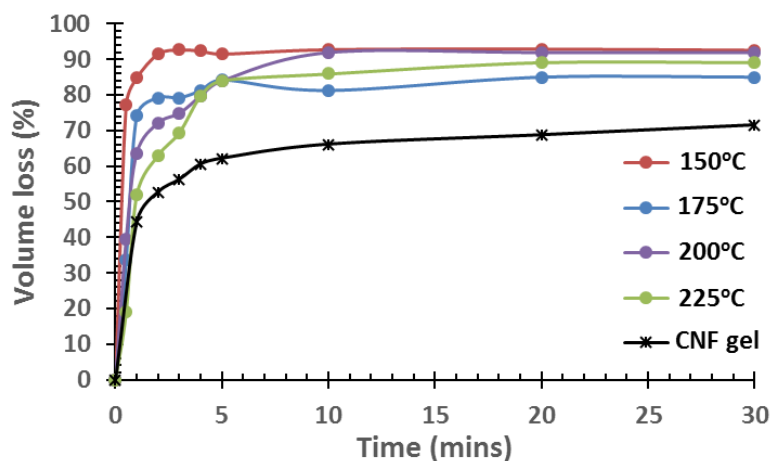


Figure 5. Thermal actuation of PNIPAm-alginate porous hydrogels with variation in shellac fibre casting temperature (150°C- 225°C), and a non-porous CNF-PNIPAm-alginate hydrogel (black) [26], represented as volume loss as a function of time. Data series are representative of experiments performed in triplicate.

Table 2. Mechanical (elastic modulus) and rheological (storage modulus) analysis for each PNIPAm-alginate hydrogel-fibre combination.

Fibre-spinning temp	Elastic Modulus (kPa)	Storage Modulus (kPa)
150°C	17 ± 1	4.4 ± 0.4
175°C	29 ± 2	5.2 ± 0.5
200°C	36 ± 4	2.2 ± 0.2
225°C	51 ± 3	3.8 ± 0.4

The effect of shellac fibre loading into the PNIPAm-alginate hydrogel on thermal actuation was examined for amounts between 100 mg - 300 mg (0.5 % -1.5 % w/v, fibres spun at 150°C). Negligible variation between the measured pore quantities was observed (Figure S1, supporting information), which was unexpected. This result suggests that either the difference in shellac loadings was too small or that the movement of interstitial water through the introduced channels was already at a maximum in the 0.5% w/v sample (100 mg shellac in 20 mL gel). Mechanical properties did not vary between measured samples.

To further investigate the actuation mechanism of porous gels, the shrinkage of a considerably smaller hydrogel (6mm x 6mm x 5mm) was measured under the same conditions. Volume loss of

the smaller porous sample was similar (70% after 30 seconds) to the larger cylindrical gel samples, when immersed in a 60°C water bath (Figure S2, supporting information). This demonstrates that by incorporating a porous network into the hydrogel, the usual rate-limiting factor of sample dimensions is overcome, i.e. interstitial water need only diffuse to the nearest pore, rather than to the nearest surface, in order to be efficiently expelled from the sample (Figure 6).

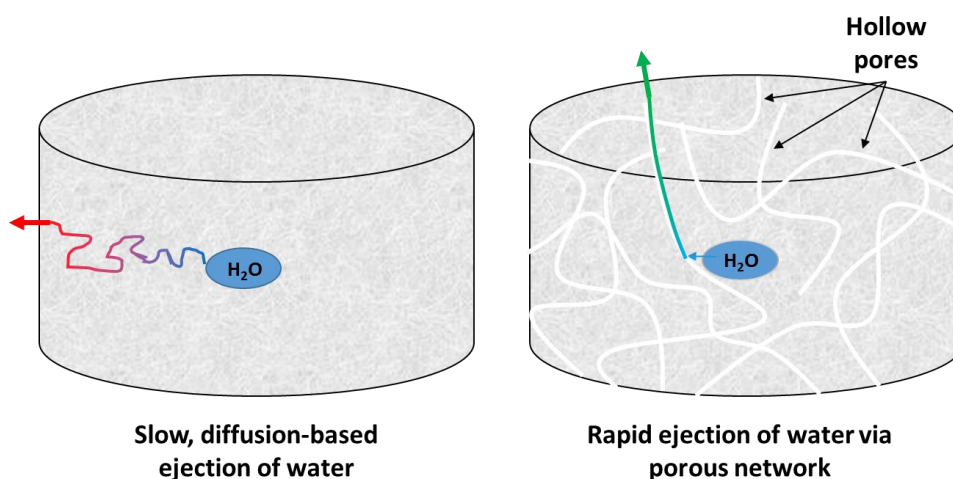


Figure 6. Schematic representation of interstitial water ejection in a hydrogel with and without hollow pores.

Conclusions:

Actuation (volume change) of PNIPAm-based hydrogels depends on the ability of interstitial water to be successfully removed from the polymer network. Introducing macroscopic channels into the gel structure, allowing for efficient water flow to the surface, is one method of achieving this end goal. This is an intuitive step from the tactic of increasing the surface area and hence decreasing the distance which water needs to travel in order to be expelled from the network, i.e. the channels behave like surfaces inside the bulk gel.

Shellac fibres which were spun via a centrifugal melt spinning technique were used to create a sacrificial network in PNIPAm host gels, resulting in a highly thermally responsive, channelled hydrogel. Incorporation of this resultant porous network was shown to drastically decrease the actuation response time of PNIPAm-alginate interpenetrating hydrogel networks, when compared to previous research on identical sample size and shape [26]. A 77% volume reduction was recorded upon immersion of a porous PNIPAm-alginate cylindrical sample in a 60°C water bath for just 30 seconds.

Mechanical properties of PNIPAm-alginate hydrogels were shown to improve with addition of the shellac fibrous network, as would be expected with the addition of any reinforcing network; however, upon chemical removal of the shellac, these properties reduced to values lower than non-porous samples. This reduction in modulus was attributed to the presence of collapsible hollow pores and an increased water content/swelling ratio due to ion-exchange between divalent calcium and monovalent sodium ions. While the presence of alginate offered no accountable physical reinforcement: elastic modulus of 17 ± 2 kPa vs 16 ± 2 kPa, it was shown to modify the hydrogel surface in such a way that interstitial water was successfully removed from the gel, rather than remaining trapped under the surface; as was observed for PNIPAm hydrogels.

Since there was no notable improvement in actuation through increasing the pore concentration of the optimised hydrogel, it is assumed that the required distance which water must travel to reach a pore has already been sufficiently minimised. A drastic decrease in gel size yielded a similar response after 30 seconds at 60°C; 70% volume loss. This proves that the well-known dimensional constraint governing the swelling/de-swelling of hydrogels[9] has been overcome through introduction of a porous network of macroscopic channels, which is a significant finding. Further reduction of actuation response time could be achieved through increasing water temperature or through more direct heating techniques; making porous PNIPAm-alginate hydrogels suitable candidates for various applications in soft robotics and microfluidics.

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References:

- [1] K.J. De France, F. Xu, T. Hoare, Structured Macroporous Hydrogels: Progress, Challenges, and Opportunities, *Adv. Healthc. Mater.* 7 (2018) 1–17. <https://doi.org/10.1002/adhm.201700927>.
- [2] Y. Shi, L. Peng, G. Yu, Nanostructured conducting polymer hydrogels for energy storage applications, *Nanoscale.* 7 (2015) 12796–12806. <https://doi.org/10.1039/c5nr03403e>.
- [3] J.E. Mealy, J.J. Chung, H.H. Jeong, D. Issadore, D. Lee, P. Atluri, J.A. Burdick, Injectable Granular Hydrogels with Multifunctional Properties for Biomedical Applications, *Adv. Mater.* 30 (2018) 1–7. <https://doi.org/10.1002/adma.201705912>.

- [4] M.C.G. Pellá, M.K. Lima-Tenório, E.T. Tenório-Neto, M.R. Guilherme, E.C. Muniz, A.F. Rubira, Chitosan-based hydrogels: From preparation to biomedical applications, *Carbohydr. Polym.* 196 (2018) 233–245. <https://doi.org/10.1016/j.carbpol.2018.05.033>.
- [5] M.E. Cooke, S.W. Jones, B. ter Horst, N. Moiemmen, M. Snow, G. Chouhan, L.J. Hill, M. Esmaeli, R.J.A. Moakes, J. Holton, R. Nandra, R.L. Williams, A.M. Smith, L.M. Grover, Structuring of Hydrogels across Multiple Length Scales for Biomedical Applications, *Adv. Mater.* 30 (2018) 1–15. <https://doi.org/10.1002/adma.201705013>.
- [6] P. Calvert, Hydrogels for Soft Machines, *Adv. Mater.* 21 (2009) 743–756. <https://doi.org/10.1002/adma.200800534>.
- [7] M. Prabakaran, J.F. Mano, Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials., *Macromol. Biosci.* 6 (2006) 991–1008. <https://doi.org/10.1002/mabi.200600164>.
- [8] R. Jankaew, N. Rodkate, S. Lamlerthton, B. Rutnakornpituk, U. Wichai, G. Ross, M. Rutnakornpituk, “Smart” carboxymethylchitosan hydrogels crosslinked with poly(N-isopropylacrylamide) and poly(acrylic acid) for controlled drug release, *Polym. Test.* 42 (2015) 26–36. <https://doi.org/10.1016/j.polymertesting.2014.12.010>.
- [9] T. Tanaka, D.J. Fillmore, Kinetics of swelling of gels, *J. Chem. Phys.* 70 (1979) 1214–1218. <https://doi.org/10.1063/1.437602>.
- [10] Y. yan Pei, D. mei Guo, Q. da An, Z. yi Xiao, S. ru Zhai, B. Zhai, Hydrogels with diffusion-facilitated porous network for improved adsorption performance, *Korean J. Chem. Eng.* 35 (2018) 2384–2393. <https://doi.org/10.1007/s11814-018-0181-y>.
- [11] C.B. Highley, C.B. Rodell, J.A. Burdick, Direct 3D Printing of Shear-Thinning Hydrogels into Self-Healing Hydrogels, *Adv. Mater.* 27 (2015) 5075–5079. <https://doi.org/10.1002/adma.201501234>.
- [12] S. Nedjari, G. Schlatter, A. Hébraud, Thick electrospun honeycomb scaffolds with controlled pore size, *Mater. Lett.* 142 (2015) 180–183. <https://doi.org/10.1016/j.matlet.2014.11.118>.
- [13] R.Y. Tam, S.A. Fisher, A.E.G. Baker, M.S. Shoichet, Transparent Porous Polysaccharide Cryogels Provide Biochemically Defined, Biomimetic Matrices for Tunable 3D Cell Culture, *Chem. Mater.* 28 (2016) 3762–3770. <https://doi.org/10.1021/acs.chemmater.6b00627>.
- [14] S. Liu, M. Jin, Y. Chen, H. Gao, X. Shi, W. Cheng, L. Ren, Y. Wang, High internal phase emulsions stabilised by supramolecular cellulose nanocrystals and their application as cell-adhesive macroporous hydrogel monoliths, *J. Mater. Chem. B.* 5 (2017) 2671–2678. <https://doi.org/10.1039/c7tb00145b>.
- [15] H.G. Kang, S.B. Lee, Y.M. Lee, Novel preparative method for porous hydrogels using overrun process, *Polym. Int.* 54 (2005) 537–543. <https://doi.org/10.1002/pi.1719>.
- [16] F. Dehghani, N. Annabi, Engineering porous scaffolds using gas-based techniques, *Curr. Opin. Biotechnol.* 22 (2011) 661–666. <https://doi.org/10.1016/j.copbio.2011.04.005>.
- [17] J. El-Ali, P.K. Sorger, K.F. Jensen, Cells on chips, *Nature.* 442 (2006) 403–411. <https://doi.org/10.1038/nature05063>.
- [18] H.J. Jeon, C.G. Simon, G.H. Kim, A mini-review: Cell response to microscale, nanoscale, and hierarchical patterning of surface structure, *J. Biomed. Mater. Res. - Part B Appl. Biomater.* 102 (2014) 1580–1594. <https://doi.org/10.1002/jbm.b.33158>.

- [19] J.Y. Tan, C.K. Chua, K.F. Leong, Fabrication of channeled scaffolds with ordered array of micro-pores through microsphere leaching and indirect Rapid Prototyping technique, *Biomed. Microdevices*. 15 (2013) 83–96. <https://doi.org/10.1007/s10544-012-9690-3>.
- [20] L.M. Bellan, M. Pearsall, D.M. Cropek, R. Langer, A 3D interconnected microchannel network formed in gelatin by sacrificial shellac microfibers, *Adv. Mater.* 24 (2012) 5187–5191. <https://doi.org/10.1002/adma.201200810>.
- [21] J.B. Lee, X. Wang, S. Faley, B. Baer, D.A. Balikov, H.J. Sung, L.M. Bellan, Development of 3D Microvascular Networks Within Gelatin Hydrogels Using Thermoresponsive Sacrificial Microfibers, *Adv. Healthc. Mater.* 5 (2016) 781–785. <https://doi.org/10.1002/adhm.201500792>.
- [22] G. Shamim, D.M. Pandey, R. Ramani, K.K. Sharma, Identification of genes related to resin biosynthesis in the Indian lac insect, *Kerria lacca* (Hemiptera: Tachardiidae), *Int. J. Trop. Insect Sci.* 34 (2014) 149–155. <https://doi.org/10.1017/S1742758414000277>.
- [23] X. Zhang, C.L. Pint, M.H. Lee, B.E. Schubert, A. Jamshidi, K. Takei, H. Ko, A. Gillies, R. Bardhan, J.J. Urban, M. Wu, R. Fearing, A. Javey, Optically- and thermally-responsive programmable materials based on carbon nanotube-hydrogel polymer composites., *Nano Lett.* 11 (2011) 3239–44. <https://doi.org/10.1021/nl201503e>.
- [24] Y.-Q. Wang, Y.-Y. Zhang, X.-G. Wu, X.-W. He, W.-Y. Li, Rapid facile in situ synthesis of the Au/Poly(N-isopropylacrylamide) thermosensitive gels as temperature sensors, *Mater. Lett.* 143 (2015) 326–329. <https://doi.org/10.1016/j.matlet.2014.12.132>.
- [25] S. Bakarich, R. Gorkin, G. Spinks, 4D Printing with Mechanically Robust, Thermally Actuating Hydrogels, *Macromol. Rapid Commun.* 36 (2015) 1211–1217. <http://onlinelibrary.wiley.com/doi/10.1002/marc.201500079/full> (accessed June 25, 2015).
- [26] H. Warren, M. in het Panhuis, G.M. Spinks, D.L. Officer, Thermal actuation of hydrogels from PNIPAm, alginate, and carbon nanofibres, *J. Polym. Sci. Part B Polym. Phys.* 56 (2018) 46–52. <https://doi.org/10.1002/polb.24430>.
- [27] L. Stevens, P. Calvert, G.G. Wallace, M. in het Panhuis, Ionic-covalent entanglement hydrogels from gellan gum, carrageenan and an epoxy-amine, *Soft Matter*. 9 (2013) 3009–3012. <https://doi.org/10.1039/c3sm27413f>.
- [28] S. Naficy, S. Kawakami, S. Sadegholvaad, M. Wakisaka, G.M. Spinks, Mechanical properties of interpenetrating polymer network hydrogels based on hybrid ionically and covalently crosslinked networks, *J. Appl. Polym. Sci.* 130 (2013) 2504–2513. <https://doi.org/10.1002/app.39417>.