EXTRUSION FREEFORM FABRICATION OF BONE-LIKE MINERALIZED HYDROGELS AND MUSCLE-LIKE ACTUATORS

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Abstract

Extrusion freeform fabrication has been used to build shapes from agarose, polyacrylamide and polyacrylic acid hydrogels. Contraction and bending can be induced by pH change or application of a voltage between embedded electrodes. Mineral reinforcement can be induced by incorporating salts into the gels and allowing them to react.

Introduction

The growth of organisms normally occurs as the deposition of new material by a layer of cells at an external or internal surface in an aqueous supporting medium. The layerwise deposition makes the process quite analogous to freeform fabrication. The cells would, however, correspond to a highly parallel multiple head system rather than one with a single moving write head. As with some freeforming processes, the actual deposition is a chemical conversion of liquid to solid and the initial deposit may be later transformed to a harder or tougher structure. With this analogy in mind we have been exploring methods by which materials similar to biological tissues might be freeformed.

We have thus developed methods to make shapes from water-swollen hydrogels using extrusion freeform fabrication and have been exploring the application of this method to the production of synthetic bone and muscle-like actuators.

Extrusion Freeform Fabrication of Hydrogels.

A hydrogel is a cross-linked solution of a water-soluble polymer with properties similar to Jell-o [1]. Agarose is a polysaccharide that is used as an electrophoresis medium for biopolymer separation. A 5% solution in water melts at about 80°C and solidifies rapidly below 60°C to a strong gel. The solidification process is a phase transition to the formation of helical agglomerates of chains. Using our extrusion system, solids can be written using a heated syringe depositing material onto a cooled plate.

Polyacrylamide gels are much used as a medium for electrophoresis of proteins and are also used to retain water for houseplants. In this case a solution of acrylamide monomer and about 5% of methylenebisacrylamide crosslinking agent is polymerized by a free radical system. The solution reacts slowly at room temperature but polymerizes in 5-10 minutes at 60°C. Since the monomer solution is very fluid, it is necessary to add a gelling agent to render the solution pseudo plastic and thus allow the parts to hold their shape while polymerization occurs. This is achieved with the addition of 12 wt.% of fumed silica as a thickening agent.

It is also possible to form gels by writing into a liquid medium. A solution of acrylamide, bisacrylamide, potassium persulfate free radical catalyst and silica is deposited below the surface of an aqueous solution of tetramethylethylenediamine (TEMED) co-catalyst. As the monomer

solution contacts the TEMED solution, the TEMED reacts with the persulfate and initiates the polymerization. To prevent the two liquids from mixing, the TEMED solution is thickened with sodium carboxymethylcellulose. In these circumstances the hydrogel forms in a liquid support medium. It has, however, proved difficult to anchor the hydrogel and stop it drifting during the process. Osmotic pressure differences tend to lead to swelling of the gel so that good resolution is hard to achieve.

Gel muscles

Muscle functions by myosin crawling along an actin track driven by the energy of hydrolysis of ATP which induces a cyclic kinking and straightening of the myosin head group. Like a man running on a slippery floor, the force depends on the rate of reaction with ATP and there is no force in the absence of energy input. The electrical stimulation of excised muscle functions by inciting the cells to release ATP, not by directly powering the contraction.

Gel muscles depend on the contraction of cross-linked ionic gels as they change temperature, pH or solvent [2, 3]. These contractions can be large and the forces exerted are comparable to muscle but the response time is very long because water must diffuse from the gel as it contracts. Bending or contraction can also be induced electrically as the field drives ions into or out of the gel. The effect tends to be weak because it depends on establishing an ion concentration gradient within the gel or between the gel and its surroundings. Steep ion gradients cannot be established at the low fields that can be set up in aqueous media. A second problem with these gels is that they generally function immersed in water, which is incompatible with simple machinery.

We envisage using freeforming methods to make stacks of gels with interdigitated electrodes, both to speed the response and to provide internal reservoirs in which the extruded water can be stored. As a first step toward this, we have formed stacks of six layers of gels using both cross-linked polyacrylic acid and polyacrylamide. These have been tested for their response on transfer from acid to base and to field applied between electrodes placed between layers 2 and 3 and between layers 4 and 5.

Figure 1 shows the swelling response on being moved from acid solution to base of a series of 6-layer gels varying from all acrylamide to all acrylic acid via 1+5, 2+4 etc.. Acrylamide gels respond little to pH changes and acrylic acid gels swell strongly in base. It was expected that the mixed gels would curve very strongly as the acrylic acid side expanded. In fact they curved only slightly and the acrylamide portion of the mixed gels swelled equally in the x,y and z directions, driven by the acrylic acid. Apparently the strong swelling of the acrylic acid gel stretches the acrylamide along x and y. The concurrent swelling along z is interpreted as a Poisson's ratio of -1/2 for the acrylamide gel. Such anomalous Poisson's ratios have been previously found for gels [4, 5].

Electrical stimulation of similar multilayer stacks is being studied. Figure 2 shows the bending response of six layer stacks with one or two outer acrylamide gel layers either side of two or four acrylic acid gel layers. These samples are run in air with electrodes embedded between the outer acrylic acid layers and the acrylamide. We envision the acrylamide layers as providing a

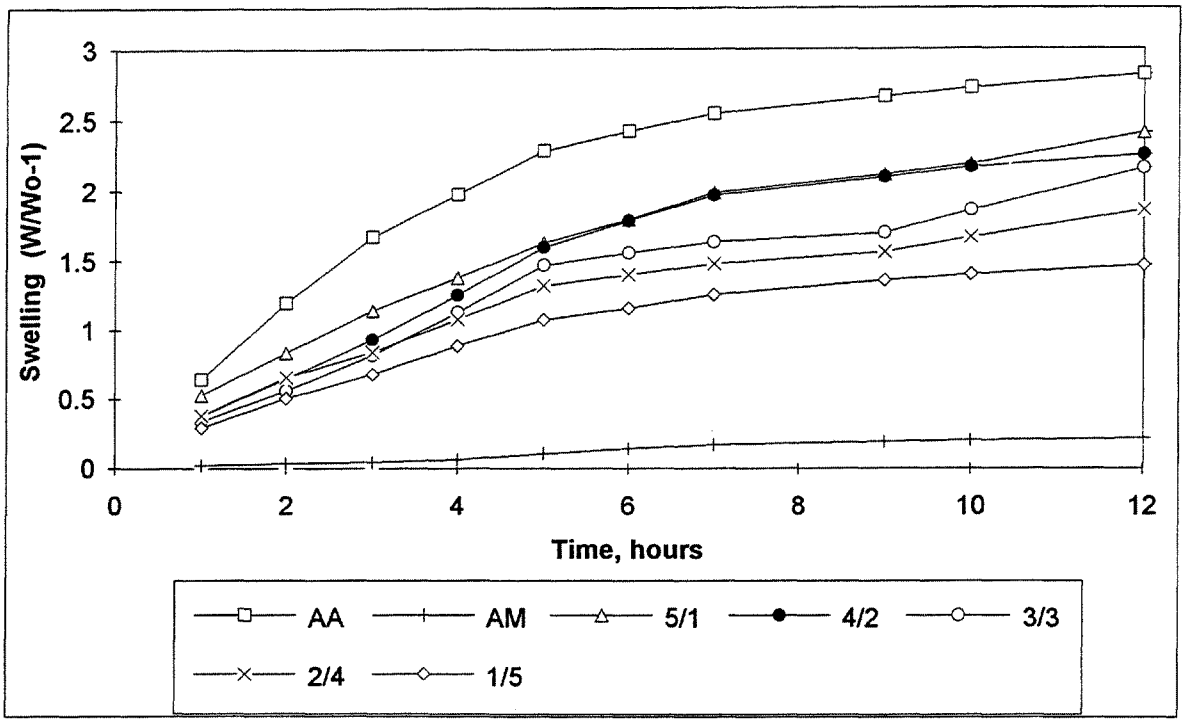

Figure 1) Water absorption by multilayer gel samples soaking in 0.1M NaOH after treatment in 0.1M HCl for 2 days. 6 layers: 0/6, 1/5,2/4,3/3,4/2,5/1,6/0 acrylamide gel/acrylic acid gel.

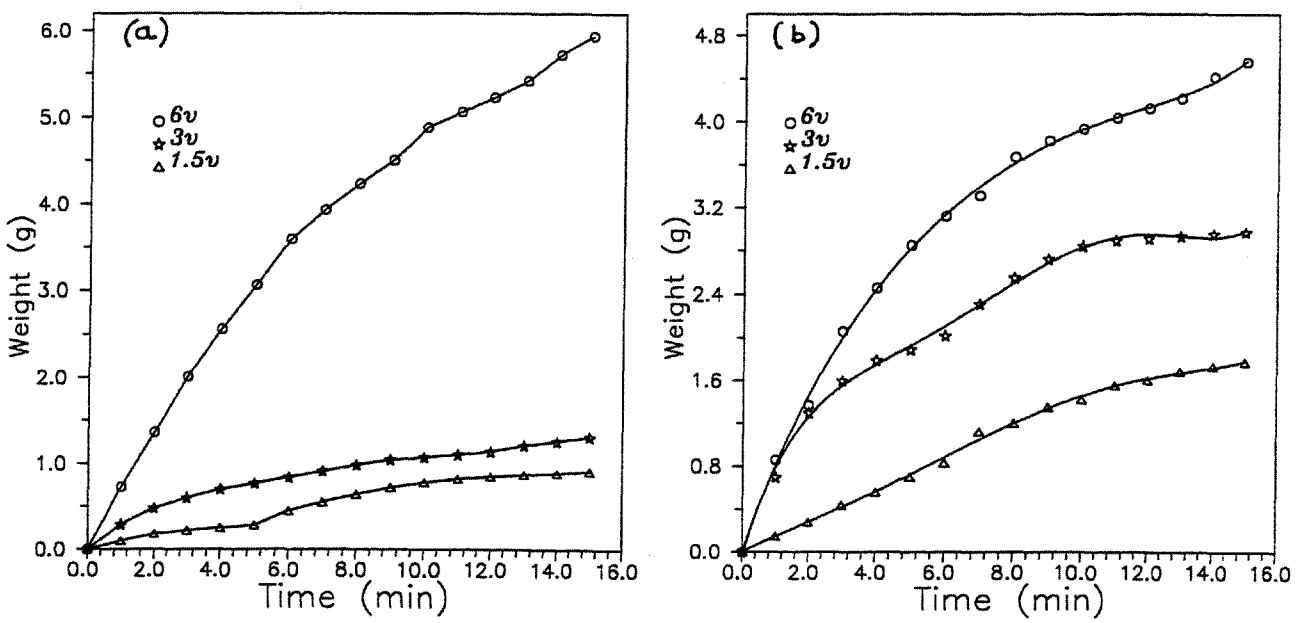

Figure 2 Force exerted by bending a 10 cm. long six layer gels when subjected to 1.5V, 3V or 6V across embedded electrodes. (a) MAAAAM: Acrylamide/electrode/acrylic acid x 4/electrode/acrylamide (b) MMAAMM: Acrylamide x 2/electrode/acrylic acid x 2/electrode/acrylamide x 2

passive reservoir for water and ions driven out of the contracting acrylic acid.

Gel bones

Bone is a composite material with 40-50 vol.% of a mineral, hydroxyapatite, embedded in a tough collagen matrix. Because the mineral grows in situ in the polymer, it is in the form of fine, highly oriented ribbons, which give bone remarkable stiffness and toughness for a particle-reinforced polymer. We hope to freeform similar materials, both for medical applications and as tough synthetic composites.

During puberty, the long bones grow by addition of new material to the "growth plate". This is a soft zone extended across the bone near one end. Here a layer of cells continuously forms new cartilage, which has a soft gel-like structure. As the cartilage thickens away from the cells, it becomes mineralized with hydroxyapatite and converts to bone over a distance of 5-10 microns from the cell front. In this way the bone progressively lengthens [6].

We hope to produce bone-like composites by a similar process of freeforming gel structures and then inducing mineralization. As a first step, agarose gels were freeformed with a high level of calcium chloride dissolved in the gel. These gels are then immersed in carbonate or phosphate solution to induce mineralization. In initial studies have focussed on carbonates because the precipitation chemistry is much simpler than for phosphate. When the carbonate solution is less concentrated than the calcium chloride in the gel, osmotic pressure drives solution into the gel, which mineralizes, as calcite, with little loss of calcium. In this way up to 14 wt.% mineral can be formed within the gel. While this fraction is too low to give a stiff structure, most of the mass of the composite is water and the mineral/polymer ratio is high. On drying, a hard bony structure with up to 70 wt.% mineral is formed, table 1.

To induce internal mineralization, multilayer gels have been formed with calcium and carbonate in alternating layers. Precipitation normally occurs uniformly in the layer with the higher initial ionic strength, rather than at the interface. The mineral content is limited by precipitation of the gel structure at high ionic strengths, especially with divalent anions. We are now reaching higher mineral contents by forming multiple layers containing dispersed powders of slightly soluble minerals, which then reprecipitate as calcium carbonate, leaving a soluble salt.

These studies raise a question that is, so far, unanswered. When bone forms, water is lost during mineralization. There is no obvious physical reason for this because the mineral forms inside the collagen fibers while the water is in a surrounding gel matrix. An understanding of this would help us drive the water out of our gels as they mineralize.

Conclusions

A freeformed gel matrix provides a medium within which subsequent or concurrent chemical processes can be used to make new materials with complex microstructures. There are many parallels between this approach and biological growth processes.

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References

1. Dagani, R., "Intelligent Gels", *Chem. Eng. News* 26-37, June 9, 1997.
2. Osada, Y., Okuzaki, H. and Hori, H., *Nature* 355 , 242-244 , 1992.
3. Gong, J. P., Nitta, T. and Osada, Y. "Electrokinetic modeling of the contraction of polyelectrolyte gels." in *Proceedings of the Second International Conference on Intelligent Materials. ICIM '94 (1994)* (eds. Rogers, C.A. & Wallace, G.G.) (Technomic Publishing Co., Lancaster, PA, 1994) pp.556-564.
4. Li, C., Hu, Z. and Li, Y., "Poisson's ratio in polymer gels near the phase-transition point", *Phys. Rev. E* 48, 603-606, 1993.
5. Takigawa, T., Morino, Y., Urayama, K. and Masuda, T., "Osmotic Poisson's ratio and equilibrium stress of Poly(acrylamide) gels", *Polymer Journal* 28, 1012-1013, 1996.
6. Vaughan, J. A. *The physiology of bone.* 1-265 (Oxford : Clarendon Press, 1981).

Table 1

Calcium carbonate mineralization of agarose gels. Maximum possible carbonate content : 77 wt.% at 2M $CaCl_2$, 62 wt.% at 1M

$CaCl_2$ in gel, M	Na_2CO_3 in solution, M	wt.% $CaCO_3$, dry gel
2	0.5	42
	1	32
	1.5	41
	2	39
	2.5	36
	0.5 $NaHCO_3$	69
	1 $NaHCO_3$	72
1	0.5	46
	1	49
	1.5	54
	2	40
	2.5	30
	0.5 $NaHCO_3$	57
	1 $NaHCO_3$	59