

Thermoresponsive Hydrogel with Embedded Magnetic Nanoparticles for the implementation of Shrinkable Medical Microrobots and for Targeting and Drug Delivery Applications

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Abstract—The paper describes the choice of a thermoresponsive material for the implementation of untethered medical microrobots and other microdevices. These entities can be propelled at a specific location in the body using a Magnetic Resonance Imaging (MRI) system while being actuated for various functions such as the release of drugs by a volume change induced by hyperthermia of nanoparticles embedded in the hydrogel. This paper presents some preliminary results showing that poly(N-isopropylacrylamide) (PNIPA) might be an interesting choice, because of the important volume decrease when temperature is increased of some degrees. Using PNIPA based microparticles could be an interesting choice because, according to our results, volume changes are more important when the volume is smaller, and shrinking rate is higher when there are nanoparticles embedded in the hydrogel.

I. INTRODUCTION

OUR group has been working for many years on a project called MR-Sub (Magnetic Resonance Submarine), which is a new approach that has been validated *in-vivo* [1] to steer and propel magnetic entities including but not limited to drug delivery devices, biosensors or microrobots with a clinical Magnetic Resonance Imaging (MRI) system. Targeting in inaccessible regions of the human body, impossible to reach by Minimally Invasive Surgeries (MIS), could be possible by this technique. An MRI system is composed of three magnetic gradient coils (typically used for image slice selection) that can be used to create a 3D displacement forces on a ferromagnetic core. The forces created depend on the magnetization of the ferromagnetic object, its volume, and the intensity of the magnetic gradients. This approach would allow some minimally invasive diagnostics, therapeutics or interventional applications. It also provides many advantages, such as an external source for actuation, which eliminates the need to integrate it to the microdevice, leading to a much simpler design with potential for further miniaturization. It also

allows an agglomeration of magnetic entities drifted by blood flow to be steered and comply to various blood vessel geometries and sizes, so that highly localized targeting can be achieved. Moreover, MRI systems can already be found in most hospitals, and can be adapted to be used as a propulsion system while maintaining their imaging functionalities [1-3].

But ferromagnetic objects alone are not very useful if they don't have a defined function. Our primary goal is to design a multifunctional microdevice, which could be targeted with a method similar to the one described in [4], where a MRI system has been used to propel, track and control in real-time a simple ferromagnetic sphere in the carotid artery of a living swine. As such, our group is working on integrating functionalities to such MRI-navigable devices and hydrogels could be an interesting material.

Hydrogels possess a tridimensional network with hydrophilic groups, allowing them to absorb large volumes of water, without losing their structure. Hydrogels properties like permeability, mechanical properties, surface properties and biocompatibility are affected by these large amounts of absorbed water, and make them particularly interesting for biomedical applications. They are largely studied and they are already used for applications such as implants, soft contact lenses, scaffolds, matrices for cell and tissue repairs and regenerations, and in drug delivery systems [5].

Moreover, some polymers, called "smart polymers" show sensitivity to external stimuli, like temperature, pH, magnetic fields or ionic strength. The most studied of these smart polymers for drugs delivery applications are the thermoresponsive polymers. These polymers have some hydrophobic groups in their structure, like methyl, ethyl or propyl groups. They all have a transition temperature, which is the temperature where they show a discontinuous phase change.

Thermoresponsive hydrogel can be either positively responsive or negatively responsive. It means that their volume will increase or decrease respectively, with an increase of the temperature. In both cases, there is a transition temperature, at which a discontinuous volume change occurs. In our case, negatively responsive hydrogels are interesting for our initial applications, because of the possibility of liberating some substances while shrinking with an increase of the temperature.

The most studied thermoresponsive polymer is poly(N-isopropylacrylamide), or PNIPA, because its Lower Critical Solution Temperature (LCST) is close to body temperature.

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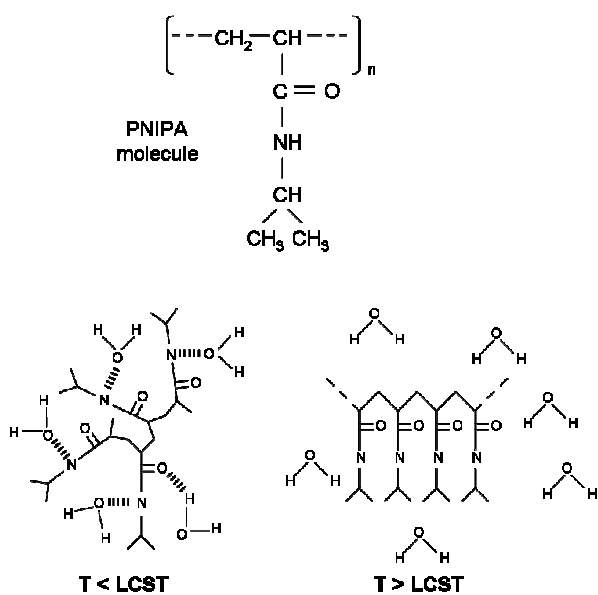


Fig. 1. Configuration change for temperature below and above LCST

PNIPA chains in solution are soluble at low temperatures, and become insoluble when temperature is increased over the LCST. This behavior is contrary to most of the non-polymer material, which normally become soluble when temperature is increased. When PNIPA is synthesized in a hydrogel form, by adding a cross-linking agent during the polymerization process, this behavior becomes a discontinuous volume change when temperature is increased under the LCST. This can be explained by the hydrophilic/hydrophobic balance within the hydrogel. At temperature below LCST, hydrogen bonds between hydrophilic groups of polymer chains are dominant, i.e. increase of water absorption. These bonds become weak and hydrophobic interactions become stronger when temperature is elevated over the LCST. The molecules change configuration, as seen in Figure 1, and water molecules are expelled outside of the hydrogel, which result in shrinkage of the hydrogel. This phenomenon is reversible, and hydrogel return in his swollen state when temperature is lowered under the LCST [6]. Swelling of these hydrogels depends of the interactions between the polymer and the solvent, which is mostly water. It also depends of the elastic forces in the polymer network, which act against the swelling.

Thermoresponsive hydrogels might be particularly interesting if they are used with magnetic nanoparticles, because those are known to produce heat by hysteresis energy loss and eddy-current energy loss, when they are placed in an external time-varying magnetic field [7]. Thus, we can synthesize a microdevice which could be externally actuated by the heat dissipated by these nanoparticles. Some groups are studying the possibility of using hyperthermia directly to heat the cells they want to threat. This is a strategy widely studied, but nanoparticles have to be targeted, and even though they are injected directly in the region of the tumor, there is a risk of damaging healthy tissues with

excessive heat. Another group uses a thermoresponsive material which is liquid at temperatures under 20°C and become a gel at temperatures under 30°C, i.e. immediately when the mixture is injected in the body. They use this material to hold the magnetic nanoparticles in place for the time required for the treatment. But even with this technique, problem of damaging good cells still remains. Furthermore, nanoparticles have to be injected directly where we want them to have an effect, and no specific non-invasive targeting of deeper regions is possible [7].

Instead, we are using nanoparticles to trigger an event, which could be, with the thermoresponsive hydrogel, the release of a substance, by inducing a volume change of the hydrogel when temperature is elevated under a determined transition temperature. This temperature could be under the temperature where cells are damaged, eliminating the problem of damaging healthy tissues.

The main goal of this particular project is to develop a thermo-responsive hydrogel, with nanoparticles embedded. These nanoparticles would allow us to guide and steer hydrogel-based entities with an MRI system, and also to for various types of actuation functions including but not limited to drug liberation, by heating the hydrogel by hyperthermia. This microdevice would allow highly localized drug delivery, eliminating side effects and drug losses anywhere else in the body.

In this study, PNIPA hydrogel have been synthesized by free radical polymerization, with and without nanoparticles, and some tests have been done to get preliminary results of the shrinking as well as the shrinking rate of the different samples.

II. MATERIALS AND METHODS

A. Hydrogel Synthesis

N-isopropylacrylamide (NIPA), N,N'-methylenebisacrylamide, N,N,N',N'-tetramethylenethylenediamine (TEMED) and ammonium persulfate (APS) were all purchased from Sigma-Aldrich Canada and were used as received.

Hydrogel samples were prepared by free radical polymerization, as describe in [8] and in [9], by dissolving 7.5 mmol of the monomers (NIPA), 0.075 mmol of accelerator (TEMED) and 0.075 mmol of cross-linker (BIS) in 10 ml of water. The solution was bubbled with nitrogen for 10 minutes. 1 ml of the solution has been poured in a vial of 1.5 ml capacity, and 0.001 mmol of initiator (APS) has been added to start the polymerization process. Gelation occurred at ambient temperature for 24 h.

For the samples with nanoparticles embedded, monomers, cross-linker and accelerator were dissolved in 6 ml of water instead of 10 ml, and, after bubbling with nitrogen for 10 minutes, 0.6 ml of this solution was added to 0.4 ml of a solution of Fe₃O₄ nanoparticles coated with dextran to get 1 ml of solution. These nanoparticles were coated with dextran and were in solution in water, at a concentration of 50 mg/ml. Gelation took place with the same amount of initiator, and for the same time. At the end, the final volume

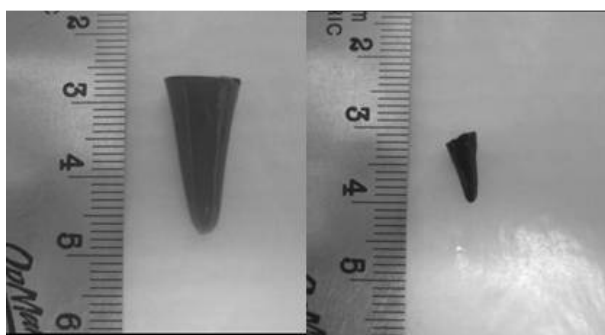


Fig. 2. Illustration of the shrinking of a PNIPA sample with magnetic nanoparticles embedded.

of all the samples were 1 ml, with some samples with nanoparticles, and some with no nanoparticle embedded.

B. Volume changes measurements

Different samples have been incubated in a water bath, at constant temperature higher than the transition temperature (36-37°C) for at least 24 hours. The samples were weighed at regular interval to see how much time each sample took to stabilize and also to measure mass changes for each sample at temperature higher than the LCST. Masses of the sample with nanoparticles embedded were also measured, for each temperature, from ambient to 36°C, to show the behavior of the hydrogel with temperature changes.

III. RESULTS AND DISCUSSION

Figure 2 shows the volume loss obtained with the sample with nanoparticles embedded. The sample has been weighed at room temperature and after 6 hours at a temperature around 36°C. The sample lost more than 90% of its mass. For samples with no nanoparticles embedded, the volume losses could be as high as 88%.

Figure 3 shows the curve of volume changes of two samples, with initial masses of 0.45g and 0.07g. The curve show the mass divided by the initial mass, at regular interval of time. Both samples were at the same temperature, around 35°C ($\pm 1^\circ\text{C}$) for two days. After that, masses didn't change significantly. These results show that volume changes are more important when volumes are smaller. We can explain that by the skin layer effect. When temperature is increased, polymer molecules change configuration, and water is expelled outside of the hydrogel. Water on the surface is expelled more rapidly, so a denser skin layer is formed, and

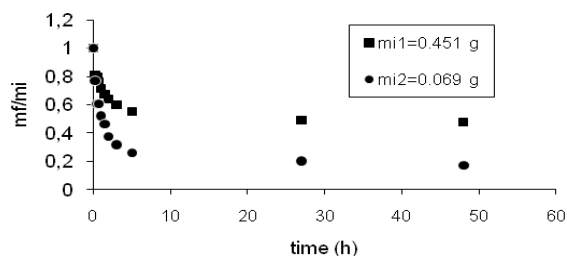


Fig. 3. Mass loss rate for 2 samples, with 2 different initial masses

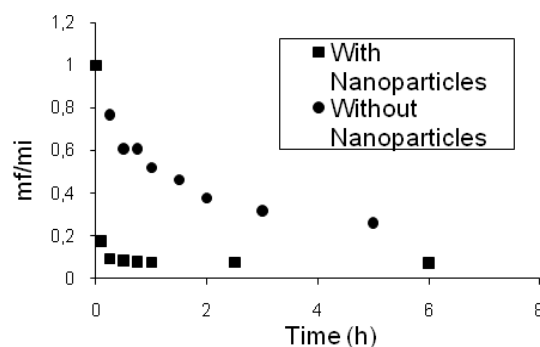


Fig. 4. Mass loss rate for 2 samples, with and without nanoparticles embedded

blocks water molecules from the middle of the hydrogel to go outside. For smaller volume, the surface/volume ratio is smaller, so water can be evacuated more effectively. That could explain why volume change is more important for smaller hydrogel volume [10].

Response times of two samples, one with nanoparticles embedded, and the other without nanoparticles are shown on Figure 4. The curves show that it takes about half an hour for hydrogel with nanoparticles embedded to stabilize, while stabilization takes more than 24 hours for the sample without nanoparticles. Thus, samples with nanoparticles embedded have by far shorter stabilization time. This phenomenon has been explain in [11] by the fact that the internal structure and the surface properties of the hydrogel is changed by nanoparticles, and there is no surface layer formation as in pure PNIPA hydrogel.

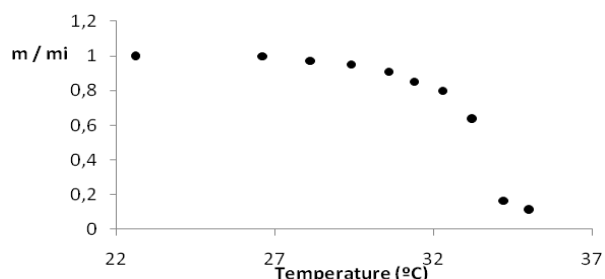


Fig. 5. Volume change of the hydrogel with nanoparticles embedded, for temperatures from ambient to 36°C

Finally, the behavior of the hydrogel when temperature is elevated is illustrated by Figure 5. Hydrogel with nanoparticles embedded have been allowed to stabilize at each temperature for 15 minutes. As seen on Figure 4, there is no significant volume change after 15 minutes, so it is enough time for the sample to stabilize. We can clearly see the discontinuous volume change that occurs around 34°C.

As mentioned earlier, our goal is to design hydrogel microparticles with magnetic nanoparticles embedded. These results are interesting because they gave us acceptable volume changes and response rates for our application. So even if these tests have been done with samples of 0.5 to 1 cm³, we can expect to get better results, i.e. larger volume changes and faster response rates with smaller particles.

IV. CONCLUSION

We showed that response time of hydrogel with embedded nanoparticles is higher than response time of pure PNIPA. We also showed that relative volume changes are larger for smaller volumes. These preliminary results showed that PNIPA is an interesting material for the application, and are positive for further experiments using smaller particles with nanoparticles embedded. We are going to have time response small enough for our application. Moreover, volume changes are going to be relatively important and they could lead to more versatile applications. The results obtained encouraged us to continue our investigation of this material for this application.

The next step would be to set the transition temperature to a temperature higher than the body temperature. Otherwise, the volume change will occur as soon as the hydrogel will be injected in the body. The LCST will also have to be lower than the temperature at which cells are damaged. So we need to get a maximum volume change, in an interval of temperature as small as 4 or 5 degrees, between 37.5°C and 43°C. This transition temperature will allow us to control exactly the moment at which we want the volume to change. A way to modify the LCST would be to copolymerize it with a specific concentration the PNIPA with acrylic acid, a hydrophilic monomer. This will modify the hydrophilic-hydrophobic balance and the LCST will become higher [12], [13]. We also have to find a way to synthesize the hydrogel in microparticles, with size as uniform as possible, with right relative concentration of monomers, to get the right transition temperature and with the right magnetic nanoparticles concentration to be able to reach this transition temperature in a time interval relatively small and to be able to target the microdevice effectively with the magnetic gradient intensity we can get with our MRI system.

Development technique would lead to a new hydrogel-based multifunctional devices and microrobots. With our MRI-based propulsion and steering approach, being part of the MR-Sub project, it will be possible to target these hydrogel-based entities effectively, providing a new therapeutic tool against cancer tumor.

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