Rapid Screening of the Lower Critical Solution Temperature of Injectable Hydrogels for Tissue Engineering Applications

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Introduction

The thermo-responsive behavior of N-isopropylacrylamide (NIPAAm) hydrogels allows us to create lightly crosslinked injectable biomaterials that collapse after reaching body temperature, releasing large amounts of water to form stiff polymer scaffolds that support tissue formation.[1,2] In this screening study, we are examining the effect of a wide array of potentially biodegradable crosslinking monomers on the lower critical solution temperature (LCST) of NIPAAm hydrogels, a unique and critical property of this material. Subsequent experiments will investigate other critical hydrogel properties such as injectability, viscoelasticity, biocompatibility and biodegradability.

Experimental*

Several novel crosslinkers were used to prepare the hydrogels, including those in Figure 1 (whose synthesis has been recently described[3]) and *N,N'*-methylene bisacrylamide (as a control). Solutions of NIPAAm/ammonium persulfate in deionized water were added to a 96 well glass microplate containing the crosslinkers (serially diluted to create a range of concentrations) and *N,N,N',N'*-tetramethylene diamine (TEMED). The plate was covered and allowed to react at room temperature for 24 h.

The microplate containing the hydrogels was then placed on a specially modified hotplate to control heating. At specific time intervals, the temperatures of the microplate were recorded and the microplate was placed in a Wallac Victor^2 1420 multilabel counter. The UV transmission value at 490 nm was then recorded. The LCST was defined as the temperature at which the transmission at 490 nm is equal to 0.5.

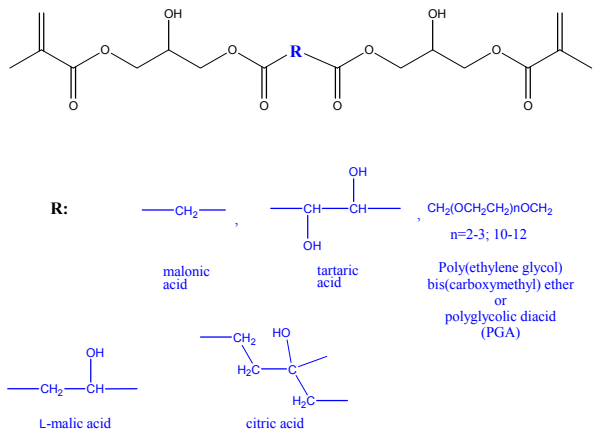

Figure 1. Synthesized crosslinkers used to created temperature-sensitive hydrogels.

Results and Discussion

Figure 2 illustrates the calculated LCST of the various hydrogels. The molar ratio of NIPAAm to crosslinker was varied from 10:1 to 2560:1. For all crosslinkers studied, the LCST was found to be between 28 °C and 31 °C, with little difference in temperature when altering crosslinker structure and/or concentration. Although the hydrophilic-hydrophobic balance has a crucial effect on the LCST of hydrogels, the concentration of crosslinker in the systems studied creates such a loosely crosslinked network that changes in the hydrophilicity of the crosslinker have a negligible effect on the LCST. Although preliminary indications are that there is little effect on phase transition temperatures of the hydrogels when changing the type or concentration of crosslinker, there appears to be significant effect on the time required for gel collapse and subsequent release of water. Even at these low concentrations, the gels created from the more hydrophilic crosslinkers appear to have a decreased water release rate, while the gels with more hydrophobic crosslinkers exhibit a relative increase in water release rate. Ongoing studies of injectability, viscoelasticity (at body temperature), biodegradability and biocompatibility are in progress.

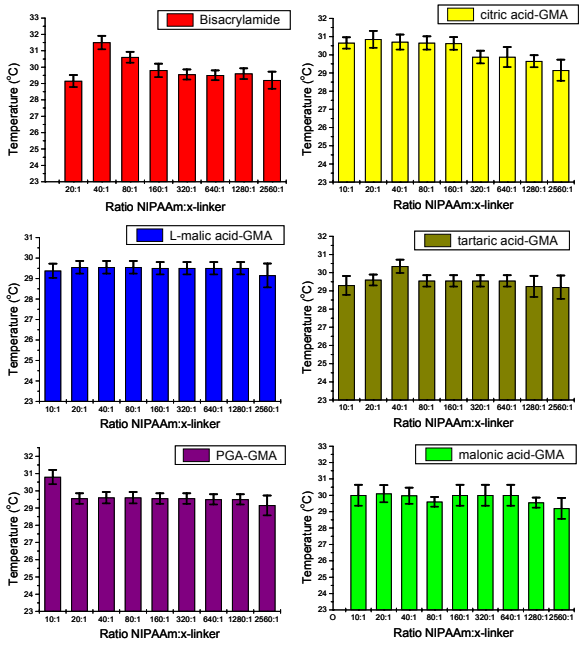

Figure 2. LCST of NIPAAm hydrogels based on UV transmission at 490 nm as a function of type of crosslinker and crosslinker concentration. Error bars in the graph represent the best estimate of two standard deviations in the experimental uncertainty.

Conclusions

An efficient method to screen a large number of hydrogels based on their LCST has been developed. Because of the relatively low crosslinker concentration there was little change in the LCST of any of the hydrogels. However, this preliminary screening does indicate that all hydrogels studied are within an acceptable temperature range for use as injectable polymeric scaffolds in tissue engineering. Additionally, gel collapse and water release rate appeared to be related to the hydrophilicity of the crosslinker.

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References

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[3] Weir, M.D.; Khatri, C.A.; Antonucci J.M. *ACS Polymer Preprints* **2001**, *42(2),* 131.

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