Injectable Temperature-Sensitive Hydrogels for Cartilage Tissue Engineering

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Statement of Purpose: Hydrogels have been recognized as promising cell carriers for tissue engineered cartilage repair. Specifically, temperature-sensitive gels, such as methylcellulose (MC), are advantageous as gelation occurs upon environmental temperature change without involving chemical reactions. By mixing MC with hyaluronic acid (HA), a major component of natural cartilage, the new material (MCHA) became more biomimetic and faster gelling [1]. In this study, we hypothesized that hydrogels based on MCHA could serve as injectable and *in situ* gelable scaffold for delivering chondrocytes for tissue engineered cartilage repair. We designed different formulations of MCHA-based hydrogels by incorporating cell adhesive molecules, i.e., gelatin, collagen I, and collagen II, to optimize the gelation time, mechanical properties, cell viability and proliferation ability.

Methods: Chondrocytes were isolated from knee joints of young equines and expanded in tissue culture flasks in DMEM-F12 medium supplemented with 10% fetal bovine serum, 1x antibiotic-antimycotic, and 50µg/ml vitamin C. When 100% confluence was reached, the cells were trypsinized and washed for further use in other tests.

HA (MW ~2MDa) and MC materials were dissolved in Dulbecco's Phosphate Buffered Saline (DPBS) at designed concentration by stirring and heating. The solution was immediately cooled down and stored at 4°C. Designed amounts of sterile gelatin, collagen I, and/or collagen II were added and mixed well. The four formulations are tabulated in Table 1. To encapsulate chondrocytes, defined volume of 4°C gel solution was added to a pellet of a known number of cells to suspend the cells, reaching density of 5million/ml. The cell suspension was added to a tissue culture plate and incubated at 37°C in a humidified environment with 5% CO_2 for 1 hour to form hydrogels. After incubation, warm medium was added on top to continue the culture.

Table 1. *Different formulations MCHA-based hydrogels*

Formulation ID	Components in 400 ml solution				
	MC (g)	HA (g)	Gelatin (mg)	Collagen I (mg)	Collagen II (mg)
MCHA	32	2			
MCHA-Col I	32	2		100	
MCHA-Gelatin	32	2	400		
MCHA-Gelatin-Col II	32	2	400		10

The rheological properties of the hydrogels were evaluated on a rheometer equipped with 25mm parallel plates. Storage Modulus G' and Loss Modulus G" were recorded at 1% strain, within the frequency range of 0.1 to 100 s^{-1}, and Complex Modulus G* at 10 s^{-1} were compared among the samples at 37°C. The rheometer was also used to determine the gelation times of the hydrogel formulations at 35°C. Gelation onset time is defined as the time point when G' becomes dominant over G"; while complete gelation time is when G' and G" reach plateaus. Viability of the cells encapsulated in the hydrogel was evaluated by using Live/Dead® cytotoxicity staining kit.

Results/Discussion: Gelation onset times and complete gelation timse of the four MCHA formulations, as well as 8% MC alone, were compared in Table 2. It shows that the incorporation of HA into MC gel indeed expedited the gelation process; and the introduction of cell adhesive molecules further shortened the gelation times.

Table 2*. Gelatin times determined by rheological tests*

	Gelation Onset Time (min)	Complete Gelation time(min)
8% MC	7	48
MCHA	4.75	30
MCHA-Col I	3.25	31
MC-Gelatin	4.25	32
MCHA-Gelatin-Col II	1.5	21

Figure 1 shows the mechanical properties of the blank hydrogels incubated in 37° DPBS at different time points. While all the formulations showed decreased mechanical strength over time due to degradation, the two gelatin-containing hydrogels seemed to have higher strength than the other two formulations. After the cell-encapsulated hydrogels had been cultured *in vitro* for seven days, the cell viability was evaluated with Live/Dead® staining method. The majority of the chondrocytes remained viable in all the hydrogels, though MCHA-Gelatin-Col II gel (Fig. 2) showed the best cell viability. At Day7, cell clusters were seen in MCHA-Gelatin-Col II gel (Fig. 2), which did not occur in the other three formulations.

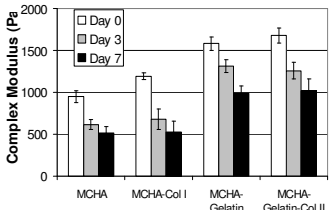

Figure 1. Mechanical strength of hydrogels incubated in DPBS at 37°C.

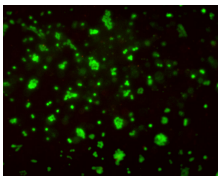

Figure 2. Chondrocytes in MCHA-Gelatin-Col II gel maintained high viability at Day7 of *in vitro* culture.

Conclusions: In this study, to develop an injectable and *in situ* gelable scaffold as a potential cell carrier for cartilage tissue engineering, the temperature-sensitive MCHA hydrogel was modified into different formulations by incorporating cell adhesive molecules. Results suggested that gelation behavior and mechanical properties of the hydrogels could be readily optimized. In addition, by choosing appropriate cell adhesive molecules to mimic the ECM of natural cartilage tissue, cell viability and proliferation could also be enhanced. Among the four formulations studied, MCHA-Gelatin-Col II was the best candidate for further detailed evaluations.

Reference: Gupta, D., et al., *Biomaterials*, 2006, 27, 2730.

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