Injectable Hydrogel을 이용한 인공 Nucleus Pulposus의 제조

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Injectable Hydrogel as an Artificial Nucleus Pulposus in a Degenerative Intervertebral Disc

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1. Introduction

The intervertebral disc is a composite structure made up of the nucleus pulposus (NP) core surrounded by the multi-layered fibers of the annulus fibrosis (AF)[1]. Water is drawn into the NP by the presence of hydrophilic proteins called proteoglycans [2]. The AF, with successive layers oriented in alternating directions, surrounds the NP. These layers are placed under tension as the NP absorbs water and swells [3]. Superiorly and inferiorly are two thin layers of vertebral cartilage end-plates. When the load on the disc is increased and kept at a high level, the pressure within the NP will also increase and it will cause the water in the NP to be squeezed into the adjacent end-plates and removed by the vertebral capillaries. Beginning at approximately 30 years of age, there is a gradual change in the types of proteoglycans and a loss of overall water content [4,5]. As the NP dehydrates and shrinks, the load on the AF increases [6]. The multi-layered structure of the AF is susceptible to delamination and demage when it is flat. Radical tears, cracks, and fissures occur first within the AF [7]. The NP may migrate from the center of the disc to the periphery through the tear. This expansion of the NP within and between the fibers of the AF causes stretching and delamination of the annulus layers and results in back pain by stimulation of the sinu-vertebral nerve. The main purpose of this study is to replace the degenerative NP with a polymeric hydrogel which can be formed by the *in situ* process in the body without surgery. Thus, the hydrogel should be injectable, not implantable. Besides, the hydrogel should be biocompatible, and have high affinity to water and proper mechanical strength. In this study, poly(hydroxyethyl methacrylate) (PHEMA) hydrogel were prepared using redox initiation system to

satisfy such complex requirements. Polymerization behavior, physical properties, and biocompatibility were checked to verify the possible utility of the hydrogel as an artificial NP.

2. Synthesis and Properties of Hydrogels

2.1. Materials

Hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were purified by distillation under vacuum. Crosslinking agent, ethylene glycol dimethacrylate (EGDMA) was purified by filtration through an inhibitor-removing column purchased from Aldrich. Sodium methacrylate (SMA), another crosslinking agent, methylene bisacrylamide (MBAAm), and redox initiators, potassium persulfate (KPS) and *N,N,N,N*-tetramethyethylenediamine (TMEDA) were used without further purification.

2.2. Synthesis of hydrogels

In this study, for the *in situ* formation of hydrogel in an intervertebral disc, KPS and TMEDA were used as redox initiators. After the redox initiators were added to the mixtures of monomers, it was filtered promptly through a sterile filter to remove bacteria and polymerized at 36.5 ℃ for 24 hr to produce PHEMA or poly(hydroxyethyl methacrylate-co-sodium methacrylate) (P(HEMA-co-SMA)) or poly(hydroxyethyl methacrylate-co-sodium methacrylate-co-methyl methacrylate) (P(HEMA-co-SMA-co-MMA)) hydrogels.

2.3. Polymerization behaviors

As shown in Figure 1, the solutions turned to gels at 36.5 ℃ within 30 min, indicating this system is suitable for the *in situ* gelation.

2.4. Water content and mechanical properties of hydrogels

To increase the water content, hydrophilic monomer, SMA was incorporated by copolymerization. Figure 2 denotes the effect of copolymerization on the water content. By addition of the small amount of SMA, the water content of P(HEMA-co-SMA) hydrogel increased up to 84.9%. In case of P(HEMA-co-SMA-co-MMA) hydrogel, the water content is lowered a little to 83.5%.

To check the mechanical property of hydrogel, compressive modulus was measured. Compressive modulus was enhanced by incorporating comonomers as shown in Figure 3. Although P(HEMA-co-SMA-co-MMA) hydrogel revealed the superior mechanical properties, P(HEMA-co-SMA) hydrogel was adopted as an injectable material as an artificial nucleus in intervertebral disc. This is based on the fact that the gelation speed of P(HEMA-co-SMA-co-MMA) hydrogel is so high as to cause some difficulties in injection during surgery and the compressive

modulus of P(HEMA-co-SMA) hydrogel is higher than that of the healthy NP(5.0-97.5 kPa) estimated by indentation tests [8].

3. Injection of Hydrogel to a Degenerative Disc of Rabbit

3.1. Degeneration of disc

In this study, 24 skeletally matured female New Zealand white rabbits were used. In order to obtain a degenerative model, a transverse stab incision was made into the disc through the anterolateral annulus in L4/5 disc. 4 months later after the operation, the lumbar spines of rabbits were checked by x-ray and MRI for the detection of the disc degeneration.

3.2. Injection of hydrogel and harvest of disc

100 $\mu\ell$ of 0.9% normal saline solution was injected into the degenerated NP level of control group and 100-200 $\mu\ell$ of hydrogel was injected to that of study group. 1 week and 1 month later after the injection, the discs were harvested. The NP in a disc of control group grew scanty and fibrous as degeneration proceeded. On the contrary, it is clearly identified that the core of a disc of study group was filled with hydrogel, showing a closer appearance to the normal disc.

Table 1 shows the water content of nucleus pulposus. In progress of degeneration, the water content fell down. When the hydrogel was injected, the water content was less decreased because the hydrogel helped a disc contain a certain amount of water even if the NP lost water-containing ability by degeneration.

The histological study was conducted after to check the morphological change and the inflammatory evidence in the gross specimen of the disc. Figure 4 shows the H&E stained NP. There is no finding about inflammatory reaction in Figure 4b, suggesting that P(HEMA-co-SMA) hydrogel, formed by the *in situ* process, is compatible enough to avoid an acute infection in a disc.

4. References

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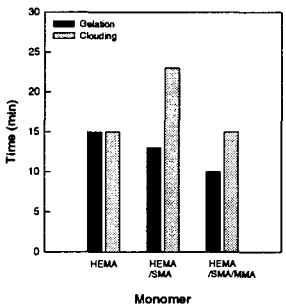

Figure 1. Gelation and clouding times of PHEMA, P(HEMA-co-SMA), and P(HEMA-co-SMA-co-MMA) hydrogels. [HEMA] = 3.5 mol/L_{water}; [SMA] = 0.175 mol/L_{water}; [MMA] = 0.1 mol/L_{water}; [KPS] = 2 x 10^{-3} mol/$mol_{monomer}$; [TMEDA] = 2 x 10^{-3} mol/$mol_{monomer}$; [EGDMA] = 1 x 10^{-2} mol/$mol_{monomer}$.

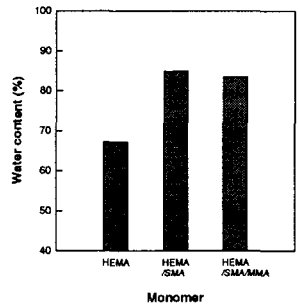

Figure 2. Water content of PHEMA, P(HEMA-co-SMA), and P(HEMA-co-SMA-co-MMA) hydrogels. [HEMA] = 3.5 mol/L_{water}; [SMA] = 0.175 mol/L_{water}; [MMA] = 0.1 mol/L_{water}; [KPS] = 2 x 10^{-3} mol/$mol_{monomer}$; [TMEDA] = 2 x 10^{-3} mol/$mol_{monomer}$; [crosslinker] = 1 x 10^{-2} mol/$mol_{monomer}$.

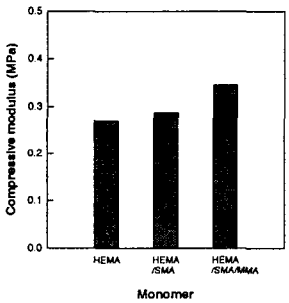

Figure 3. Compressive moduli of PHEMA, P(HEMA-co-SMA), and P(HEMA-co-SMA-co-MMA) hydrogels. [HEMA] = 3.5 mol/L_{water}; [SMA] = 0.175 mol/L_{water}; [MMA] = 0.1 mol/L_{water}; [KPS] = 2 x 10^{-3} mol/$mol_{monomer}$; [TMEDA] = 2 x 10^{-3} mol/$mol_{monomer}$; [EGDMA] = 1 x 10^{-2} mol/$mol_{monomer}$.

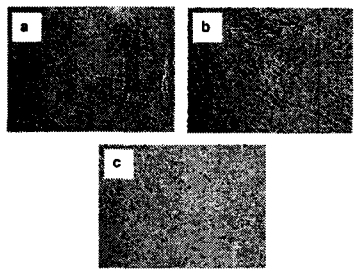

Figure 4. H&E stained nucleus pulposus in rabbits lumbar disc: a, control (1 month); b, hydrogel-injected (1 month); c, normal.

Table 1. Water content (%) of nucleus pulposus in rabbits lumbar disc

Normal	Control		Hydrogel-injected	
	1 week	1 month	1 week	1 month
72.19	77.60	66.29	75.21	72.12