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Materials Science and Engineering C 28 (2008) 270–273
MATERIALS SCIENCE & ENGINEERING C
www.elsevier.com/locate/msec

The evaluation of collagen gel with various connection states by using MRI

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Received 10 October 2005; accepted 12 October 2006
Available online 24 January 2007

Abstract

To noninvasively evaluate the connection states of collagen fiber, a characterizing factor of the physical property, is considered to be helpful in the evaluation of cartilage functions. The purpose of this study was to examine how the connection states of collagen influence the MRI parameters by evaluating the collagen gel with various connection states using MRI. MRI was performed to six type I collagen gel samples with various connection status and a water sample. The evaluation parameters included T1 relaxation time, T2 relaxation time, and diffusion coefficient. With regard to gel samples with cross-links, the T2 relaxation time was shortened in proportion to the dose of glutaraldehyde. It is considered that as the glutaraldehyde concentration increases, the distance between protons in water molecules decreases; this is followed by a stronger bipole–bipole interaction, resulting in a shorter T2 relaxation time. The diffusion coefficient for gel samples with cross-links also decreased with increasing glutaraldehyde concentrations. However, gel samples without glutaraldehyde were almost the same as that of the water. This result suggested that the degree of entrapment of water inside the gel samples without cross-links, even when it converted into gel, was found to be nearly equal to that of the free water.

Keywords: Magnetic Resonance Imaging; Collagen gel; cross-link; T1 relaxation time; T2 relaxation time; Diffusion coefficient

1. Introduction

The major components of the extracellular matrix of an articular cartilage are collagen fibers and proteoglycans. Inside cartilaginous tissues, a large amount of water is retained by hydrated proteoglycans located in the gaps of strong collagen fiber networks. The strong collagen networks provide kinetic support to the cartilaginous tissues, and their entrapments of proteoglycans containing a large amount of water establish the physical and functional properties of articular cartilages, such as anti-extensibility and anti-compressibility. The collagen fibers maintain their network structures by forming cross-links [1–3]. It is known that when the collagen networks begin to collapse such as degeneration, proteoglycans increase their degree of freedom and retained water content, causing a change in the cartilage viscoelasticity.

With regard to magnetic resonance imaging (MRI), different types of imaging procedures have been developed until now;

Table 1
Composition of the samples

Sample	0.5%type‡Tcollagen (ml)	PBS (ml)	Glutaraldehyde (ml)	Water (ml)	Total (ml)
1)	12	1.5	1.5	0	15
2)	12	1.5	0.75	0.75	15
3)	12	1.5	0.15	1.35	15
4)	12	1.5	0.075	1.425	15
5)	12	1.5	0	1.5	15
6)	12	0.75	0	2.25	15
7)	0	0	0	20	20

1)–4): collagen samples with cross-links.
5), 6): collagen samples without cross-links.
7): Ultrapure water.

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0928-4931/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.msec.2006.10.012

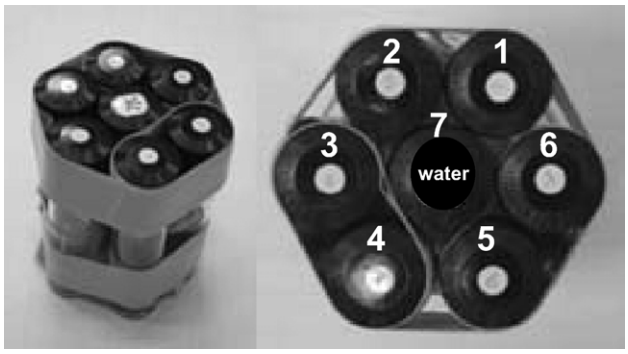

Fig. 1. Collagen gel samples and ultrapure water sample for MR imaging. Each sample 1)–7) in the test tube with the screw entrance were grouped for MR imaging.

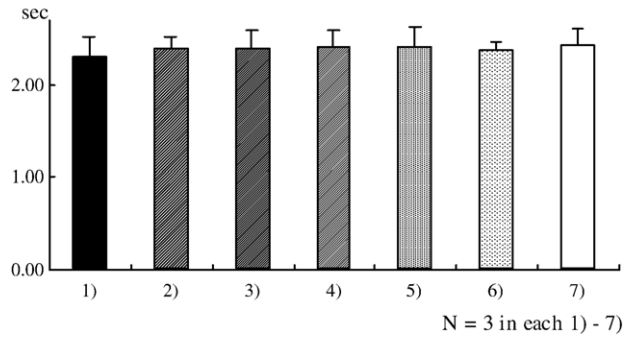

Fig. 2. T1 relaxation time for gel samples and ultrapure water. There are no significant difference between each gel samples and the ultrapure water.

these imaging procedures enable a more detailed morphological observation of articular cartilages, showing the laminar structures by T2 weighted images [4–7] and changes in the T1 relaxation time and diffusion coefficient due to the decrease in proteoglycans [8]. However, the evaluation of the connection states of collagen has not yet been reported. To noninvasively evaluate the connection states of collagen fiber, a characterizing factor of the physical property is considered to be helpful in the evaluation of cartilage functions.

The purpose of this study was to evaluate collagen gel in various connection states with MRI and to examine how the connection states of collagen influence the MRI parameters.

2. Materials and methods

2.1. Materials

Three sets of seven gel and water samples 1)–7) were used in this study (Table 1) (Fig. 1). Gel samples 1)–4) with cross-links were prepared using 0.5% of pepsin-soluble type I collagen prepared from bovine skin, phosphate buffered saline (PBS), glutaraldehyde, and water. Gel samples 5) and 6) without cross-links were prepared using collagen, PBS, and water. The sample 7) comprised degassed ultrapure water. Gel samples 1)–4) differed in the number of cross-links with four increased glutaraldehyde contents, and gel samples 5) and 6) were prepared by changing the PBS amount. The collagen concentration in each gel sample remained constant at 0.4%.

Table 2
Results of T1, T2 relaxation time and diffusion coefficient

Sample	T1 relaxation time s (mean/SD)	T2 relaxation time s (mean/SD)	Diffusion coefficient $\times 10^{-3}$ mm^2/s
1)	2.30/0.22	0.91/0.02 [a]	1.90/0.05
2)	2.38/0.13	0.99/0.06 [a]	1.97/0.05
3)	2.39/0.19	1.37/0.31 [a,*,**]	2.06/0.07*,**
4)	2.40/0.18	1.58/0.12 [a,*,**]	2.09/0.06*,**
5)	2.40/0.22	1.57/0.10 [a,*,**]	2.12/0.04*,**
6)	2.38/0.08	1.55/0.22 [a,*,**]	2.12/0.03*,**
7)	2.43/0.18	2.05/0.11	2.12/0.03*,**

**Significantly different from gel sample 1) ($p<0.05$).
***Significantly different from gel sample 2) ($p<0.05$).
[a] Significantly different from ultrapure water 7) ($p<0.05$).

2.2. MRI

MR imaging was performed with 2.0T superconducting magnet (Biospec 20/30 system; Bruker Inc., Karlsruhe, Germany) and a 72-mm birdcage coil. ParaVision (Bruker Inc., Karlsruhe, Germany) software was employed in this study. The evaluation parameters included T1 relaxation time, T2 relaxation time, and diffusion coefficient. The saturation recovery method was employed for calculating the T1 relaxation time. Seventeen phases of repetition time (TR), namely, 100, 150, 200, 250, 300, 350, 400, 500, 750, 1000, 1500, 2000, 3000, 5000, 7500, 10,000, and 15,000 ms, were set along with an echo time (TE) of 15 ms and a matrix of 128 × 128. To measure the T2 relaxation time, imaging was performed using multi-spin echo sequence with a TR of 15,000 ms; thirty phases of TE, namely, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, 285, 300, 315, 330, 345, 360, 375, 390, 405, 420, 435, and 450 ms; and a matrix of 64 × 64. Conventional diffusion weighted spin echo sequence was performed for calculating the diffusion coefficient. Since the water diffusion phenomenon in the samples were isotropic self-diffusion,

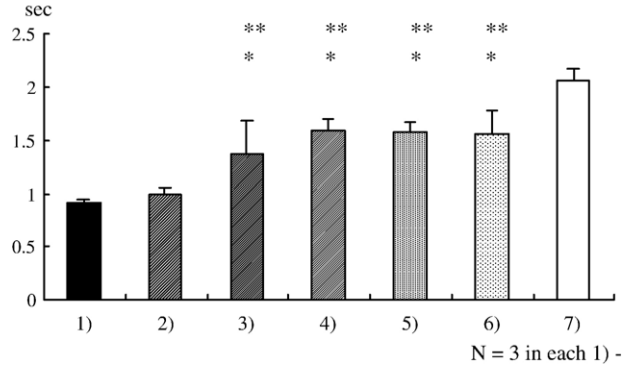

Fig. 3. T2 relaxation time for gel samples and ultrapure water. Significant difference between gel samples 1) – 6) and 7) is indicated ($p<0.05$). *Significantly different from sample 1) ($p<0.05$). **Significantly different from sample 2) ($p<0.05$).