Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 3105–3111
Biomaterials
www.elsevier.com/locate/biomaterials

Poly(*N*-isopropylacrylamide) (PNIPAM)-grafted gelatin hydrogel surfaces: interrelationship between microscopic structure and mechanical property of surface regions and cell adhesiveness

Shoji Ohya[a,*], Satoru Kidoaki[b], Takehisa Matsuda[b]

[a]*Department of Bioengineering, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan*
[b]*Division of Biomedical Engineering, Graduate School of Medicine, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan*

Received 24 May 2004; accepted 23 August 2004
Available online 30 September 2004

Abstract

Poly(*N*-isopropylacrylamide)-grafted gelatin (PNIPAM-gelatin) serves as a temperature-induced scaffold at physiological temperature. This study was aimed at determining the effect of the graft architecture of thermoresponsive PNIPAM-gelatin on the surface topography and elastic modulus of the hydrogels prepared with different architectured PNIPAM-gelatins: the surface topography and elastic modulus were determined by atomic force microscopy (AFM). PNIPAM-gelatin surfaces showed an irregularly concavo-convex structure with a vertical interval of approximately 1 μm regardless of the weight ratio of PNIPAM to gelatin (P/G: 5.8, 12, and 18). The elastic moduli of hydrogels varied at measured sites. The mean elastic moduli of PNIPAM-gelatin with the lowest P/G were low, but increased with increasing P/G. Human umbilical vein endothelial cells adhered and spread on PNIPAM-gelatin hydrogels with the highest P/G, whereas reduced adhesion and nonspreading, round-shaped cells resided on the hydrogels with lower P/Gs. Interrelationship between elastic modulus and cell adhesion and spreading potentials were discussed from physicochemical and cellular biomechanical viewpoints.

Keywords: Poly(*N*-isopropylacrylamide)-grafted gelatin; Atomic force microscopy; Microscopic structure; Mechanical property; Cell adhesiveness

1. Introduction

Tissue engineering has recently been proposed as a promising therapeutic discipline for repairing or to replace diseased or lost tissues; moreover, some engineered tissues have been used for clinical applications [1–4]. Such tissues are fabricated ex vivo or in vivo with or without using synthetic or biologically derived macromolecules so as to provide an appropriate extracellular milieu. To reconstruct a functional tissue, the micro-extracellular environment for incorporated cells is essential. The extracellular space should be incorporated with a cell adhesion matrix and/or structural platform, and interconnected (microscopic) voids in order to facilitate a supply of oxygen and nutrients to cells, cell migration and tissue ingrowth.

We recently prepared a thermoresponsive artificial extracellular matrix (ECM), poly(*N*-isopropylacrylamide)-grafted gelatin (PNIPAM-gelatin) [5–8], which is prepared by quasi-living radical graft polymerization initiated from a gelatin molecule [9,10], and evaluated how the viability and proliferation of cells entrapped in a three-dimensional (3D) hydrogel depended on the graft architecture, including graft chain density and graft chain length of PNIPAM-gelatin [8]. As a rough approximation, bovine smooth muscle cells proliferated well in hydrogels prepared using PNIPAM-gelatin with a high weight ratio of PNIPAM to gelatin (P/G).

In this study, we investigated how cell adhesion on a hydrogel surface is influenced by the graft architecture

*Corresponding author. Tel.: +81-6-6833-5012; fax: +81-6-6872-8090.
E-mail address: ohya@ri.ncvc.go.jp (S. Ohya).

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.08.006

of PNIPAM-gelatin. The effects of surface topography and surface mechanical strength, both of which are determined by atomic force microscopy (AFM), on cell adhesion and spreading potentials were evaluated and discussed from the physicochemical and biomechanical viewpoint.

2. Materials and methods

2.1. Materials

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSC) was obtained from Dojindo Laboratories (Kumamoto, Japan). *N*-isopropylacrylamide (NIPAM) and 4-chloromethyl benzoic acid were obtained from Tokyo Chemical Industry Ltd. (Tokyo, Japan). NIPAM was used after recrystallization using a toluene–hexane solution. Gelatin (molecular weight: approximately 9.5×10^4 g/mol, from bovine bone) and sodium *N*,*N*-diethyldithiocarbamate trihydrate were obtained from Wako Pure Chemical Industry Ltd. (Osaka, Japan). Solvents and other reagents, all of which are of reagent grade, were purchased from Wako and used after conventional purification.

2.2. Preparation of PNIPAM-gelatin hydrogel

PNIPAM-gelatins with different graft densities (approximately 11, 23, and 34 graft chains per gelatin molecule; average molecular weight of the graft chain: approximately 5.0×10^4 g/mol) were prepared according to the procedure described previously [5–8]. Briefly, the amino groups of gelatin were reacted with the carboxyl group of 4-dithiocarbamylmethyl benzoic acid using the condensation reagent, WSC. The degree of dithiocarbamylation was adjusted with reaction time. Subsequently, NIPAM was polymerized from dithiocarbamate-derivatized gelatin in water under UV irradiation for 10 min (400 W Hg lamp, AH400RP, UV, Saitama, Japan; light intensity at 250 nm: 4.0 mW/cm^2). By graft density, three different PNIPAM-gelatins were prepared (PNIPAM/gelatin weight ratios (P/G) = 5.8, 12, and 18). Hydrogels were prepared from PNIPAM-gelatins with different P/G at 5 and 20 w/v% of aqueous solution (Chemical structures are shown in Fig. 1). The aqueous or M199 solutions of PNIPAM-gelatins (concentration: 5 and 20 w/v%, 100 μL) placed on the tissue culture dish (diameter: 35 mm, Iwaki Glass, Tokyo, Japan) fixed on the stage of AFM equipment were warmed to 37 °C to form white opaque hydrogels. The water or M199 was added onto the hydrogel, which were subjected to surface topological and mechanical strength characterizations described below. The formed hydrogel tightly adhered on the bottom of the dish.

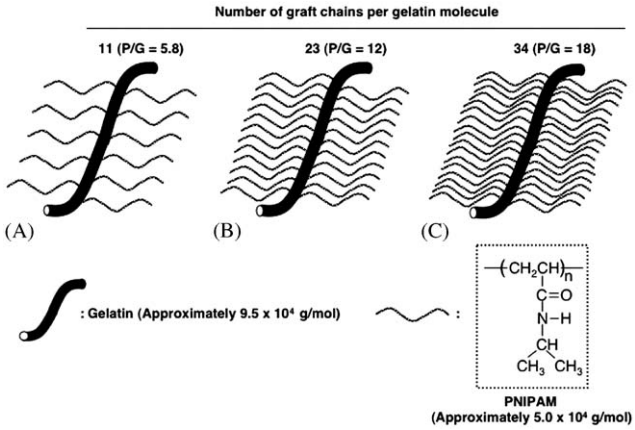

Fig. 1. Schematic structure of PNIPAM-gelatins with different graft chain densities.

2.3. Surface observation and elastic modulus

The surface images and force-versus-indentation (F–δ) curves of the hydrogels in water or M199 at 37 °C were measured by AFM (Nanoscope IIIa, Dimension 3000, Digital Instruments), using a commercial Si_3N_4 probe tip (manufacturing spring constant: 0.06 N/m (the value was used for the calculation of elastic modulus without further validation. Since the same tip was used throughout the study, the discussion was made on basis of the relative values); Digital Instruments, Santa Barbara, CA, USA). The probe tip was equipped with a commercial fluid cantilever folder (Digital Instruments), and immediately immersed into water or M199. The measurement was performed in water or M199 at 37 °C. To control sample temperature, AFM equipment was placed in a box maintained at 37 °C using heater with a thermocontroler (Nikon, Japan). Surface images (Scanning size: 10 μm × 10 μm) were obtained by a tapping mode (resonance frequency: 7.0 kHz).

F–δ curves were measured linearly every 100 nm (total of 101 points measured) by a contact mode. Elastic moduli were calculated from the F–δ curves according to the Hertz model described below [11]. If the tip is infinitely stiff and conical in shape, the Hertz model predicts

$$F = \frac{2\tan\alpha}{\pi}\frac{E}{1-\upsilon^2}\delta^2, \quad (1)$$

where F is the loading force, E is the elastic modulus, υ is the Poisson ratio, α is the open angle of the tip, and δ is the indentation depth. Here, open angle α was 35° and Poisson ratio υ was fixed to 0.5 for simplification. Therefore, elastic moduli were calculated from F–δ curves fitted to the equation using a linear least-squares method.