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ScienceDirect
Reactive & Functional Polymers 67 (2007) 1388–1397
REACTIVE & FUNCTIONAL POLYMERS
www.elsevier.com/locate/react

Novel cell sheet carriers using polyion complex gel modified membranes for tissue engineering technology for cell sheet manipulation and transplantation

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Received 11 June 2007; received in revised form 25 July 2007; accepted 26 July 2007
Available online 10 August 2007

Dedicated to Professor Teiji Tsuruta on the occasion of his 88th birthday (Beiju).

Abstract

A novel hydrogel membrane composed of polyion complex gels was developed to transfer cultured cell sheets from thermoresponsive cell culture dishes. Polyion complexes have been prepared by mixing poly(*N*,*N*-dimethylacrylamide-*co*-2-acrylamido-2-methylpropane sulfonic acid) (P(DMAAm-*co*-AMPS)) as anionic water-soluble polymers and poly(*N*,*N*-dimethylacrylamide-*co*-2-acryloxyethyltrimethylammonium chloride) (P(DMAAm-*co*-AETA-Cl)) as cationic water-soluble polymers. Various polyion complexes have been prepared by changing the composition of two polyelectrolytes. Through a set of Teflon® ring device, polyion complexes have been modified on porous membranes by electron beams irradiated cross-linking and covalent grafting technique. NIH/3T3 mouse cells as demo cells have been cultured to prepare cell sheets. This cell sheets could be recovered by using the resulting polyion complex gels as cell sheet carriers from temperature-responsive cell culture surfaces and be transferred to new collagen-coated tissue culture polystyrene dishes. This new technique has potential applications not only for transplantation but for creation of three dimensional layered cell sheet tissues.

Keywords: Polyion complex gel; Electron beam irradiated immobilization; Cell sheet carrier; Regeneration medicine

1. Introduction

Recently a novel approach of tissue engineering, cell sheet engineering, was proposed by our laboratory [1]. Poly(*N*-isopropylacrylamide) (PIPAAm) was introduced on the surfaces of tissue culture polystyrene (TCPS) dishes by electron beams (EB) irradiated polymerizations [2–4]. PIPAAm is a temperature-responsive polymer and has the lower

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doi:10.1016/j.reactfunctpolym.2007.07.058

critical solution temperature (LCST) at 32 °C [5]. At the cell culture condition of 37 °C, where PIPAAm dish surface was hydrophobic, cells adhered and grew to confluency. By lowering temperature below the LCST, the dish surface became hydrophilic and all of cells detached from the dish surfaces and formed an intact cell sheet [4]. Since cell sheet could keep the cell–cell junctions and deposited extracellular matrix (ECM) underneath of the cell sheet [6], physiological functions and bioactivity of cell sheet are similar to the cells in tissue, which could provide potential applications as an engineered tissue. Many kinds of tissues have been reconstructed using cell sheet engineering technology successfully, such as skin [7], corneal epithelium [8,9], bladder [10], cardiac tissue [11,12], and so on. In particular, the corneal reconstruction has been applied on clinical application [8,9].

Such cell sheets usually shrunk, wrinkled and folded without any support after detached from the PIPAAm dishes. This is sometimes troublesome in applicating such cell sheets for tissue engineering. Moreover, to construct a heterotypic tissue and/or a multiple layer tissue, it is also necessary to develop cell sheet carrier to keep sizes of cell sheets as well as to transfer them. A hydrophilic modified poly(vinylidene difluoride) (PVDF) membrane was used as such support membrane (carrier) [13]. But some kinds of cell sheets could not physically stick to hydrophilic modified PVDF membrane surfaces even at low temperature because of the weak interaction between cell sheet and the supporter membrane to use as cell sheet carriers. Biological hydrogel such as fibrin gel coated membrane was another choice. However, due to strong interaction of cell sheet with fibrin gel, the cell sheet is not released from fibrin gel coated membrane after transplanted. Inflammation reaction would occur during the gel degradation in vivo, which leads to unwanted damage to transplanted sites. Additionally, the fibrin gel must be prepared just before use. Thus, new cell sheet carriers with three major requirements should be necessary for advanced tissue engineering using cell sheets. These requirements are: (i) cell sheet should be physically attached to the carrier surfaces and be peeled off from the PIPAAm dish completely; (ii) cell sheet should be detached and released from the carrier surfaces after transplanted; (iii) carrier membranes will be stored for long time without any damage for possibility of mass production and preparative of industrialization.

In this study, a novel approach was developed to transfer cell sheets by using polyelectrolyte complex gels. Polyelectrolytes are special polymers, which bind with the oppositely charged surfaces or associate to form complexes with other oppositely charged polymer molecules. Moreover, in some case, the viscosity of the polyelectrolyte complex solution is increased because the movements of polymer chains are limited by the electrostatic interactions. Since similar biochemical changes are found in many biological systems present in extracellular and intracellular environments, artificial polyelectrolytes complexes may have remarkable potential applications in biotechnology. Cell encapsulation is one of the important applications of polyelectrolytes complexes, in which cell was encapsulated through complexation of oppositely charged polyelectrolytes complexes [14]. Multilayered films obtained by laying polyelectrolytes complexes were prepared as cell culture substrates [15,16]. Furthermore, polyelectrolyte shows a good biocompatible. For example, zwitterionic polyelectrolytes were used as blood compatible material studied by Ishihara and co-workers [17,18]. Thus, polyelectrolyte complexes provide a potential application for support membrane of cell sheet.

2. Experimental

2.1. Materials

2-Acrylamido-2-methylpropane sulfonic acid (AMPS) (Alfa Aser Ltd., Ward Hill, MA, USA), 2-acryloxyethyltrimethylammonium chloride (AETA-Cl, 80% solution in water) (Polysciences Inc., Warrington, PA, USA) and *N*,*N*′-azobis-4-cyanovaleric acid, V-501, (Wako Pure Chemical Industries, Co. Ltd., Osaka, Japan) were used as received. *N*,*N*-dimethylacrylamide (DMAAm) was purchased from Wako and was purified by distillation under reduced pressure (b.p. 51 °C/1 mmHg) to remove polymerization inhibitor. 2,2′-Azobisisobutyronitrile (AIBN, Wako) was purchased from Wako and recrystallized from ethanol before use. *N*,*N*-dimethylformamide (DMF, Wako) was distilled under reduced pressure (b.p. 45 °C/20 mmHg).

2.2. Synthesis of anionic water-soluble polymers

A known amount of AMPS, DMAAm, and AIBN as an initiator were dissolved in 50 mL of DMF in a round-bottomed flask connected with a two-way stopcock. The mixture was degassed by freeze-