Polyacrylamides containing sugar residues: synthesis, characterization and hepatocyte attachment studies

Raman Bahulekar¹, Takayoshi Tokiwa¹, Junko Kano¹, Toshiharu Matsumura², Isao Kojima³ and Makoto Kodama¹*

¹ Bionic Design Group, National Institute for Advanced Interdisciplinary Research, ³ National Institute of Materials and Chemical Research, 1–1–4 Higashi, Tsukuba, Ibaraki 305, Japan
² Cell Technology Center, Meiji Institute of Health Sciences, Meiji Milk Products Co. Ltd., 540 Naruda. Odawara, Kanagawa 250, Japan
* Fax +81–298–54–2560, Email: mkodama@nair.go.jp

Homo polymers of acrylamide having glucose (PAAm-glucose) and galactose (PAAm-galactose) as pendent groups were synthesized. Tissue culture polystyrene (TCPS) plates coated with these polymers showed increased surface wettability. Coating of PAAm-glucose and PAAm-galactose on to TCPS plates was also confirmed by X-ray photoelectron spectroscopic (XPS) characterization. Rat hepatocytes in primary culture attached to the surfaces of PAAm-galactose homopolymer, but not to those of PAAm-glucose homopolymer.

Key words: polyacrylamide, galactose, hepatocyte, attachment

Introduction
Glycotechnology has been widely accepted as one of the most important tools for a variety of biomedical applications (Kochetkov, 1984; Duncan et al., 1989). It has been realized that the synthetic polymers bearing sugar residues recognize cells through the marker molecules present on cell surface (Schnaar, 1994; Weigel et al., 1979). Such polymers also offer good surface for cell attachment in culture. Much attention has been paid in recent years synthesizing polymers having sugar residues for cell attachment. Most of the methods reported hitherto were based on the functionalization of a sugar moiety by introducing an olefinic polymerizable group (with or without spacer) to form a monomer and subsequent polymerization using a free radical initiator. We investigated the synthesis and characterization of polyacrylamides having simple sugar residues such as glucose and galactose. Preliminary results on attachment of rat hepatocytes to these polymer surfaces in primary culture are presented in this communication.

Materials and methods
Synthesis of polymers
Glucosamine and galactosamine hydrochloride were reacted with acryloyl chloride in alkaline medium, such as 0.1 M bicarbonate buffer (pH 8.5), to form monomers. Polymerization of N-acryloylated glycosylamines was carried out in water at 30°C±0.1°C for 18 h with (NH₄)₂S₂O₈/TEMED initiator system. Polymers were precipitated by pouring the reaction mixture into methanol. The polymers were purified by repeated dissolution and precipitation (3 times) and finally freeze-dried from aqueous solution.

Preparation of polymer coated plates
Aqueous solutions of these polymers (1.0 ml, 0.1 % w/v) were contacted with 35 mm tissue culture polystyrene (TCPS) plates (area = 9.62 cm²) (Iawaki, Tokyo, Japan) for 48 h at room temperature. The plates were thoroughly dried under vacuum at room temperature. Crosslinking of adsorbed polymer was done by exposing the plates to UV radiation (UV cross linker CL-1000, UVP Inc., Upland, USA) of 1.2 × 10⁵ mJ/cm² for 30 min. Although data are not shown, without crosslinking of the polymers, hepatocytes attached poorly to PAAm-galactose surfaces, suggesting an effective crosslinking of the polymers synthesized in this study. The polymer coating was confirmed by surface characterization techniques such as X-ray photoelectron spectroscopy (XPS) and contact angle and by hepatocyte attachment studies.
Measurements

XPS spectra were measured on an ESCAlab 220i (Fisons Instruments) with a monochromatized Al/Kα X-ray radiation at pass energy of 100 eV. Water contact angle measurements were done on FACE contact angle goniometer (Kyowa Scientific Instruments, Japan).

Preparation of hepatocytes and cell attachment test

Hepatocytes were prepared from male Wistar rats (150–200g) by a collagenase perfusion method (Seglen, 1976). The liver cells suspended in Hanks balance salt solution (HBSS) were filtered through a 50 mm nylon mesh. The cell pellet was collected by centrifugation for 1 min at 50 × g. Cells were further purified by repeating the centrifugation. The cell pellets were suspended in Percoll solution (density 1.07 g/ml) and centrifuged for 10 min at 50 × g. More than 90% of the isolated cells were viable as measured by Trypan Blue dye exclusion test. The resulting parenchymal cells were suspended in Williams E medium containing 50 ng epidermal growth factor (EGF)/ml (R&D system, Minneapolis, USA), 0.1 mM insulin (Wako) and 1 mM dexamethasone (Sigma, St. Louis, MO., USA). Isolated cells were inoculated at 2.4 × 10^5 cells/plate with 2 ml serum-free culture medium and incubated for 24 h at 37°C in a humidified incubator with 5% CO_2 in air. In this study, type I collagen-coated plates (Iwaki) were used as controls. Cell attachment was evaluated by taking the photographs of cells on Olympus IMT-2 phase contrast microscope equipped with camera. Furthermore, cell nuclei were stained with hematoxylin (H-stain) after fixation with methanol.

Results and discussion

Two polyacrylamide derivatives bearing glucose and galactose sugar moieties were synthesized from the corresponding N-acryloyl glucosamine and galactosamine monomers. N-acryloylation of glucosamine/galactosamine hydrochloride was carried out in bicarbonate buffer (pH 8.5) and methanol (1:1 v/v) solvent. It has been reported previously that N-acryloylation of glucosamine proceeds best around pH 8.5 in bicarbonate:methanol (1:1 v/v) solvent (Kallin et al., 1989). The two sugars are isomers of each other. These polyacrylamide-sugar derivatives are hydrophilic and miscible with water in all proportions. The transmission FT-IR spectrum of PAAm-glucose in KBr pellets (Figure 1) shows 1651 cm⁻¹ band characteristics of amide carbonyl groups.

![Figure 1](image1.png)

**Figure 1** FT-IR spectrum (KBr pellet) of homo polymers of acrylamide having glucose (PAAm-glucose).

![Figure 2](image2.png)

**Figure 2** X-ray photoelectron spectroscopy survey spectra of polyacrylamide (PAAm), homo polymers of acrylamide having glucose (PAAm-glucose) and those of acrylamide having galactose (PAAm-galactose).