Biomaterials and Biomechanics in Bioengineering, *Vol. 1, No. 3 (2014) 163-174*
(Formerly, Biomaterials and Biomedical Engineering)
DOI: http://dx.doi.org/10.12989/bme.2014.1.3.163

Cross-linkable and water-soluble phospholipid polymer as artificial extracellular matrix

Eri Maeta[1] and Kazuhiko Ishihara[*1,2]

[1]*Department of Materials Engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*
[2]*Department of Bioengineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*

(Received July 29, 2014, Revised August 12, 2014, Accepted September 17, 2014)

Abstract. The objective of this study is to prepare an artificial extracellular matrix (ECM) for cell culture by using polymer hydrogels. The polymer used is a cytocompatible water-soluble phospholipid polymer: poly[2-methacryloyloxyethyl phosphorylcholine (MPC)-n-butyl methacrylate-p-nitrophenyloxycarbonyl poly(ethylene oxide) methacrylate (MEONP)] (PMBN). The hydrogels are prepared using a cross-linking reaction between PMBN and diamine compounds, which can easily react to the MEONP moiety under mild conditions. The most favorable diamine is the bis(3-aminopropyl) poly(ethylene oxide) (APEO). The effects of cross-linking density and the chemical structure of cross-linking molecules on the mechanical properties of the hydrogel are evaluated. The storage modulus of the hydrogel is tailored by tuning the PMBN concentration and the MEONP/amino group ratio. The porous structure of the hydrogel networks depends not only on these parameters but also on the reaction temperature. We prepare a hydrogel with 40-50 μm diameter pores and more than 90 wt% swelling. The permeation of proteins through the hydrogel increases dramatically with an increase in pore size. To induce cell adhesion, the cell-attaching oligopeptide, RGDS, is immobilized onto the hydrogel using MEONP residue. Bovine pulmonary artery endothelial cells (BPAECs) are cultured on the hydrogel matrix and are able to migrate into the artificial matrix. Hence, the RGDS-modified PMBN hydrogel matrix with cross-linked APEO functions as an artificial ECM for growing cells for applications in tissue engineering.

Keywords: phospholipid polymer; cytotoxicity; artificial extracellular matrix; hydrogel; cell culture

1. Introduction

Polymeric matrices for cell culture are of interest in the fields of tissue engineering and regenerative medicine (Garg and Goyal 2014, Place *et al.* 2009, Stock and Mayer 2001). Generally, aliphatic polyester derivatives are used for this purpose; however, more functionalized polymers are required for advanced cell engineering applications. Cytocompatibility is one of the most important properties required for this purpose.

A novel cell culture matrix should fulfill two criteria: cytocompatibility and cell-specific adhesive properties. Natural extracellular matrix (ECM) is composed of hydrophilic matrix

*Corresponding author, Professor, E-mail: ishihara@mpc.t.u-tokyo.ac.jp

http://www.techno-press.org/journals/bme&subpage=7 ISSN: 2288-3738 (Print), 2288-3746 (Online)

soluble phospholipid polymer with phenylboronic acid moiety", *Biomater.*, **28**(10)**,** 1770-1777.
Konno, T., Watanabe, J. and Ishihara, K. (2003), "Enhanced solubility of paclitaxel using water-soluble and biocompatible 2-methacryloyloxyethyl phosphorylcholine polymers", *J. Biomed. Mater. Res.*, **65A**(2), 210-215.
Konno, T., Watanabe, J. and Ishihara, K. (2004), "Conjugation of enzymes on polymer nanoparticles covered with phosphorylcholine groups", *Biomacromolecules*, **5**(2), 342-347.
Lewis, A.L. (2000), "Phosphorylcholine-based polymers and their use in the prevention of biofouling", *Colloids Surf. B: Biointerfaces*, **18**(3), 261-275.
Lutolf, M.P. and Hubbell, J.A. (2005), "Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering", *Nat. Biotechnol.*, **23**(1), 47-55.
Makgoba, M.W., Bernard, A. and Sanders, M.E. (1992), "Cell adhesion/signalling: biology and clinical applications", *Eur. J. Clin. Invest.*, **22**(7), 443-453.
Mo, Y., Kubota, K. and Nishinari, K. (2000), "Rheological evidence of the gelation behavior of hyaluronan-gellan mixtures", *Biorheology*, **37**(5-6), 401-408.
Nishizawa, K., Takai, M. and Ishihara, K. (2011), "A bioconjugated phospholipid polymer biointerface with nanometer-scaled structure for highly sensitive immunoassays", *Methods Mol. Biol.*, **751**, 491-502.
Oda, H., Konno, T. and Ishihara, K. (2013), "The use of the mechanical microenvironment of phospholipid polymer hydrogels to control cell behavior", *Biomater.*, **34**(24), 5891-5896.
Place, E.S., George, J. H., Williams, C.K. and Stevens, M.M. (2009), "Synthetic polymer scaffolds for tissue engineering", *Chem. Soc. Rev*., **38**(4), 1139-1151.
Ross,o F, Giordano, A, Barbarisi, M and Barbarisi, A. (2004), "From cell-ECM interactions to tissue engineering", *J. Cell Physiol.*, **99**(2),174-180.
Rowley, J.A., Madlambayan, G. and Mooney, D.J. (1999), "Alginate hydrogels as synthetic extracellular matrix materials", *Biomater.*, **20**(1), 45-53.
Sambu, S., Xu, X., Schiffer, H.A., Cui, Z.F. and Ye, H. (2011), "RGDS-fuctionalized alginates improve the survival rate of encapsulated embryonic stem cells during cryopreservation", *Cryo. Lett.*, **32**(5), 389-401.
Sandvig, I., Karstensen, K., Rokstad, A.M., Aachmann, F.L., Formo, K., Sandvig, A., Skjåk-Braek, G. and Strand, B.L. (2014) "RGD-peptide modified alginate by a chemoenzymatic strategy for tissue engineering applications", *J. Biomed. Mater. Res. A.*, doi: 10.1002/jbm.a.35230.
Seo, J.H., Matsuno, R., Takai, M. and Ishihara, K. (2009), "Cell adhesion on phase-separated surface of block copolymer composed of poly(2-methacryloyloxyethyl phosphorylcholine) and poly(dimethylsiloxane)", *Biomater.*, **30**(29), 5330-5340.
Silva, E.A and Mooney, D.J. (2014), "Synthetic extracellular matrices for tissue engineering and regeneration", *Curr. Top. Dev. Biol.*, **64**, 181-205.
Stock, U.A. and Mayer, J.E. Jr. (2001), "Tissue engineering of cardiac valves on the basis of PGA/PLA co-polymers", *J. Long Term Eff. Med. Implants*, **11**(3-4), 249-260.
Ueda, T., Oshida, J., Kurita, K., Ishihara, K. and Nakabayashi, N. (1992), "Preparation of 2-methacryloyloxyethyl phosphorylcholine copolymers with alkyl methacrylates and their blood compatibility", *Polym. J.*, **24**, 1259-1269.
Walter, N., Busch, T., Sefferlein, T. and Spatz, J.P. (2011), "Elastic moduli of living epithelial pancreatic cancer cells and their skeletonized keratin intermediate filament network", *Biointerphases*, **6**(2), 79-85.
Xu, Y., Sato, K., Mawatari, K., Konno, T., Jang, K., Ishihara, K. and Kitamori, T. (2010), "A microfluidic hydrogel capable of cell preservation without perfusion culture under cell-based assay conditions", *Adv. Mater.*, **22**(28), 3017-3021.

YC