Novel surface-modification technique of collagen film for alteration of its surface property

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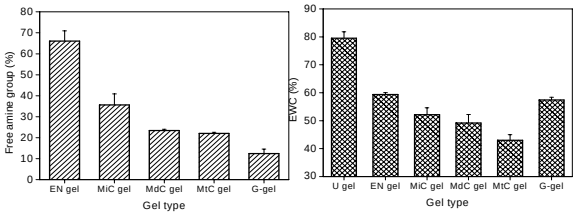
Statement of Purpose: Collagen gel is often applied for diverse biomaterial, especially for cell related products, possesses high thrombogenicity and low mechanical strength which limits its use as a biomaterial [1]. In order to use collagen gel as a biomaterial, such as a blood vessel, prevention of thrombus formation, and reinforcement of physical and mechanical properties is required. 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, a well-known material for its blood compatibility [2], was immobilized on the collagen gel to develop a mechanically reinforced and biocompatible collagen hybrid gel [3]. However, using the conventional method, high polymer immobilization rate could not be obtained and therefore, could not be used as a biomaterial. In this study, we suggest a new technique of immobilization using well-known cross-linker *N*-(3-dimethylamino-propyl)-*N′*-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). With this collagen hybrid gel, the physical and biological property was characterized.

Methods: Collagen film was fabricated (*l*=0.018mm) and was immobilized with poly(MPC-*co*-methacrylic acid) (PMA) using EDC and NHS in ethanol:water=5:5 for 48 hours at 4°C to make a MPC-immobilized collagen gel (MiC gel). Then PMA was re-immobilized on the MiC gel to obtain a higher density MPC immobilized collagen gel (MdC, MtC gel). X-ray photoelectron spectroscopy (XPS), atomic force microscope, static contacting angle (SCA) and was used to characterize the surface of the hybrid gel. Swelling ratio, free amine group analysis, shrinkage temperature, and mechanical test was used to characterize the cross-linking efficiency. And cell adhesion test was executed to characterize the cell compatibility of the collagen hybrid gel. Collagen gels that are interhelically cross-linked with EDC and NHS (EN gel), and glutaraldehyde (G-gel) was prepared by conventional method [1,4] to compare the physical and biological property of the collagen gel.

Results / Discussion: PMA was successfully immobilized on the surface of the collagen gel. The collagen hybrid gels were transparent, except for G-gel which was yellow. XPS result showed that the phospholipids group was mainly deposited on the surface of the collagen hybrid gel. The surface morphology shows the smooth, homogenous surface for the PMA-immobilized collagen hybrid gels, implying that the gels are completely covered with PMA. SCA showed that the hydrophilicity is increasing as more MPC is immobilized, indicating that the density of MPC is increasing by re-immobilizing process.

The free amine groups exist in the microfibrils was about 65% for EN gel and 15% for G-gel (Figure 1). The percentage of unreacted amine group lays between 20% and 40% for MPC immobilized gels. The decrease in the free amine group by MPC re-immobilization implies that higher density of MPC immobilization can be achieved.



(a) (b)

Figure 1. Free amine groups (a) and equilibrium water content (b) of respective collagen gels.

The cross-link of the collagen gel using EDC and NHS is known stop reacting after 1 hour, but the re-activation of carboxylic group by EDC and NHS made the collagen to cross-link with PMA and formed much denser network. Equilibrium water content (EWC) (Figure 1 (b)) shows that the network of the collagen hybrid gel is becoming denser as the MPC is immobilized. G-gel, which has lower free amine group content, shows relatively higher EWC indicating that the interhelical cross-link has limit in dimensional stability.

The shrinkage temperature increased for the collagen hybrid gel compared to uncross-linked collagen gel. This implies that immobilization of PMA occurred on the surface of the collagen hybrid gel, but made the gel much tougher and protect the gel from the thermal degradation by forming much denser network. Formation of the denser network increased the tensile strength also. The strong network formed between polymer and the collagen fibrils made the tensile strength much stronger.

The protein adsorption and cell adhesion test using fibrinogen and L929 showed that the number of protein and cell adhered on the surface decreases as the density of PMA increased (Figure 2). The toxicity for all of collagen gels, except for G-gel, was not shown. When the morphology of the cells was observed, cell adhered on the surface of MPC-immobilized collagen gel were kept round, indicating the interaction between the cell and surface was suppressed.

Conclusions: The high rate of MPC polymer was immobilized on the collagen hybrid gel, and it was stably cross-linked with collagen microfibrils. This increased the mechanical property and stabilized the collagen hybrid gel. Possession of MPC polymer on the surface suppressed the adsorption of the protein and adhesion of the cell.

References: 1) Wissink MJB et al., Biomaterials 2001;22:151-163. 2) Ishihara K. Sci Technol Adv Mater 2000;1:131-138. 3) Nam K et al. Biomaterials 2006;27:1-8. 4) Olde Damink LHH *et al*., *Biomaterials* 1996;17:765-773.