Preparation of an artificial skin by replication of its native structure using collagen-GAG complex

KwangwooNam, Tsuyoshi Kimura, AkioKishida
Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, JAPAN.

Statement of Purpose: The most frequently used method for the development of biocompatible skin replacement is development of artificial skin using collagen, for the collagen composes major part of the native skin. For this, we focused on the structural aspect of native skin. The native skin possesses a distinguishable chemical structure from other native tissues, where the high content of glucosaminoglycan (GAG) which forms complex with collagen in the dermis. This induces good water sustainability, promotes cell adhesion and proliferation, and controls the immunogenicity. [1-2)] Therefore, a method for reconstruction of an artificial skin using GAG and collagen is necessary.

Our group found that it is possible to obtain a collagen gel with stabilized collagen fibril structure by using fibrillogenesis and gelling contemporarily.[3)] Based on this, it is possible to prepare a collagen-GAG complex by placing the collagen molecules through the GAG molecules and then fibrillize. However, it is not clear whether the phase-separation would occur during this process. In this study, we are reporting on preparation of the collagen-GAG complex with fibrillzed collagen structure, and characterized its physical and biological properties targeting the reconstruction of the artificial skin.

Methods: Hyaluronic acid (HA), chondroitin-4-sulfate (CS), and heparin (Hep) was dissolved in 1.8wt% of NaCl and 0.04M of Na2HPO4 aqueous solution. Then, the collagen aqueous solution was added to the GAG solution at the ratio of 1:1 v/v. The ratio of GAG to collagen was controlled at 8:1, 4:1, 2:1 and 1:1. The mixture was stirred until they are fully homogenized forming a viscose sol. Then, the mixture was kept in the frezzer (4^{o}C) for 24 h until the mixture solidified. Then the water/ethanol solution containing 1-ethyl-3-(3-dimethyl aminopropyl)-1-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) was added to the solidified collagen-GAG complex. The EDC and NHS was washed with water/ethanol solution and then lyophilized. The structure of the complex was observed using atomic force microscope (AFM), scanning electron microscope (SEM) and GAG staining kit. The physical characterization of the collagen-GAG complex was executed by measuring water absorption ratio, shrinkage temperature and mechanical strength to define the physical role of GAG in the complex. The cell behavior was observed using fibroblast to observe the biological role of GAG within the complex.

Results: Basically, all complex showed collagen fibrillization with D-peridicity except for collagen-Hep, where the dispersion of heparin was shown, limiting the fibrillogensis of the collagen (fig 1). We could observe by the GAG staining using alcian blue that the GAG is located within the complex. However, the amount of the GAG within the complex was lower showing less than 50% of adopted GAG would remain. The gelling and fibrilligenesis process occurs contemporarily showing that

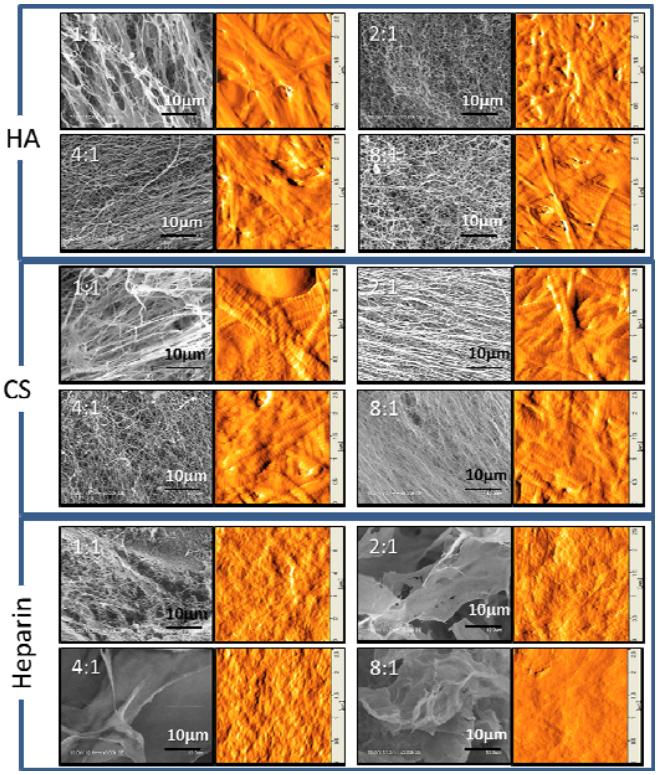

Figure 1. The structure of collagen-GAG observed with SEM and AFM. Arrows imply the fibrillogensis of the collagen.

the rapid initial water absorption then slow absorption for 72 hrs. The existence of GAG showed relatively higher water absorptivity. The exception was shown for collagen-Hep complex. The mechanical strength test executed by compression showed that the GAG is contributing to the increase in the mechanical strength. Interstingly, collagen-HA and collagen-CS complex showed 100% recovery after full compression. Furthermore, the increase in the shrinkage temperature was also observed for the complexes. The cell adhesion and proliferation was shown to be promoted for the collagen-GAG complex. The infiltration of the cells could be seen, showing the possibility of 3-dimensional cell culture suitable for the reconstruction of an artificial skin.

Conclusions: We have successfully prepared a collagen-GAG complexes. They possesses higher mechanical strength, higher water absorptivity, dimensional stability, and good cell compatibility. We believe that this complex would provide a good platform for the artificial skin

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References 1) A. Van der Smissen et al., *Biomaterials* **32** (2011) 8938. 2) S. Franz et al., *Biomaterials* **32** (2011) 6692. 3) K. Nam et al., *Soft Matter* **8** (2012) 472