Collagen Matrix possessing similar structure of that of native extracellular matrix

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Statement of Purpose: Collagen is one of the most actively studied biomolecules that is being used for the tissue engineering and regenerative medicine. The use of collagen is based on the fact that collagen is the major component of the extracellular matrix (ECM). Mostly, collagen structure is fabricated in gel or sponge type which can be applied for two or three dimensional cell culture. The collagen gel or sponge is usually chemically or physically cross-linked inter- or intrahelically. The advantage of the cross-linking is that the mechanical strength can be enhanced and biological functions can be controlled. However, there are certain drawbacks such as encapsulation or foreign body response *in vivo*, brittle mechanical strength, and toxicity. So, we tried to prepare a collagen matrix which possesses good elastic modulus, yet shows good biocompatibility *in vivo* by mimicking the structure of a native ECM. In this study, we are going to discuss on preparation of the collagen matrix which possesses the similar structure of that of native ECM to regenerate its physical and biological property.

Materials and Method: Bovine collagen type I aqueous solution was used to prepare a collagen matrix. Preparation of the collagen matrix which possesses the similar structure of that of native ECM was executed via fibrillogeneses [1]. In short, the collagen aqueous solution was inserted into a dialysis cassette and was put into the NaCl 0.9wt% aqueous solution to execute fibrillogensis of collagen. After 24 hrs, the dialysis cassette was taken out from the aqueous solution and the collagen matrix was obtained (collagen matrix I). This collagen matrix was air-dried for 48 hrs to obtain a much thinner collagen matrix (collagen matrix II). The dry collagen matrix was washed with distilled water before executing characterization. The observation of the collagen matrix structure was executed using scanning electron microscope (SEM) and atomic force microscope (AFM). Furthermore, the mechanical strength test and in vivo evaluation of the collagen matrices was executed. Chemically cross-linked gel using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) as cross-linker and physically cross-linked collagen gel using alkaline solution as cross-linking agent was used for the comparative study.

Results and Discussion: The collagen aqueous solution would precipitate in NaCl 0.9wt% aqueous solution due to the fibrillogenesis. The SEM image of the collagen matrices showed that the matrix is consisted of microfibrils entangled with each other. The microfibrils possesses fibril band with thickness of approximately 67nm, which implies that regulated *D*-periodicity was obtained. This result is important in the aspect that unregulated *D*-periodicity may cause the cardiovascular-related diseases [2]. In the case of collagen matrix II, the microlayers, which is thought to be formed by the slow evaporation of water was shown. This microlayer of the collagen matrix II was similar to that of native ECM. The regulated *D*-periodicity has remained after the drying process, implying that the air drying process did not affect the microstructure of the collagen matrix. The distance between the microlayers could be controlled by the concentration of the collagen solution used for the fibrillogenesis and the evaporation speed.

The mechanical strength of the collagen matrix II showed that high tensile strength can be achieved. For the chemically cross-linked collagen gel, it showed brittle elongational modulus and for physically cross-linked collagen gel. In the case of collagen matrix II, the elongation modulus increased approximately 9 times compared to that with collagen gel. Since the cross-linker was not used, the strain % was extended approximately 2 times from that of cross-linked collagen gel. All collagen matrices were degraded by collagenase within 3 hours, implying that the toughness is not due to the cross-linking, but it is the specific character of collagen fibrils.

In vivo results showed that the implantation of the collagen matrices would not cause any inflammatory response for 8 weeks. No capsulation was observed, and the integration to the native tissue was promoted, implying that the collagen matrix is bioinert. The collagen matrix II showed no degradation after 8 weeks of implantation, but there was no inflammatory response or any capsulation observed around the dry matrix. In the case of collagen gel, the collagen degraded, but showed high rate of inflammatory response. This indicates that the mimicking the structure of native tissue is very important for the regeneration of the biological property of native tissue.

Conclusion: Form this, study, we showed that simple fibrillogenesis would alter the physical and biological property of the collagen matrix. The physical property of the collagen matrix was much tougher than that of collagen gel, due to the formation of the collagen fibrils. No inflammatory response was observed for 8 weeks in vivo. These imply that in order to apply the collagen construct into the living body, construction of the structure which is similar to that to the native ECM is very important

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References

[1] Gross, J. J Biol Chem. 1958,;233: 355-360.
[2] Starborg, T. Method 2008; 45: 53-64.