EFFECT OF PREPARATION CONDITION ON TENSILE STRENGTH OF HAP-DEPOSITED COLLAGEN FIBERS

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Abstract
The aim of this paper is to evaluate the effects of the microstructural factors on the tensile strength of HAp/collagen complex fibers. We prepared the collagen fibers with the varied concentration of the cross-linking agent by the bio-inspired method, where the fibrosis and the cross-linking are generated at the same time. Then, the HAps were deposited on the collagen surface by the biomimetic deposition method, where the collagen fibers are dipped into the pseudo body fluid alternatively. Here, the alternative dipping time and preparation temperature were varied. In addition, HAp/collagen complex fibers were coated with the adhesive protein before the HAp deposition. As a result, it is suggested that the concentration of the cross-linking agent, where the fibrosis and cross-linking induce a synergistic effect, could maximize the tensile strength of collagen fibers. Secondary, the longer dipping and the lower temperature increased the tensile strength of the HAp/collagen complex fibers. Finally, the HAp deposition by the alternating immersion method after corting with the adhesion protein decreased the tensile strength of the HAp/collagen complex fibers, within the range covered in this paper. This means that the individual optimization of the microstructural factors would not necessarily maximize the tensile strength of the HAp/collagen complex fibers.

1. Introduction

In recent years, we have been investigating to realize the hydroxyapatite (HAp)/collagen composite materials, which possess the optimally composed and layered microstructures mimicking the real bone, using a biomimetic bottom-up approach. Here, the optimum preparation conditions could be hierarchically determined based on the micro-tensile tests, from the microscopic structures toward the macroscopic structures. This parametric study would enable the creation of the artificial bone cartridges to bone defects. Previously, we had evaluated the effect of the synthesis condition on the mechanical properties of the collagen fibers as the unit structure of the HAp/collagen complex fibers [1]. Here, we developed a micro-tensile testing device utilizing the deflection of a cantilever beam. In addition, we have tried to develop the HAp-deposition method on the prepared collagen fibers.

In this study, we prepared the collagen fibers by the bio-inspired method, where the fibrosis and the cross-linking are generated at the same time. The effect of the preparation condition of collagen fibers was evaluated. And then, HAps were deposited on the surface of the collagen fibers by the biomimetic deposition method, where the collagen fibers are dipped into the pseudo body fluid alternatively. The effect of the deposition condition of HAps on the tensile strength of the HAp/collagen complex fibers was evaluated.

2. Experimental Procedures

2.1. Preparation of collagen fibers

The collagen solution of 0.1 [wt%] was prepared using the fish collagen peptides (Tsuji Oil Mill Co. Ltd.) and distilled water. The sodium phosphate buffer of 40 [mmol/L] was prepared as the pH control agent using sodium dihydrogen phosphate (Wako Pure Chemical Industries, Ltd.) and disodium hydrogen phosphate (Wako Pure Chemical Industries, Ltd.). These solutions were mixed with the sodium chloride solution of 1.00-2.00 [mol/L] (Wako Pure Chemical Industries, Ltd.). To generate the cross-linking between the collagen molecules, the solution of 1-ethyl-3-carbodiimide hydrochloride (EDC) (Dojindo Molecular Technologies, Inc.) of 0-50 [mmol/L] was added. The mixed solution was maintained at 25 degree C for 6 days using a water bath. After the fibrosis is progressed, collagen fibers will be entangled, resulting in the difficulty to extract single fibers. Therefore, the fibrosis was delayed by the cold storage after their preparation.

2.2. HAp deposition

The deposition of HAps on the collagen surface was carried out using the alternating immersion method [2]. The tris-HCl buffer was prepared by mixing tris(hydroxymethyl)aminomethane hydrochloride (Sigma-Aldrich Co. LLC.), tris base (Sigma-Aldrich Co. LLC.) and distilled water. The "Ca solution" was prepared by mixing the tris-HCl buffer and calcium chloride (Wako Pure Chemical Industries, Ltd.) of 200 [mmol]. The "P solution" was prepared by mixing disodium hydrogen phosphate (Wako Pure Chemical Industries, Ltd.) of 120 [mmol] and distilled water. The HAp-deposition on the surface of the collagen fibers prepared under the EDC concentration of 5 mmol/L was generated using a biomimetic deposition method. Here, the collagen fibers were alternatively dipped into the "Ca solution" and the "P solution" as the pseudo body fluids. The dipping time was varied as 30, 150 and 300 [s]. In addition, the temperature of the Ca and P solutions was maintained as 10, 25 and 37 degree C using a digital desktop constant temperature water bath.

2.3. Preparation of HAp/collagen complex coated by adhesive protein

Osteonectin (SPARC/BM-40, originated from human being, recombinant) (Wako Pure Chemical Industries, Ltd.) was selected as a representative adhesive protein. First, osteonectin of 50 [μg] and sterilized distilled water of 50 [μL] were mixed. This solution was applied to the extracted collagen fibers prepared under the condition of 37 degree C and the dipping time of 300 [s] using the micro bullets to conduct the coating of the collagen fibers, before the HAp deposition.

2.4. Micro-mechanical testing procedure

Figure 1 illustrates the micro-tensile test developed by the authors previously [1]. An extracted collagen fiber was adhered to one end of a glass fiber (WFA230 100BS6, ϕ = 8.50 [μm]) (Nitto Boseki Co., Ltd.) and a boron fiber (KPSI500, ϕ = 100 [μm]) (AVCO), in turn. The boron fiber was fixed to the spindle of the micrometer. Here, the boron fiber was arranged parallel to the spindle axis. Then, the another end of the glass fiber was fixed on the acrylic plate. Here, the glass fiber was arranged perpendicularly to the boron fiber. The deflection was applied to the glass fiber by pulling the boron fiber using the micrometer. The deflection of the glass fiber was observed using a digital microscope (VHX-500) (KEYENCE Corporation) with a zoom lens (VHZ-100) (KEYENCE Corporation). Here, the magnification of used zoom lens was 100-1000 times. The tensile strength was calculated using the deflection of the glass fiber at the breakage of HAp/collagen complex fibers, by assuming the deflection of a cantilever beam.

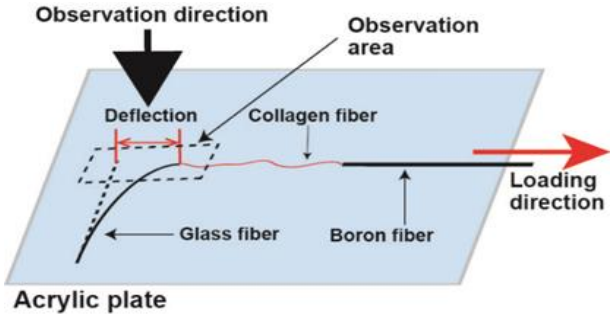


Figure 1. Schematic drawing for micro-tensile test.

3. Results and Discussion

3.1. Effect of preparation condition on tensile strength of collagen fibers

Figure 2 shows the measured tensile strength of the prepared collagen fibers with the varied EDC concentration. This indicates that the average tensile strength of the prepared collagen fibers peaked at the EDC concentration of 5 mmol/L. In addition, the EDC concentration of 60 mmol/L did not derive the fibrosis. Therefore, it is suggested that the EDC concentration, where the fibrosis and cross-linking induce a synergistic effect, could maximize the tensile strength of the collagen fibers.

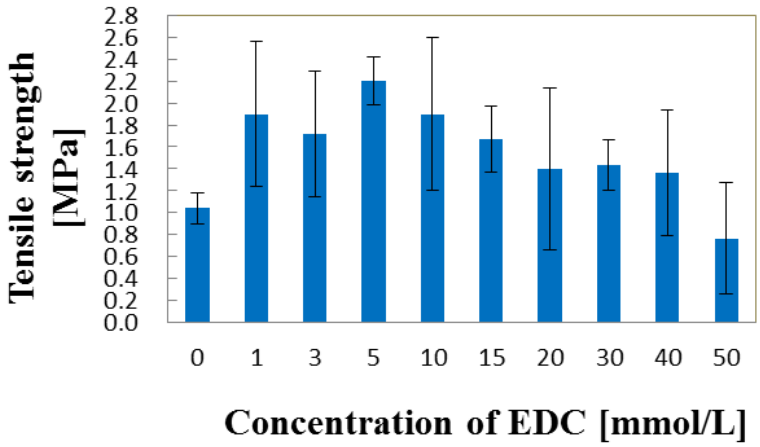


Figure 2. Tensile strength of prepared collagen fibers with varied EDC concentration.

3.2. Effect of HAp deposit condition on tensile strength of HAp/collagen complex fibers

Figure 3 shows the tensile strength of the collagen fibers with EDC of 5 [mmol/L] after the HAp deposition. This indicates that longer dipping and the lower temperature increase the tensile strength of the HAp/collagen complex fibers. Thus, the longer HAp deposition induced the higher tensile strength of the HAp/collagen complex fibers. This might be attributed to the increase of the stiffness caused by the increase of the deposited HAp crystals. In addition, the lower temperature increased the tensile strength of the HAp/collagen complex fibers. It is known that the higher temperature decreases the lattice defects of the HAp crystals, resulting in the increase of the crystal size [2]. From the observation using a scanning electron microscope shown in Figures 4 and 5, it seams that the smaller crystals were generated under the condition of the lower temperature. Therefore, it is considered that the smaller HAp crystals were deposited uniformly under the lower temperature, resulting in the increase of the tensile strength of the HAp/collagen complex fibers.

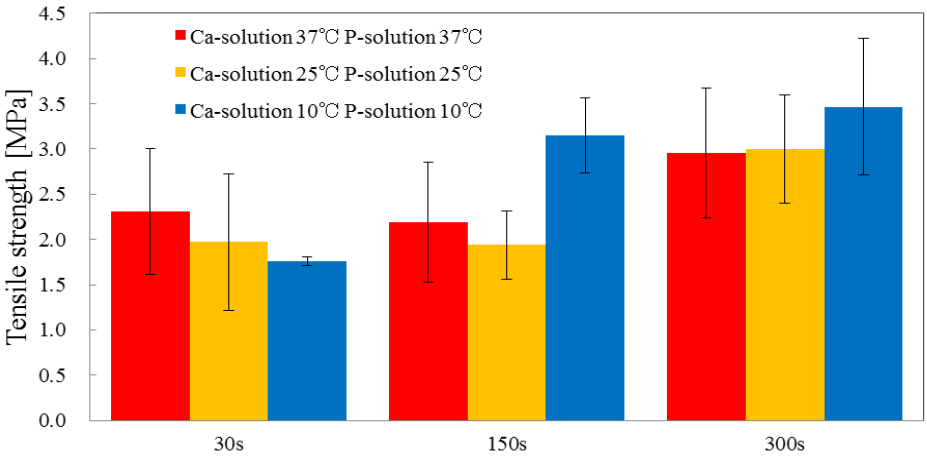
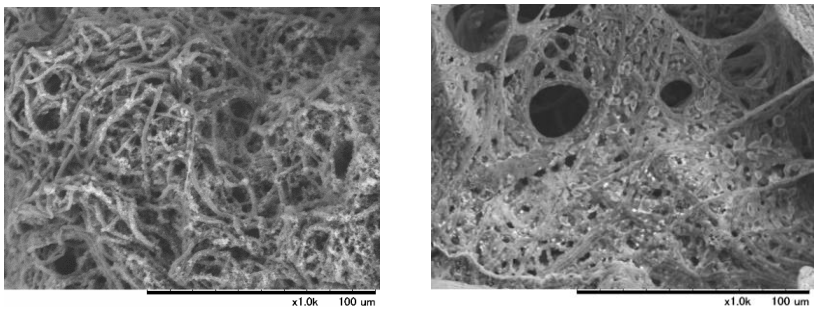
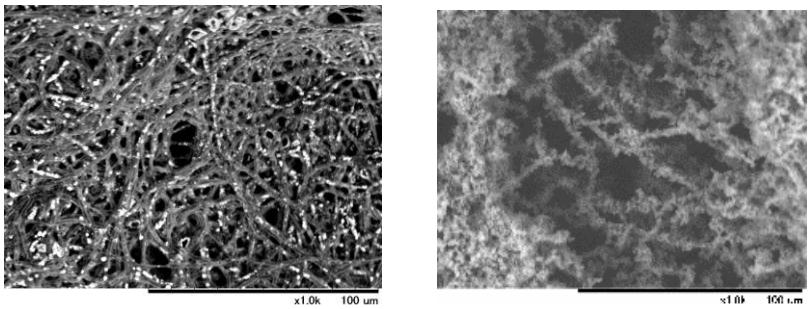


Figure 3. Tensile strength of collagen fibers with EDC of 5 [mmol/L] after HAp deposition.



(a) After 30 [s] immersion (b) After 300 [s] immersion

Figure 4. SEMs of collagen fibers with HAps deposited at 37 degree C.



(a) After 30 [s] immersion (b) After 300 [s] immersion

Figure 5. SEMs of collagen fibers with HAps deposited at 10 degree C.

3.3. Effect of adhesion protein on tensile strength of HAp/collagen complex fibers

Figure 6 shows the tensile strength of the collagen fibers with EDC of 5 [mmol/L] and HAp-deposited at 37 degree C for 300 [s] with and without coating of osteonectin after dipping into calcium solution and phosphorus solution. This indicates that the HAp deposition by the alternating immersion method after corting with the adhesion protein decreased the tensile strength of the HAp/collagen complex fibers. It is known that osteonectin used as the adhesion protein has the high affinity to the collagen fibers and the HAp crystals [3]. Thus, it can be considered that the excessive bonding between the collagen fibers and the HAp crystals induced the embrittlement of the HAp/collagen complex fibers. This means that the individual optimization of the microstructural factors, such as the collagen cross-linking, the HAp deposition and the adhesion between the collagen fibers and the HAp crystals, would not necessarily maximize the mechanical properties of the HAp/collagen complex fibers. Therefore, as the next step, the optimum microstructure of the HAp/collagen complex fibers should be investigated by the parametric study.

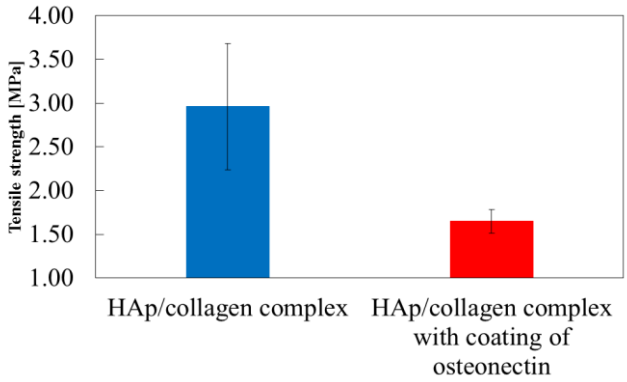


Figure 6. Comparison on tensile strength of collagen fibers with EDC of 5 [mmol/L] with and without coating of osteonectin after dipping into calcium solution and phosphorus solution.

3. Conclusions

We can summarize the obtained results as follows.
1. The EDC concentration of 5 mmol/L maximized the tensile strength of the collagen fibers, suggesting that the EDC concentration, where the fibrosis and cross-linking induce a synergistic effect, could maximize the tensile strength of collagen fibers.
2. The longer HAp deposition induced the higher tensile strength of the HAp/collagen complex fibers, within the range covered in this paper.
3. The HAp deposition by the alternating immersion method after corting with the adhesion protein decreased the tensile strength of the HAp/collagen complex fibers, within the range covered in this paper. This means that the individual optimization of the microstructural factors would not necessarily maximize the tensile strength of the HAp/collagen complex fibers.

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