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Design of Collagen-Based Hemostatic Material for Use in Plastic and Reconstructive Surgery

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Abstract

This study aims to develop collagen-based hemostatic materials. The sheet-shaped collagen sponge was manufactured by freeze-vacuum drying the aqueous solution of collagen (Col) and heat-denatured collagen (Col') at a composition ratio of 2/1. The both sides or one side of sponge was treated with ultraviolet (UV) irradiation for 15 minutes to introduce intermolecular crosslinks between collagen molecules. The elution behavior of collagen sponge was investigated by immersing the sponge in water for a predetermined time and then by measuring the weight change. The double-sided UV-irradiated sponge showed very slow elution properties. On the other hand, the single-sided UV-irradiated sponge showed initially fast elution and subsequent very slow elution properties. Such initially fast elution of collagen molecules from the surface without UV-irradiation allows an adhesion of collagen sponge to the wound surface and results in hemostatic effect. In addition, the water absorption and retention properties of sponge were investigated by placing the hydrated sponge on a mesh for a predetermined time and then by measuring the weight change. The double-sided UV-irradiated sponge absorbed 81 times more water than own weight and showed a value of 45 times even after 7 days. The single-sided UV-irradiated sponge absorbed 80 times more water than own weight and showed a value of 39 times even after 7 days. The sponge with high water absorption and retention properties allows a wound healing effect because such sponge can absorb large amounts of blood plasma and exudates containing various cell growth factors. The double-sided UV-irradiated sponge is a good candidate for the wound dressing. On the other hand, the single-sided UV-irradiated sponge is a good candidate for the hemostatic material.

Keywords

Sheet-Shaped Collagen Sponge, Hemostatic Material, Water Absorption and Retention Property, Hemostatic Effect, Wound Healing Effect

1. Introduction

The design of hemostatic material and wound dressing needs to understand the mechanism of wound healing [1]. In general, the events of wound healing are divided into four phases: vascular response, blood coagulation, inflammation and new tissue formation. As medical products related to blood coagulation, several types of hemostatic materials are commercially available. A typical hemostatic material is a fibrin adhesive. By the action of coagulation factors, fibrinogen is converted to fibrin and exerts tissue adhesion to stop bleeding. Another typical hemostatic material is sponge-shaped, sheet-shaped or cotton-like collagen. Platelets are activated when they come into contact with collagen. Activated platelets promote platelet aggregation to form platelet thrombus. In addition, activated platelets release coagulation factors. By the action of these factors, fibrinogen is converted to fibrin and exerts a hemostatic effect. These collagen-based hemostatic materials provide a place for blood coagulation and exert a hemostatic effect. This hemostatic material is made from the collagen derived from cowhide that has the original fiber structure. Unfortunately, the antigenic determinants of the collagen molecule have not been removed. Other hemostatic materials are gelatin sponge and cotton-like oxidized cellulose. These materials do not have a direct hemostatic effect. These materials absorb blood in the sponge or cotton-like structure and cause blood clotting. Commercially available hemostatic materials are designed to promote blood coagulation, but not designed to promote new tissue formation.

Regarding new tissue formation, several types of wound dressings composed of collagen (Col) have been developed and used in the field of plastic and reconstructive surgery [2] [3] [4]. One of the excellent wound dressings is the sheet-shaped collagen sponge with a silicone film attached to the top surface [5]. This wound dressing is manufactured by freeze-vacuum drying a mixed aqueous solution of fibrous Col and heat-denatured collagen (Col'), followed by thermal dehydration to introduce intermolecular crosslinks between collagen molecules. In the treatment of severe burns, this wound dressing is applied to deep tissue defect wounds after excision of necrotic tissue. The treatment with this wound dressing can form excellent transplant beds for autologous split-thickness skin grafts. In addition, several types of collagen-based wound dressings and cultured skin substitutes have been used to prepare the wound bed for autologous split-thickness skin grafts [6] [7]. However, the importance of treatment for the wound surface caused by collecting autologous split-thickness skin has not been noticed. Such a shallow wound is thought to heal with regular wound dressings despite a lot of

bleeding. It is an important task to cleanly heal such a wound surface. Exactly, it is necessary to develop a covering material that has a hemostatic effect and a wound healing effect.

It is well known that Col has an excellent hemostatic effect and an excellent wound healing effect [8] [9]. In the previous study [10], we designed collagen-based sponge devices for use in the field of oral surgery. These devices are the sheet-shaped collagen sponge to cover mucosal and gingival small tissue defect wounds. These sponges are manufactured by freeze-vacuum drying a mixed aqueous solution of Col and Col', followed by UV irradiation to introduce intermolecular crosslinks between collagen molecules. The results of previous study showed that the sheet-shaped sponge composed of Col and Col' with a composition ratio of 2/1 is the top candidate for the devises used in oral surgery. Based on these findings, we designed a large size sheet-shaped collagen sponge having a hemostatic effect and a wound healing effect for use in plastic and reconstructive surgery. Especially we focus on clean healing of bleeding wound surfaces caused by collecting autologous split-thickness skin. In addition, we focus on clean healing of facial trauma caused by traffic accidents and fall accidents.

2. Materials and Methods

2.1. Manufacture of Sheet-Shaped Collagen Sponge

The sheet-shaped collagen sponge was manufactured by the method described in our previous article [10]. Purified granular collagen (Col) derived from porcine skin was purchased from Nippon Meat Packers (Osaka, Japan). Terror peptides of collagen molecule that are antigenic determinants have been removed by enzyme degradation. The sheet-shaped collagen sponge was manufactured as follows. Col (32 g) was dissolved in distilled water (4000 mL) to obtain Col aqueous solution (0.8 w/v%, pH2.8). Aside from this, Col (16g) was dissolved in distilled water (2000 mL) and then heated at 60˚C for 1 hour to obtain heat-denatured collagen aqueous solution (0.8 w/v%, pH2.8). This heat-denatured collagen is referred to as Col'. The Col aqueous solution was mixed with the Col' aqueous solution by stirring and then adjusted to the conditions of pH4.5 by dropping sodium hydroxide aqueous solution. This clear mixture (20 mL) was poured into a plastic tray (80 mm × 50 mm) and refrigerated at 4˚C for 6 hours or more, and then was frozen at −85˚C overnight and followed by freeze-vacuum drying to obtain a sheet-shaped sponge. These sponges are referred to as Sponge-CC'21. The composition ratio of Col and Col' is 2/1. The surface of sponge was irradiated using a 15 W ultraviolet lamp with a wavelength of 253.7 nm at a distance of 20 cm to introduce crosslinks between collagen molecules. Both surfaces of the sponge were treated with UV irradiation for 10, 15 and 20 minutes. They were named as Sponge-CC'21-10x2, Sponge-CC'21-15x2 and Sponge-CC'21-20x2. These are double-sided UV-irradiated sponges. One surface of the sponge was treated with UV irradiation for 10, 15 and 20 minutes. They were named as Sponge-CC'21-10x1, Sponge-CC'21-15x1 and Sponge-CC'21-20x1. These are single-sided

UV-irradiated sponges.

2.2. Weight Change of Collagen Sponge When Immersed in Water

The weight change of Sponge-CC'21 was investigated as follows. The sponge was immersed in a plastic container filled with distilled water (200 mL) and then this container was placed in an incubator at 37˚C for a predetermined time. The hydrated sponges were collected using a 10-mesh size stainless steel mesh after immersion in water for 3 hour, 6 hour, 1 day, 3 days, 5 days and 7 days and then returned to a plastic tray (80 mm × 50 mm). These hydrated sponges were frozen at −85˚C overnight and followed by freeze-vacuum drying to obtain dry sponges. The weight of the dry sponge was measured and compared with the original weight before immersion in water. This experiment was performed using 8 containers. The average value was calculated by measuring the weight of each dry sponge collected from each container.

2.3. Water Absorption and Retention Properties of Collagen Sponge

The water absorption and retention properties of Sponge-CC'21 were investigated as follows. The sponge was immersed in a plastic container filled with distilled water (200 mL) at 37˚C. After 10 minutes, the hydrated sponge was collected using a 10-mesh size stainless steel mesh and then placed on this mesh for 30 minutes to remove excess water and then weighed. This hydrated sponge placed on the mesh was put in the plastic container and then this container was placed in an incubator at 37˚C. The hydrated sponge placed on the mesh was taken out as it was and weighed at predetermined time intervals. This experiment was performed using 8 containers. The average value was calculated by measuring the weight of each hydrated sponge.

2.4. Enzymatic Degradation of Collagen Sponge by Collagenase

Collagenase powder was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The enzymatic degradation of Sponge-CC'21 by collagenase was investigated as follows. Collagenase (32 mg or 64 mg) was dissolved in distilled water (1600 mL). The enzyme concentration was adjusted to 0.002 w/v% or 0.004 w/v%. The weight change of each sponge was investigated by immersing a sponge in each plastic container filled with distilled water containing collagenase (200 mL) and then placing it in an incubator at 37˚C for a specified period of time. These hydrated sponges were collected using a 10-mesh size stainless steel mesh after immersion for 3 days and then returned to a plastic tray (80 mm × 50 mm). These hydrated sponges were frozen at −85˚C overnight and followed by freeze-vacuum drying to obtain dry sponges. The weight of dry sponge was measured and compared with its original weight before immersion in water. This experiment was performed using 8 containers. The average value was calculated by measuring the weight of each dry sponge collected from each container.

2.5. Statistical Evaluation

Data were expressed as means ± standard error. Statistical analysis was performed using Student's t-test for comparison between two groups. Each experiment was performed 8 times to examine statistically significant differences in the measured values (n = 8).

3. Result

3.1. Manufacture of Sheet-Shaped Collagen Sponge

The important key in the manufacturing process is how to prepare a clear mixed aqueous solution of Col and Col' [10]. The Col aqueous solution (pH 2.8) precipitates when the pH value reaches 4 under 25˚C conditions. On the other hand, the Col' aqueous solution does not precipitate even in the neutral pH range. The mixed aqueous solution of Col and Col' with a composition ratio of 2/1 partially precipitates when the pH value reaches 5 or higher under 25˚C conditions. Therefore, this mixture was adjusted at pH4.5 to maintain transparency. The sheet-shaped collagen sponge was manufactured by freeze-vacuum drying this clear mixed solution. The sponge has a size of 80 mm × 50 mm and a thickness of 3 mm. The both sides or one side of sponge was treated with UV irradiation. The sponge slightly turns yellow when the UV irradiation time is 20 minutes or more. The shape of collagen sponge does not change when immersed in water (**Figure 1(A)**). The hydrated sponge has moderate physical properties that can be easily collected using a stainless steel mesh and placed on the mesh (**Figure 1(B)**). Such properties are suitable for use as a hemostatic material and a wound dressing. It is an advantage that the sheet-shaped collagen sponge can maintain its hydrated shape when placed on the wound surface.

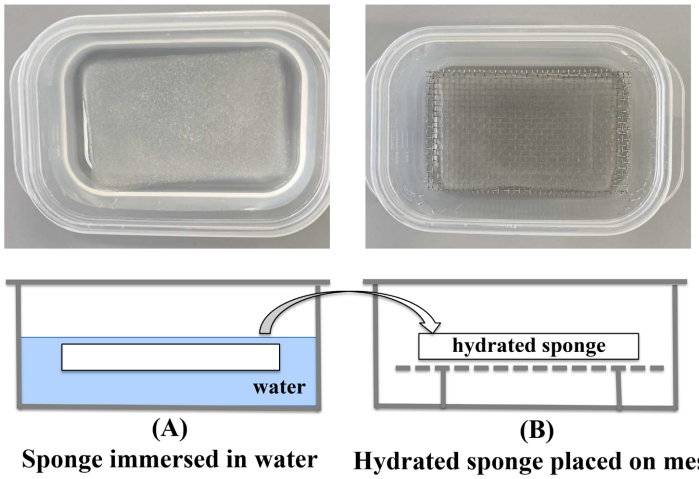

Figure 1. Experimental system for measuring weight change and water absorption and water retention of collagen sponge: (A) Sheet-shaped sponge was immersed in water for a predetermined time and then the weight of hydrated sponge was measured. This experiment was performed using 8 containers (n = 8). (B) Hydrated sponge was placed on a 10-mesh size stainless steel mesh and weighed at predetermined time intervals. This experiment was performed using 8 containers.

3.2. Weight Change of Collagen Sponge When Immersed in Water

It is important that collagen sponge is biodegraded and absorbed by the time new tissue formation is completed. As an in vitro experiment, the weight change of collagen sponge when immersed in water was investigated. The weight change of collagen sponge when immersed in water is related to the degree of crosslinks between collagen molecules. The degree of crosslinks is related to UV irradiation time. Fist, the weight change of the collagen sponge treated with UV irradiation for 10 minutes was examined. The weight change rate of Sponge-CC'21-10x2 measured after immersion in water for 3 hours, 6 hours, 1, 3, 5 and 7 days was 90%, 86%, 82%, 79%, 78% and 74% of the original weight before immersion in water, respectively. On the other hand, the weight change rate of Sponge-CC'21-10x1 measured after immersion in water for 3 hours, 6 hours, 1, 3, 5 and 7 days was 86%, 79%, 71%, 57%, 54% and 54% of the original weight before immersion in water, respectively (Figure 2). Second, the weight change of the collagen sponge treated with UV irradiation for 15 minutes was examined. The weight change rate of Sponge-CC'21-15x2 measured after immersion in water for 3 hours, 6 hours, 1, 3, 5 and 7 days was 91%, 86%, 85%, 83%, 80% and 77% of the original weight before immersion in water, respectively. On the other hand, the weight change rate of Sponge-CC'21-15x1 measured after immersion in water for 3 hours, 6 hours, 1, 3, 5 and 7 days was 88%, 78%, 75%, 69%, 65% and 63% of the original weight before immersion in water, respectively (Figure 3). Third, the weight change of the sponge treated with UV irradiation for 20 minutes was examined. The weight change rate of Sponge-CC'21-20x2 measured after immersion in water for 3 hours, 6 hours, 1, 3, 5 and 7 days was 88%, 88%, 86%, 83%, 82% and 79% of the original weight before immersion in water, respectively. On the other hand, the weight change rate of Sponge-CC'21-20x1 measured

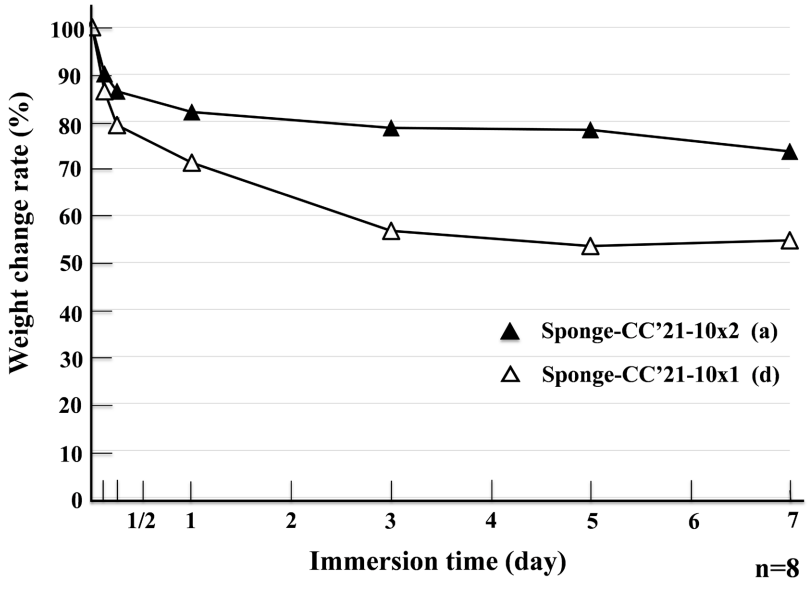

Figure 2. Weight change rate (%) of Sponge-CC'21 when immersed in water: (▲) Sponge-CC'21-10x2 (a); (△) Sponge-CC'21-10x1 (d).

after immersion in water for 3 hours, 6 hours, 1, 3, 5 and 7 days was 85%, 82%, 66%, 59%, 55% and 52% of the original weight before immersion in water, respectively (Figure 4).

The double-sided UV-irradiated sponge showed very slow elution properties. On the other hand, the single-sided UV-irradiated sponge showed initially fast elution and subsequent very slow elution properties. This phenomenon is considered to be due to the initially fast elution of collagen molecules from the surface without UV-irradiation. This fast elution of collagen molecules allows adhesion of the collagen sponge to the wound surface and results in hemostatic effect.

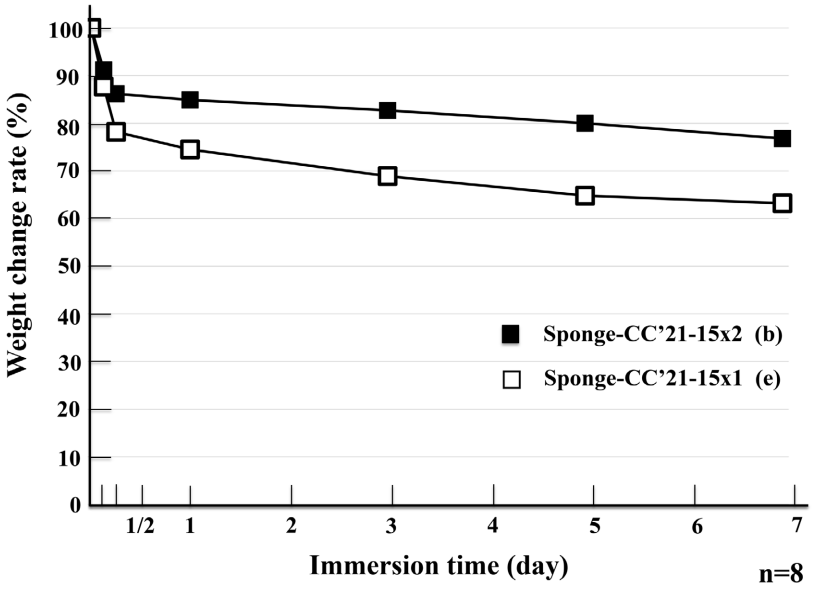

Figure 3. Weight change rate (%) of Sponge-CC'21 when immersed in water: (■) Sponge-CC'21-15x2 (b); (□) Sponge-CC'21-15x1 (e).

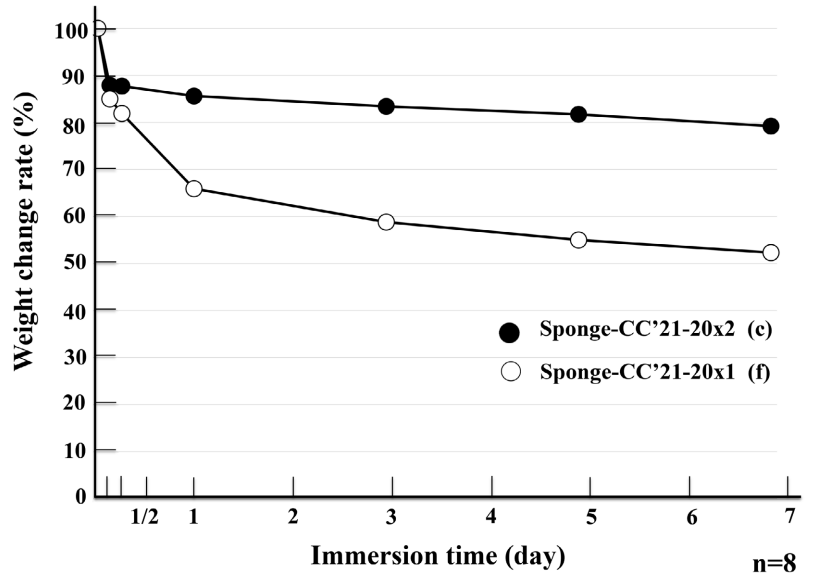

Figure 4. Weight change rate (%) of Sponge-CC'21 when immersed in water: (●) Sponge-CC'21-20x2 (c) (○) Sponge-CC'21-20x1 (f).

In case of double-sided UV-irradiated sponges, the crosslinks between collagen molecules was successfully introduced with UV irradiation time of 10 minutes or more. The weight change rate of Sponge-CC'21-15x2 was 77% of the original weight even after immersion in water for 7 days. In case of single-sided UV-irradiated sponges, the crosslinks between collagen molecules was best introduced with a UV irradiation time of 15 minutes. The weight change rate of Sponge-CC'21-15x1 was 63% of the original weight even after immersion in water for 7 days (Figure 5). On the other hand, the weight change rate of Sponge-CC'21-20x1 was 52% of the original weight after immersion in water for 7 days. Optimal UV irradiation time is required because UV irradiation induces intermolecular crosslinks but causes molecular damage.

3.3. Water Absorption and Retention Properties of Collagen Sponge

It is important that the collagen sponge has high water absorption and retention properties. In clinical use, it is a great advantage to be able to absorb a large amount of blood plasma and exudates containing various cell growth factors. In addition to that, being able to maintain a moist environment is also a great advantage to promote wound healing. For this reason, the water absorption and retention properties of Sponge-CC'21 were examined as follows. The hydrated sponge was placed on a 10-mesh size stainless steel mesh and then its weight was measured at predetermined time intervals. First, the weight of hydrated Sponge-CC'21-10x2 was 79, 54, 52, 50, 40 and 25 times that of the dry sponge after 30 minutes, 1, 2, 3, 5 and 7 days, respectively. The weight of hydrated Sponge-

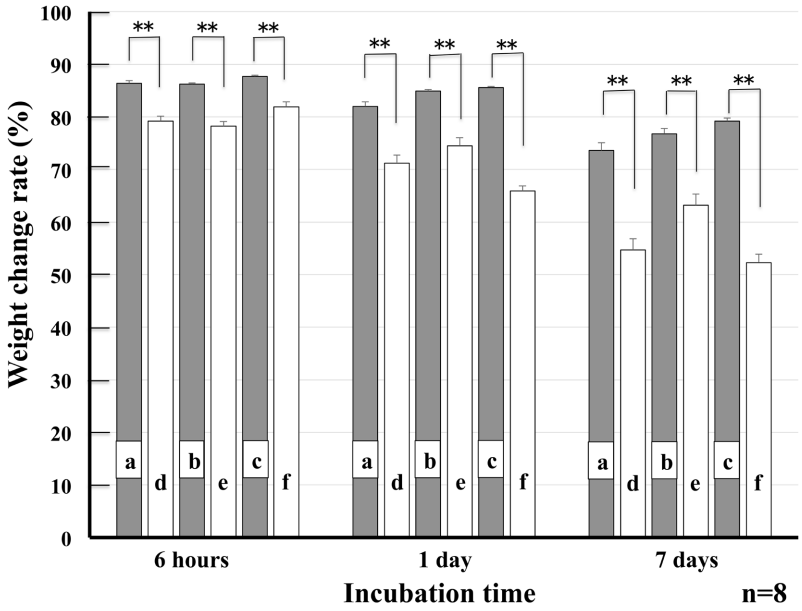

Figure 5. Comparison of weight change rate (%) of double-sided UV-irradiated sponges and single-sided UV-irradiated sponges: (a) Sponge-CC'21-10x2 (d) Sponge-CC'21-10x1; (b) Sponge-CC'21-15x2 (e) Sponge-CC'21-15x1; (c) Sponge-CC'21-20x2 (f) Sponge-CC'21-20x1; **P < 0.01 [Student's t-test].

CC'21-10x1 was 80, 46, 39, 33, 26 and 19 times that of the dry sponge after 30 minutes, 1, 2, 3, 5 and 7 days, respectively (Figure 6). Second, the weight of hydrated Sponge-CC'21-15x2 was 81, 63, 62, 60, 56 and 45 times that of the dry sponge after 30 minutes, 1, 2, 3, 5 and 7 days, respectively. The weight of hydrated Sponge-CC'21-15x1 was 80, 55, 51, 47, 43 and 39 times that of the dry sponge after 30 minutes, 1, 2, 3, 5 and 7 days, respectively (Figure 7). Third, the weight of hydrated Sponge-CC'21-20x2 was 83, 62, 57, 55, 52 and 47 times that of the dry sponge after 30 minutes, 1, 2, 3, 5 and 7 days, respectively. The weight of hydrated Sponge-CC'21-20x1 was 83, 55, 50, 46, 41 and 35 times that of the dry sponge after 30 minutes, 1, 2, 3, 5 and 7 days, respectively (Figure 8).

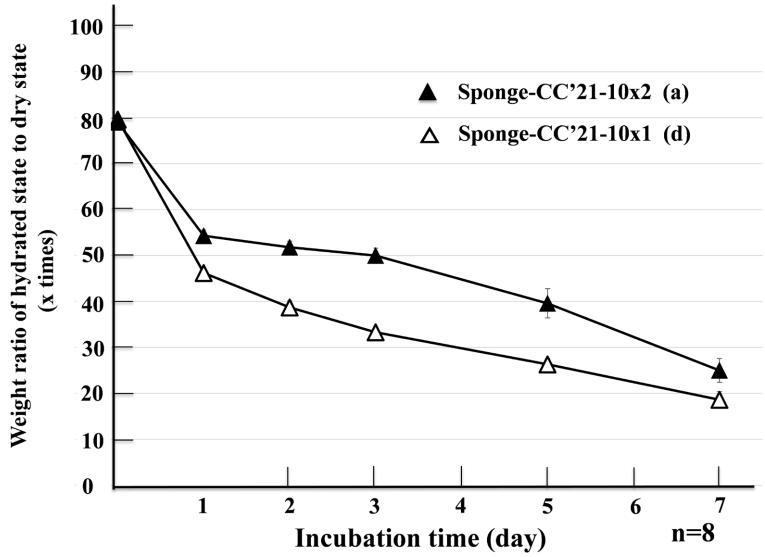

Figure 6. Weigh ratio of hydrated state to dry state of sponges (x times): (▲) Sponge-CC'21-10x2 (a); (△) Sponge-CC'21-10x1 (d).

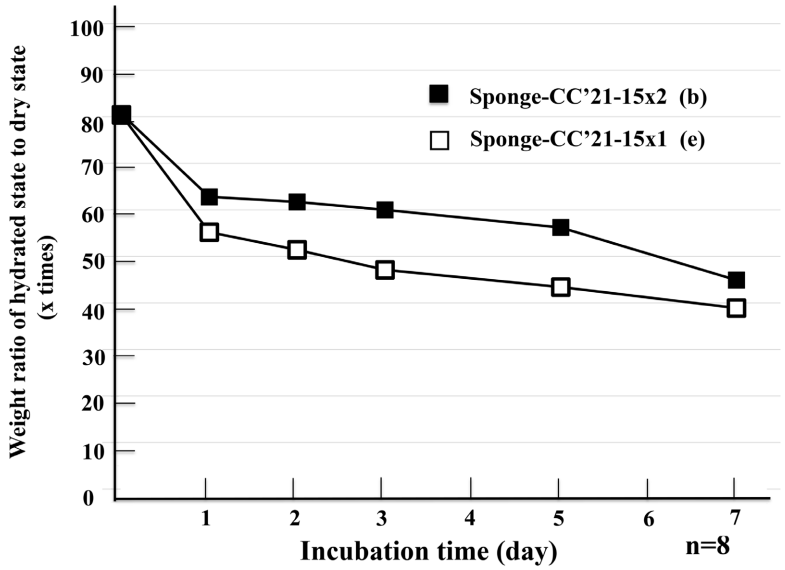

Figure 7. Weigh ratio of hydrated state to dry state of sponges (x times): (■) Sponge-CC'21-15x2 (b); (□) Sponge-CC'21-15x1 (e).

The single-sided UV-irradiated sponges partially dissolve in the water absorbed in the sponge and flow out under the mesh. The single-sided UV-irradiated sponges showed a lower value in water retention than the double-sided UV-irradiated sponges. Despite such collagen elution, the weight of hydrated Sponge-CC'21-15x1 was 39 times that of the dry sponge even after 7 days (Figure 9). Such high water absorption and long-term water retention properties are suitable for use as a covering material with a hemostatic effect and a wound healing effect.

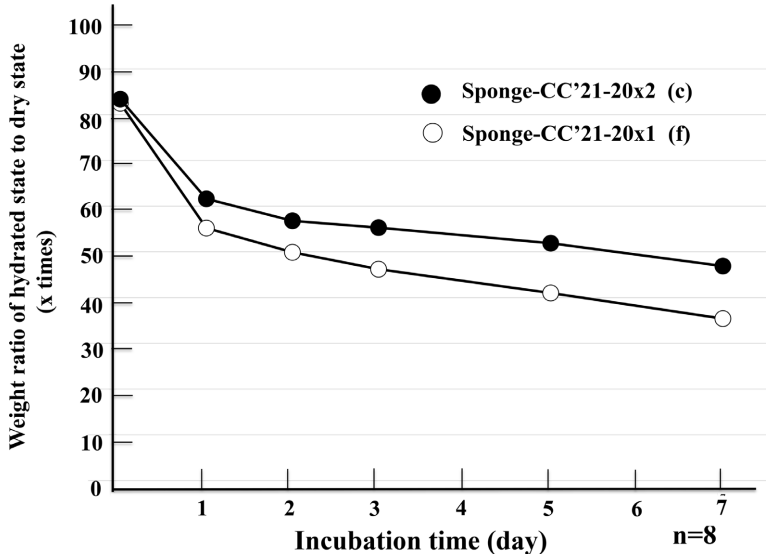

Figure 8. Weigh ratio of hydrated state to dry state of sponges (x times): (●) Sponge-CC'21-20x2; (c) (○) Sponge-CC'21-20x1 (f).

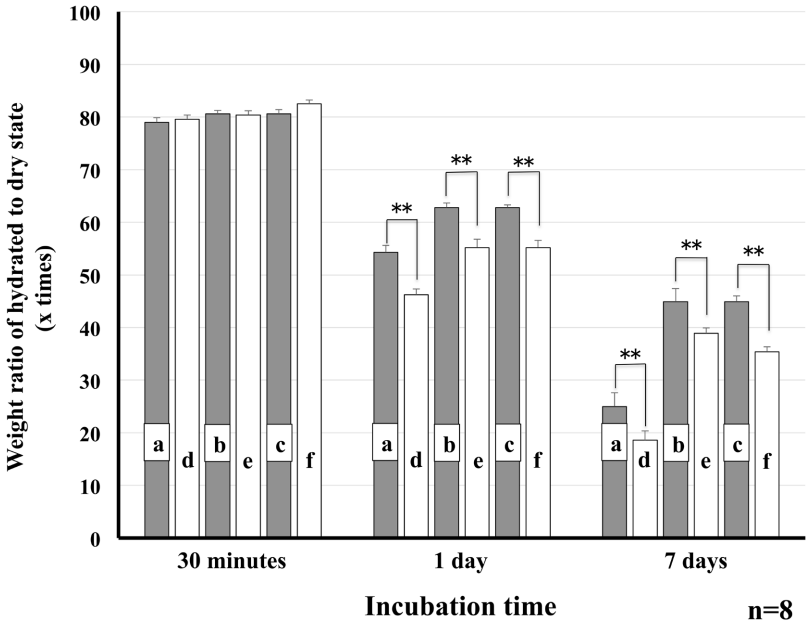

Figure 9. Comparison of weight ratio of hydrated state to dry state of sponge (x times): (a) Sponge-CC'21-10x2; (b) Sponge-CC'21-15x2; (c) Sponge-CC'21-20x2; (d) Sponge-CC'21-10x1; (e) Sponge-CC'21-15x1; (f) Sponge-CC'21-20x1; **P < 0.01 [Student's t-test].

3.4. Enzymatic Degradation of Sponge by Collagenase

It is important that collagen sponge is biodegraded and absorbed by the time new tissue formation is completed. As an in vitro experiment, the enzymatic degradation properties of collagen sponge by collagenase were investigated (Figure 10). As comparison data, the weight change rates of Sponge-CC'21-15x2 and Sponge-CC'21-15x1 when immersed in water for 3 day were 83% and 69% of the original weight, respectively. On the contrary, the weight change rates of Sponge-CC'21-15x2 and Sponge-CC'21-15x1 when immersed in water containing collagenase (0.002 w/v%) for 3 day were 81% and 59% of the original weight, respectively. In addition, the weight change rates of Sponge-CC'21-15x2 and Sponge-CC'21-15x1 when immersed in water containing collagenase (0.004 w/v%) for 3 day were 80% and 53% of the original weight, respectively. These results suggest that the intermolecular crosslinks in these collagen sponges can effectively delay enzymatic degradation by collagenase. Such biological properties are suitable for as a covering material with a hemostatic effect and a wound healing effect. It is expected that Sponge-CC'21-15x2 and Sponge-CC'21-15x1 is biodegraded and absorbed by the time new tissue formation is completed.

4. Discussion

In the previous study [10], we designed collagen-based sponge devices for use in the field of oral surgery. These sponges are the small size sheet-shaped collagen

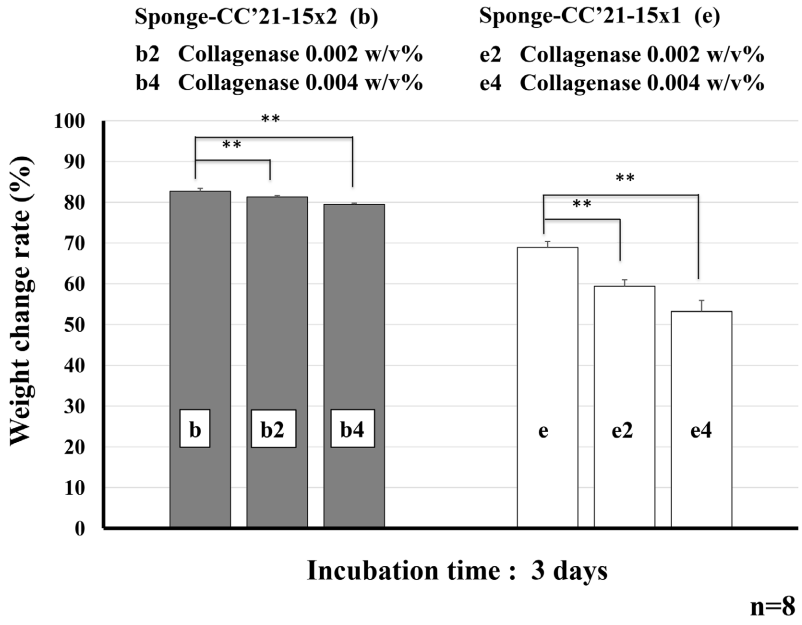

Figure 10. Enzymatic degradation behavior of sponge by collagenase: Group1: Weight change rate (%) of sponge when immersed in water for 3 days: (b) Sponge-CC'21-15x2; (e) Sponge-CC'21-15x1; Group2: Weigh change rate (%) of sponge when immersed in water contacting collagenase (0.002 w/v%) for 3 days: (b2) Sponge-CC'21-15x2; (e2) Sponge-CC'21-15x1; Group 3: Weigh change rate of sponge when immersed in water contacting collagenase (0.004 w/v%) for 3 days: (b4) Sponge-CC'21-15x2 (e4) Sponge-CC'21-15x1. **P < 0.01 [Student's t-test].

sponge to cover mucosal and gingival defect wounds in oral surgery. In this study, we designed a large size sheet-shaped collagen sponge for use in the field of plastic and reconstructive surgery. The target is the sheet-shaped collagen sponge to cover acute wounds. In the treatment of severe burns, autologous split-thickness skin grafting is performed. The treatment of the wound surface caused by collecting split-thickness skin requires a covering material that has a hemostatic effect and a wound healing effect. Another target is the sheet-shaped collagen sponge to cover outer surface wounds. Multiple trauma injuries are mainly due to traffic accidents and fall accidents and often associated with facial trauma. The treatment of these facial wounds also requires a covering material that has a hemostatic effect and a wound healing effect. What should be emphasized is the following. The wound healing process should proceed smoothly and result in minimal scar formation. The most important task is to heal the wound cleanly. Exactly, it is necessary to develop a covering material that has a hemostatic effect and a wound healing effect.

The method of introducing crosslinks between collagen molecules by UV irradiation allows the design of unique structures. When both surfaces of sheet-shaped sponge are treated with UV irradiation, the both outer layer is sufficiently cross-linked, while the intermediate layer is insufficiently cross-linked because UV rays do not reach the intermediate layer. When only upper surface of sponge is irradiated with UV, the upper layer is sufficiently cross-linked, but the lower layer is not cross-linked.

In this study, we investigated the characteristics of the double-sided UV-irradiated collagen sponge and the single-sided UV-irradiated collagen sponge. First, the elution properties of collagen molecules from the sponge were examined. It is thought that some collagen molecules in the intermediate layer gradually dissolved in water when the double-sided UV-irradiated sponge was immersed in water. As a typical example, the weight change rate of Sponge-CC'21-15x2 was 77% of the original weight even after immersion in water for 7 days. This sponge showed very slow elution properties. From such characteristics, Sponge-CC'21-15x2 is considered to be suitable as a wound dressing. On the other hand, it is thought that collagen molecules in the lower layer easily dissolved in water when the single-sided UV-irradiated sponge was immersed in water. Despite such elution of collagen molecules, the weight change rate of Sponge-CC'21-15x1 was 63% of the original weight even after immersion in water for 7 days. Such elution properties are due to sufficient cross-linking of the upper layer. This sponge showed the initially fast elution of collagen molecules from the sponge surface without UV-irradiation. This fast elution of collagen molecules allows adhesion of the collagen sponge to the wound surface and results in hemostatic effect. From such characteristics, Sponge-CC'21-15x1 is considered to be suitable as a hemostatic material. When the single-sided UV-irradiated collagen sponge is attached on a wound surface, a part of collagen sponge can dissolve quickly in blood plasma and exudates and results in adhesiveness onto a wound surface. At

the same time, a fibrin network is formed, resulting in a hemostatic effect (Figure 11).

Second, the water absorption and retention properties of the collagen sponges were examined. The double-sided UV-irradiated collagen sponges and the single-sided UV-irradiated collagen sponges can absorb about 80 times more water than the own weight. As a typical example, the weight of hydrated Sponge-CC'21-15x2 was 45 times that of the dry sponge even after placing on the mesh for 7 days. Similarly, the weight of hydrated Sponge-CC'21-15x1 was 39 times that of the dry sponge even after placing on the mesh for 7 days. These high water absorption and long-term water retention properties are suitable for use as a covering material with a hemostatic effect and a wound healing effect.

Third, the biological properties of the collagen sponges were examined by immersing a sponge in water containing collagenase under the condition of 0.002 w/v% and 0.004 w/v%. The weight of Sponge-CC'21-15x2 reduced slightly. On the other hand, the weight of Sponge-CC'21-15x1 reduced at a moderate rate. As a basic design, the sheet-shaped collagen sponge should be biodegraded by the time new tissue formation is completed. Too slow biodegradation is a physical obstacle to new tissue formation. The double-sided UV-irradiated collagen sponge is thought to be able to maintain its structure for 2 weeks or more in clinical use. Therefore, Sponge-CC'21-15x2 is considered to be suitable as a wound dressing. The single-sided cross-linked collagen sponge allows initially fast elution of collagen molecules from the surface without UV irradiation. However, this sponge is thought to be able to maintain its structure for at least a week in clinical use. Therefore, Sponge-CC'21-15x1 is considered to be suitable as a hemostatic material. In actual use, the single-sided UV-irradiated collagen sponge is used to attach the surface without UV irradiation to the wound surface.

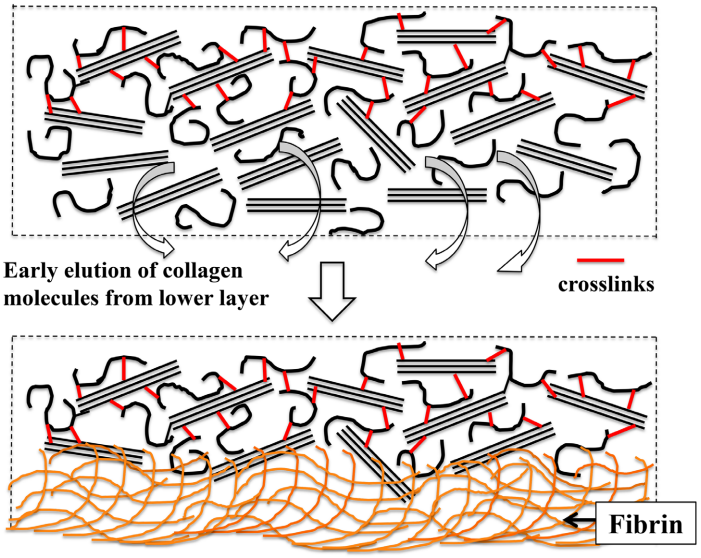

Figure 11. Schematic diagram of single-sided UV-irradiated sheet-shaped sponge: non-cross-linked collagen molecules in the lower layer elute first, and then the fibrin network structure is formed.

In case of the double-sided UV-irradiated collagen sponge, both sides can be applied to the wound surface. The conventional treatment is to attach the sheet-shaped collagen sponge to the wound surface and fix it with conventional non-stickiness gauze.

More effective treatment can be considered. In the previous study [11], we examined the properties of the sheet-shaped hyaluronic acid (HA) sponge containing epidermal growth factor (EGF). In the experiment of the previous study, the cultured dermal substitute was prepared by incorporating human skin fibroblasts into collagen gels. To make a wound surface model, this cultured dermal substitute was elevated to the air-culture medium interface. The HA sponge containing EGF was placed on this wound surface model. The incorporated EGF increased the production of vascular endothelial growth factor and hepatocyte growth factor from fibroblasts in the cultured dermal substitute. Both growth factors have the effect of promoting wound angiogenesis. In addition to that, EGF itself has the effect of promoting epidermis formation [12]. In addition to them, HA itself has a wound healing effect [13]. The more effective treatment is to attach the sheet-shaped collagen sponge such as Sponge-CC'21-15x2 to the wound surface, and attach a HA sponge containing EGF on it, and then fix these sponges with conventional non-stickiness gauze. We look for such a more effective treatment in the next study.

5. Conclusion

The sheet-shaped collagen sponge was manufactured by freeze-vacuum drying the aqueous solution of Col and Col' at a composition ratio of 2/1. Double-sides or single-side of the sponge was treated with UV irradiation for 15 minutes to introduce crosslinks between collagen molecules. As a basic design, the sheet-shaped collagen sponge should be biodegraded and absorbed by the time new tissue formation is completed in clinical use. The double-sided UV-irradiated collagen sponge, Sponge-CC'21-15x2 is a good candidate for the wound dressing to cover a deep wound surface. On the other hand, the single-sided UV-irradiated collagen sponge, Sponge-CC'21-15x1 is a good candidate for the hemostatic material to cover a shallow wound surface with significant bleeding.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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