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Design of Novel Wound Dressing Composed of Collagen and Hyaluronic Acid Containing Epidermal Growth Factor

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Abstract

This research aims to develop a wound dressing composed of collagen (Col) and hyaluronic acid (HA) containing epidermal growth factor (EGF). First important issue is to contain EGF in the wound dressing in a stable state. The sheet-shaped sponge was manufactured by freeze-vacuum drying an aqueous solution of Col. Both sides of sponge were treated with ultraviolet (UV) irradiation to introduce intermolecular cross links between collagen molecules. This sponge was named Sponge-Col. Another sheet-shaped sponge was manufactured by freeze-vacuum drying an aqueous solution of HA containing EGF. This sponge was named Sponge-HA/EGF. The wound dressing was manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom to create a sandwich structure. This method can prevent the reducing of EGF activity due to UV irradiation for intermolecular cross-linking. Second important issue is to enable gradual release of EGF from the wound dressing. The elution behavior of this wound dressing was investigated by measuring the weight change after immersion in water for a predetermined time. This wound dressing showed initially fast elution and subsequent very slow elution properties. The upper layer and lower layer Sponge-Col enabled gradual release of the middle layer Sponge-HA/EGF. This result suggests that EGF contained in the wound dressing is gradually released together with HA from the wound dressing. Third important issue is to provide moist wound-healing environment. The upper layer and lower layer Sponge-Col can provide the wound dressing with high water absorption and long-term water retention properties.

Keywords

Collagen, Hyaluronic Acid, Epidermal Growth Factor, Wound Dressing

1. Introduction

The practical design of wound dressing needs to understand the general wound healing process [1]. The key function of wound dressing is to promote the healing process under the impaired conditions such as burn injury and intractable skin ulcer. The superior function of wound dressing depends on the properties of material itself [2] [3] [4]. Hyaluronic acid (HA) is identified as a major extracellular component having the characteristics such as unique hygroscopic, rheological and viscoelastic properties. HA creates an excellent environment for wound healing. HA is an important biomaterial for wound healing, because it has various biological activities [5] [6]. Collagen (Col) is a primary connective tissue component providing strength and integrity to all tissues. Col is also an important biomaterial for wound healing, because it has various biological activities [7]. It is well known that growth factors accelerate wound healing by regulating various cell functions such as proliferation, differentiation and migration [8] [9]. It has been reported that endogenous levels of growth factors are reduced in some chronic wounds [10]. To improve this situation, the potential benefits of exogenous growth factors in the treatment of wounds have been emphasized. The combined use of wound dressing and growth factor has great advantages. It is important how to stably contain growth factor in the wound dressing. It is also important how to gradually release growth factor from the wound dressing.

As the first study [11], we have developed wound dressing composed of HA containing epidermal growth factor (EGF) [11]. EGF is a potent stimulator of cell proliferation of various cells including keratinocytes, fibroblasts, and vascular endothelial cells [12] [13]. The sponge of HA containing EGF dissolves in water. Therefore, it was needed to improve for better utility. As the second study [14] [15], the wound dressing was manufactured by freeze-vacuum drying an aqueous solution of Col and HA containing EGF, followed by UV irradiation to introduce crosslinks between collagen molecules. The efficacy of this wound dressing was evaluated in clinical trials. Most clinical cases were assessed as having good or excellent results [16]. The manufacturing process of this wound dressing is very practical. However, this method needs some improvement, because EGF becomes less effective to some extent due to UV irradiation in the process of introducing intermolecular crosslinks between collagen molecules. It is necessary to improve for better utility.

In this study, we designed a sandwich structure consisting of three layers of sheet-shaped sponge. The sheet-shaped sponge was manufactured by freeze-vacuum drying an aqueous solution of Col. Both sides of sponge were treated with UV irradiation to introduce intermolecular crosslinks between collagen

molecules. This was named Sponge-Col. Aside from this, another sheet-shaped sponge was manufactured by freeze-vacuum drying an aqueous solution of HA containing EGF. This was named Sponge-HA/EGF. The wound dressing was manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom to make a sandwich structure. This method can prevent the reducing of EGF activity due to UV irradiation for intermolecular cross-linking. The main purpose of this study is to investigate whether the upper layer and lower layer Sponge-Col can delay the elution of the middle layer Sponge-HA/EGF when immersed in water.

2. Materials and Methods

2.1. Manufacture of Col Sponge

The sheet-shaped Col sponge was manufactured by the method described in our previous paper [17] [18]. The method was briefly described below. 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. Col (25.6 g) was dissolved in distilled water (3200 mL) to obtain Col aqueous solution (0.8 w/v%, pH2.8 at 25˚C). Aside from this, Col (12.8 g) was dissolved in distilled water (1600 mL) and then heated at 60˚C for 1 hour, and then cooled to room temperature to obtain heat-denatured collagen aqueous solution (0.8 w/v%, pH2.8 at 25˚C). This heat-denatured collagen was named Col'. Both aqueous solutions were mixed and then adjusted to the conditions of pH4.5 by dropping sodium hydroxide aqueous solution. The composition ratio of Col and Col' is 2/1. This clear mixture (30 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. Both surfaces of the sponge were irradiated using a 15 W ultraviolet lamp with a wavelength of 253.7 nm for 15 minutes at a distance of 20 cm to introduce intermolecular crosslinks between collagen molecules. This sponge was named Sponge-Col.

2.2. Manufacture of HA Sponge with EGF

The sheet-shaped HA sponge containing EGF was manufactured by applying the method described in our previous paper [11]. The method was briefly described below. HA powder (Bio Sodium Hyaluronate SF 20, molecular weight; 2000 kDa) was purchased from SHIN-EI CHEMICAL CO. LTD (Osaka, Japan). Recombinant human EGF was purchased from Shanghai Haohai Biological Technology (Shanghai, China). HA powder (32 g) was dissolved in distilled water (2000 mL) under stirring using a screw propeller to obtain HA aqueous solution (1.6 w/v%, pH 6.8 at 25˚C). This HA aqueous solution was treated in an autoclave at 105˚C for 10 min, and then cooled to room temperature to obtain sterilized high molecular weight HA solution. Aside from this, HA powder (6.4 g)

was dissolved in distilled water (400 mL) under stirring using a screw propeller to obtain HA aqueous solution (1.6 w/v%, pH 6.8 at 25˚C). This HA aqueous solution was treated in an autoclave at 120˚C for 60 min, and then cooled to room temperature to obtain sterilized low molecular weight HA solution. Both aqueous solutions were mixed. The composition ratio of high molecular weight HA and low molecular weight HA was 5/1. A freeze-dried product of EGF (4000 μg) was dissolved in 20 ml of distilled water. This EGF aqueous solution was sterile filtered and added into the mixed HA aqueous solution. This HA aqueous solution containing EGF (24.1 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 HA sponge containing EGF. This sponge contains EGF at a concentration of 1 μg/cm^2. This sponge was named Sponge-HA/EGF.

2.3. Manufacture of Wound Dressing

During the manufacture of Col sponge by freeze-vacuum drying, the side in contact with air was called the upper surface. This upper surface formed a thin film. On the other hand, the side in contact with the plastic tray was called the lower surface. This lower surface formed a porous sponge structure. Here, an aqueous solution for adhesion was prepared. HA powder (0.5 g) was dissolved in distilled water (50 mL) to obtain HA aqueous solution. This HA aqueous solution was treated in an autoclave at 105˚C for 10 min, and then cooled to room temperature. This viscous aqueous solution (about 0.02 mL) was dotted in 9 places on this upper surface of Col sponge. The positions of the dots were the following 9 places; upper left, upper center, upper right, and middle left, middle center, middle right, and lower left, lower center, lower right. The wound dressing was manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom, and then by lightly pressing these 3 layers of sponge to adhere using HA aqueous solution in 9 dots (**Figure 1**). This three-layered sponge was named C/H/C-wound dressing. As a sample for comparison, another wound dressing was manufactured by laminating Sponge-Col on the top and Sponge-Col on the bottom, and then by lightly pressing these 2 layers of sponge to adhere using HA aqueous solution in 9 dots. This two-layered sponge was named C/C-wound dressing.

2.4. Weight Change of Wound Dressing When Immersed in Water

The weight change of C/H/C-wound dressing was investigated by applying the method described in our previous papers [17] [18]. The C/H/C-wound dressing was placed in a basket made of a 10-mesh size stainless steel mesh and then was immersed in a container filled with distilled water (200 mL) at room temperature. The sponge was completely submerged in water (**Figure 2**). This container was placed in an incubator at 37˚C for a predetermined time. The hydrated sponge placed in a basket was taken out as it is after immersion in water for 1/4,

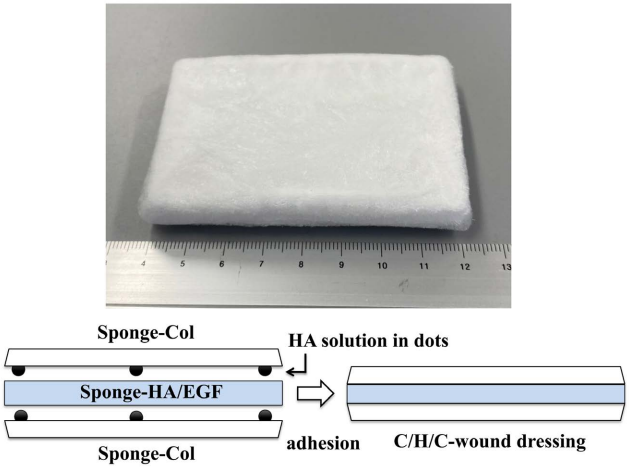

Figure 1. C/H/C-wound dressing manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom to create a sandwich structure: Each sheet-shaped sponge was adhered with HA solution in 9 dots. C/H/C-wound dressing has a size of 80 mm × 50 mm and a thickness of 8 mm.

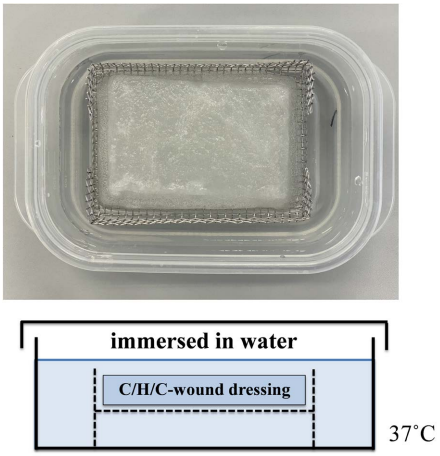

Figure 2. Experimental system for measuring weight change of C/H/C-wound dressing: This wound dressing was immersed in water for a predetermined time, and then freeze-vacuum dried to collect the residual sponge. This shows the wound dressing immersed in water for 7 days.

1, 3, and 7 days. This hydrated sponge was replaced in a plastic tray (80 mm × 50 mm), and then frozen at −85˚C overnight and followed by freeze-vacuum drying to collect the residual dry sponge. The weight of the dry sponge was measured and compared with the original weight before immersion in water. This experiment was performed using 5 containers. The average value was calculated by measuring the weight of each dry sponge. As a comparative experiment, the weight change of C/C-wound dressing was investigated in a similar way.

2.5. Water Absorption and Retention Properties of Wound Dressing

The water absorption and retention properties of C/H/C wound dressing were

investigated by applying the method described in our previous papers [17] [18]. The C/H/C wound dressing was placed in a mesh basket, and thenimmersed in aplastic container filled with distilled water (200 mL) for 30 minutes at room temperature. The sponge was completely submerged in water. This hydrated sponge placed in a mesh basket was taken out as it is, and then placed on a plate for 30 minutes to remove excess water. The weight of hydrated sponge was measured together with a mesh basket. This hydrated sponge placed in a mesh basket was put in a plastic container to keep it in a lifted up state. This container was placed in an incubator at 37˚C. The hydrated sponge placed in a mesh basket was taken out as it is, and then weighed at predetermined time intervals (**Figure 3**). This experiment was performed using 5 containers. The average value was calculated by measuring the weight of each hydrated sponge. As a comparative experiment, the water absorption and retention properties of C/C-wound dressing were investigated in a similar way.

2.6. Enzymatic Degradation of Wound Dressing by Collagenase

Collagenase powder was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The enzymatic degradation of C/H/C-wound dressing by collagenase was investigated by applying the method described in our previous papers [17] [18]. Collagenase (20 mg or 100 mg) was dissolved in distilled water (1000 mL). The enzyme concentration was adjusted to 0.002 w/v% or 0.010 w/v%. The weight change of C/H/C-wound dressing was investigated as follows. The C/H/C-wound dressing was placed in a mesh basket, and then was immersed in a plastic container filled with distilled water containing collagenase (200 mL) at room temperature. The sponge was completely submerged in water. This container was placed in an incubator at 37˚C. The hydrated sponge placed in a mesh basket was taken out as it is after immersion for 3 days. This hydrated

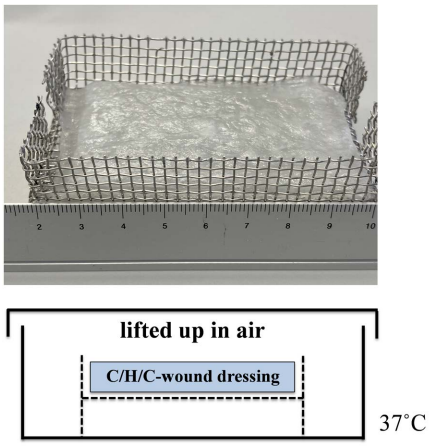

Figure 3. Experimental system for measuring water absorption and water retention of C/H/C-wound dressing: This wound dressing was immersed in water, and placed in a stainless steel mesh basket, and lifted up in air, and then weighed at predetermined time intervals. This shows the hydrated wound dressing lifted up for 7 days.

sponge was replaced in a plastic tray (80 mm × 50 mm), and then frozen at −85˚C overnight and followed by freeze-vacuum drying to collect the residual dry sponge. The weight of the dry sponge was measured and compared with the original weight before immersion in water containing collagenase. This experiment was performed using 5 containers. The average value was calculated by measuring the weight of each dry sponge. As a comparative experiment, the weight change of C/C-wound dressing was investigated in a similar way.

2.7. 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 5 times to examine statistically significant differences in the measured values (n = 5).

3. Result

3.1. Manufacture of Col Sponge

The important key in the manufacturing process is how to prepare a clear mixed aqueous solution of Col and Col' [17] [18]. 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 pH 4.5 to maintain transparency. The sheet-shaped Col sponge was manufactured by freeze-vacuum drying this clear mixed solution (0.8 w/v%). The Col sponge has a size of 80 mm × 50 mm and a thickness of 3 mm. The both sides of sponge were treated with UV irradiation for 15 minutes. The shape of cross-linked Col sponge does not change when immersed in water. The hydrated sponge has moderate physical properties. It can be easily picked up with tweezers. Such physical properties are suitable for use as a wound dressing.

3.2. Manufacture of HA Sponge with EGF

The important key in the manufacturing process is how to prepare a sheet-shaped HA sponge having appropriate physical properties. It is difficult to pour high molecular weight HA aqueous solution into the plastic tray due to its high viscosity. It is difficult to make sponges having appropriate physical properties using low molecular weight HA aqueous solution. In this study, a highly concentrated and moderately viscous HA solution was prepared. The HA aqueous solution (1.6 w/v%) was treated in an autoclave at 105˚C for 10 min and cooled to room temperature. This high molecular weight HA aqueous solution has high viscosity. Aside from this, another HA aqueous solution (1.6 w/v%) was treated in an autoclave at 120˚C for 60 min and cooled to room temperature. This low molecular weight HA aqueous solution has low viscosity. Both HA aqueous so-

lutions were mixed. The composition ratio of high molecular weight HA and low molecular weight HA was 5/1. This mixed HA aqueous solution showed a moderate viscosity of about 100 dPa·s at 25˚C. This viscosity was convenient for pouring the HA aqueous solution into the plastic tray. Since the pH of HA aqueous solution is neutral (pH 6.8 at 25˚C), EGF can be stably mixed. Moreover, since the concentration of HA aqueous solution is as high as 1.6 w/v%, a sheet-shaped HA sponge having appropriate physical properties can be manufactured. The HA sponge with EGF has a size of 80 mm × 50 mm and a thickness of 3 mm.

3.3. Manufacture of Wound Dressing

The most important key in the manufacturing process is how to prepare wound dressing without reducing the activity of EGF. C/H/C-wound dressing with 3-layered structure was manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom. EGF activity is preserved in this laminating step, because UV irradiation process can be avoided in the manufacturing process of Sponge-HA/EGF. Another important key is how to adhere without changing each sponge structure. It is possible to adhere without changing each sponge structure using highly concentrated and moderately viscous HA aqueous solution (1.0 w/v%) in dots. Actually, HA aqueous solution was dotted in 9 places on this upper surface of Sponge-Col. This adhesion method was simple and convenient. C/H/C-wound dressing has a size of 80 mm × 50 mm and a thickness of 8 mm. As a comparison product, C/C-wound dressing with 2-layered structure was manufactured by laminating Sponge-Col on the top and Sponge-Col on the bottom in a similar way. C/C-wound dressing has a size of 80 mm × 50 mm and a thickness of 6 mm.

3.4. Weight Change of Wound Dressing When Immersed in Water

The weight change of Col 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. In our previous study [17] [18], the Col sponge with double-sided UV-irradiation for 15 minutes showed very slow elution properties when immersed in water. These experimental results demonstrated that intermolecular cross-linking by UV irradiation for 15 minutes was sufficient.

The main purpose of this study is to investigate whether the upper layer and lower layer Sponge-Col can delay the elution of the middle layer Sponge-HA/EGF when immersed in water. The weight of C/C-wound dressing after immersion in water for 1/4, 1, 3, and 7 days was 0.49, 0.48, 0.45 and 0.43 g, respectively (**Figure 4**). The original weight before immersion in water was 0.53 g. The weigh change rate of C/C-wound dressing after immersion in water for 7 days was 81% of the original weight. This result demonstrated that intermolecular cross-linking by UV irradiation for 15 minutes was sufficient to retain the sponge structure even after immersion in water for 7 days. The weight of C/H/C-wound dressing

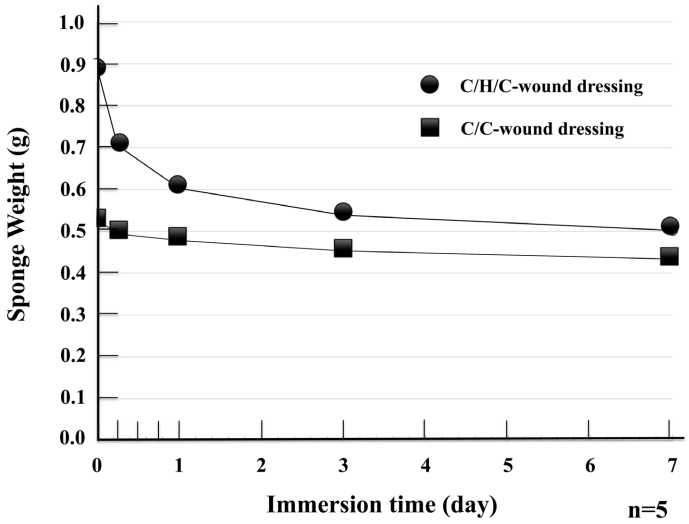

Figure 4. Weight change of C/H/C-wound dressing and C/C-wound dressing when immersed in water for 1/4, 1, 3, and 7 days, and then freeze-vacuum dried to collect the residual sponge: This experiment was performed using 5 containers (n = 5).

after immersion in water for 1/4, 1, 3 and 7 days was 0.71, 0.60, 0.54 and 0.50 g, respectively (**Figure 4**). The original weight before immersion in water was 0.89 g. The weigh change rate of C/H/C-wound dressing after immersion in water for 7 days was 56% of the original weight. The weight difference between C/H/C-wound dressing and C/C-wound dressing was compared (**Figure 5**). The original weight difference between the both wound dressings is 0.36 g. The weight difference between the both wound dressings after immersion for 1/4, 1 and 7 days was 0.22, 0.12 and 0.07 g, respectively. These results suggest that the upper layer and lower layer Sponge-Col can effectively delay the elution of the middle layer Sponge-HA/EGF.

3.5. Water Absorption and Retention Properties of Wound Dressing

One of the important properties of wound dressings is their high water absorption and retention. In clinical use, it is a great advantage to absorb a large amount of blood plasma and exudates containing various cell growth factors. In addition to that, being able to maintain moist environment is also a great advantage to promote wound healing. For this reason, the water absorption and retention properties of C/H/C-wound dressing were examined. The initial weight of hydrated C/H/C-wound dressing after lifting was 35.0 g. The weight ratio of the hydrated sponge and the dry sponge was 39 times. The hydrated sponge weight after 1 and 7 days was 20.6 and 14.2 g, respectively (**Figure 6**). On the other hand, the initial weight of hydrated C/C-wound dressing after lifting was 28.4 g. The weight ratio of the hydrated sponge and the dry sponge was 54 times. The hydrated sponge weight after 1 and 7 days was 22.0 and 13.6 g, respectively (**Figure 6**). There was no difference in water holding capacity between C/H/C-wound

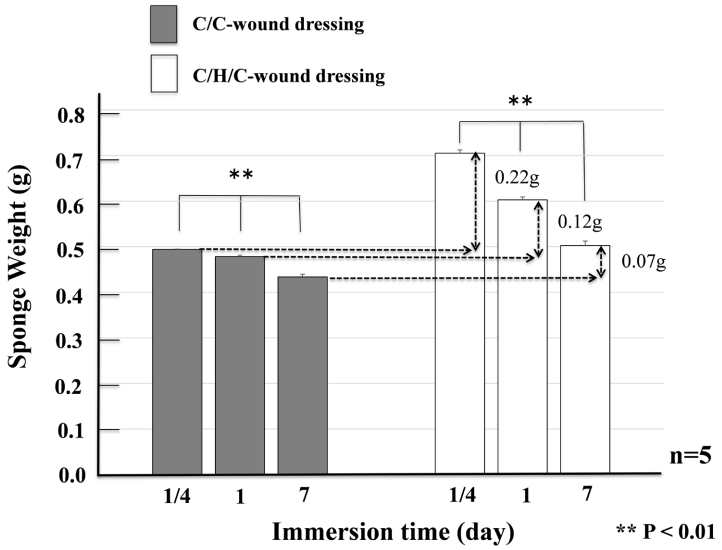

Figure 5. Comparison of weight change of C/H/C-wound dressing and C/C-wound dressing when immersed in water for 1/4, 1, and 7 days, and then freeze-vacuum dried to collect the residual sponge: This experiment was performed using 5 containers (n = 5). **P < 0.01 [Student's t-test].

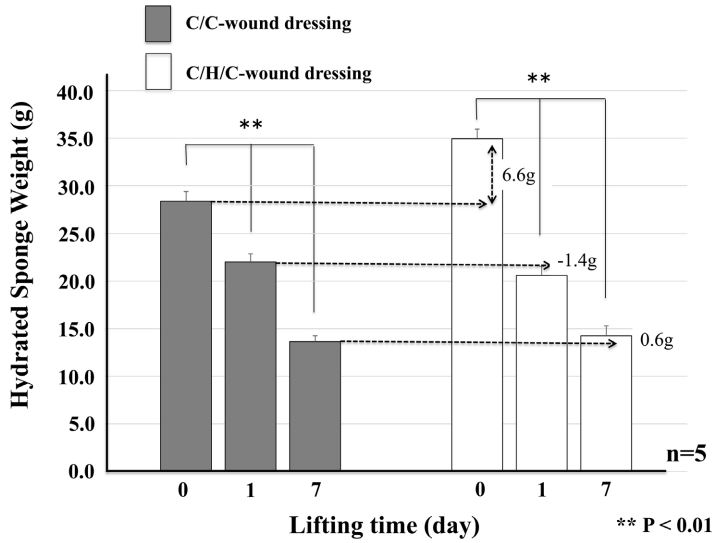

Figure 6. Comparison of weight change of hydrated C/H/C-wound dressing and hydrated C/C-wound dressing when immersed in water, and then lifted up for 0, 1, and 7 days: This experiment was performed using 5 containers (n = 5). **P < 0.01 [Student's t-test].

dressing and C/C-wound dressing after 1 and 7 days. These results suggest that the high water holding capacity of C/H/C-wound dressing is due to the high water holding capacity of the upper layer and lower layer Sponge-Col. The upper layer and lower layer Sponge-Col can maintain high water absorption and long-term water retention properties of C/H/C-wound dressing. Actually, the weight ratio of the hydrated state and the dry state of C/H/C-wound dressing even after 7 days was 16 times. These properties are suitable for providing moist wound-

healing environment.

3.6. Enzymatic Degradation of Wound Dressing by Collagenase

Ideally, collagen sponge should be biodegraded and absorbed until new tissue is formed. As an in vitro experiment, the enzymatic degradation properties of C/C-wound dressing by collagenase were investigated. As comparison data, the weight of C/C-wound dressing when immersed in water for 3 day was 0.45 g. This is 85% of the original weight (0.53 g). The weight of C/C-wound dressing when immersed in water containing collagenase at a concentration of 0.002 w/v% or 0.010 w/v% for 3 days was 0.44 g and 0.44 g, respectively (Figure 7). These are 83% of the original weight. These results indicate less weight loss due to collagenase under this concentration condition. On the other hand, the weight of C/H/C-wound dressing when immersed in water for 3 day was 0.54 g. This is 61% of the original weight (0.89 g). The weight of C/H/C-wound dressing when immersed in water containing collagenase at a concentration of 0.002 w/v% or 0.010 w/v% for 3 day was 0.51 g and 0.45 g, respectively (Figure 7). These are 53% and 46% of the original weight, respectively. The weight difference between C/C-wound dressing and C/H/C-wound dressing at collagenase concentration of 0.002 w/v% was 0.07 g. This indicates that the upper layer and lower layer Sponge-Col can effectively delay the elution of the middle layer Sponge-HA/EGF. However, the weight difference between the both wound dressings at collagenase concentration of 0.010 w/v% was almost 0. This indicates that the upper layer and lower layer Sponge-Col cannot delay the elution of the middle layer Sponge-HA/EGF. There was no change in the weight of C/C-wound dressing at low and high collagenase concentrations. It is thought that there was structural change

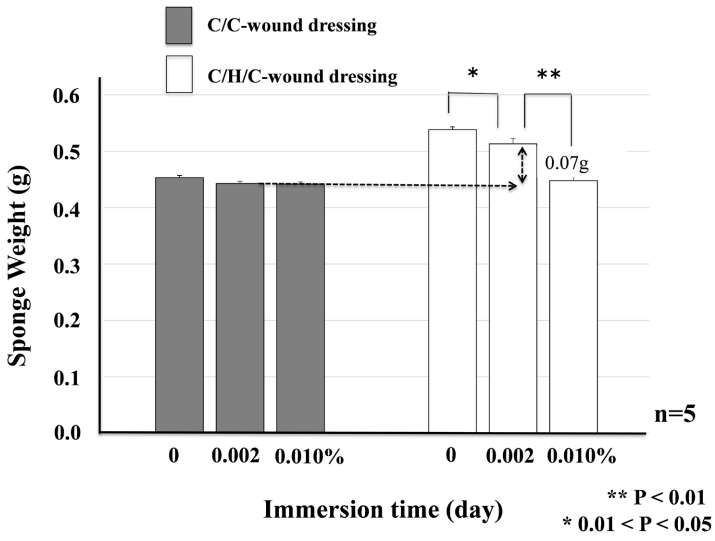

Figure 7. Comparison of weight change of C/H/C-wound dressing and C/C-wound dressing when immersed in water contacting collagenase (0.002 w/v% and 0.010 w/v%) for 3 days, and then freeze-vacuum dried to collect the residual sponge: This experiment was performed using 5 containers (n = 5). *P < 0.05, **P < 0.01 [Student's t-test].

in the porous structure under conditions of high collagenase concentration. As a result, the elution of Sponge-HA/EGF increased. This suggests that the application of C/H/C-wound dressing needs to change more frequently for wound surfaces with high collagenase concentration.

4. Discussion

As the first study [11], the properties of the sheet-shaped HA sponge containing EGF was examined. In this experiment, the cultured dermal substitute was prepared by incorporating human skin fibroblasts into collagen gel. 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 (VEGF) and hepatocyte growth factor (FGF) 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. In addition to them, HA itself has a wound healing effect. However, the HA sponge containing EGF is water soluble and poorly handled as a wound dressing. Therefore, it was needed to improve for better utility.

As the second study [14] [15], we developed a wound dressing composed of Col and HA containing EGF in order to improve handling as a wound dressing. This wound dressing was manufactured by freeze-vacuum drying a mixed aqueous solution of heat-denatured Col and HA containing EGF, followed by UV irradiation to introduce intermolecular crosslinks between collagen molecules. It was found that the wound dressing enables gradual release of HA and EGF from the network structure of Col. The function of this wound dressing was investigated in a wound surface model using cultured dermal substitute. The results demonstrated that the incorporated EGF increased the production of VEGF and HGF from fibroblasts in the cultured dermal substitute. This wound dressing was proven effective in clinical trials [16]. It is necessary to improve for better utility, because EGF activity is reduced to some extent due to UV irradiation for intermolecular crosslinks between collagen molecules.

The purpose of this study is to find a manufacturing method not to reduce the EGF activity. Actually, C/H/C-wound dressing was manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom. This method can prevent the reducing of EGF activity due to UV irradiation for intermolecular crosslinks between collagen molecules. The results of this study demonstrate that the upper layer and lower layer Sponge-Col can effectively delay the elution of middle layer Sponge-HA/EGF (**Figure 8**). In addition, the upper layer and lower layer Sponge-Col can maintain high water absorption and long-term water retention properties of C/H/C-wound dressing. These properties are a great advantage for providing moist wound-healing environment.

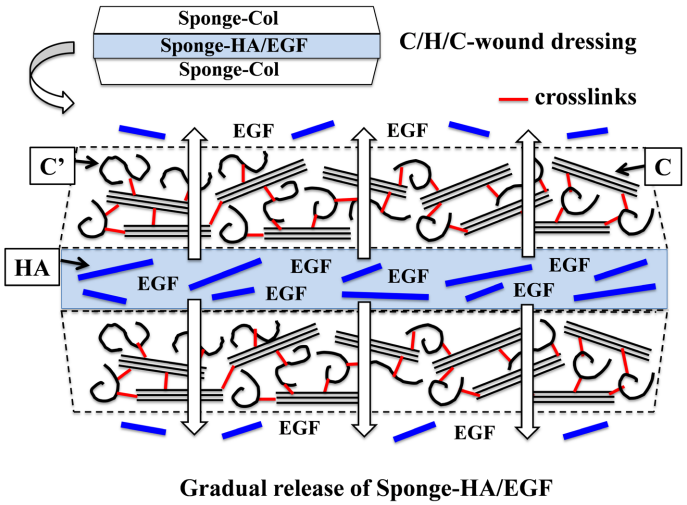

Figure 8. Schematic diagram of C/H/C-wound dressing: The upper layer and lower layer Sponge-Col allows a gradual release of the middle layer Sponge-HA/EGF.

This study focused on the design of wound dressing containing EGF. It is also possible to design wound dressings containing other cell growth factors such as basic fibroblast growth factor (bFGF) applying this sandwich structure. The results of our previous study [19] demonstrated that bFGF has the same effect as EGF.

Freeze-dried EGF and bFGF preparations are commercially available. These products are used by dissolving in a special dissolving solution and then applied daily by spray to wound surface. It is necessary to improve the trouble of daily administration by spray. Daily spray administration is not an efficient application of EGF or bFGF. EGF or bFGF-containing wound dressings are expected to be commercially available. Efficient and convenient application of EGF or bFGF can reduce overall costs by applying the sandwich structure composed of HA and Col spongy sheets.

5. Conclusion

The novel wound dressing can be manufactured by laminating Sponge-Col on the top, Sponge-HA/EGF in the middle, and Sponge-Col on the bottom. This process to create a sandwich structure has a great advantage. It is possible to prevent the reducing of EGF activity due to UV irradiation for intermolecular cross-linking. The upper layer and lower layer Sponge-Col enabled a gradual release of the middle layer Sponge-HA/EGF when immersed in water. It is also possible to maintain high water absorption and long-term water retention properties of C/H/C-wound dressing.

Conflicts of Interest

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

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