Advanced Treatment of Burns and Skin Ulcers Using Tissue-Engineered Products

Misato Kuroyanagi1 and Yoshimitsu Kuroyanagi2,3

1Department of Emergency and Critical Care Medicine, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Chiba, Japan
2Kitasato University, Minami-ku, Sagamihara, Kanagawa, Japan
3Technosurg Ltd., Midori-ku, Sagamihara, Kanagawa, Japan

Abstract

Three types of tissue-engineered products have been introduced in this review. The first product is an allogeneic cultured dermal substitute. This product is manufactured by incorporating allogeneic fibroblasts into a spongy sheet of hyaluronic acid and collagen. Allogeneic fibroblasts are rejected gradually in immune system. However, they are able to release some growth factors that are essential for wound healing. Hyaluronic acid and collagen also have a potential to promote wound healing. The clinical study demonstrated that this cultured dermal substitute is useful for the treatment of burns and skin ulcers. The second product is a functional wound dressing. This product is manufactured by freeze-drying an aqueous solution of hyaluronic acid, collagen, and epidermal growth factor. Epidermal growth factor has a potential to promote wound healing by enhancing both keratinocyte and fibroblast proliferation, and also by stimulating fibroblasts to synthesize vascular endothelial growth factor and hepatocyte growth factor that are essential for angiogenesis. The clinical study demonstrated that this wound dressing is useful for the treatment of burns and skin ulcers. The third product is a skin care product. This product is manufactured by freeze-drying an aqueous solution of hyaluronic acid, collagen, epidermal growth factor, and other ingredients for skin care. The experiment using a culture system demonstrated that this skin care product stimulates fibroblast to synthesize an increased amount of vascular endothelial growth factor and hepatocyte growth factor. This skin care product is aimed to use for post-treatment of chemical peeling and laser therapy used in aesthetic dermatology.

Keywords: Cultured dermal substitute; Wound dressing; Skin care product; Fibroblasts; Epidermal growth factor

Introduction

In some cases, burns and skin ulcers fail to proceed through a normal wound healing process. One of the causes is deficiency of growth factors at the wound site. The application of growth factors is useful to improve such a wound condition. Several types of tissue-engineered products have been developed for the treatment of burns and skin ulcers [1,2]. Three types of skin substitutes using autologous or allogeneic cells are manufactured by some culture techniques. Cultured epidermal substitute (CES) is manufactured by using keratinocytes. Cultured dermal substitute (CDS) is manufactured by using fibroblasts and biomaterial. Cultured skin substitute (CSS) is manufactured by using keratinocyte, fibroblast, and biomaterial (Figure 1). The pioneering research of Green and co-workers demonstrated that it is possible to grow keratinocytes as a stratified sheet [3,4]. Autologous CES has been evaluated in many hospitals and proved very useful for the treatment of severe burns [5-11]. Another pioneering research of Bell and co-workers demonstrated that CSS manufactured by using keratinocyte, fibroblast, and collagen gel sheet has an ideal skin-equivalent structure [12-14]. Allogeneic cells are rejected gradually in immune system. However, they are able to release some types of growth factors that are necessary for wound healing to promote. Allogeneic CSS has been evaluated in many hospitals and proved very effective for the treatment of skin ulcers [15].

As the first research strategy, we developed an allogeneic CDS. This product is manufactured by incorporating allogeneic fibroblast into a two-layered spongy sheet of hyaluronic acid (HA) and collagen (Col) [16-23]. The biomaterial itself has a potential to promote wound healing. This spongy sheet has an intermolecular cross-linking among HA and Col molecules, and therefore this CDS is able to be cryopreserved for a long period and then thawed prior to clinical application. This CDS can release various types of growth factors even after cryopreserving and thawing process. As the second research strategy, we developed a functional wound dressing that is composed of a spongy sheet of HA and Col containing epidermal growth factor (EGF) [24-26]. EGF is able to facilitate both keratinocyte and fibroblast proliferation. In addition, it is capable of stimulating fibroblast to synthesize an increased amount of angiogenic growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) (Figure 2). In such a reason, EGF is considered as one of the ideal growth factors for

Figure 1: Structure of tissue-engineered products: CES manufactured by using keratinocyte, CDS manufactured by using fibroblast and biomaterial, and CSS manufactured by using keratinocyte, fibroblast and biomaterials.

*Corresponding author: Yoshimitsu Kuroyanagi, (Emeritus Professor of Kitasato University), Technosurg Ltd., 5-4-30, Nishishishimoto, Midori-ku, Sagamihara, 252-0131, Japan, Tel: 01142-770-9920, Fax: 01142-770-9920, E-mail: drs.yoshi@technosurg.co.jp

Received: November 27, 2017; Accepted: January 10, 2018; Published: January 17, 2018

Citation: Kuroyanagi M, Kuroyanagi Y (2018) Advanced Treatment of Burns and Skin Ulcers Using Tissue-Engineered Products. Dermatol Case Rep 3: 137.

Copyright: © 2018 Kuroyanagi M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
wound healing. As the third research strategy, we developed EGF-incorporating skin care product. This skin care product is aimed to use for post-treatment of chemical peeling and laser therapy.

Materials and Methods

Cultured dermal substitute using cell and biomaterial

Properties of biomaterial used in cultured dermal substitute: HA is a useful biomaterial for wound healing, as it has various biological activities [27-31]. HA is able to create an excellent wound healing environment. HA also stimulates cell migration and angiogenesis. Col is also a useful biomaterial for wound healing. Col-derived peptides are able to act as a chemoattractant for fibroblasts in vitro and may have a similar activity in vivo [32].

Preparation of allogeneic CDS: Allogeneic CDS (10 cm × 10 cm) was manufactured by seeding fibroblasts on the Col layer of two-layered spongy sheet of HA and Col, followed by cryopreserving. This spongy sheet of cross-linked HA is soaked into an aqueous solution of Col, followed by freeze-drying to obtain a two-layered spongy sheet of HA and Col. Finally, Col spongy side is irradiated with an ultraviolet lamp in order to induce intermolecular cross-linking among Col molecules [17].

Cell banking was established in accordance with the procedure described in our article [16]. A small piece of skin (1 cm × 1 cm) was obtained from patients younger than one-year-old during surgical excision of excrescence. The skin donors were checked to be free from infectious viruses such as HBV, HCV, HIV and HTLV, and also negative on the treponema pallidum hemagglutination test. Fibroblasts were isolated from a skin and then cultured successively. These fibroblasts were suspended in a cryopreservation medium and then cryopreserved in liquid nitrogen according to a conventional procedure. Prior to manufacturing CDS, a part of these cryopreserved fibroblasts was thawed and then cultured successively to get an adequate number of cells. Cryopreserving and thawing CDS were performed in accordance with the method described in the article [17]. The amounts of VEGF, basic fibroblast growth factor (bFGF), and HGF were measured [18,19]. This experiment showed that the cryopreserved CDS is able to keep the potential to release VEGF, bFGF, and HGF at appreciable levels.

Clinical study using allogeneic CDS: A multi-center clinical study using allogeneic CDS was conducted at 31 hospitals as a project of Regenerating Medical Millennium Projects of the Ministry of Health, Labour and Welfare. During this period of the project, 4700 sheets of allogeneic CDS were manufactured and cryopreserved at -153°C at the R&D Center for Artificial Skin, School of Allied Health Sciences, Kitasato University. The clinical study was carried out in compliance with the ethical guidelines of the participating hospitals. The cryopreserved CDS were delivered to the participating hospitals using a box filled with dry ice and then continuingly cryopreserved in a freezer at -85°C. Prior to clinical application, the cryopreserved CDS was thawed and rinsed with lactated Ringer’s solution.

In practice, wound surface was debrided as necessary and rinsed with physiological saline solution. CDS was placed cell-seeded side down on the wound surface. CDS was applied to the wound surface, on which a conventional ointment-gauze dressing was placed to fix the CDS. This treatment using CDS together with ointment-gauze dressing was applied repeatedly at an interval of 5 to 7 days for a period of 6 weeks, or longer if necessary. The efficacy of this treatment was assessed in 415 cases, including severe burns and intractable skin ulcers. Excellent result was assessed in 62.6%, good result was assessed in 30.0%, fair result was assessed in 6.7%, and poor result was assessed in 0.7% of the all cases in total clinical evaluation. Very safe result was assessed in 89.4%, mostly safe result was assessed in 8.9%, problem result was assessed in 1.0%, and unsafe result was assessed in 0.7% of the all cases in safety evaluation (Figure 4). The representative clinical results have been reported in the articles [20-23].

The representative clinical results are shown here:

A 95-year-old woman with an intractable skin ulcer was treated with

Figure 2: Functions of EGF: (1) Enhancing keratinocyte and fibroblast proliferation (2) Enhancing VEGF and HGF production by fibroblast.

Figure 3: CDS manufactured by seeding and culturing fibroblasts within the Col layer of two-layered spongy sheet of HA and Col, followed by cryopreserving. Prior to clinical application, the cryopreserved CDS is thawed and rinsed with lactated Ringer’s solution. Fibroblasts in CDS synthesize and release VEGF, bFGF and HGF.

Figure 4: Results of total clinical evaluation and safety evaluation in 415 clinical cases, including severe burns and intractable skin ulcers. Excellent result was assessed in 62.6%, good result was assessed in 30.0%, fair result was assessed in 6.7%, and poor result was assessed in 0.7% of the all cases in total clinical evaluation. Very safe result was assessed in 89.4%, mostly safe result was assessed in 8.9%, problem result was assessed in 1.0%, and unsafe result was assessed in 0.7% of the all cases in safety evaluation (Figure 4). The representative clinical results have been reported in the articles [20-23].
allogeneic CDS. The wound condition improved 1 month later, showing a reduction in wound size associated with a good granulation tissue formation. The skin ulcer healed mostly 2 months later (Figure 5).

A 90-year-old woman with a squamous cell carcinoma was treated with allogeneic CDS. CDS was applied to the debrided wound surface. The wound condition improved, showing a good granulation tissue formation, and the debrided wound surface healed completely 28 days later (Figure 6).

A 65-year-old man with a necrotic lesion on the right foot was treated with allogeneic CDS. At 39 days, a favourable wound bed was prepared, on which a split-thickness auto-skin graft was performed. At 83 days after auto-skin grafting, the condition of grafted skin was excellent (Figure 7).

A 60-year-old man with an intractable ulcer on the left leg was treated with allogeneic CDS. At 3 months, a favourable wound bed was prepared, on which a 3-fold extended mesh split-thickness auto-skin graft was performed. At 10 days after auto-skin grafting, the condition of grafted skin was excellent (Figure 8).

An 81-year-old woman who suffered a third-degree burn on the left leg was treated with an auto-skin graft and allogeneic CDS. In this case, a 6-fold extended mesh split-thickness auto-skin graft was applied, on which CDS was placed. CDS was applied repeatedly at an interval of 5 to 7 days. This extended mesh graft took successfully, and showed excellent epithelialization 18 days later, despite poor wound conditions (Figure 9).

Wound dressing using growth factor and biomaterial

Property of EGF: EGF has some useful effects for wound healing. EGF is able to enhance both keratinocytes and fibroblasts proliferation, thus facilitating granulation tissue formation as well as epithelialization [33,34]. In addition, EGF is able to stimulate fibroblasts to synthesize an increased amount of VEGF and HGF that are important to facilitate angiogenesis. Another research demonstrated that simultaneous administration of VEGF and HGF is able to promote synergistically new blood vessel formation compared with administration of each factor alone [35]. HGF has a useful effect for angiogenesis as well as epithelialization [36]. Because of these reasons, EGF is a promising factor in order to promote wound healing.
Preparation of EGF-incorporating wound dressing: EGF-incorporating wound dressing was manufactured by freeze-drying an aqueous solution of high molecular weight HA, low molecular weight HA, and Col containing EGF. High molecular weight HA is useful to create an excellent wound healing environment, while low molecular weight HA is useful to promote angiogenesis. Both side of spongy sheet was irradiated with an ultraviolet lamp in order to induce intermolecular cross-linking among Col molecules [24-26]. This wound dressing (5 cm × 8 cm) contains EGF at a concentration of 2.0 µg/cm².

Clinical study using EGF-incorporating wound dressing: The fundamental study showed an excellent result [24.25]. Based on this result, the efficacy of EGF-incorporating wound dressing was assessed in 16 clinical cases, including burns, donor sites, traumatic skin defects, and intractable skin ulcers. Wound surface was debrided as necessary and rinsed with physiological saline solution. As basic usage, EGF-incorporating wound dressing was placed on the wound site, on which a commercially available polyurethane film dressing was placed as a top dressing. In the case of wounds with excess amounts of exudate, a conventional ointment-gauze dressing was used as a top dressing. This treatment using EGF-wound dressing together with each top dressing was applied repeatedly at an interval of 3 to 5 days for a period of 6 weeks, or longer if necessary. Good or excellent result was assessed in 93.7% of the all cases in total clinical evaluation [26].

The representative clinical results are shown below:

A 74-year-old woman with a traumatic ulcer on the left leg was treated with EGF-incorporating wound dressing, basically twice a week. Wound size decreased significantly 6 weeks later. The wound healed mostly 3 months later

A 65-year-old woman with a skin ulcer on the right knee was treated with EGF-incorporating wound dressing, basically twice a week. At 6 weeks, a favourable wound bed was prepared, showing a reduction in wound size associated with a good granulation tissue formation, on which a split-thickness auto-skin graft was performed. At 2 months after auto-skin grafting, the condition of grafted skin was excellent.

Skin care product using growth factor and biomaterials

Properties of ingredients for skin care: Arginine (Arg) has useful biological properties. One report demonstrated that Arg is necessary for T lymphocyte maturation [37]. Another report demonstrated that Arg is a major substrate for the production of nitric oxide, which is toxic to tumors and infected cells. Other reports indicated that Arg and Arg-derived nitric oxide are useful for wound healing [38-42]. Vitamin C (VC) has useful biological properties. One report demonstrated that VC has a potential to enhance HGF secretion by fibroblasts, when VC is applied together with EGF [43]. For this reason, the combination uses of EGF and VC have a higher potential to stimulate fibroblasts to synthesize an increased amount of VEGF and HGF [44]. Poly-y-glutamic acid (PGA) has a potential to enhance production of natural moisturizing factors. PGA has a superior film-forming property. By such reason, PGA is able to keep moisture conditions by coating a skin surface. Glucosyl ceramide (GC) has a potential to connect epidermal keratinocytes themselves, and enhancing moisture-keeping environment by controlling water evaporation from skin. Because of these reasons, EGF-incorporating skin care product was manufactured by freeze-drying an aqueous solution of high molecular weight HA, low molecular weight HA, Col, Arg, VC-derivative, GC, PGA, and EGF. This skin care product is water-soluble, because of no intermolecular cross-linking among HA and Col. This skin care product (5 cm × 8 cm) contains EGF at a concentration of 1.0 µg/cm² [45].

Post-treatment of chemical peeling and laser therapy: A partially damaged skin is capable of healing through the repair mechanisms of skin. Based on this regenerative potential, chemical peel has been widely used in aesthetic dermatology. Chemical agents cause exfoliation of the skin. This treatment leads to the skin rejuvenation through a normal wound healing process. However, the wound conditions depend on the concentration of the acid and the duration of exposure [46-48]. This skin damage is similar to a superficial chemical burn. Therefore, excellent skin care products are needed for post-treatment of chemical
The application of allogeneic fibroblast is useful, because this cell is able to synthesize various types of growth factors capable of enhancing wound healing. The application of HA and Col is also useful, because these biomaterials have a potential to facilitate wound healing. The clinical study demonstrated that allogeneic CDS is useful for the treatment of burns and skin ulcers. Another clinical study showed that EGF-incorporating wound dressing is useful for the treatment of burns and skin ulcers. The experiment using a culture system demonstrated that EGF-incorporating skin care product can enhance the VEGF and HGF production by fibroblast. This skin care product is promising for post-treatment of chemical peeling and laser therapy used in aesthetic dermatology.

**Future Perspectives**

As future clinical research, it is worth examining the efficacy of allogeneic CDS combined with EGF-incorporating skin care product. As basic usage, CDS was applied to the wound surface, on which a conventional ointment-gauze dressing was used to fix the CDS. In future clinical study, CDS is applied to the wound surface, on which EGF-incorporating skin care product is placed, and more a conventional ointment-gauze dressing is used as a top-dressing. This additional EGF can enhance VEGF and HGF production by fibroblasts in CDS.

**Acknowledgments**

The authors are grateful to the doctors who participated in the project of Regenerating Medical Millennium Projects of the Ministry of Health, Labour and Welfare.

**References**

1. Kuroyanagi Y (2016) Tissue-engineered products for skin regenerative medicine. Open J Regenerative Med 5: 61-84.
2. Kuroyanagi M, Kuroyanagi Y (2017) Tissue-engineered products capable of enhancing wound healing. AIMS Materials Sci 4: 561-581.
3. Rheinwald JG, Green H (1977) Epidermal growth factor and the multiplication of cultured human epithelial keratinocytes. Nature 265: 421-424.
4. Green H, Kehinde O, Thomas J (1979) Growth of cultured human epithelial cells into multiple epithelia suitable for grafting. Proc Natl Acad Sci USA 76: 5665-5668.
5. O’Connor NE, Muliken JG, Banks-Schlegel S, Kehinde O, Green H (1982) Grafting of burns with cultured epithelium prepared from autologous epithelial cells. Lancet 1: 75-78.
6. Gallico GG, O’Connor NE, Compton CC, Kehinde O, Green H (1984) Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 311: 446-451.
7. Cuono CB, Langdon R, McGuire J (1986) Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. Lancet 1: 1123-1124.
8. Compton CC, Gilt JM, Bradford DA, Regauer S, Gallico GG, et al. (1989) Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting. Lab Invest, 60: 600-612.
9. Gallico GG, O’Connor NE, Compton CC (1989) Cultured epithelial autografts for giant congenital nevi. Plast Reconstr Surg 84: 1-9.
10. De-Luca M, Albanese E, Bondanza S, Megna M, Ugozzi L, et al. (1989) Multicenter experience in the treatment of burns with autologous and allogenic cultured epithelium, fresh or preserved in a frozen state. Burns 15: 303-309.
11. Odessy R (1992) Multicenter experience with cultured epidermal autograft for treatment of burns. J Burn Care Rehabil 13: 174-180.
12. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T (1981) Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science 211: 1052-1054.
13. Bell E, Ehrlich HP, Sher S, Merrill C, Sarber R, et al. (1981) Development and use of a living skin equivalent. Plast Reconstr Surg 67: 386-392.
14. Bell E, Sher S, Hull B, Merrill C, Rosen S, et al. (1983) The reconstitution of living skin. J Invest Dermatol 81: 2-10.
15. Parenteau N, Naughton G (1999) Skin: the first tissue-engineered products. Sci Am April 1: 59-61.
16. Kuroyanagi Y, Kubo K, Matsui H, Kim HJ, Numari S, et al. (2004) Establishment of banking system for allogeneic cultured dermal substitute. Artif Organs 28: 13-21.
17. Kubo K, Kuroyanagi Y (2004) Development of a cultured dermal substitute composed of a spongy matrix of hyaluronic acid and atelo-collagen combined with fibroblasts: Cryopreservation. Artif Organs 28, 182-188.
18. Kubo K, Kuroyanagi Y (2005) A study of cytokines released from fibroblasts in cultured dermal substitute. Artif Organs 29: 845-849.
19. Hashimoto A, Kuroyanagi Y (2008) Standardization for mass production of allogeneic cultured dermal substitute by measuring the amount of VEGF, bFGF, HGF, TGF-β, and IL-8. J Artif Organs 11: 225-231.
20. Kashiwa N, Ito O, Ueda T, Kubo K, Matsui H, et al. (2004) Treatment of full-thickness skin defect with concomitant grafting of 8-fold extended mesh auto-skin and allogeneic cultured dermal substitute. Artif Organs 28: 444-450.
21. Ohnishi T, Okamoto K, Kaminaka C, Kishi T, Sakurane M, et al. (2004) Digital gangrene associated with idiopathic hypereosinophilia; treatment with allogeneic cultured dermal substitute (CD5). Eur J Dermatol 14: 168-171.
22. Mori Y, Fujita S, Fukagawa S, Machino T, Goto T, et al. (2004) Clinical evaluation of allogeneic cultured dermal substitutes for intractable skin ulcers after tumor resection. Eur J Dermatol 14: 172-176.
23. Yamada N, Uchinuma E, Kuroyanagi Y (2008) Clinical trial of allogeneic cultured dermal substitute for intractable skin ulcers of the lower leg. J Artif Organs 11: 100-103.
24. Kondo S, Kuroyanagi Y (2012) Development of wound dressing composed of hyaluronic acid and collagen sponge with epidemial growth factor. J Biomater Sci 23: 629-643.
25. Kondo S, Niijima H, Yu A, Kuroyanagi Y (2012) Evaluation of a wound dressing composed of hyaluronic acid and collagen sponge containing epidemial growth factor in diabetic mice. J Biomater Sci 23: 1729-1740.
26. Yu A, Takeda A, Kumazawa K, Miyoshi H, Kuroyanagi M, et al. (2015) Preliminary clinical study using a novel wound dressing composed of hyaluronic acid and collagen containing EGF. Open J Regenerative Med 4: 6-13.
27. Chen WYJ, Abatangelo G (1999) Functions of hyaluronan in wound repair. Wound Repair and Regeneration 7: 79-89.
28. West DC, Hampson IN, Arnold F, Kumar S (1985) Angiogenesis induced by degradation products of hyaluronic acid. Science 228: 1324-1326.
29. Sattar A, Rooney P, Kumar S, Pye D, West DC, et al. (1994) Application of angiogenic oligosaccharides of hyaluronan increases blood vessel numbers in rat skin. J Invest Dermatol 103: 576-579.
30. Lees VC, Fan TP, West DC (1995) Angiogenesis in a delayed revascularization model is accelerated by angiogenic oligosaccharides of hyaluronan. Lab Invest 73: 259-266.
31. Pardue EL, Ishibashi S, Ramamurthi A (2008) Role of hyaluronan in angiogenesis and its utility to angiogenic tissue engineering. Organogenesis 4: 203-214.
32. Postlethwaite AE, Seyer JM, Kang AH (1978) Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. Proc Natl Acad Sci USA 78: 871-875.
33. Carpenter G, Cohen S (1976) Human epidermal growth factor and the proliferation of human fibroblasts. J Cell Physiol 88: 227-237.
34. Carpenter G, Cohen S (1979) Epidermal growth factor. Annu Rev Biochem 48: 193-216.
35. Xin X, Yang S, Ingle G, Zlot C, Rangell L (2001) Hepatocyte growth factor enhances vascular endothelial growth factor-induce angiogenesis in vitro and in vivo. Am J Pathol 158: 1111-1120.
36. Conway K, Price P, Harding KG, Jiang WG (2006) The molecular and clinical impact of hepatocyte growth factor, its receptor, activators, and inhibitors in wound healing. Wound Repair and Regeneration 14: 2-10.
37. Ochoa JB, Strange J, Kearney P, Dellin G, Endean E, et al. (2001) Effects of L-arginine on the proliferation of T lymphocyte subpopulations. J. Parenter. Enteral Nutr 25: 23-29.
38. Kirs SJ, Barbul A (1999) Role of arginine in trauma, sepsis, and immunity. J Parenter Enteral Nutr 14: 226-229.
39. Shi HP, Efron DT, Most D, Tantry US, Barbul A, et al. (2000) Supplemental dietary arginine enhances wound healing in normal but not inducible nitric oxide synthase knockout mice. Surgery 128: 374-378.
40. Zhu H, Ka B, Murad F (2007) Nitric oxide accelerates the recovery from burn wounds. World J Surg 31: 624-631.
41. Witte MB, Barbul A (2003) Arginine physiology and its implication for wound healing. Wound Repair and Regeneration 11: 419-423.
42. Matsumoto Y, Arai K, Momose H, Kuroyanagi Y (2009) Development of wound dressing composed of hyaluronic acid sponge containing arginine. J Biomed Sci Polymer Edn 20: 993-1004.
43. Wu YL, Gohda E, Iwao M, Matsunaga T, Nagao T, et al. (1998) Stimulation of hepatocyte growth factor production by ascorbic acid and its stable 2-glucoside. Growth Horm IGF Res 8: 421-429.
44. Niijima H, Kuroyanagi Y (2014) Development of novel wound dressing composed of hyaluronic acid and collagen sponge containing epidemial growth factor and vitamin C derivative. J Artificial Organs 17: 81-87.
45. Yamamoto A, Ohno H, Kuroyanagi Y (2016) Evaluation of epidermal growth factor-incorporating skin care product in culture experiment using human fibroblasts. Open J Regenerative Med 5: 44-54.
46. Coleman WP III, Brody HJ (1997) Advances in chemical peeling. Dermatol Clin 15: 19-26.
47. Dinner MI, Artz JS (1998) The art of the trichloroacetic acid chemical peel. Clin Plast Surg 25: 53 62.
48. Brody HJ, Monheit GD, Resnik SS, Alth TH (2000) A history of chemical peeling. Sci Am April 1: 59-61.