Ointment vehicles regulate the wound-healing process by modifying the hyaluronan-rich matrix

Yusuke Murasawa, PhD[1]; Katsunori Furuta, BS[2]; Yasuhiro Noda, PhD[3]; Hiroyuki Nakamura, DDS, PhD[4]; Satoshi Fujii, MD, PhD[5]; Zenzo Isogai, MD, PhD[1]

1. Department of Advanced Medicine,
2. Department of Pharmacy, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan,
3. Department of Pharmacy, College of Pharmacy, Kinjo Gakuin University, Nagoya, Japan,
4. Department of Dental Regenerative Medicine, Center of Advanced Medicine for Dental and Oral Diseases, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan,
5. Department of Laboratory Medicine, Asahikawa Medical University, Asahikawa, Japan

Reprint requests:
Zenzo Isogai, MD, PhD, 7-430 Morioka-cho, Obu, Aichi 474-8511, Japan.
Tel: +81-562-46-2311 Ext. 7156
Fax: +81-562-48-2373
Email: zenzo@ncgg.go.jp

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ABSTRACT

Topical ointment consists of an active ingredient and vehicle, and the vehicle largely comprises the volume of the ointment. During the treatment of chronic wounds, such as pressure ulcers, the vehicle has been considered inactive, only serving as a carrier of the main pharmaceutical. However, recent reports have indicated that the vehicle has distinct clinical effects that depend on its physicochemical properties. Therefore, an understanding of the action mechanism of the ointment vehicle in wound tissue is necessary. In this study, we established a mouse model to analyze tissue reactions induced by the following ointment vehicles, an oil-in-water emulsion (EM) vehicle; a macrogol ointment (MO), which is a water-soluble, hydrophilic vehicle; and a MOs containing sucrose (MS). EM-treated wounds exhibited an inflammatory reaction characterized by tissue edema and thick granulation tissue; however, MO- and MS-treated wounds did not exhibit this reaction. Moreover, EM-treated wounds exhibited infiltration of inflammatory cells unlike MO-treated wounds. In contrast, the formation of collagenous tissue was dominantly observed in MO-treated wounds. Because the vehicle regulates the water environment of the wound, the water-holding extracellular matrix molecules, including hyaluronan (HA) and proteoglycan, were examined using immunohistochemical and biochemical methods. The versican G1 fragment, serum-derived HA-associated protein (SHAP) and HA (the VG1F-SHAP-HA) complex characteristically found in inflammatory conditions of pressure ulcers was found in EM-treated wounds. To histologically analyze the mechanism of action of the vehicle, we evaluated the ointment vehicle-wound tissue interface in an en bloc manner. Formation of the HA-containing complex was observed locally between the vehicle and wound surface. On the basis of these data, ointment vehicles regulate the wound-healing process through the formation of HA-rich extracellular matrices at the ointment-wound interface. This study provides a better understanding of the treatment of deep-pressure ulcers with focus on ointment vehicles.

INTRODUCTION

Several topical ointments are listed in the Japanese guidelines for the treatment of pressure ulcers.[1,2] An ointment consists of an active ingredient and vehicle, and the vehicle comprises the majority of the ointment volume. The active ingredients of an ointment are designed for use in specific stages of wound healing, and the vehicle is considered inactive because it only carries the main ingredient. However, recent studies have suggested that some ointment vehicles exhibit distinct regulatory behavior in terms of the capacity of water absorption.[3,4] The water absorption capacity of the ointment vehicle has been characterized by its mode of absorption using the Franz cell model with a semipermeable membrane, which allows physicochemical assessment of an ointment in vitro.[3,4] The differences in water absorption seem to depend on the chemical composition of each ointment vehicle, particularly the pattern of the oil and water mixture.

Wound healing occurs in the following order: hemostasis, inflammation, repair, and remodeling. A dynamic change in the extracellular matrices (ECM) is required for the wound healing process to occur.[5,6] In particular, hyaluronan (HA)-rich matrices are abundant during the early stage of wound healing.[7–10] HA is a simple polysaccharide that holds a large amount of water in its structure. Therefore, an HA-containing ECM may regulate the balance of water during the wound-healing process. To have such a role in tissue, HA must interact with the ECM network through HA-binding molecules, including versican, which is a

chondroitin sulfate proteoglycan. Another HA-binding molecule, serum-derived HA-associated protein (SHAP), which corresponds to the heavy chains of inter-α-trypsin inhibitors (IαI) is supplied from the serum.[11] Therefore, HA and its associated molecules are present in chronic wounds such as pressure ulcers.[12,13] We have recently found the HA-containing complex composed of versican G1 fragment (VG1F), SHAP, and HA (the VG1F-SHAP-HA complex) in the granulation tissue of pressure ulcers.[13] Furthermore, the VG1F-SHAP-HA complex is characteristically present in edematous granulation tissue.[13] Therefore, the HA-containing ECM complex may be a desirable interface between the ointment and wound.

To date, little attention has been focused on the biological effect of the ointment vehicle in regulating the wound-healing process through appropriate adjustment of the moist environment.[1,14] For instance, cream in the form of an oil-in-water emulsion (EM) vehicle has less water absorption capacity than other vehicles and often induces an excessively moist environment for wound healing,[4] resulting in edematous granulation tissue. In contrast, a macrogol ointment (MO), which is a water-soluble, hydrophilic vehicle, absorbs exudates, and creates an excessively dry wound environment.[14] Furthermore, MOs containing sucrose (MS), which is available as a povidone-iodine sugar ointment, can absorb excessive amounts of exudates from the wound.[4] Therefore, povidone-iodine sugar ointment and MO have the capacity to absorb water, and osmotic pressure due to sucrose and/or polyethylene glycol may be the main force in active absorption.

According to in vitro studies that focused on the water absorption capacity of ointments and clinical experience with using ointments for pressure ulcers, ointment vehicles may regulate the water environment through their own physicochemical properties.[4] However, the pharmacological effects of these ointments on pressure ulcers are largely based on the opinions of experts. Moreover, the tissue reactions of each ointment vehicle have not been clarified. Therefore, interactions between the ointment vehicle and wound surface should be clarified to further understand the pharmaceutical effects of these vehicles on pressure ulcers.

In the present study, using a mouse wound-healing model, we evaluated distinct biological responses to EM, MO and MS vehicles to clarify their regulatory mechanisms.

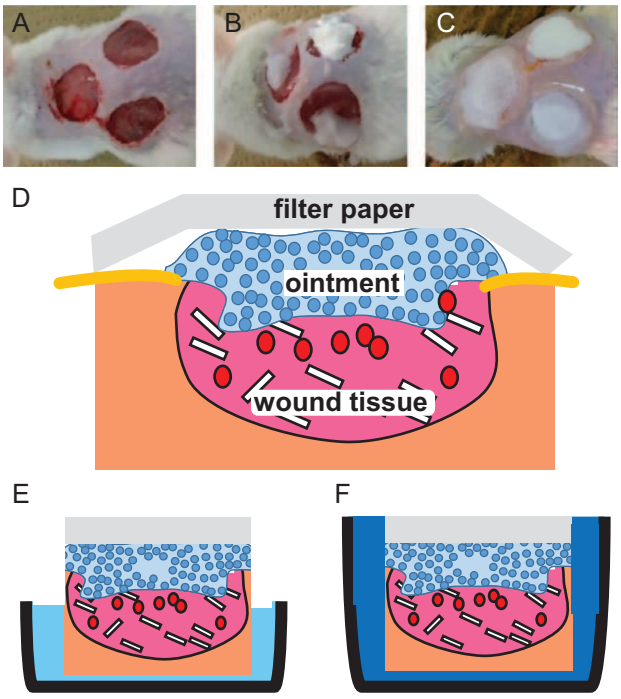

Figure 1. Mouse model of the topical application of ointment vehicles. (A) Wounds were created on the back of mice using a 6-mm trepan. (B) Different ointment vehicles were applied to each wound. Ointment vehicles were EM, MO, and MS. (C) After applying ointment vehicles, the wounds were covered with filter paper and sealed with glue. Wound tissue was obtained with a biopsy. (D) Topical application of the ointments to the wounds. In this model, each ointment stably interacted with the wound tissues. (E) For en bloc preparation of tissue, the ointment-wound tissue complex was processed using a specific procedure. Initially, a portion of the tissue sample was fixed without eluting the ointment layers. (F) Whole samples, including wound tissue, ointment, and filter paper, were immersed and embedded in solution. EM, cream in the form of an oil-in-water emulsion; MO, macrogol ointment; MS, MO containing sucrose.

METHODS

Ointment vehicles

EM, (Shinsui-Cream) and MO are used in clinical practice in Japan. The samples used in this study were purchased from Hoei (Osaka, Japan). Although povidone-iodine ointment (U-pasta, Kowa-soyaku, Tokyo, Japan) is available in Japan, MO and purified sucrose were mixed in a ratio of 1:1 (w/w) to eliminate the effect of povidone-iodine for the substitution of MS. The ointment vehicles were stored at 4 °C until use. A clean procedure for mixture of vehicles was performed according to the protocol of our clinical pharmaceutical facility.

Mouse model for the ointment application

A mouse model was established to recreate the wound-healing process of pressure ulcers in humans under common situation. Three wounds were created on the skin of the backs of retired ICR mice older than 20 weeks (n = 4 for each experiment, Japan SLC) using a 8-mm trepan (Kai Industries, Seki, Japan) under appropriate anesthesia (Figure 1A). Then, the wounds were treated with the ointment vehicles (Figure 1B), covered with filter paper (Whatman, 3MM Chr, GE Healthcare, Little Chalfont, UK) and sealed with a glue-like dressing (Ekiban-A; Taihei-Yakuhin, Japan; Figure 1C). The procedure enabled us to effectively apply the ointment onto the wound for a certain period of time. This protocol was approved by the animal ethics committee of the National Center for Geriatrics and Gerontology and was conducted in accordance with the Japanese guidelines for animal care.

Samples were obtained for histochemical and biochemical analyses on days 1, 3, and 6 by reexcising the wound tissues while the mice were under anesthesia. The thickness of each granulation tissue was measured at the time of biopsy. For conventional pathological analyses, the tissue samples were fixed in 10% (v/v) formalin in phosphate-buffered saline (PBS) and embedded in paraffin. Some samples were stored at −80 °C and used for the biochemical analyses.

En-bloc *tissue preparation*

To examine the reaction between the ointment vehicle and wound (Figure 1D), we prepared some histological samples in an en bloc manner. For the initial fixation procedure, only part of the tissue was immersed in solution (Figure 1E). The obtained wound tissues were incubated in 0.5% (w/v) glutaraldehyde and 4% (w/v) paraformaldehyde in PBS in a 35-mm dish for 1 hour. Then, the cubed tissues were washed twice with ice-cold PBS for 5 minutes. The object cubes were transferred to prepared glasses, dehydrated, and incubated for 10 minutes in an ascending series of ethanol solutions (60%, 90%, and 100%). Thereafter, the ethanol was replaced by an embedding solution of 50% (v/v) LR White Resin (14381-CA; Electron Microscopy Science, Hatfield, PA) in ethanol and incubated in this solution for 2 hours. Following this, the samples were incubated in 100% LR White Resin for 3 hours and placed on ice to prevent the ointment layer from being immersed in the solution. Finally, to polymerize the en bloc sample, we immersed the entire sample, including the wound tissue, ointment, and filter paper, in a 100% LR White Resin-containing accelerator (50 μL of the accelerator for 10 mL of LR White Resin) for 20 minutes (Figure 1F).

For the "wound smear" experiments, we used cotton swabs (Nihon Menbou, Tokyo, Japan) to obtain protein samples from the wound's surface. The samples were smeared on glass slides (Platinum; Matsunami, Osaka, Japan), air-dried for 60 minutes, and fixed in 10% (v/v) formalin (Wako, Osaka, Japan). Subsequent immunostaining was performed as described previously.[13]

Immunohistochemistry

The polyclonal antibodies pAb 6084 (VG1) and pAb 8,531 (an antibody that recognizes the DPEAAE neoepitope generated with ADAMTS proteolysis of V1) have been characterized in our previous study.[15] A rabbit pAb against IαI (all Heavy Chains) was purchased from Sigma-Aldrich (St. Louis, MO). A monoclonal antibody (mAb) that acted against the macrophage marker CD68 (STJ-6572; St John's Laboratory Ltd., London, UK) was used to detect macrophages. A rabbit pAb that acted against neutrophils (ab68672) was purchased from Abcam (Cambridge, UK). To detect HA, we used a biotin-conjugated HA-binding protein ([bHABP]; Seikagaku Kogyo, Tokyo, Japan). A rabbit pAb that acted against type I collagen (R1038) was purchased from Acris Antibodies (San Diego, CA). The following secondary probes were used to detect the primary probes: Alexa Fluor 633-conjugated streptavidin (Invitrogen, Carlsbad, CA), Alexa Fluor 568-conjugated goat anti-mouse IgG (Invitrogen), and Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen).

Biochemical assay

Wound tissues were extracted with 6 M guanidine hydrochloride (GdnHCl), 50 mM Tris–HCl, 1 mM phenylmethylsulfonyl fluoride, and a 1% (v/v) protease inhibitor (Sigma-Aldrich) at a pH of 7.5 for 72 hours at 4°C with shaking. The supernatant was collected by centrifugation at 4°C. The extracts were subjected to molecular-sieve chromatography using Sepharose CL-2B which was equilibrated and eluted with 4 M GdnHCl, 50 mM Tris–HCl, pH 7.5, as previously described.[13] Protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce/Thermo Fisher, Waltham, MA), with bovine serum albumin as the standard. For dot blot and blot overlay analyses, 5 μL fraction samples were spotted onto a nitrocellulose membrane (Bio-Rad Laboratories Inc., Des Plains IL). Horseradish-peroxidase (HRP)-conjugated anti-rabbit IgG (Dako, Glostrup, Denmark) and HRP-conjugated streptavidin (Invitrogen) were used to detect the primary probes.

Statistical analyses

Statistical analysis was performed using the Mann–Whitney test. ImageJ software (version 1.46, http://imagej.nih.gov/ij/) was used to quantify the fluorescent intensity of immune-cell staining for each antibody that was detected with fluorescence. The area of each cell was selected, and the maximum fluorescent intensity, minus the background of each cell, was used for data analysis. The fluorescent signals in 1,500 × 1,500 (2.25×10^6) pixels were calculated. A p-value < 0.05 indicated statistical significance.

RESULTS

Different ointment vehicles induced distinct tissue reactions in the mouse model of wound healing

Different macroscopic findings of the wound tissues treated with EM, MO, or MS were found (Figure 2). Wounds that were treated with EM appeared pinki and edematous on day 3 (Figure 2A). In contrast, wounds that were treated with MO had thin, white granulation tissue on day 3 (Figure 2B). Furthermore, wounds that were treated with MS appeared thinner and white (Figure 2C). Granulation tissues thickness was significantly different among the different ointment vehicles (Figure 2D). Prominent changes in the wound size were not seen during the observation period (data not shown).

During the histopathological analyses, the EM-treated wounds showed edematous interstitial changes with a less collagenous ECM, particularly on day 3 (Figure 3A–C). In contrast, the connective tissue of the MO-treated wounds had a denser and rich collagenous ECM throughout the wound-healing process (Figure 3D–F). In particular, a collagenous ECM was prominent in the tissue of MO-treated wounds on day 6 (Figure 3F). Furthermore, there was remarkable infiltration of the inflammatory cells in the

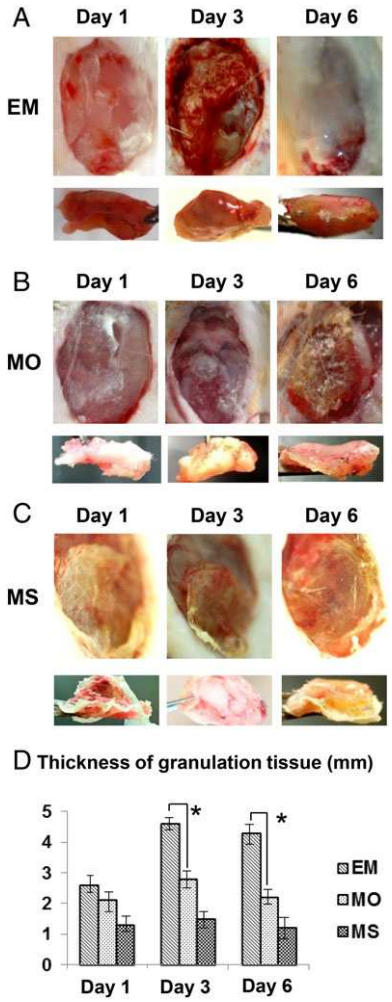

Figure 2. Macroscopic findings of wounds treated with distinct ointment vehicles. (A–C) Representative photographs of wounds in the ointment application model Wounds were treated with each ointment on days 1, 3, and 6 are shown. Representative macroscopic findings are shown in the upper panel. A horizontal image of the tissues obtained during biopsy is shown in the lower panel. (D) Granulation tissue thickness was measured using the biopsy-obtained samples. The average thickness (mm) of the granulation tissues is indicated as the mean ± standard deviation of four independent experiments. On days 3 and 6, there was a significant difference in the thickness of the tissues treated with EM and those treated with MO treatments. *$p < 0.05$.EM: cream in the form of an oil-in-water emulsion; MO, macrogol ointment.

granulation tissue of EM-treated wounds (Figure 3A and B), whereas MO-treated wounds exhibited relatively few inflammatory cells (Figure 3D and E).

The VG1F-SHAP-HA complex was characteristically present in EM-treated wounds

During the immunohistochemical analyses, we found that EM-treated wounds had surface lesions containing both VG1F and HCs on day 3 (Figure 4A). Furthermore, on day 3, VG1F and HA colocalized in the EM-treated wounds (Figure 4C). In contrast, the coexistence of VG1F, HC, and HA was not observed MO-treated wounds at any stage (Figure 4B and D). In particular, HCs were not observed in MO-treated wounds (Figure 4B). Similarly, the coexistence of VG1F and HC was not observed in EM-treated wounds on day 1 or 6 (Figure 4A). Immunohistochemical methods showed that type I collagen was dominant in MO-treated wounds; however, less type I collagen was found in EM-treated wounds (data not shown).

During biochemical analyses, extracts from the wound tissue were subjected to gel filtration under 4 M guanidine-HCl solution. Immuno-reactivity with VG1F, HCs, and HA was observed in fractions that were eluted around the void volume (Figure 4E). In contrast, VG1F, HCs, and HA were not eluted around the void volume of the MO-treated wound fractions even though HA and VG1F were independently detected throughout the fractions (Figure 4F). In contrast, high molecular-weight species consisting of type I collagen were observed specifically in MO-treated wounds (Figure 4E and F).

The VG1F-SHAP-HA complex formed in the interface between the wound tissue and ointment

Because the ointment vehicle directly interacts with wound tissue during the wound healing process, the interface between the ointment vehicle and wound surface was analyzed using the smear experiment and histochemical analyses using en bloc specimens. During the smear experiments of the wound surface, the coexistence of VG1F, HCs, and HA was found specifically in EM-treated wound samples (Figure 5A), indicating that the complex tended to form at the wound. In contrast, this complex was not found in smear samples from MO-treated wounds; however, VG1F and HA were located individually (Figure 5A).

The interface between the ointment and wound was visualized using histological specimens. On day 3, island-like eosinophilic staining was observed in the space between the wound's surface and ointment of EM-treated wounds (Figure 5B, broken box). In contrast, a similar finding was not observed in MO-treated wounds (Figure 5B). On day 6, differences between the ointment vehicles were not more prominent than those on day 3. In EM-treated wounds, the VG1F-SHAP-HA complex located in the lesion was equivalent to that in the eosinophilic material in the space between the wound surface and ointment (Figure 5B and C). However, VG1 and HA did not colocalize in MO-treated wounds. In addition, HCs were not seen in the interface lesion of MO-treated wounds (Figure 5C).

Inflammatory reactions during the wound-healing process were affected by ointment vehicles

On day 3, when compared with MO-treated wounds, macrophages were dominant in EM-treated wounds (Figure 6A). In contrast, neutrophils were abundant in MO-treated wounds on day 3 (Figure 6B). Quantitative analyses of this area demonstrated remarkable differences between ointment vehicles in terms of macrophages (Figure 6C) and neutrophils infiltration (Figure 6D).

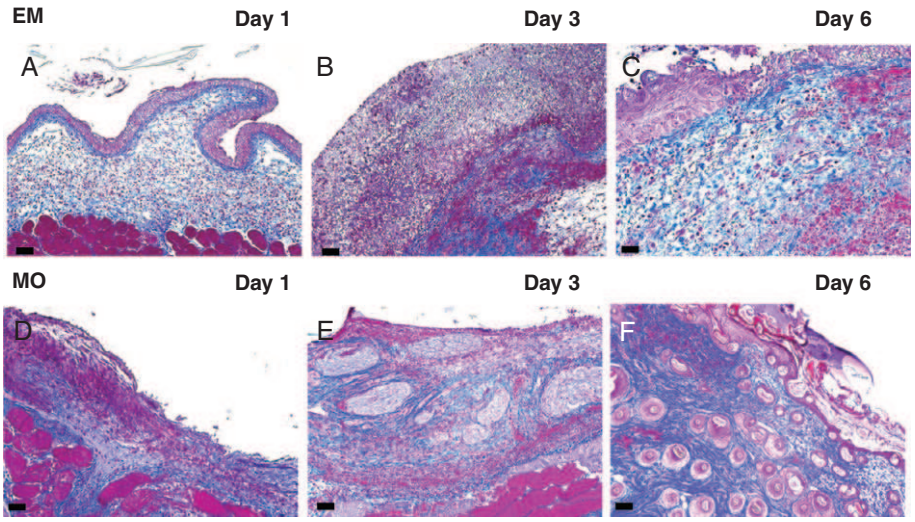

Figure 3. Microscopic findings of wounds treated with distinct ointment vehicles. (A-F) Representative microscopic images of wounds treated with each ointment vehicle on days 1, 3, and 6. Tissue specimens were stained with Masson trichrome staining. Bars = 20 μm. EM, cream in the form of an oil-in-water emulsion; MO, macrogol ointment.

DISCUSSION

In this study, we demonstrated that different ointment vehicle without active ingredients induced distinct tissue reactions. Although the ointment vehicle was considered inactive, this study elucidated the biological effects of the ointment vehicle on the wound-healing process. The mouse model used in this study showed a significant difference in the healing process of EM-treated and MO-treated wounds. The guidelines of the Japanese Dermatological Association and Japanese Society of Pressure Ulcers[1,2] suggested selecting an ointment on the basis of the physiochemical properties of its vehicle, and this study provides the basic scientific background for these guidelines.

The pathogenic mechanism of action of the ointment vehicle has not been completely clarified. However, we hypothesized that the mechanisms of action are as follows: the ointment vehicle directly interacts with minerals, growth factors and ECM proteins in a selective manner; EM adds water to the wound and possibly acts as a biological modulator; and MO absorbs water from the wound, which is concentrated with soluble molecules such as minerals, growth factors, proteases, and ECM proteins. These mechanisms may occur simultaneously during the wound-healing process.

The ointment vehicle regulates the water environment[3,4] and possibly acts as a counterpart of the wound tissue. Therefore, in this study, we focused on water-holding molecules, including HA and versican, in wound tissue.[7,10,16] During immunohistochemical analyses, the VG1F-SHAP-HA complex was characteristically observed in wounds treated with EM, but not in wounds treated with MO. The results were in accordance with the results of other studies that evaluated the characteristic appearance of the VG1F-SHAP-HA complex in edematous pressure ulcer wounds in humans[13] and SHAP-HA and versican under other inflammatory conditions, including rheumatoid arthritis and colitis.[17–20] Because the VG1F-SHAP-HA complex is associated with inflammatory reactions,[13] macrophages and neutrophils were stained and quantified in wounds treated with different vehicles. The dominance of macrophages, rather than neutrophils, in EM-treated wounds was consistent with the results of previous studies showing an association between versican-HA and macrophages.[19,20] Although, the details of this mechanism remain unclear, our results were consistent with the finding that changes in the HA-containing ECM affects the inflammatory response during wound healing.[21,22] Modifying the HA-containing ECM may also be relevant because HA-containing biomaterials modify the wound-healing process.[23–26]

The physicochemical properties of the vehicle, such as its water-absorbing capacity, may be critical factors in the interaction of the vehicle and wound surface. In previous studies, the ECM of the wound surface was likely to be overlooked because of technical reasons; conventional pathological procedures did not allow visualization of the interface between the ointment and pressure ulcer wounds.[27] Therefore, we analyzed the tissue reaction in the interface using a "smear experiment." Furthermore, a new en bloc histochemical method was performed to analyze the mechanism of action. Our new technique enabled us to visualize the VG1F-SHAP-HA complex between the wound surface and EM. Therefore, the HA-containing ECM may be a critical regulator that collaborates with the ointment vehicle at the wound surface. The ointment vehicle may act as a temporal and/or external ECM in the wound's environment.

During the healing of deep-pressure ulcers, formation of granulation tissue is required at a certain stage.[1] In contrast, prolonging the inflammation stage with excessive granulation tissue sometimes slows the entire healing process. In this study, EM and MO caused distinct types of granulation tissue formation. Granulation tissue that was treated with

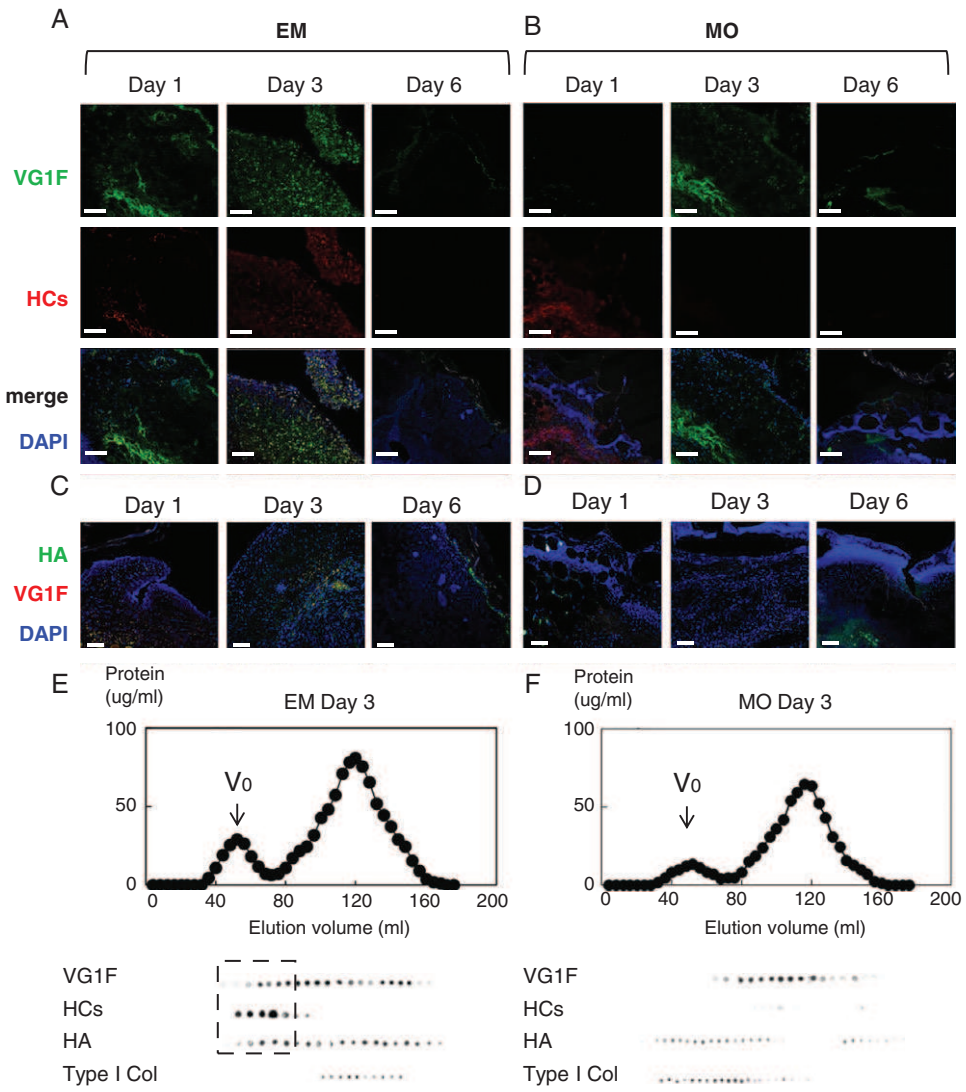

Figure 4. HA-containing ECMs in wounds treated with EM and MO. (A–D) Localization of VG1F, interalpha-inhibitor HCs, and HA in the wound tissues treated with different ointment vehicles. Tissue specimens were stained with anti-VG1F (pAb 8531; anti- DPEAAE neoepitope), anti-Iαl (HCs), and bHABP (HA) as indicated. The merged images of three antibodies are shown. Scale bars = 50 μm. The color of the letter that indicates the molecules represents the color of staining. (E, F), Molecular-sieve chromatography and dot blot/blot overlay analyses. Samples of wound tissues treated with ointments were extracted with 6 M guanidine-HCl and separated on CL-2B Sepharose under dissociative conditions. The representative elution profile was obtained, and dot blot/blot overlay analyses of the fractions were performed. The dot blot samples were probed with the following antibodies: pAb 8531 (VG1F), anti-Iαl (HCs), and anti-type I collagen (type I col). HA was detected using a blot overlay analysis using bHABP. Formation of the VG1F-SHAP-HA complex is indicated by the broken line. V_0, = void volume. EM, cream in the form of an oil-in-water emulsion; MO, macrogol ointment; HA, hyaluronan; VG1F, versican G1 fragment; HC, heavy chain; Iαl, inter-α-trypsin inhibitor; bHABP, biotin-conjugated hyaluronan-binding protein; SHAP, serum-derived HA-associated protein; pAb, polyclonal antibody; col, collagen.

EM grew rapidly and, occupies much larger volume than tissue treated with MO in our experimental model, however, granulation tissue of MO-treated wounds was denser and more packed. Although, it has not been concluded whether an edematous or flat wound is favorable during the wound-healing process, in practice, both types of wounds transiently appear during certain stages of the healing process of deep-pressure ulcers.[14] Furthermore, in practice, a blended

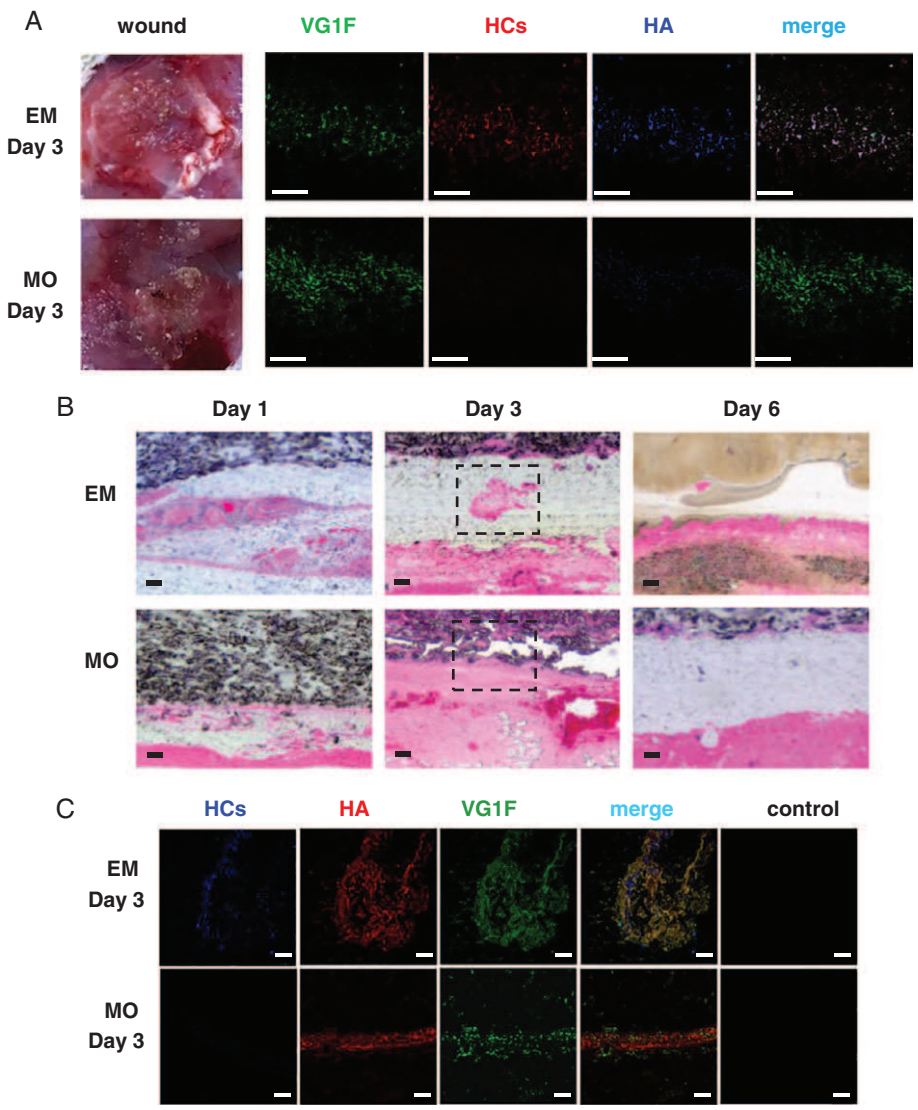

Figure 5. Characterization of HA-containing ECMs at the interface between the ointment and wound. (A) The "smear experiment" invloving the wound surface were treated with EM and MO. Samples of the wound surface were obtained on day 3 and spread onto a glass slide. Samples were then incubated with pAb 8,531 (VG1F), anti-Iαl (HCs), and bHABP (HA). Individual and merged images of representative results of three independent experiments are shown. Scale bars = 100 μm. (B, C) en bloc histochemistry of the tissue specimens including the ointment vehicle and wound tissue. B, Specimens were stained with hematoxylin and eosin. Tissues were obtained on days 1, 3, and 6. Serial sections adjacent to the boxed area were subjected to immunofluorescence (C). Scale bars = 20 μm. (C) Immunofluorescence of wounds treated with each ointment on day 3. Tissues were stained with anti-Iαl (HCs), pAb 8,531 (VG1F), and bHABP (HA). Merged images of three antibodies and the negative control (omitting primary probes) are shown. Representative results from three independent experiments are shown. Scale bars = 20 μm.EM: cream in the form of an oil-in-water emulsion; MO, macrogol ointment; HA, hyaluronan; ECM: extra-cellular matrix; Iαl, inter-α-trypsin inhibitor; HC, heavy chain; pAb: polyclonal antibody; VG1F, versican G1 fragment; bHABP, biotin-conjugated hyaluronan-binding protein.

ointment comprising MO and EM appropriately regulates the amount of exudate and modifies the water-rich ECM in wound tissue.[28,29]

This study has some limitations. This was not an interventional study of human pressure ulcers. However, pathological findings of actual pressure ulcers are limited and individual wound conditions are highly variable.[27] Therefore, this mouse model would be useful for understanding the mechanism of action of ointment vehicles.

In conclusion, our results may provide a better understanding of the treatment of deep-pressure ulcers by focusing on the ointment vehicle.

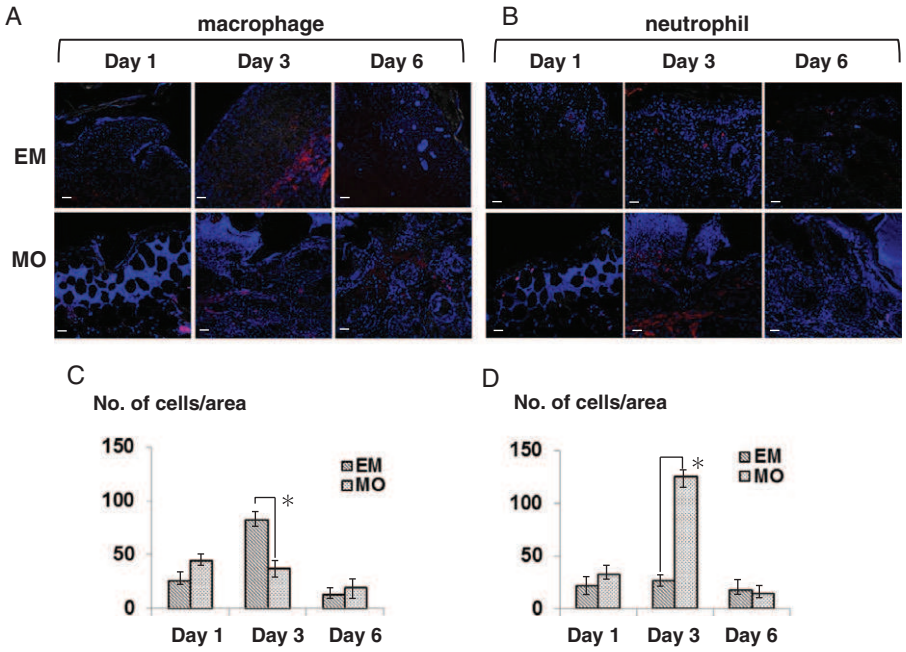

Figure 6. Different inflammatory reactions in wounds treated with an ointment vehicle.Immunofluorescent images of macrophages (A) and neutrophils (B) in wound tissue. The tissue samples were obtained from wounds treated with EM (upper panel) or MO (lower panel). Tissue samples were obtained on days 1, 3, and 6. Specimens were stained with antimacrophage and antineutrophil antibodies. Representative results of three independent experiments are shown. Scale bars = 50 μm. (C, D) Immunofluorescence of the infiltrated cells was quantified with ImageQuant software. The numbers of macrophages and neutrophils per 2.25×10^6 pixels were counted in 10 areas. There was a significant difference between wounds treated with different vehicles. C: $p = 0.024$ to <0.05, D: $p = 0.013$ to <0.05. EM, cream in the form of an oil-in-water emulsion; MO, macrogol ointment.

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