Sanyo Chemical

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Development of a novel medical material using silk-elastin, a functional protein

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"The Sanyo Chemical News" (No. 488) introduced that silk-elastin, a functional protein, has a potential in the treatment of wound such as bedsores (pressure ulcers) and burn injury (heat injury). In medical practice, there is a need for wound healing promoting material that helps to protect against infection and promote wound healing. The need is particularly great for patients with deep wounds and those who have wounds with poor blood flow due to diabetes etc. Sanyo Chemical is developing the use of silk-elastin as a novel biomedical material that can promote healing of wounds including those with risk of infection. Here, I introduce the characteristics of silk-elastin and the development states as a wound healing material, and describe the future prospects.

What is silk-elastin?

Silk-elastin is an artificial protein manufactured by combining human elastin and silkworm to generate fibroin-specific sequences, using DNA recombinant technology (Figure 1). Silk-elastin is considered suitable for the treatment of wounds since it contains many elastin-derived sequences in the molecule and thus has a high cellular affinity (it fits to skin without causing inflammation) and has a high elasticity (provides skin tension). In water solution, silk-elastin is aggregated at low temperature due to hydrogen bonding between silk fibroin-derived sequences, but the hydrogen bonding decreases at higher temperature, and it turns into a gel form after absorbing water and being swollen by elastin-derived sequences with hydrophilic character (Figure 2, 3). The gelled silk-elastin solution is an irreversible gel. The gel has an elasticity similar to that of soft tissue including skin.

Wound healing process

The skin covering the human body accounts for 16% of the body weight and has a variety of function. Skin is made of subcutaneous tissue (together with the muscles and underlying bones), dermis, and epidermis (the utmost surface). The wounds such as pressure ulcer and heat injury are caused by tissue necrosis due to pressure or heat.

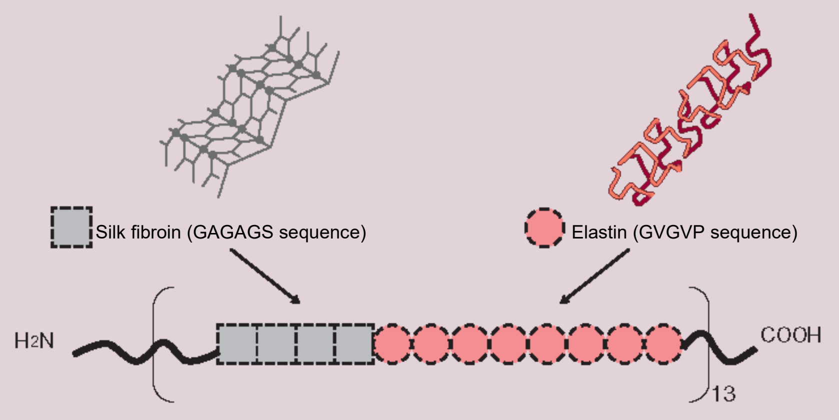

Figure 1 Amino-acid sequence of silk-elastin

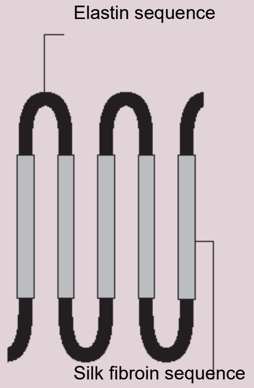

Figure 2 Structure of silk-elastin

The wounds such as scratches and cuts are caused by physical destruction of skin tissue. Wounds are classified into four groups according to wound depth (wounds of epidermis, wounds involving dermis, wounds involving subcutaneous tissue, and wounds involving muscles/bones). The wounds where skin barrier function is absent or those with poor blood flow due to diabetes etc. are associated with a higher risk of infection.

Wound healing process begins with hemostasis via platelet aggregation and is achieved by vasoconstriction. Then, body fluid (exudate) infiltrates into the surface of the wound, and inflammatory cells (macrophage etc.) are recruited (migration) to the surface of the wound by cytokines (proteins that play a role in cell signalling) in exudate, and ingestion of necrotic tissue and removal of bacteria occurs <inflammatory stage>. After inflammation is resolved, fibroblasts (producing collagen to form granulation tissue) migrate and proliferate in the wound surface also by cytokines, and formation of granulation tissue occurs <proliferation stage>. After granulation tissue is formed, the epithelial cells that make up the epidermis extend, and granulation tissue evolves into scar tissue (organization) <mature stage>. The healing process ends when epithelialization is completed.

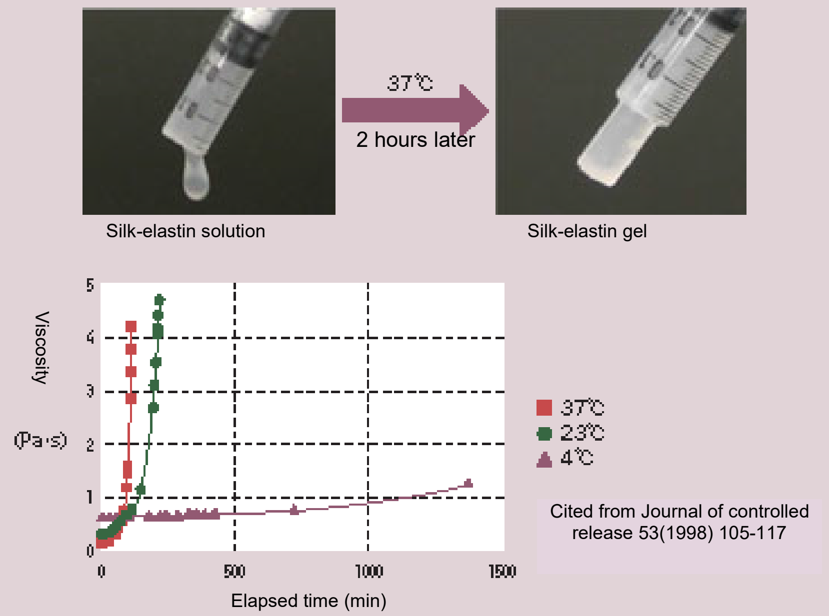

Figure 3 **Changes in viscosity in silk-elastin**

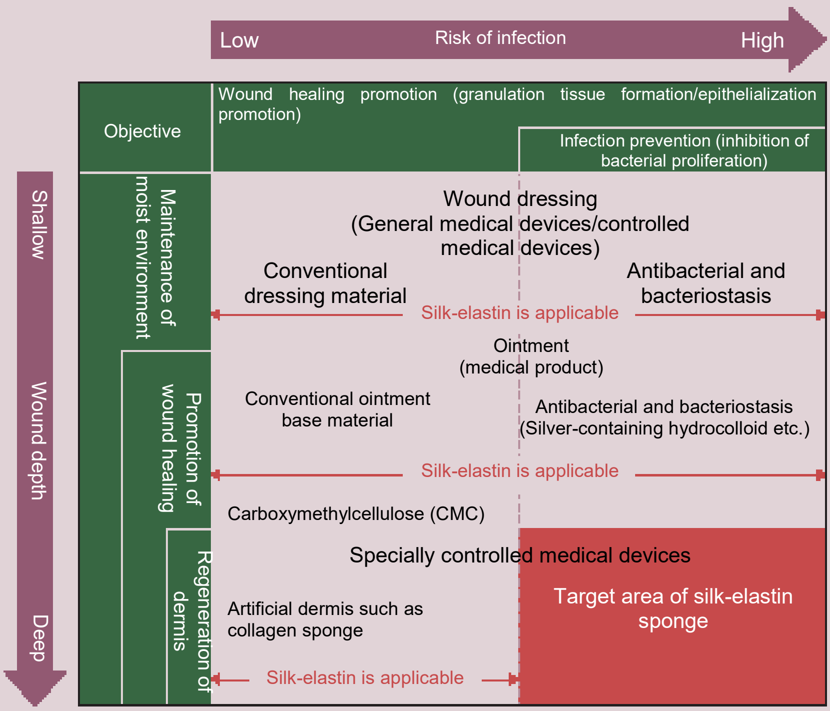

Figure 4 **proper uses of wound healing materials**

Silk-elastin and silk-elastin sponge are currently under development, and they are not commercially available at the time of this writing.

Development of wound healing materials

The conventional medical devices for wound treatment are classified according to wound depth and the level of infection risk as shown in Figure 4. Wound dressing or ointments are used on relatively shallow wounds for the purpose of "maintenance of moist environment." Carboxymethylcellulose (CMC) etc. are used on wounds with low risk of infection, and bacteriostatic agents such as silver-containing hydrocolloid are used on wounds with high risk of infection.

Silk-elastin as a wound healing material is characterized by its high adhesiveness and compliance for complex wounds compared with wound dressing and ointments because the solution spreads over the lesion and turns into a gel at body temperature.

Silk-elastin sponge

Silk-elastin film

Figure 5 **Forms of silk-elastin**

Silk-elastin gel has a function not only in "maintenance of moist environment" but also in "protection of wound" for appropriate wound surface conditions. In addition, because silk-elastin has a high cellular affinity, it promotes migration and proliferation of cells required in the inflammation stage and proliferation/mature stages ("wound healing promotion"). Furthermore, silk-elastin has a high resistance to infection, and thus it can be an effective wound healing material in patients with high risk of infection.

On the other hand, sponge-like materials using collagen (with high cellular affinity) such as collagen sponge are expected to act as a scaffold of cells, and are used for the purpose of "wound healing promotion" in wounds with greater depth but low risk of infection. However, collagen sponge is vulnerable to infection and thus not suitable for wounds with greater depth and high risk of infection. In such cases, currently, ointments are used instead although their wound healing promotion effects are inferior to collagen sponge. Therefore, there is a need of wound healing material that is resistant to infection and effective for wound healing promotion in clinical practice.

In order to address the need, our company succeeded in processing of the sponge-like material (silk-elastin sponge) and film-like material (silk-elastin film) with a variety of density and thickness by our original interface control technique (Figure 5). Since silk-elastin has a high resistance to infection, silk-elastin sponge can be an effective material for wounds with greater depth and high risk of infection.

Wound healing effects of silk-elastin sponge

The mechanism of action is assumed to be as follows. By using the sponge-like material instead of solution, exudate from the wound surface is absorbed by the sponge, resulting in dissolution of silk-elastin. The fluid including high-density exudate spreads over the wound surface, and turns into a gel (Figure 6). Since the gel containing exudate that is formed in the wound surface has an extremely high adhesiveness, it is effective in "protection of wound" and "maintenance of moist environment." In addition, through the effects of cytokines derived from the exudate included in the gel and of silk-elastin with high cellular affinity, migration of macrophage (that is essential in the inflammatory stage) and migration and proliferation of fibroblasts (that facilitates skin regeneration) are promoted in the entire wound. It is assumed that these actions contribute to "inhibition of bacterial proliferation" and "promotion of wound healing."

Figure 7 shows the measurements of the concentration of exudate-derived cytokines in silk-elastin gel. The experiment was carried out on healthy guinea pigs in which a skin defect (involving all layers) was created on the back. Then, silk-elastin sponge was administered on the wound surface, and polyurethane film was applied. At 12 hours after administration, gelled silk-elastin on the wound surface was extracted, and concentration of cytokines related to proliferation of fibroblasts (bFGF) and those related to anti-inflammatory response (IL-1β and TNF-α) were measured. The results showed that the concentrations of the cytokines in the silk-elastin group were approximately 2 to 10 times higher compared with those

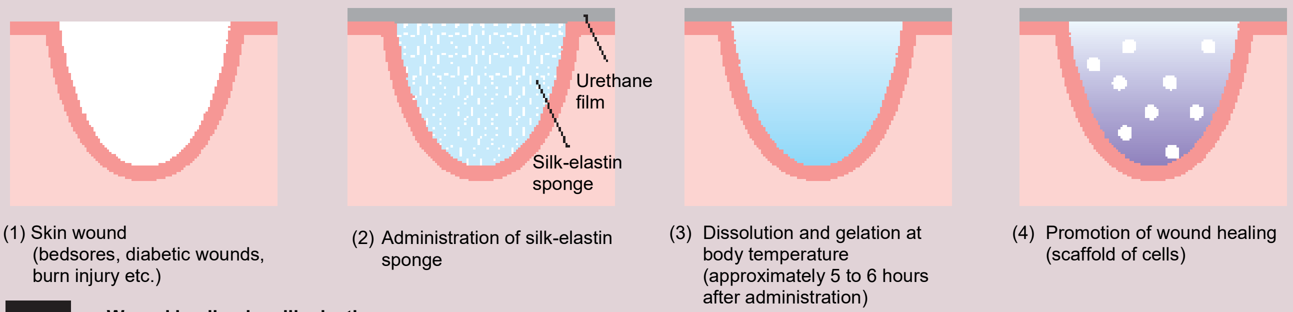

Figure 6 **Wound healing by silk-elastin sponge**

included in the fluid collected by washing the wound surface with phosphoric acid buffer solution (PBS). Figure 8 shows the results of experiment using animal models similar to those described in Figure 7. Pseudomonas aeruginosa was seeded on the wound surface and then silk-elastin was administered. At 3 days of treatment, the wound surface was removed, and the number of bacteria on the wound surface was counted using bacterial colony. As a result, conventional materials (CMC gel products) failed to inhibit the proliferation of bacteria, whereas silk-elastin inhibited the proliferation.

Figure 9 shows the results of experiment using animal models similar to those described in Figure 7. At 5 days of treatment, granulation tissue formation was evaluated by histological assessment (HE staining). Granulation tissue formation was almost not observed in the polyurethane film alone, whereas granulation tissue formation was observed in the whole wound surface when silk-elastin sponge was used, demonstrating the effectiveness of silk-elastin in granulation tissue formation.

Based on these results, we consider that silk-elastin sponge, as a wound healing material, can be an extremely effective medical material that can be applied to wounds with greater depth and high risk of infection for which the application of conventional medical devices has been difficult. We are planning to establish the clinical proof of concept (POC) by biological safety testing and subsequent investigator-initiated trial. With the aging population, the numbers of elderly patients with pressure ulcer and those with intractable skin ulcer such as diabetic skin ulcer are increasing. Silk-elastin is expected to promote wound healing in these conditions and contribute to improvement in quality of life (QOL) of elderly patients.

This study is supported by the Acceleration of Transformative Research for Medical Innovation (ACT-M) in 2016 of Japan Agency for Medical Research and Development (AMED), and the study is being conducted in collaboration with the Department of Plastic and Reconstructive Surgery, Postgraduate School of Medicine, Kyoto University.

[Contact (about the product)]

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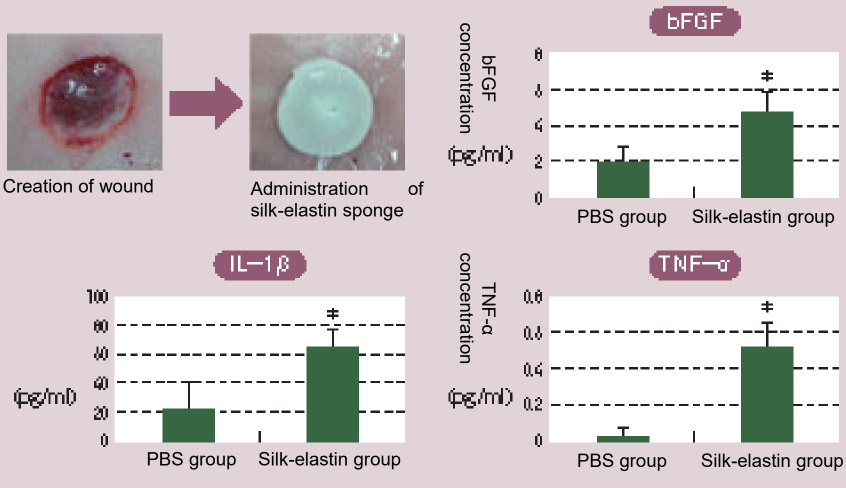

Figure 7 **Effects of silk-elastin on cytokine production**

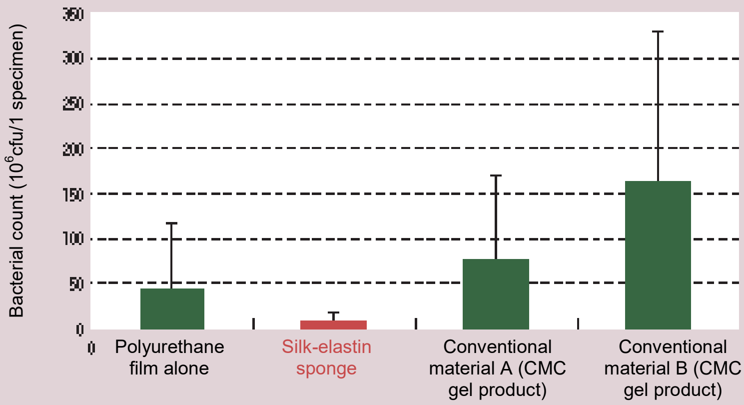

Figure 8 **An experiment on the effects of silk-elastin on the inhibition of bacterial proliferation**

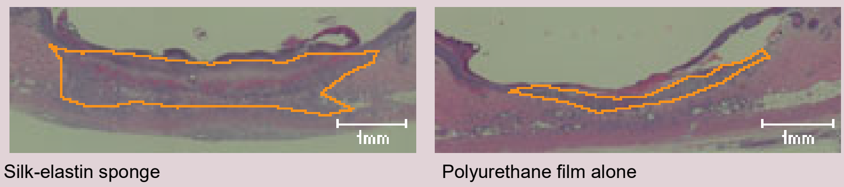

Figure 9 **Effects of silk-elastin on granulation tissue formation**