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Development of New Medical Materials Using Functional Protein Silk-Elastin

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Treatment options for wounds such as pressure ulcers (bedsores) and burns include surgical treatment (e.g., skin grafting) and conservative treatment (e.g., use of wound dressings or topical agent). Usually, conservative treatment is provided first, and if wound healing does not occur, surgical treatment is provided. Silk-elastin, a biocompatible protein, has the potential for conservatively treating several types of wounds that conventionally require surgical treatment as well as improving outcomes of subsequent surgical treatment. Therefore, at our company, we are developing new medical materials using silk-elastin that can reduce the psychological, physical, and economic burden of patients. This paper presents the features of silk-elastin, the progress of its development as a wound healing material, and future prospects.

Features of silk-elastin

In 2009, our company signed a license agreement with Protein Polymer Technologies Inc., a bio-venture company in the United States (US), and introduced the technology of silk-elastin, a functional protein with high cell affinity, expected to be highly effective for the treatment of wounds. Silk-elastin is an artificial protein manufactured by genetic recombination consisting of elastin, a protein found in human skin, and silk fibroin, a component of silk **[Figure 1]**.

Silk-elastin is considered suitable for wound therapy because its molecular structure contains many elastin sequences, thus possessing

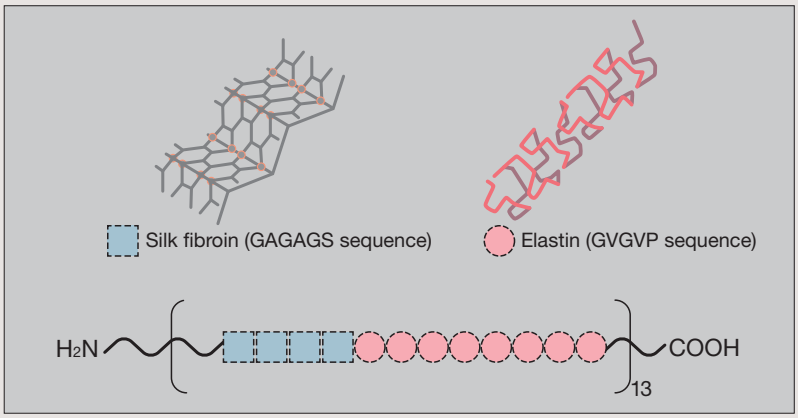


Figure 1 • Amino acid sequence of silk-elastin

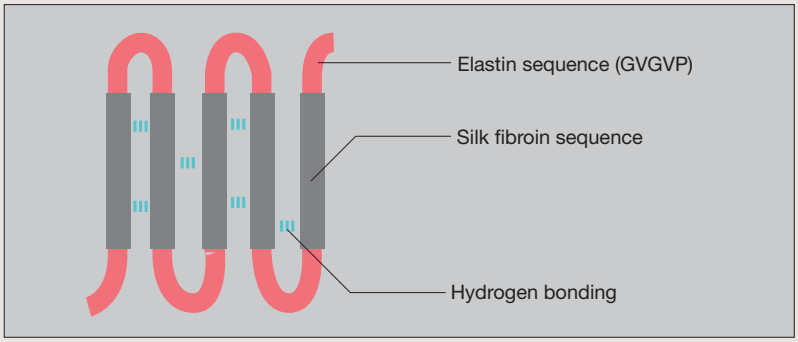


Figure 2 • Structure of silk-elastin

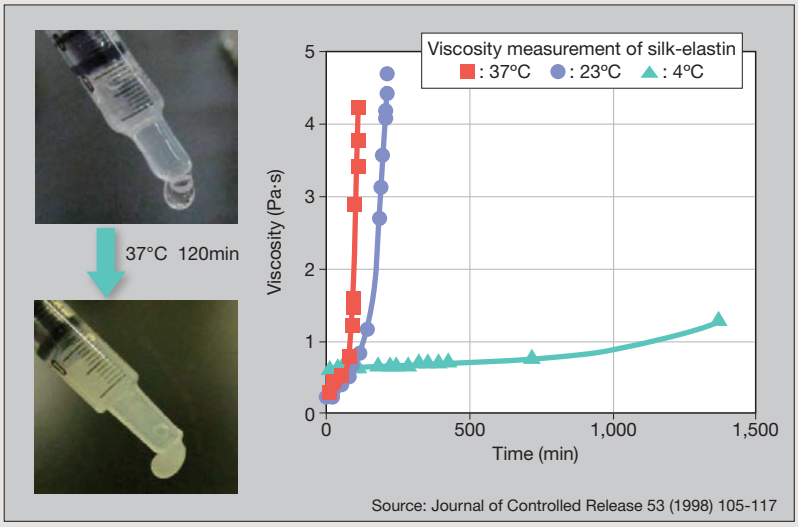
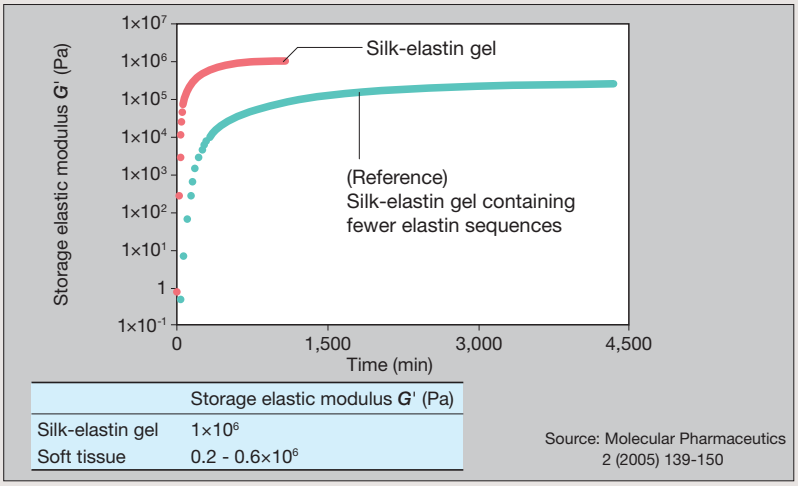


Figure 3 • Changes in viscosity of silk-elastin



	Storage elastic modulus *G'* (Pa)
Silk-elastin gel	1×10^6
Soft tissue	$0.2 - 0.6\times10^6$

Source: Molecular Pharmaceutics 2 (2005) 139-150

Figure 4 • Measurement of storage elastic modulus of silk-elastin gel

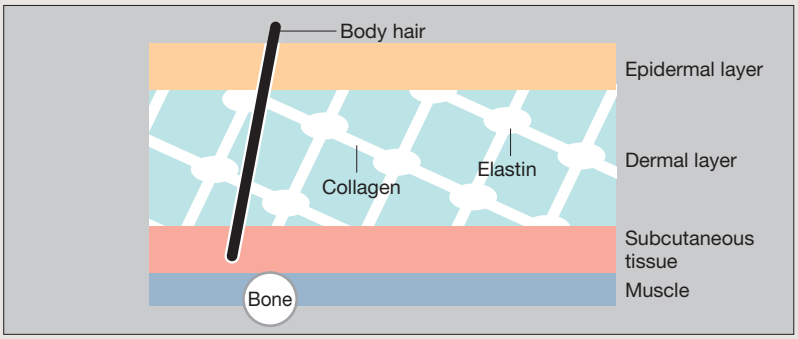


Figure 5 • Skin structure

high cellular affinity (the property to be applied to the skin without causing inflammation) and high elasticity (the property to add resilience to the skin). In terms of physical properties, silk-elastin solution turns into a gel when heated. This gelation is caused by structural changes owing to temperature changes. While silk-elastin is aggregated at low temperatures because of hydrogen bonding of the silk fibroin sequences, this hydrogen bonding weakens with heat, and highly hydrophilic elastin sequences retain water and swell, resulting in a gel **[Figures 2 and 3]**. Once gelated, silk-elastin becomes an irreversible gel that does not return to aqueous solution even at low temperatures and has an elasticity resembling a soft tissue, such as the skin, owing to its elastin sequences **[Figure 4]**. The time required for gelation can be controlled by the heating temperature, silk-elastin concentration, pH, salt concentration, etc.

Development of a wound treatment material

Skin covering the entire human body accounts for 16% of body weight and has a variety of functions, including (1) retaining and preventing loss of water, (2) regulating body temperature, (3) defending the body from external stimuli, and (4) serving as a sensory organ. Over muscles and bones, the skin structure comprises the subcutaneous tissue, dermal layer, and epidermal layer on the outermost surface **[Figure 5]**. Wounds such as pressure ulcers and burns are caused by the necrosis of skin tissue owing to pressure or heat. In addition, wounds such as scratches and cuts cause physical destruction of skin tissue. Wounds are divided into four groups according to the depth (wounds reaching the epidermis, wounds reaching the dermis, wounds reaching the subcutaneous tissue, and wounds reaching the muscles/bones). Common wound-healing processes include platelet aggregation and hemostasis by vasoconstriction, followed by incorporation of dead tissue and removal of bacteria by inflammatory cells (e.g., macrophages) (inflammatory phase). After the inflammatory phase ends, fibroblasts (producing collagen that forms granulation tissue) migrate and proliferate within the wound surface to form granulation tissue (proliferation phase). Once granulation tissue is formed, the epithelial cells that make up the epidermis extend, and the granulation tissue is transformed into scar tissue (development of a wound mark) (the mature stage). When epithelialization is completed, complete wound healing is achieved. In wounds reaching the epidermis/dermis, granulation tissue is regenerated in the defective dermis layer with a bandage or by natural healing force, and epithelialization (epidermal regeneration) is eventually achieved. For wounds reaching the subcutaneous tissues, muscles, and/or bones, on the other hand, it is difficult to achieve healing only with natural healing power; conservative treatment and surgical treatment are generally combined. The medical devices used for conventional wound healing can be roughly divided into wound dressings for skin defects and other similar devices. Wound dressing for skin defects is a medical device intended to "protect the wound" and "maintain a moist environment." Various materials are used in such situations, such as alginic acid, chitin, and carboxymethylcellulose (CMC). Other similar medical devices include collagen sponge, which is a dermal graft for skin defects, and dextranomer, which is a polymer absorbent for removing dead tissues. These two materials are intended for "promoting formation of granulation tissue" to actively

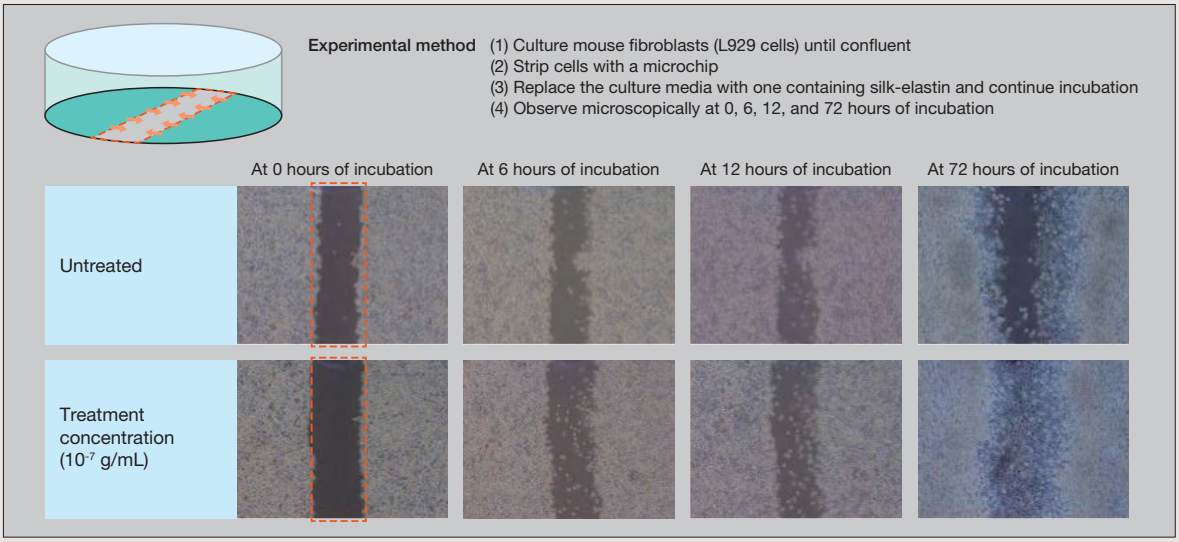


Figure 6 • Cell migration capability of silk-elastin with fibroblasts

promote skin regeneration and are used for different purposes from the aforementioned wound dressings for skin defects.

◇Features of silk-elastin

Silk-elastin as a wound-healing material is characterized by high adhesion and followability suitable for complicated wounds as the silk-elastin solution fills the skin defect and gelates at the body temperature. Therefore, the aqueous gel formed at the affected area enables optimal adhesion and supports the intended functions of the wound dressing for skin defects: "protect the wound" and "maintain a moist environment." Furthermore, as a protein with high cellular affinity, silk-elastin helps to "promote formation of granulation tissue" because it promotes migration and proliferation of the cells responsible for skin regeneration, namely fibroblasts.

Figure 6 shows the cell migration capability of silk-elastin with fibroblasts. The experiment was conducted by first proliferating mouse fibroblasts on a cell culture petri dish, then scraping them off over one line with a microchip, and culturing them again on a new medium containing silk-elastin before observation. After 6 hours of culturing, the culture medium with silk-elastin resulted in greater migration of cells from both ends of the stripped area, compared with the medium without silk-elastin. After 72 hours, it was confirmed that fibroblasts were growing again over the entire surface.

Silk-elastin is expected to not only "promote formation of granulation tissue" but also "inhibit wound contraction," "prevent spread of bacterial infection, add elasticity to the affected area, and effectively treat wounds in combination with drugs" owing to its gel properties.

[Inhibition of wound contraction]

In general, if scar contracture occurs at the moving parts, such as joints, patients' movements may be restricted by a diminished range of motion. Scar contracture can be cured only through surgical treatment (e.g., scar removal surgery), and it imposes a burden on patients even after wound healing is completed. Since its ability to inhibit wound contracture can prevent excessive contracture of the closed wound around the joint, silk-elastin is expected to help mitigate a decrease in range of motion and prevent tightly pulled skin after healing.

[Prevention of spread of bacterial infection]

Conventional medical devices (particularly gel products such as CMC) can absorb exudates and weaken adhesion between the wound and the gel, allowing bacterial proliferation and adversely affecting skin regeneration. On the other hand, silk-elastin gel can prevent invasion by bacteria around the wound through close adherence and can physically contain existing bacteria to inhibit their proliferation.

[Addition of skin elasticity to the affected area]

The elastin component of silk-elastin provides skin elasticity, which prevents recurrence of pressure sore and improves esthetic appearance.

[Combination therapy with drugs]

Based on the gel properties of silk-elastin, it can be formulated with pharmaceutical products with pharmacological and therapeutic effects, such as cell growth factors, enabling slow release of the substance along with decomposition of the silk-elastin gel. In general, cell growth factors are substances that have a short half-life because biomaterial (particularly enzymes) break down or inactivate them. Therefore, formulation of cell growth factors in silk-elastin gel can effectively prevent their degradation and inactivation.

◇Effects observed in animal tests

In actual animal tests, silk-elastin has been demonstrated to help promote formation of granulation tissue. The experiment was

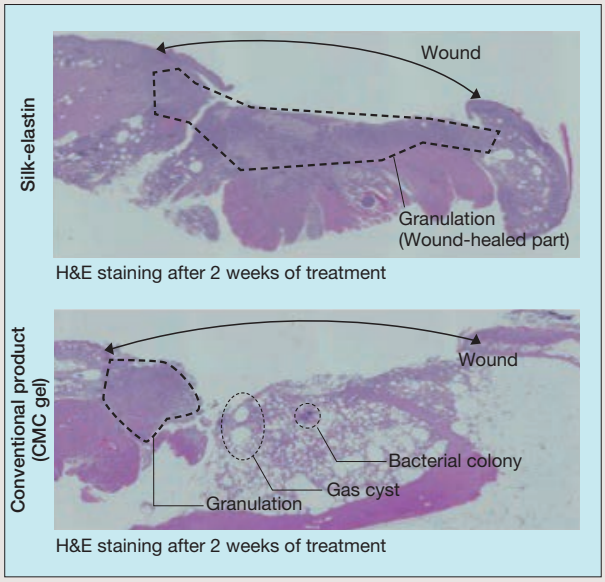


Figure 7 • Effect of silk-elastin on promoting formation of granulation tissue

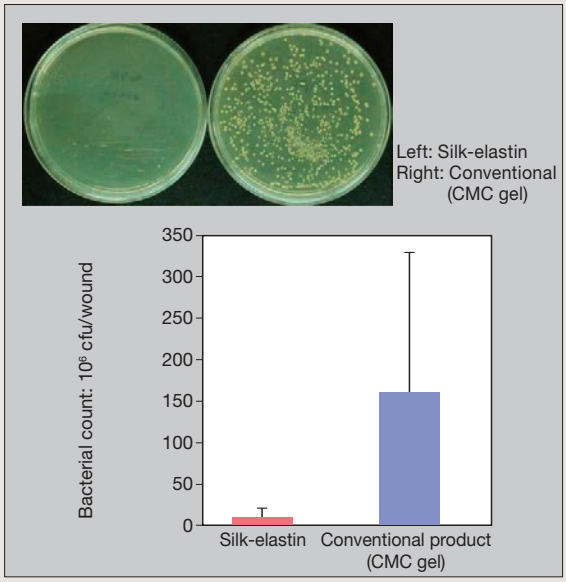


Figure 8 • Verification experiment of the effect of silk-elastin on "preventing spread of bacterial infection"

conducted by first making a wound reaching the muscle/bone at the thigh of genetically diabetic mice and then administering silk-elastin solution. Silk-elastin solution gelates at the mouse body temperature and covers the wound surface. A polyurethane film was applied, and after 2 weeks following the treatment, formation of granulation tissue was evaluated macroscopically and histologically (hematoxylin and eosin [H&E] stain). As shown in **Figure 7**, in the group treated with the conventional product (CMC gel), granulation tissue was formed only on a part of the wound surface and was deemed insufficient in the histological evaluation. In addition, on the wound surface where granulation tissue was not formed, a gas cyst and bacterial infection were observed. In the silk-elastin group, on the other hand, healing of the wound surface was clearly evident macroscopically.

Furthermore, formation of granulation tissue was observed throughout the wound surface in the histological evaluation, supporting the strong ability of silk-elastin to promote formation of granulation tissue.

Silk-elastin's ability to "prevent spread of bacterial infection" was also demonstrated in the verification experiment. During the experiment, a wound (full thickness wound) reaching the subcutaneous tissue was made on the back of healthy guinea pigs. Then, Pseudomonas aeruginosa was spotted on the wound surface at 10^6 cfu/wound before administering silk-elastin solution or a conventional product (CMC gel) and applying a polyurethane film. On day 3 of treatment, the wound surface was removed and the number of bacteria present on the wound surface was determined using the bacterial colony technique **[Figure 8]**. Results showed that the conventional product (CMC gel) failed to inhibit bacterial growth, whereas silk-elastin successfully inhibited bacterial growth.

Based on these results, we consider that silk-elastin, as a wound-healing material, offers a new conservative treatment method different from conventional medical devices and becomes a new medical device with the possibility of expanding the range of conservative treatment. In the future, development will be accelerated for clinical trials and regulatory application/approval through conduct of biological safety studies. As refractory skin ulcers such as pressure sore and diabetic skin ulcers in the elderly are expected to increase with the aging population of industrialized countries, silk-elastin is expected to promote wound healing and contribute to improvement in quality of life (QOL) of the elderly.

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A-STEP: This technology transfer support program aims to support application of technologies in the research and development phase inspired from research achievements of universities and public research institutions, etc. regarding sciences and technologies that are important for the national economy.

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