Meniscus Injury
Development of a novel treatment method

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Japan, with the highest longevity in the world, has entered a super-aging society with fewer children that no country has ever experienced. However, in recent years, stress has been placed on increasing "healthy life expectancy" rather than merely increasing life expectancy. Healthy life expectancy is defined as the length of life that a person can live with good physical and mental health. It is reported that healthy life expectancy is approximately 10 years shorter than the overall life expectancy in Japan.[1] The factors that reduce healthy life expectancy (requiring long-term nursing care) include locomotor disability (accounting for 20% of all causes), of which "joint disease" is the most common (accounting for 10.9% of all causes).

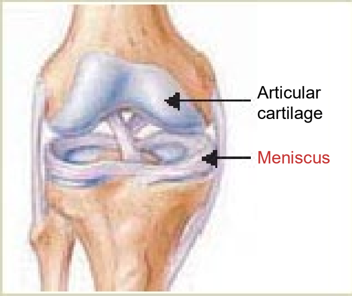
Osteoarthritis (OA) is the most common "joint disease" in the elderly population. The number of OA patients is estimated at 25.3 million, of which approximately 8 million patients have symptoms such as pain. Osteoarthritis of the knee (knee OA) occurs secondary to cartilage loss, meniscus injury, or anterior cruciate ligament injury, all of which are caused by the aging process and by traumatic injury. In particular, the meniscus has a role as a cushion, and its injury is a major contributory cause of knee OA. Therefore, repair of meniscus can contribute to prevention of knee OA. However, repair of meniscus is perceived as difficult.

Our company is developing the use of silk-elastin, an artificial protein, as a wound healing material. On the other hand, the Department of Orthopedic Surgery National University Corporation Hiroshima University has long been involved in the treatment of knee joint diseases including prevention and treatment of knee OA. Currently, we are jointly developing the use of silk-elastin as meniscus regeneration material. Here, I introduce the characteristics of silk-elastin and the development states as meniscus regeneration material, and describe the future prospects.

Meniscus injury

The components of the knee joint include ligaments, tendons, and muscles. In addition to these structures there are elastic "articular cartilage" that covers the surface of bones and provides near-frictionless mechanical motion and "meniscus" that has a role as a cushion absorbing shocks.

Articular cartilage and meniscus decrease friction and absorb shocks and thus they are important for smooth joint function.



Provided by the Department of Orthopedic Surgery Hiroshima University

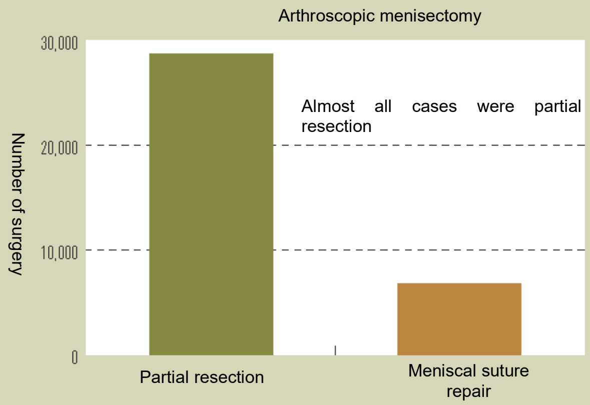
Figure 1 Structure of Knee

The meniscus has a half moon shape, and there are two in each side (medial and lateral sides) (**Figure 1**).

However, these structures are susceptible to injury and deformation by repetitive loading especially in the elderly and athletes. When these structures are injured or deformed, it can cause a wide variety of symptoms such as pain and discomfort during walking, difficulty in bending and stretching, inflammation, and retention of fluid or blood.

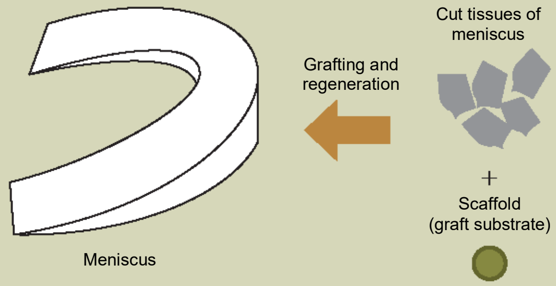
Regeneration of meniscus

Human body has an ability to repair tissue damage; however, it is recognized that repair of articular cartilage and meniscus is extremely difficult because these tissues receive less blood and lymph flow and that they are subjected to high loading.



Health Insurance Claims, Specific Health Checkups and Specific Health Guidance Database by the Ministry of Health, Labour and Welfare
(From April 2014 to March 2015)

Figure 2 **Current status of treatment of meniscus**



Provided by the Department of Orthopedic Surgery Hiroshima University

Figure 3 **Concept of meniscal repair**

Approach to tissue engineering for articular cartilage using the patient's own cells (autologous cells) has been developed in recent years; however, the meniscus repair and regeneration technique has not been established.

Currently, symptomatic treatment with "arthroscopic meniscus partial resection" is the mainstay of treatment for injury and deformation of meniscus (**Figure 2**). However, it is known that even partial resection of the meniscus has a great influence on knee function and later induces knee OA, resulting in difficulty in bending, stretching, and walking. Therefore, meniscal suture repair has been performed increasingly to preserve meniscal functions. However, the success of meniscal suture repair depends on the degree and extent of the injury, and therefore its indication is limited. In addition, the effectiveness of meniscal suture repair is limited. It was reported that complete recovery was difficult in patients with lower healing ability and having lesions with deformation.

Under these circumstances, our company and the Department of Orthopedic Surgery Hiroshima University are jointly developing the use of silk-elastin as "graft substrate," considering that the material has a potential to improve treatment outcomes with meniscal repair. Furthermore, we aim to establish a more simple and effective technique (both efficacy and safety) for the treatment of meniscus injury with a combination use of "cut tissues of meniscus" (under development by Hiroshima University) which allows effective use of autologous tissue (including autologous cells) (**Figure 3**).

* Two several millimeters incisions are made on the knee joint. An arthroscope is inserted into one of the incisions, and the injured portion is resected by inserting the surgical tool into the other incision.

What is silk-elastin?

Silk-elastin is an artificial protein manufactured by combining elastin, a naturally occurring protein polymer, and silk fibroin-specific sequences, using genetic recombination (**Figure 4, 5**).

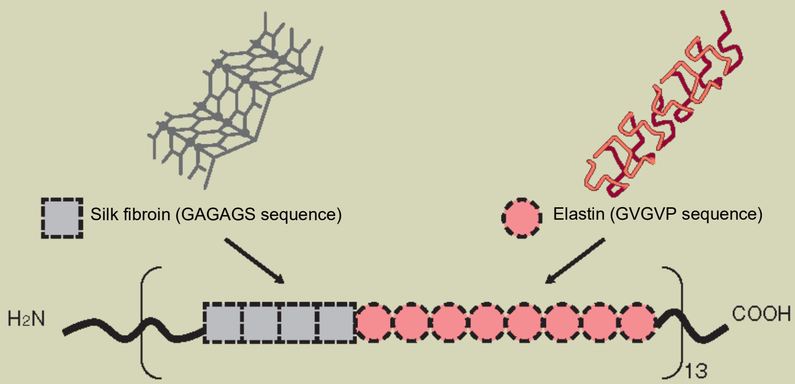


Figure 4 **Amino-acid sequence of silk-elastin**

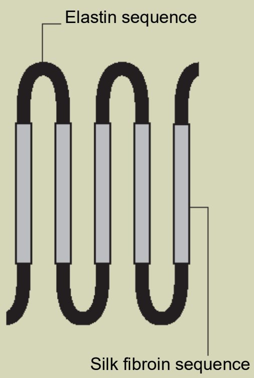


Figure 5 **Structure of silk-elastin**

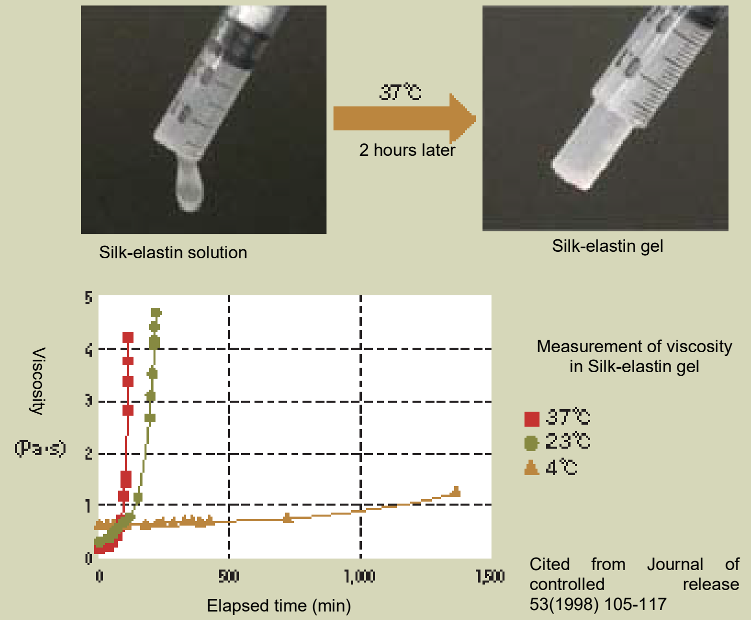


Figure 6 **Changes in viscosity in silk-elastin**

Silk-elastin is considered suitable for wound treatment 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 (it 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 6**).

The gelled silk-elastin solution is an irreversible gel. The gel has an elasticity similar to that of soft tissue including skin. In addition, 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 7**). We consider that this allows to provide optimal biological and mechanical environment as a graft substrate.

Evaluation of silk-elastin as a meniscal graft substrate

Here we performed histological evaluation of silk-elastin as a graft substrate using small animals. An anterior horn defect model using the meniscus of Japanese white domestic rabbits was created and silk-elastin sponge was grafted into the defect (**Figure 8**). Rabbits with untreated defects were used as a control group. At 4 weeks after operation, the control group did not show significant tissue formation, whereas the silk-elastin graft group showed filling of the tissue defects (hematoxylin-eosin staining) and regeneration of tissue significantly expressing cartilage matrix (safranine O staining).

Figure 7 **Forms of silk-elastin**

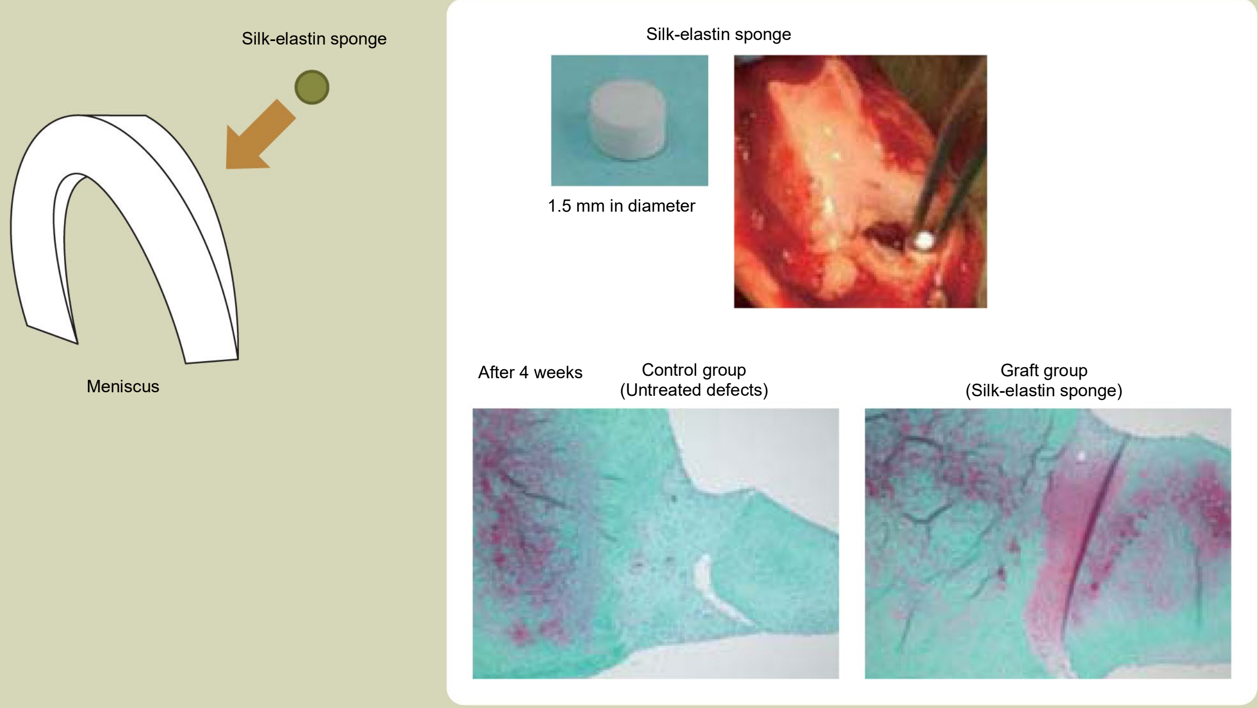


Figure 8 **Efficacy evaluation of silk-elastin**

Development of meniscus regeneration material

We were able to confirm the effectiveness of silk-elastin as a "graft substrate" that has a potential to improve treatment outcomes with meniscal repair. In addition, Hiroshima University is developing a simultaneous grafting method to graft the patient's own meniscal tissue and silk-elastin (**Figure 9**). Traditionally, the resected meniscal tissue has been discarded. This is an inventive, world's first approach in which the meniscal tissue including substrate and cells is grafted into the defect area.

Figure 9 **Cut tissues of meniscus**

This approach does not require culture; therefore, reoperation (after culture) is not needed. This one-time operation is one of the advantages of taking this approach. It is also expected that adding this approach to meniscus suture repair may lead to better outcomes in meniscal repair and degeneration.

Future planning

This study is supported by the Acceleration of Transformative Research for Medical Innovation (ACT-MS) in 2018 of Japan Agency for Medical Research and Development (AMED), and the study is being conducted in collaboration with Hiroshima University. We are planning to establish the clinical proof of concept (POC) as the set-up company by obtaining the non-clinical data in collaboration with Hiroshima University. The meniscus regeneration is our first-step. We further aim to provide a total treatment solution targeting the radical cure of knee osteoarthritis.

We are also developing a wound treatment material for bedsores and refractory skin ulcer of lower leg etc. by utilizing the characteristics of silk-elastin in collaboration with Kyoto University. We continuously develop the use of new medical materials and pursue our biomedical project.

References
1) Ministry of Health, Labour and Welfare「健康寿命のあり方に関する有識者研究会報告書」(March 2019)

[Contact (about the product)]
Marketing Department of Biotechnology & Medical Division
https://www.sanyo-chemical.co.jp/eng/