Silk Fibroin Bombyx Mori Yarns for Bio-Resistant Woven Aortic Prostheses

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Abstract. **The main aim of this research is to study the possibility of applying natural silk yarn for composite structure of woven bio-resistant blood vessel prosthesis, which has good anti-thrombotic and anti-inflammatory properties. During the initial phase of research, the modern world experience of practical application of natural silk in surgical practice has been considered. The second phase of theoretical research has been devoted to the further study of modern science and practical experience of laboratory, pre-clinical and clinical application of natural silk fibres in different areas of cardiac surgery, including designing, manufacturing and implantation of vascular prostheses of various fibrous structure and geometric designs. In the process of implementation of the practical part of this scientific work, the authors propose an innovative woven tubular structure, consisting of three different types of warp and weft yarns: polyester, polyurethane and natural silk. Natural silk yarns needed for this purpose are made of silk fibroin fibres Bombyx mori. In the laboratory, the authors of the article have studied the mechanical and deformation properties of natural silk yarns needed to manufacture innovative implants of the blood vessels. The properties of polyurethane and polyester yarns were examined before, in the previous studies. The laboratory tests have confirmed the possibility of the use of natural silk yarn in the designing and manufacturing of woven tubular prosthesis. It should be noted that the structure of walls of the blood vessel prosthesis designed and proposed in this paper is unique. The further research will be aimed at producing the prototypes of aortic prosthesis and determining its basic biomechanical properties.**

Keywords: Bombyx mori, silk fibroin, natural silk fibre, vascular implant, woven prosthesis.

I. Introduction

Atherosclerosis often damages the abdominal aorta. In such a case, the reconstruction of the aortic segment is required to resume normal blood flow in the cardiovascular system. The cross-sectional diameter of healthy aorta changes during pulsations by 11% compared to diastole. Therefore, the pathologically damaged segment should be replaced with the implant made of long-term elastic materials. Properties of synthetic implants must be close to the natural human blood vessels. Unfortunately, modern commercial prostheses do not exhibit such characteristics with completely similar properties of natural blood vessels. Scientists also do not offer such prototypes at this time.

Many scientists continue to actively develop various multidisciplinary scientific directions for improving the quality of vascular prostheses. For example, this research can be divided into five main scientific directions:

- development of synthetic absorbable materials with precisely known dates of destruction;
- development of non-absorbable materials with good bio-mechanical qualities and minimal adverse effects on tissue;
- development of antibacterial materials;
- development of materials that stimulate tissue repair processes.

Currently, the majority of vascular implants available on the medical market are made of polyester fibres with a low level of biological affinity.

Scientists regularly look for new solutions for the application of new materials and modern technologies.

The aim of this research is to create the modified structure of wall of woven vascular implants and use natural silk yarns in combination with polyester and polyurethane.

Bombyx Mori Silk Fibroin Fibres

Yarn made of natural silk fibres has been specially selected for this research. Firstly, natural silk yarns usually have good strain and other mechanical properties. The main advantage of natural silk yarn is a very good flexibility and very high strength at low thickness. Secondly, these fibres and yarns are natural and used in medicine. Natural silk fibres have very strong anti-inflammatory properties in the tissue. In this case, yarns were made of twisted fibres of silk fibroin.

Silk fibroin is a natural polymer from *Bombyx mori*, which has been used in textile production clinical sutures, and more recently as a scaffold for tissue regeneration [1–3].

Silk fibroin exhibits impressive mechanical properties, as well as biocompatibility making it an attractive biomaterial and scaffold for tissue engineering. The fibroin protein is one of biological materials used for artificial skin and other medical applications. As a result of its biodegradability [4], silk fibroin was evaluated for several biomedical applications.

It is well known that natural silk yarn has unique physical properties and this material is considered the gold standard in surgery. For example, a natural silk thread has been successfully used for surgical stitch.

Natural silk emitted by the silkworm consists of two main proteins: sericin (20-30%) and fibroin (70-80%). Fibroin is the structural centre of the silk, and sericin is the sticky material surrounding it.

Fibroin is a type of protein created by *Bombyx mori* silkworms in the production of silk. The fibroin protein consists of layers of antiparallel β-sheet. Its primary structure mainly consists of the recurrent amino acid sequence.

Sericin is a type of protein created also by *Bombyx mori* silkworms in the production of silk. Chemical nature of serecin is $C_{30}H_{40}N_{10}O_{16}$.

These substances stimulate the metabolism in the human body. However, the natural silk filament is elastic, soft and smooth. It has very good deformation and strength indicators.

Silk fibres typically have a triangular cross-section, or in the form of a trapezoid (See Fig. 1).

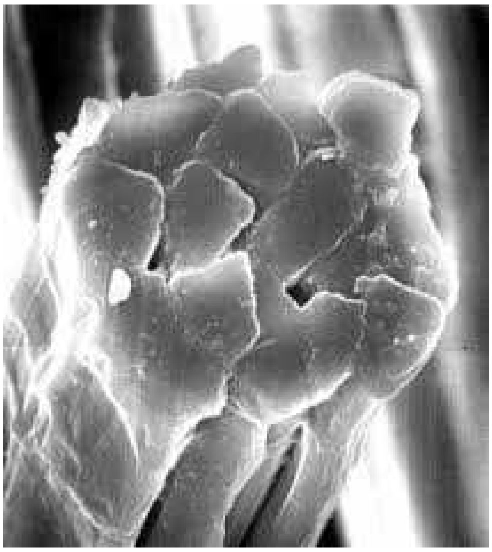

Fig. 1. Bombyx mori silk fibre thickness of 10 μm, SEM

It is well known that *Bombyx mori* silk is composed of filament core protein, silk fibroin and a glue-like coating consisting of nonfilamentous protein – sericin. Silk fibroin is characterized by repetitive hydrophobic and hydrophilic peptide sequences and consists of heavy and light chain polypeptides of ~390 kDa and ~26 kDa, respectively, linked by a disulfide bond at the C-terminus of the two subunits. The primary structure of *Bombyx mori* silk fibroin protein is characterized by the presence of three amino acids in a roughly 3:2:1 ratio: glycine (~45%), alanine (~30%) and serine (~12%). The sequence is dominated by [Gly-Ala-Gly-Ala-Gly-Ser]n. Silk fibroin chains also contain amino acids with bulky and polar side chains, in particular tyrosine, valine, and acidic amino acids. The repetitive sequence in hydrophobic residues dominates the β-sheet structure, forming crystalline regions in silk fibroin fibres and films. The formation of these β-sheets results in insolubility in water [5].

II. Previous experience

Scientists offer several methods for obtaining solutions, mixtures and gels based on silk fibroin, for example, bio-compatibility films, special impregnations, new bio-materials, porous materials, drug delivery platform and substances for tissue-engineering applications [6-9].

Other scientists also conducted similar research to find new solutions for the modification of vascular implants [10-12].

In 2010, the Japanese Professor Tetsuo Asakura used the natural silk yarn for production of specimens of small diameter vascular implants (less than d = 4 mm). These prostheses were implanted in the area of the abdominal aorta of animals [13-15]. After one year, the prostheses were explanted and then histological studies were conducted. The results of this study showed that cells had migrated within the silk fibre and formed a close-knit vascular structure. The lumen-side endothelial cells and surrounding smooth muscle cells were aligned in an orderly manner, the nutrient vessels of the outer membrane were formed. The structure of prosthesis very closely resembled the original blood vessel. Furthermore, after the examination of the post-transplantation small-diameter silk vascular prostheses in detail, the scientists established that the silk content was decreasing and after a year a new vascular structure formed. In addition to the increase in cellular components, it was also discovered that over time the the collagen content increased and the silk fibre content decreased.

Professor Tetsuo Asakura and his colleagues also carried out pre-clinical studies of comparing the patency of the two different types of small-diameter implants. Silk fibroin-based grafts (1.5 mm in diameter, 10 mm in length) were implanted into the abdominal aorta of male 10 to 14-week-old Sprague Dawley rats by end-to-end anastomosis.

Polytetrafluoroethylene (PTFE)-based grafts were used as the control. Patency of the fibroin grafts one year after implantation was significantly higher than that of PTFE grafts (85.1% vs 30%, $p < 0.01$). These findings have demonstrated that a small-diameter silk fibroin-based vascular graft has excellent patency [16].

A group of researchers from China (Guoping Guan, Lu Wang, Quan Zhan, Xiaoyuan Yang, Yuling Li) together with researchers from the United States (Robert Guidoin) and Canada (Martin W. King) also involved in research in the field of production of vascular implants from silk fibroin [17]. They used natural silk fibroin fibres from *Bombyx mori* and *polyethylene terephthalate* (PET) filaments as raw materials to weave a silk fibroin-PET based small diameter vascular graft, whose inner diameter was about 4 mm. Furthermore, in order to improve the cyto-compatibility and hemo-compatibility of the graft, the inner surface was chemically modified through UV irradiation and grafting methods. In addition, the cyto-compatibility and hemo-compatibility were examined through in vitro cell culture and thrombus detection tests, respectively. The results showed that the properties of the graft are suitable for further *in vivo* study and could prove valuable in future clinical applications. As a result, the graft has demonstrated superior performance in terms of cell proliferation and thrombus detection.

For example, we can select next positive practical application of silk fibroin in medicine. It is silk fibroin suture use in surgery. These sutures are made by twisting of silk fibres. Then these sutures must be processed and covered by natural wax or silicone.

The main parameters of sutures should be standardized in the USP/EP. The manufacturing process of suture shall be certified in accordance with the GMP standard. Suture manufacturer must have WHO, ISO, CE, USFDA, PCT certificates or other local documents. These regulations are valid also for producers of implants.

III. The Innovative Structure of Wall of the Woven Implant

In woven practice, the most commonly used natural silk yarn is twisted from eight filaments.

In our case, we have developed and proposed a new structure of wall of the vascular implant. We have used polyester yarns (T = 10 tex), polyurethane yarns (T = 9.5 tex) and natural silk yarns (T = 10 tex) in the ratio 1:2:1. Thus, the hollow tubular structure is formed in the process of weaving. This structure of wall of the implant must be effective in the pulsating hemodynamics (see Fig. 2).

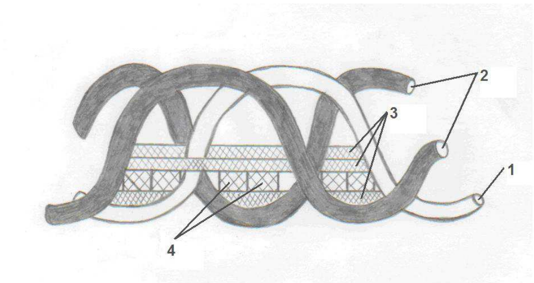

Fig.2. The structure of wall of the implant: 1 – the polyester warp yarns; 2 – the natural silk warp yarns; 3 – the polyurethane warp yarns; 4 – the polyurethane weft yarns

This design of wall of the vascular prosthesis is unique. Main conditions of such a design are as follows. Combined structure consists of three different types of yarn. The wall of the prosthesis has very good elastic properties in the longitudinal and radial directions. Natural silk yarn helps change the bio-resistant properties of the vascular implant.

Natural silk yarn helps to change the bio-resistant properties of the vascular implant. It is one of the main and fundamental ideas of this research. As a result, such a combined structure helps provide a good long-term hemodynamic process and good integration of prosthesis in the body tissue.

IV. The Study of Biomechanical Properties

Silk yarn testing has been carried out at the next stage of scientific research. For this purpose, we have used Zwick/Roell testing machine and a special computer program to record the experiment data. This program is also needed for the integrated analysis of experimental data, including charting (see Fig. 3). In general, the biomechanical study shows very good results.

Final results of the study have been processed using special software. For example, the deformation properties of silk yarns have been tested, and then the mean and maximum values have been examined.

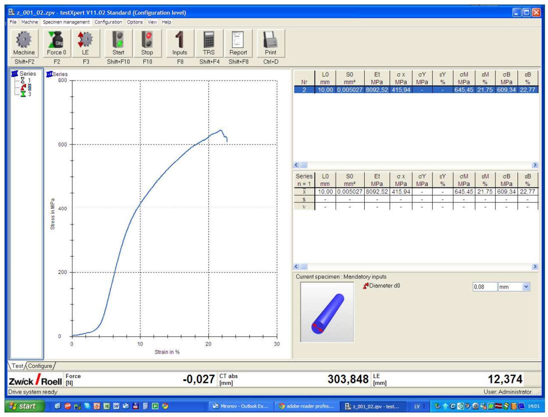

Fig.3 Natural silk yarn stress (Mpa) / strain (%) diagram

The final table shows the basic information on the natural silk yarn (see Table 1).

Table 1. Main indicators (the average value) of silk yarn

Yarn length (mm)	Average diameter of yarn (mm)	Maximum stress σM (Mpa)	Maximum strain εM (%)
10.00	0.08	645.45	21.75

V. Conclusions

A unique woven structure and excellent long-term physical properties of yarns are the main factors of this success. As a result, new woven prosthesis has also very good elastic deformation properties in longitudinal and radial directions.

Silk yarns improve bio-compatibility with living tissues and improve the integration of the implant. These prostheses are better resistant to microorganisms. Implants made of natural silk significantly help reduce the toxin production after implantation.

These implants help restore the normal long-term process of hemodynamics in human beings after the operation. Within one year, natural silk fibres have gradually degraded in the organism and in their place cells have formed a new vascular structure of the living tissue.

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Viktorija Kanceviča, Andrejs Lukjančikovs. Dabiskā zīda pavedieni no silk fibroin Bombyx mori šķiedrām biorezistentā austā asinsvadu protezē
Darba galvenais mērķis radīt asinsvadu protēzes, kurās iestrādāti silk fibroin Bombyx mori šķiedru pavedieni, kas ar laiku uzsūksies organismā un tiks aizvietoti ar pacienta dzīvajiem audiem. Šādās protēzes sieniņu struktūru veido kompozītie šķiedru materiāli ar labām antitrombogēnām un pretiekaisuma īpašībām. Darba gaitā tika izpētīta mūsdienu pasaules zinātnieku pieredze dabiskā zīda pavedienu izmantošanā ķirurģiskajā praksē: veterinārijā un klīniskajā sfērā. Šī darba autori piedāvā unikālu, inovatīvu, pulsēt spējīgu asinsvadu protēzes sieniņas struktūru, kurā būs iestrādāti poliestera, poliuretāna un dabiskā zīda pavedieni no fibroin Bombyx mori šķiedrām. Dabiskā zīda pavedienu īpašības tika izpētītas laboratorijas apstākļos un rezultāti pierādīja, ka tos var veiksmīgi izmantot aušanas tehnoloģijā, radot jaunas konstrukcijas implantus. Turpmāk tiek plānots izgatavot aortas asinsvadu protēžu prototipus, izmantojot inovatīvo sieniņu struktūru un dabiskā zīda pavedienus, kas ar laiku inkorporējoties dzīvā organismā uzsūksies, radot ilgtermiņā darboties spējīgu protēzi ar lielāku dzīvo audu klātbūtni tās konstrukcijā.

Виктория Канцевича, Андрей Лукьянчиков. Нити из натуральных шелковых волокон silk fibroin Bombyx mori для изготовления биорезистентных тканых протезов аорты
Главной целью данной работы являлось изучение возможности применения нитей натурального шелка для разработки композитной структуры стенки тканого биорезистентного протеза кровеносного сосуда, обладающего хорошими антитромбогенными и противовоспалительными свойствами. В ходе начального этапа исследовательской работы был изучен современный мировой опыт практического применения натуральных шелковых нитей в хирургической практике. На втором этапе теоретического исследования, был дополнительно изучен современный научный и практический опыт лабораторного, преклинического и клинического применения натуральных шелковых волокон в различных областях кардиохирургии, в том числе, при проектировании, изготовлении и имплантации сосудистых протезов различной волокнистой структуры и геометрической конструкции. В процессе реализации практической части данной научной работы авторами была предложена инновационная структура тканой трубчатой конструкции, состоящей из трех различных видов основных и уточных нитей: полиэфирных, полиуретановых и шелковых. Натуральные шелковые нити, предназначенные для этой цели, были изготовлены из волокон silk fibroin Bombyx mori. В ходе проведенных лабораторных исследований были изучены механические и деформационные свойства натуральной шелковой нити, необходимой для изготовления инновационных имплантов кровеносных сосудов. Свойства полиэфирных и полиуретановых нитей изучались ранее, в ходе предыдущих исследований. Результаты лабораторных тестов подтвердили возможность использования нитей натурального шелка при проектировании и изготовлении тканого трубчатого протеза. Следует отметить, что разработанная и предлагаемая в данной работе структура стенки протеза кровеносного сосуда является уникальной. Дальнейшей целью данной работы является изготовление прототипов протезов аорты и определение их основных биомеханических свойств.