

# Spider Silk in Medicine

## Weaving the Way for Innovative Medical Applications

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### Abstract

Materials have specific properties which make them useful for certain applications. Medicine is an area in which various material characteristics find a role in the human body. Spider silk fibre has revolutionary properties which make it an excellent new biomaterial. This review article will explore the chemistry, physics and biology of this material. Spider silk fibre has more recently also been reverse engineered into the physical state of an aqueous and ultra-thin film state. This opens up additional possibilities. It also allows implantation of silk material based devices within the human body in a manner that seamlessly conforms to biotic-abiotic interface. The versatile uses of spider silk in medicine include micro sutures (a type of joint found in the skull) with more strength, sturdy bio-scaffolds for regenerative medicine and tissue engineering for artificial skin and nerve grafts, tendon and ligament repair with the required strength and elasticity, weight-bearing artificial knee menisci in Orthopaedic surgery, liquid silk for biological wound dressings, silk micro particles for drug delivery and finally silk optics, bio-photonics, biosensors and bio electronics. The practical challenges and scope of research will revolve around the mass production and genetic engineering of spider silk, and reinventing a material that has existed for millennia.

### Introduction

The origin of my idea to explore the potentials of Spider Silk in Medicine came about while I was researching into materials for my A Level Physics Project. As an aspiring medic, I was interested in the vast use of biomaterials within the field of medicine. Spider silk particularly stood out with its amazing physical properties.

The material spider silk must not be confused with silkworm silk, which is a traditional silk biomaterial used since ancient times. Silkworm silk is produced by the caterpillar larvae of the moth *Bombyx mori* and is found on mulberry trees. Both spider silk and silkworm silk share a limited similarity in basic protein structure but differ in configuration.

Spider silk has incredible strength. It has been stated that “a pencil-thick spider’s silk thread is capable of stopping a Boeing-747 in full flight”<sup>1</sup>. A single strand of dragline silk is stronger than steel and the man-made super material Kevlar<sup>2</sup>. This is surprising at first; we do not usually think of silk as being strong, or being able to easily brush aside webs that the spider spent hours scrupulously spinning. There is a scene in the film ‘Spider-man 2’, in which Spider-man holds back an out of control train using ropes of silk strands. Perhaps this is not simply a superhero fantasy by Marvel Comics, defying the laws of physics. It may be illustrating a scientific fact.

The bar graph illustrates the published<sup>345</sup> mechanical properties of current suture materials in medicine in comparison to dragline silk.

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**Fig. 1: Figure 1**

Bar graph comparing physical properties of spider silk to current surgical suture materials

### CHEMISTRY – The structural building blocks

Dragline silk consists of the protein spidroin. As indicated in Figure 2, at a primary structure level, the spidroin consists of a central repetitive region of the amino acids glycine and alanine, enclosed by two terminal regions.

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<http://archive.ysjournal.com/wp-content/uploads/2015/01/Gly-Ala-Structure-300x108.png>

## Fig. 2: Figure 2

Chemical structure of a spidroin strand

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In spider silk, at a secondary level, a single strand consists of beta sheet crystallites, formed as chains of the amino acids (polypeptide molecules) arranged into an ordered and crystalline manner. These crystalline sheets are embedded in an amorphous (not crystalline; lacking a clear structure with random orientations) glycine matrix, consisting of helical and beta turn structures (structures in which the protein can reverse the direction of the peptide chain), interlinked with hydrogen bonds (Figure 2). It is the interaction between the hard crystalline segments formed by polypeptide molecules arranged in an orderly crystalline manner, and the elastic semi-amorphous regions that give spider silk its properties.

Structure of spider silk inside a typical fibre

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<https://archive.archive.ysjournal.com/wp-content/uploads/2015/01/Secondary-Structure--300x232.jpg>

## Fig. 3: Figure 3

Structure of spider silk inside a typical fibre

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Strong intermolecular interactions (disulfide/hydrogen/ionic bonds) exist between the crystallites and the randomly oriented amorphous segments <sup>6</sup>.

The distinction between this and silkworm silk must be made. Silkworm silk consists of two parallel strands of protein called fibroin. Similar to the spidroin in spider silk, the fibroin also consists of repeating units of glycine and alanine, but also consists of serine in the pleated  $\beta$ -sheets. Additionally, the filaments of fibroin are coated in a glue-like layer of another protein, sericin<sup>7</sup>.

## PHYSICS – The strength

Definitions

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If the silk is stretched beyond its yield point, these intermolecular and intramolecular interactions would be broken first; therefore the amorphous protein chains can be extended and straightened out. The ability of the silk to be straightened out without losing its strength under tension gives it its **DUCTILE** property. It can plastically elongate without losing strength under increasing tensile stress (any cracks on the surface in a ductile material do not become larger under stress)<sup>7</sup>

The large forces required to break these strong inter/intramolecular interactions and to stretch out and fully untangle the amorphous protein chains give dragline silk its **HIGH TENSILE STRENGTH**; it requires a large force of  $1.1 \times 10^9$  N per  $m^2$  area for it to break under tension when being stretched out at either end.

This combination of being able to withstand high stress (as silk has a high tensile strength) yet experiencing little strain due to untangling of amorphous chains means spider silk has a high stress-strain ratio. This high Young's modulus of 10 GPa gives it the property of **STIFFNESS**, meaning for a large stress applied to the silk ( $10 \times 10^9$  N per  $m^2$  area) there is a very small strain (1%). It is resistant to bending or stretching so does not easily deform.

The hydrogen bonds in the  $\beta$ -sheets break by a 'stick-slip' motion in which energy is dissipated through the amorphous matrix. This makes the silk **TOUGH** as a large energy can be absorbed per unit volume and cracks are unable to spread through the fibre. Dragline silk has a toughness of  $160 \text{ MJm}^{-3}$ , meaning it is difficult to break as it absorbs a large energy ( $160 \times 10^6$  Joules) per  $m^3$  volume before breaking. It tends to stretch before breaking.

## BIOLOGY – A time tested material

The most common species of spider which produces the spider dragline silk is the **Araneus diadematus** (Orb Weaving

Spider/European Garden Spider), found in Europe and some parts of North America.

#### Araneus diadematus

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<https://archive.archive.ysjournal.com/wp-content/uploads/2015/01/Araneus-diadematus-283x300.jpg>

#### Fig. 4: Figure 4

Araneus diadematus

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It has six to seven glands, each from which a different type of silk is secreted. The silk useful as a biomaterial is MA (Major Ampullate) silk, used for mooring threads, framework and radial thread of the spider web. Its average diameter is  $2.53 \pm 0.04$  micrometres. **8** A human hair ranges from 20 to 150 micrometres.

#### Types of spider silk

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<https://archive.archive.ysjournal.com/wp-content/uploads/2015/01/Types-of-Silk-300x225.jpg>

#### Fig. 5: Figure 5

Types of spider silk

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There are over 41,000 species of spiders. The mechanical properties of each of their silk vary, reflecting the evolutionary and ecologically diverse spider lineages. Dragline silk is used as a lifeline by spiders and forms the supporting framework of the web in which spiders catch prey. The material properties of dragline silk itself differ among various evolutionary lineages of spiders. The silk of the *Araneus diadematus* is much stronger and tougher than the silk of other spiders. This could reflect the ecology of the spider as the threads of the *Araneus diadematus* may need to stop the high kinetic energy of flying insect prey. Therefore, by studying the evolutionary and ecological nature of spiders, the silk with the most advanced mechanical properties can be distinguished and consequently used for biomedical applications. **9**

Agnarsson et al highlight the ecological impact on the properties of spider silk. In extreme river ecosystems in Madagascar, where the web bridge line must be 10-14 m to span across the riverbank, and withstand force from water droplets, the silk would exhibit high-performance properties of strength and toughness. **9**

In addition to its superior physical qualities, it is a natural material. It has, therefore, biological properties which make it compatible with the body. Being a natural material, it is also cheap, so easier to use. Spider silk has antimicrobial properties and is non-immunogenic, non-reactive and biodegradable. This makes it an excellent biomaterial; enhancing its invaluable nature.

A research article by Wright and Goodacre from the University of Nottingham discusses the evolutionary origins of the antibacterial nature of silk. Silk was treated with Proteinase K, an enzyme which breaks down proteins. It was observed that the addition of the enzyme reduced the ability of the silk to inhibit bacterial growth. This experimental evidence suggests an associated protein in silk is responsible for its antimicrobial property. The antimicrobial property may have evolved in order that silk can resist microbial decomposition. This is an advantage as the necessity for web maintenance and the amount of harmful microbes that the spider is exposed to is reduced. Additionally, this reduced exposure to harmful microbes would be a protective mechanism for the developing eggs. Reducing the bacteria present on the silk would also make the web harder to detect by predators that use visual or olfactory signals. **10**

## Medical Applications

The understanding of silk using the basic sciences can be translated into a variety of medical applications, which are conceptualized in the table below.

#### Table 1

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In the spider's silk gland, the solution of silk protein and water is spun into a fibre and it is extruded or secreted from the gland. In the search for better materials, scientists have reverse-engineered the silk fibre back to its water and protein form. Consequently, silk in the form of solutions opens up new and exciting possibilities. Through design and implementation of these newer silk platforms, it allows the integration of the soft curvilinear world of biology and medicine with the rigid planar one of traditional electronics and optics.

Table 2

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**Fig. 7: Figure 7**

### **Micro-sutures: ocular and nerve repair**

In Medicine, the suture materials commonly used are silkworm silk, stainless steel, synthetic absorbable polymers such as polyglycolic acid (Dexon), polyglactin (Vicryl) and synthetic non-absorbable polymers such as polyamide (Nylon), polyester (Dacron) and polypropylene (Prolene).<sup>11</sup> Although the structure of silk produced by silkworms used in sutures currently is similar to dragline silk, silkworm silk has fewer intramolecular  $\beta$ -sheets in the amorphous region so it is less extensible and its yield point is reached sooner. Therefore, silkworm silk lacks the superior mechanical properties of spider silk. It does not have the flexibility, especially when higher grade thicker sutures are used for their tensile strengths. Sutures require more tensile strength to maintain the edges of the wound together until the body heals the edges. Spider silk is superior for use in sutures as it is thinner and yet has a greater tensile strength (higher than mammalian tendons), toughness, stiffness and ductility. This means that it can withstand a relatively large tensile force per unit area with little strain when used in securing wounds. It can also be stretched out into sutures of large length. It is biocompatible with human cells, not irritant (unlike silkworm silk which has had problems with body rejection and irritation) and it is absorbable, so sutures do not have to be removed after the wound has healed. Since dragline silk is very thin and non-irritant it can be used for micro-sutures, in general microscopic anastomosis (reconnection of two streams that previously branched out, such as blood vessels) and ocular surgery. Also, its fine structure means it can be used in nerve repair to bring the cut ends of the nerve together. Several nerve fibres form a nerve. A fine suture is required so that when suturing the nerve, it does not damage large sections of the constituent nerve fibres.

### **Musculoskeletal system: accommodating movement and various forces**

Spider silk can be used to make thicker durable sutures for tendon repair. Hennecke et al discussed the potential use of silk to replace current materials in flexor tendon repair. Issues with existing sutures include the risk of infection, body reactions and mechanical instability.<sup>12</sup> Braided spider silk sutures could be an excellent alternative, with high tensile strengths and Young's modulus values and the strength to withstand reoccurring movement of the tendon. The use of spider silk in micro-sutures used in, for example, eye surgery and in thicker sutures used in tendon repair demonstrates its versatility as a biomaterial. The German company AMSilk has produced Biosteel, a perfect thread formed from spider silk.<sup>13</sup>

### **Hernia mesh**

Hernias occur due to the protrusion of part of an organ through a weakness of the cavity wall which contains it. A repair technique used is a mesh, placed either under or over the defect and held in place by sutures. Mesh allows a tension-free repair to bridge the hernia defect as opposed to sewing the two sides of the incision above the hernia together with stitches. The mesh can be in the form of a patch that goes under or over the weakness, or it can be in the form of a plug that goes inside the hole. It acts as a scaffold for growth of a patient's own tissue, which then incorporates the mesh into the surrounding area. The current hernia meshes are commonly made of Marlex, a polypropylene material.<sup>14</sup> It is foreign to the body and has the risk of infection and tissue reactions, complications that are much feared. Dragline silk's strength and fine nature make it a very suitable material to create a rigid mesh to support the hernia and prevent further protrusion. Its ductility and flexibility mean it can conform to the body's size, position and movement. Spider silk's biocompatibility means infection rate and risk of rejection are eliminated.

### **Bio-scaffold for Tissue engineering**

The strong, fine, biocompatible nature of silk together with different physical states allow a matrix platform to act as a strong support framework which would enable cells and tissues to grow through, without causing any tissue reactions or harmful effects.

### **Artificial skin**

Cutaneous constructs could be utilised when there is skin and tissue loss due to major burns. The perfect artificial skin would

have to form a bilayer construct, consisting of the epidermis and dermis. The scaffold should mimic the structure and functions and provide mechanical support and regulate cell activities. It should support cell attachment and migration and guide cell differentiation. In vitro investigation has shown that several types of human cells, including epidermal keratinocytes, dermal fibroblasts and endothelial cells, can be successfully cultured on silk scaffolds in various forms to form a single layer or bilayered skin model if conditioned appropriately with nutrients, warmth and air. The silk film was prepared by dissolving the silk fibres in lithium bromide. It was biocompatible with minimal inflammatory reaction, haemocompatible with the underlying blood vessels and oxygen and water permeable, which is necessary as the skin acts as an interface. It supported the attachment and proliferation of cells.

Current research has looked into synthetic polymers like polyglycolic acid and polylactic acid as scaffold materials. These materials have poor mechanical properties. This has been evidenced by the deficiency of regenerated axons in nerve grafts. Collagen, a structural protein which is currently used, can lose its mechanical properties. Spider silk has better mechanical properties as well as being more biocompatible with no risk of rejection.<sup>15</sup> However, there are still challenges in creating artificial skin. It is difficult for a construct to restore all layers of skin which include the functions of touch and temperature sensation, excretion, perspiration, thermoregulation, protection from ultraviolet light, synthetic function and its aesthetic function. Currently, silk is more feasibly used in the construction of the epidermal layer alone. This paper from the University of Brighton, England illustrates in great detail some of the currently marketed and clinically available tissue-engineered skin substitute products.<sup>16</sup>

### **Nerve graft**

This same concept of silk being used as a support mesh on which cells grow back can be used in nerve grafts and silk filled conduit for larger nerve defects. Research is aiming to lead to treatment for damaged nerves with a large defect which require a scaffold support that allow nerve tissues to grow back and bridge the gap.<sup>17</sup>

The fine and strong nature of spider silk combined with its high biocompatibility and the ability to guide the migration of regenerating nerve cells and supporting (Schwann) cells make it a very useful material for regenerative medicine. The ultimate aim would be spinal cord repair using this approach.

### **Heart Muscle regeneration**

Bio-scaffolds can also be applied to regenerating heart cells in myocardial reconstruction. Currently, a magnesium fluoride coated magnesium alloy LA63 is used to form the scaffold onto which heart cells are cultured and proliferate. This can surgically revitalize the heart whose muscles have died following a heart attack. LA63 lattice does have very high mechanical properties to withstand the continuous contraction of the myocardial muscle for a period of time. But these metallic constructs need precise engineering techniques.<sup>18</sup> The high tensile strength, high Young's Modulus and high toughness of spider silk could perhaps make it a more advanced material for use in the myocardial scaffolds that are easily conformable to the moving contours and forces of the heart.

### **Tendon and Ligament regeneration**

Tissue engineering can also be applied to tendon and ligament repair. Both formed from collagen which forms a fibrous connective tissue, tendons connect muscle to bone and ligaments connect two bones. Collagen can lose its fibrous and mechanical properties when manipulated for use in repair. Spider silk could serve as an improved alternative scaffold material for cells to proliferate and form the collagen fibrous tissue tendons and ligaments. Bone marrow-derived mesenchymal stem cells and anterior cruciate ligament fibroblasts from the knee can be cultured on a porous silk scaffold for tissue engineering. Research is also ongoing to develop a tendon and ligament scaffold by the synergistic incorporation of the silk and collagen. Furthermore, its advanced mechanical properties of high tensile strength and a high Young's Modulus will enable it to withstand motion experienced when tendons and ligaments move. Its toughness also means it can withstand movement as it will absorb a large amount of energy before fracturing. However, tissue engineered tendons and ligaments face challenges. The long-term stability and physical integrity of the silk scaffold constructs is not certain.<sup>19</sup>

### **Biological wound dressing**

The people of the Carpathian Mountains across Central and Eastern Europe used spider silk for wounds.<sup>10</sup> The antimicrobial silk will mean it will not cause any reactions or immune response. Biological wound dressings such as hydrogels can be made as spider silk can be liquefied into a spidroin solution by the addition of acids or other additives. Hydrogels are three-dimensional polymer networks which are physically durable to swelling in aqueous solution but do not dissolve in these solutions. The biocompatible porous silk bioscaffold facilitates cell in-growth for wounds to heal.

### **Drug delivery**

The liquid silk can be made, mixed with a medical drug and the solution can be converted into a solid particle state or very fine microsphere structures. This can be for the delivery of small molecule drugs to a particular site in the body. It will not cause immune reactions as it travels through the body to the site needed.<sup>20</sup>

## Biophotonics/ silk optics

Added to these benefits is the recent discovery that silk is a gifted manipulator of light. Light travels through silk almost as easily as it travels through glass fibres. The science of **photonics** includes the generation, emission, transmission, modulation, signal processing, switching, amplification, and detection/sensing of light. The term has an analogy to electronics. The term photonics developed as an outgrowth of the first practical semiconductor light emitters LED invented in the early 1960s and optical fibres developed in the 1970s. Nano-texturing allows the use of this silk platform as biosensors that utilises the behaviour of light along nano wavelength distances or barriers. The brilliant blue colour of the morpho butterfly is a natural example of the manipulation of light by the non-silk natural material of this butterfly. Diffraction refers to the phenomenon that occurs when a wave encounters an obstacle or a slit that is comparable in size to its wavelength. Maximum diffraction occurs when the aperture is equal to or smaller than the light wavelength (400 – 600 nm). In optics, a diffraction grating is an optical component with a periodic structure, which splits and diffracts light into several beams travelling in different directions. The directions of these beams depend on the spacing of the grating and the wavelength of the light so that the grating acts as the dispersive element. Silk films can be nano-textured or imprinted to manipulate light. These can then be used as biosensors. Light transmitted into the blood or cells will be modified by the incident substrate such as proteins, DNA, cells etc. The interaction of this light with the silk film can be used to bio-sense the biologic media. Silk photonics can be used to detect cellular real time data and may offer clues to cancer cell initiation, metabolic status, oxygenation status etc. Silk can alternatively be doped with semiconductors or integrated with nanoelectronic circuitry to yield bio-electronics. This can lead to implantable bioelectronic sensors.<sup>21</sup> Silk in the form of tubes offers new possibilities compared to glass based fibre-optic technology. Silk is not as efficient as glass fibre in conducting light, but researchers are working on coating the silk fibre with appropriate chemicals to overcome this limitation. Glass based fibre optics requires precise manufacturing technology while silk is manufactured for us by the spider! Natural silk is only five microns in diameter, less than a tenth of the width of a human hair (50 microns), while glass fibres vary from about 10-60microns internal diameter.<sup>22</sup> Silk optics opens up new avenues for medical imaging.

## Liquid silk coating

The biological evolution of silk into a non-immunogenic, non-toxic and non-reactive material offers the possibility of coating medical implants to help reduce the human body's ability to recognise the foreign material in the implants. Silicone breast implants can elicit an adjacent tissue inflammatory response that can distort the implant, affect the consistency and feel of the implant or produce pain to the patient. AMSilk has produced silicone implants coated with silk protein BioShield-S1 which are currently in pre-clinical trials.<sup>23</sup>

## Orthopedic surgery – Artificial Meniscus

Meniscal implants using dragline silk could withstand movement and compressive forces. A meniscus is a fibrocartilaginous structure that partly divides a joint cavity such as in the knee. This is a common injury in football players and can become a major health problem. The founder of Oxford Silk Group, Professor Fritz Vollrath, along with his team is currently developing implants for this. By dissolving in lithium bromide, the dragline silk becomes firmer. The resulting implant has high strength and toughness. A large force and energy are required before fracture so it can support the weight of the body. It will absorb a large energy before any damage can occur. It integrates much better potentially with the human body than any plastic material. According to Vollrath, the meniscal implants, which are undergoing animal trials, could be used in humans in one to two years.<sup>24</sup>

## PRACTICAL ASPECTS AND FUTURE AVENUES FOR RESEARCH

Translating the ideas in academia to clinical practice unearths many practical challenges of using spider silk in the various medical fields mentioned above. The fact that dragline silk is a natural material is one practical advantage. Current materials involving petrochemical processes are not sustainable in the long run, but the use of silk would be.

Potential commercial mass production for many spider silks is still extremely finite. This arises from the difficulty of high-density spider farming due to their cannibalistic behavior. Furthermore, only approximately 12 m of silk can be obtained from a spider web. This is an extremely small quantity compared to other materials such as from a silkworm cocoon, from which 600-900 m of silk can be yielded. This means alternative methods of manufacture are required.

Current research involves genetic engineering to produce silk similar to dragline silk on a mass scale. So far, it has not been possible to recreate an authentic copy of spider silk.

The difficulty of this can be understood to some extent by comparison to the current mass production of human insulin by genetic engineering. Gone are the days of pig or porcine insulins. The fundamental requisite is understanding the genetic code. The complexity of the genetic code in turn depends on the complexity of the proteins being coded for. The primary structure of insulin was characterized in the late 1950s by Frederick Sanger. Insulin is a smaller protein made of two polypeptide chains comprising of 51 amino acids in total and molecular weight of 5808 Da. The relatively simple structure meant in 1978, the first genetic engineering company Genetech, could mass produce genetically engineered human insulin.

In comparison, dragline silk presents a much more complex situation. The molecular weight is 200-350 KDa. The secondary



structure is a complex co-polymer of alanine crystallites and glycine amorphous chains. The knowledge of spider silk genes coding for this structure is incomplete. Therefore, an exact mimic of a native dragline silk fibre has not been possible thus far. All transgene constructs for recombinant silk proteins have relied on partial cDNA. These truncated cDNAs encode only a fraction (typically 20% or less) of the repetitive region and the C-terminal domain.<sup>25</sup>

Research must centre on alternative methods. Teulé et al outline research into the use of vectors to create transgenic silkworms encoding chimeric silkworm/spider silk protein genes. The engineered silk produced by the silkworm was a composite of chimeric silkworm and spider silk. It was tougher than the parental silkworm silk and equally tough as dragline silk. The experimental evidence demonstrates that silkworms can be genetically engineered to manufacture silk with properties close to native dragline silk, although exact replication has still not been achieved.<sup>26</sup> Bioengineering firm NexiaBiotech have genetically engineered goats to secrete spider silk proteins into its milk, which can then be separated off.<sup>27</sup>

Some other companies that have embraced this new material challenge include ARAKNITEK collaborating with Utah State University,<sup>28</sup> AMSilk<sup>13</sup> and Spiber Technologies.<sup>29</sup>

## CONCLUSION

The scientific world is always searching for novel biomaterials that could revolutionise our daily lives. The multifarious applications of spider silk in medicine have been clearly demonstrated in an array of medical areas. The potential applications of spider silk indeed stretch beyond the field of medicine. It is also being considered as a material for use in bulletproof clothing, ropes, nets, seat belts, parachutes and composite materials in some vehicles including aircraft. The day may arrive when a coffee cup made of spider silk can be thrown away without the fears from the current non-degradable polystyrene cups! It is remarkable how such a super material exists naturally, carefully mastered over thousands of years by evolutionary processes with superlative chemical, biological and physical properties. Perhaps mastering of the proteins and the permutative possibilities offered by the 20 amino acids should be the subject of tomorrow's material science research. Most of us are unaware of the fact that spiders, a creature which for many of us triggers trepidation, contain the material that could revolutionise our way of life. Perhaps arachnophobes should reconsider their fear, for it could be a spider silk stitch or bendy spider silk bioelectronics that may save them in a hospital of the future!

## Glossary

**Amorphous**– A structure without a clear shape or form.

**Antimicrobial**– An agent that kills microorganisms or inhibits their growth.

**Hernia**- When a section of an organ moves out of where it is usually located.

**Orthopaedic Surgery**-Surgery concerning the musculoskeletal system.

**Immunogenicity**- The ability of a substance to cause an immune response.

**Young's Modulus**-The relationship between stress and strain.

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