

**THE POSSIBLE ROLE OF SUTURE MATERIALS OBTAINED
FROM GENETICALLY MODIFIED SILK**

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1. Abstract

The use of silk from mulberry silkworms as a suture material has been a traditional medical practice, dismissed in last decades due to a number of concerns, mainly allergic reactions from sericin, which is one of the proteins composing silk. The possibility of using genetically modified silk from either mulberry silkworms or other species of spiders, such as *Nephila Clavipes*, has been proposed in the last few years for a number of applications, including also suture materials. Genetic modification has mainly been aimed at improving silk characteristics, especially from the point of view of mechanical properties and durability, although a better bio-compatibility has also been regarded as a consequent objective.

This work is aimed at understanding whether an effective combination of bio-compatibility, bio-degradation and low risk can be obtained on genetically modified silk, in order to propose it as a suture material. This evaluation required obtaining and discussing suitable definitions, adaptable to the application on study, of biocompatibility and risk from genetically modified items. In the specific case of suture materials, biocompatibility includes technical concepts, such as rapid infiltration and surgical system integration. In addition, GM risk on non-food material is still a quite new idea, and the discussion on suitable definitions are still very active.

It is suggested in conclusion that thorough knowledge of the material to be implanted is necessary to lead to a successful application of GM silk as a suture material. This knowledge is not always available so far, in particular long term effects. This lack of knowledge heavily contributes to the extent of risk perception, and is ultimately determining the acceptance/refusal of the material in itself, sometimes with no further considerations on the advantages that its use would have on surgical practice.

2. Introduction

In medicine, the materials used to close a wound, ideally until it heals, are in general defined as *suture materials*. Suture materials may be *absorbable* (which dissolve in the living tissue), *non-absorbable* (to be removed after some time); also, they may contribute

actively to the healing e.g., by being impregnated with drugs, such as antibiotics, in which case they are defined as *bio-active* [1]. In medical applications for suture, biocompatible materials are needed, which can be suitable for implantation. Biocompatibility can be defined in general as not producing a toxic, injurious, or immunological response in a living tissue [2]. However, more specific definitions can be necessary, when dealing with particular application of biomaterials, so that the discussion on more technical biocompatibility definitions will be resumed in Section 3.2 and 3.3. In the particular case of suture, a material is suitable for this application once it is biocompatible, biodegradable and presents low risks also in the long term from any of the components or processes used in its fabrication. Such processes would include, if relevant, genetic modification.

Silk from silkworm (*Bombyx mori*) has been for many years an obvious choice for suture materials. Its use was discontinued in the 60s because of sericin (silk's gum), which is a powerful allergene, able to contaminate suture material [3]. It is worthy to note that silkworm is not the only producer of silk; a number of spiders, such as e.g., *Nephila Clavipes*, are also producers of silk, which provides for a number of theoretical possibilities of replacement from nature. Difficulties and limitations of the application of natural silk as a biomaterial, particularly the issue of optimising degumming, and the need to dispose of a material of constant properties, possibly multi-functional, have in recent years suggested genetic modification of spider and mulberry silkworm silk to fulfil this aim. This practice requires the knowledge of the molecular structure of silk fibroin. In the particular case of silkworm silk, fibroin is a single protein with a repeated aminoacid structure, including glycine, alanine and serine, whilst sericin is a mixture of proteins with a large number of aminoacids containing hydroxyl (OH⁻) groups: sericin is therefore more soluble in water. Fibroin is disposed in sheets, connected by hydrogen bonds (see Figure 1): such an arrangement is defined as β -strand conformation [4]. The modification of this sequence is able to widely change the properties of silk, especially its crystallinity [5]. Alternative methods, not involving GM, are also possible, as for example the grafting (chemical insertion on the molecular chain of proteins) of other chemical groups on the silk structure [6]. However, this may create other sources of risk, connected to the presence of chemicals, as will be discussed in Section 3.1.

This suggests that ultimately a risk evaluation between different materials, measuring long-term biocompatibility, including therefore also genetic modification issues, would be needed. A major difficulty of this analysis includes the need to define a GM risk procedure for non-edible body interacting items, such as suture materials. These should balance the envisaged advantages of the modified materials, and subsequently evaluate perceived and real risk.

In this work, the possibilities of genetically modified silk to be applied as a suture material are examined, according to the requirements given above (effective combination of bio-compatibility, bio-degradation and low risk). This goal will be reached by the fulfilment of five objectives. These are:

- Defining which is the typical composition of silk obtained from silkworm and how silk from silkworm silk is genetically modified
- Evaluating if the present level of scientific knowledge about silkworm's silk genetics is likely to be sufficient to prevent post-implant risks
- Investigating to what extent genetically modified silkworm silk can also be used to manufacture bio-degradable materials, offering some examples of possible applications
- Finding out whether the need for bio-implantable materials has been communicated to the public, especially in the particular case of suture materials and if so how effectively this has been carried out
- Evaluate whether the concern over genetically modified bio-silk has in some ways hindered the research over this possible bio-implantable material, or not

This literature review is divided in four main parts, and is followed by a discussion: the first part concentrates on the typical composition and properties of silk obtained from mulberry silkworm, stressing the main differences between this material and spider's silk. The second part involves a discussion about risk from GM species, in the particular case of non-food items: the concept of the present EU regulations is presented, and some literature about the interpretation of risk in this case is reviewed and commented. In the third part, the present interpretation of the concept of bio-compatibility is introduced and clarified, on the specific subject of suture materials. The fourth part includes more details about the techniques proposed for modification (genetic or not) for silk from different

spiders and silkworm: the specific properties of the materials obtained from each process are compared and briefly discussed from the point of view of their possible use as suture materials. The discussion concentrates on the communication and decision-making aspects, in particular trying to offer information on the possible links between communicating the need for bio-implantable materials to the public and possible production of the material. In this regard, some emphasis is given on the need for the communication to be successful to include some ethical content in an appropriate and comprehensive framework, dealing with all the possible causes for concern.

3. Literature review

3.1 Silk from silkworm and spiders and issues of its application in suture materials

Silk is a natural material: its properties, such as mechanical strength and length of the spun wire, chemical composition, and degree of crystallinity, are very variable, both between species and between individuals of the same species. The lack of consistency and the consequent difficulty of providing end-users with precise product specifications are probably the main reasons why the possibility of modifying silk has found some interest: as suggested above, suitable routes appear to be chemical modification or genetic modification. Chemical modification is more often concerned with the increase of weight (weighting) of silk, to compensate for the loss of weight and mechanical resistance, due to degumming. Weighting was originally obtained by metallic salts, such as tin phosphate and silicate in mineral form, which allowed silk to be dimensionally stable, not soluble in water and resistant to oil contamination [7]. More recently, grafting of large chemical groups, such as methacrylates or acrylamides, are used for weighting [7-8], which has a beneficial effect also on mechanical properties of silk and possibly also on its biocompatibility. However, the presence of acrylamide poses some risks, in that its neurotoxicity has been clearly demonstrated in humans, whilst its carcinogenicity has only been revealed from animal studies, but has not yet being excluded on humans [9] One also possibly needs to observe that inherent variability in properties leads to the

possibility of unpredictable reactions from the application of natural silk as a material for medical use. In particular, evidence of positive immune response to silkworm silk as a suture material has been revealed, which has been attributed to the fact that sericin, a component of silk before degumming, is classified as a type 1 allergene, including common immune disorders, such as asthma, hay fever, eczema [3]. Also silk fibroin, although more rarely, has been reported as giving some positive immune response [10].

In the case of medical materials, the introduction of chemicals to modify silk properties, as e.g., more thorough degumming through the action of enzymes [11], does not usually improve the mechanical properties, and may not always be recommendable, as regards the biocompatibility of the material. It appears therefore that genetic modification represents the easiest way for the achievement of better biocompatibility. Modifying the structure of sericin, in order e.g., to improve its solubility in water, would by itself ease degumming. As a matter of fact, this was attempted and led to further knowledge on the nature of sericin, which is different from fibroin, since the two proteins are extracted from different glands in the silkworm [12]. While the amino acid sequence motifs present in spider fibroins are now quite well known, as is the molecular architecture of spider silks, still there is some uncertainty about which mechanical and structural properties make silk as we know it [13]. The structural characteristics of silk do not change abruptly from one species to another, involving always triple helices hierarchical structures: in particular e.g., an oriented beta-sheet crystal structure is common to silkworm and spider (dragline) silks [4]. In spite of this, as noted before, the properties of silk may change considerably within and between species, possibly because the sequence and genetic layout of aminoacids that form it.

However, a kind of "third way" may also be possible between chemical and genetic modification, which is the artificial reeling of silkworm silk from immobilised silkworms. The resulting silk appears to have higher properties of the naturally spun fibres, approaching the strength of spider silk, but preserving the flexibility of silkworm silk [14]. In other words, genetic engineering of silk might not be necessary to improve the tensile strength of silk, because promising results have been obtained by simply forcing bave, which eventually forms silk, out of the silkworm [15]. This outcome was attributed to the fact that degumming of the cocoon has a detrimental effect on the

mechanical properties of silk obtained, for the effect of chemicals on fibroin [16]. Since both sericin and fibroin have a protein structure, it is unlikely that a chemical aggressive with sericin would have no effect on fibroin [17].

3.2 Risk assessment of genetically modified non-edible items in the specific case of silk

Risk assessment for GM implantable devices, such as genetically modified silk for suture materials, has become a necessary requirement for the large quantities of this material introduced into the market. There are a number of difficulties nevertheless: in particular, most studies on the traceability of genetically modified organisms concern GM food [18]. However, the concept of European Union regulations on GM food would in general apply to whatever GM product, since it involves two main aspects: safety and freedom of choice. Safety is defined in a twofold sense: first, not posing threats to human or animal health, and second, being safe for the environment [19]. Freedom of choice means that people using, or being subjected to the use, of GM items, need to be offered the possibility of the application of the alternative products [20]. As will be discussed in Section 4.2, this is not always the case for suture materials.

In general, GM risk can be measured by the extent of genetic modification is based on the number of successful transformation events e.g., insertions of an extraneous gene (transgene) in a specific location on a chromosome with no reject from the cell. During cell replication and therefore species development, the transgene is also replicated. A significant issue in this regard is the need for the creation of international rules for attributing to 'transformation events' unambiguous names and of detection methods capable of identifying the fragments of DNA that mark the specificity of each 'event' [21]. In this regard, the most used method is fluorescence detection. In this method, an enzymatic DNA sequence analysis is first executed using an oligonucleotide (a short sequence of DNA or RNA bases) as a primer. To the oligonucleotide then a different coloured molecule (chromophore) is attached for each of the four bases forming DNA (adenosine, cytosine, guanine and thymine) [22].

Only after traceability is achieved, the evaluation of total risk from genetically modified

organisms can be successfully carried out. It has also been suggested elsewhere [23] that a global risk assessment on genetically modified organisms (GMO) would need to include environmental considerations, for example the larger use of pesticides in cultivations, and not be limited to health risks. This is relevant also in the case of GM silk, since one of the reasons why it was developed is also to reduce the use of chemicals, especially in the above mentioned case of acrylamide.

A further aspect to be introduced in the picture is biocompatibility. To this avail, an agreed definition of biocompatibility for suture materials would be needed. In practice, a biocompatible suture material would need to present the general property of offering "predictable interactions with contacting biological phases" [24]. In the case of suture materials, the biological phases involved are those found in the wound to be healed: what "predictable interactions" mean in practice is to be explained and developed in Section 3.3.

In summary, from what discussed above, it appears that a possible risk assessment on non-food GM crops, such as GM silk in sutures, would be divided in three interconnected regions of concern: one regarding the amount of genetic modification, the second the (higher or lower) use of chemicals, and the third the (equally higher or lower) biocompatibility achieved as effects of genetic modification. These three factors together would finally be determinant for the acceptance of the new material.

3.3 The concept of biocompatibility as applied to suture materials

In general, natural silk fibres appear to be biocompatible, since human tissue culture cells can grow on and attach to them. This result indicates that, at least in principle, silk should not pose any threat to the patient, being stable and not behaving as a possible foreign antigene [24]. However, more specific definitions of biocompatibility may be required, as highlighted in the Introduction. In this sense, suture materials obtained from silk, which have to be intended primarily as non-absorbable, are required to resist degradation owed to the host and/or pathogenic organisms, which may produce proteolytic (i.e., protein-dissolving) enzymes. In this respect, further analyses for the assessment of biocompatibility are needed, as reported below. In addition, biocompatibility is by no

means sufficient to guarantee that a suture material can be successfully used, because a large number of other characteristics are required for the. These include tensile strength, knot strength, elasticity, low memory (i.e., low change of stiffness over time), degradability, time reactivity, and absence of infections [25].

In practice, biocompatibility of suture materials is often assessed by means of implantation in animals, such as rabbits and mice, as well as, at a later stage of development, in humans. The inflammatory tissue reaction is then measured [26]. This poses ethical issues, and reducing as much as possible the risk of immune reaction and the subsequent need for *in vivo* tests (on animals and/or humans) would be highly beneficial. On the other side, *in vitro* tests (on tissue samples) may in some cases be sufficient: in particular, these tests have shown the dependence on the capability of absorption of silk fibroin on a number of factors. These include in particular the morphology (fibres, film, sponge) and conformation (α -helix, random coil, β -sheet) [27]. The importance of geometry and structure over properties gives some ground for genetic modification, because it suggests that silk characteristics can be optimised, regardless of the species it is obtained from. For example, in [28] polypeptide sequences based on glycine and alanine, which constitute the basis for dragline spider silk, were altered, in order to form a β -strand conformation, persisting in a wide range of pH and temperature conditions. This would confer to the silk dimensional stability and properties, for example the length of spun wire, perfectly comparable to mulberry silk.

Possibly ignoring or understating all the above considerations, commercial sites that propose bio-silk materials can claim that bio-silk is absolutely safe, due of the long-time track record of safety [29]. This appears to exclude the existence of allergy problems and at the same time does not clarify why, if the scenario is so positive, GM of silk has been attempted in the last decades. In general, however, this offers also a good point for discussion, in the sense that, despite the claims, cladding or embedding of silk fibres is always suggested for suture applications. These materials can in some cases also be used for other functions: a range of possible directions for research will follow below.

3.4 New materials obtained from bio-silk and their characteristics

A number of new biomaterials have been developed, based on modified silk, in the last few years [30-33]. A general consideration on silk biomaterials is that pure silk does not in reality exist in the first place, because degumming, even when not practiced with aggressive chemicals, does damage the material. Typical remedies, as discussed above, are silk weighting or grafting. Some alternatives were sought in the last years, especially with the objective of providing new functionalities for GM silk, which would increase its value and possibly ease their acceptance from users and public. In general, it is significant to note that, in spite of what is suggested e.g., in [3], genetic modification on silk is not normally intended to eliminate allergies by easing removal of sericin or providing alternative non-allergenic composition. Rather, it appears that the emphasis of research in this field is concentrated on obtaining multi-functional materials, for example enabling the combination of silk properties with additional biological, chemical, or technical features. New directions investigated in research include blended silk/cellulose fibres [30], coating of sericin-free silk with gelatin [31], silk fibroin scaffolds to support stem cells adhesion [32], and silk bio-polymers for drug delivery [33]. The common ground of these studies is that silk would pass from non-absorbable to absorbable suture material. In this case, degradation becomes a required factor, either because the material has to dissolve in the new tissue, such as in forming the scaffold, or because it has to deliver right doses of a drug on the body, and therefore show a known rate of degeneration with time.

The next step of this process of using silk as an absorbable suture material is the ability for it to provide self-assembly (and possibly regenerative) capabilities, which are actively sought after in biomaterials [27]. Self-assembly, a well-known process for some biological organisms, such as in lizards, would ideally imply that no need for absorption in the tissue, because it gradually builds itself, when in contact with the host tissue, therefore in the right conditions of pH, temperature, humidity and in presence of chemicals able to trigger this process.

In addition, multifunctionality, which is of interest in suture materials, because it basically means that the material is able to work in different environmental conditions, is

most recently achieved through genetic hybridisation across the whole spectrum of proteins, not being limited to those present in silk [34-36]. An interesting work at this subject concerns the development of a clone between the major protein in the spider dragline silk of *Nephila clavipes* fused to the carboxyl (COOH) terminal of dentin (teeth protein). This is aimed at providing a flexible and easily re-structured scaffold material [37]. However, also for achieving multifunctionality, it is probably not realistic to say that genetic modification is absolutely necessary: a recent study allowed preparing a mulberry silk fabric with one-side hydrophobic and the other side hydrophilic, simply by treatment with fluorocarbon and irradiation through a vacuum particular ultraviolet lamp [38].

The conclusion of the research can be twofold: first, there are no reliable ways of predicting what would be the long term effect of genetic modification over protein structure, and second, the resolution of allergy problems due to sericin seems to represent only a minor objective in the research over silk. More general goals of genetically modifying silk are mainly the improvement of mechanical properties and the reduction of variability in characteristics, trying to make it a "designed" material, suitable for surgical applications, more than a "natural" material [34b]. In this sense, the distinction between spider silk and silkworm silk tends to disappear in the current research trend, which appear more based on speculations on proteins, as extracted from different arthropods. It is to be seen if further literature research will confirm or not these conclusions.

4. Discussion: research on improvement of suture materials and the role of GM silk in it

4.1 Communication of the need for innovative suture materials and of the related risks

According to specialised literature (see for example [39]), no ideal suture material so far exists, so that surgeons strive to use a material as close as possible to the ideal. However, there are still considerable differences between suture materials used in practice and an ideal one, as shown in Table 1. These differences are mainly concentrated on the present issue of guaranteeing that a suture material is non-allergenic and non-carcinogenic.

Therefore, operators working in the health sector are supposed to be well aware of this difficulty, which means in the end that further research will be needed to achieve these objectives. As a matter of fact, research on basic topics in suture materials is still going on, so reaching an optimal suture practice is also in no way a resolved question [see e.g., 40].

Communication of the need for research in suture materials to the larger public encounters more difficulties. In clinical practice, it is an ethical requirement that patients are informed about the treatment they will be submitted to (informed consent), and are offered alternatives, when available, unless an emergency situation arises, in which the patient cannot be informed [41]. However, informed consent does not usually apply to the application of suture materials, which are considered as consumables, and not drugs (although they have undoubtedly an effect on healing) [42]. In addition, the public is probably more sensitive to environmental tolerance (e.g., recyclability, biodegradability) of materials, although it might not be informed that this is an essential requirement for GM items, as seen in Section 3.3.

In this regard, suture materials obtained from cellulose e.g., containing recycled cellulose fibres (Lyocell), would seem more environmentally-friendly: however, untreated Lyocell suture materials present a larger amount of fibres breaking and moving to the surface, as an effect of service (a phenomenon called *fibrillation*) [43]. This example clarifies that moving towards a more bio-degradable material for sutures may need on the other side the treatment with aggressive chemicals, such as sodium hydroxide.

In conclusion, communication of the need for new suture materials would need to know in detail the characteristics required from them: apart from some general properties for surgery instruments and materials (e.g., sterility), which are on the other side the ones achieved so far, these are not obvious. In addition, needs arising from surgical practice may be easily hidden or overwhelmed by the interest for bio-degradable materials and/or materials free from noxious chemicals. It may be therefore necessary first to clarify which is going to be (if any) the role of GM silk as an environmentally-friendly material and only after selection of a specific artificial silk, evaluate the extent of risk from genetic modification.

4.2 Decision-making and GM silk: perceived risk and materials development

There appears to be limited interest in the development of GM silk as a specific material for sutures. In general, a hindrance which appears to be in the way of its success is the scarce development of dedicated guidelines for GM non-food items, which on one side do not allow a repeatable and reliable risk assessment, and on the other side may contribute to an excessive or inappropriate evaluation of long term risk. In addition, the surgical practice would require "informed consent" procedures to be introduced also for suture materials, if the genetic modification risk is to be assessed in the long run.

It is very likely that in the future, considering the value from the mechanical and structural point of view that GM silk may present as a suture material and reflecting on the fact that alternative suture materials (e.g., biodegradable cellulose ones) have often to be treated with harsh chemicals, such as alkali, this development will be resumed.

A possible route for this development, as indicated by the literature review, will be trying to increase the value of GM silk by providing alternative high-profile applications: this procedure is typically followed on new materials, which present problems of acceptability for high cost and/or poor image (see for example the case of polylactic acid for the production of bio-degradable plastics [44]). In this sense, further possibilities could be introduced by comparative life cycle analysis (LCA) studies of the environmental performance on alternative suture materials, including of course the GM aspect (if relevant), which may prove that bio-silk is not globally inferior to other materials in this sector.

5. Conclusions

The application of genetically modified silk as a suture material has been investigated. The principal factors contributing to the possible success of this application are acquiring new properties in the way of ideal suture materials (particularly as regards obtaining better mechanical properties with reduced chemical treatment). Advantages, which would compensate for the genetic modification, are the intrinsic bio-degradability of the material, and the possibility of a broader field of application for it in medicine (e.g., as

scaffolding material for tissue engineering) and engineering (as innovative composite material for plastic replacement). However, the assessment and the communication of the involved risks are still in their infancy and would need also a modification of the ethical consent procedure in the case of suture materials. These are nowadays still considered as medical consumables: this indicates a considerable undervaluing of their importance for wound healing: in this sense, applying a specifically designed material, such as artificial silk, would confer to them the deserved place in the surgical practice.

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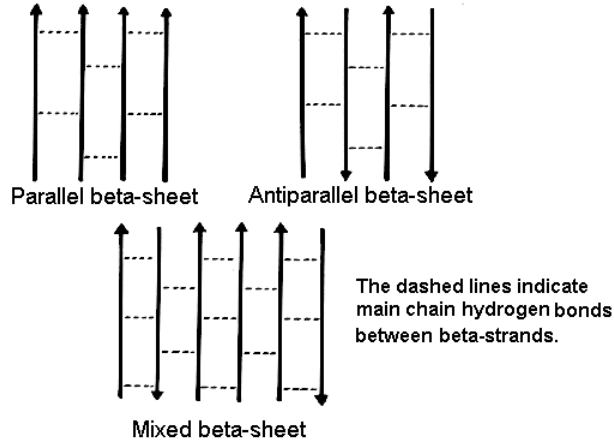


Figure 1 Beta strand conformation of protein chains

Ideal suture material	Suture materials commonly used in practice
Sterile	Sterile
Non-electrolytic (no electrostatic discharge)	High and uniform tensile strength (permits use of finer sizes)
non-capillary (non permeable to fluids)	High tensile strength retained in vitro, holding securely the wound throughout the critical healing period;
Non-allergenic	Consistent and uniform diameter
Non-carcinogenic	Pliable (for ease of handling and knot security)
Non-ferromagnetic	Free from substances or impurities (optimum tissue acceptance)
Easy to handle, minimally reactive in tissue and not predisposed to bacterial growth	Predictable performance
Capable of holding securely tissue layers throughout the healing period (when knotted no fraying or cutting)	
Resistant to shrinking in tissues	
(When absorbable) Absorbed completely with minimal tissue reaction	

Table 1 Comparison of the properties sought for an ideal suture material and those obtained from a real suture material