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Review Article

Silk proteins and its biomedical and dental application: A Review

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Abstract

Silk is a group of fibrous proteins that has been used for centuries in the textile industry and as surgical sutures. Silk, in addition to its unique mechanical properties, silk possesses other properties, such as biocompatibility, biodegradability, anti – bacterial properties, thermal stability, controlled degradation features, and ease of sterilization, ability to self-assemble make it a promising material for biomedical applications. Although silk forms only fibers in nature, synthetic techniques can be used to control the processing of silk into different morphologies, such as scaffolds, films, hydrogels, microcapsules, and micro- and nanospheres. Moreover, the biotechnological production of silk proteins broadens the potential applications of silk. With this background, this review describes about silk proteins, its properties, synthesis and uses.

KEYWORDS: Silk, silk proteins, sericin, fibroin, dental

Introduction

Silk, a structural protein, represents a distinct class of biocompatible and green polymers. It has been the focus of biomedical research for its biodegradability, low immunogenic response and ease of processing [1]. Silk can be considered to be one of the oldest materials known to mankind, documented as early as 131-211 AD by the Greek physician Aelius Galenus [2] for its use as a medical suture. The US Pharmacopoeia (USP) classifies the conventional silk sutures still in use today as non-degradable and non-adsorbent, primarily because of the wax coating that protects silk fibroin (SF) from proteolytic digestion in vivo [3]. In addition to silk proteins, which are a prime candidate for medical applications such as tissue engineering and drug delivery applications, silk proteins have also gained prominence in new frontiers. Gentle processing of silk fibers to obtain water-derived regenerated silk fibroin supports the feasibility of manufacturing SF-based photonic devices or biosensors for various biomedical applications [4,5]. Silk proteins have also been shown to be an effective stabilizing agent, extending the shelf life of fruits [6] and biopharmaceuticals such as vaccines and antibiotics [7]. For a material to be termed bioresorbable, it must be physiologically acceptable to the body and degrade in order to be assimilated or safely eliminated from the body without causing any adverse reaction.

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Silk proteins meet these criteria because of their biocompatibility and biodegradability. In addition, the versatile properties of silk make it an ideal candidate for various biomedical applications. This overview describes silk, silk proteins, properties and uses in the medical and dental fields.

Definition:

Silk is a thread spun by the caterpillars of various butterflies. Silk is a natural protein filament. Its filament density is 1.34 g/cm, making it a medium-weight fiber. Silk is the result of the secretion of silk glands. They are a pair of long tubular and convoluted glands, one lying on each side of the caterpillar's alimentary canal. Fibroin, a type of fibrous protein, is secreted by each gland, which is initially in a liquid state. These glands are connected to a very narrow tube-like structure known as the spinneret, which is part of the pituitary gland. The liquid secretion of two glands flows through the spinnerets, turning them into a single thread. Sericin, which causes the two fibroin fibers to unite, is secreted by a pair of accessory glands located in the front of the silk gland. Due to the contraction and expansion of the caterpillar body, two streams of fibroin are ejected through the spinneret and sericin. Upon contact with air, this sticky secretion turns into a fine, long and solid silk thread [8].

Different types of silk: [8]

Silk is commonly known as the queen of fibers. It is a byproduct of the silk cocoon's life cycle. There are two major types of silk: cultivated silk and wild silk.

The manufacturing steps of silk are,

1. Sericulture
2. Sorting cocoons
3. Softening the sericine
4. Reeling
5. Throwing

Chemical composition of silk:

- Silk Gum or Sericine → 22-25%
- Silk or Fibrin →62.5-67%
- Water →10-11%
- Salts →1-1.5%

Fibrin is composed of a number of α-amino acids in which the most important are.

- Glycine →38%.
- Alanine →22%
- Serine →15%
- Tyrocine →9%
- Other →16 %

Structure of silk

Domestication of the silkworm dates back thousands of years. The cocoon is enveloped in a continuous silk thread that can exceed 1 km [9] in length. Normal silk fibers are composed of two types of self-assembled proteins: fibroin and sericin [10,11]. These two proteins have the same 18 amino acids, with variable quantities of glycine, alanine, and serine. Nuclear fibroins are surrounded by sericin, a family of hydrophilic proteins that binds two fibroin fibers [12,13]. P25, a 25 kDa glycoprotein, is a type of protein that binds these proteins non-covalently [11,14]. Fibroin is a large molecule consisting of approximately two-thirds crystalline portion and one-third amorphous portion. The crystalline part of the fiber comprises repeating amino acids (-Gly-Ala-Gly-Ala-Gly-Ser-) that create an antiparallel sheet and contribute to the fiber's stability and mechanical qualities [9,15-17].

Hydrogen bonds between neighbouring peptide chains generate the secondary structures of fibroin, which are a random coil, an amorphous type, and an antiparallel sheet type. These structures date back to [18] and are classified as random coil, amorphous type, and antiparallel sheet. Silk fibroins are natural block copolymers consisting of hydrophobic blocks with short side-chain amino acids, such as glycine and alanine, and hydrophilic blocks with longer side-chain amino acids, in addition to charged amino acids [19]. Hydrogen bonding gives rise to - layers or crystals from the former blocks. Silk I and silk II are the two primary structural components of silk fibroin.

There are regions of a random coil and amorphous structure in silk I. The antiparallel sheet structure is used to describe the silk II structural type of silk fibroins [20]. The former structure is water-soluble, whereas the latter structure excludes water and is insoluble in multiple solvents, including mildly acidic and alkaline environments and several chaotropic techniques, such as methanol [21-23] treatment.

Silk Fibroin

Silk Fibroin is composed of an H-chain, an L-chain, and a glycoprotein (fibrohexamerin fhx/P25) in a molar ratio of 6:6:1. The H-chain and L-chain are connected by a disulfide bond, and six hetero-dimers are associated with a single P25 at the H-chain moiety via hydrophobic interactions [24]. The N-terminal is glycosylated with residues of mannose and glucosamine [25]. Poly-(glycine-alanine) repeats (GAGAGS, GAG AGY) are the principal repeat sequences responsible for the reported ß-sheet crystallite areas in silk. The crystallite regions are encased in amorphous-

membranes, microparticle

Silk protein compatibility with biological systems

Silk fibrion-based materials displayed promising biocompatibility due primarily to their cytocompatibility and decreased immunogenic potential compared to collagen, PLGA, and a multitude of other polymers.

Protein degradation of silk

According to the classification provided by the US Pharmacopeia, silk is classified as nondegradable. However, based on the available literature, it can be regarded a biodegradable substance. This could be due to the fact that silk breakdown is typically mediated by a foreign body response. In contrast to synthetic materials, silk fibroins' biodegradability does not induce an immunogenic response. Biodegradation is the decomposition of polymeric substances into smaller molecules. The mechanics are intricate and the procedures differ considerably. Typically, the factors include physical, chemical, and biological components. Silk fibroins can be categorized as enzymatically degradable polymers depending on the route of degradation. Significantly contribute to the destruction of silk fibroins are enzymes. Due to their enzymatic degradability, silk fibroins' unusual physicochemical, mechanical, and biological features have been widely studied. The breakdown of biomaterials by enzymes is a two-step process. The first step is the adsorption of the enzyme onto the surface of the substrate via the surface-binding domain, followed by the hydrolysis of the ester bond.

Figure 4. Properties of silk proteins (Fibroin)

Application of Silk proteins in the Healthcare Industry [30]

Silk proteins for Tissue Engineering Scaffolds and Constructs - A three-dimensional scaffold for tissue engineering must enable cell recruitment, adhesion, proliferation, and differentiation as in vivo. The slower disintegration rates and minimal inflammatory response of silk fibroin make it beneficial for biodegradable scaffolds where gradual tissue development is required.

Skin Grafts and Wound Dressings - Due to their great susceptibility to damage and wear, tissue engineering solutions for skin have been extensively investigated. Until now, several biomaterials like chitosan, collagen, cellulose, alginate, silk fibroin, dextran, polylactic acid (PLA), elastin, polyethene glycol (PEG), polycaprolactone (PCL), and silicone have been used to create acellular scaffolds for wound healing. B. Mori silk fibroin has gained widespread acceptance as a wound dressing material due to its outstanding biodegradability, biocompatibility, cost-effectiveness, and minimal immunological reaction.

Silk Fibroin for Cartilage Tissue Heal - Silk fibroin sponge scaffolds offered mechanical stimulation to the chondrocytes when cultivated in a bioreactor, and such cartilage grafts might be employed to repair knee joint abnormalities. The evaluation of Agarose–silk fibroin blended hydrogels for cartilage regeneration revealed that they support chondrogenesis and cartilage-like native extracellular matrix formation.

Vascular Grafts - The use of B. Mori silk fibroin in vascular tissue engineering has been thoroughly investigated. Multilayered vascular grafts created by rolling patterned mulberry silk and non-mulberry silk films imitated the native blood arteries.

Cardiac Tissue Patches - The greatest obstacle in cardiac tissue engineering is to effectively imitate the original extracellular matrix so that it may be used to repair damaged heart muscles. Silk is an ideal natural biomaterial for such applications because its matrix stiffness may be precisely matched to the native muscle rigidity. It has been demonstrated that both B. Mori and A. Mylitta silk fibroin scaffolds can treat myocardial infarction. It has been proposed that three-dimensional cardiac constructs created by stacking cell-laden patterned silk sheets provide an appropriate substrate for heart tissue regeneration.

Hepatic illnesses, particularly liver cirrhosis, have posed a major threat to the population. In recent years, numerous bioartificial liver devices and cell therapies have been created to treat liver problems. Implantable hepatic tissues were created by loading hepatocytes onto 3D scaffolds composed of polymers such as polylactide-co-glycolide, polycaprolactone, polyethene glycol, polyethene, alginates, and cellulose. It has been proven that silk fibroin–blended collagen films stimulate the growth of rat hepatocytes.

Muscle Tissue Regeneration - Muscle tissue engineering requires biomaterials with the necessary mechanical strength. To create functional muscle structures, silk fibroin and poly(aniline-co-N-(4-sulfophenyl) aniline) (PASA) have been combined. These scaffolds revealed the rapid in vitro growth of C2C12 cells. In addition, electrospun nanofibrous scaffolds comprised of silk fibroin/PLA/collagen demonstrated increased myoblast adhesion, proliferation, and maturation.

Tendon and Ligament Grafts - Tendon and ligament tissue damage is increasingly widespread in sports injuries, resulting in mobility impairment. Due to their weak regenerative capacity, restoring the function of such tissues requires extensive tissue engineering. Silk is an attractive candidate for tendon/ligament tissue engineering scaffolds due to its high tensile strength. A matrix of braided silk fibroin mimics the human anterior cruciate ligaments (ACL). It encouraged the proliferation of human bone marrow mesenchymal stromal cells and possessed the same mechanical strength as the native ACL.

Engineered Intervertebral Disc - Intervertebral disc (IVD) degeneration in the form of lower back discomfort, spinal stenosis, and radiculopathy affects the backbone's posture and stability. None of the available medicines can restore the function of the IVD274 gene. Ideal IVD scaffold biomaterial characteristics include biocompatibility, high tensile strength, and resemblance to the native extracellular matrix. Due to its miraculous characteristics, silk fibroin has been utilized in this area. Successful tissue-engineered IVD should mirror the morphology and function of both the nucleus pulposus (NP) and annulus fibrosus (AF) components of IVD (AF). Silk fibroin/fibrin/hyaluronic acid was utilized to create a biphasic hybrid scaffold that mimics both the NP and AF.

It has been demonstrated that electrospun silk fibroin–nerve guiding conduits are efficient for peripheral nerve healing.

Bioartificial Pancreas - As the number of diabetic patients continues to rise, there is a growing need to focus on alternative treatment methods to insulin therapy, medication therapy, and islet transplantation. Multiple hydrogels, nanoparticles, and microspheres have been manufactured to provide prolonged insulin release. The survival rate of pancreatic islets encased in silk hydrogel with laminin, collagen, and mesenchymal stromal cells was increased.

Cancer Therapeutics and Drug Screening Models - Due to its biocompatibility, biodegradability, and lack of immunological rejection, silk is an effective biomaterial for cancer therapy. As the drug carrier, silk formulations such as films, hydrogels, capsules, silk-coated liposomes, and nanoparticles can solve the greatest challenge faced by an anti-cancer therapy, which is sustained release leading to its target destination. It has been demonstrated that B. Mori silk films may deliver doxorubicin when applied intratumorally in an orthotopic human breast cancer model. Silk fibroin can also be used to create 3D cancer models that more closely resemble in vivo circumstances than 2D culture dishes, as well as for drug development applications.

Tissue On Chip for High Throughput Screening - The growing demand for newer technologies to reduce failures in pre-clinical trials of drug discovery has led to the development of tissue-on-chip or organ-on-chip (TOC/OOC), which is an innovative approach towards three-dimensional microfluidic devices that mimic a functional tissue/organ and can replace animal models for drug screening and drug development applications. Several such chips for engineering the heart, skin, lungs, kidneys, and arteries have already been developed. Even the emergence of a human-on-chip model is attributable to technological advancements. Recently, silk hydrogel microfluidics has been in the spotlight due to its improved biological activities that result in tissue reproduction.

Advances in Silk-Based Biosensing and Biomedical Imaging - Silk fibroin has been utilized in formulations to create bioinks that can be doped with components to create inkjet-printable functional devices for sensing, therapy, and regenerative medicine..

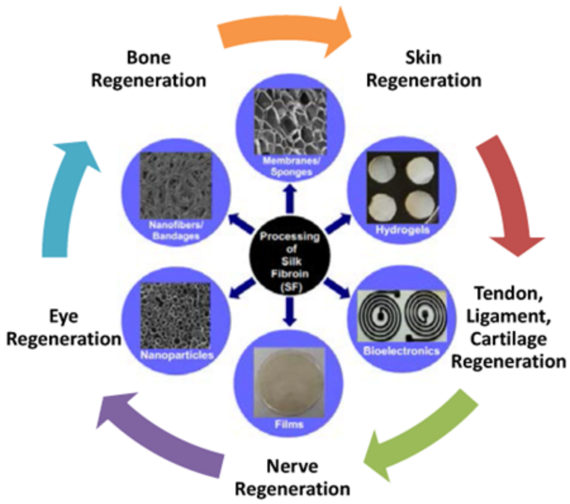

Figure 5. Application of Silk Fibroin in medical field

Applications of Silk in Allied Healthcare Applications [30]

Food technology - Silk fibroin adsorbed with olive leaf antioxidants is proposed as a biopolymer for the production of antioxidant and antimicrobial functional foods and nutritional supplements. Bombyx mori silk sericin in food is said to alleviate constipation, inhibit the development of colon cancer, and enhance mineral absorption.

Reported benefits of employing silk sericin in the food business include its ready availability, non-toxicity, great moisture-retaining capacity, antioxidant, and effectiveness as an emulsifier and antifrosting agent. Reportedly, bread containing a specified amount of silk sericin is an optimal processed food that impacts digestion and absorption. Silk protein has also been utilized in the production of infant food that is purported to prevent and lessen skin diseases like atopic asthma and atopy. Silk protein has also been utilized in the production of purportedly preventative or therapeutic foods for Parkinson's disease.

Electronics - Some of the defining characteristics of next-generation electronics include flexibility, stretchability, and wearability. Recently, electronic implantable medical devices have been created for therapies or purposes like cardiovascular regulation, drug delivery, and augmentation of biological structure348. Due to its unique structure and qualities, silk has multiple advantages, including durable mechanical capabilities, tunable deterioration, and the ability to be fabricated into various forms. Silk has been approved by the Food and Drug Administration (FDA) for use in electronic devices with implantable biomedical and therapeutic applications.

Biomedical Textiles - Silk-based biomaterials have been utilized in clinical settings for a number of years and are now regarded as a possible alternative material for biomedical textiles. Additional modifications have been made to silk-based biomaterials in order to make them appropriate for biomedical textile applications. Different silk-based biomaterials for biomedical textiles can be classified as non-implantable, implantable, extracorporeal, and healthcare materials.

• Silk-Based Non-Implantable Materials - Non-implantable materials in biomedical textiles include wound dressings, pressure garments, orthopaedic bandages, and prosthetic socks, among others. Silk materials have frequently been utilized as wound treatments. Recently, a two-layered wound dressing comprised of wax-coated SF woven fabric, a sericin sponge, and a bioactive layer of glutaraldehyde crosslinked silk fibroin gelatin has been produced. These wound dressings demonstrated wound reduction, epithelialization, and collagen production.

• Implantable Materials Based on Silk - Implantable materials in biomedical textiles are utilized for wound closures during skin procedures, vascular implants, artificial tendons/ligaments, and artificial heart valves, among others. For a number of years, sutures constructed from natural silk strands have been utilized.

• Silk-Based Extracorporeal Implants - Extracorporeal organs are artificial organs engaged in the purification of blood. Extracorporeal organs consist of the artificial kidney, artificial liver, and mechanical lung. Using a urease-immobilized SF membrane and polymer-based spherical carbonaceous adsorbent, a silk-based wearable artificial kidney system for peritoneal dialysis has been created lately.

• Other Silk-Based Healthcare Materials - Silk-based healthcare/hygiene biomedical textiles have been in clinical applications in the operating theatre, including surgeon's gowns, masks, caps, patient drapes, and cover cloths, as well. Among the several benefits of these compounds are their mechanical qualities, softness, and antibacterial capabilities.

Silk has been utilized in cosmetics for a number of years. Sericin and its combination with SF have been utilized in cosmetics for the skin, hair, and nails. Silk sericin-based lotion, cream, and ointment have been produced. It has been reported that they have skin-elasticizing, anti-wrinkle, and anti-ageing properties.

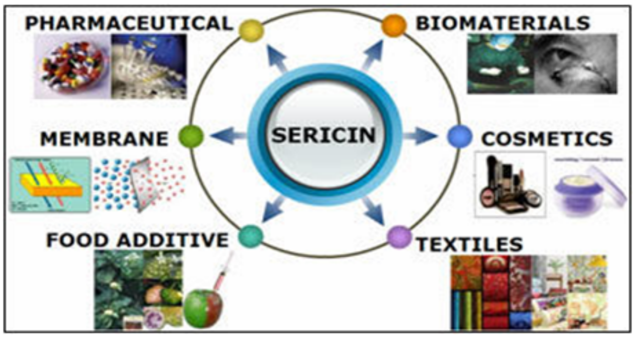

Figure 6. Application of Silk Sericin in Medical and Non – Medical Field

Bioremediation - Remediation of polluted land, air and groundwater is a major environmental issue having worldwide significance. Silk has been widely utilized alone or in conjunction with other polymers for the removal of heavy metals from aqueous solution, the purification of water, the adsorption of hazardous pigments, and air filtration [30].

Silk biomaterials for dental applications [31]

Silk biomaterials have been utilized for many years in biological applications; nevertheless, there are only a few uses in dentistry, such as suture materials and dental tissue regeneration

Tissue engineering and regenerative medicine are active research fields for the regeneration of oral and dental human tissues. Silk-based composite scaffold materials are being employed for hydroxyapatite biomineralization experiments, and natural spider silk was used as a template for the nucleation and development of hydroxyapatite crystals.

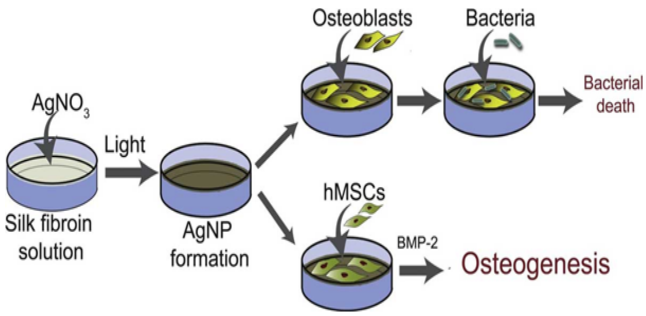

Figure 7. Silk Fibroin in Dental Tissue engineering

Recently, there has been an increase in the use of coating technology for biomaterials to improve their surface properties. From the biological point of view, the material coating could enhance or reduce cellular adhesion for biomedical implants. The biocompatibility and low immunogenicity of natural silk make it a good candidate for coating applications. In addition, antibacterial properties can be included in silk-based composite materials, such as silk nano-composites containing silver nanoparticles. Titanium nanoparticles have been shown to stop the growth of microorganisms such as Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.

Damrongrungruang et al. characterized electrospun silkworm silk scaffolds for gingival tissue regeneration applications and found that gingival fibroblasts attached and proliferated on electrospun fibres, confirming the non-toxicity of silk. In a study with mouse fibroblast cells in vitro, polypropylene and polyamide foams coated with silkworm silk supported the cells and allowed them to proliferate. [32]

Conclusion

Silk is a protein fiber derived from silkworms and is the only natural filament material. Caterpillars, spiders, and mussels can spin these threads. Significant effort has been made to biodegradable silk biomaterials over the past several decades. Silk fibroins are extracted from the cocoons of Bombyx mori, the mulberry silkworm. Silk materials are increasingly used as biodegradable materials due to their high tensile strength, tunable biodegradability, hemostatic capabilities, non-cytotoxicity, low antigenicity, and non-inflammatory features.

Typically, silk fiber is composed of two types of self-assembled proteins: fibroin and sericin. Fibroin is a key component of silk fiber that serves as the core, whereas sericin is a small component that functions as the protein coating. In comparison to fibers of comparable tensile integrity, the former is composed of highly structured -sheet crystal regions and semi-crystalline areas responsible for silk's elasticity. The development of therapeutic devices such as temporary prostheses, three-dimensional porous structures as scaffolds for tissue engineering, and controlled/sustained release drug delivery vehicles favours biodegradable materials. The medical application necessitates biomaterial with unique characteristics, such as biodegradability. According to the classification provided by the US Pharmacopeia, silk is classified as non-degradable. According to the literature, however, it can be considered a biodegradable material, albeit over a longer period of time. Silk fibroins can be categorized as enzymatically degradable polymers depending on the route of degradation. Lastly, a better knowledge of the biodegradation behaviour of silk proteins will shed light on the design of silk biomaterials for future medical applications.

Future prospective

Researchers are developing surprising new medical applications for silk. Silk, which is manufactured by silkworms and spiders, has long been prized as a clothing material. It is strong, elastic, and safe to use even inside the human body, so scientists are investigating how to weave it into bulletproof vests, use it to heal wounds, support bones, and maybe replace tendons.

Purdue University scientists have developed silk that may destroy germs when activated by light. DNA from silkworms was injected with a naturally occurring protein that can be triggered to induce a pathogen-killing chemical reaction. The silk generated by the genetically engineered silkworms was crimson and glowed. When scientists placed E. coli bacteria to silk and exposed it to a green LED light for one hour, the bacterium's survival rate decreased by 45%. The reaction is comparable to using hydrogen peroxide to disinfect a cut, according to Young Kim, co-author of the article published in Advanced Science. In the future, the material might be included in devices that filter air and water, or it could be utilized to create enhanced bandages, given that silk already has cooling properties that aid in the treatment of inflammation.

According to materials scientist Mei Wei of the University of Connecticut, silk can potentially be used to support our bones. Typically, doctors implant metal supports to stabilize a fractured or broken bone, but the metal can induce more fractures and must be removed. The team led by Wei developed a kind of silk that can support wounds and degrades naturally within the body after a year.

Silk may one day be developed to replace our tendons, particularly if combined with a nanocellulose found within trees that is both strong and inexpensive. Researchers at the KTH Royal Institute of Technology in Stockholm are investigating a hybrid material that combines the strength (of nanocellulose) with the tensile strength and elasticity of aramid fibers (of silk). The outcome could one day be employed in protective vests or to replace tendons. Nanocellulose does not degrade within the human body, but cells can grow on and around it, and its unusual elasticity makes it a perfect match.

Meet the Future's Surprising Medical Material: Silk (popularmechanics.com)

"Activated Silk, manufactured from pure silk protein, is the first technology to enhance dermal filler biomaterials since HA and lidocaine," said Dr Greg Altman, CEO & Co-Founder of Evolved by Nature. Our present trial results indicate that we are well on our approach to developing solutions that outperform and redefine current benchmarks.

Silk Medical Aesthetics has licensed Evolved by Nature's comprehensive patent portfolio for hyaluronic acid and lidocaine with Activated Silk for use in injectable aesthetic medicine and is developing the next-generation dermal filler platform to address all needs in volume restoration and the improvement of the skin's appearance and texture. Silk Medical Aesthetics is a Boston-based firm with the objective of developing the next-generation dermal filler platform by utilizing the power of natural silk.

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