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Technological innovations in sericulture

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Abstract

Sericulture has undergone a major transformation due to emerging technologies in the field of biotechnology leading to the emergence of newer technologies that have the potential to impact the various aspects of silkworm. Technologies such as gene therapy, gene editing, nano-biotechnology, transgenic technology etc. are being used to address a variety of challenges in silkworm such as producing transgenic silkworms with increased cocoon quality and quantity, to use silk as a biomaterial in medical and pharmaceutical industry etc. The unique characteristics of silk such as strength, elasticity, biodegradability, biocompatibility and mechanical robustness regarding different biomedical uses make it remarkable biomaterial for a wide variety of technical and medical applications. Micrococoons derived from silkworm are an efficient vehicle for drug delivery, a potential approach to treat osteomyelitis and dermal diseases. Silk fibroin and sericin component of silk possesses excellent biocompatibility, controllable biodegradability, remarkable mechanical strength, and low immunogenicity. Therefore, it has been widely used in tissue engineering and regenerative medicine applications such as bone, cartilage, and cornea repair. Bio-nanotechnology advancements lead to the production of silk structures that can be used in water filtration devices, in optics, photonics, in sensors and in drug delivery.

Keywords: silk, sericulture, sericin, fibroin and bioengineering

Introduction

Sericulture is an age-old, labour extensive, cottage-based rural industry that eradicates unemployment by providing employment to around 8.7 million people in the country. Silk cocoon which is the natural dome of architecture and bioengineering is the ultimate end product of silkworm rearing and basic material for silk industry. Silk is commonly known as "Queen of Textile" due to its properties such as elegance, versatility, coolness, elasticity, tensile strength, lustrous and bio-steel because of its strength (Anonymous, 2021) [1]. Silk represents a unique family of structural proteins that are biocompatible, biodegradable, mechanically superior, offer a wide range of biomedical applications. Chemically, silk fibre consists of fibroin, sericin, carbohydrates, inorganic matter, waxy material and pigments. Silk fibroin shows remarkable mechanical, degradation and biocompatibility properties, favoring its use to generate highly loaded grafts, especially in the musculoskeletal field. The sericin is a natural polymer, which acts as an adhesive joining two fibroin filaments in order to form silk yarn. Sericin has unique properties such as an antioxidant, moisturizing, healing, antibacterial, antimicrobial protection against ultraviolet radiation and anti-tumor. The advent of genetic engineering techniques such as transgenic technology, bioengineering, nanotechnology etc has enabled us to transform the silkworm's genetic makeup in a variety of novel ways. The silkworm, *Bombyx mori* L has become a useful model of the Lepidoptera, with an increasingly important role in basic biological research. Silk produced from silkworm cocoons exist in fibre form in nature but the artificial engineering techniques facilitate the fabrication of silk solutions from silk fibre inversely. These silk nanostructures can be utilized to fabricate structurally and functionally efficient materials such as hydrogels, fibres, films, coatings and 3D matrices. Silk materials possess manifold applications in biomedicine and emerging fields such as water treatment, environmentally adaptive materials, and optical and electronic devices. After the invention of the *piggyBac* transposon mediated technology to make silkworm transgenic, efforts have been made to study the silk gland of the silkworm and to make it a suitable efficient system for the production of the foreign protein. In past decade more than 10 foreign proteins were successfully expressed in transgenic silkworm which includes model protein, human or animal derived pharmaceutical proteins and silk- based proteins.

Although, silk gland is considered to be a bioreactor system for pharmaceutical protein production and genetically engineered proteins, that are cost effective, easy-scale-up and simply processed with improved mechanical properties and new bio-functionalities.

Fig 1: Fifth instar silkworm Larvae.

Major technological innovations in Sericulture

Micrococoons and their use in biotechnology and medicines

Micrococoons are micron-scale capsules comprised of a solid and tough shell of silk nano-fibrils that surround and protect a center of liquid cargo. Micrococoons are more than a thousand times smaller than those created by silkworms. The micrococoons are prepared from native silk feedstocks extracted from the silk glands of 5th instar larvae of *Bombyx mori* that had just started to construct cocoon (Laity *et al.*, 2015) [26]. Other materials required to prepare micrococoon are fluorinert FC-70 (Sigma-Aldrich, UK) and N, N bis (n-propyl) polyethylene oxide-bis (2-trifluoromethyl polyperfluoroethylene oxide) amide surfactant. The silk glands are spliced out from rest of the body using dissection microscope and transferred to cold distilled water to peel off the epithelial membrane using tweezers. This operation is carried instantly to curtail the dilution of silk gland contents. The highly coiled posterior region and thin anterior region of silk gland are removed and discarded off to synthesize native silk cocoons. The Native Silk Feedstocks (NSF) micrococoons are fabricated by a specially designed microfluidic system with 20 mm diameter of channels (Shimanovich *et al.,* 2017) [46].

These silk nanostructures are utilized to fabricate structurally and functionally efficient materials such as hydrogels, fibers, films, coatings and 3D matrices (Wang *et al.,* 2018) [18]. There are certain instances of silk nanoparticles being utilized in systemic applications including drug-delivery (Solomun *et al.,* 2020) [48] and solid tumor targeting (Mottaghitalab *et al.,* 2015) [40]. The artificial spinning of native silk by microfluidic strategy provides long term storage and aggregation prone biomaterials as micron-scale shapes or capsules often termed as ‘micrococoons’ possessing a wide range of potential applications in medicine and biotechnology.

Potential applications of Micrococoons

Micrococoons as a vehicle for drug delivery system

The silk based nanomaterials are extremely versatile in drug delivery. The fibroin molecules possesses amine, hydroxyl, carboxyl and thiol functional groups of amino acids which can anchor many biomolecules or antibodies as markers for active targeting and can be modified to manage the entrap drug properties (Phama and Tiyaboonchaia, 2020) [45].

Small molecule delivery: Although the pharmaceutical products are available as small molecules in market yet there are certain limitations such as hydrophobicity, low permeability, short half-life, non-specific targeting etc that curtails their commercial utilization. The silk fibroin nano-particles are found promising to fix the obstacles of drug delivery. Fibroin nanoparticles have gained much importance as it can incorporate natural compounds such as curcumin (Gou *et al.*, 2019) [16], quercetin (Lorzano-Perez *et al.,* 2017) [32], alpha mangostin (Phama *et al.,* 2019) triptolide and celastrol (Ding *et al.,* 2017) which are more beneficial to treat many diseases. Although the entire drug loaded fibroin nanoparticles signified high entrapment efficiency, increased drug solubility and stability, decreased drug degradation, reduced undesirable toxicity, controllable release profile and retained drug therapeutic efficiency (Phama *et al.,* 2019). The silk based nanoparticles drug delivery has been extensively studied for anticancer drugs.

Protein delivery: The growth factors and enzymes are extensively used therapeutic proteins to revitalize cell growth and differentiation during tissue regeneration. However, the clinical application of enzymes has adverse limitations such as short half-life, low stability, limited tissue penetration, and potential toxicity. Fibroin nanoparticles (FNPs) have the potential to deliver big molecules to achieve therapeutic protein delivery. FNPs have been used in delivering bovine serum albumin (Chen *et al.,* 2018), vascular endothelial growth factor (VEGF) (Kundu *et al.,* 2010), b-glucosidase (Cao *et al.,* 2014), etc. It can be assumed that micrococoons can serve as potential carrier for protein delivery in therapeutics.

Gene delivery: Due to verified higher transfection efficiency as well as higher degree of specificity fibroin has been explored to have potential gene delivery system. Numata *et al.* (2009) reported that silk altered by insertion of PLL (Poly L-lysine) sequences revealed that high transfection efficiency of plasmid DNA in human embryonic kidney (HEK) cells. While figuring out all the facts related to utilization of silk based nanoparticles in gene delivery, micrococoon is supposed to prove potential vector in gene delivery.

Vaccine delivery: Vaccine is the introduction of biological preparation consisting of the killed causative agent or toxins that acts as antigen and used to induce production of antibodies to enhance the immunity against particular disease. The simple, cheap, easy to manufacture and thermostable vaccine is the need of the hour (Phama and Tiyaboonchaia, 2020) [45]. Fibroin nanoparticles are promising candidates to safeguard the vaccines from degradation and have improved accessibility to antigen presenting cells (APCs). Liu *et al.* (2014) [30] investigated fibroin/chitosan microsphere possess higher loading capacity of 89.14% and an average particle size of 1.98 mm that effectively prevents the loading vaccine from DNase digestion with higher immunization. NSF micrococoons have the potential for long term storage with enhanced stability of aggregation prone proteins. Shimanovich *et al.* (2017) [46] revealed that micrococoons could increase the stability and lifetime of an antibody and have potential to treat the challenging neurodegerative diseases.

Ocular drug delivery: Ocular drug delivery is considered as challenging task in pharmaceutical sciences. Dong *et al.* (2015) reported that the better cell adhesion in human corneal epithelial cells (HCEC), when silk fibroin coated liposomes loaded with ibuprofen were used for ocular delivery. The drug release and permeation rates could be modulated by calibrating the concentration of silk fibroin. Tran *et al.* (2018) reported that fibroin films and hydrogels have been used to treat ocular diseases since long time as a delivery platform. It is assumed that silk micrococoons could be the potential nanoparticles to treat the ocular diseases with high efficacy and stability of the drug delivered.

Micrococoons as potential approach to treat osteomyelitis

The latest parenteral antibiotics, hyperbaric oxygen, and surgery are inadequate to treat osteomyelitis due to many limitations such as short half-life of antibiotics, systematic toxicity of drugs, and shortage of blood supply and bone necrosis. The orthopedic surgeons are still struggling to treat the disease effectively. Fibroin nanoparticles possess non-toxic, biodegradable and mucoadhesive properties and can upgrade the particle retention time at the infected area (Phama and Tiyaboonchaia, 2020) [45]. Zhou *et al.* (2011) [59] reported the controlled release of vancomycin antibiotic as a best method to treat osteomyelitis.

Micrococoons as a promising approach to treat dermal diseases:

Filon *et al.* (2015) reported fibroin nanoparticles serve as a promising vehicle for drug delivery through transdermal surfaces. It can easily penetrate and permeate due to amphiphilic properties and can control over the particle size. Mao *et al.* (2017) reported that fibroin (Hydrogel state) incorporated with curcumin in polymeric nanoparticles improved the transdermal delivery efficiency in psoriatic mouse model. Wang *et al.* (2006) reported that silk fibroin films, fibres, nets, meshes, membranes, yarns and sponges stimulated cell adhesion, proliferation and differentiation in controlled conditions and initiated tissue repair in vivo conditions. It was explored that 3D silk fibroin scaffolds proved effective biomaterial to engineer the skeleton tissues and connective tissues including skin as well. Shimanovich *et al.* (2017) [46]. reported to Media Line, if the skin cells and tissues are damaged or dead, “Silk can give it a second life; it can help it to divide and live again”. The researcher further added, “Silk could help to regenerate skin after a burn”. It is assumed that micrococoons have the potential transdermal applications in near future.

Micrococoons in the context of neurodegenerative conditions

The neurodegenerative conditions, Alzheimer’s disease (AD) and Parkinson’s disease are the major protein misfolding disorders that cause degeneration of neural complex. Yonesi *et al.* (2021) reported that according to 2019 census Alzheimer’s disease is considered as the sixth leading source of mortality in the United States. Micrococoons have potential to improve the stability and lifetime of an antibody. Shimanovich *et al.* (2017) [46]. revealed the effect of micrococoons with an antibody against the protein alpha-synuclein which is involved in Parkinson’s disease.

Insect silk – Biotech approaches for medical applications

Silk can be credited as one of the most ancient materials known to man which has been documented for its use as a medical suture, as early as 131-211 A. D. by Greek Physician Aelius Galenus (Goel, 2015). Insect silk is mainly produced by various species of class Insecta of phylum Arthropoda. These belong to order Lepidoptera (family Bombycidae and Saturnidae). Non-insect silk is mainly produce by class Arachnida (spiders, ticks and mites) and Myriapoda (centipedes). Silk fibroin and sericin from various silkworm species have been researched for their potential applications in the healthcare industry such as tissue engineered grafts, cancer therapeutics, biomedical imaging, bio-sensing, biomedical textiles, implants, and bioremediation products. Silk fibres are biocompatible, several silks show no cytotoxicity, evoke nearly no immune response if implanted into the body, and degrade into non-toxic by-products. Advances in biotechnological production of silk enabled large scale production of silk proteins for the development of new materials for various applications including biomedical applications as drug carriers, sensors or scaffolds for tissue engineering.

Medical applications

Seri surgical scaffolds: These are surgical meshes manufactured by unwinding *Bombyx mori* silk cocoons and working with the silk thread. These are used in plastic surgery applications, including total body contouring, brachioplasty, abdominoplasty, mastopexy, and breast reconstruction.

Silk solution: *Bombyx mori* silk in its solubilized aqueous form has been investigated for a range of therapeutic applications, including treatment of diabetes, chronic wounds and inflammation. Recent studies have investigated the utility of regenerated silk fibroin solution in preclinical animal models for the treatment of ocular conditions, including dry eye and corneal injuries. Silk fibroin treatment inhibited detachment of corneal epithelial cells, increased the number of conjunctival goblet cells, and inhibited the secretion of inflammatory factors in the lacrimal gland of the eye, resulting in recovery of the tear film and mucus layer of the eye, improved corneal health, and reduced dry eye symptoms.

Silk films: Silk films are also being investigated as a substrate for the development of retinal prostheses. Recently reported retinal prostheses consisting of semi-conductive poly (3-hexylthiophene) and conductive poly (3,4-ethylene-dioxythiophene)-poly (styrenesulfonate) layers spin-coated onto silk films were shown to restore light sensitivity and visual acuity of the primary visual cortex. Cast from the Indian non-mulberry tasar silkworm, *Antheraea mylitta* extensively explored for engineering corneal stroma and their innervations.

Electrospun silk: Electrospun silk fibroin has been explored for a range of applications, including wound dressings, bone and ligament replacements, and vascular grafts.

Hydrogels: Self-assembling silk hydrogels are emerging as useful tools for the therapeutic delivery of stem cells. For example, pancreatic islet transplantation is plagued by a functional decline and decreased viability of the islets during the peri-transplantation period, so self-assembling silk fibroin hydrogels have been examined as a potential delivery system.

Nanoparticles: Nanoparticles are often proposed for solid tumor targeting, as they can exploit the leaky vasculature and reduced lymphatic drainage associated with tumors, which results in enhanced permeability and retention (EPR) at the target tissue (Nakamura *et al.,* 2015) [41]. Nanoparticles generated from *Bombyx mori* and *Antheraea mylitta* silks were stable, spherical, negatively charged, and 150-170 nm in diameter, and they showed no cytotoxicity at the tested concentration (Kundu *et al.,* 2012).

Table 1: Different biomaterials derived from insect silk with their applications

Material Format	Silk Type	Features	Applications	Reference
Films	Silk fibroin and silk sericin	2D solvent casted, transparent films with nano-features ranging from 1 to 12 nm; patterned silk films by PDMS stamping	Bone, corneal, vascular tissue engineering, biomaterial design	Gupta *et al.* (2016)
Sponge	Silk fibroin and silk sericin	Porous silk sponges, with highly interconnected pores ranging from 60 to 300 µm; silk fibre reinforced scaffolds with compressive strength up to 13MPa; processed through freeze drying, salt-leaching techniques; ease in making composites with inorganic or organic additives	Bone, cartilage, inter-vertebral disc, meniscus liver, pancreas tissue engineering and drug delivery	Bhunia *et al.* (2018)
Fibres/Suture	Silk fibroin	Monofilament or multifilament degummed silk fibres (woven or non-woven) biospun silkworm filaments	Tendon tissue engineering, nerve guides, stents, suture material	Liu *et al.* (2007) [30]
Electrospun mat	Silk fibroin	Fibre diameter ranging from 50 to 500 nm; ease in making composites with other polymers, inorganic or organic additives	Bone, osteochondral, nerve, ocular skin, skeletal muscle tissue engineering, vascular conduits, wound dressings, drug delivery, biofilters	Chouhan *et al.* (2018)
Microparticles	Silk fibroin	Particle size ˜ 3 µm prepared by salvation or mechanical comminution	Bioactive molecule/drug delivery	Bessa *et al.* (2010)
Nanoparticles	Silk fibroin	Nanoparticles ranging from 2 to 500 nm, fabricated via an array of methods such as capillary microdot desolvation, electrospraying, micro emulsion, supercritical fluid application	Tissue engineering, drug, bioactive molecule delivery	Mehrotra *et al.* (2019)
Hydrogels	Silk fibroin	Highly tuneable hydrogels with respect to elasticity, degradability developed through self-assembly, use of green cross linkers, or physical cross linkers	Drug delivery, tissue engineering	Chouhan *et al.* (2018)

Silk proteins and their commercial use

Silk derived from the silkworm, *Bombyx mori* is a natural protein that is mainly made of sericin and fibroin proteins. Silk filament is a double strand of fibroin, which is held together by gummy substances called silk sericin. Silk is fibrous protein synthesized in specialized epithelial cells that line glands in silk producing organisms. Silk represents a unique family of structural proteins that are biocompatible, biodegradable, mechanically superior, offer a wide range of properties, have a wide range of biomedical applications. Silk consist of two main proteins, sericin and fibroin, fibroin being the structural centre of the silk and sericin being the sticky material surrounding it.

Sericin: The sericin is a natural polymer, which acts as an adhesive joining two fibroin filaments in order to form silk yarn (Mondal *et al.,* 2007) [38]. Structurally, sericin is a globular protein consisting of random coil and β-sheets. At lower temperatures, the solubility is reduced and the random coil structure is converted into β-sheets, resulting in the formation of a gel (Zhou *et al.,* 2000) [59]. The molecule is highly hydrophilic with a molecular weight that ranges from 20 to 400 kDa and consists of 18 amino acids, including essentials. The polar groups (carboxyl, hydroxyl and amine groups) of amino acid side chains and its organic composition, solubility, and structural organization enable cross-linking, co-polymerizations, and combinations with other polymers, which together convey unique properties to sericin as an antioxidant, moisturizing, healing, antibacterial, antimicrobial protection against ultraviolet radiation, and anti-tumour (Aramwit *et al.,* 2009) [6].

Sericin Applications

Immunological response: Aramwit *et al.* (2009) [6] investigated the inflammatory mediators induced by sericin *in vitro* and *in vivo*. When the sericin was added to the culture media of mouse monocyte and alveolar macrophage cell lines, there was an increase in cell proliferation and the generation of tumour necrosis factor (TNF-α) and interleukin1 beta (IL-1β). However, this increase in cytokines does not activate other inflammatory cascades. In their *in vivo* assay, the authors used an 8% sericin cream, which was applied topically on wounds on the back of rats. After 7 days of treatment, there was a reduction of the expression of TNF-α and IL-1β in tissue and the overall wound healing was accelerated in treated animals. In this way, sericin promoted wound healing without exacerbating the inflammatory process. Sericin can be considered as a biocompatible protein, since it presents very low immunogenicity (Lamboni *et al.,* 2015).

Antioxidant: Dietary antioxidants have been of great interest, especially due to the findings on the effect of free radicals in the body, which can have serious consequences if their products are not neutralized by an efficient antioxidant system (Sorg, 2004). Cocoons of *Bombyx mori* can provide natural pigments typically flavonoids and carotenoids that accumulate in sericin layers (Kurioka *et al.,* 2002). These pigments are known for their biological properties as antioxidants and anti-tyrosinase. Micheal *et al.* (2014) suggested that the main constituent amino acids of sericin protect the mid-gut epithelial cells of *Bombyx mori* and haemocytes from oxidative damage, probably by the ability of sericin to eliminate ROS (Reactive oxygen species). Li *et al.* (2008) observed a protective effect of sericin in hepatic and gastric injuries caused by alcohol in mice.

Cosmetology: The use of sericin in cosmetic formulation, such as creams and shampoos, leads to an increase in hydration, elasticity, cleaning with less irritation, and anti-aging and anti-wrinkle effects and also prevents nails from chapping and brittleness (Singh *et al.*, 2014). These applications are especially due to the presence of amino acids with hydrophilic side groups (80%), such as serine (30 to 33%), which has large capacity to absorb water. The sericin may also form a soft and smooth film on the surface of the skin, preventing the loss of water (Patel and Modasiya, 2011).

Supplement in culture media and cryopreservation: Sericin obtained from the cocoon was added alone or combined with BSA in the culture media of mammalian cell lines (murine hybridomas 2E3-0, human hepatoblastoma HepG2, human epithelial HeLa cells, and human embryonal kidney 293 cells). The use of small sericin, increased cell proliferation in four lines, with positive results in concentrations from 0.01% to 0.1%, while higher concentrations (1%) were potentially dangerous. The sericin promoted an increase in cell viability, which became more pronounced when added to the BSA. Furthermore, the activity of the sericin did not change after autoclaving, showing its use as a supplement in culture media to stimulate cell proliferation (Terada *et al.*, 2005) [51].

Wound healing: Several studies provide evidence of the healing properties of sericin, since it operates in stimulating the migration, proliferation, and production of collagen (Aramwit *et al.,* 2007). Tsubouchi *et al.* (2005) [54] examined human fibroblasts cultured in culture media containing sericin (400kDa, known as sericin M) and observed cell growth of 250% in 72 hours, due to ease in connection of cell and media, which is dependent on repeated domains rich in serine found in the sericin. In a clinical study, (Aramwit *et al.,* 2013) [6] used the standard antibiotic cream (silver sulfadiazine) with 8% sericin for the treatment of open wounds resulting from second-degree burns. The 29 patients had their burns treated with topical application of sericin, and blind evaluation showed that sericin accelerated wounds closure. The average time required to reach 70% of epithelialization of the burn surface to complete healing in treatment group was significantly shorter than control (without sericin), about 5–7 days. There was also a decrease in length of hospitalization and patients' pain, improving their quality of life.

Anti-tumor effect: Kaewkorn *et al.* (2012) [21] studied the effect of sericin in the proliferation and apoptosis of colon tumour cells. The small-size sericin, 61–132kDa, showed an inhibitory effect on human colorectal cancer cells (SW480) when compared to normal human fetal colonic mucosal cells (FHC). Further, sericin caused a reduction in cell viability by inducing apoptosis of tumour cells with increased activity of caspase-3 and reduction of Bcl-2 expression, an anti-apoptotic protein. The sericin did not induce apoptosis in control cells, acting as chemo-protector against colon cancer cells.

Fibroin: Fibroin is the 'core' protein secreted from the posterior part of the silk gland. Silk fibroin, is a hydrophobic glycoprotein fibers are about 10-25 µm in diameter and consist of two proteins: a light chain (26 kDa) and heavy chain (390 kDa) which are present in a 1:1 ratio and linked by a disulfide bond (Zhou *et al.*, 2000) [59]. The amino acid compositions of silk fibroin from *Bombyx mori* consist primarily of glycine (43%), alanine (30%) and serine (12%). Silk fibroin shows remarkable mechanical, degradation and biocompatibility properties, favouring its use to generate highly loaded grafts, especially in the musculoskeletal field (Teuschl *et al.,* 2013) [52].

Applications of Silk fibroin

Non-woven silk fibroin mats: silk fibroin has been used to generate non-woven silk mats from reprocessed native silk fibers or by electrospinning. Non-woven silk mats were prepared by partial solubilization of native silk fibers, usually in formic acid and small amounts of calcium chloride. Silk fibroin non-woven mats electrospun from a 98% silk formic acid solution were implanted in calvarial defects of rabbits for bone regeneration and resulted in complete healing with new bone at 12 weeks (Kim *et al.,* 2005).

Silk fibroin films: Silk fibroin film has been cast from aqueous or organic solvent systems, as well as after blending with other polymers. Microstructure in films, which are advantageous for increasing surface roughness for cell attachment, were formed via blending of silk with PEO (Jin *et al.*, 2004) [20].

Silk films, employed for healing full thickness skin wounds in rats, healed in seven days faster with a lower inflammatory response than traditional porcine-based wound dressings.

Silk fibroin hydrogels: Hydrogels are three-dimensional polymer networks which are physically durable to swelling in aqueous solutions but do not dissolve in these solutions. Hydrogel biomaterials provide important options for the delivery of cells and cytokines. The pH of the silk fibroin solution impacted the rate of solution gelation. An increase in silk fibroin concentration, increase in temperature, decrease in pH and an increase in Ca++ concentration decreased the time of silk fibroin gelation. Osteoblast-like cells that attached when cultured on 2% silk fibroin hydrogels showed adherence and biocompatibility. Addition of 30% glycerol to the hydrogel increased the proliferation of the cells (Motta *et al.,* 2004) [39].

Silk fibroin porous sponges: Porous sponge scaffolds are important for tissue-engineering applications for cell attachment, proliferation, and migration, as well as for nutrient and waste transport. Regenerated silk fibroin solutions, both aqueous and solvent, have been utilized in the preparation of porous sponges. Sponges have been formed using porogens, gas foaming and lyophilization. Tissue engineered silk sponges were useful for healing critical size femur defects in rats (Meinel *et al.,* 2005) [35].

Biological applications of silk fibroin: Silk fibroin possesses excellent biocompatibility, controllable biodegradability, remarkable mechanical strength, and low immunogenicity. Therefore, it has been widely used in tissue engineering and regenerative medicine applications such as bone, cartilage, and cornea repair. Silk fibroin has also been explored as a biomaterial for skin repair due to its haemostatic properties, low inflammatory potential, and permeability to oxygen and water vapour. *In vivo* studies have further shown that silk fibroin film dressing or spongy dressing promotes faster wound healing and better skin regeneration, compared to the hydrocolloid dressing or porcine dermis/dermal matrix in small animal models.

Silk films for optics, photonics and medicine

Silk fibres are long, thin, opaque, flexible and tough- ideal for a cocoon (or a silk scarf), but unworkable in optical devices. Using silk in optical devices requires reverse engineering of the natural fiber generation process, dissolving the native silk fibers to obtain a purified silk solution (water and fibroin) that closely resembles the material in the caterpillar's silk gland. This versatile solution forms the starting material for silk films, gels, sponges, blocks and other materials. In addition to mechanical strength and toughness, silk fibroin has remarkable optical properties. Silk films are transparent to visible light of all wavelengths and have extremely low (less than 5 nm rms) surface roughness (Applegate *et al.,* 2014) [3]. Silk films are probably the simplest and most versatile materials from which regenerated silk can be transformed. The process is simple, silk fibroin solution is cast onto a surface and allowed to dry. As the water evaporates, the silk proteins self-assemble into a transparent sheet that can be lifted free of the surface. The volume and concentration of solution controls the thickness, which can range from a few nanometers to several hundred microns. When the liquid silk solution is cast onto a patterned substrate, the liquid will fill any crevices on that surface, so that the resulting film conforms to the shape of the mold, leaving a perfect inverse replica with high fidelity and resolution. Suitably, designed masters can allow formation of a wide variety of optical devices. On the downside, the simple cast and dry process is relatively slow in ambient conditions, it usually takes 12 to 36 hours for the solution to fully dry. Films can also shrink while drying and can become deformed when the film is removed from the master. This faster technique makes it possible to rapidly produce large numbers of devices (Mehrotra *et al.,* 2019) [34].

Applications of silk based nanoparticles

Micro/Nano-fluidics: The expedition of silk from its territory of textile industry to the realm of micro fluidics merits special mention. It is pertinent to note the fact that silk spinning gears of both spiders and silk worms operate on the extraordinary principles of micro fluidics. It is also relevant to mention that micro/ nano fluidic channels within silk scaffolds ensure optimal nutrient and oxygen supply apart from serving as precursors for vascularization, an issue of pertinence in the domain of tissue engineering and regenerative medicine.

Bioresists: Progress in energy-efficient logic and memory applications has been catalyzed by innovative electronic circuit designs. Hota *et al.* (2012) [18] reported bipolar memristive switching using *Bombyx mori* silk cocoon fibroin film.

Investigation of the local conduction behavior at nanoscale (using scanning tunneling microscopy) evinced a filamentary switching mechanism. Researchers are optimistic about plausible application of the bioengineered silk based Bioresists (with multiple arbitrary customizations of material composition) for micro/nanoscale bioelectric stimulation, "live" (real-time) and "in situ" cell micro/nanomanipulation.

Sensing Applications: Apart from the tissue engineering applications, Silk fibroin nano fibers have been recently utilized as a platform for biosensor applications. Silk fibroin nano fibers have also been utilized as chemo sensors by a simplistic approach where organic dyes were added in the silk solution prior to electro spinning. The fluorescent silk nanofibrous membranes developed by Min *et al.* (2018) were ecofriendly and disposable. They could be utilized for the detection of acidic vapors including hazardous and volatile hydrochloric acid vapors.

Biocatalyst Applications: Separation and adsorption of enzymes using biocatalysts is a great challenge. Recently, silk fibroin nano fibrous matrices have been utilized in such applications owing to the high absorption capacity and their high surface area to volume ratio. Yi *et al.* (2018) demonstrated a cost-effective strategy by fabricating SF nano fibrous membranes functionalized with sulfated group surface.

Environmental Applications: Silk based nano materials have also fetched applications in the environmental domain. The researchers have proposed the prospective application of the same as a plausible answer to heavy metal pollution of water, a global environmental menace.

Filtration Applications: Min *et al.* (2018) developed ecofriendly and multifunctional air purification filters using SF-PEO nano fibrous membranes that were highly effective for reducing air pollution. The translucent silk nano fibrous air filters also provided view ability giving an idea of presence of contaminants in the air. The filtration efficiencies of the silk based air filters also outperformed the commercially used semi high-efficiency particulate air (semi-HEPA) filters for particulate matters (PMs) of sizes under 2.5 and 10μm. Silk being a biodegradable polymer with long shelf life further provided benefits as the developed air filter could be naturally degraded after use. Thus, the silk based air filter system could be an alternative to currently used plastic based filters which are non-disposable and non-degradable.

Bio-nanotechnology for silk based materials and devices

Bio-nanotechnology is an emerging field that sits at the convergence of biology, material science and nanotechnology. This field holds exciting opportunities to foster high-impact advances in bioengineering and medicine. Recent progresses in biochemical engineering, such as gene-splicing, protein engineering, site-specific chemistry, self-assembly and high-throughput screening, enable the optimization, integration and tuning of the function sand properties of biological materials. Advances in nanotechnology, like high-resolution cryo-electron microscopy, top-down and bottom-up nanofabrication, lab-on-a-chip and microfluidic technology, offer the power to take advantage of the structures, functions and processes of biological systems to supply novel functional nano-structured biological materials and systems.

Applications of silk-based materials in bio-nanotechnology

Tissue engineering: In tissue engineering, biomaterial scaffolds play a pivotal role by defining the three dimensional extracellular matrix templates for tissue regeneration. Besides excellent biocompatibility and biodegradability, matching mechanical properties to native tissue extracellular matrix may be a primary consideration during the choice of biomaterials. Silk hydrogels are attractive biomaterials for tissue engineering applications, as their stiffness are often fine-tuned and that they can incorporate growth factors and other cell signaling factors to optimize cell functions. The micro-structured and elastic silk hydrogels supported somatic cell growth and proliferation for greater than 3 weeks. These hydrogels have also been used for somatic cell encapsulation and for in vivo implants. Aligned silk nanofibers fabricated by electro-spinning provide topographical templates leading to elongated and oriented cellular morphologies, which can provide interesting avenues to use silk fiber scaffolds for the de novo engineering of structurally aligned tissues (Altman *et al.*, 2003) [2].

Nanostructured optics: Silk-based biomaterials are utilized in optical materials thanks to favourable properties, such as mechanical robustness, transparency, surface flatness and degradability. With the development of both soft-lithography and nano-imprinting processes, geometries and topologies are often replicated right down to tens of nano-meters for bio-photonic applications using silk fibroin solutions and films. Silk protein has been used as a biopolymer substrate for flexible photonic devices thanks to its attractive mechanical and optical properties. ZnO nano-rod array hybrid photo-detectors on Au nanoparticle-embedded silk protein were fabricated for flexible optoelectronics. These novel ZnO nano-rod array photo-detectors on a natural silk protein provide a platform to understand flexible and self-powered bio-photonic devices for medical applications (Ketan *et al.*, 2010).

Electronics: Silk-based materials, thanks to their flexibility and biocompatibility, are suitable candidates for advanced flexible electronic devices.

The fabrication of flexible silk-based electronics systems incorporating electrophysiological, temperature, and strain sensors was demonstrated so as to live the electrical activity produced by the heart, brain, and skeletal muscles. Advances in silk-based electronic devices would open new avenues for employing biomaterials within the design and integration of high-performance, bio-integrated electronics for future applications in consumer electronics, computing technologies, and biomedical diagnosis, also as human-machine interfaces (Huang *et al.*, 2018) [19].

Sensors: Harnessing silk proteins to get stimuli-responsive biomaterials supported protein folding-unfolding for biomedical applications offers tremendous opportunities for fine tuning control, responses, and utility, like for biosensors and controlled drug delivery devices. The insight gained also will allow enhanced understanding of structure-function relationships with protein designs, thus guiding genetic designs of dynamic hydrogels that respond predictably to virtually any desired environmental input to trigger changes within the material (Tao *et al.*, 2014) [3, 50].

Filtration: Membranes for water treatment have developed to deal with global challenges of water pollution and to get rid of molecular level contaminants. Liquid-exfoliated silk nano-fibrils (SNFs) were utilized to get filtration membranes with high water flux and efficient separation for dyes, proteins, and nanoparticles. The SNF membranes are sustainable, and fewer expensive compared to existing commercial membranes. Multilayer architectures in water purification membranes enable increased water throughput, high filter efficiency, and high molecule loading capacity (Ling *et al.*, 2017).

Conclusion

Silk is a fibrous protein produced by spiders and insects. Silk filament is a double strand of fibroin held together by a protein called silk sericin. The development of pharmaceutical and medical applications by means of the domesticated silkworm, *Bombyx mori* L was recognized in 2000 by using innovative transgenic technology. Transgenic technology had revolutionized the production of biopharmaceuticals products with efficiencies far superior than any conventional microbial or cell culture production systems. Silk fibres are biocompatible, show no cytotoxicity, evoke nearly no immune response if implanted into the body, and degrade into non-toxic by-products. Advances in biotechnological production of silk enabled large scale production of silk proteins for the development of new materials for various applications including biomedical applications as drug carriers, sensors or scaffolds for tissue engineering. A large amount of silk sericin goes into waste from silk industry and increases pollution load in industrial effluent. But it may be recovered and reused and sericin may be isolated from silk in various ways. All sericin applications proves that sericin, a by-product of silk industry can play a key role as an effective biomaterial of future. Silk fibroin has been increasingly considered for biomedical applications due to its biocompatibility, low immunogenicity, slow degradation and mechanical properties.

Silk is the versatile biomaterial and possesses remarkable properties at micron scale too. Micrococoon is the silk based nanoparticles devised by microfluidic strategy and is the potential solution to sensitive molecules having health and nutritional properties. The fibroin nanoparticles possess the biotechnological applications and have been investigated for the delivery of small molecules, protein drugs, genes and vaccines. Micrococoons are the potential candidates for drug delivery and have been recently investigated for drugs such as alpha-synuclein in Parkinson's and ibuprofen in ocular diseases. Need of further studies should be required to explore the bioactive properties and frontiers in life science to improve the need of silk products for socio-economic welfare.

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