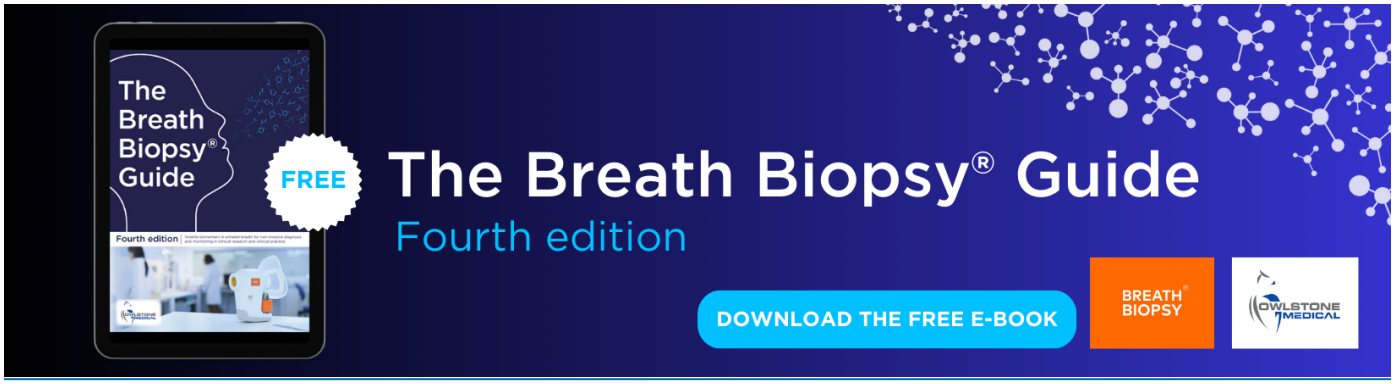
Nonmulberry silk proteins: multipurpose ingredient in bio-functional assembly

To cite this article: Deboki Naskar *et al* 2021 *Biomed. Mater.* **16** 062002



IOP Publishing Biomed. Mater. 16 (2021) 062002 https://doi.org/10.1088/1748-605X/ac20a0

Biomedical Materials

TOPICAL REVIEW

Nonmulberry silk proteins: multipurpose ingredient in bio-functional assembly

Deboki Naskar[1,4,6], **Sunaina Sapru**[1,5,6], **Ananta K Ghosh**[1], **Rui L Reis**[3], **Tuli Dey**[2,*] and **Subhas C Kundu**[1,3,*]

[1] Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal 721302, India
[2] Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, Pune, Maharashtra 411007, India
[3] 3Bs Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark—4805-017 Barco, Guimaraes, Portugal
[4] Present address: Cambridge Institute for Medical Research, School of Clinical Medicine, University of Cambridge, Hills Road, Cambridge CB2 0XY, United Kingdom
[5] Present address: Robert H. Smith Faculty of Agriculture, Food and Environment, The Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot, IL, Israel
[6] Contributed equally.
[*] Authors to whom any correspondence should be addressed.

E-mail: tulidey@unipune.ac.in **and** Kundu@i3Bs.uminho.pt

RECEIVED
23 February 2021

REVISED
22 July 2021

ACCEPTED FOR PUBLICATION
24 August 2021

PUBLISHED
22 September 2021

Keywords: Indian silks, nonmulberry, fibroins, sericins, biomedical materials, tissue engineering, regenerative medicine

Supplementary material for this article is available online

Abstract

The emerging field of tissue engineering and regenerative medicines utilising artificial polymers is facing many problems. Despite having mechanical stability, non-toxicity and biodegradability, most of them lack cytocompatibility and biocompatibility. Natural polymers (such as collagen, hyaluronic acid, fibrin, fibroin, and others), including blends, are introduced to the field to solve some of the relevant issues. Another natural biopolymer: silkworm silk gained special attention primarily due to its specific biophysical, biochemical, and material properties, worldwide availability, and cost-effectiveness. Silk proteins, namely fibroin and sericin extracted from domesticated mulberry silkworm *Bombyx mori*, are studied extensively in the last few decades for tissue engineering. Wild nonmulberry silkworm species, originated from India and other parts of the world, also produce silk proteins with variations in their nature and properties. Among the nonmulberry silkworm species, *Antheraea mylitta* (Indian Tropical Tasar), *A. assamensis/A. assama* (Indian Muga), and *Samia ricini/Philosamia ricini* (Indian Eri), along with *A. pernyi* (Chinese temperate Oak Tasar/Tussah) and *A. yamamai* (Japanese Oak Tasar) exhibit inherent tripeptide motifs of arginyl glycyl aspartic acid in their fibroin amino acid sequences, which support their candidacy as the potential biomaterials. Similarly, sericin isolated from such wild species delivers unique properties and is used as anti-apoptotic and growth-inducing factors in regenerative medicines. Other characteristics such as biodegradability, biocompatibility, and non-inflammatory nature make it suitable for tissue engineering and regenerative medicine based applications. A diverse range of matrices, including but not limited to nano-micro scale structures, nanofibres, thin films, hydrogels, and porous scaffolds, are prepared from the silk proteins (fibroins and sericins) for biomedical and tissue engineering research. This review aims to represent the progress made in medical and non-medical applications in the last couple of years and depict the present status of the investigations on Indian nonmulberry silk-based matrices as a particular reference due to its remarkable potentiality of regeneration of different types of tissues. It also discusses the future perspective in tissue engineering and regenerative medicines in the context of developing cutting-edge techniques such as 3D printing/bioprinting, microfluidics, organ-on-a-chip, and other electronics, optical and thermal property-based applications.

Abbreviations

Am	*Antheraea mylitta*
Aa	*Antheraea assamensis/assama*
Sr	*Samia cynthia ricini/Philosamia ricini*
Ap	*Antheraea pernyi*
Ay	*Antheraea yamamai*
3D	Three dimension
RGD	Arginylglycylaspartic acid
ECM	Extracellular matrix
PLGA	Poly(lactic-co-glycolic acid)
PLA	Polylactic acid
PEG	Polyethylene glycol
DTA	Differential thermal analysis
TGA	Thermogravimetric analysis
PCL	Polycaprolactone
HUVEC	Human umbilical vein endothelial cells
α-SMA	Alpha smooth muscle actin
SM-MHC	Smooth muscle myosin heavy chain
ABCG2	ATP binding cassette subfamily G member 2
TGF-β	Transforming growth factor beta
BMP-2	Bone morphogenetic protein 2
ALP	Alkaline phosphatase
VDR	Vitamin D receptor
RunX2	Runt-related transcription factor 2
Col1A2	Collagen, type I, alpha 2
OPN	Osteopontin
GLI-1/2	Glioma-associated oncogene homolog 1 and 2
Shh	Sonic hedgehog
BSA	Bovine serum albumin
FITC	Fluorescein isothiocyanate
DPPH	Hydroxyl, superoxide, 1, 1-diphenyl-2- picrylhydrazyl
COX-2	Cyclooxygenase-2 protein
4-HNE	4-hydroxynonenal
PCNA	Proliferating cell nuclear antigen
FBS	Foetal bovine serum
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
PVA	Poly(vinyl alcohol)
TNF-α	Tumour necrosis factor alpha
HepR21	Liver hepatocellular carcinoma cell line
4-MU	4-Methylumbelliferone
pAKT	Phosphorylated protein kinase B
PKC	Protein kinase C
MDA-MB-231	Adenocarcinoma breast epithelial cell
GAG	Glycosaminoglycan
DSC	Differential scanning calorimetry
TMDSC	Temperature-modulated DSC
FTIR	Fourier-transform infrared spectroscopy
PEDOT	Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate
CLUSTAL	Multiple sequence alignment program
CD	Circular dichroism
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
AFM	Atomic force microscopy
µ-CT	Micro computed tomography

1. Introduction

Scope and utilisation of tissue engineering and regenerative medicine are exponentially increasing over the last few decades (Shafiee and Atala 2017, Gaharwar *et al* 2020). The potential of replacing or rejuvenating the damaged tissue or organ system is realised long back (Langer and Vacanti 1993, Mao and Mooney 2015). The primary purposes of such research are to restore or improve the diseased/damaged tissue functions through many diverse techniques, including direct implantation of cells or cell-seeded constructs, controlled release of drug/growth factors, and creating an artificial niche for better understanding of cellular/tissue function (Griffith 2002, Stoddart 2017, Koons *et al* 2020).

Active research in the tissue engineering field includes the formulation, fabrication, and evaluation of potential scaffold (s)/construct (s), which can support cell proliferation, tissue regeneration and meet other critical criteria such as transport of nutrients or secretory products. In the last two decades, several potential and clinically relevant scaffold structures are being fabricated. They are utilised to investigate cell-biomaterial interactions along with the *in vitro* cell proliferation and differentiation and *in vivo* validation. A few characteristics such as controllable degradation, optimum porosity, swelling, rigidity, compliance, and cytocompatibility are needed to develop for tailoring an appropriate scaffold. Different synthetic (both nondegradable and biodegradable) and natural polymers are used widely to fabricate constructs of different dimensions and compositions (Jagur-Grodzinski 2006). They are the most diverse class of biomaterials, whose surface property can be physically, chemically or biochemically modified. They are formattable in any size and in complex shapes according to the application (Dhandayuthapani *et al* 2011). Due to the high mechanical strength, semi-crystalline nondegradable polymers such as polyethylene, polypropylene, polytetrafluoroethylene and polyethylene terephthalate are used as orthopaedic implants, suture, dressings, woven fabrics, vascular grafts, heart valve sewing rings and membranes for hemodialyzers. Among amorphous nondegradable soft polymers, polyvinyl chloride, rubbers, silicones, polyurethanes, polyacrylates and poly sulfonates/carbonates are used for tubing, blood storage bags, soft tissue implants, drug delivery system, contact lens, dental fillings, instrument coating, membranes for extracorporeal devices and their spare parts. Biodegradable synthetic polymers include, poly(a-hydroxy acids), poly(e-caprolactone), poly(orthoesters), poly(a-amino acids), poly(alkyl 2-cyanoacrylates), poly(anhydrides), polyphosphazanes and polyhydroxyalkanoates (Piskin 1995). Diverse structures such as thin-films (Zelikin 2010),

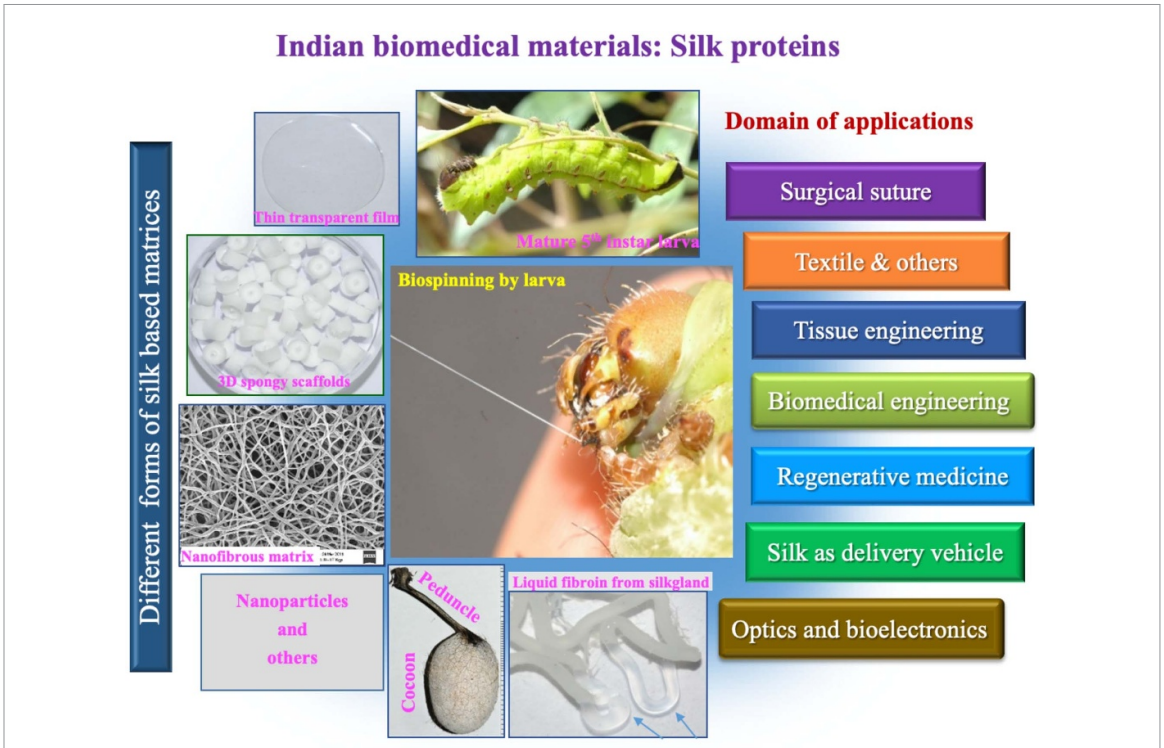


Figure 1. This schematic presentation, along with original photographs, indicates how we can use silkworm silk proteins (sericins and fibroins) for different scaffold formats like 2D films, 3D matrices, 3D electrospun nanofibre mats, nanoparticles, and other matrices for different types of applications, as biomedical materials for different kinds of 2D and 3D cell cultures for tissue engineering and regenerative medicine due to their essential and crucial properties as biomaterials. Further, the graphic vividly speculates the future trends and progress of silk-based engineered natural, biomimetic and advanced biomaterials as implantable biopolymers, delivery platforms, electronic devices, optics, biosensors, nanotechnological platforms, *in vitro* disease modelling, stem and cancer cells research. For details, please refer to this article and other reviews mentioned in this paper.

particles (Wilczewska *et al* 2012, Bulutoglu *et al* 2020), layer by layer assembly, gel formation, printing and 3D scaffolds (Mouriño *et al* 2013, Calori *et al* 2020) are successfully used to deliver therapeutic molecules. However, synthetic polymers face the question of delayed biodegradability (Dhandayuthapani *et al* 2011), short term cytocompatibility (Ter Horst *et al* 2018), extractables leaching, which may incite unwanted immunological/foreign body responses in the host. Such problems can be avoided by considering natural polymer, or ECM based constructs (Lu *et al* 2011). Natural polysaccharides based biomaterials include starch and cellulose (from plant), agar and alginates (from algae), chitin, chitosan, hyaluronic acid, glycosaminoglycans and cellulose (from animals), dextran, polygalactosamine, xanthan and cellulose (from bacteria) (Torres *et al* 2019).

Regarding natural protein based biomaterials, type I collagen (from porcine and murine tissue), and silk proteins (from silkworms (figure 1) and insects) are the most popular natural biomaterials. Other than these, fibronectin, tropoelastin, laminin, entactin, and gelatine are also used as biomaterials either alone or in combination with each other. These proteins lack the material property for biomaterials, so they need other structural/filler proteins to fabricate more structurally stable biocomposite materials. Still, they lack the *in vivo* like tissue complexity and spatial presentation of growth factors found *in vivo* (Aamodt and Grainger 2016). Williams (2019) discusses the merits and demerits of some natural biopolymers in detail, highlighting the characteristics required in a potential biomaterial, including the availability, cost-effectiveness, scalability, mechanical strength, degradation, minimal inflammation, and others, along with the compatibility for bioprinting. A more accessible way to have a native-like structure is to isolate the ECM and decellularise it to have a cell-free native construct that can act as a support to modulate cell functions. However, optimal decellularisation and digestion protocol need to be followed. Otherwise, different forms and concentrations of ECM will not serve the purpose for desired application (Catoira *et al* 2019). Recent studies showed ECM-derived materials can also trigger innate host immune responses following implantation (Xing *et al* 2020).

Among the options for naturally occurring polymers exhibiting such desired qualities, the silk proteins originated from silkworms are favoured for many reasons such as cost-effectiveness, ease of availability, biodegradability, and minimal inflammation (Naskar *et al* 2014,

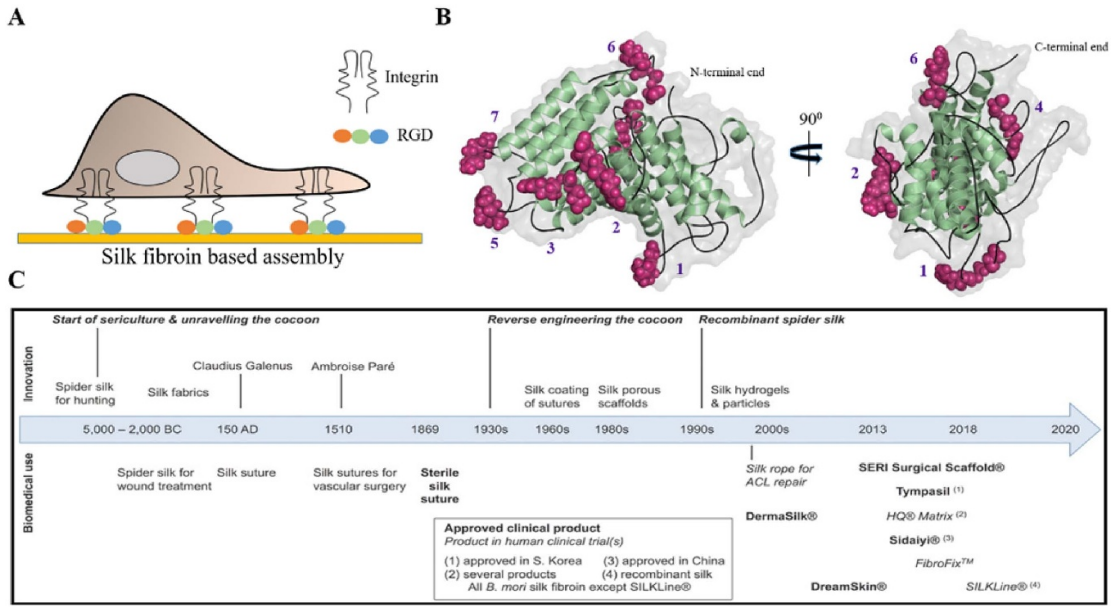


Figure 2. RGD motif and its abundance in *A. mylitta* silk fibroin 3D predicted model. Historical timeline of the application of silk in the field of biomedicine. (A) Schematic diagram depicting the interaction between RGD (present in or grafted on silk protein based extracellular matrices) and integrin molecules of the mammalian cells. (B) The 3D structure model of *A. mylitta* fibroin is predicted from the amino acid sequence (NCBI Accn: AAN28165, partial CDS) of N-terminal domain (1–507aa) using TrRefineRosetta modelling method in Robetta server (https://robetta.bakerlab.org/). The best model is chosen among the five predicted models based on the error estimate, compactness, and B-factor of the residues. The molecular graphics of the cartoon diagram is prepared using the program Pymol (www.pymol.com). The transparency of the surface representation is kept at 80% for clear visualisation of the cartoon diagram. The RGD motifs on the loops are labelled in magenta and are numbered sequentially from the N-terminal domain in both 0 and 90 degree orientations. (C) Milestones in the evolution of silk from first sericulture, to the thread for textile, for suturing patients, recent biomaterials and clinically approved products in the field of tissue engineering and clinical biomedicine. Holland *et al* (2019) John Wiley & Sons.

Kundu *et al* 2012, Cao and Wang 2009). Silk fibroin has travelled a long path of evolution as a biomaterial, starting from the thread for textile and suturing patients to recent use as scaffolding/biomaterial in the tissue engineering field (figure 2(C)). Medicinal use of silk fibre can be traced long back as early as the second century CE (Muffly *et al* 2011). In recent times, tremendous development in the fabrication and utilisation of *Bombyx mori* (Bm) silk-based assemblies can be observed in tissue engineering and regenerative medicines. Fibroin-based biomaterials have shown as suitable supporting scaffold for tissue replacement, repair, and regeneration and for studying different *in vitro* and *in vivo* pathophysiological, toxicological, and immunological aspects. The readers are encouraged to explore some seminal reviews in these fields (Vepari and Kaplan 2007, Rockwood *et al* 2011, Holland *et al* 2019) to get a glimpse of the same.

Silk obtained from the domesticated silkworm, *Bombyx mori*, is colloquially called 'mulberry silk' as the worms are primarily fed with mulberry leaves. Similarly, wild silkworms (*Antheraea* species) and semi-domesticated silkworms (Eri, *Samia* species) are polyphagous, i.e. they feed on different leaves and are called 'nonmulberry/wild silk' in most of the literature. The interest in wild or nonmulberry silk is increased in the last few years due to its untapped potential as biomaterials for tissue engineering and regenerative medicine (Mandal and Kundu 2008c, Kar *et al* 2013, Pal *et al* 2013). This report is intended to illustrate the participation of Indian origin nonmulberry wild *Antheraea mylitta* (Am) silk fibroin and sericin (figure 1), along with other nonmulberry species, in the current biomedical and tissue engineering fields as a particular highlight. These specific recent references (across the span of the last 20 years, focusing particularly 2000–2020) will move through the ideas of how we should think and proceed in the immediate future for utilising this and other wild species as nature-made biofunctionalised biomaterials. We further discuss the established protocols, fabrication technologies, and applications in these fields, along with the encouraging results, pitfalls, and futuristic goals.

1.1. Silk proteins as biomaterials

1.1.1. Sources of silk proteins

Naturally occurring raw silk proteins, in the form of very fine and lustrous thread, is the primary structural component of small protective scrotiform (cocoon) woven by the silkworm (figure 1 and 9). This cocoon, especially the rigid, non-woven structures observed in *Antheraea* species evolved to optimally protect the pupa (plural—pupae) as they transform into a moth, from the extreme climatic and natural conditions as

well as from different external threats. Apart from fibroin, which provides structural integrity to the silk fibre in a pair of continuous threads, the other protein is the glue-like sericin, which conglutinates both the fibroin threads (Sehnal and Zurovec 2004). Wild cocoons of *A. mylitta* exhibit ten times more work-of-fracture indicating their robust mechanical strength (Zhang *et al* 2013).

1.1.2. Nonmulberry silk sources in India

India is the second largest producer of silk in the world and the only country producing all the five commercial varieties of silks, including both mulberry (*B. mori*) and nonmulberry wild (*Antheraea* species: Tropical Tasar, Figure 1, Oak Tasar and Muga) and semi-domesticated (Eri) silkworms (figure 9). Considering total raw silk production in India in 2016–17: Mulberry contributed 71.7%, Tasar 9.9%, Eri 17.8% and Muga 0.6% of the total 30 348 metric ton production (Neog 2020). For textile purposes, silk threads are primarily isolated from the cocoons, however, for tissue engineering and biomedical purposes, fibroin and sericin are isolated from both cocoon and larvae. Among all, *A. mylitta,* (Am), *A. assamensis/A. assama,* (Aa), *Samia ricini/Philosamia ricini,* (Sr), *A. pernyi,* (Ap), and *A. yamamai* (Ay) are explored for engineering biomaterials in the last few years. Silkworms feeding on nonmulberry trees such as Castor (*Ricinus communis*), Shal (*Shorea robusta*), Tapioca (*Manihot esculenta*), Arjun (*Terminalia arjuna*), Kesseru (*Heteropanax fragrans*) and others are found in central India and mainly in the north-east region of India in dispersed clusters and remain yet under-utilised for biomaterials (like *A. roylei. A. frithi,* and *A proylei).*

1.1.3. Characteristics of silk proteins

The components of silk fibre (fibroin and sericin) are identified and are evaluated as the potential biomaterials for regenerative tissue engineering. Silk fibroin-based constructs hold an important place due to their unique qualities such as biocompatibility, biodegradation, ease of fabrication, aqueous based processing flexibility, non-toxicity, lower antigenic/non-inflammatory response, tailorable degradation rate for regulation of bioactive molecule release, oxygen/water permeability, low cost compared to other ECM proteins such as collagen, fibrin, laminin, fibronectin, and elastin (Vepari and Kaplan 2007, Holland *et al* 2019, Nguyen *et al* 2019, DeBari *et al* 2021). Similarly, silk protein sericins of silkworms show several biochemical and biophysical properties facilitating the use of sericins based matrices in different purposes (Kundu *et al* 2008b, Kunz *et al* 2016, Das *et al* 2021). Both the proteins from various nonmulberry sources in different versatile, functional architecture are exploited in different types of tissue engineering applications, as discussed in the following sections.

1.1.4. Advantages of nonmulberry silk proteins-based biomaterials over mulberry ones

The worldwide availability of this mulberry silk protein makes it a cost-effective material for research and medical applications. However, mulberry fibroin lacks integrin-binding tripeptide RGD motifs (table 2 and SI available online at stacks.iop.org/BMM/16/062002/mmedia), which is identified as an integrin (of cell) binding sequence present in most of the extracellular proteins influencing cell-matrix interaction (figure 2(A)) (Minoura *et al* 1995). A recent study (Baba *et al* 2019) has shown that silk fibroin from an RGD overexpressing Bm strain has a more profound effect on wound closure, granule formation and cell proliferation in cutaneous wound healing than the original Bm fibroin. This phenomenon leads to additional efforts to create a recombinant protein with RGD peptides or chemical coupling/biofunctionalisation of such motifs on materials (Sofia *et al* 2001, Wohlrab *et al* 2012, Vidal *et al* 2013, Saotome *et al* 2015). Fibroin isolated from nonmulberry origins, such as Indian *A. mylitta* (Datta *et al* 2001a), Indian *A. assama* (Gupta *et al* 2015), Chinese *A. pernyi* (Yukuhiro *et al* 1997), Japanese *A. yamamai* (Hwang *et al* 2001) are found to contain such RGD motifs inherently in their amino acid sequences (table 2 and SI). Considering Am, we have managed to predict the *ab initio* 3D structure of the fibroin as well as the presence and location of the RGD motifs in it using TrRefineRosetta modelling method in the Robetta server (Yang *et al* 2020; https://robetta.bakerlab.org/). Due to the unavailability of a model template for structural homology, we have used this *ab initio* approach based on the available amino acid sequence of the protein only. The partial amino acid sequence (ACCN: AAN28165, partial CDS of 507 amino acids) from the N-terminal domain of Am fibroin is used for this prediction (SI). The RGD motifs are predicted on the loop regions that are exposed on the protein surface (figure 2(B)). Such presence of these integrin-binding motifs on protein surfaces directly influence improved cell adhesion, subsequent proliferation, and differentiation on the nonmulberry fibroin-based matrices, where no additional biofunctionalisation is required (Mandal and Kundu 2008b, 2009a, Mandal *et al* 2010b, Acharya *et al* 2009a, Patra *et al* 2012, Silva *et al* 2019). The discovery of inherent RGD sequences on the nonmulberry silk fibroin ignites the interest in utilising these proteins for regenerative medicine.

Other advantageous nonmulberry fibroin features over mulberry include good yield, mechanical strength, biomineralisation, osteoconductivity, and osteogenic properties. Am has the largest yield of silk proteins compared to other varieties (Naskar *et al* 2014). Depending upon the amino acid composition of the fibroin, which varies from species to species, the mechanical property also differs. The poly-alanine sequence of nonmulberry is more hydrophobic than

poly-glycine-alanine sequence in mulberry fibroin, resulting in their higher binding energy and mechanical strength along with long-term structural stability in *in vitro*/*in vivo* enzymatic environment (You *et al* 2015). Although in terms of Young's modulus and breaking stress, nonmulberry is inferior, they sustain more breaking strain than mulberry (Fang *et al* 2016). Recent studies of Am fibroin-based scaffolds for bone tissue engineering reveal one of its crucial properties, i.e. osteogenic potential (Sahu *et al* 2015; Behera *et al* 2017, Midha *et al* 2017; Naskar *et al* 2017b). Nonmulberry fibroin has the potential to nucleate hydroxyapatite on its surface in the presence of simulated body fluid (SBF) and initiate mineralisation on it. This is the critical requirement for a biomaterial to be bioactive and can potentially support *in vivo* bone bonding. The apatite crystal formation on the matrix surface is thought to be due to the enhanced exposure of the $–COO^-$ group of Asp and Glu amino acids of the Am fibroin, which attracts free Ca^{2+}, HPO^{2-} and OH^- ions in the SBF. These advantageous properties of the Am fibroin over other silk fibroins make it a preferred candidate for load-bearing tissue regeneration, particularly bone tissue engineering.

1.2. Nonmulberry fibroin

1.2.1. Structural properties

The silkworm spins the cocoon surrounding itself using the thread of fibroin along with another silk protein, sericin. Fibroin is synthesised inside the silk glands of silkworms. While spinning, silkworms produce very thin twin fibroin strands from their spinneret, which are simultaneously glued together with sericin. The thickness of fibres varies depending upon mulberry and nonmulberry species, season and conditions of the growing environment. The fibre becomes more robust and harder once spun outside the body. At the molecular level, hydrophobic glycoprotein fibroin exists as a semi-crystalline state. The highly ordered crystalline anti-parallel β-sheet contributes to the tensile properties (strength and toughness), while less ordered β-sheet amorphous spacers contributes to the physical properties (flexibility, elasticity, moisture regain, dyability and chemical resistance) to the fibres (Naskar *et al* 2014, Nguyen *et al* 2019). Depending upon the crystallinity of the fibroin, silkworms are divided into three major subfamilies i.e. Bombycidae (*B. mori*), Saturniidae (*A. mylitta*) and Thaumetopoeinae (*Anaphe moloneyi*). The polypeptide side chain influences the dimensional difference among the orthorhombic unit cells of fibroin (Warwicker 1956). The fine structure of fibroin varies considerably from one species to another. The stability or degradability of the fibroins depends on the amino acid composition and accessibility. For example, Saturniidae's crystalline part is mainly composed of poly-alanine chains. The bonds are naturally more resistant to any chemical attack and simultaneously hydrophobic in comparison to *B. mori* and *A. pernyi*, whose crystalline part is composed of several repetition of poly-glycine-alanine-glycine-serine/tyrosine motif (Shaw and Smith 1961, You *et al* 2015). The amino acid composition of nonmulberry fibroin mostly contains glycine, alanine, serine and arginine (Kricheldorf *et al* 1983). The amorphous regions contain the bulky and polar side chain, which are more abundant in wild silk fibroin than mulberry fibroin and are responsible for maintaining silk properties under various external treatments (Mori and Tsukada 2000). The proteins are glycosylated by O-linked oligosaccharides via N-acetylgalactosamine. Am fibroin is a homodimer protein with molecular mass ~395 kDa, whereas Aa fibroin has two fragments: 220 and 20 kDa, Sr has 97 and 45 kDa fragments, Ap has 26, 45 and 67 kDa fragments and finally, Ay has 450 kDa fragment. These proteins do not contain any light chain or P25 polypeptide homolog of Bm fibroin.

1.2.2. Fibroin solubilisation and regeneration

Natural fibroin (whether wild or domesticated) can be extracted from two sources namely directly from the live silkworm gland or indirectly from cocoon fibres. Because of the structural stability of wild silk strains, it is difficult to isolate or purify fibroin from the cocoon. Fibroin from nonmulberry wild silkworm is generally isolated by dissolving the silk gland (figure 1) in anionic surfactant (lithium dodecyl sulphate or sodium dodecyl sulphate) and stabilising the protein (Datta *et al* 2001b, Mandal and Kundu 2008a) and subsequently reformatted into various scaffolding forms (Konwarh *et al* 2017). A few studies attempt to prepare hydrogels from the solution obtained by dissolving nonmulberry cocoons in an ionic solution called 1-butyl-3-methyl imidazolium acetate (Goujon *et al* 2013, Silva *et al* 2013, 2020, Srivastava *et al* 2019). The cocoons first need to be devoid of sericin, which can be achieved using a method called degumming (figure 9), which includes boiling in water or acid/alkali treatment (Kunz *et al* 2016). Another recent study reported a facile method to dissolve Am fibroin directly from the cocoon using 10% $CaCl_2$-formic acid-based solvent (Srivastava and Purwar 2018, Zhang *et al* 2019).

1.2.3. Biophysical properties

The regenerated silk fibroins can self-assemble in the aqueous solution into the beta-sheet rich network at room temperature. External mechanical forces such as ultrasonication can accelerate this process by altering the hydrophobic hydration and initiating beta-sheet formation (Liu *et al* 2013). Wild silk fibroins are substantially different from mulberry fibroin in thermal properties (such as stability, degradation, bound water sustaining ability, and molecular mobility during the glass transition). Fibroins are different in terms of other structural properties, such as ratios of bulky/non-bulky, hydrophilic/hydrophobic,

and glycine/alanine. They behave differently under different parameters, such as moisture regain and intrinsic viscosity (Sen and Babu 2004a). From DTA and TGA analyses of fibroin fibres, it is observed that nonmulberry silks are more stable in high temperature than the mulberry one (Babu and Sen 2007, Wang *et al* 2015). Moreover, Saturniidae fibroin with lower tensile strength provides superior compressive strength, toughness and elasticity required for many specific functions (Bandyopadhyay *et al* 2019). However, their structure versus mechanical property correlation is quite similar (Sen and Babu 2004b).

1.3. Nonmulberry sericin

The silk consists of another gel-like component known as sericin, and the separation of sericin from the fibroin fibre is known as degumming. Sericin yield depends on the extraction procedure, age, and species/races used with the wild species. Another noticeable and vital component is the peduncle (figure 1), a very hard or very soft incomplete structure, mainly observed in the wild silkworms, including *A. mylitta* (toughest one). This very hard peduncle of Am, a wood-like substance, facilitating the attachment of the cocoons with the branch of the tree forming a very tight and robust ring-like structure, is primarily composed of 200 kDa sericin (Dash *et al* 2006). This peduncle supports these wild cocoons not to be taken away by the predators, like birds while hanging in nature. In textile industries, harsh detergents and soaps are used for degumming purpose, which usually destroys sericin's structural and functional properties (Yun *et al* 2013).

The domesticated *B. mori* silk contains more than 20% sericin, whereas semi-domesticated or wild silkworms, including *A. mylitta* silk, usually has less than 10% sericin. The amount of sericin obtained mainly depends on the extraction methods and species of mulberry and nonmulberry silk cocoons. Moreover, wild cocoon sericin is less stable under high temperatures but supports better water exchange than mulberry sericin (Mazzi *et al* 2014). Sericin is a globular glycoprotein composed of 18 different amino acids (primarily hydrophilic), with serine being the predominant amino acid (Dash *et al* 2007, Kundu *et al* 2014a, Silva *et al* 2019). The other primary amino acids are those having polar side chains with amino, carboxyl, or hydroxyl groups like glutamic acid, aspartic acid, glycine, threonine, and tyrosine, which facilitate the crosslinking, blending, and copolymerisation with other polymers (Dash *et al* 2007, Aramwit *et al* 2009a). The complete molecular details of wild Am sericin are still unverified except that sericin isolated from the cocoon of Am exhibits three major protein bands in SDS-PAGE (116, 97, and 66 kDa) (Dash *et al* 2007). However, many studies observe the peptides of different molecular weights ranging from <50 to >250 kDa. The genome sequence of *A. yamamai* exhibits five sericin genes, namely AySer1 (8549 bp), AySer2 (9413 bp), AySer3 (6691 bp), AySer4 (8317 bp), and AySer5 (4510 bp) (Kim *et al* 2017).

The domesticated, semi-domesticated, and wild silk sericins exhibit β-sheets, random coils, and α-helix in some instances (Kumar and Mandal 2017). They reveal a difference in their secondary structures (Teramoto and Miyazawa 2005, Kunz *et al* 2016), which depends on the method of sericin extraction, moisture content, temperature, and the physical form of sericin used (solution, gel, and others). These chemical and structural features impart diverse beneficial traits to this silk protein. The crystallinity of sericin can be induced or enhanced by adding ethanol or other crosslinking agents (Teramoto and Miyazawa 2005, Oh *et al* 2011, Siritientong *et al* 2011, Siritienthong *et al* 2012, Nayak *et al* 2012).

The potential applications of nonmulberry fibroins and sericins in tissue engineering and regenerative medicine are discussed in the next section.

2. Silk based structures and assembly relationship

Whenever considering polymer-based biomaterials, the assembly or hierarchical organisation of the material at different molecular scale levels and its structure-function-property relationship must be comprehended. The first step of assembling a biopolymer or protein-small molecule is initiated within a cell membrane inside an organism, which can be engineered at the genetic level. In the next step, outside the cell membrane, the small molecules communicate to form a longer length scale and eventually form macroscopic material. Understanding each architectural layer provides an insight into designing a polymer with targeted structural and functional features. These assemblies translate and contribute to their thermal, electrical, mechanical, and biochemical properties (Johansson *et al* 2019).

As already mentioned, silk is a fibrous polymeric protein produced and used by insects and spiders for structural support purposes for their survival. Before exploring silk protein-based matrices, the structure-function relationship needs to be fully understood, or the knowledge gap can limit the use of biomaterials. Silk primary amino acids are arranged in block copolymers. There are several intermediate stages of the transition of fibroin from the random coil/alpha-helix in the gland to the regenerated assembly into beta-sheet crystallites in the scaffold/matrix. These structural conformations are confirmed using different biophysical techniques, such as FTIR, CD, x-ray diffraction, microscopic studies (SEM, TEM, and AFM) and mechanical testing. The stages are from random coil/alpha-helix to beta-sheet precursors (silk I and silk II) and finally to a stable beta-sheet by ethanol/methanol treatment. These stages are not controlled at the molecular level,

and as a result, environment-dependent polymorphism can be observed (Valluzzi *et al* 2002). On another note, although the secondary structure of fibroin sequences is similar across the nonmulberry strains, the minor differences in the basic amino acid motifs and domains give rise to the diversity of the silks and related properties (mainly mechanical properties) of the protein-based matrices. More details of the assembly function can be found in the mentioned literature in table 1.

3. Fibroin based assemblies and their applications in tissue engineering

Although the fabrication of different types of matrices or assemblies from mulberry fibroin are documented as early as 1994, nonmulberry protein-based assemblies are standardised in the last ∼15 years following mulberry protein's path (Ap—since 2006, Am—since 2008 and Sr/Aa—since 2009 (Source: NCBI)). The standardisations are performed through fibroin isolation followed by regeneration to engineer different functional scaffolding formats by tuning the surface and seeing its effect on the cell-matrix interface. A growing interest has been observed since the late 90s and early 2000 about how molecular recognition, self-assembly, and a well-defined architecture of material ultimately imply cellular metabolism. Thus, silk proteins are utilised to fabricate different dimension scaffolds (figure 1), such as (zero: bare or coated/tagged nanoparticles; 2D: coating, thin-film, electrospun mat on a surface; 3D: sponge, hydrogel). A list of all the major assemblies is presented in table 1. Mechanically more robust and more elastic than mulberry scaffolds and biocompatible thin films are fabricated by solution casting and air-drying method (Mandal and Kundu 2008c, Acharya *et al* 2009a, Kar *et al* 2013, Pal *et al* 2013, Mai-Ngam *et al* 2011). Nanofibrous fibroin-polymer (PCL) matrices (Kim *et al* 2003) are fabricated using the electrospinning technique (Schneider *et al* 2009). Three-dimensional scaffolds are prepared using nano-fibres (Chen *et al* 2020). Blended fibres or films of fibroin and other cytocompatible biopolymers (collagen, gelatin, chitosan and others) are prepared to maximize their uses and tune the degradability (Nguyen *et al* 2019). Three-dimensional porous scaffolds of pure fibroin protein can be prepared by freeze-drying (Lv and Feng 2006) or salt leaching method (Yao *et al* 2012). Tunable pore size and porosity of the scaffolds can alter cellular functionality (Mandal and Kundu 2008b). Similarly, customised fibroin based patterned matrices can be fabricated using micropatterning/micromolding (Mandal *et al* 2009b). Incorporation of other components such as carbon nano fibre can be used to enhance different characteristics including tensile strength, electrical conductance and biocompatibility (Naskar *et al* 2017a). Biospun Am fibroin matrices (figure 1 and 3) are shown to be more stable and biocompatible (Mandal and Kundu 2010a). Microparticles from the Am, Aa and Sr fibroins can be fabricated using wet milling and spray drying techniques (Bhardwaj *et al* 2015). All such investigations highlight the potential of fibroin protein-based structures for culturing diverse types of cells and their potential use in different types of tissue engineering.

We discuss in the following sections the reported research works, which used nonmulberry fibroin based assemblies in different avenues of tissue engineering and regenerative medicine.

3.1. Cardiovascular tissue engineering

Cardiovascular disease is one of the leading causes of human fatality worldwide. As cardiomyocytes cannot regenerate by natural homeostasis, the potential of silk fibroin-based films, sponges and patches are investigated to support the cells for proper heart function. Am fibroin matrices are preferred over Bm fibroin or gelatine in terms of sarcomere maturation and alignment, cell–cell communication and synchronous beating without affecting their response to the external stimuli. The tissue on the scaffold is found to be stable and beat synchronously for at least 20 d. The findings, together with the known *in vivo* properties of silk, particularly RGD containing Am fibroin matrices (figure 4) suggest that these 3D scaffolds may be suitable for the establishment of therapies for cardiac disease requiring mechanical support. (Patra *et al* 2012). With a similar aim, Aa fibroin-based biomaterial ink is prepared to print anisotropic cardiac constructs. Using a gel embedding-based bioprinting method, vascularised myocardial tissue is fabricated. The constructs are found to be nonimmunogenic both *in vitro* and *in vivo*. Moreover, upon maturation in culture, vascularised myocardial tissue shows maturation dependent expression of proteins for both cardiomyocytes and HUVEC cells (Mehrotra *et al* 2020). Considering the vascular part, multi-layered small-diameter vascular grafts are fabricated using Bm, Am and Sr fibroins (Park *et al* 2015). Patterned silk films of Am and Sr fibroin mimic vascular conduit. Hemocompatible and stronger Am and Sr fibroin films show more significant proliferation of vascular cells. Additionally, patterns on the films favour the functional contractility of the smooth muscle cells with elevated markers (α-SMA and SM-MHC) (Gupta *et al* 2016b).

3.2. Skeletal muscle tissue engineering

Human adult skeletal muscle has limited ability to regenerate. The suitability of different fibroin-based scaffolds is studied for supporting the adult skeletal muscle growth as tissue ECM (figure 5). Although human primary skeletal muscle myoblast adheres, proliferate and deposit ECM on all the matrices. The analysis of gene expression shows a different variety of myotube formation on the matrices. It is found

Table 1. List of research articles showing a wide range of nonmulberry protein-based assemblies and their purpose and biomedical relevance.

Assembly	Purpose/biomedical relevance	Research works
Cocoon fibre surface	A biocompatible potential substratum for supporting *in vitro* cell adhesion and proliferation.	Darshan *et al* (2017)
Biospun fibre-based matrix	Human pre-osteoblast differentiation to osteocyte on silk braids; Biocompatibility of biospun matrix.	Midha *et al* (2017), Mandal and Kundu (2010a)
Membrane mimetic environment	Biocompatibility studies for a wide range of mammalian cells: mouse/human fibroblast, human bone marrow derived mesenchymal stem cells, cornea cells, muscle cells, vascular cells, and skin graft on a rat model.	Mandal and Kundu (2008c), Acharya *et al* (2009a), Kar *et al* (2013), Pal *et al* (2013), Hazra *et al* (2016), Park *et al* (2015), Gupta *et al* (2016b), Srivastava and Purwar (2017), Srivastava *et al* (2017), Sen *et al* (2020), Luan *et al* (2006)
Biomimetic micron cell fibres, nanofibrous membranes	*In vitro* biocompatibility studies using fibroblast cells; different surface modifications of the matrices and subsequent *in vitro* and *in vivo* studies for bone regeneration; as wound dressing for *in vitro* and *in vivo* wound healing; promotes osteogenic differentiation of mesenchymal stem cells; encapsulation efficiency and release kinetics.	Bhattacharjee *et al* (2015a), Bhattacharjee *et al* (2015b), Bhattacharjee *et al* (2016a), Bhattacharjee *et al* (2016b), Bhattacharjee *et al* (2016c), Bhattacharjee *et al* (2016e), Srivastava *et al* (2019), Srivastava and Purwar (2018), Chouhan *et al* (2019a), Chouhan *et al* (2019b), Panda *et al* (2015b), Li *et al* (2016)
Porous spongy scaffolds	Substratum that supports *in vitro/in vivo* osteogenesis, adipogenesis, *in vitro* cardiomyocyte and muscle myofibroblast differentiation; Support for *in vitro* tumor model.	Mandal and Kundu (2008b), Mandal and Kundu (2009a), Patra *et al* (2012), Chaturvedi *et al* (2017), Talukdar *et al* (2011b), Singh *et al* (2018), Saha *et al* (2013), Sahu *et al* (2015), Kundu *et al* (2013), Talukdar *et al* (2011a), Talukdar and Kundu (2012), Talukdar and Kundu (2013)
Hydrogel	Support for hepatocarcinoma model; substratum with chondrogenic potential.	Kundu and Kundu (2013), Singh *et al* (2016)
Substrate for biomolecule immobilisation	Metal (titanium) surface modification for faster osseointegration.	Naskar *et al* (2015), Sharma *et al* (2016)
Nanoparticle for biomolecule loading and delivery	Entrapment efficiency and release kinetics of the model drugs, enzyme, small molecule, curcumin, and growth factor; targeted drug delivery to the cancer cells and sustained release.	Sundar *et al* (2010), Zhang *et al* (2018), Panja *et al* (2017), Mottaghitalab *et al* (2015), Subia *et al* (2014), Subia *et al* (2015), Kundu *et al* (2010)
Blends and composite materials	Enhanced compressive modulus for cartilage, bone regeneration; antithrombogenic potential.	Singh *et al* (2017), Gupta *et al* (2016a), Naskar *et al* (2017a, 2017b), Behera *et al* (2017), Yang *et al* (2014), Bäcker *et al* (2017), Srivastava and Purwar (2017), Bhardwaj and Kundu (2012), Choudhury *et al* (2016), Moses *et al* (2018)
Microparticles	Potential drug delivery vehicle/microcarrier with enhanced encapsulation efficiency and release kinetics.	Bhardwaj *et al* (2015), Li *et al* (2013), Wang *et al* (2014)
Bioprinting	Cartilage and bone bioink supporting osteochondral differentiation and angiogenesis; anisotropic cardiac construct for vascularised myocardial tissue.	Mehrotra *et al* (2020), Moses *et al* (2020)

(Continued.)

Table 1. (Continued.)

Assembly	Purpose/biomedical relevance	Research works
Micropatterning/ micromolding	Guided cellular adhesion on the matrix for bio-device or implant fabrication.	Mandal *et al* (2009b)
Assemblies explained by Electronics and Physics	In regards of memory devices, optical wave guide devices, bio-photonic devices, bioelectronic devices, and sensors.	Hu *et al* (2020), Zhu *et al* (2016), Shivananda *et al* (2020), Hota *et al* (2012), Wang *et al* (2016), Perotto *et al* (2017), Gogurla *et al* (2016), Pradhan *et al* (2020), Guo *et al* (2020)

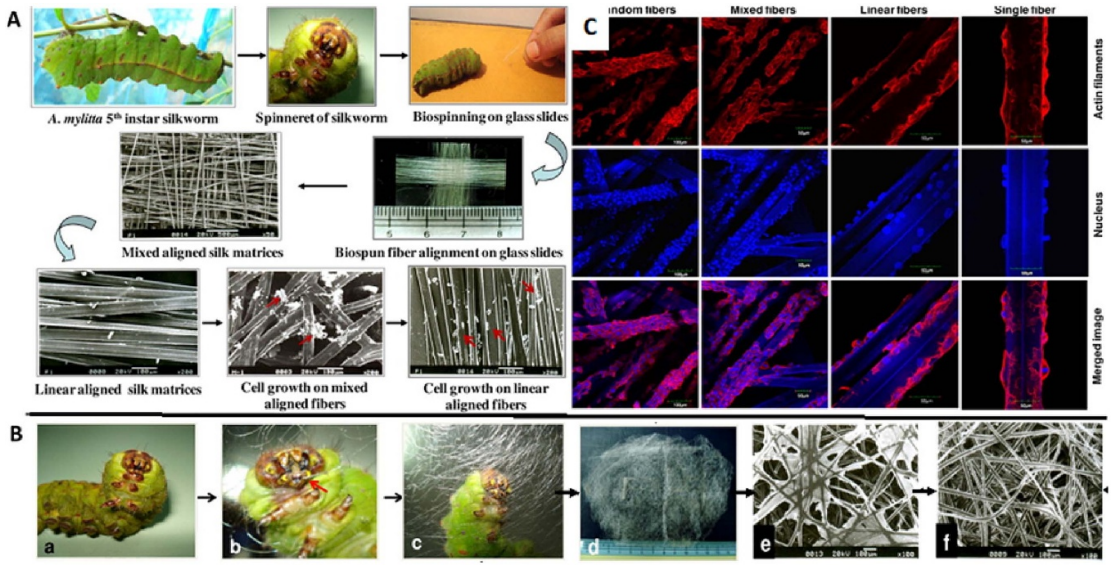


Figure 3. Biocompatibility of fibrous fibroin matrices fabricated using biospinning by *A. mylitta* silkworm. Step by step process of biospinning of silk fibre from mature *A. mylitta* 5th instar larva into (A) linear fibre, mixed fibre and (B) random fibre containing matrices along with respective scanning electron microscopic images of fibres with and without cells (AH927) grown on it. (C) Confocal microscopic images of 5 d cellular growth on linear, mixed, random fibres, and single fibre. The cells are stained with rhodamine-phalloidin (red) for actin and Hoechst 33 342 (blue) for nuclei. Reprinted from Mandal and Kundu (2010a), Copyright (2010), with permission from Elsevier.

that the length, orientation, alignment and maturation of the cells are directly related to the mechanical property of the matrices and not to the composition of the matrices (Chaturvedi *et al* 2017).

3.3. Skin tissue engineering

Another major silk fibroin-based application is focused on skin tissue engineering. Purwar group has published some work on this topic using non-mulberry cocoon fibroin. The source of regenerated fibroin for most of their work is ionic solution solubilised cocoon fibres. In one study, they have fabricated nonwoven composite wound dressing film by solution casting method followed by finishing with different concentrations of chitosan solution. Chitosan (2%) modified films show sufficient porosity, permeability, and suitable mechanical and thermal property, with potent antimicrobial activity. Also, chitosan finishing further improves cellular growth and favourable morphological features of fibroblast cells (Srivastava and Purwar 2017).

Similarly, upon incorporating 5%–15% dextrose as plasticiser on the Am and Aa films, the mechanical property of the films are enhanced significantly. Other surface biophysical properties are also improved, including sustained antibiotic release, and as a result, skin fibroblast attachment and proliferation are also improved (Srivastava *et al* 2017). In another study, the regenerated fibroin is used to fabricate nanofibrous matrices. To enhance the therapeutic efficacy, upon coating of silver nanoparticles (from dandelion: *Tridax procumbens* leaf extract), the matrices show good mechanical strength, water absorption with adequate porosity, excellent antimicrobial activity and biocompatibility for dermal fibroblast cells (Srivastava *et al* 2019). Following a similar experimental approach, they have also explored the self-assembled nanofibrous nonwoven mats for their biocompatibility for dermal fibroblast cells and wound dressing applications (Srivastava and Purwar 2018). Cocoons themselves can be used as biomaterial exhibited in this study (Darshan

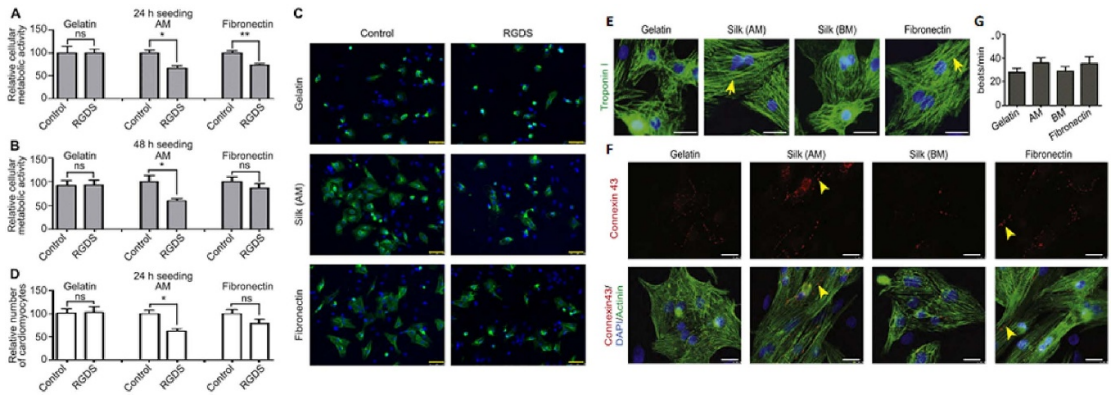


Figure 4. RGD dependency of cardiomyocyte attachment, cellular interaction and differentiation on *A. mylitta* fibroin matrix. The presence of RGD in Am fibroin assist cardiomyocyte attachment, cell–cell communication and contractility. Attachment of preincubated cardiomyocytes (for 30 min in control peptide RGES or RGDS) on gelatine, Am, and fibronectin surfaces for (A) 24 h and (B) 48 h are monitored in terms of metabolic activity, (C) number of cells attached using fluorescent labelling of green troponin I vs blue DAPI (Scale bars: 50 μm) and (D) its subsequent quantitation. Comparatively smaller number of cells are observed on Am and fibronectin surface due to free RGD blocking on cell surface. (E) Presence of well-established and aligned sarcomere (yellow arrow) on Am and fibronectin in comparison to gelatine and Bm. (F) Enhanced expression of cell–cell connection marker connexin 43 on Am in comparison to rest. (G) Quantitative beating frequency per minute shows more beating on Am and fibronectin than rest. Reprinted from Patra *et al* (2012), Copyright (2012), with permission from Elsevier.

Table 2. Availability and number of RGDs in different mulberry and non-mulberry species fibroin amino acid sequences (source: NCBI database and SI). The bold column indicates the number of RGD motifs in non-mulberry/wild Indian tropical Tasar silkworm, *Antheraea mylitta* silk fibroin after partial sequencing. This provides us an additional information that if full sequence of this fibroin is carried out, a greater number of RGD motifs are expected.

Species name	Accession no.	Amino acid length	Group (Reference)	Year	Journal	No. of RGD present
B. mori	CAA35180 (light chain)	262	Mizuno, S. (Yamaguchi *et al*, 1989)	1989	J. Mol. Biol. 210 (1), 127–139	0
B. mori	NP_001106733 (heavy chain)	5263	Xia, Q.Y. (Zhao *et al*, 2015)	2015	J. Biol. Chem. 290 (2), 972–986	0
A. mylitta	AAN28165 (partial CDS)	507	Kundu, S. C. (Datta *et al*, 2001a)	2001	Comp. Biochem. Physiol., B 129 (1), 197–204	**7**
A. assama	AIN40502	2809	Nagaraju, J. (Gupta *et al*, 2015)	2015	Sci Rep 5, 12706 (2015)	3
S. ricini	BAQ55621	2880	Sezutsu, H.	2015	Unpublished	1
A. pernyi	AAC32606	2639	Yukuhiro, K. (Hideki and Yukuhiro, 2000)	2000	J. Mol. Evol. 51 (4), 329–338	10
A. yamamai	BAJ11925	2856	Yukuhiro, K. (Sezutsu *et al*, 2010)	2010	Int. J. Wild Silkmoth & Silk	12

et al 2017) where cocoon mats, both pristine and degummed ones, from Am and Bm are used for culturing epidermal cells. Am mats without degumming or collagen coating promote the adhesion of keratinocytes in contrast to Bm mats. In another study, polyurethane—Am fibroin scaffolds are fabricated using blending and immobilisation techniques. Epidermal growth factor treated immobilised scaffolds show better healing prospects than blend ones. The former offers complete healing in either type of burn wound (hyperglycaemic burn vs typical burn) in the rat model (Sen *et al* 2020). In another study Aa fibroin is used to fabricate nanofibrous mat followed by coating with two recombinant spider silk (containing fibronectin and lactoferricin) to create multifunctional wound dressing. Combined layer of two spider silks shows better wound healing efficiency compared to the single separate coatings or noncoated counterparts both *in vitro* and *in vivo* (Chouhan *et al* 2019a). In *in vivo* rat model of 3rd-degree burn, fibronectin coated Aa scaffolds demonstrate accelerated wound healing in comparison to the uncoated Aa

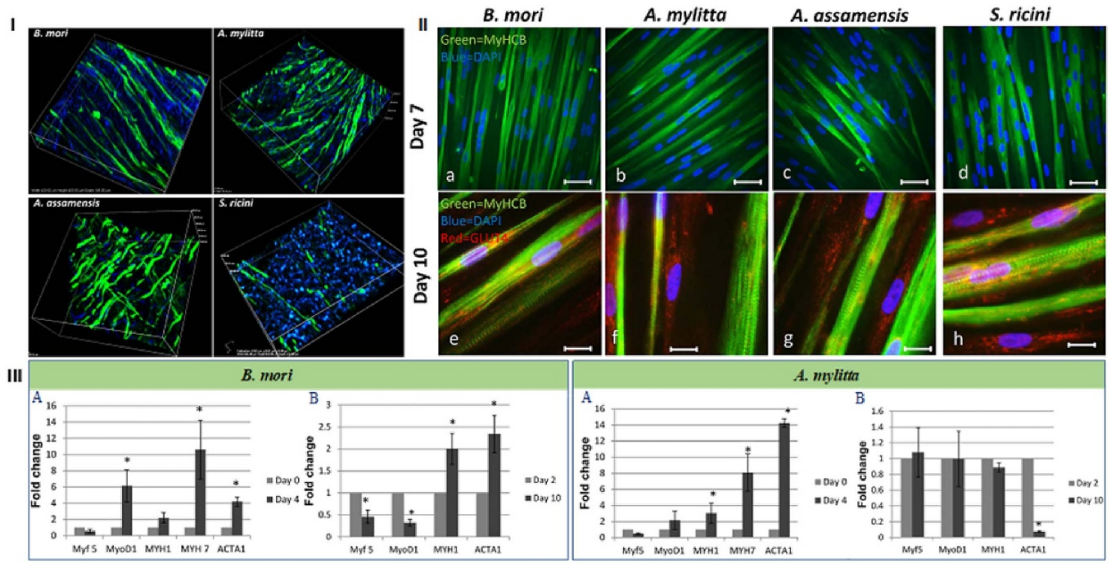


Figure 5. Differentiation of human muscle myofibroblasts on different fibroin 2D/3D matrices. Confocal microscopic images of (I) formation of muscle like tissue by human skeletal muscle myoblasts (HSMMs) after culturing for 4 d in proliferation medium followed by 4 d in differentiation medium on different 3D silk fibroin scaffolds. The cells are stained with anti-myosin monoclonal (mAb) antibody (NOQ7.5.4D)/goat anti-mouse IgG-AF-488. Fluorescence microscopic images of (II) Differentiation of HSMMs to form myotube on different fibroins coated etched glass surfaces. Cells are cultured for 4 d in growth media, 2 d in proliferation media and finally 7/10 d in differentiation media. (a)–(d) Expression of slow muscle myosin, which is stained with MyHCB (anti-myosin heavy chain mAb) and (e)–(h) differentiation to myotube, which is stained by MyHCB and anti-glucose transporter 4 (GLUT4)/goat anti-rabbit IgG-AF546. Scale bar: 50 μm (a)–(d) and 25 μm (e)–(h). All nuclei (I & II) are stained with DAPI. (III) Expression analysis of five different muscle markers (MYF5, MYOD1, MYH1, MYH7, and ACTA1) on Bm and Am 3D scaffolds using qRT-PCR. The expression levels are normalised to TBP as a reference gene, and also day 4 and day 10 expression levels are normalised to day 0 and day 2, respectively. For a level of significance analysis, Mann-Whitney U-test is performed. Data represented as mean± SE for 4 biological replicates. Chaturvedi *et al* (2017) John Wiley & Sons.

matrix and commercially used DuoDERM dressing patch during 14 d treatment course (Chouhan *et al* 2019b).

3.4. Cornea and liver tissue regeneration

Am transparent thin films are developed to replace the use of traditional amniotic membrane for the treatment of damaged corneal surfaces (figure 6). The thin films support sprouting, migration, attachment and growth of epithelial cells and keratocytes from rat corneal explants, and supported limbal stem cells as evidenced by enhanced expression of ABCG2. After implantation, the rabbit cornea remains transparent with normal tear formation and intraocular pressure without any inflammatory response or neovascularisation (Hazra *et al* 2016).

To prepare a matrix, which can support a liver tissue, Aa fibroin is used to fabricate cryogel. The cryogels can absorb a large amount of media and are mechanically stable. The matrices can also support the viability, proliferation and healthy metabolic activity of human hepatocarcinoma cells (Kundu and Kundu 2013).

3.5. Cartilage tissue engineering

Am fibroin-based sponge matrices being mechanically robust and porous, naturally becomes a better candidate for osteo- or chondrogenesis based applications. Evaluation of the osteogenic and adipogenic potential of the scaffolds exhibit that Bm scaffolds act as a better substratum for adipogenesis. In contrast, Am scaffolds favour better osteogenesis due to its mechanical strength (Mandal and Kundu 2009a). To establish a successful functional cartilaginous model construct, the biomechanical property of the Am sponge scaffold is correlated with cell (immature bovine chondrocytes) seeding density and matrix accumulation followed by assessment of the cartilaginous differentiation of the cells (Talukdar *et al* 2011b). Fibre reinforced fibroin composite matrices from Aa are investigated for their tissue engineering potential for cartilage regeneration. The matrix with increased compressive modulus and stiffness stimulate the cells to deposit enhanced sGAG, collagen type II and up regulated the expression of the cartilage-specific gene markers (collagen type II, aggrecan, and sox-9) (Singh *et al* 2017).

3.6. Osteochondral tissue engineering

To explore the osteochondral potential of silk fibroin, a hierarchically biphasic scaffold is fabricated using Aa and Bm fibroin along with fibre reinforced phase. With its promising compressive modulus, the scaffold supports the growth and proliferation of chondrocytes and osteoblasts, as evident from *in vitro* and *in vivo* studies (Singh *et al* 2018). Further, the feasibility of developing a multifunctional similar silk fibroin (Aa and Bm fibroin together) based cartilage and

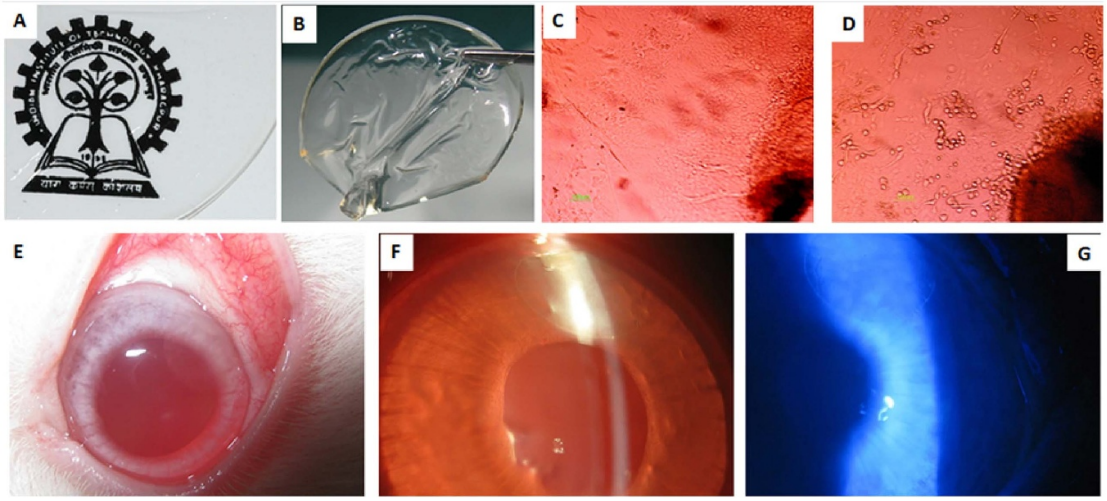


Figure 6. Transparent *A. mylitta* fibroin thin films for corneal regeneration. (A) As fabricated transparent thin film from Am regenerated fibroin solution and (B) same film after ethanol treatment to induce crystallinity and insolubility. The films are 94.4% transparent and thickness is $30 \pm 9.7\mu$m. Cellular outgrowth from corneal explants on (C) Am film and (D) amniotic membrane after 8 d of culture. (E) Gross examination of the cornea after 2 months of implantation of Am film, and (F) slit lamp biomicroscopy shows that films remain stable, transparent and well tolerated by the animal model. (G) Absence of fluorescein in the cornea implies no sign of ulceration and erosion in the eye. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Scientific Reports Hazra *et al* (2016) (c) 2016.

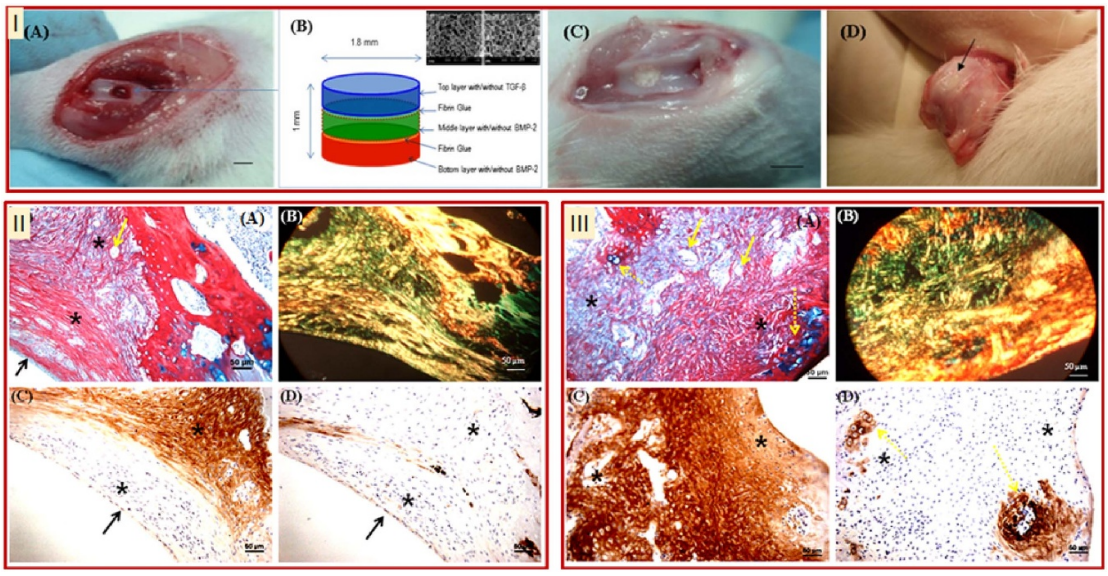


Figure 7. Layered *A. mylitt*a vs *B. mori* fibroin sponge scaffolds for *in vivo* osteochondral regeneration. (I) (A) Osteochondral defect (1.8 mm × 1 mm) is created in rat patella-femoral groove (Scale bar—2 mm). (B) The implant is prepared as a layered construct with a different layer being loaded with different bone-related growth factors (e.g. TGF-β and BMP-2) (inset: A scanning electron microscopic images of both Bm and Am fibroin sponges) and (C) implanted in the osteochondral defect. (D) The appearance of repair site 8 weeks post-implant, where normal cartilage formation can be seen on the implantation site (arrowhead). (II and III) Histological and immunohistochemical analysis of the growth factor loaded Bm and Am fibroin explants: (A) AB/SR staining (B) Birefringence of AB/SR section (C) Type I collagen and (D) Type II collagen immunostaining. Black stars: scaffold and yellow arrow: formation of the blood vessel within the explants. Scale bars −50μm. These analyses indicate, Am scaffolds support cells towards chondrogenic lineage, while Bm scaffolds support towards osteogenic lineage in similar conditions. Reproduced from Saha *et al* (2013). CC BY 4.0.

bone bioinks are studied by analysing their *in vitro* and *in vivo* capacity of osteochondral differentiation and angiogenesis (Moses *et al* 2020). With the similar aim, Aa fibroin-based sol derived 70S bioactive glass (a biphasic composite) is fabricated to study its coherent interface for osteochondral regeneration. Aa composite mats were found to perform better than their Bm counterpart mats, as evidenced by the enhanced expression of crucial cartilage and bone markers (Joseph *et al* 2017). The potential of Bm and Am silk fibroin scaffolds for *in vitro* and *in vivo* osteochondral regeneration is investigated (figure 7). It is observed that the cell free multi-layered Am/Bm fibroin scaffolds loaded with TGFb3 or recombinant

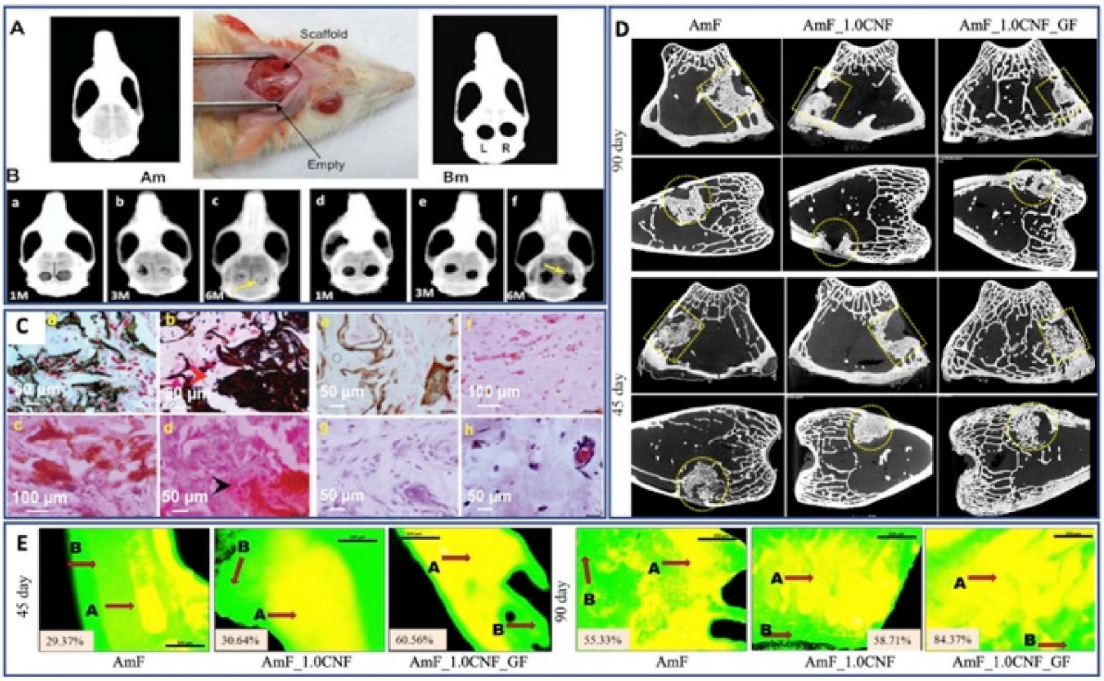


Figure 8. *In vivo osteoconductivity of A. mylitta fibroin porous scaffold in bone regeneration. In vivo* bone regeneration assessment of Am 3D porous, spongy scaffolds using rat calvarial and rabbit distal femoral defect models. (A) X-ray radiography of normal calvaria (left), 5 mm bilateral defects (right), and surgical site with implanted scaffold vs empty defect (center). (B) Comparative radiographical images after one (a), three (b), and six (c) months of surgery showing gradual progression of defect healing for Am and same time points (d)–(f) for Bm. After 6 months, the defect was covered entirely with neo-bone formation on Am scaffold, while no bone formation is observed on Bm scaffold. (C) Further histological examination of von Kossa (a) and (b) for Am and (e) and (f) for Bm for 1 and 3 month post-surgery each) and Alizarin Red S (c) and (d) for Am and (g) and (h) for Bm for 1 and 3 month post-surgery each) staining show enhanced matrix deposition on Am after 3 months as shown by arrowhead, while some deposition is observed after 1 month, which disappeared after 3 months on Bm. (D) Comparative micro-CT 2D projection images of the distal femoral defect sites at two day points (45 and 90 d) for Am (AmF), carbon nano fibre reinforced Am (Am_1.0CNF) and TGF-β and BMP-2 growth factors loaded AM/CNF (AmF_1.0CNF_GF) scaffolds show that osseointegration process starts from the scaffolds by using its porous microarchitecture as a mould. Growth factor loaded scaffold mainly promote complete osteogenesis in comparison to the other two groups in terms of periosteum formation by mimicking the trabecular network of the bone during 90 d study. Upper and lower panel: side and top tomographic slice views of the bone at the same defect point, yellow dotted border: area of an implanted scaffold. (E) Further fluorochrome (oxytetracycline) labelling images of the same study at 45 and 90 d post-surgery show increased area of new bone formation, which indicates that the GF loaded matrix induces higher mineral apposition than the rest two groups (AmF and AmF_1.0CNF). (A) bright yellow region is representing the new bone formed, and (B) green region representing the local bone. Scale bar: 500 μm. Inset: % area of new bone formation. Sahu *et al* (2015) John Wiley & Sons. Reprinted from Naskar *et al* (2017b), Copyright (2017), with permission from Elsevier.

human BMP-2 support *in vivo* neo-osteochondral tissue formation on them. A good biointegration is observed between native and neo tissue within the osteochondral defect in patellar grooves of Wistar rats (Saha *et al* 2013).

3.7. Bone tissue engineering

For their enhanced mechanical strength, different Am fibroin based pure and composite matrices are engineered and studied extensively for their bone regeneration potential. Am scaffold's potential for bone tissue engineering is investigated through its efficiency at repairing rat calvarial bone defect (figure 8(B) and 8(C)). After 3 months of implantation, a complete ossified regeneration with increased mineralisation is observed in case of implant site of Am scaffold while no bone formation is observed on Bm scaffold for the same period (Sahu *et al* 2015). With the aim of enhancing mechanical strength further, different strategy based composite matrices are engineered and studied extensively for their bone regeneration potential. For example, hydroxyapatite and Aa fibroin fibre reinforced Aa fibroin tricomposite matrix (Gupta *et al* 2016a), dual growth factor loaded carbon nano fibre reinforced Am fibroin composite (figure 8(D) and 8(E)) (Naskar *et al* 2017b), bioactive glass-reinforced Aa silk fibroin composite (Moses *et al* 2018), hydroxyapatite reinforced Am fibroin composite (Behera *et al* 2017) scaffolds are studied focusing to *in vitro* as well as *in vivo* osseointegration, osteoinduction and osseogenesis investigations. Different nanofibrous matrices fabricated using electrospinning process are also studied for a similar aim. For example, mesenchymal stem cells from human cord blood are better differentiated towards osteogenic lineage on Sr and Am fibroin blended fibrous scaffolds in comparison to Bm fibroin or gelatine scaffolds (Panda *et al* 2015b). With the similar aim, Am fibroin blended or grafted poly (ϵ-caprolactone) or polyvinyl alcohol-based nanofibrous matrices with or without hydroxyapatite and/or osteogenic growth factors are experimented in detail, focusing both

in vitro and *in vivo* (Bhattacharjee *et al* 2015a, 2015b, 2016a, 2016b, 2016c, 2016e). The osseointegration and osteogenesis potential of Am fibroin immobilised or antibiotic-loaded Am fibroin nanoparticle immobilised implant metal titanium are investigated for faster bone-implant integration (without implant loosening), immune response or nosocomial infections (Naskar *et al* 2014, Sharma *et al* 2016).

3.8. Differential molecular mechanism and disease modelling

In order to test the hypothesis, that Am native braids with mechanical stiffness in the range of trabecular bone can regulate osteocyte differentiation, human pre-osteoblast differentiation to osteocyte is inspected on the braids. Interestingly, Wnt signalling promoted osteocyte differentiation in terms of upregulated β-catenin expression in the presence of pro-osteogenic supplements and TGF-β (Midha *et al* 2017). Further insights into the direct regulatory role of the Am fibroin braids on the hedgehog and parathyroid signalling pathways in controlling osteogenic differentiation of human foetal osteoblasts show enhanced expression of osteogenic markers (ALP, VDR, RunX2), matrix proteins (Col1A2, OPN), and signalling molecules (GLI1, GLI2, Shh) along with terminal osteocytic phenotype (Midha *et al* 2018).

Being a very conducive environment for adhesion, growth and proliferation of diverse cell types, fibroin and sericin based matrices are also utilised to harbour disease related cells such as cancerous cells to mimic the tumour microenvironment. Am fibroin based 3D effective hepatocarcinoma niche model is established to study tumour microenvironment. This *in vitro* 3D model is examined for multicellular (HepR21) aggregation resulting in tumour formation followed by treatment with 4-MU (hyaluronan synthase inhibitor), which reduces HA level and downregulates the tumour growth promoting factors (pAKT and PKC) while upregulating the tumour suppressing p53 gene (Kundu *et al* 2013). Another breast cancer (MDA-MB-231 cells) model is constructed using Am fibroin scaffold to mimic *in vivo* tumour microenvironment. The cytotoxicity of three different drugs (Paclitaxel, Celecoxib, and ZD6474) are studied in this model and a combinatorial treatment strategy of these drugs at IC_{50} resulted up to 84% death of cancer cells (Talukdar and Kundu 2012, 2013). It was also observed that Am fibroin scaffold is advantageous over Bm fibroin scaffold in terms of cell viability and proliferation and glucose consumption and lactate production by the breast cancer/prostate cancer cells (Talukdar *et al* 2011a).

3.9. Drug and nanoparticle deliveries

Silk matrices of different dimensions embedded with the drug are fabricated to evaluate the release kinetics of embedded model drugs (BSA and FITC-inulin). This single model can be tuned for controlled and sustained release of a wide range of bioactive molecules and enzymes with different release kinetics (Sundar *et al* 2010). Nanoparticles from Bm and Am silk fibroin are utilised as drug delivery (Zhang *et al* 2018), anticancer agent curcumin delivery (Panja *et al* 2017), and growth factor delivery system (Mottaghitalab *et al* 2015) considering the ease of fabrication and biodegradation. The efficiency of folate conjugated Am fibroin nanoparticles loaded with anticancer drug doxorubicin is investigated for cell viability, proliferation and endocytosis. The nanoparticles are found to be nontoxic, targeted to cancer cells, easily endocytosed, and capable of sustained drug release (Subia *et al* 2014, 2015). Silk fibroin nanoparticles are formulated using Bm and Am fibroin and standardised for their size to be 150–170 nm, surface charge to be negative, with stable and spherical morphology along with its cellular uptake as confirmed by FITC accumulation inside cells and release of growth factors for over 3 weeks (Kundu *et al* 2010).

3.10. Other applications

Presence of inherent RGD sequences on fibroin protein makes it an important tool to study directional cell adhesion and controlled cell proliferation. Upon revelations of its intriguing biological, mechanical, and physical properties in recent years, fibroin has attracted interest from other multidisciplinary areas. In a pioneer study, nonmulberry fibroin protein is used as a lithographic ink to create micro patterns (Mandal *et al* 2009b). Directional adhesion of fibroblasts is promoted on such patterns, which can be utilised for bio-device or implant fabrication. Recent reports show other optical (Hu *et al* 2020) and electrical (Zhu *et al* 2016, Shivananda *et al* 2020) properties of fibroins, which may help to fabricate memory devices (Hota *et al* 2012, Wang *et al* 2016, Zhang *et al* 2021). They also have different optical properties (i.e. refractive index), which may enable the fibroins to be used for optical wave guide devices (Perotto *et al* 2017), bio-photonic devices (Gogurla *et al* 2016), bioelectronic devices, sensors, and different new nature-inspired functional materials (Pradhan *et al* 2020) using new silk processing methods such as bio-inspired spinning and additional advanced biopolymer processing (Guo *et al* 2020).

4. Sericin: a bio-medically relevant molecule and its role in tissue engineering

A. mylitta silkworms are grown wild and hence, are required to be protected from different biotic and abiotic stress (protection from temperature fluctuations, water, fungi, bacteria and other predators) (Halder *et al* 2015) (movie 1). The constituents of such stronger cocoons possess many valuable traits, making them a potential candidate for biomedical engineering (figure 9 and 10). Antibacterial properties of extracted sericin are demonstrated

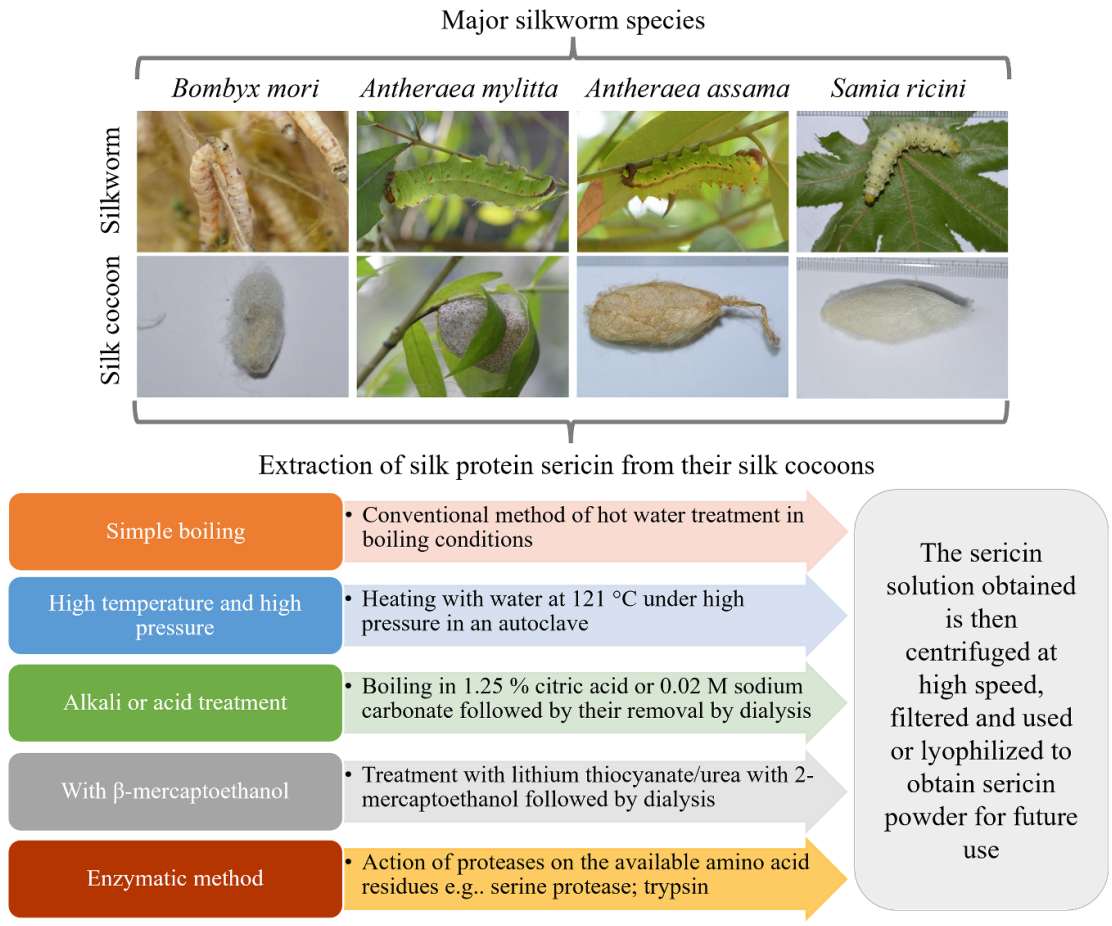


Figure 9. Different species of silkworms and the methods of extractions of sericin from their cocoons. The primary two kinds, i.e. mulberry (*Bombyx mori*) and the major nonmulberry (*Antheraea mylitta, Antheraea assama, Samia ricini*) silk cocoons are cut into small pieces. These cut pieces are then subjected to different isolating procedures to obtain either the aqueous sericin solutions or dried sericin powders.

against gram-positive bacteria and gram-negative bacteria in different studies (Senakoon *et al* 2009), highlighting its promising role in wound dressing, antibacterial soaps, and mouthwash and others. The mechanism of action of sericin on the morphology, cellular integrity, and growth of gram-negative bacteria (*E. coli*) are analysed in detail (Xue *et al* 2016). Antioxidant properties of *A. mylitta* sericin are evaluated in detail by Dash *et al* (2008a). The protective nature of sericin against oxidative stress are also analysed (Dash *et al* 2008a), which encourages the use of natural sericin protein as a non-enzymatic antioxidant. The antioxidant activity is also observed in the sericin of wild African species of silk moths (Manesa *et al* 2020). The inclusion of sericin as a dietary supplement exhibits the decreased activity of intestinal proteases and suppression of colon cancer (Kato and Iwami 2002) and improves the effects of hypercholesterolemia (Deori *et al* 2016). Diverse methods of sericin extraction were also observed to influence its beneficial properties. Alkali-treated sericin of Am silkworms demonstrates the maximum reduction in the lipid peroxidation and antioxidant activity than other methods of sericin extraction (such as urea, autoclave, and boiling methods) (Kumar and Mandal 2017). Wild *A. mylitta* has different eco races depending on their geographical locations within Indian Central states. A comparative study conducted with other such eco races, namely Daba, Modal and Raily, shows that all these eco races exhibit the beneficial properties of sericin, including the free-radical scavenging activity and inhibition of lipid peroxidation (Jena *et al* 2018). The anti-apoptotic and photo-protective effects of sericin against ultraviolet B (UVB) through reduced expression of COX-2, 4-HNE and PCNA are established (Zhaorigetu *et al* 2003). These experiments emphasize the protective effect of sericin against UV irradiated damage of the skin. Sericin also inhibits UVB mediated apoptosis by activating the anti-apoptotic machinery in human keratinocytes (Dash *et al* 2008b). Based on the natural defensive properties of sericin, its role as a cell culture additive (Sahu *et al* 2016) is also examined. Am sericin (0.05% w/v) supplemented media exhibits comparable growth of fibroblasts as observed in media supplemented with 10% FBS marking its promising nature as serum substitute for growth supplement (Sahu *et al* 2016).

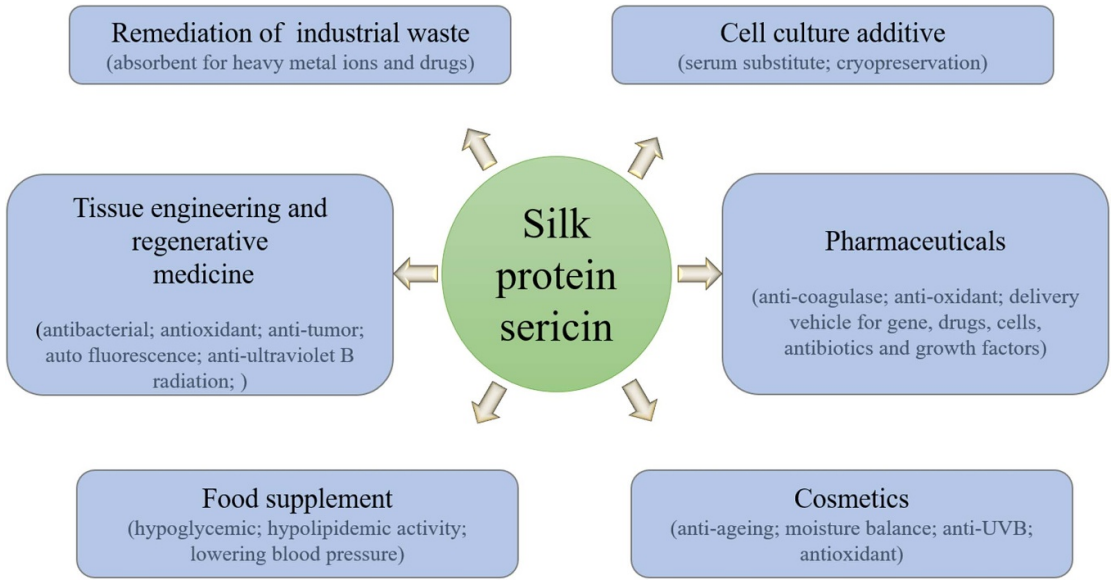


Figure 10. Beneficial properties of the sericins are directly applicable to human welfare. Silk protein sericin has numerous essential features that are valuable in diversified areas, including cosmetics, food supplements, industrial waste remediation, cell culture additives, pharmaceuticals, tissue engineering, and regenerative medicine. Each application is attributed to the specific property of sericins, which augments their efficacy.

4.1. Sericin based assemblies

Silk industries are focused on obtaining silk threads (fibroin), and sericin is considered a waste by-product. Along with the cocoon, sericin can also be isolated directly from the silk glands of the mature silkworms (Dash *et al* 2009) and the peduncle of the cocoons (Dash *et al* 2006). There are various methods of extraction (figure 9) (Kundu *et al* 2008b, Kundu *et al* 2014b) and fabrication of sericin based structures (figure 10). Some of these methods are summarised below in brief as general extraction procedures for any species of silks. Sericin can be isolated directly from the middle silk gland of the mature *A. mylitta* (Dash *et al* 2009). Sericin from Am cocoons can be separated as described before (Dash *et al* 2006, Sahu *et al* 2016) through high temperature and high-pressure treatment. The time required for wild cocoons is higher than domesticated or semi-domesticated (20–30 min) as wild cocoons are comparatively more rigid and thus require more time for degumming. Many prefer this procedure as this does not involve the use of any chemical and its subsequent removal (Aramwit *et al* 2010). The isolation of sericin can also be performed by boiling the silk cocoons pieces in robust acidic or alkaline solutions (1.25% citric acid or 0.02 M sodium carbonate) for 30–60 min depending on the type of silkworm species (Kurioka *et al* 2004, Dash *et al* 2007, Aramwit *et al* 2010, Yun *et al* 2013). Another method involving 8 M urea, 1% SDS, and 2% β-mercaptoethanol works quite well with the Am peduncles (cut into small pieces) and yields a good amount of sericin with the appearance of distinct protein bands in SDS-PAGE (Dash *et al* 2007). Several enzymes such as trypsin, papain, and alkalis are also used to degum cocoons (Freddi *et al* 2003).

Attributing to the valuable properties of sericin mentioned in the previous section, this protein is being explored as a biomaterial to address various tissue engineering and biomedical applications (figure 11). Sericin in the different formulations is fabricated and is examined for the potential in tissue repair. Aqueous silk sericin solution promotes cellular growth and viability with increased collagen production without any toxicity up to the sericin concentration of 40 μg ml^{-1} when incubated for 24 h (Aramwit *et al* 2010). Various types of matrices are fabricated using sericin. Being extremely hydrophilic, sericin is often combined with other polymers to achieve the desired mechanical stability as per the need of the application. One of the significant applications of this protein is skin tissue engineering. For this, various matrices are used, including thin films or membranes (Akturk *et al* 2011, Nayak *et al* 2012), sponges/scaffolds (Napavichayanun *et al* 2016, Ampawong and Aramwit 2017), hydrogels (Jiao *et al* 2017, Qi *et al* 2018, Sapru *et al* 2019), microcapsules/microspheres (Nayak *et al* 2014, Aramwit *et al* 2016), nanoparticles (Khampieng *et al* 2015), electrospun mats (Bhowmick *et al* 2018, Gilotra *et al* 2018, Sapru *et al* 2018) and cream-based formulations (Aramwit *et al* 2013a, 2013b). Hydrogels are primarily prepared as 3D matrices of sericin, as this protein is highly hydrophilic and can absorb moisture. Therefore, this can be exploited as dermal filler or sealant. In addition, such materials are more suitable for skin tissue application considering the properties of sericin, which are more beneficial for skin as

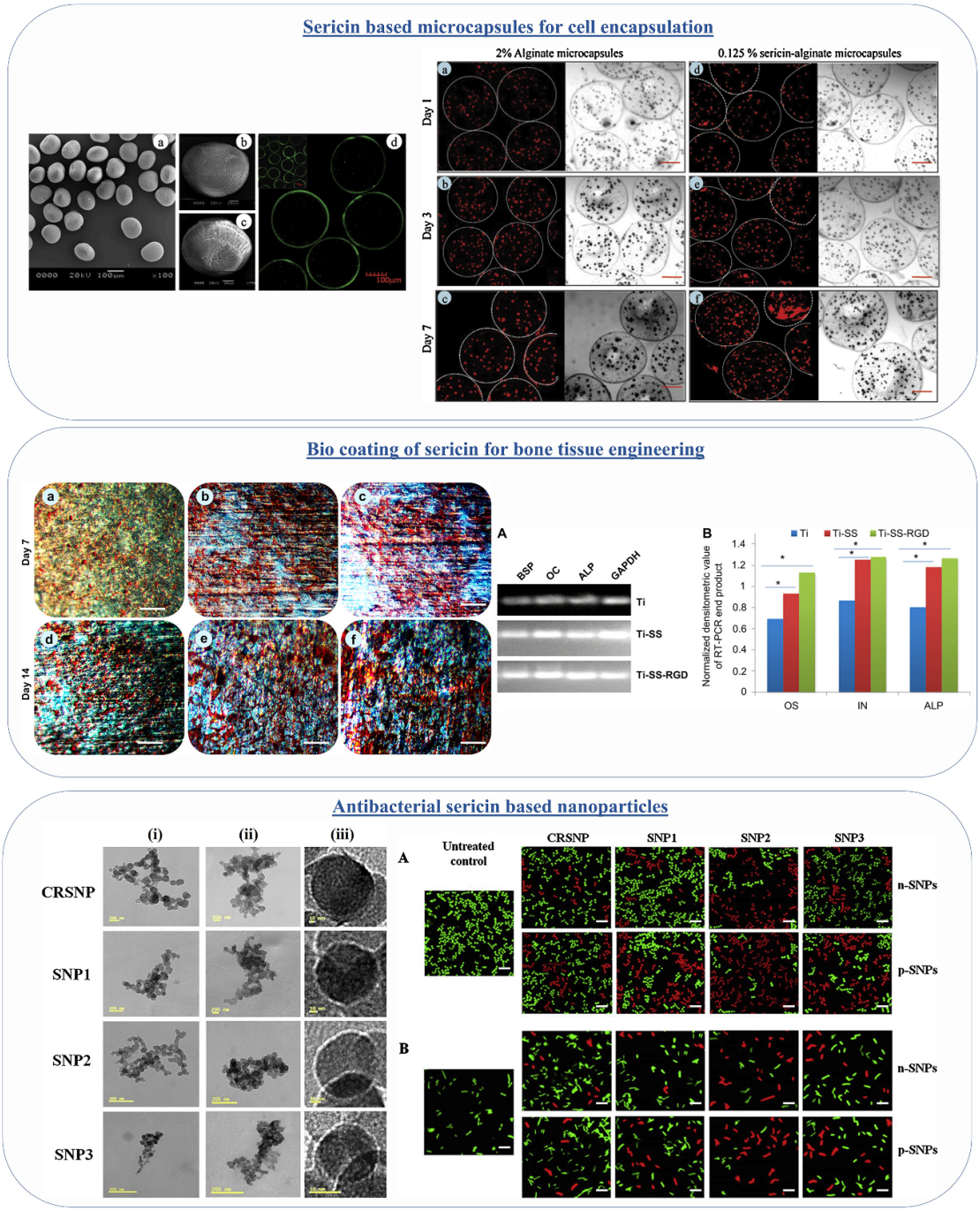


Figure 11. Various formulations and applications of sericin matrices. Sericin microcapsules: (a) scanning electron microscopic images of the sericin based microcapsules, (b) upper view, (c) lateral view and (d) confocal images of the microcapsules crosslinked with genipin. Assessments of the cell proliferation by live staining and confocal microscopy for days 1, 3 and 7. The cells are stained before encapsulations. Alginate–chitosan microcapsules (a)–(c), and 0.125% sericin containing alginate–chitosan microcapsules (d)–(f) at days 1, 3 and 7 with corresponding differential interference contrast images. Magnification bar = 100 μm. Reprinted from Nayak and Kundu (2014), Copyright (2014), with permission from Elsevier. Sericin bio coating: optical microscopy images of Alizarin Red stained osteoblasts after culturing for 7 and 14 d on (a), (d) Ti, (b), (e) Ti-SS and (c), (f) TieSSeRGD, respectively. Scale bar ¼ 100 mm. (A) Expression of bone-related genes in osteoblastic cultures on different Ti surfaces. Representative electrophoretic images from the reverse transcriptase-polymerase chain reaction (RT-PCR) showed for bone sialoprotein (BSP), osteocalcin (OC), alkaline phosphatase (ALP) and GAPDH. (B) GAPDH normalised densitometric quantification of PCR product using Image j software. Reprinted from Nayak *et al* (2013), Copyright (2013), with permission from Elsevier.Sericin nanoparticles: Morphological characterisation of different sericin nanoparticles (SNPs). (i) Negative surface potential nanoparticles (n-SNPs), (ii) positive surface potential nanoparticles (p-SNPs) and (iii) individual p-SNP measured by HRTEM. SNPs prepared with *A. mylitta* crude sericin (CRSNPs) and individual sericin fraction-1 (50–300 kDa), SNP1; fraction-2 (30–50 kDa), SNP2; and fraction-3 (10–30 kDa) SNP3 with negative (n-SNP) or positive (p-SNP) surface potential evaluated for anti-bacterial potential against (A) *S. aureus* and (B) *E. coli* cells treated with n-SNPs and p-SNPs at 50 μg ml^{-1}. Green and red in the confocal images represent viable and dead cells, respectively. Scale bars are 10 μm. Reprinted from Dutta *et al* (2020), Copyright (2020), with permission from Elsevier.

hydrogels are considered the appropriate matrices. Hence, apart from the use of fibroin in skin repair (Gholipourmalekabadi *et al* 2020), sericin is gaining importance in this field (Das *et al* 2021). Sericin, alginate and chitosan-based microcapsules prepared by ionotropic gelation and high voltage for cellular encapsulation offers great potential in cell-based therapeutics (Nayak *et al* 2014). Bio-coating helps in functionalising the surface with valuable features of sericin (Nayak *et al* 2014). Microcapsules or microencapsulation facilitates the entrapment of cells, growth factors, antibodies or other necessities as needed for the regeneration process (figure 11). This formulation can deliver cells in an injectable manner and makes it possible to reach farther sites as well without the need for any implantation.

In the following sections, we summarise the research investigations conducted *in vitro* and *in vivo* with a specific focus on the sericin of nonmulberry silkworm, *A. mylitta.* This narrates other than *B. mori* sericins, the importance of wild sericins as biomedical materials due to their specific biochemical characteristic being grown as wild in nature.

4.2. Skin tissue engineering

Am sericin is explored in different formulations for different applications including membranes/electrospun matrices as dressing, hydrogels as fillers or sealers, microcapsules for cellular encapsulation, and direct coating as bioactive additive.

Membranes formed with native sericin isolated from the middle silk gland of Am and without the use of any crosslinker are cytocompatible with minimal immunogenicity (Dash *et al* 2009). For most of the research works, Am sericin is isolated from silk cocoons. Glutaraldehyde crosslinked sericin membranes, and sericin/gelatine membranes are found to be non-toxic and support cell growth (Mandal *et al* 2009c, Nayak *et al* 2012). Sericin and gelatine porous scaffolds (optimum pore size of 170 ± 20 μm) prepared using Am silk cocoon sericin protein are also cytocompatible and robust to handle (Mandal *et al* 2009c). Nanofibrous sericin based matrices support the growth of human keratinocytes and, when loaded with antibiotics are potential candidates as a wound dressing (Sapru *et al* 2018).

Hydrogel matrix of sericin and poly (vinyl alcohol) with glutaraldehyde as crosslinker exhibit improved adhesion and proliferation of fibroblasts (Mandal *et al* 2011). Hydrogels of Am sericin peptides and chemically modified PVA with methacrylate groups also exhibit better cellular adhesion than those prepared with Bm (Lim *et al* 2012). Semi-interpenetrating Am sericin and polyacrylamide-based hydrogels (pore size 23–52 μm) offer rapid gelation (∼5 min at 37 °C), high absorbance ability (∼112% uptake potential) and good mechanical strength (61 kPa). The growth of fibroblasts indicating their potential as dermal sealants with enhanced ability to absorb exudates (Kundu *et al* 2012). Porous hydrogel matrices of Am sericin and carboxymethyl cellulose with glutaraldehyde and aluminium chloride as crosslinkers support the growth of human keratinocytes with low inflammation as indicated by the levels of TNF-α produced (Nayak *et al* 2014). Sericin based porous (57.23–75.22 μm, pore size) hydrogels with chitosan as a support polymer and genipin as crosslinker retain the inherent features i.e. antioxidant (196.1 ± 17.7 M Fe (II) mg^{-1}) and anti-bacterial (8–15 mm zone of inhibition) attributing to its components (sericin and chitosan) (Sapru *et al* 2017). The sericin-based hydrogels are non-immunogenic and support the growth of human fibroblasts, indicating their potential as an affordable dermal substitute (Sapru *et al* 2017). Antibiotic loaded Am sericin based nanofibrous matrices (80–400 nm fibre thickness) support the proliferation of human keratinocytes *in vitro* and accelerates the full-thickness wound healing *in vivo* with minimal inflammation, restoration of epithelial tissue, generation of blood vessels and dense collagen-rich ECM (Sapru *et al* 2018). Bi-layered skin constructs fabricated with Am silk sericin and chitosan hydrogels by co-culture of human dermal fibroblasts and keratinocytes exhibit improved cellular attachment, growth and migration with the generation of ECM components, including metalloproteinases and collagen *in vitro* (Sapru *et al* 2019). These hydrogels when implanted subcutaneously in rats leads to dense collagen and matured blood vessels formation, minimal immune response and infiltration of host cells into the implanted hydrogels indicating their non-inflammatory nature and potential to support the repair of skin tissue (Sapru *et al* 2019). Sericin based hydrogels incorporated with glycosaminoglycans further enhance the efficacy of such hydrogels in skin repair (figure 12) (Sapru *et al* 2021).

4.3. Liver tissue engineering

Encapsulation of hepatocytes in sericin, alginate and chitosan-based microcapsules exhibits enhanced cell viability and distribution while maintaining their metabolic activities as indicated by intracellular albumin content, glucose consumption rate and urea secretion rate (Nayak *et al* 2014).

4.4. Drug delivery

Am sericin has the potential to form self-assembled micro and nano structures (Khire *et al* 2010). One such example is the self-assembled nanostructures (100–110 nm diameter) of Am sericin with pluronic F-127 and F-87 exhibiting the potential to successfully deliver both hydrophilic and hydrophobic drugs to the target sites *in vitro* (Mandal and Kundu 2009). In a recent study, sericin nanoparticles fabricated

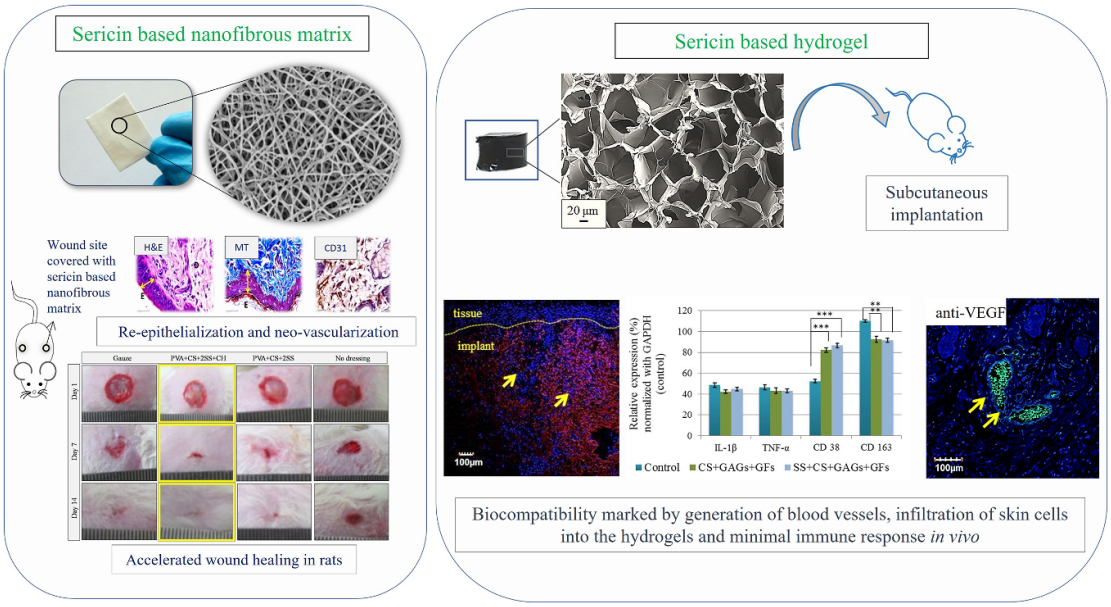


Figure 12. Prospects of *Antheraea mylitta* sericin in skin repair evaluated *in vivo*. Hydrogel and nanofibrous matrix fabricated with *A. mylitta* sericin exhibit potential as skin repair and wound healing. Minimal inflammation observed with these matrices support their biocompatibility and non-immunogenic nature. Reprinted from Sapru *et al* (2021), Copyright (2021), with permission from Elsevier.

with three different fractions of sericin protein, i.e. 50–300 kDa, 30–50 kDa and 10–30 kDa, resulting in varied sized nanoparticles exhibits other reactive oxygen species generation and antibacterial activity depending on their molecular size and surface charge potential (Dutta *et al* 2020a) (figure 11). Poly-l-lysine coated sericin nanoparticles with positive surface potential and size 33–49 nm shows maximum antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* amongst all sized and charged nanoparticles made in the study (Dutta *et al* 2020) (figure 11).

4.5. Hard tissue engineering

Sericin use is not limited to soft tissue engineering, but it offers applications in hard tissue engineering as well such as in bone tissue engineering. However, sericin does not offer the mechanical strength required by such application, but a coating of sericin of orthopaedic implants enhances their prospects for osseointegration. Such biological coatings influence cellular behaviour, including differentiation and matrix remodelling, which in turn controls the fate of the implant. Am sericin, when immobilised on the titanium using glutaraldehyde as crosslinker, upregulates the expression of bone-specific proteins, including bone sialoprotein, osteocalcin and alkaline phosphatase with no inflammatory response (Nayak *et al* 2013). Additionally, integrin-binding peptide sequence Arg-Gly-Asp (RGD) when conjugated to sericin-coated titanium also exhibits similar results with enhanced potential of implant for bone tissue engineering (Nayak *et al* 2013)

5. Different other indigenous nonmulberry silks in the biomedicine field

5.1. Other indigenous silk

Research interest in not-so-common strains of silkworm and their potential in the biomedical field piqued in the last few years primarily due to the encouraging results obtained from *A. mylitta*, as discussed in this review. Other nonmulberry silks such as *A. pernyi* are different in structure and property compared to Bm silk. Ap silk has higher toughness as a strain-stiffening material due to the abundance of beta-sheets in the amorphous domain like Am silk. (Guo *et al* 2017). Regenerated Bm scaffolds show significantly higher mass loss and free amino acid content release than nonmulberry (Ap and Ay) scaffolds *in vitro* and *in vivo* conditions (You *et al* 2015). Like Am cocoon, Ap cocoon still lacks exploitation for a probable bulk source of fibroin due to its poor solubility in common aqueous-based solvents. However, these cocoons can be dissolved using ionic liquid (1-butyl-imidazolium acetate).

The sponges made from this solution blended with chitin are found to be stable. The biophysical and biochemical properties exhibited by these matrices imply their promising potential as a support for cartilage regeneration (Silva *et al* 2019, 2020). Another method for dissolving degummed cocoon fibre is using molten $Ca(NO_3)_2$ solution (You *et al* 2015, Li *et al* 2016). After successfully regenerating fibroin, similar biomaterials are fabricated from the mentioned nonmulberry silk proteins, such as thin fibroin films, to study the attachment and growth of human

bone marrow-derived mesenchymal stem cells (Luan *et al* 2006). Fibroin nanofibrous matrices are made using aqueous-based electrospinning technique and the encapsulation efficiency and the release kinetics of the matrices are carried out (Li *et al* 2016). Similarly, the encapsulation efficiency and the release kinetics of lysozyme or rhodamine loaded Ap fibroin microspheres are also studied (Li *et al* 2013, Wang *et al* 2014). Ap sericin-based mineralised composite material is investigated to observe biomineralisation property and cell viability and osteogenic differentiation of human bone marrow stem cells (Yang *et al* 2014). Ap silk fibroin fibres are found to be superior in assisting calcium deficient hydroxyapatite formation in the presence of SBF (Zhang *et al* 2020). Ap fibroin-based composite scaffolds are also explored for their potential to serve as 3D support for the spheroid model. For example, these composite scaffolds of Ap fibroin enhance the growth of prostate epithelial cancer cells and aid the formation of stronger glandular-like prostate spheroids compared to the synthetic polymer scaffolds (Bäcker *et al* 2017). Hence, further probing of such untapped potential biomaterials is required, providing us with some unique abilities and drawing financial empowerment to the cottage industry.

5.2. Transgenic silkworm producing spider fibroin

The spider dragline silk gene is cloned in *B. mori* silkworm using different gene replacement strategies to enhance the mechanical property of silk fibroin. For example, a transcription activator-like effector nuclease-mediated homology-directed repair mechanism is adopted to express major ampullate spidroin-1 gene from *Nephila clavipes* (Xu *et al* 2018). In another study, a partial sequence of spider (*Araneus ventricosus*) dragline silk gene (SpA) is cloned under *B. mori* fibroin heavy chain promoter for expression of both the protein in fused form in cocoon silk (Kuwana *et al* 2014). A piggyBac vector-based cloning of 2.4 kbp A2S814 synthetic spider silk sequence encoding repetitive flagelliform-like $(GPGGA)_8$ elastic and prominent ampullate spidroin-2 (linker-alanine8) crystalline motifs in *B. mori* fibroin is achieved with the same purpose (Teule *et al* 2012). As a result of these strategies, the toughness of the transgenic silkworm's chimeric silk improved significantly depending on the quantity of spider dragline protein expressed in fibroin. Thus, this transgenic silk offers a wide possibility to be explored as a new high-performance biopolymer for biomedical applications and can be used directly in the textile industry.

5.3. RGD containing polymer as a functional biomaterial

A wide variety of non-toxic polymeric materials are constantly investigated for their potential of mechanical stability, elasticity, and stability towards degradation. Most of these polymers lack biocompatibility. One of the most popular approaches for material modification is incorporating the cell recognition motif (RGD: R: arginine; G: glycine; D: aspartic acid) to obtain controlled cell-material interaction. A very detailed discussion on different RGD functionalisation methods of various polymers and *in vitro* evaluation is documented by Hersel *et al* (2003).

6. Silk based hybrid/blended assembly for tissue engineering

Silk proteins are compatible for hybrid or blend formulation. Such hybrid compositions can complement the functionality of each of the components and add diverse physical attributes to the structures, which may not be achieved otherwise.

6.1. Fibroin-polymer blends

When fibroin supply is limited, or a particular tissue type needs to be mimicked, different polymers can be used to complement such functionality. The potential of such structures for tissue engineering are investigated in detail. A blending of PEG 4000 with Bm or Am fibroins alter the mechanical and thermal properties as compared to the pure matrices. Blended Am matrices show better *in vitro* activity of HOS osteosarcoma cells than Bm blend matrix (Acharya *et al* 2009b). Hydrogels of Aa and Bm fibroin with agarose are fabricated separately and evaluated for their chondrogenic potential. Biochemical studies reveal significantly higher levels of sulphated GAGs, collagen, aggrecan, and sox-9 expression in blended hydrogels, which indicates their promising role in chondrogenesis (Singh *et al* 2016).

A blend of Am fibroin and chitosan is explored for its *in vitro* chondrogenesis potential. *In vitro* differentiation study with rat bone marrow stem cell for 3 weeks exhibited the proper chondrogenic phenotype of the cells in high expression of GAG, collagen, and cartilage-specific gene markers expressions (Bhardwaj and Kundu 2012). In another study chitosan-Am fibroin, nonwoven composite films are investigated for their potential for wound dressing. The blended matrices indicate higher as well as dynamic mechanical properties along with good hemocompatibility, cytocompatibility and biodegradation compared to the pure matrices (Srivastava and Purwar 2017). Another blend with similar components (surface functionalisation of plasma induced chitosan grafted Aa fibroin yarn) exhibit enhanced antithrombogenic property and antimicrobial activity compared to Aa yarn, which is further improved upon impregnation of antibiotic drug penicillin G sodium salt (Choudhury *et al* 2016).

6.2. Inter-species fibroin blends

Inter-species fibroins are blended particularly between mulberry and nonmulberry types (for example, Bm with Am or Bm with Aa or Bm with Sr) to fine-tune their performances such as gelation rate, mechanical performance and degradation rate according to the application required (Li *et al* 2018). An elaborative study of thermal analysis (DSC and TMDSC) and FTIR of these blends is conducted. It is observed that Bm fibroin is fully miscible with Am, Aa, Sr and Thai silk fibroins. The contents of α-helix and random coil are tuneable. Also, the glass transition and degradation temperature are customisable by tuning the blending ratio as per requirement (Xue *et al* 2017). A nanofibrous scaffold containing 70:30 blend of Sr and Am fibroin is fabricated using electrospinning, and its physiochemical properties and supportive cell potential are assessed. The scaffolds show superior hydrophilicity and mechanical strength as well as enhanced human cord blood mesenchymal stem cell adhesion, proliferation and metabolic activity as compared to Bm (Panda *et al* 2015a).

7. Limitations of current platform

In the last few decades, an enormous number of studies are reported on bio-medical and tissue engineering related to the prospect of silk-based biomaterials and different types of scaffold matrices. However, the practice involved in the protocols observed to vary worldwide. The absence of a standard operating protocol for either fibroin and sericin extraction from nonmulberry sources and fabrication of different matrix types makes it difficult to establish an uniform platform for a true comparison. Secondly, most of the reports relied heavily on *in vitro* experimentation. Hence further understanding of such matrices under *in vivo* conditions is warranted. A few evaluations reported contradictory data between mulberry and nonmulberry matrices, which need further probing. A few limitations are discussed in this section as this may generate strategies for designing further experiments.

7.1. Absence of standardised operating protocol for nonmulberry sericin

A. mylitta, being grown wild, are hypothesised to have more protective nature than the domesticated *B. mori*. This is noted in the *in vitro* and *in vivo* experimentations conducted with this silk protein. Batch to batch variations is commonly observed in many natural substances, including the widely used foetal bovine serum in cell maintenance and culture. Such variations are also observed in the silk proteins isolated from domesticated silkworm *B. mori*. Growing them under varied environmental conditions can influence their properties (Offord *et al* 2016). As grown in wild conditions, *A. mylitta* also exhibits batch to batch variation, reflecting in the silk proteins isolated. It is crucial to maintain consistency in the extraction of proteins and sources of materials. Sericin, in general, has less mechanical strength as opposed to silk protein fibroin. It should be noted that sericin isolated using the previously described procedures varies in terms of their chemical and physical properties, including the composition of amino acids, zeta potential and size of molecules (Aramwit *et al* 2010). Their biological effects on fibroblasts, such as viability and the production of collagen, also differ. Sericin isolated from any of the methods (heat, alkali, acid or urea) is non-toxic to cells if used within a concentration of 40 $\mu g\ ml^{-1}$. Heat degraded sericin leads to maximum production of collagen and more viability of the cells compared to other methods (Aramwit *et al* 2009a, 2010). On the other hand, sericin isolated by the urea method is relatively more toxic to the cell, influencing less collagen production (Aramwit *et al* 2010). Amino acids content in the sericin influences its behaviour in cell culture. The higher amounts of methionine and cysteine promote the proliferation of cells and the production of type I collagen and protein synthesis (Aramwit *et al* 2009a). It also leads to the synthesis of a relatively higher amount of nitric oxide. However, the level produced is non-toxic to cells. This indicates that this silk protein is safe for biological use (Aramwit *et al* 2009a). For extracting Am sericin, the highest yield is noted with the alkali method (0.02 M sodium carbonate, 60 min) or conventional industrial methods of combining soap and alkali (Yun *et al* 2013). Hence, a set of the standardised protocol is required to reproduce the results and carry forward the investigation.

7.2. Contradictory reports of *in vitro* and *in vivo* inflammatory responses of sericins

Earlier, sericin is considered an inflammatory agent. Recent several studies supported by *in vivo* investigations and clinical trials indicate that sericin is safe to use and only cause inflammation when present in combination with fibroin (Aramwit *et al* 2009b, 2013a, 2013b, Thurber *et al* 2015). Although Bm sericin is explored in most *in vivo* experimentations, including sericin-based cream formulation tested clinically, there are limited *in vivo* reports with *A. mylitta* (figure 12). However, the investigations show the promising nature of Am sericin as a useful biomaterial. Still, more in-depth *in vivo* inspections need to be carried out to understand the further potential of different sericin matrices for biomedical applications.

8. Future outlook

Several research groups have spent a couple of decades of research and developments on nonmulberry fibroins and sericins; still, certain avenues are left to discover. In the future, the researchers are expected to explore the following topics.

8.1. Opportunity for *A. mylitta* fibroin-based microfluidics and bioprinting

Bm fibroin is accepted for diverse applications ranging from fabrication of matrices for growing primary cells or cell lines to deliver cells or therapeutic molecules to the diseased tissue. Successful utilisation of silk fibroin for microfluidics device fabrication is reported almost a decade ago (Bettinger *et al* 2007, Kinahan *et al* 2011), which is continued to date (Zhao *et al* 2016, Li *et al* 2020, 2021, Lu *et al* 2019). After that, others also reported the potential of silk fibroin to fabricate micro-architectural structures through protein-based photoresist (Kurland *et al* 2013) inkjet printing (Tao *et al* 2015), hydrogel elastomers (Yuk *et al* 2016) and soft-lithography (Kumar *et al* 2018). Recent development includes the development of silk fibroin based bioink for memory implants (Costa *et al* 2017), 3D bioprinting to achieve tissue engineering applications (Kim *et al* 2018) and Silk/PEDOT sensors (Pradhan and Yadavalli 2021). In future, all such developments could pave the way for Am fibroin too for microfluidics and bioprinting applications.

8.2. *A. mylitta* sericin for hard tissue engineering

Sericin from different silkworms has variations in terms of amino acid compositions and degree of bioactivities. For this reason, it is mainly required to be combined with other polymers or use crosslinker to achieve the desired strength. This low mechanical stability can be a limitation to use sericin for hard tissue engineering such as bone. However, as mentioned earlier, sericin coating has shown promising titanium implants (Nayak *et al* 2013). More studies with Am sericin matrices for such application need to be conducted.

8.3. Immediate attention needed for *A. mylitta* fibroin sequencing

Another important task is left out for molecular biologist for unveiling the complete gene sequence of Am silk fibroin. This will reveal the length of fibroin, which is expected to have around 3555 amino acids or so and the number of RGD motifs present in it. Currently, a partial sequence of Am fibroin (507 amino acids from N-terminal domain) is available with seven RGDs in it (Datta *et al* 2001a). We have predicted the 3D appearance of the protein structurally using TrRefineRosetta modelling method in Robetta server (https://robetta.bakerlab.org/) without using any homology template (figure 2(B)). From the *ab initio* predicted structure, the RGD motifs can be observed to be present on the loop regions, which is exposed on the protein's surface (figure 2(B)). This phenomenon might have influenced the cells (both *in vitro* and *in vivo*) to attach their integrins on Am fibroin-based matrices, resulting in their superior cytocompatibility and preference over other synthetic/natural polymeric matrices. Multiple sequence alignment in CLUSTAL 2.1 unveils that Am (partial sequence) has 82% amino acid sequence similarity with Ap (10 RGDs) and Ay (12 RGDs), 75% with Aa (3 RGDs), and 61% with Sr (1 RGD). So, there is a possibility to reveal more RGDs in the new complete sequence of Am fibroin. The analysis of RGD peptide density, spacing, availability on the protein surface, and subsequent impact on cell spreading, migration, and other cellular parameters will open new avenues of cell biology research.

9. Conclusion

Silk-based materials have been used for medical purposes from time immemorial. Extensive scientific studies related to the evaluation and validation of silk-based assemblies have been initiated in the early 1900s to develop silk-based sutures and scaffolds. With the development of tissue engineering techniques and advancement of cell culture protocols, the involvement of silk protein-based hydrogels, thin films, and other structures has become an inseparable part of recent tissue engineering and regenerative medicine labs. In the past, silk protein-related works mainly involved mulberry silks (*Bombyx mori*) due to their worldwide availability. However, in the early 2000s, many other potential silk candidates have been introduced to scientists. The discovery of 'inherent 'RGD' or 'integrin-binding motifs' present in the protein sequence of nonmulberry silks created an invigorating interest in the silk protein based research. This unique feature of the nonmulberry fibroin isolated from *A. mylitta* offers considerable potential to support the researchers to accelerate/enhance their 2/3D cell culture for studying growths, proliferation, and differentiation of all most any kind of cells (viz; any types of stem cells, fibroblasts, and cancer cells). Furthermore, these materials do not need to be biofunctionalised with RGD peptides with an additional tedious procedure of conjugation, which is an essential ingredient for extracellular matrices. Additionally, the 3D matrices provide *in vitro* 3D cancer model platforms for screening the therapeutics for *in vitro* efficacy and toxicity without using *in vivo* animal models.

Decade-long research with such assemblies has given the scientists an insight into the untapped potential of these materials. Therefore, such materials are predicted to be a part of the biomedical engineering field without significant and costly post-isolation modifications in the future. It is expected that their functional superiority would be reflected in the *in vivo* platform. This development might provide the necessary support for regenerative medicine and tissue engineering research. Investigation for other potential candidates originating from the Indian subcontinent, namely Eri (*Samia cynthia ricini*) and Muga (*A. assamensis*), along with their international counterparts from China (*A. pernyi*) and Japan (*A. yamamai*) should be continued in the future. With

such success of *A. mylitta* silk protein-based structures, other neglected ecoraces from the Indian subcontinent stood a chance to be recognised in the international scientific community and embraced for further evaluation.

Data availability statement

No new data were created or analysed in this study.

The 3D structure model of *A. mylitta* fibroin is predicted from the amino acid sequence (NCBI Accn: AAN28165, partial CDS) of N-terminal domain (1–507aa) using TrRefineRosetta modelling method in Robetta server (https://robetta.bakerlab.org/). And subsequently the molecular graphics is prepared using the program Pymol (www.pymol.com).

Acknowledgments

Financial supports are obtained from MHRD, Govt. of India (DN & SS) and from Department of Biotechnology (DBT) (BT/PR10941/MED/32/333/2014) and Indian Council of Medical Research (ICMR) (5/13/12/2010/NCD-III), Govt. of India (SCK). TD acknowledge DST SERB and RUSA for financial support. SCK presently holds a European Research Area Chair and Full Professor position at 3Bs Research Group, University of Minho, Portugal supported by the European Union Framework Programme for Research and Innovation HORIZON 2020 under Grant Agreement No. 668983—FoReCaST to RLR. The FCT-Portugal project (PTDC/BTM-ORG/28070/2017) supported SCK. We are grateful to Dr Prasun Kundu, Cambridge Institute for Medical Research, Cambridge, UK, for suggestions and scientific inputs, particularly with *A. mylitta* silk fibroin 3D predicted structure model based on amino acid sequences improve the manuscript.

Conflict of interest

The authors declared no conflict of interest and agreed to the final version of the manuscript.

Ethical permission

No ethical permission is required for this article.

ORCID iDs

Deboki Naskar https://orcid.org/0000-0003-0902-3869
Sunaina Sapru https://orcid.org/0000-0003-1501-1071
Rui L Reis https://orcid.org/0000-0002-4295-6129
Tuli Dey https://orcid.org/0000-0003-0413-0729
Subhas C Kundu https://orcid.org/0000-0002-7170-2291

References

Aamodt J M and Grainger D W 2016 Extracellular matrix-based biomaterial scaffolds and the host response *Biomaterials* **86** 68–82

Acharya C, Ghosh S K and Kundu S C 2009a Silk fibroin film from non-mulberry tropical tasar silkworms: a novel substrate for *in vitro* fibroblast culture *Acta Biomater.* **5** 429–37

Acharya C, Kumary T V, Ghosh S K and Kundu S C 2009b Characterisation of fibroin and PEG-blended fibroin matrices for *in vitro* adhesion and proliferation of osteoblasts *J. Biomater. Sci. Polym. Ed.* **20** 543–65

Akturk O, Tezcaner A, Bilgili H, Deveci M S, Gecit M R and Keskin D 2011 Evaluation of sericin/collagen membranes as prospective wound dressing biomaterial *J. Biosci. Bioeng.* **112** 279–88

Ampawong S and Aramwit P 2017 *In vivo* safety and efficacy of sericin/poly (vinyl alcohol)/glycerin scaffolds fabricated by freeze-drying and salt-leaching techniques for wound dressing applications *J. Bioact. Compat. Polym.* **32** 582–95

Aramwit P, Kanokpanont S, De-Eknamkul W, Kamei K and Srichana T 2009a The effect of sericin with variable amino-acid content from different silk strains on the production of collagen and nitric oxide *J. Biomater. Sci. Polym. Ed.* **20** 1295–306

Aramwit P, Kanokpanont S, De-Eknamkul W and Srichana T 2009b Monitoring of inflammatory mediators induced by silk sericin *J. Biosci. Bioeng.* **107** 556–61

Aramwit P, Kanokpanont S, Nakpheng T and Srichana T 2010 The effect of sericin from various extraction methods on cell viability and collagen production *Int. J. Mol. Sci.* **11** 2200–11

Aramwit P, Palapinyo S, Srichana T, Chottanapund S and Muangman P 2013a Silk sericin ameliorates wound healing and its clinical efficacy in burn wounds *Arch. Dermatol. Res.* **305** 585–94

Aramwit P, Towiwat P and Srichana T 2013b Anti-inflammatory potential of silk sericin *Nat. Prod. Commun.* **8** 501–4

Aramwit P, Yamdech R and Ampawong S 2016 Controlled release of chitosan and sericin from the microspheres-embedded wound dressing for the prolonged anti-microbial and wound healing efficacy *Assoc. Am. Phys. Surgeons J.* **18** 647–58

Baba A, Matsushita S, Kitayama K, Asakura T, Sezutsu H, Tanimoto A and Kanekura T 2019 Silk fibroin produced by transgenic silkworms overexpressing the arg-gly-asp motif accelerates cutaneous wound healing in mice *J. Biomed. Mater. Res.* B **107** 97–103

Babu M and Sen K 2007 Thermal behaviour of silk *Res. J. Textile Apparel* **11** 21–27

Bäcker A, Erhardt O, Wietbrock L, Schel N, Göppert B, Dirschka M, Abaffy P, Sollich T, Cecilia A and Gruhl F J 2017 Silk scaffolds connected with different naturally occurring biomaterials for prostate cancer cell cultivation in 3D *Biopolymers* **107** 70–79

Bandyopadhyay A, Chowdhury S K, Dey S, Moses J C and Mandal B B 2019 Silk: a promising biomaterial opening new vistas towards affordable healthcare solutions *J. Indian Inst. Sci.* **99** 445–87

Behera S, Naskar D, Sapru S, Bhattacharjee P, Dey T, Ghosh A K, Mandal M and Kundu S C 2017 Hydroxyapatite reinforced inherent RGD containing silk fibroin composite scaffolds: promising platform for bone tissue engineering *Nanomedicine* **13** 1745–59

Bettinger C, Cyr K, Matsumoto A, Langer R, Borenstein J and Kaplan D 2007 Silk fibroin microfluidic devices *Adv. Mater.* **19** 2847–50

Bhardwaj N and Kundu S C 2012 Chondrogenic differentiation of rat MSCs on porous scaffolds of silk fibroin/chitosan blends *Biomaterials* **33** 2848–57

Bhardwaj N, Rajkhowa R, Wang X and Devi D 2015 Milled non-mulberry silk fibroin microparticles as biomaterial for biomedical applications *Int. J. Biol. Macromol.* **81** 31–40

Bhattacharjee P, Kundu B, Naskar D, Kim H-W, Bhattacharya D, Maiti T K and Kundu S C 2016a Potential of inherent RGD containing silk fibroin–poly (Є-caprolactone) nanofibrous matrix for bone tissue engineering *Cell Tissue Res.* **363** 525–40

Bhattacharjee P, Kundu B, Naskar D, Maiti T K, Bhattacharya D and Kundu S C 2015a Nanofibrous Non-mulberry silk/PVA scaffold for osteoinduction and osseointegration *Biopolymers* **103** 271–84

Bhattacharjee P, Naskar D, Kim H-W, Maiti T K, Bhattacharya D and Kundu S C 2015b Non-mulberry silk fibroin grafted PCL nanofibrous scaffold: promising ECM for bone tissue engineering *Eur. Polym. J.* **71** 490–509

Bhattacharjee P, Naskar D, Maiti T K, Bhattacharya D and Kundu S C 2016b Investigating the potential of combined growth factors delivery, from non-mulberry silk fibroin grafted poly(ε-caprolactone)/hydroxyapatite nanofibrous scaffold, in bone tissue engineering *Appl. Mater. Today* **5** 52–67

Bhattacharjee P, Naskar D, Maiti T K, Bhattacharya D and Kundu S C 2016c Non-mulberry silk fibroin grafted poly (Ie-caprolactone)/nano hydroxyapatite nanofibrous scaffold for dual growth factor delivery to promote bone regeneration *J. Colloid Interface Sci.* **472** 16–33

Bhattacharjee P, Naskar D, Maiti T K, Bhattacharya D and Kundu S C 2016e Non-mulberry silk fibroin grafted poly(ε-caprolactone) nanofibrous scaffolds mineralised by electrodeposition: an optimal delivery system for growth factors to enhance bone regeneration *RSC Adv.* **6** 26835–55

Bhowmick S, Thanusha A V, Kumar A, Scharnweber D, Rother S and Koul V 2018 Nanofibrous artificial skin substitute composed of mPEG-PCL grafted gelatin/hyaluronan/chondroitin sulfate/sericin for 2nd degree burn care: *in vitro* and *in vivo* study *RSC Adv.* **8** 16420–32

Bulutoglu B, Devalliere J, Deng S L, Acun A, Kelangi S S, Uygun B U and Yarmush M L 2020 Tissue scaffolds functionalised with therapeutic elastin-like biopolymer particles *Biotechnol. Bioeng.* **117** 1575–83

Calori I R, Braga G, De Jesus P C C, Bi H and Tedesco A C 2020 Polymer scaffolds as drug delivery systems *Eur. Polym. J.* **129** 109621

Cao Y and Wang B 2009 Biodegradation of silk biomaterials *Int. J. Mol. Sci.* **10** 1514–24

Catoira M C, Fusaro L, Di Francesco D, Ramella M and Boccafoschi F 2019 Overview of natural hydrogels for regenerative medicine applications *J. Mater. Sci. Mater. Med.* **30** 115

Chaturvedi V, Naskar D, Kinnear B F, Grenik E, Dye D E, Grounds M D, Kundu S C and Coombe D R 2017 Silk fibroin scaffolds with muscle-like elasticity support *in vitro* differentiation of human skeletal muscle cells *J. Tissue Eng. Regen. Med.* **11** 3178–92

Chen Y, Shafiq M, Liu M, Morsi Y and Mo X 2020 Advanced fabrication for electrospun three-dimensional nanofibre aerogels and scaffolds *Bioactive Mater.* **5** 963–79

Choudhury A J, Gogoi D, Kandimalla R, Kalita S, Chaudhari Y B, Khan M R, Kotoky J and Chutia J 2016 Penicillin impregnation on oxygen plasma surface functionalised chitosan/*Antheraea assama* silk fibroin: studies of antibacterial activity and antithrombogenic property *Mater. Sci. Eng.* C **60** 475–84

Chouhan D, Das P, Thatikonda N, Nandi S K, Hedhammar M and Mandal B B 2019a Silkworm silk matrices coated with functionalised spider silk accelerate healing of diabetic wounds *ACS Biomater. Sci. Eng.* **5** 3537–48

Chouhan D, Lohe T-U, Thatikonda N, Naidu V G M, Hedhammar M and Mandal B B 2019b Silkworm silk scaffolds functionalised with recombinant spider silk containing a fibronectin motif promotes healing of full-thickness burn wounds *ACS Biomater. Sci. Eng.* **5** 4634–45

Costa J B, Silva-Correia J, Oliveira J M and Reis R L 2017 Fast setting silk fibroin bioink for bioprinting of patient-specific memory-shape implants *Adv. Healthcare Mater.* **6** 1701021

Darshan G H, Kong D, Gautrot J and Vootla S 2017 Physico-chemical characterisation of *Antheraea mylitta* silk mats for wound healing applications *Sci. Rep.* **7** 10344

Das G, Shin H-S, Campos E V R, Fraceto L F, Del Pilar Rodriguez-torres M, Mariano K C F, De Araujo D R, Fernández-Luqueño F, Grillo R and Patra J K 2021 Sericin based nanoformulations: a comprehensive review on molecular mechanisms of interaction with organisms to biological applications *J. Nanobiotechnol.* **19** 30

Dash B C, Mandal B B and Kundu S C 2009 Silk gland sericin protein membranes: fabrication and characterisation for potential biotechnological applications *J. Biotechnol.* **144** 321–9

Dash R, Acharya C, Bindu P C and Kundu S C 2008a Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts *BMB Rep.* **41** 236–41

Dash R, Ghosh S K, Kaplan D L and Kundu S C 2007 Purification and biochemical characterisation of a 70 kDa sericin from tropical tasar silkworm, *Antheraea mylitta Comparative Biochem. Physiol.* B **147** 129–34

Dash R, Mandal M, Ghosh S K and Kundu S C 2008b Silk sericin protein of tropical tasar silkworm inhibits UVB-induced apoptosis in human skin keratinocytes *Mol. Cell. Biochem.* **311** 111–9

Dash R, Mukherjee S and Kundu S C 2006 Isolation, purification, and characterisation of silk protein sericin from cocoon peduncles of tropical tasar silkworm, *Antheraea mylitta Int. J. Biol. Macromol.* **38** 255–8

Datta A, Ghosh A K and Kundu S C 2001a Differential expression of the fibroin gene in developmental stages of silkworm, *Antheraea mylitta* (Saturniidae) *Comparative Biochem. Physiol.* B **129** 197–204

Datta A, Ghosh A K and Kundu S C 2001b Purification and characterisation of fibroin from the tropical Saturniid silkworm, *Antheraea mylitta Insect Biochem. Mol. Biol.* **31** 1013–8

DeBari M K, King C I, Altgold T A and Abbott R D 2021 Silk fibroin as a green material *ACS Biomater. Sci. Eng.* **7** 3530–44

Deori M, Devi D, Kumari S, Hazarika A, Kalita H, Sarma R and Devi R 2016 Antioxidant effect of sericin in brain and peripheral tissues of oxidative stress induced hypercholesterolemic rats *Front. Pharmacol.* **7** 319

Dhandayuthapani B, Yoshida Y, Maekawa T and Kumar D S 2011 Polymeric scaffolds in tissue engineering application: a review *Int. J. Polym. Sci.* **2011** 1–19

Dutta S, Chowdhury T and Ghosh A K 2020 Green synthesis of poly-L-lysine-coated sericin nanoparticles and their molecular size-dependent antibacterial activity *Colloids Surf.* B **188** 110822

Fang G, Sapru S, Behera S, Yao J, Shao Z, Kundu S C and Chen X 2016 Exploration of the tight structural-mechanical relationship in mulberry and non-mulberry silkworm silks *J. Mater. Chem.* B **4** 4337–47

Freddi G, Mossotti R and Innocenti R 2003 Degumming of silk fabric with several proteases *J. Biotechnol.* **106** 101–12

Gaharwar A K, Singh I and Khademhosseini A 2020 Engineered biomaterials for *in situ* tissue regeneration *Nat. Rev. Mater.* **5** 686–705

Gholipourmalekabadi M, Sapru S, Samadikuchaksaraei A, Reis R L, Kaplan D L and Kundu S C 2020 Silk fibroin for skin injury repair: where do things stand? *Adv. Drug Deliv. Rev.* **153** 28–53

Gilotra S, Chouhan D, Bhardwaj N, Nandi S K and Mandal B B 2018 Potential of silk sericin based nanofibrous mats for wound dressing applications *Mater. Sci. Eng.* C **90** 420–32

Gogurla N, Sinha A K, Naskar D, Kundu S C and Ray S K 2016 Metal nanoparticles triggered persistent negative

photoconductivity in silk protein hydrogels *Nanoscale* **8** 7695–703
Goujon N, Rajkhowa R, Wang X and Byrne N 2013 Effect of solvent on ionic liquid dissolved regenerated *Antheraea assamensis* silk fibroin *J. Appl. Polym. Sci.* **128** 4411–6
Griffith L G 2002 Tissue engineering–current challenges and expanding opportunities *Science* **295** 1009–14
Guo C, Li C, Mu X and Kaplan D L 2020 Engineering silk materials: from natural spinning to artificial processing *Appl. Phys. Rev.* **7** 011313
Guo C, Zhang J, Wang X, Nguyen A T, Liu X Y and Kaplan D L 2017 Comparative study of strain-dependent structural changes of silkworm silks: insight into the structural origin of strain-stiffening *Small* **13** 1702266
Gupta A K, Mita K, Arunkumar K P and Nagaraju J 2015 Molecular architecture of silk fibroin of Indian golden silkmoth *Antheraea Assama. Sci. Rep.* **5** 12706
Gupta P, Adhikary M, Joseph Christakiran M, Kumar M, Bhardwaj N and Mandal B B 2016a Biomimetic, osteoconductive non-mulberry silk fibre reinforced tricomposite scaffolds for bone tissue engineering *ACS Appl. Mater. Interfaces* **8** 30797–810
Gupta P, Kumar M, Bhardwaj N, Kumar J P, Krishnamurthy C S, Nandi S K and Mandal B B 2016b Mimicking form and function of native small diameter vascular conduits using mulberry and non-mulberry patterned silk films *ACS Appl. Mater. Interfaces* **8** 15874–88
Halder P K, Naskar D, Kumar A, Yao J, Kundu S C and Ghosh A S 2015 Potential mode of protection of silkworm pupae from environmental stress by harbouring the bacterial biofilm on the surfaces of silk cocoons *Curr. Microbiol.* **70** 228–34
Hazra S, Nandi S, Naskar D, Guha R, Chowdhury S, Pradhan N, Kundu S C and Konar A 2016 Non-mulberry silk fibroin biomaterial for corneal regeneration *Sci. Rep.* **6** 21840
Hersel U, Dahmen C and Kessler H 2003 RGD modified polymers: biomaterials for stimulated cell adhesion and beyond *Biomaterials* **24** 4385–415
Hideki S and Yukuhiro K 2000 Dynamic rearrangement within the *Antheraea pernyi* silk fibroin gene is associated with four types of repetitive units *J. Mol. Evol.* **51** 329–38
Holland C, Numata K, Rnjak-Kovacina J and Seib F P 2019 The biomedical use of silk: past, present, future *Adv. Healthcare Mater.* **8** 1800465
Hota M K, Bera M K, Kundu B, Kundu S C and Maiti C K 2012 A natural silk fibroin protein-based transparent bio-memristor *Adv. Funct. Mater.* **22** 4493–9
Hu F, Lin N and Liu X Y 2020 Interplay between light and functionalised silk fibroin and applications *IScience* **23** 101035
Hwang J S *et al* 2001 Cloning of the fibroin gene from the oak silkworm, *Antheraea yamamai* and its complete sequence *Biotechnol. Lett.* **23** 1321–6
Jagur-Grodzinski J 2006 Polymers for tissue engineering, medical devices, and regenerative medicine. concise general review of recent studies *Polym. Adv. Technol.* **17** 395–418
Jena K, Pandey J P, Kumari R, Sinha A K, Gupta V P and Singh G P 2018 Tasar silk fibre waste sericin: new source for anti-elastase, anti-tyrosinase and antioxidant compounds *Int. J. Biol. Macromol.* **114** 1102–8
Jiao Z *et al* 2017 *In vivo* characterisations of the immune properties of sericin: an ancient material with emerging value in biomedical applications *Macromol. Biosci.* **17** 1700229
Johansson U, Widhe M, Shalaly N D, Arregui I L, Nilebäck L, Tasiopoulos C P, Åstrand C, Berggren P-O, Gasser C and Hedhammar M 2019 Assembly of functionalised silk together with cells to obtain proliferative 3D cultures integrated in a network of ECM-like microfibres *Sci. Rep.* **9** 6291
Joseph C M, Reardon P J T, Konwarh R, Knowles J C and Mandal B B 2017 Mimicking hierarchical complexity of the osteochondral interface using electrospun silk-bioactive glass composites *ACS Appl. Mater. Interfaces* **9** 8000–13
Kar S, Talukdar S, Pal S, Nayak S, Paranjape P and Kundu S C 2013 Silk gland fibroin from Indian muga silkworm *Antheraea assama* as potential biomaterial *Tissue Eng. Regen. Med.* **10** 200–10
Kato N and Iwami K 2002 Resistant protein; its existence and function beneficial to health *J. Nutr. Sci. Vitaminol.* **48** 1–5
Khampieng T, Aramwit P and Supaphol P 2015 Silk sericin loaded alginate nanoparticles: preparation and anti-inflammatory efficacy *Int. J. Biol. Macromol.* **80** 636–43
Khire T S, Kundu J, Kundu S C and Yadavalli V K 2010 The fractal self-assembly of the silk protein sericin *Soft Matter* **6** 2066–71
Kim K, Yu M, Zong X, Chiu J, Fang D, Seo Y-S, Hsiao B S, Chu B and Hadjiargyrou. M 2003 Control of degradation rate and hydrophilicity in electrospun non-woven poly(D,L-lactide) nanofibre scaffolds for biomedical applications *Biomaterials* **24** 4977–85
Kim S H *et al* 2018 Precisely printable and biocompatible silk fibroin bioink for digital light processing 3D printing *Nat. Commun.* **9** 1–14
Kim S-R *et al* 2017 Genome sequence of the Japanese oak silk moth, *Antheraea yamamai*: the first draft genome in the family Saturniidae *GigaScience* **7** 1–11
Kinahan M E, Filippidi E, Köster S, Hu X, Evans H M, Pfohl T, Kaplan D L and Wong J 2011 Tunable silk: using microfluidics to fabricate silk fibres with controllable properties *Biomacromolecules* **12** 1504–11
Konwarh R, Bhunia B K and Mandal B B 2017 Opportunities and challenges in exploring indian non-mulberry silk for biomedical applications *Proc. Indian Natl Sci. Acad.* **83** 85–101
Koons G L, Diba M and Mikos A G 2020 Materials design for bone-tissue engineering *Nat. Rev. Mater.* **5** 584–603
Kricheldorf H R, Müller D and Ziegler K 1983 Secondary structure of peptides—9. 13C NMR CP/MAS investigation of silk proteins *Polym. Bull.* **9** 284–91
Kumar G B, Melikov R, Aria M M, Yalcin A U, Begar E, Sadeghi S, Guven K and Nizamoglu S 2018 Silk-based aqueous microcontact printing *ACS Biomater. Sci. Eng.* **4** 1463–70
Kumar J P and Mandal B B 2017 Antioxidant potential of mulberry and non-mulberry silk sericin and its implications in biomedicine *Free Radic. Biol. Med.* **108** 803–18
Kundu B and Kundu S C 2013 Bio-inspired fabrication of fibroin cryogels from the muga silkworm *Antheraea assamensis* for liver tissue engineering *Biomed. Mater.* **8** 055003
Kundu B, Kurland N E, Bano S, Patra C, Engel F B, Yadavalli V K and Kundu S C 2014a Silk proteins for biomedical applications: bioengineering perspectives *Prog. Polym. Sci.* **39** 251–67
Kundu B, Kurland N E, Yadavalli V K and Kundu S C 2014b Isolation and processing of silk proteins for biomedical applications *Int. J. Biol. Macromol.* **70** 70–77
Kundu B, Saha P, Datta K and Kundu S C 2013 A silk fibroin based hepatocarcinoma model and the assessment of the drug response in hyaluronan-binding protein 1 overexpressed HepG2 cells *Biomaterials* **34** 9462–74
Kundu J, Chung Y-I, Kim Y H, Tae G and Kundu S C 2010 Silk fibroin nanoparticles for cellular uptake and control release *Int. J. Pharm.* **388** 242–50
Kundu S C *et al* 2012 Invited review: non-mulberry silk biopolymers *Biopolymers* **97** 455–67
Kundu S C, Dash B C, Dash R and Kaplan D L 2008b Natural protective glue protein, sericin bioengineered by silkworms: potential for biomedical and biotechnological applications *Prog. Polym. Sci.* **33** 998–1012
Kunz R I, Brancalhão R M C, De Ribeiro L F C and Natali M R M 2016 Silkworm sericin: properties and biomedical applications *Biomed. Res. Int.* **2016** 1–19
Kurioka A, Kurioka F and Yamazaki M 2004 Characterisation of sericin powder prepared from citric acid-degraded sericin

polypeptides of the silkworm, *Bombyx mori Biosci. Biotechnol. Biochem.* **68** 774–80
Kurland N E, Dey T, Kundu S C and Yadavalli V K 2013 Precise patterning of silk microstructures using photolithography *Adv. Mater.* **25** 6207–12
Kuwana Y, Sezutsu H, Nakajima K, Tamada Y and Kojima K 2014 High-toughness silk produced by a transgenic silkworm expressing spider (*Araneus ventricosus*) dragline silk protein *PLoS One* **9** e105325
Langer R and Vacanti J 1993 Tissue engineering *Science* **260** 920–6
Li G J, Wang J, Sun S and Lu S Z 2013 Study on *Antheraea pernyi* silk fibroin microspheres carried drug *Adv. Mater. Res.* **796** 117–20
Li S, Hang Y, Ding Z, Lu O, Lu G, Chen H and Kaplan D L 2020 Microfluidic silk fibres with aligned hierarchical microstructures *ACS Biomater. Sci. Eng.* **6** 2847–54
Li X, Li M, Zhang Q, Yan S and You R 2016 Aqueous-based electrospinning of regenerated *Antheraea pernyi* silk fibroin *Fibres Polym.* **17** 1421–7
Li X, Zhang J, Feng Y, Yan S, Zhang Q and You R 2018 Tuning the structure and performance of silk biomaterials by combining mulberry and non-mulberry silk fibroin *Polym. Degrad. Stabil.* **147** 57–63
Lim K S, Kundu J, Reeves A, Poole-Warren L A, Kundu S C and Martens P J 2012 The influence of silkworm species on cellular interactions with novel PVA/silk sericin hydrogels *Macromol. Biosci.* **12** 322–32
Liu G, Xiong S, You R, Wang L and Li M 2013 Ultrasonication-induced rapid gelation of wild silkworm silk fibroins *Adv. Mater. Res.* **634–638** 1165–9
Lu L, Fan S, Geng L, Yao X and Zhang Y 2021 Low-loss light-guiding, strong silk generated by a bioinspired microfluidic chip *Chem. Eng. J.* **405** 126793
Lu L, Fan S, Niu Q, Peng O, Geng L, Yang G, Shao H, Hsiao B S and Zhang Y 2019 Strong silk fibres containing cellulose nanofibres generated by a bioinspired microfluidic chip *ACS Sustain. Chem. Eng.* **7** 14765–74
Lu P, Takai K, Weaver V M and Werb Z 2011 Extracellular matrix degradation and remodelling in development and disease *Cold Spring Harb. Perspect. Biol.* **3** a005058–a005058
Luan X-Y, Wang Y, Duan X, Duan Q-Y, Li M-Z, Lu S-Z, Zhang H-X and Zhang X-G 2006 Attachment and growth of human bone marrow derived mesenchymal stem cells on regenerated *Antheraea pernyi* silk fibroin films *Biomed. Mater.* **1** 181–7
Lv Q and Feng Q 2006 Preparation of 3D regenerated fibroin scaffolds with freeze drying method and freeze drying/foaming technique *J. Mater. Sci. Mater. Med.* **17** 1349–56
Mai-Ngam K, Boonkitpattarakul K, Jaipaew J and Mai-Ngam B 2011 Evaluation of the properties of silk fibroin films from the non-mulberry silkworm *Samia cynthia ricini* for biomaterial design *J. Biomater. Sci. Polym. Ed.* **22** 2001–22
Mandal B B, Das S, Choudhury K and Kundu S C 2010b Implication of silk film RGD availability and surface roughness on cytoskeletal organisation and proliferation of primary rat bone marrow cells *Tissue Eng.* A **16** 2391–403
Mandal B B, Das T and Kundu S C 2009b Non-bioengineered silk gland fibroin micromolded matrices to study cell-surface interactions *Biomed. Microdevices* **11** 467–76
Mandal B B, Ghosh B and Kundu S C 2011 Non-mulberry silk sericin/poly (vinyl alcohol) hydrogel matrices for potential biotechnological applications *Int. J. Biol. Macromol.* **49** 125–33
Mandal B B and Kundu S C 2008a A novel method for dissolution and stabilisation of non-mulberry silk gland protein fibroin using anionic surfactant sodium dodecyl sulfate *Biotechnol. Bioeng.* **99** 1482–9
Mandal B B and Kundu S C 2008b Non-bioengineered silk fibroin protein 3D scaffolds for potential biotechnological and tissue engineering applications *Macromol. Biosci.* **8** 807–18
Mandal B B and Kundu S C 2008c Non-bioengineered silk gland fibroin protein: characterisation and evaluation of matrices for potential tissue engineering applications *Biotechnol. Bioeng.* **100** 1237–50
Mandal B B and Kundu S C 2009a Osteogenic and adipogenic differentiation of rat bone marrow cells on non-mulberry and mulberry silk gland fibroin 3D scaffolds *Biomaterials* **30** 5019–30
Mandal B B and Kundu S C 2009d Self-assembled silk sericin/poloxamer nanoparticles as nanocarriers of hydrophobic and hydrophilic drugs for targeted delivery *Nanotechnology* **20** 355101
Mandal B B and Kundu S C 2010a Biospinning by silkworms: silk fibre matrices for tissue engineering applications *Acta Biomater.* **6** 360–71
Mandal B B, Priya A S and Kundu S C 2009c Novel silk sericin/gelatin 3D scaffolds and 2D films: fabrication and characterisation for potential tissue engineering applications *Acta Biomater.* **5** 3007–20
Manesa K C, Kebede T G, Dube S and Nindi M M 2020 Profiling of silk sericin from cocoons of three southern African wild silk moths with a focus on their antimicrobial and antioxidant properties *Materials* **13** 5706
Mao A S and Mooney D J 2015 Regenerative medicine: current therapies and future directions *Proc. Natl Acad. Sci.* **112** 14452–9
Mazzi S, Zulker E, Buchicchio J, Anderson B and Hu X 2014 Comparative thermal analysis of Eri, Mori, Muga, and Tussar silk cocoons and fibroin fibres *J. Therm. Anal. Calorim.* **116** 1337–43
Mehrotra S, De Melo B A G, Hirano M, Keung W, Li R A, Mandal B B and Shin S R 2020 Non-mulberry silk based ink for fabricating mechanically robust cardiac patches and endothelialised myocardium-on-a-chip application *Adv. Funct. Mater.* **30** 1907436
Midha S, Chameettachal S, Dey E and Ghosh S 2017 Non-mulberry silk braids direct terminal osteocytic differentiation through activation of wnt-signaling *ACS Biomater. Sci. Eng.* **3** 1062–74
Midha S, Chawla S, Chakraborty J, Chameettachal S and Ghosh S 2018 Differential regulation of hedgehog and parathyroid signalling in mulberry and non-mulberry silk fibroin textile braids *ACS Biomater. Sci. Eng.* **4** 595–607
Minoura N, Aiba S I, Higuchi M, Gotoh Y, Tsukada M and Imai Y 1995 Attachment and growth of fibroblast cells on silk fibroin *Biochem. Biophys. Res. Commun.* **208** 511–6
Mori H and Tsukada M 2000 New silk protein: modification of silk protein by gene engineering for production of biomaterials *Rev. Mol. Biotechnol.* **74** 95–103
Moses J C, Nandi S K and Mandal B B 2018 Multifunctional cell instructive silk-bioactive glass composite reinforced scaffolds toward osteoinductive, proangiogenic, and resorbable bone grafts *Adv. Healthcare Mater.* **7** 1701418
Moses J C, Saha T and Mandal B B 2020 Chondroprotective and osteogenic effects of silk-based bioinks in developing 3D bioprinted osteochondral interface *Bioprinting* **17** e00067
Mottaghitalab F, Farokhi M, Shokrgozar M A, Atyabi F and Hosseinkhani H 2015 Silk fibroin nanoparticle as a novel drug delivery system *J. Control. Release* **206** 161–76
Mouriño V, Cattalini J P, Roether J A, Dubey P, Roy I and Boccaccini A R 2013 Composite polymer-bioceramic scaffolds with drug delivery capability for bone tissue engineering *Expert. Opin. Drug Deliv.* **10** 1353–65
Muffly T M, Tizzano A P and Walters M D 2011 The history and evolution of sutures in pelvic surgery *J. R. Soc. Med.* **104** 107–12
Napavichayanun S, Yamdech R and Aramwit P 2016 The safety and efficacy of bacterial nanocellulose wound dressing incorporating sericin and polyhexamethylene biguanide: *in vitro, in vivo*, and clinical studies *Arch. Dermatol. Res.* **308** 123–32
Naskar D, Barua R R, Ghosh A K and Kundu S C 2014 Introduction to silk biomaterials *Silk Biomaterials for Tissue Engineering and Regenerative Medicine* (Amsterdam: Elsevier) pp 3–40

Naskar D, Bhattacharjee P, Ghosh A K, Mandal M and Kundu S C 2017a Carbon nanofibre reinforced non-mulberry silk protein fibroin nanobiocomposite for tissue engineering applications *ACS Appl. Mater. Interfaces* **9** 19356–70

Naskar D, Ghosh A K, Mandal M, Das P, Nandi S K and Kundu S C 2017b Dual growth factor loaded Non-mulberry silk fibroin/carbon nanofibre composite 3D scaffolds for *in vitro*and *in vivo* bone regeneration *Biomaterials* **136** 67–85

Naskar D, Nayak S, Dey T and Kundu S C 2015 Non-mulberry silk fibroin influence osteogenesis and osteoblast-macrophage cross talk on titanium-based surface *Sci. Rep.* **4** 4745

Nayak S, Dey S and Kundu S C 2014 Silk sericin–alginate–chitosan microcapsules: hepatocytes encapsulation for enhanced cellular functions *Int. J. Biol. Macromol.* **65** 258–66

Nayak S, Dey T, Naskar D and Kundu S C 2013 The promotion of osseointegration of titanium surfaces by coating with silk protein sericin *Biomaterials* **34** 2855–64

Nayak S and Kundu S C 2014 Sericin-carboxymethyl cellulose porous matrices as cellular wound dressing material *J. Biomed. Mater. Res.* A **102** 1928–40

Nayak S, Talukdar S and Kundu S C 2012 Potential of 2D crosslinked sericin membranes with improved biostability for skin tissue engineering *Cell Tissue Res.* **347** 783–94

Neog K 2020 Gap for sustainable production of Eri and Muga *Integrated Farming System for Sustainable Agriculture (April, 2020 Assam Agricultural University, Jorhat, Assam, India)*

Nguyen T P, Nguyen Q V, Nguyen V H, Le T H, Huynh V Q N, Vo D V N, Trinh Q T, Kim S Y and Le Q V 2019 Silk fibroin-based biomaterials for biomedical applications: a review *Polymers* **11** 1933

Offord C, Vollrath F and Holland C 2016 Environmental effects on the construction and physical properties of *Bombyx mori* cocoons *J. Mater. Sci.* **51** 10863–72

Oh H, Lee J Y, Kim M K, Um I C and Lee K H 2011 Refining hot-water extracted silk sericin by ethanol-induced precipitation *Int. J. Biol. Macromol.* **48** 32–37

Pal S, Kundu J, Talukdar S, Thomas T and Kundu S C 2013 An emerging functional natural silk biomaterial from the only domesticated non-mulberry silkworm *Samia ricini*: an emerging functional natural silk biomaterial *Macromol. Biosci.* **13** 1020–35

Panda N N, Biswas A, Pramanik K and Jonnalagadda S 2015b Enhanced osteogenic potential of human mesenchymal stem cells on electrospun nanofibrous scaffolds prepared from eri-tasar silk fibroin *J. Biomed. Mater. Res.* B **103** 971–82

Panda N, Bissoyi A, Pramanik K and Biswas A 2015a Development of novel electrospun nanofibrous scaffold from *P. ricini* and *A. mylitta* silk fibroin blend with improved surface and biological properties *Mater. Sci. Eng.* C **48** 521–32

Panja S, Behera S, Kundu S C and Halder M 2017 Optical spectroscopic and morphological characterisations of curcuminised silk biomaterials: a perspective from drug stabilisation *ACS Omega* **2** 6755–67

Park A R, Park Y H, Kim H J, Kim M K, Kim S G, Kweon H Y and Kundu S C 2015 Tri-layered silk fibroin and poly-ε-caprolactone small diameter vascular grafts tested *in vitro* and *in vivo Macromol. Res.* **23** 924–36

Patra C, Talukdar S, Novoyatleva T, Velagala S R, Mühlfeld C, Kundu B, Kundu S C and Engel F B 2012 Silk protein fibroin from *Antheraea mylitta* for cardiac tissue engineering *Biomaterials* **33** 2673–80

Perotto G, Zhang Y, Naskar D, Patel N, Kaplan D L, Kundu S C and Omenetto F G 2017 The optical properties of regenerated silk fibroin films obtained from different sources *Appl. Phys. Lett.* **111** 103702

Piskin E 1995 Biodegradable polymers as biomaterials *J. Biomater. Sci. Polym. Ed.* **6** 775–95

Pradhan S, Brooks A K and Yadavalli V K 2020 Nature-derived materials for the fabrication of functional biodevices *Mater. Today Bio.* **7** 100065

Pradhan S and Yadavalli V K 2021 Photolithographically printed flexible silk/PEDOT:PSS temperature sensors *ACS Appl. Electron. Mater.* **3** 21–29

Qi C, Liu J, Jin Y, Xu L M, Wang G B, Wang Z and Wang L 2018 Photocrosslinkable, injectable sericin hydrogel as 3D biomimetic extracellular matrix for minimally invasive repairing cartilage *Biomaterials* **163** 89–104

Rockwood D N, Preda R C, Yücel T, Wang X, Lovett M L and Kaplan D L 2011 Materials fabrication from *Bombyx mori* silk fibroin *Nat. Protoc.* **6** 1612–31

Saha S, Kundu B, Kirkham J, Wood D, Kundu S C and Yang X B 2013 Osteochondral tissue engineering *in vivo*: a comparative study using layered silk fibroin scaffolds from mulberry and non-mulberry silkworms *PLoS One* **8** e80004

Sahu N *et al* 2015 Non-mulberry silk fibroin scaffold shows superior osteoconductivity than mulberry silk fibroin in calvarial bone regeneration *Adv. Healthcare Mater.* **4** 1709–21

Sahu N, Pal S, Sapru S, Kundu J, Talukdar S, Singh N I, Yao J and Kundu S C 2016 Non-mulberry and mulberry silk protein sericins as potential media supplement for animal cell culture *Biomed. Res. Int.* **2016** 13

Saotome T, Hayashi H, Tanaka R, Kinugasa A, Uesugi S, Tatematsu K, Sezutsu H, Kuwabara N and Asakura T 2015 Introduction of VEGF or RGD sequences improves revascularisation properties of *Bombyx mori* silk fibroin produced by transgenic silkworm *J. Mater. Chem.* B **3** 7109–16

Sapru S, Das S, Mandal M, Ghosh A K and Kundu S C 2018 Prospects of non-mulberry silk protein sericin-based nanofibrous matrices for wound healing—*in vitro* and *in vivo* investigations *Acta Biomater.* **78** 137–50

Sapru S, Das S, Mandal M, Ghosh A K and Kundu S C 2019 Non-mulberry silk protein sericin blend hydrogels for skin tissue regeneration-*in vitro* and *in vivo Int. J. Biol. Macromol.* **137** 545–53

Sapru S, Das S, Mandal M, Ghosh A K and Kundu S C 2021 Sericin-chitosan-glycosaminoglycan hydrogels incorporated with growth factors for *in vitro* and *in vivo* skin repair *Carbohydr. Polym.* **258** 117717

Sapru S, Ghosh A K and Kundu S C 2017 Non-immunogenic, porous and antibacterial chitosan and *Antheraea mylitta* silk sericin hydrogels as potential dermal substitute *Carbohydr. Polym.* **167** 196–209

Schneider M R, Schmidt-Ullrich R and Paus R 2009 The hair follicle as a dynamic miniorgan *Curr. Biol.* **19** R132–42

Sehnal F and Zurovec M 2004 Construction of silk fibre core in Lepidoptera *Biomacromolecules* **5** 666–74

Sen K and Babu K M 2004a Studies on Indian silk. I. macro characterisation and analysis of amino acid composition *J. Appl. Polym. Sci.* **92** 1080–97

Sen K and Babu K, M 2004b Studies on Indian silk. II. structure-property correlations *J. Appl. Polym. Sci.* **92** 1098–115

Sen S, Basak P, Sinha P B, Maurye P, Jaiswal K K, Das P and Mandal T K 2020 Anti-inflammatory effect of epidermal growth factor conjugated silk fibroin immobilised polyurethane ameliorates diabetic burn wound healing *Int. J. Biol. Macromol.* **143** 1009–32

Senakoon W, Nuchadomrong S, Sirimungkararat S, Senawong T and Kitikoon P 2009 Antibacterial action of Eri (*Samia ricini*) sericin against *Escherichia coli* and *Staphylococcus aureus Asian J. Food Agro-Industry* **2** S222–8

Sezutsu H, Uchino K, Kobayashi I, Tamura T and Yukuhiro K 2010 Extensive sequence rearrangements and length polymorphism in fibroin genes in the wild silkmoth, *Antheraea yamamai* (Lepidoptera, Saturniidae) *Int. J. Wild Silkmoth Silk* **15** 35–50

Shafiee A and Atala A 2017 Tissue engineering: toward a new era of medicine *Annu. Rev. Med.* **68** 29–40

Sharma S, Bano S, Ghosh A S, Mandal M, Kim H W, Dey T and Kundu S C 2016 Silk fibroin nanoparticles support *in vitro*

sustained antibiotic release and osteogenesis on titanium surface *Nanomedicine* **12** 1193–204
Shaw J T B and Smith S G 1961 Comparative studies of fibroins. IV. The composition and structure of chemically resistant fractions from some silk fibroins *BBA—Biochimica Et Biophysica Acta* **52** 305–18
Shivananda C S, Rao B L and Sangappa 2020 Structural, thermal and electrical properties of silk fibroin–silver nanoparticles composite films *J. Mater. Sci.: Mater. Electron.* **31** 41–51
Silva S S, Gomes J M, Vale A C, Lu S, Reis R L and Kundu S C 2020 Green pathway for processing non-mulberry *Antheraea pernyi* silk fibroin/chitin-based sponges: biophysical and biochemical characterisation *Front. Mater.* **7** 135
Silva S S, Kundu B, Lu S, Reis R L and Kundu S C 2019 Chinese Oak Tasar silkworm *Antheraea pernyi* silk proteins: current strategies and future perspectives for biomedical applications *Macromol. Biosci.* **19** 1800252
Silva S S, Popa E G, Gomes M E, Oliveira M B, Nayak S, Subia B, Mano J F, Kundu S C and Reis R L 2013 Silk hydrogels from non-mulberry and mulberry silkworm cocoons processed with ionic liquids *Acta Biomater.* **9** 8972–82
Singh Y P, Adhikary M, Bhardwaj N, Bhunia B K and Mandal B B 2017 Silk fibre reinforcement modulates *in vitro* chondrogenesis in 3D composite scaffolds *Biomed. Mater.* **12** 045012
Singh Y P, Bhardwaj N and Mandal B B 2016 Potential of agarose/silk fibroin blended hydrogel for *in vitro* cartilage tissue engineering *ACS Appl. Mater. Interfaces* **8** 21236–49
Singh Y P, Moses J C, Bhunia B K, Nandi S K and Mandal B B 2018 Hierarchically structured seamless silk scaffolds for osteochondral interface tissue engineering *J. Mater. Chem.* B **6** 5671–88
Siritienthong T, Ratanavaraporn J and Aramwit P 2012 Development of ethyl alcohol-precipitated silk sericin/polyvinyl alcohol scaffolds for accelerated healing of full thickness wounds *Int. J. Pharm.* **439** 175–86
Siritientong T, Srichana T and Aramwit P 2011 The effect of sterilisation methods on the physical properties of silk sericin scaffolds *Aaps Pharmscitech.* **12** 771–81
Sofia S, McCarthy M B, Gronowicz G and Kaplan D L 2001 Functionalised silk-based biomaterials for bone formation *J. Biomed. Mater. Res.* **54** 139–48
Srivastava C M and Purwar R 2017 Chitosan-finished *Antheraea mylitta* silk fibroin nonwoven composite films for wound dressing *J. Appl. Polym. Sci.* **134** 44341
Srivastava C M and Purwar R 2018 Fabrication of 3D self-assembled non-mulberry *Antheraea mylitta* (Tasar) fibroin nonwoven mats for wound dressing applications *Macromol. Res.* **26** 872–81
Srivastava C M, Purwar R and Gupta A P 2019 Enhanced potential of biomimetic, silver nanoparticles functionalised *Antheraea mylitta* (Tasar) silk fibroin nanofibrous mats for skin tissue engineering *Int. J. Biol. Macromol.* **130** 437–53
Srivastava C M, Purwar R, Gupta A and Sharma D 2017 Dextrose modified flexible Tasar and Muga fibroin films for wound healing applications *Mater. Sci. Eng.* C **75** 104–14
Stoddart A 2017 Hydrogels: a less than swell time *Nat. Rev. Mater.* **2** 17018
Subia B, Chandra S, Talukdar S and Kundu S C 2014 Folate conjugated silk fibroin nanocarriers for targeted drug delivery *Integrat. Biol.* **6** 203–14
Subia B, Dey T, Sharma S and Kundu S C 2015 Target specific delivery of anticancer drug in silk fibroin based 3D distribution model of bone–breast cancer cells *ACS Appl. Mater. Interfaces* **7** 2269–79
Sundar S, Kundu J and Kundu S C 2010 Biopolymeric nanoparticles *Sci. Technol. Adv. Mater.* **11** 014104
Talukdar S and Kundu S C 2012 A non-mulberry silk fibroin protein based 3D *in vitro* tumour model for evaluation of anticancer drug activity *Adv. Funct. Mater.* **22** 4778–88
Talukdar S and Kundu S C 2013 Engineered 3D silk-based metastasis models: interactions between human breast adenocarcinoma, mesenchymal stem cells and osteoblast-like cells *Adv. Funct. Mater.* **23** 5249–60
Talukdar S, Mandal M, Hutmacher D W, Russell P J, Soekmadji C and Kundu S C 2011a Engineered silk fibroin protein 3D matrices for *in vitro* tumour model *Biomaterials* **32** 2149–59
Talukdar S, Nguyen Q T, Chen A C, Sah R L and Kundu S C 2011b Effect of initial cell seeding density on 3D-engineered silk fibroin scaffolds for articular cartilage tissue engineering *Biomaterials* **32** 8927–37
Tao H, Marelli B, Yang M, An B, Onses M S, Rogers J A, Kaplan D L and Omenetto F G 2015 Inkjet printing of regenerated silk fibroin: from printable forms to printable functions *Adv. Mater.* **27** 4273–9
Ter Horst B, Chouhan G, Moiemen N S and Grover L M 2018 Advances in keratinocyte delivery in burn wound care *Adv. Drug Deliv. Rev.* **123** 18–32
Teramoto H and Miyazawa M 2005 Molecular orientation behaviour of silk sericin film as revealed by ATR infrared spectroscopy *Biomacromolecules* **6** 2049–57
Teule F, Miao Y-G, Sohn B-H, Kim Y-S, Hull J J, Fraser M J, Lewis R V and Jarvis D L 2012 Silkworms transformed with chimeric silkworm/spider silk genes spin composite silk fibres with improved mechanical properties *Proc. Natl Acad. Sci.* **109** 923–8
Thurber A E, Omenetto F G and Kaplan D L 2015 *In vivo* bioresponses to silk proteins *Biomaterials* **71** 145–57
Torres T, Troncoso O P, Pisani A, Gatto F and Bardi G 2019 Natural polysaccharide nanomaterials: an overview of their immunological properties *Int. J. Mol. Sci.* **20** 5092
Valluzzi R, Winkler S, Wilson D and Kaplan D L 2002 Silk: molecular organisation and control of assembly *Philos. Trans. R. Soc.* B **357** 165–7
Vepari C and Kaplan D L 2007 Silk as a biomaterial *Prog. Polym. Sci.* **32** 991–1007
Vidal G, Blanchi T, Mieszawska A J, Calabrese R, Rossi C, Vigneron P, Duval J L, Kaplan D L and Egles C 2013 Enhanced cellular adhesion on titanium by silk functionalised with titanium binding and RGD peptides *Acta Biomater.* **9** 4935–43
Wang F, Wolf N, Rocks E-M, Vuong T and Hu X 2015 Comparative studies of regenerated water-based Mori, Thai, Eri, Muga and Tussah silk fibroin films *J. Therm. Anal. Calorim.* **122** 1069–76
Wang H, Zhu B, Wang H, Ma X, Hao Y and Chen X 2016 Ultra-lightweight resistive switching memory devices based on silk fibroin *Small* **12** 3360–5
Wang J, Li G J, Jiang R, Yin Z P and Lu S Z 2014 *Antheraea pernyi* silk fibroin microspheres carried lysozyme *Adv. Mater. Res.* **915–916** 875–8
Warwicker J O 1956 The crystal structure of silk fibroins *Trans. Faraday Soc.* **52** 554–7
Wilczewska A Z, Niemirowicz K, Markiewicz K H and Car H 2012 Nanoparticles as drug delivery systems *Pharmacol. Rep.* **64** 1020–37
Williams D F 2019 Challenges with the development of biomaterials for sustainable tissue engineering *Front. Bioeng. Biotechnol.* **7** 1–10
Wohlrab S, Müller S, Schmidt A, Neubauer S, Kessler K, Leal-Egaña A and Scheibel T 2012 Cell adhesion and proliferation on RGD-modified recombinant spider silk proteins *Biomaterials* **33** 6650–9
Xing H, Lee H, Luo L and Kyriakides T R 2020 Extracellular matrix-derived biomaterials in engineering cell function *Biotechnol. Adv.* **42** 107421
Xu J, Dong Q, Yu Y, Niu B, Ji D, Li M, Huang Y, Chen X and Tan A 2018 Mass spider silk production through targeted gene replacement in *Bombyx mori*. *Proc. Natl Acad. Sci.* **115** 8757–62
Xue R, Liu Y L, Zhang Q S, Liang C C, Qin H Z, Liu P F, Wang K, Zhang X Y, Chen L and Wei Y 2016 Shape changes and interaction mechanism of *Escherichia coli* cells treated with sericin and use of a sericin-based hydrogel for wound healing *Appl. Environ. Microbiol.* **82** 4663–72

Xue Y, Jao D, Hu W and Hu X 2017 Silk-silk blend materials: a comparative study of Mori-Tussah, Mori-Muga, Mori-Eri, and Mori-Thai silk films *J. Therm. Anal. Calorim.* **127** 915–21

Yamaguchi K, Kikuchi Y, Takagi T, Kikuchi A, Oyama F, Shimura K and Mizuno S 1989 Primary structure of the silk fibroin light chain determined by cDNA sequencing and peptide analysis *J. Mol. Biol.* **210** 127–39

Yang J, Anishchenko I, Park H, Peng Z, Ovchinnikov S and Baker D 2020 Improved protein structure prediction using predicted interresidue orientations *Proc. Natl Acad. Sci.* **117** 1496–503

Yang M, Shuai Y, Zhang C, Chen Y, Zhu L, Mao C and Ouyang H 2014 Biomimetic nucleation of hydroxyapatite crystals mediated by *Antheraea pernyi* silk sericin promotes osteogenic differentiation of human bone marrow derived mesenchymal stem cells *Biomacromolecules* **15** 1185–93

Yao D, Dong S, Lu Q, Hu Q, Kaplan D L, Zhang B and Zhu H 2012 Salt-leached silk scaffolds with tunable mechanical properties *Biomacromolecules* **13** 3723–9

You R, Xu Y, Liu Y, Li X and Li M 2015 Comparison of the *in vitro* and *in vivo* degradations of silk fibroin scaffolds from mulberry and non-mulberry silkworms *Biomed. Mater.* **10** 015003

Yuk H, Zhang T, Parada G, Liu X and Zhao X 2016 Skin-inspired hydrogel–elastomer hybrids with robust interfaces and functional microstructures *Nat. Commun.* **7** 12028

Yukuhiro K, Kanda T and Tamura T 1997 Preferential codon usage and two types of repetitive motifs in the fibroin gene of the Chinese oak silkworm, *Antheraea pernyi Insect Mol. Biol.* **6** 89–95

Yun H, Oh H, Kim M K, Kwak H W, Lee J Y, Um I C, Vootla S K and Lee K H 2013 Extraction conditions of *Antheraea mylitta* sericin with high yields and minimum molecular weight degradation *Int. J. Biol. Macromol.* **52** 59–65

Zelikin A N 2010 Drug releasing polymer thin films: new era of surface-mediated drug delivery *ACS Nano* **4** 2494–509

Zhang F, Yang R, Zhang P, Qin J, Fan Z and Zuo B 2019 Water-rinsed non-mulberry silk film for potential tissue engineering applications *ACS Omega* **4** 3114–21

Zhang H, You R, Yan K, Lu Z, Fan Q, Li X and Wang D 2020 Silk as templates for hydroxyapatite biomineralisation: a comparative study of *Bombyx mori* and *Antheraea pernyi* silkworm silks *Int. J. Biol. Macromol.* **164** 2842–50

Zhang J, Kaur J, Rajkhowa R, Li J L, Liu X Y and Wang X G 2013 Mechanical properties and structure of silkworm cocoons: a comparative study of *Bombyx mori, Antheraea assamensis, Antheraea pernyi* and *Antheraea mylitta* silkworm cocoons *Mater. Sci. Eng.* C **33** 3206–13

Zhang Y, Han F, Fan S and Zhang Y 2021 Low-power and tunable-performance biomemristor based on silk fibroin *ACS Biomater. Sci. Eng.* **7** 3459–68

Zhang Y, Sun T and Jiang C 2018 Biomacromolecules as carriers in drug delivery and tissue engineering *Acta Pharm. Sinica* B **8** 34–50

Zhao S, Chen Y, Partlow B P, Golding A S, Tseng P, Coburn J, Applegate M B, Moreau J E, Omenetto F G and Kaplan D L 2016 Bio-functionalized silk hydrogel microfluidic systems *Biomaterials* **93** 60–70

Zhao X-M, Liu C, Jiang L-J, Li Q-Y, Zhou M-T, Cheng T-C, Mita K and Xia Q-Y 2015 A juvenile hormone transcription factor Bmdimm-Fibroin H chain pathway is involved in the synthesis of silk protein in silkworm, *Bombyx mori J. Biol. Chem.* **290** 972–86

Zhaorigetu S, Yanaka N, Sasaki M, Watanabe H and Kato N 2003 Inhibitory effects of silk protein, sericin on UVB-induced acute damage and tumour promotion by reducing oxidative stress in the skin of hairless mouse *J. Photochem. Photobiol.* B **71** 11–17

Zhu B, Wang H, Leow W R, Cai Y, Loh X J, Han M-Y and Chen X 2016 Silk fibroin for flexible electronic devices *Adv. Mater.* **28** 4250–65