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REVIEW

Can the venerated silk be the next-generation nanobiomaterial for biomedical-device designing, regenerative medicine and drug delivery? Prospects and hitches

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Abstract
Of late, the relevance of silk in a myriad of material science and biotechnological realms has been realized, as attested by the incessantly clambering number of reports and patents in the scientific repositories. The write-up is geared off with a scrutiny into the pertinence of the basic nano-structural features of silk, christened as the 'queen of textile' for exemplary bioengineering applications including designing and fabrication of devices for microfluidics, optofluidics, chemo/bio sensing, etc. Then, the major thrust of this short review is directed towards comprehending the prospects of using silk-based biomaterials (e.g. scaffolds, electrospun membranes, films, hydrogels, bioinks) for tissue engineering and regenerative medicine as well as targeted delivery of various biomolecular cargoes/therapeutic agents, etc., as vouched by few *avant-garde* endeavours of the recent years. The write-up is entwined with a discussion on the various factors that could plausibly hinder the realization of silk as the next-generation nanobiomaterial, suggestions for some approaches to dodge and deal with the practical snags and what lies ahead!

Keywords Drug delivery · Microfluidics · Nanobiomaterial · Regenerative medicine · Silk · Tissue engineering

Introduction

Witnessing the continually growing pertinence of silk as a material of choice in the biomedical domain is quite invigorating. In recent years, there has been commendable addition to the records in the scientific repositories, vouching for the immense prospects of silk in the niche of tissue engineering and regenerative medicine (TERM). Revered with the sobriquet of 'queen of textile', silk is making a larger-than-life journey from the traditional looms and the ramp walks to the operation theatres as well as its foray into the nano/bioengineering labs for fabrication of innovative optical, electronic, energy and microfluidic devices demand special mention [1–3]. A myriad of insects (e.g. silkworms, spiders etc.) are known to synthesize silk, the synthesis being dictated by microfluidic principles [4]. Silk represents nature's quintessential nanotechnological manoeuvre, attesting the evolutionary intricacies of nano-viscoelastic, nano-roughness, nano-stiffness, nano-electric and nano-tribological facets [5, 6]. Dictates of intermediate hydrophobic/hydrophilic block ratios and longer chain lengths have been corroborated with the formation of spider silk fibre and its high strength, as attested by mesoscopic modelling in concert with genetic block copolymer synthesis [7]. Furthermore, functioning of spider silk peptide as linear nano-spring for intracellular tension-sensing has also been proposed [8]. On the other hand, covalently integrated repetitive heavy and non-repetitive light chains as well as the crystalline and amorphous domains (with varied compositional abundance of amino acids) are the signature attributes of silkworm silk [9]. Features of biocompatibility, resilience, mechanical robustness and tunable biodegradation profile as well as ease of processibility into multiple formats (e.g. nanoparticles, films, electrospun mats, hydrogels, etc.) have conferred special niche to silkworm silk (constituted by fibroin (SF) and the sticky hydrophilic sericin (SSC)) in particular in the domains of soft and hard tissue engineering, cosmetics, food

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preservation, etc. [1, 2, 9, 10]. Perusal of recent publications and patents unveils that exploration of the nanoscale attributes as well as the unique biomaterial properties of silk has paved the way to numerous advanced biomedical applications, amongst others. In the succeeding sections of this article, I have tried to cite a few exemplary endeavours that stand in testimony to the afore-stated statement, interlaced with highlights on the prospects and challenges in the niche of silk-based nanobiomaterials.

From micro/nanofluidics to optofluidics

Pertinently, the early morning sight of condensing water droplets on the spider silk-web's robust fibres (constituted by hierarchically organized bundles of nano-confined flaw-tolerant fibrils, embodying β-crystallites in specific orientations) may be bracketed together with the nanoscale topography of alternating conical spindle knots and joints [11]. The concert of surface energy gradient and alteration in Laplace pressure in the droplet core dictates the movement of the droplet over the fibre. Elucidation of differential roughness and surface wettability due to such unique nanofibrillar organization has propelled research for the fabrication of microfluidic devices for oil–water separation, filtration, fog-collection as well as cost-effective, easy-to-use and field-deployable devices, particularly for diagnostic and environmental applications [4, 11, 12]. As an exemplary evidence, Bhandari et al. [13] had adeptly evaluated the prospects of a silk fabric-chip for direct immunoassay, based on capillary action mediated sample flow and visual detection. Fabrication of 'fab-chips' (*zari* silk, roughened with silver nanoparticles), impregnated as wearable pathogen sensors in clothing [14] has also been documented. The researchers had used adenine (associated with the major SERS signal of DNA—a pathogenic biomarker) to demonstrate the practical feasibility of the chips in diagnostic applications. In another report, application of microfluidic thread-based analytical device (μTAD) was attested by the facile separation of weak and normal ABO blood type (Fig. 1I), based on antibody treated silk threads [15], circumventing the issues of transfusion inaccuracies as well as facilitating easier migration of erythrocytes, thereby, preventing agglutination in comparison to cotton fibres. At this juncture, it is crucial to note that albeit natural silk spinning has inspired the fabrication of artificial innovative spinning devices, component fine-tuning still remains elusive [4]. Furthermore, achieving optimal solution flow-attributes as well as enhanced user control of the microfluidic devices demands simulation and optimization studies. On the other hand, high fidelity nano-patterned optical arrangements (with tunable signature-spectral profile for sensing bioactive substances) in silk have paved the way to fabrication of number of optofluidic devices, permitting straightforward readout [16]. Embossed photonic nanometre scale patterns into fibroin films and haemoglobin, incorporated into silk diffraction gratings for oxygen sensing [17]; highly sensitive silk plasmonic absorber sensor (SPAS) (based on silk-gold nanostructure) (Fig. 1II) for glucose sensing [18], etc. merit special mention. In this backdrop, investigation of the optical attributes of non-mulberry (e.g. *Philosamia ricini*, *Anatheraea assama*) silk proteins with unique amino acid sequences [9] is expected to open up new avenues to explore.

Prospects for TERM and biocargo-delivery

As mentioned in the introductory section, silk has received an unprecedented impetus in the domain of TERM and allied biomedical applications. Some of the exemplary reports are cited underneath. To start off, silk-based approaches to address skin damage has been at the forefront of research in the last few years [19]. As an exemplary evidence, Jadi et al. [20] had demonstrated that non-mulberry silk sericin could function as an effective antioxidant in ameliorating UV A and UV B radiation-induced skin damage, thereby raising the prospects of its inclusion in skin care products (Fig. 1III). Needless to say that a number of pathophysiological conditions (e.g. burns, diseases like diabetes, etc.) perturb the otherwise highly orchestrated process of wound healing. In situ 3D printing, in situ forming hydrogels, electrospraying, use of microRNA (miRNA) and small interfering RNA (siRNA)-based skin therapeutics and so on have been investigated for accelerated wound healing [19]. Amongst others, electrospun silk-based mats have garnered considerable research thrust in the recent years. Lately, a simple coating technique was employed for functionalization of silkworm silk fibroin-based electrospun matrices with recombinant spider silk fusion proteins [21]. Functionalization of the spider silk fusion proteins with cell-binding RGD (Arg-Gly-Asp) peptide, growth factor peptide and antimicrobial peptide conferred multifaceted features to the fabricated SF-based wound dressings (Fig. 1IV). In an extended work to this report, the researchers had recently demonstrated substantial skin tissue regeneration (in comparison to commercial Duoderm dressing and untreated wounds), mediated by spider silk fusion proteins functionalized silkworm silk-based skin dressings in alloxan-treated diabetic rabbit model [22]. Thus, these studies attest the prospective candidature of such hybrid silk matrices to address diabetic wounds and burns.

On the other hand, although islet transplantation is projected as an apt strategy to address type 1 diabetes, however, islet dysfunction in long-term culture hinders the clinical success of the approach [23]. In this backdrop, Kumar et al. [23] had reported mulberry and non-mulberry silk fibroin

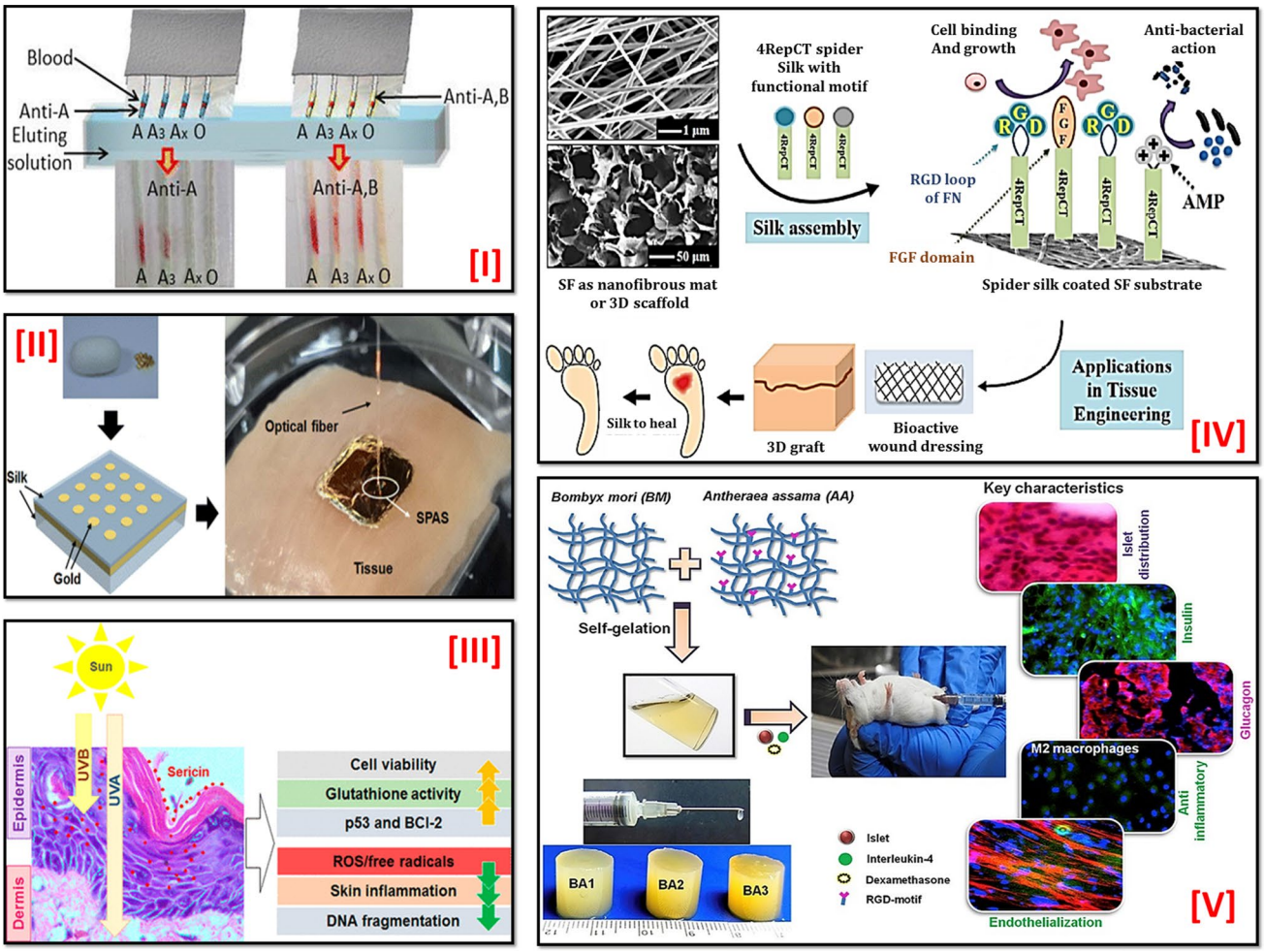

Fig. 1 **I** Silk thread-based microfluidics assay for weak ABO blood typing. (Reproduced from [15], Copyright© 2014, American Chemical Society). **II** Silk-based nanoplasmonic optical sensor (Reproduced from [18], Copyright© 2015, American Chemical Society). **III** Downregulation of oxidative stress confers sericin-mediated protection against UV-induced skin damage (Reproduced from [20], Copyright© 2018, American Chemical Society). **IV** Hybrid silk-protein matrix as bioactive wound dressings and skin grafts (Reproduced from [21], Copyright© 2018, American Chemical Society). **V** Immunomodulatory injectable silk hydrogels for functional islets' maintenance (Reproduced from [23], Copyright© 2018, Elsevier)

blend-based immuno-informed biomimetic, injectable hydrogel, exhibiting local modulation of in vivo inflammatory responses, for islet encapsulation and prospective transplantation for insulin-dependent type 1 diabetes (Fig. 1V). Pertinently, the 3D micro-niche of the hydrogels (compatible with endothelial cells) supported a persistent islet viability and insulin secretion. On a similar vein, mulberry (*Bombyx mori*) and non-mulberry (*Philosamia ricini* and *Antheraea assamensis*) silkworm silk protein were exploited through sequential rolling of cell-laden patterned films for a simple biofabrication of an affordable and biodegradable vascular graft (Fig. 2I) [24]. Translating research of such biofabricated small diameter graft into an 'off the shelf' product for replacement of occluded/diseased coronary artery and arteriovenous fistula for hemodialysis access, as proposed in the study, demand further in vivo assessments. Similarly, gold nanoparticle-doped electrospun silk protein-based nerve conduit, pre-seeded with Schwann cells has been adroitly employed for structural and functional regeneration of severed sciatic nerves in Sprague–Dawley rats, further vouched by the restoration of stretching and jumping capacity as well as muscular regeneration in them [25].

In another recent endeavour, a bioengineered silk-blend-based full thickness angle-ply construct (Fig. 2II) was reported to mimic the native intervertebral disc (IVD) complexity [26]. Proliferation, alignment and maturation of primary annulus fibrosus (AF) cells (porcine-origin) and differential mechanical features mediated guidance of extracellular matrix turnover were documented. This study is pertinent in the context of the fact that the conventional surgical interventions (which are highly case-dependent and not applicable to all patients) are good enough only in

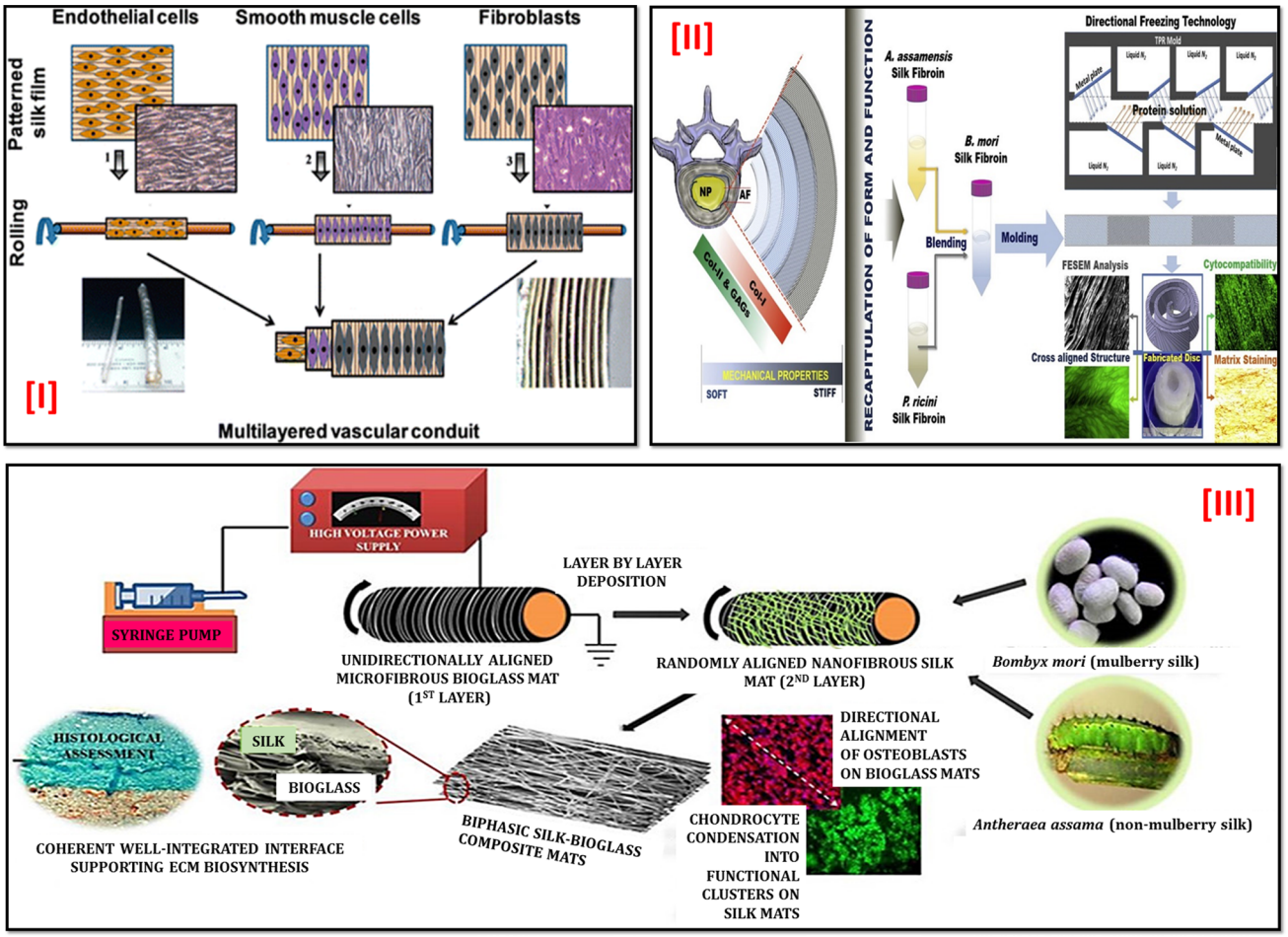

Fig. 2 **I** Patterned silk films-based small diameter vascular conduits (Reproduced from [24], Copyright© 2016, American Chemical Society). **II** Engineered silk-based angle-ply intervertebral disc construct (Reproduced from [26], Copyright© 2018, Elsevier). **III** Mimicking of the hierarchical complexity at the osteochondral interface using mulberry and non-mulberry silk-bioactive glass composites (Reproduced from Ref. [27] under Creative Commons Attribution (CC-BY) License, Copyright© 2017, American Chemical Society)

symptomatic pain relief, with no restitution of the biomechanical functions [26] and possibly eventual degeneration of adjacent parts.

On a similar note, the current treatment approaches of autologous chondrocyte implantation, matrix-assisted chondrocyte implantation or mosaicplasty to address osteochondral defects (OCD) are challenged in the context of obtainability of suitable donor tissue, donor site morbidity and the consequential poor resistance of the fibrocartilage towards shear and clinical robustness [27]. In this regard, exploitation of biphasic scaffolds for OCD has been envisaged as a prospective solution. At this juncture, I would like to cite one of our works wherein, we had demonstrated that a silk–bioglass-based inexpensive, implantable biphasic electrospun nanofibrous composite, mimicking the osteochondral interfacial micro-niche (Fig. 2III) could offer a plausible solution for conditions like osteochondral degeneration and arthritis [27]. The biphasic scaffold provided a bioactive, mechanically robust and porous bioglass-based osteogenic matrix to emulate the micro-milieu of the subchondral bone (that assisted directional alignment of osteoblasts) while chondrocyte condensation into functional clusters was evident on the silk mats.

Pertinently, of late, silk-based 3D bio-printing has given a whole new perspective to the endeavours to mimic the microarchitecture of native tissues [28]. In the realm of silk-based 3D bio-printing, rheological adjustment of silk fibroin solution is a serious practical issue. Use of toxic chemical cross-linkers may usher in negative trade-off with respect to the cell-viability while use of organic solvents (e.g. 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)) to augment viscosity could lead to unintended trimming of the fibroin protein [28]. In this context, researchers have fabricated a hybrid silk fibroin–gelatin polymer-based bioink (free from any toxic chemical cross-linker), exhibiting excellent 3D bio-printability for prospective cartilage tissue engineering

(Fig. 3) [28]. The work also attested a commendable print fidelity of the bioink for creating anatomical structures, exemplified by printing of a human ear under optimized extrudability. In another recent report, an immunocompatible 3D bio-printed (based on silk–gelatin bioink) scaffold was fabricated to mimic the internal and bulk architecture of the menisci for plausible patient specific implantation [29].

In another recent endeavour, researchers have established the prospects of non-invasive magnetic actuation of iron oxide nanoparticles loaded SF nanofibrous matrix to engineer functional cardiac tissue construct and achieving actuation frequency-dependent tuning of drug release [30]. Furthermore, recent endeavours have also been directed towards exploitation of both silk fibroin and sericin nanoparticles for the therapeutic delivery of various bioactive moieties/drugs/anticancer agents like curcumin, insulin, doxorubicin, quercetin, atorvastatin, etc. (Table 1). To cite for evidence, in vivo reduction in tumour size (bracketed together with the plausible reactive oxygen species mediated apoptosis) was registered for curcumin and 5-fluorouracil loaded silk fibroin nanoparticles (in comparison to free drug usage) in an endeavour to address breast cancer [38]. Similarly, a mechanically strong injectable hydrogel system, comprising a blend of silk protein and doxorubicin loaded folic acid functionalized single walled carbon nanotube was fabricated for on-demand, localized, targeted drug release, post intermittent exposure to near- infrared light (Fig. 4) [39]. The pH and temperature responsive hydrogel system was endocytosed by folic acid receptor positive (FR^{+ve}) oral cancer cell line KB, eventually inducing the apoptotic pathway. A qualitative understanding as well as accessibility of quantitate data on intracellular trafficking besides unmasking of the delivery via endosomotropic or lysomotropic ways are pre-requisite for the successful application of such silk-based systems for delivery of therapeutic agents [3, 40].

On the other hand, formation of strong complexes of silk with DNA is hindered due to non-cationic nature of the former. In this context, nanoscale complexes of silk chains with

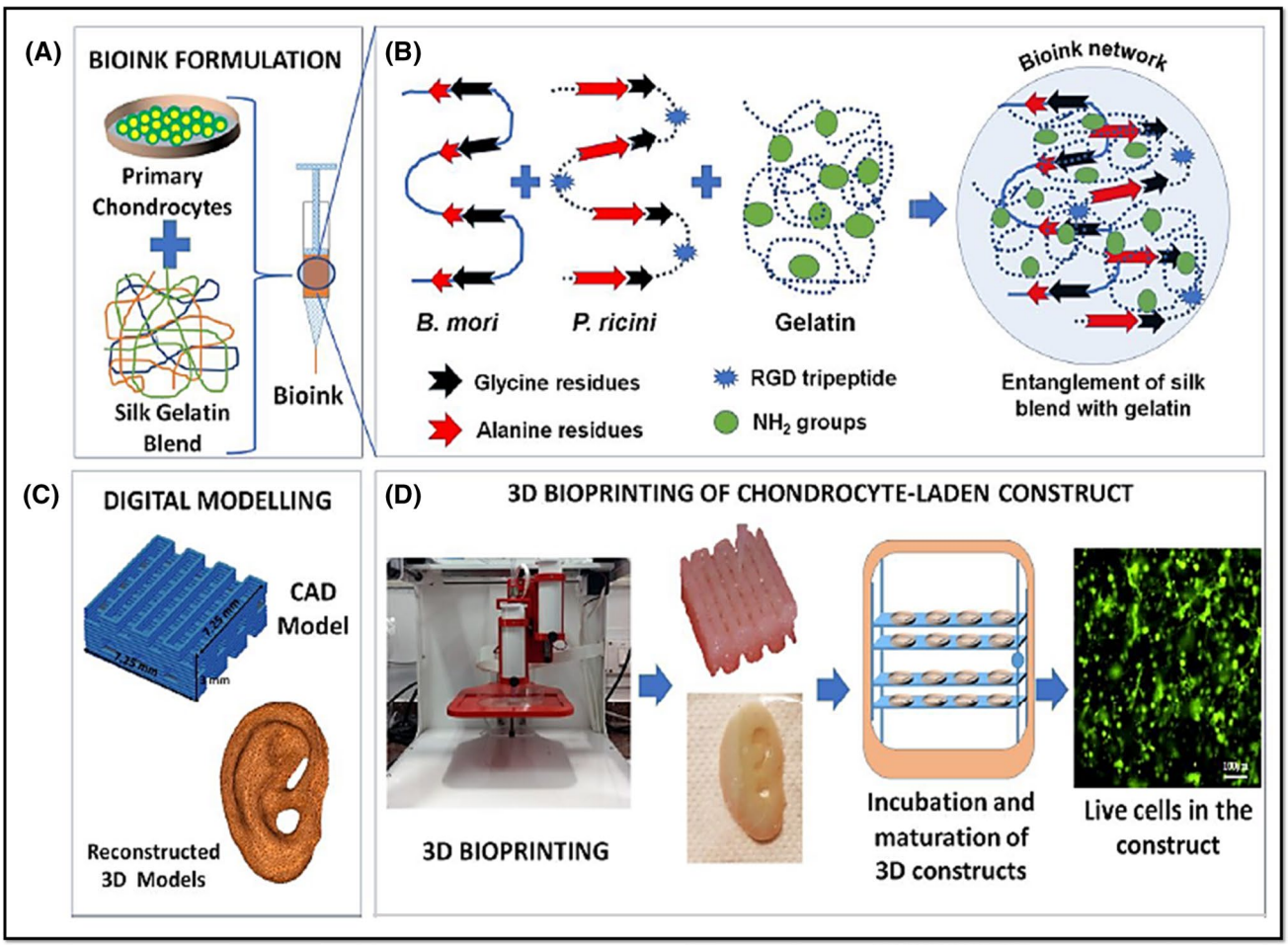

Fig. 3 Cross-linker-free silk–gelatin bioink for 3D bio-printing for prospective application in cartilage tissue engineering. Scheme of the study showing **a** bioink formulation, **b** entanglement and interaction of biopolymers, **c** digital modelling and, **d** 3D bio-printing and maturation of cell-laden construct (reproduced from [28], Copyright© 2019, American Chemical Society)

Table 1 Representative nanoscale silk-based therapeutic agent delivery systems

Therapeutic agent	Silk protein used for fabrication of the nano-delivery system	Outcome, observed at the bio-interface	References
Insulin	Silk fibroin	Bound insulin exhibited augmented resistance to trypsin digestion and prolonged half-life than native insulin; in vitro stability in human serum	[31]
Paclitaxel (PTX)	Silk fibroin	Speedier killing of gastric cancer cell lines BGC-823 and SGC-7901; better antitumor activity than the free PTX	[32]
Cisplatin	Silk fibroin	Apoptosis in A549 lung cancer cells	[33]
Atorvastatin	Silk sericin	Increased hyperlipidemic activity	[34]
Quercetin	Silk fibroin	Quercetin's sustained release at pH 7.4 (phosphate buffer saline) and pH 6.8 (simulated intestinal fluid)	[35]
Doxorubicin (Dox)	Silk sericin	pH responsive, surface charge reversal nanoparticles with augmented cellular entry and Dox delivery	[36]
Curcumin	Silk fibroin	Increased cytotoxicity in neuroblastoma cells in comparison to hepatocarcinoma cells	[37]

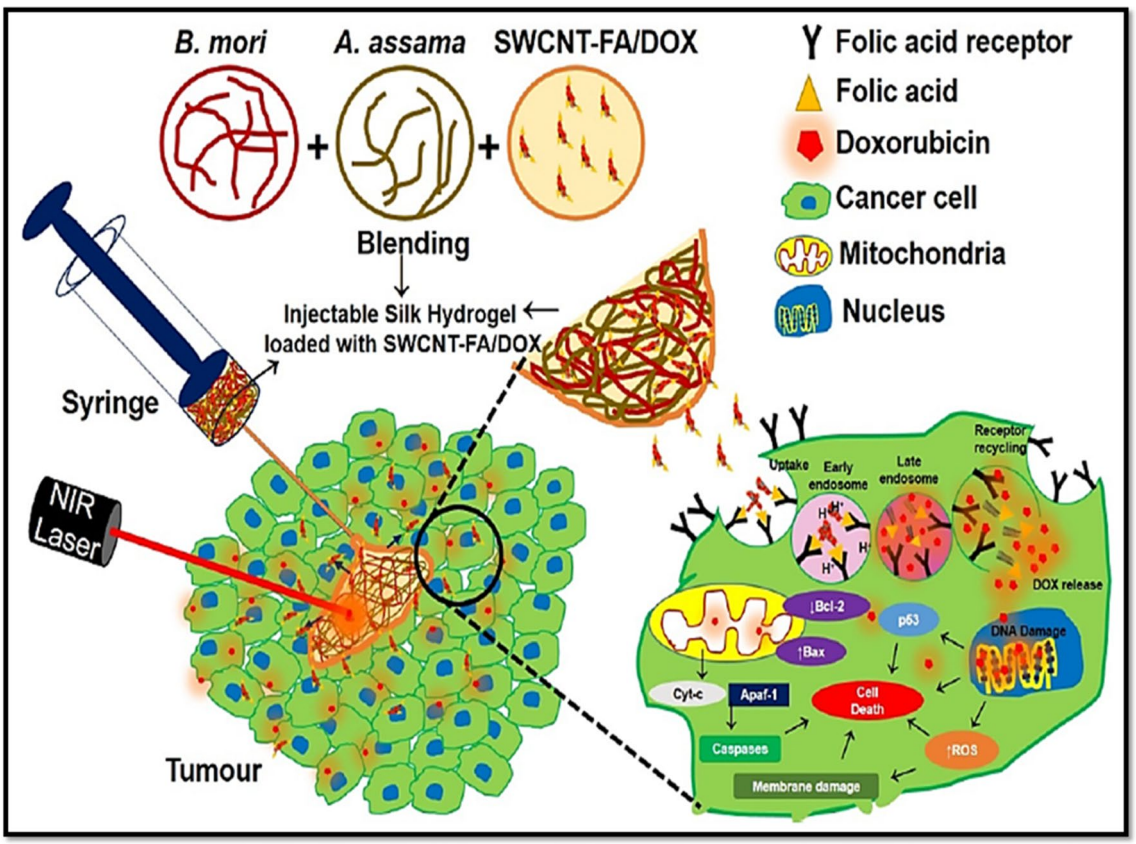

Fig. 4 Carbon nanotube loaded silk hydrogel for on-demand anticancer drug delivery (reproduced from [39], Copyright© 2019, American Chemical Society)

poly(L-lysine) copolymers, displaying electrostatic interaction with plasmid DNA (pDNA) were employed for gene-delivery into human embryonic kidney cells [41]. Similarly, *Antheraea pernyi* silk fibroin (rich in cell-binding RGD sequences)/poly(ethylene imine)/DNA ternary complex exhibited higher transfection efficacy in human colorectal carcinoma (HCT 116) and human embryonic kidney (HEK 293) cells with augmented transfection being displayed in the former, attributed to copiousness of RGD binding integrins [42]. These are just a few representative studies to

illustrate the potential of silk for regenerative medicine and allied biomedical applications.

The challenges and the future direction

Albeit, there exist tremendous prospects for bench-to-bed side technology transfer of silk-based nanobiomaterials, a number of pertinent issues need to be addressed tactfully. The prime requisite lies for a scalable, economically feasible-production protocol (with quality-control) for the silk-based nanobiomaterials, propelling clinical trials and applications. In particular, some of the practical snags while resorting to wild non-mulberry silk (endowed with RGD sequence that promotes cell adhesion and proliferation) are the slow-paced seed production and assaults to the worms by a myriad of pests, predators and diseases. Indoor rearing of such worms could be a safer alternative. From the laboratory-experiments' perspective, the conventional (toxic) lithium salts (used for mulberry silk fibroin extraction) do not hold good enough for cocoon-solubilization for non-mulberry silk protein extraction while harvesting of gland proteins is challenged by concerns of rapid aggregation and precipitation. Anionic detergents like sodium dodecyl sulphate have been forwarded as effective substitutes [9]. On the other hand, territorial nature and hostile attribute of spiders obstruct the collection of ample volumes of their silk proteins. In this context, seri-informatics, complemented by genomic/molecular tools would be instrumental in conservation and improvement in the production of silk of superior quality. In a similar vein, deeper insight into the biochemical machinery of the silk worms (particularly the endemic varieties) and the spiders along with metabolic and cellular engineering of appropriate hosts in the context of genetic engineering could pave the way to the production of novel silk-based nanobiomaterials. Needless to say that comprehending the physicochemical characteristics of the silk proteins with respect to the genomic and proteomic information of the silkworms is of utmost importance [1, 43]. Another issue is the current incipient status of the clinical trials of silk-based nanobiomaterials [3, 44]. There lies a plethora of reports on the successful in vitro or preclinical assessments of silk-based nanobiomaterials for regenerative medicine as well as prospective delivery of biomolecular cargoes; however, in vivo studies and clinical trials are just a handful. Detailed unveiling of the pharmacokinetics, pharmacodynamics and off-target outcomes in bench studies is pre-requisite for commercial translation of silk-based nanobiomaterials.

Amalgamation of bioreactors and stem cell technology is envisaged to pave the way to more realistic applications of silk-based nanobiomaterials for TERM. *What next*? Possibly, the simulation and optimization strategies to fabricate transplantable bioengineered organs with befitting performance/price equilibrium. Furthermore, silk-based scaffold systems could be possibly employed for the enhanced production of medically important biocatalysts like urokinase under optimized media in bioreactors. Current research also seems to be buoyant towards organ-on-a-chip/multichannel 3D microfluidic silk-based cell culture chip platforms in near future. The realization would imply a route to evade the use of animals in drug assessments and toxin evaluation. As a conclusive statement, I would like to stress upon the fact that silk-based nanobiomaterial-research demands inter-institutional concerted activities and incentives for an innovation ecosystem to ensure a 'silken-health for all'.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Ethical approval No studies with human or animal subjects were conducted afresh by the author for inclusion in this review article.

References

1. Janani G, Kumar M, Chouhan D, Moses JC, Gangrade A, Bhattacharjee S, Mandal BB (2019) An insight into silk-based biomaterials: from physico-chemical attributes to recent biomedical applications. ACS Appl Biol Mater. https://doi.org/10.1021/acsabm.9b00576
2. Bandyopadhyay A, Chowdhury SK, Dey S, Moses JC, Mandal BB (2019) Silk: a promising biomaterial opening new vistas towards affordable healthcare solutions. J Indian Inst Sci. https://doi.org/10.1007/s41745-019-00114-y
3. Konwarh R, Dhandayuthapani B (2019) Sustainable bio-resource, silk at the nanoscale for biomedical applications. In: Karak N (ed) Dynamics of advanced sustainable nanomaterials and their related nanocomposites at the bio-nano interface. Elsevier, Amsterdam, pp 125–145
4. Konwarh R, Gupta P, Mandal BB (2016) Silk-microfluidics for advanced biotechnological applications: a progressive review. Biotechnol Adv 34:845–858. https://doi.org/10.1016/j.biotechadv.2016.05.001
5. Silva EL, Rech EL (2013) Unravelling the biodiversity of nanoscale signatures of spider silk fibres. Nat Commun 4:3014. https://doi.org/10.1038/ncomms4014
6. Nova A, Keten S, Pugno NM, Redaelli A, Buehler MJ (2010) Molecular and nanostructural mechanisms of deformation, strength and toughness of spider silk fibrils. Nano Lett 10:2626–2634. https://doi.org/10.1021/nl101341w
7. Lin S, Ryu S, Tokareva O, Gronau G, Jacobsen MM, Huang W, Rizzo DJ, Li D, Staii C, Pugno NM, Wong JY, Kaplan DL,

Buehler MJ (2015) Predictive modelling-based design and experiments for synthesis and spinning of bioinspired silk fibres. Nat Commun 6:6892. https://doi.org/10.1038/ncomms7892
8. Brenner MD, Zhou R, Conway DE, Lanzano L, Gratton E, Schwartz MA, Ha T (2016) Spider silk peptide is a compact, linear nanospring ideal for intracellular tension sensing. Nano Lett 16:2096–2102. https://doi.org/10.1021/acs.nanolett.6b00305
9. Konwarh R, Bhunia BK, Mandal BB (2017) Opportunities and challenges in exploring Indian nonmulberry silk for biomedical applications. Proc Indian Natl Sci Acad 83:85–101. https://doi.org/10.16943/ptinsa/2017/41288
10. Mehrotra S, Chouhan D, Konwarh R, Kumar M, Kumar JP, Mandal BB (2019) A comprehensive review on silk at nanoscale for regenerative medicine and allied applications. ACS Biomater Sci Eng 5:2058–2074. https://doi.org/10.1021/acsbiomaterials.8b01560
11. Ju J, Zheng Y, Jiang L (2014) Bioinspired one-dimensional materials for directional liquid transport. Acc Chem Res 47:2342–2352. https://doi.org/10.1021/ar5000693
12. Chen Y, Zheng Y (2014) Bioinspired micro/nanostructure fibers with a water collecting property. Nanoscale 6:7703–7714. https://doi.org/10.1039/c4nr02064b
13. Bhandari P, Narahari T, Dendukuri D (2011) 'Fab-chips': a versatile, fabric-based platform for low-cost, rapid and multiplexed diagnostics. Lab Chip 11:2493–2499. https://doi.org/10.1039/c1lc20373h
14. Robinson AM, Zhao L, Alam MYS, Bhandari P, Harroun SG, Dendukuri D, Blackburn J, Brosseau CL (2015) The development of "fab-chips" as low-cost, sensitive surface-enhanced Raman spectroscopy (SERS) substrates for analytical applications. Analyst 140:779–785. https://doi.org/10.1039/c4an01633e
15. Nilghaz A, Zhang L, Li M, Ballerini DR, Shen W (2014) Understanding thread properties for red blood cell antigen assays: weak ABO blood typing. ACS Appl Mater Interfaces 6:22209–22215. https://doi.org/10.1021/am505849e
16. Domachuk P, Perry H, Amsden JJ, Kaplan DL, Omenetto FG (2009) Bioactive "self-sensing" optical systems. Appl Phys Lett 95:253702. https://doi.org/10.1063/1.3275719
17. Amsden JJ, Kaplan DL, Omenetto FG (2014) Tufts University, nanoimprinting of silk fibroin structures for biomedical and biophotonic applications. US Patent 8,747,886
18. Lee M, Jeon H, Kim S (2015) A highly tunable and fully biocompatible silk nanoplasmonic optical sensor. Nano Lett 15:3358–3363. https://doi.org/10.1021/acs.nanolett.5b00680
19. Chouhan D, Dey N, Bhardwaj N, Mandal BB (2019) Emerging and innovative approaches for wound healing and skin regeneration: current status and advances. Biomaterials 216:119267. https://doi.org/10.1016/j.biomaterials.2019.119267
20. Kumar JP, Alam S, Jain AK, Ansari KM, Mandal BB (2018) Protective activity of silk sericin against UV radiation-induced skin damage by downregulating oxidative stress. ACS Appl Biol Mater 1:2120–2132. https://doi.org/10.1021/acsabm.8b00558
21. Chouhan D, Thatikonda N, Nilebäck L, Widhe M, Hedhammar M, Mandal BB (2018) Recombinant spider silk functionalized silkworm silk matrices as potential bioactive wound dressings and skin grafts. ACS Appl Mater Interfaces 10:23560–23572. https://doi.org/10.1021/acsami.8b05853
22. Chouhan D, Das P, Thatikonda N, Nandi SK, Hedhammar M, Mandal BB (2019) Silkworm silk matrices coated with functionalized spider silk accelerate healing of diabetic wounds. ACS Biomater Sci Eng 5:3537–3548. https://doi.org/10.1021/acsbiomaterials.9b00514
23. Kumar M, Gupta P, Bhattacharjee S, Nandi SK, Mandal BB (2018) Immunomodulatory injectable silk hydrogels maintaining functional islets and promoting anti-inflammatory M2 macrophage polarization. Biomaterials 187:1–17. https://doi.org/10.1016/j.biomaterials.2018.09.037
24. Gupta P, Kumar M, Bhardwaj N, Kumar JP, Krishnamurthy CS, Nandi SK, Mandal BB (2016) Mimicking form and function of native small diameter vascular conduits using mulberry and non-mulberry patterned silk films. ACS Appl Mater Interfaces 8:15874–15888. https://doi.org/10.1021/acsami.6b00783
25. Das S, Sharma M, Saharia D, Sarma KK, Sarma MG, Borthakur BB, Bora U (2015) *In vivo* studies of silk based gold nano-composite conduits for functional peripheral nerve regeneration. Biomaterials 62:66–75. https://doi.org/10.1016/j.biomaterials.2015.04.047
26. Bhunia BK, Mandal BB (2018) Modulation of extracellular matrix by annulus fibrosus cells on tailored silk based angle-ply intervertebral disc construct. Mater Des 158:74–87. https://doi.org/10.1016/j.matdes.2018.08.015
27. Moses JC, Reardon PJ, Konwarh R, Knowles JC, Mandal BB (2017) Mimicking hierarchical complexity of the osteochondral interface using electrospun silk-bioactive glass composites. ACS Appl Mater Interfaces 9:8000–8013. https://doi.org/10.1021/acsami.6b16590
28. Singh YP, Bandyopadhyay A, Mandal BB (2019) 3D bioprinting using cross-linker free silk-gelatin bioink for cartilage tissue engineering. ACS Appl Mater Interfaces 11:33684–33696. https://doi.org/10.1021/acsami.9b11644
29. Bandyopadhyay A, Mandal BB (2019) 3D printed silk-based biomimetic tri-layered meniscus for potential patient specific implantation. Biofabrication. https://doi.org/10.1088/1758-5090/ab40fa
30. Chouhan D, Mehrotra S, Majumder O, Mandal BB (2018) Magnetic actuator device assisted modulation of cellular behavior and tuning of drug release on silk platform. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.8b00240
31. Yan HB, Zhang YQ, Ma YL, Zhou XL (2009) Biosynthesis of insulin-silk fibroin nanoparticles conjugates and in vitro evaluation of a drug delivery system. J Nanopart Res 11:1937. https://doi.org/10.1007/s11051-008-9549-y
32. Wu P, Liu Q, Li R, Wang J, Zhen X, Yue G, Wang H, Cui F, Wu F, Yang M, Qian X (2013) Facile preparation of paclitaxel loaded silk fibroin nanoparticles for enhanced antitumor efficacy by locoregional drug delivery. ACS Appl Mater Interfaces 5:12638–12645. https://doi.org/10.1021/am403992b
33. Qu J, Liu Y, Yu Y, Li J, Luo J, Li M (2014) Silk fibroin nanoparticles prepared by electrospray as controlled release carriers of cisplatin. Mater Sci Eng, C 44:166–174. https://doi.org/10.1016/j.msec.2014.08.034
34. Kanoujia J, Singh M, Singh P, Saraf SA (2016) Novel genipin crosslinked atorvastatin loaded sericin nanoparticles for their enhanced antihyperlipidemic activity. Mater Sci Eng, C 69:967–976. https://doi.org/10.1016/j.msec.2016.08.011
35. Lozano-Pérez AA, Rivero HC, Hernández MDCP, Pagán A, Montalbán MG, Víllora G, Cénis JL (2017) Silk fibroin nanoparticles: efficient vehicles for the natural antioxidant quercetin. Int J Pharm 518:11–19. https://doi.org/10.1016/j.ijpharm.2016.12.046
36. Hu D, Xu Z, Hu Z, Hu B, Yang M, Zhu L (2017) pH triggered charge-reversal silk sericin-based nanoparticles for enhanced cellular uptake and doxorubicin delivery. ACS Sustain Chem Eng 5:1638–1647. https://doi.org/10.1021/acssuschemeng.6b02392
37. Montalbán MG, Coburn JM, Lozano-Pérez AA, Cenis JL, Víllora G, Kaplan DL (2018) Production of curcumin loaded silk fibroin nanoparticles for cancer therapy. Nanomater 8:126. https://doi.org/10.3390/nano8020126
38. Li H, Tian J, Wu A, Wang J, Ge C, Sun Z (2016) Self-assembled silk fibroin nanoparticles loaded with binary drugs in the treatment of breast carcinoma. Int J Nanomed 11:4373. https://doi.org/10.2147/IJN.S108633

39. Gangrade A, Mandal BB (2019) Injectable carbon nanotube impregnated silk based multifunctional hydrogel for localized targeted and on-demand anticancer drug delivery. ACS Biomater Sci Eng 5:2365–2381. https://doi.org/10.1021/acsbiomaterials.9b00416
40. Seib FP (2017) Silk nanoparticles-an emerging anticancer nanomedicine. AIMS Bioeng 42:239–258. https://doi.org/10.3934/bioeng.2017.2.239
41. Numata K, Subramanian B, Currie HA, Kaplan DL (2009) Bioengineered silk protein-based gene delivery systems. Biomaterials 30:5775–5784. https://doi.org/10.1016/j.biomaterials.2009.06.028
42. Liu Y, You R, Liu G, Li X, Sheng W, Yang J, Li M (2014) *Antheraea pernyi* silk fibroin-coated PEI/DNA complexes for targeted gene delivery in HEK 293 and HCT 116 cells. Int J Mol Sci 15:7049–7063. https://doi.org/10.3390/ijms15057049
43. Malay AD, Sato R, Yazawa K, Watanabe H, Ifuku N, Masunaga H, Hikima T, Guan J, Mandal BB, Damrongsakkul S, Numata K (2016) Relationships between physical properties and sequence in silkworm silks. Sci Rep 6:27573. https://doi.org/10.1038/srep27573
44. Holland C, Numata K, Rnjak-Kovacina J, Seib FP (2018) The biomedical use of silk: past, present, future. Adv Healthc Mater. https://doi.org/10.1002/adhm.201800465