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The relationship of rheological properties and the performance of silk fibroin hydrogels in tissue engineering application

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ABSTRACT

Hydrogel-based systems are widely used for conventional 3D-cell culture, where cells can be seeded on or embedded in 3D-matrix gels for cultivation. Several new approaches have emerged to develop innovative more performing polymeric biomaterials for tissue regeneration. Within this class of biomaterials, land and marine based-polymers (including among other collagens, silk, chitosan/chitin and alginates) have been explored to date. The best-known example of silk to date is the fiber produced by land-based animals like silkworms for the production of their cocoon. There are many successful studies already proving the empirical evidence of biomaterials from land-based silk in biomedical applications. Generally, silk-based hydrogels are mainly involved in the fabrication of different implants for skin, bone, cartilage and vascular-regeneration. The ideal silk fibroin hydrogels for skin, cosmetic and wound healing purposes should exhibit enhanced biological response which is mainly regulated by its tailored mechanical, rheological, viscoelastic properties, effective tissue regeneration ability, controllable swelling, hemostasis and biocompatibility. Accordingly, this review summarizes the rheological and viscoelastic properties of silk-fibroin based composite hydrogels obtained from various raw materials/composites, highlighting the relation of its rheological response to hydrogel biomaterial functions aiming biomedical applications.

1. Introduction

Hydrogels are soft water-rich three-dimensional polymer networks that are fabricated by physical and chemical crosslinking of polymers. For decades, hydrogels are playing a major role in cosmetics and pharmacological applications. Several kinds of hydrogels are developed by various fabrication techniques such as stirring, sol-gel, drop-casting, freeze-drying, electrospinning and 3D printing [1–13]. Due to their hydrophilic nature, hydrogels are favorable for structural and functional modification, water retention, easy to make composites with other polymers and flexi handling for cell cultures[14]. Besides, the transparent nature of hydrogels facilitates easy read-out of culture cells such as histological, immunological and fluorescence staining methods [15–18], unlike other forms of polymers such as scaffolds and sponges. Due to their excellent bioactivity and biocompatibility, hydrogels are used in different applications such as drug delivery, tissue engineering, tissue adhesives, biosensors, soft robots, electronic skin, wearable electronic devices and self-healing materials [19–22].

In recent times, many polymers both synthetic and natural materials have tried to adopt fabrication techniques to produce better hydrogels for tissue engineering applications [23–27]. For instance, natural materials such as collagen [28–31], chitosan [32–35], alginate [36–38],

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silk fibroin [39–41], Aloe vera [42–44] and gelatin[45–47] and synthetic materials such as polyvinyl alcohol (PVA) [48–50], poly(lactic acid) (PLA) [51–53], poly(lactic-co-glycolic acid) (PLGA) [54–56], ethylene dimethacrylate (EDMA) [57,58] and so on. Among the different polymers, silk fibroin attracts more due to its easy processability accompanied by its unique biocompatibility, biodegradability and fibrillary nature [59–61]. Silk folds in β-sheet structures due to the hydrophobic domains of short-side chain amino acids and is commercially available as filaments[62,63]. The properties and shape of worm silk may vary from their origin[64]. In most cases, the silk fibroin is extracted from cocoons of the domestic silkworm, *Bombyx mori*, however, it can be extracted from other animals such as spiders and insects [65,66]. Spider silk does not contain sericin and has different mechanical properties than worm silk[67]. The major structure of silk fibroin consists of two polypeptide chains such as a ~350 kDa heavy chain and ~25 kDa light chain, connected by a disulfide link [68,69]. The amino acids such as alanine, glycine and serine of silk fibroin are responsible for the formation of antiparallel beta-sheets during the hydrogelation process from random coil structure (Fig. 1) [65,70–72].

Many works reported the potential use of silk fibroin-based hydrogels in wound healing, cell culture and tissue engineering [27,39,73–75]. Interestingly, a bibliometric work in the SCOPUS data base, by using the key word "silk-fibroin" offer almost 10,000 documents reported so far, on the contrary, if we combine the key word Silk Fibroin + Tissue Engineering only 2400 documents are shown. The vast majority of these works were reported in the last 10 years with a clear exponential growth trend as observed in Fig. 2. The investigation of mechanical and biological properties claimed the programmable biodegradation, toughness, strength and biocompatibility of silk fibroin [40,65,76,77]. Though it has admirable properties, silk fibroin is often combined with other polymers, antibiotics and biomolecules to upregulate the biological functions.

At the same time, several crosslinking methods have been reported in recent times in order to improve the properties of silk fibroin-based hydrogel [74,78]. In this case, two widely used techniques are physical and chemical crosslinking. The physical crosslinking method by vortex shearing, heating, electric current, ultrasonication and pH triggered the structural transition of fibroin from random coil to stable β-sheet during the process of hydrogelation [79]. The physical crosslinking produce thermodynamically stable β-sheet structures to stabilize the fibroin molecules, which exhibit relatively slow degradation in vitro and in vivo [63,80–83]. On the other hand, the chemical crosslinking of fibroin is achieved by adding natural plasticizers such as glutaraldehyde, carbodiimide and genipin, (which mainly react with lysine and arginine in the protein structure) to reduce cytotoxicity and self-aggregation (β-sheet conformation) [84–89].

One of the main advantages of silk fibroin is its semi-solid nature that enables easy handling (injectable) that promotes the applicability and healing efficiency in biomedical fields for the regeneration of bones,

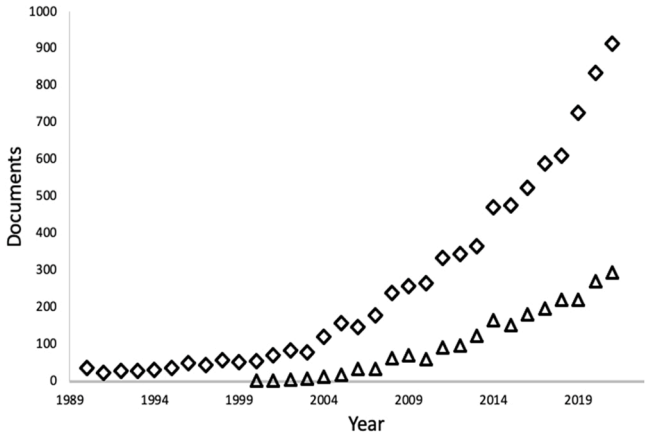

Fig. 2. Number of document per year in the SCOPUS data base (1990–2021) using the keyword Silk Fibroin (empty diamonds) and Silk Fibroin + Tissue Engineering (empty Triangles).

skins, cartilages, vascular, tendon and ligament tissues [40,65,76,77]. The injectable fibroin hydrogel comprising biological cell cultures can easily be administered to target defective tissues in the body and promote angiogenesis and tissue regeneration [77]. For instance, silk fibroin-chitosan hybrid hydrogel loaded with extract of the longan seeds promoted the differentiation of bone cells and mineralization in bone tissue engineering applications [90]. Zhang et al. [91] fabricated injectable fibroin hydrogel for augmenting the cervical tissue of pregnant rabbits and showed that the fibroin hydrogel could be the replacement of cerclage for the treatment of premature birth due to cervical insufficiency. Moreover, fibroin- polyethylene hydrogel loaded with dexamethasone was fabricated to aid hearing problems as a hearing device (in situ disc). Accordingly, the fabrication of native and hybrid form of fibroin-based hydrogels are an emerging area that has been exclusively used in many aspects of the biomedical field [78].

From all the above scientific facts, we assume that the intrinsic physicochemical and rheological properties are mainly regulated the biological properties of the silk fibroin-based hydrogel. In fact, the rheological properties of the hydrogel are highly influenced by several external factors such as fabrication method, polymer concentration, copolymers, pH, temperature, crosslinkers and molecular structures, which control the stability of β-sheet, conductivity, adhesion property, self-healing ability, 3D printability, injectability and environmental stimuli-responsiveness. It is possible that the rheological properties potentially alter the receptor interaction of biological cells by manipulating external factor, which needs to be proved by proper scientific evidence. This can be realized by studying the molecular signaling interaction between biological cells' receptors and the functional group

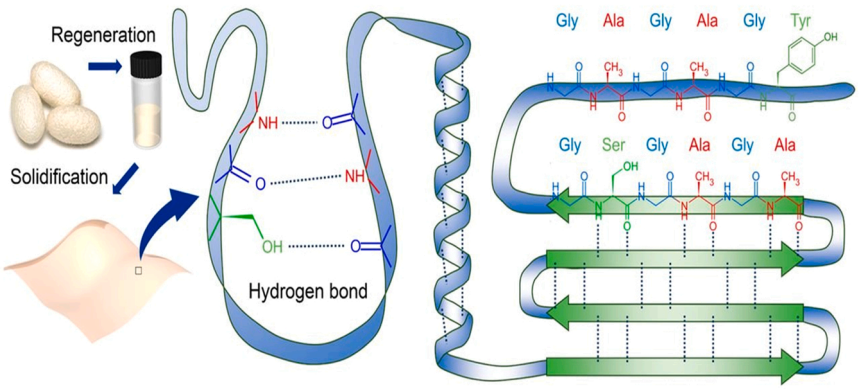

Fig. 1. Schematic of reconstituted silk-fibroin molecular chain showing the hydrogen bonding and secondary structures (random coil, β-sheet, helix) [225].

of hydrogel with varying rheological properties. Based on the above concepts, the present review reveals the major significance of the rheological properties such as viscoelastic, viscosity, gel strength, gelation time and swelling characteristics in the functional and biological behavior of silk fibroin-based hydrogel. Overall, this review addresses the scientific issues of fabricating novel hydrogels by manipulating the rheological properties in relation to the better biological functions of silk fibroin-based hydrogels.

2. Rheological properties of fibroin hydrogels

2.1. Viscoelastic properties

Aqueous hydrogels exhibit inherently interesting characteristic features when they are subjected to external stress, i.e. they can behave like a physical gel/solid or they can flow like a liquid under certain conditions. Thus, it is crucial to determine precisely its rheological features. Beside the viscosity as a function of the shear rate, temperature, ionic strength, etc. it is important to study their viscoelastic properties. In general, the viscoelastic behavior in such materials is derived from two main characteristic features of the polymer such as viscous and elastic response under mechanical stress due to liquid and solid interface. Among the different suitable features of polymers in biomedical applications such as biocompatibility, cytotoxicity, structure and so on, the viscoelastic property of aqueous-based polymer solutions plays a major role in terms of maintaining the similar elastic properties of host tissue to mimic the actual microenvironment for better healing ability. Earlier, a few authors investigated some novel experimental techniques to characterize the viscoelastic properties of the polymer [92,93]. In order to use polymer-based materials in practical applications, these polymers should be fabricated in such a way as to maintain the native tissue's rheological properties.

Silk fibroin-based hydrogel has been fabricated alone or through their association with other polymer materials with different rheological properties using different fabrication techniques (Table 1). In general, the rheological properties of hydrogels are highly variable depending on their intended use and fabrication/processing techniques. In most cases for biomedical applications, the desired rheological properties of fibroin hydrogels are balanced or improved by the addition of other biocompatible polymers such as collagen, gelatin, cellulose nanofibers, and bacterial cellulose, polyacrylamide and lignin-agarose [71,74,78].

As aforementioned, most aqueous-based hydrogels exhibit naturally a mixture of viscous and elastic behaviors. The majority of soft materials like polymer, hydrogel, and composites, have typical characteristics of elastic and viscous nature, whichare assessed experimentally through rheological measurements by computing their complex shear modulus G* and their associated storage modulus (G′) and loss modulus (G′'). G* (in Pa) describes the entire viscoelastic behavior of a sample and it is defined according to the law of elasticity for oscillatory rheological experiments are $G^* = \tau_A / \gamma_A$, where τ_A stands for the shear-stress amplitude (in Pa), γ_A stands for the dimensionless strain amplitude. G* is often referred to as the complex shear modulus. The storage modulus G' for its part represents the elastic portion of the material while the loss modulus G'' represent their viscous portion. Finally, a very important parameter is the phase shift (δ = angle between 0° and 90°), which is the time lag between the pre-set and the resulting sinusoidal oscillation for each measure. As far as the hydrogel is concerned, the storage modulus G′ refers to the deformation energy stored during physical/shear stress (i.e., the solid phase of the hydrogel) and the loss modulus G″, refers to the dissipated energy during physical/shear stress (i.e., liquid phase of the hydrogel). Another critical factor in the aqueous-based hydrogel is known as the loss factor defined as tan δ and it is computed by a simple calculation of G″/G′. Therefore, if G″ exceeds G′ (i.e., tan $\delta > 1$), the hydrogel flows like a liquid while, alternatively, when G′ exceeds G″ with tan $\delta < 1$, the hydrogel would resist flow and thus behave more like a solid [94,95].

Table 1
The viscoelastic (elastic modulus and viscous modulus) properties of silk fibroin hydrogel.

Sl. No	Concentration of Fibroin (%)	Other polymer	Elastic modulus (G') (kPa)	Viscous modulus (G") (kPa)	References
1	8		0.15	0.10	[125]
2	10		2.20 (20 °C)	1.93 (20 °C)	[205]
3	8		2.5	0.55	[170]
4	1		4.8		[100,101]
	2		7.4		
	3		22.4		
	4		33.1		
	5.2		70		
5	2.45		6.81	0.610	[171]
6	1		332.5	11	[206]
	2		2739.9	200	
	3		10,614.2	1000	
7	2.5		5	0.25	[173]
8	1–20		369–1712	63–441	[161]
9	16		1 (37 °C)	0.08 (37 °C)	[207]
10	4		7.48		[208]
	5		2.45		
12	8	Gelatin (15%)	751 (18 °C)	845 (18 °C)	[169]
			504 (28 °C)	328 (28 °C)	
			221 (37 °C)	442 (37 °C)	
13	3.8		0.08 (25 °C)	0.02 (25 °C)	[209]
14	15	Gelatin (1:2)	900–1200 (25 °C)	100–800 (25 °C)	[210]
15	0.25	10 % GM	3.2		[211]
	0.5	10 % GM	3.3		
	0.75	10 % GM	3.4		
	1	10 % GM	3.6		
16	20	–	10		[212]
	20	CNc	22		
	20	CNf	38		
	20	BC	63		
17	20	PVA	161		[213]
18	1.52	Ethanol (50 %)	3.0		[157]
	1.5	Ethanol (50 %)	1.5		
	1.23	Ethanol (50 %)	0.78		
	1	Ethanol (50 %)	0.33		
	0.75	Ethanol (50 %)	0.1		
	0.5	Ethanol (50 %)	0.03		
	0.25	Ethanol (50 %)	0.002		
19	6.1	Ethanol	4.1	2.5	[157]
		3:1	1.7	1.3	
		1:1	1.43	0.2	
		1:2			
20	3	Polyacrylamide	25	1.5	[214]
	3	7:3	48	2.8	
	3	6:4	85	5.0	
	3	5:5	200	11.5	
		0:1			
21	15	Ethanol	800	800	[158]
22	4	LG/$ZnCr_2O_4$	4.8	1.71	[105]
23	4	% GTA1.0%	7.48		[208]
	4	GTA	3.47		
	5	0.01 % GTA	2.45		
	5	0.001 % PCZ	2.75		
	5	0.005 % PCZ	1.79		
	5	0.01 % PCZ	1.60		
	5	0.05 % PCZ	1.50		
	5	0.1 % PCZ	1.33		
	5	0.5 % PCZ	0.97		
24	4	Collagen (1 %)	3	0.2	[87]
25	4	MeOH-treated	8.56	150	[205]
	4	(20 °C)	342	244	
	4	MeOH-treated (40 °C)	357	102	

(continued on next page)

Table 1 (*continued*)

Sl. No	Concentration of Fibroin (%)	Other polymer	Elastic modulus (G') (kPa)	Viscous modulus (G") (kPa)	References
26	28	10 % Gelatin	0.0014	0.00675	[122]
	5	20 % Gelatin	0.0077	0.0094	
	5	30 % Gelatin	0.448	0.145	
	5	40 % Gelatin	1.330	0.402	
	5	50 % Gelatin	2.320	0.714	
	5	5 % Gelatin	3.02	0.67	
	5	10 % Gelatin	0.371	0.0016	
	10	20 % Gelatin	2.07	0.0077	
	20		0.766	0.432	
27	3	1.5 % HA	204	56.6	[184]
	3	− 0.2 U/ml L	200.5	51.3	
	3	pH 2	97.8	33.7	
	3	pH 7	130.5	5.8	
		pH 12			

Gelatin methacrylate (GM), Cellulose nanocrystals (CNc), Cellulose nanofibers (CNf), Bacterial cellulose (BC), lignin–agarose (LG), Hyaluronic acid (HA) and Laccase (L)

Elastic modulus (G′) also provides valuable information about the storage modulus, elastic storage modulus and tensile modulus, which is defined by the stored deformation energy upon physical/shear stress (solid phase of the hydrogel) and is calculated by the ratio of the stress-to-strain curve of the material. For its part, the viscous/dynamic modulus or loss modulus G'' (which exemplifies the energy stored in the elastic structure of the material) provides valuable information by the dissipated energy upon physical/shear stress (liquid phase of the hydrogel) and is calculated by the ratio of stress to strain under vibratory conditions. Both parameters depend on the chemical structure and the molecular weight distribution of polymers[96]. It has been reported that a lower G' modulus value in SF hydrogel composites is often attributed to the prolongation of the formation of beta-sheet microcrystals [97].

The studies on silk fibroin hydrogels in tissue regeneration mainly skin, bone, cartilage, and invertebrate disc have been an increasing trend. For instance, high-strength silk fibroin hydrogel was fabricated by freezing and thawing technique using silk fibroin cross-linked with PVA. Interestingly, the mechanical and rheological properties of the silk fibroin/PVA hydrogel resemble that of ear cartilage and retained their actual shape during the entire cell culture study [97]. Silk fibroin functional hydrogel was fabricated with superparamagnetic iron oxide labeled cellulose nanocrystal and used for non-invasive monitoring of hydrogel degradation during cartilage regeneration [98]. In another study, silk fibroin fabricated with methacrylate hyaluronic acid had high mechanical strength, excellent biocompatibility and suitability for load-bearing soft tissue to culture osteoblast cells [99]. Also, hydrogels fabricated with 70 KPa using 5% silk fibroin showed better neural tissue regeneration properties than that of hydrogel with 4.8 KPa using 1% silk fibroin [100,101]. The viscoelastic properties of SF hydrogel have been proved to be of paramount importance for the fabrication of biocompatible scaffolds produced via extrusion bioprinting. Indeed, Chen et al. demonstrated that hydrogels based on the association of high-molecular-weight regenerated silk fibroin (HMWRSF) with hydroxypropyl methylcellulose (HPMC) exhibited biological response dependence on their rheological properties. They reported a 3-interval thixotropy test (3ITT) in which G' was higher than G'' during the first stage of the test i.e. the small amplitude oscillatory shear (SAOS) region, with 1% amplitude. At this stage, such a sample is in the hydrogel state. In contrast, the G' decreased abruptly until reaching lower values than G'' during the second stage of the large amplitude oscillatory shear (LAOS). In this stage, the test is running at 500% amplitude so the hydrogel shows fluidity. Finally, during the third stage of the test, applying the SAOS with a 1% amplitude, is observable that G′ increased accordingly much higher than G''. So the recovery process cycle is achieved within a very short time. Authors also claim that such 3D bioprinted scaffolds exhibited biological response dependence on the apparent density of the materials. This physical property was also related to the viscoelastic behavior of the hydrogel and its printability [97]. Similarly, Liu et al. recently reported the viscoelastic properties of SF hydrogel at different content and molecular weight aiming to fabricate bio functional hydrogels for protein drug delivery. They reported that all the hydrogels exhibited higher elastic than viscous modulus G′ > G'' with no cross-linking point, which is the signature of a semi-solid behavior. They also demonstrated that the gap between G′ and G'' increased as the content of SF increased from 5 to 30 mg/ml indicating that SF hydrogels were good elastomers with specific mechanical strength [103]. Interestingly, the drug release capacity and the degradation rate of the SF hydrogel were also dependent on the molecular weight, SF content and their intrinsic physicomechanical properties. In general, more elastic and viscous properties favor the physiological state and strength of fibroin hydrogels. Several authors reported elastic and viscous modulus of fibroin hydrogels alone and a combination of other polymers (Table 1). As shown in Table 1, different concentrations of fibroin and types of additives significantly altered the elastic modulus and viscous modulus of hydrogels. The value of elastic modulus is ranged from 0.002 to 10,614 KPa for the fibroin hydrogel with the concentration of 0.25–20% with or without other polymers. Table 1 confirms that the viscoelastic properties of hydrogel were highly depending on the fibroin content and the molecular weight. For instance, higher viscoelastic properties can be obtained by increasing the content and molecular weight of fibroin [100,101].

Skin and wound healing: The hydrophilic nature of hydrogel plays a critical role in the nourishment of damaged skins to speed up recovery. It has been demonstrated that the silk fibroin hydrogel prepared with optimum viscoelastic properties can promote cell adhesion, proliferation, blood coagulation and drug delivery to target tissue for skin regeneration and wound healing process [97,104,105].

By considering these properties, several types of pure and composite silk fibroin hydrogels were fabricated in recent times. The methacrylated chitosan silk fibroin hydrogel fabricated with 13 KPa elastic modulus and 10 KPa viscous modulus improved the mechanical, and adhesive properties and improved the healing ability in wound repair of ICR male mice [102]. Hydrogels prepared from 3% and 6% silk fibroin of *A. assama* and *B. mori* with 336.42 and 49,086 Pa elastic modulus, respectively potentially promoted skin regeneration and burn wound healing [103]. Silk fibroin combined with gelatin and adipose-derived stem cells and platelet-rich plasma with 2 KPa elastic modulus and 0.06 KPa viscous modulus improved wound healing in a murine pressure ulcer model [104]. Silk fibroin cross-linked lignin–agarose/ $ZnCr_2O_4$ nano biocomposites hydrogels had elastic modulus and viscous modulus of 4.88 KPa and 1.72KPa, respectively and tested for antimicrobial properties (inhibiting anti-biofilm activity) and healing of burn wounds of mice [105]. It has been reported that the silk fibroin- polyvinyl alcohol-borax ionic hydrogel fabricated with 100 KPa showed excellent biocompatibility, stretchability, tunable conductivity, adhesive capabilities, water retention and self-healing ability [106]. A regenerated silk fibroin/polyacrylamide double cross-linked hydrogel with high toughness and viscoelastic strength exhibited good ionic conductivity [107]. Recently, Sonamuthu et al. fabricated silk fibroin loaded with anti-inflammatory curcumin and metal-chelating dipeptide (L-carnosine) to produce antioxidant and anti-inflammatory hydrogels, which improved the healing efficiency of wounds in diabetic mice, along with anti-inflammatory and anti-oxidation functions [108]. The functionality of fibroin hydrogel in skin regeneration and wound healing can be improved by fabricating hydrogel with specific adhesion to the wound and incorporating growth factors, cytokines and drugs [79,109]. In general, the fibroin hydrogel fabricated with high viscoelastic properties retains more adhesive properties, which substantially promote the wound healing process [110].

Drug release: The viscoelastic properties potentially regulate the drug delivery characteristics of silk fibroin hydrogel. For instance,

changes in the viscoelastic properties potentially affect the drug-releasing behavior of fibroin hydrogel. More specifically, the hydrogel with higher viscoelastic characteristics could increase the releasing rate of target drugs [111,112]. In general, silk fibroin hydrogels possess good drug-loading ability and biodegradation. Due to the functions of injectability, in situ gelation and thixotropy, silk fibroin hydrogels have fascinated important consideration in the field of drug release. For instance, the injectable silk fibroin/hydroxypropyl cellulose hydrogel loaded doxorubicin and curcumin with elastic modulus 100 KPa and storage modulus 0.2 Pa had simultaneous and long-term sustained release for the treatment of local cancer [111]. The release rate of doxorubicin (DOX) in silk fibroin hydrogel depends on injectability and pH responsiveness. Several studies have reported the influence of viscoelastic properties in drug delivery efficiency of silk fibroin hydrogel, especially loaded with DOX [112,113], dexamethasone [114], salinomycin [115] and other drugs.

2.2. Viscosity properties

Viscosity controls the physiological, functional properties and gelation behavior of hydrogel. The viscosity of polymer solution is one of the most substantial constraints for the preparation of hydrogel [116]. The viscous nature of hydrogel is highly influenced by the polymer concentration, type of crosslinking (both physical and chemical), temperature, pH, ionic strength, and the presence of surface active agents (surfactant) and/or ethanol treatments. The major challenging properties for silk fibroin hydrogel fabrication are optimum intrinsic physical properties (surface tension, viscosity, and rheology) along with the material properties (swelling ratio). Hence, it is necessary to find an optimum viscosity of polymers to fabricate innovative hydrogels. Hence, many studies were carried-out to find the optimum viscosity of fibroin to fabricate an ideal hydrogel for practical application. Table 2 summarizes the viscosity of different hydrogels prepared by different fibroin concentrations. In general, the hydrogels were fabricated with 0.25–20% of fibroin concentration and reported the viscosity ranging from 0.09 to 10, 000 Pa. The viscosity of hydrogel differs at varying silk fibroin due to polymer interactions [117]. It was reported that the viscosity was in the range of 103–108 Pa s obtained from native silkworm silk, *B. mori* [118, 119].

The strength and gelation time of the hydrogels are purely dependent on the viscosity of the fibroin solution used for fabrication. In other words, the higher viscosity of hydrogel promotes an increase in the gel strength and gelation time [8,120]. According to the available literature, the average viscosity of silk fibroin aqueous solution was 0.3–30 P for gelation of hydrogels [96,121], however, the lower viscosity can be useful for hydrogel formation with additional treatment such as temperature, pH and ethanol treatment.

The viscosity of silk fibroin hydrogel can be affected by additives such as glycerol, 1,3-propanediol, 1,2,6-hexanetriol, adonitol, and erythritol, which are reported to affect the content of β-sheet in silk [96]. For instance, the addition of 10 % gelatin and 1 % glycerol in 5% silk fibroin produce the most robust structures with more viscosity (Dynamic 4677 and plastic viscosity 1978 cP), which was 349–412 times higher viscus than silk alone [96,122–124].

The viscosities of Muga silk fibroin hydrogel in the concentration ranging from 5 %, 7 %, and 10 % were 1145, 1243 and 1446 cP, respectively and the biodegradation, biocompatibility (using L929 fibroblast cells) and antimicrobial activity confirmed that the muga silk fibroin hydrogels fabricated with the viscosities ranging from 1145 to 1446 cP could be the potential material for skin tissue engineering and regenerative medicine [116]. The authors further concluded that better biocompatibility was obtained by higher rheological properties such as viscosity, swelling behavior and gelation of fibroin hydrogel. Recently, Yao et al. fabricated silk fibroin hydrogels by applying ultra-sonication in crystalline silk fibroin (CSF) in order to produce more β-sheet-rich domain and reported the viscosities of SF hydrogels (1–5 wt%) ranging from 0 to 20 mPa.s [105]. In this work, the authors observed that the higher flowability and the higher gel formation were mainly due to low viscosity (<0.025 Pa·s) and higher viscosity (20 mPa.s) of hydrogel, respectively. More importantly, they also disclosed that the CSF hydrogel with higher viscosity showed better biocompatibility in chondrocyte cells than CSF hydrogel with low viscosity. From the empirical evidence, it was confirmed that viscosity plays a crucial role in the biocompatibility of hydrogels [105].

The hydrogel fabricated with 6 % silk fibroin for the application of skin regeneration and wound burn had a viscosity of 20 Pa [103]. Yuan et al. reported that the range of viscosity of hydrogel was increased with increasing silk fibroin content (10,12,14,16% and 18%), pH (3.5,4.0, 4.5,5.0 and 5.5) and time (0,15,30 and 60 min) [125]. In the above study, the silk fibroin hydrogel was used for biomedical adhesive purpose and reported a wide range of viscosities (0–2000 Pa) for a different formulation of fibroin and poly(ethyleneglycol) (PEG) content. Several studies also claimed that the viscosity of hydrogel is highly regulated by temperature [8,96,126–130].

The methacrylated silk fibroin (5,10 % and 15 %)-gelatin hydrogels prepared with 10–70 Pa viscosity showed excellent biocompatibility, inflammatory infiltration, angiogenesis, nerve regeneration, ulcer and wound healing [104,131].

The 2 % silk fibroin hydrogel fabricated with different

Table 2
Viscosity of Silk fibroin hydrogel.

Concentration of Fibroin %	Composites	Viscosity (Pa)	References
8		1890–892	[125]
7		0.6	[215]
16		0.5	[216]
20		1.38	[114]
10		0.09	[217]
3.8		0.2 (25 °C)	[209]
1		100	[206]
2		1000	
3		10,000	
0.25		0.14	[211]
0.5		0.18	
0.75		0.35	
1		59.73	
5		0.8–0.9	[176]
2		1200	[155]
5		1.145	[116]
7		1.243	
10		1.446	
1	1 g SSE	22.20	[132]
2	1 g SSE	25.90	
5	1 g SSE	31.90	
1		22.10	
2		25.30	
5		30.80	
8	15% Gelatin	25. (28 °C)	[169]
8	15% Gelatin	0.57 (37 °C)	
15	Gelatin (1:2)	400–500 (25 °C)	[210]
2	20% Pluronic F-127	600 (37 °C)	[218]
16	HRP	4.7	[207]
28	–	0.59	[122]
20	20 % Gelatin	23	
10	10 % Gelatin	1.04	
5	5 % Gelatin	0.16	
5	40 % Gelatin	8.67	
5	50 % Gelatin	4.87	
5	10 % Gelatin	1.19	
5	20 % Gelatin	3.3	
5	30 % Gelatin	5.43	
2	Biliverdin	3	[219]
	1 %	7.5	
	2 %	8	
	3 %		

Sesbania sesban Extract (SSE), horseradish peroxidase (HRP)

concentrations of Biliverdin (1 %, 2 % and 3 %) for antiglioma photothermal therapy and wound healing had a viscosity of 3–8 Pa. The viscosities of silk fibroin hydrogel with different concentrations (1,2 % and 5 %) were 22.20, 25.90 and 31.90 Pa, which was slightly altered (22.10, 25.30 and 30.80) with the addition of Sesbania sesban (SS) extract (1 g total phenolic the total phenolic content of the SS leaves/flowers), respectively [132]. The above study disclosed that SSE-loaded fibroin hydrogel demonstrated comparable anti-inflammatory and become a prospective for rheumatoid arthritis treatment. Zhao et al. reported that the higher viscosity of hydrogel by increasing the concentration of silk fibroin was due to the introduction of more hydrogen bonding sites between fibroin molecules [133]. Therefore, an ultimate viscosity of the hydrogel would favor the gelation strategy and thereby facilitates a beneficial environment for biological cells for tissue regeneration.

2.3. Gel strength

One of the major factors for fabricating hydrogel is gel strength (GS), which is defined as the amount of force to retain and develop a gel form during colloidal dispersion. In general, the higher gel strength is favorable for potential application in case of drug delivery, slow degradation, stability and biocompatibility. It was reported that a novel hydrogel should possess 4282–13562 g [134], however, this range may differ based on the applications. Various factors such as polymer concentration, temperature, pH and ionic state could affect gel strength, which is potentially related to different types of bonds such as ionic bonds [135–137], hydrogen bonds [138–140], hydrophilic interactions [141–143] and hydrophobic [144–147]. In short, intra- and intermolecular interactions such as hydrophobic interactions and hydrogen bonds are the major cause of the hydrogelation process [148–150]. The gel strength of hydrogel plays a critical role in biological applications such as wound healing, drug delivery, agriculture, food industries, and so on [151]. The formation of a β-sheet from a random coil leads to solidifying of the polymer solution into hydrogel [152–154].

As shown in Table 3, the GS of hydrogel is potentially varied concerning fibroin concentration. The GS of pure silk fibroin hydrogel was reported in the range of 0.0016–243708 g based on the fibroin content. However, the addition of other composites could potentially moderate the GS of fibroin hydrogel. The hydrogel used for rheumatoid arthritis treatment showed varying GS (1205, 1318 and 1276 g) based on the amount of fibroin used for hydrogel fabrication (1, 2% and 5%) [132]. The drug-releasing behavior of 2% silk fibroin-methylcellulose / 3% silk fibroin-polyacrylamide hydrogel was evaluated with the GS of 5.71 g and 1294 g, respectively [155,156]. Silk fibroin hydrogel with varying fibroin concentrations (0.25–1.5%) evaluated for photodynamic therapy had gel strength of 0.001–22 g, respectively [157]. Chen et al. reported that the regenerated silk fibroin (12%)- hydrophobic polyacrylamide hydrogels prepared with 1.24×10^6 g gel strength could be used as a touch screen pen, a strain sensor, and the electronic skin of artificial robots [107]. The ethanol (20–100%) treated silk fibroin (7.5–15%) hydrogel with 4000–34600 g gel strength facilitated the controlled network structures, toughness and favorable biodegradability [158]. For bone repair and bone engineering applications, the silk fibroin (25%)-hexafluoroisopropanol (1:1) hydrogel was fabricated with 1.3×10^5 g gel strength [159]. Numata et al. reported that superior mechanical, biodegradable and biocompatible fibroin (20%) hydrogel could be achieved with 7×10^5 g by adding 5% pectin [160]. Sonicated silk fibroin solutions at 4%, 8%, and 12% (w/v) gelled for 0.5–2 h with 254–2040 g were used for encapsulation of Human bone marrow-derived mesenchymal stem cells [161]. In another study, high-strength durable silk fibroin (8–14%) hydrogel with versatile processability was fabricated with 815–7138 g gel strength [162]. The regenerated silk fibroin hydrogel crosslinked with horseradish peroxide (HRP)/H_2O_2 showed small-sized and uniformly distributed β-sheet domains formation in the hydrogel with $0.3–3.00 \times 10^4$ g [163]. Interestingly, fabricating regenerated silk fibroin with hydroxypropyl methyl cellulose increased the GS from 3772 to 12,542 g [164]. The highest gel strength of gelatin/silk fibroin hydrogels encapsulated with adipose-derived stem cells and platelet-rich plasma was reported as 30 kPa, which evidenced the strong rigidity that improved wound healing in a murine pressure ulcer model [104]. Accordingly, all these observations proved that the higher GS of hydrogel holds great promise for applications in biomedical and pharmaceutical fields.

Table 3
The gel strength of different silk fibroin hydrogel.

Concentration of Fibroin %	Composites	Gel strength (g)	References
1		1205.15	[132]
2		1318.02	
5		1276.71	
2		5.71	[155]
1.52		22	[157]
1.5		10.65	
1.23		5.77	
1		2.66	
0.75		0.91	
0.5		0.05	
0.25		0.0016	
4		1019	[166]
8		4078	
12		10,197	
16		20,395	
4		254	[161]
8		102	
12		2040	
8–14		815–7138	[162]
3	Polyacrylamide	1294	[156]
3	7:3	1809	
3	6:4	2466	
3	5:5	2777	
	0:1		
12	HPA	1,244,035	[107]
7.5	20 (Ethanol)	296,732	[158]
15.0	40	285,516	
25.0	60	243,708	
15	80	4099	
15	100	15,907	
15		26,716	
15		33,038	
15		34,669	
25	HFIP (1:1)	132,560	[159]
20	HFIP (1:1)	82,000	[160]
20	Pectin (5%)	70,359	
10	HRP and H_2O_2/ alcohols	3161–30591	[163]
10	HMC (1:1)	3772–12542	[164]

Hydrophobic Polyacrylamide (HPA), Hexafluoroisopropanol (HFIP), horseradish peroxidase (HRP), hydroxypropyl methyl cellulose (HMC)

2.4. Gelation time

Gelatin time is simply defined as the time taken for a polymer solution to transform into a gel, a process called solidification. Due to the presence of fibrous protein (fibroin), the silk hydrogel is easy to form a gel with optimum polymer concentration. The optimum gelatin time for silk fibroin hydrogel with different concentrations of fibroin was listed in Table 4. As shown in Table 4, a wide varying gelation time was reported for different concentrations or compositions of silk fibroin hydrogels ranging from 22 s to 2 weeks.

It is worth mentioning that maintaining the gelation period for 1–3 days exhibited strong mechanical properties of the regenerated pristine silk fibroin (8–14 %) [162]. However, the self-gelation of fibroin hydrogel was reported to be slow ranging from days to weeks depending on fibroin concentration (0.6–15 %) at room temperature or 37 °C [165–167]. The actual gelation of hydrogel is achievable by keeping the pH of silk fibroin closer to its isoelectric point at 4.8–5 [168].

Previously, it was reported that the gelation time of regenerated silk

Table 4
Gelation time of different concentration/composites of silk fibroin hydrogels.

Concentration of Fibroin (%)	Composites	Gelation time	Temp	Ref
1		> 2 weeks		[206]
2		> 2 weeks		
3		> 2 weeks		
8		22 s		[125]
0.175		No		[171]
0.35		No		
0.5		No		
0.7		120 min		
1.05		30 min		
1.25		20 min		
1.75		10 min		
2.45		6.26 min		
1–8		7–12 d		[165]
2		40 h	37 °C	[220]
3		40 h		
1.25		11 h		[173]
2.5		10 h		
3.75		9.5 h		
5		9 h		
1–20		1–100 h		[161]
2		60 h	37 °C	[155]
2		30 d	37 °C	[166]
4		23 d		
6		14 d		
8		10 d		
12		9 d		
16		7 d		
20		5 d		
3 (*B. mori*)		15–20 d	37 °C	[103]
		25–30 d	RT	
3 (*A. assama*)		2–3 h	37 °C	[103]
		5–6 h	RT	
3: 3 (*B. mori*:		480 s	37 °C	[103]
A. assama)		2350 s	RT	
4		16 h	60 °C	[182]
5	Dex	533 min	37 °C	[114]
10	1 %	406 min		
15	1 %	239 min		
20	1 %	161 min		
5	1 %	497 min		
10	4 %	372 min		
15	4 %	219 min		
20	4 %	150 min		
5	4 %	478 min		
10	8 %	350 min		
15	8 %	215 min		
20	8 %	107 min		
	8 %			
8	Gelatin (15 %)	40 min	37 °C	[169]
8	Oxidized Pectin	2300 s		[170]
SF25	P75	3200 s		
SF50	P50	1700 s		
SF75	P25			
3 (*B. mori*)	50 % PEG	288.6 h	37 °C	[172]
	100 % PEG	289.2 h		
	150 % PEG	282.7 h		
	300 % PEG	265.7 h		
	500 % PEG	191.4 h		
	700 % PEG	103.1 h		
	900 % PEG	50.4 h		
		38 h		
3 (*A. pernyi*)	50 % PEG	69.3 h	37 °C	[172]
	100 % PEG	57.1 h		
	150 % PEG	50.1 h		
	300 % PEG	45.9 h		
	500 % PEG	38.6 h		
	700 % PEG	38 h		
	900 % PEG	42.7 h		
		43.5 h		
5	0.1–4 % $NaNO_3$	2–14 min		[176]
2	Ultrasonic at 20 %	22 h		[178]
	amplitude	4–5 min		
6.1				[157]
	Ethanol	24 h		
	3:1	2.14 h		
	1:1	20 min		
	1:2			
4	SDS (8–12 mM)	500 min	37 °C	[177]
		15–18 min		
75	HFIP(%)	> 10 d		[162]
50	82	> 5 d		
37.5	78	3 d		
30	74	2 d		
25	71	2 d		
21.5	68	1 d		
18.75	65	1 d		
16.67	63	1 d		
13.6	60	1 d		
	56			
5	Doxorubicin	88 min		[111]
5	Curcumin	75 min		
5	Dox-Cur	75 min		
5		87 min		
4	HRP	130 min	37 °C	[163]
4	300	30 min		
4	500	9 min		
4	700	6.5 min		
4	900	4 min		

Dexamethasone(Dex), Hexafluoroisopropanol (HFIP), polyethylene glycol (PEG).

fibroin (4 %) was decreased from 130 min to 4 min by increasing HRP concentration, however, H_2O_2 showed an increased gelation time, which might be due to excessive oxidation of polymer in presence of excessive H_2O_2 [163]. The silk fibroin (5–20 %) fabricated with Dex (1–8 %) for the therapy of cisplatin-induced ototoxicity showed 533–107 min of gelation time [114]. Cell-laden silk fibroin (8 %)–gelatin (15 %) hydrogel was fabricated with a gelation time of 40 min in order to support multilineage differentiation of stem cells [169]. However, no gelation was observed at 4 °C in sonication crosslinked silk fibroin-gelatin hydrogel and it was increased with increasing temperature from 28° to 37 °C. Further, the authors reported that the gelation time was increased from 1 h 15 min to a few seconds by increasing the sonication time of 8% silk fibroin solution from 5 to 30 s. A shorter gelation time (22 s) was reported by Yuan et al. for 8 % silk fibroin hydrogel prepared for Biomedical Adhesive application [125]. The incorporation of oxidized pectin at an optimum concentration (50:50 ratio) increased the gelation time of silk fibroin hydrogel than that of other ratios (25:75 and 75:25), which could be due to the presence of an equal ratio of aldehyde to amine groups of oxidized pectin and fibroin [170]. Buitrago et al. have done detailed research on the gelation behavior of silk fibroin and collagen based on the concentration, temperature and ionic strength of polymers [171]. More specifically, the gelling behavior of silk fibroin showed that the gelling time was decreased from 120 min to 6.26 min and 27–7 min with increasing silk fibroin concentration (0.7–2.45%) and sonication time (5–15 s), respectively, and no gelation was observed at 0.3 % and 0.5% fibroin concentration by the ultrasonication unless they were blended with collagen. At a constant collagen concentration (1 mg/ml), the gelation time was decreased significantly with more than 1% silk fibroin (6.5, 2.8 and 2.1 min for 0.5 %, 1 % and 2 % fibroin, respectively). Overall, these observations revealed that the gelation behavior of hydrogel is regulated by the physical and chemical molecular interaction of silk fibroin with other polymers. The gelation time of 1–8 % fibroin hydrogel was 7–12 days, and the gelation time was decreased with increasing fibroin content and temperature, however, the fibroin concentration of less than 1% failed to produce hydrogelation [165]. In another study, the gelation pattern of hydrogel prepared from domestic (*Bombyx mori*) and wild (*Antheraea pernyi*) silkworms was investigated. Their results showed that

the gelation behavior of hydrogel from wild silk (69.3 h) was quicker than the domestic silkworm-derived hydrogel (103.1 h), even with a higher PEG concentration (500 %) [172]. Similarly, the gelation time of hydrogel prepared from 3 % (*B. mori*), 3 % (*A. assama*) and 3 % (*B. mori*)− 3 % (*A. assama*) was 15–20 d (37 °C) and 25–30 d (RT), 2–3 h (37 °C) and 5–6 h (RT) and 480 s (37 °C) and 2350 s (RT), respectively [103]. In the same time, gelation time of silk fibroin at 37 °C was 20 min, whereas it was 45 min at 25 °C [103].

The gelation time of silk fibroin (2.5 %) hydrogel (11 h) was abruptly decreased by 50% methanol (25 s) due to the conformational changes and aggregation and subsequent gelation [173]. To support this concept, earlier studies reported that the incorporation of alcohol into silk fibroin cause dehydration of protein molecules thereby increasing intra and intermolecular interactions to stabilize hydrogel [174,175].

The silk fibroin hydrogel (4 %, 8 %, and 12 %) fabricated for encapsulation of human bone marrow-derived mesenchymal stem cells showed different gelation times (50 h to few s) based on K^{+}, Ca^{2+} and pH treatment [161]. In another study, the gelation time of silk fibroin was significantly decreased (14–2 min) by adding 0.1–4 % of $NaNO_3$ [176]. Increasing temperature from 25° to 50 °C decreased the gelation time of 2 % silk fibroin hydrogel, which occurred by a conformational transformation of a random coil structure to a β-sheet structure in the hydrogel [155]. The gelation time of Doxorubicin-Curcumin-loaded 5 % silk fibroin thixotropic hydrogel for the chemotherapy of cancer was 88–75 min [111]. Similarly, the addition of horseradish peroxide (300–900 units/ml) with 0.3 % H_2O_2 could potentially decrease the gelation time (130 min) of 4 % silk fibroin to 30–4 min at 37 [163]. The gelation time of 4 % silk fibroin hydrogel treated with a gelling agent, SDS (8–12 mM) was also decreased from 500 min to 15–18 min through the rapid formation of β-sheet structures due to hydrophobic and electrostatic interactions [177]. The ultrasonic treatment at 20 % amplitude decreased the gelation time of 2 % silk fibroin from 22 h 4–5 min [178]. Improving gelation time during the ultrasonic treatment is due to the highest Amide I/Amide II ratio, which directly denotes the available beta-sheet structure and crystalline phase of silk fibroin, a critical factor for the stronger gel structure [178].

2.5. Swelling ratio

The swelling ratio of hydrogel is purely determined by the amount of hydrophilic (water adsorption), functional groups and special interaction of polymers to maintain structure stability [179]. Due to the presence of a fibrillary structure that tends to form a 3D network in a hydrogel, the silk fibroin gets swell naturally. The swelling property is an important key factor for the drug loading and delivery mechanism and provides a spatial arrangement for the biological cells to grow similarly to native tissues.

The recombinant human fibroblast growth factor-1 (FGF1) (1 μg/ml) loaded silk fibroin hydrogels with a 20% swelling ratio were reported to promote the wound healing process in rats [41]. This study concluded that the FGF1-loaded fibroin hydrogel could be the new treatment option for wound repair and regeneration. In another study, the swelling rate of methacrylate gelatin (16.1 %) hydrogels was reduced to 8–5 % by adding 5, 10 % and 15 % methacrylated silk fibroin. The authors justified that these changes could be due to the high cross-linking reaction of methacrylated silk fibroin, which subsequently shortens the pore size and intermolecular gap. In the above study, the potential role of methacrylate gelatin-methacrylated silk fibroin composite hydrogel in inflammatory infiltration, angiogenesis, nerve regeneration, ulcer and wound healing in mice model was reported [104]. It was reported that an increasing concentration of methanol from 0 % to 50 % abruptly decreased the swelling ratio of 2.5 % silk fibroin hydrogel from 9.0 to 1.2 [173]. The swelling behavior of hydrogel was decreased (9.4, 8.6, 7.8 and 7.0) with increasing silk fibroin concentrations (1.25 %, 2.5 %, 3.75 % and 5 %). In contrast, no significant changes were observed in the swelling properties of Gelatin methacrylate hydrogel prepared with an increasing concentration of silk fibroin from 0.5 % to 2 %, however, the authors did not justify the observation in this work [180].

The swelling ratio of silk fibroin crosslinked lignin–agarose/$ZnCr_2O_4$ nano biocomposites hydrogel was 815 % [105]. The swelling behavior of silk fibroin (8 %)–gelatin (15 %) hydrogel was increased by Tyrosinase crosslinking (55–60 %) than crosslinking induced by sonication (30–35 %) [169]. The swelling rate of 4 % silk fibroin was increased from 1 to 7 with the addition of gelation from 0 % to 90 %, however, no swelling behavior of the pure silk fibroin was observed after 48 h at 20 °C in this study, which could be due to the intrinsic hydrophobicity and presence of rigidifying beta crystals in silk fibroin [181]. In addition, the swelling ratio (400 %) of silk fibroin (4 %) hydrogel was significantly improved (1000 %) by carboxymethyl chitosan (CC) (1:1) due to chemical crosslinking of hydroxyl, carboxyl and amino functional groups between two polymers to form a stable network structure and the swelling effect was stimulated by changing pH from 2 to 7 in silk fibroin (from 400 % to 600 %), respectively [182]. Wang and Zhang reported that the electropolymerized silk fibroin hydrogel with a swelling ratio of 1056.4 % is mainly comprised of a mixture of α-helix and β-sheets crystalline structures [183]. The tyramine-modified 1.5 % hyaluronic acid (catalyzed by 0.2 U/ml Laccase) loaded 3 % silk fibroin hydrogel showed the swelling ratio of 30 %, 40 % and 70 % at pH 2,7 % and 12 %, 20 %, 10 % and 5 % at 0.01, 0.08 and 0.15 M NaCl, 35 %, 10 % and 12 % at 0.01,0.08 and 0.15 M $CaCl_2$, and 25 % and 2 % at 25 % and 50 % ethanol treatment, respectively [184]. The properties of swelling–deswelling behaviors against pH, ionic, temperature and protein content were investigated by Huang et al. who reported that the rate of swelling ratio was increased in the first 24 h then attained equilibrium and the swelling ratio of 5 % silk fibroin hydrogel declines with the increase of polyurethane contents (15–30 %) i.e corresponding equilibrium swelling ratios (ESRs) decreased from 4.37 to 3.85, respectively, which was due to rise of chemical cross-linking interactions in hydrogels [185]. Not only protein content, but the molecular weight of polymers also plays a crucial factor in hydrogel network formation that controls the gel properties such as viscosity, permeability and the swelling ratio [186–188]. Previously, Kim et al. investigated the effect of the molecular weight of silk fibroin ranging from 263.1 to 82.7 kDa produced by alkaline hydrolysis on swelling properties of hydrogel and tested the suitability for human mesenchymal stem cells (hMSCs) culture [189]. Their findings showed that fibroin with low molecular weight (MW) showed only 18 % of gel formation, whereas unhydrolyzed native fibroin (3 %) formed 98 % hydrogel, which revealed that the low MW fibroin does not important to make hydrogel due to random coil structure (unable to form beta sheets due to disarrangement of the structure by hydrolysis). In contrast, the hydrogel formed by low MW fibroin had higher swelling rate (~200 %) due to loss network and intermolecular space to attract more water than native fibroin hydrogel (~30 %) [190–196]. Though, the low MW fibroin hydrogel had high swelling rate, it had very poor cell adhering properties as the number of hMSCs cells were more attached in native fibroin hydrogel than low MW hydrogel. One of the possible mechanisms is that the binding and migration behavior of cells are closed related with polymer matrix stiffness, therefore the poor stiffness and rheological properties could be the possible reason for poor hMSCs cells attachment on low MW hydrogel [197–204]. The overall concept of silk fibroin hydrogel with suitable rheological properties and biomedical application is summarized in Figs. 3 and 4.

It is noticeable that the changes in the rheological parameters potentially alter the biocompatibility and biological regeneration performance of a hydrogel. For instance, the hydrogel with higher viscosity, gel strength, and gelation, and optimum swelling behavior could achieve better biocompatibility and regenerative ability.

As the aforementioned works highlighted, the viscoelastic properties of silk fibroin hydrogel biomaterials not only serve to predict its mechanic-intrinsic properties such as the elastic/viscous modulus, the gelation time, gel strength and swelling degree but also serve to

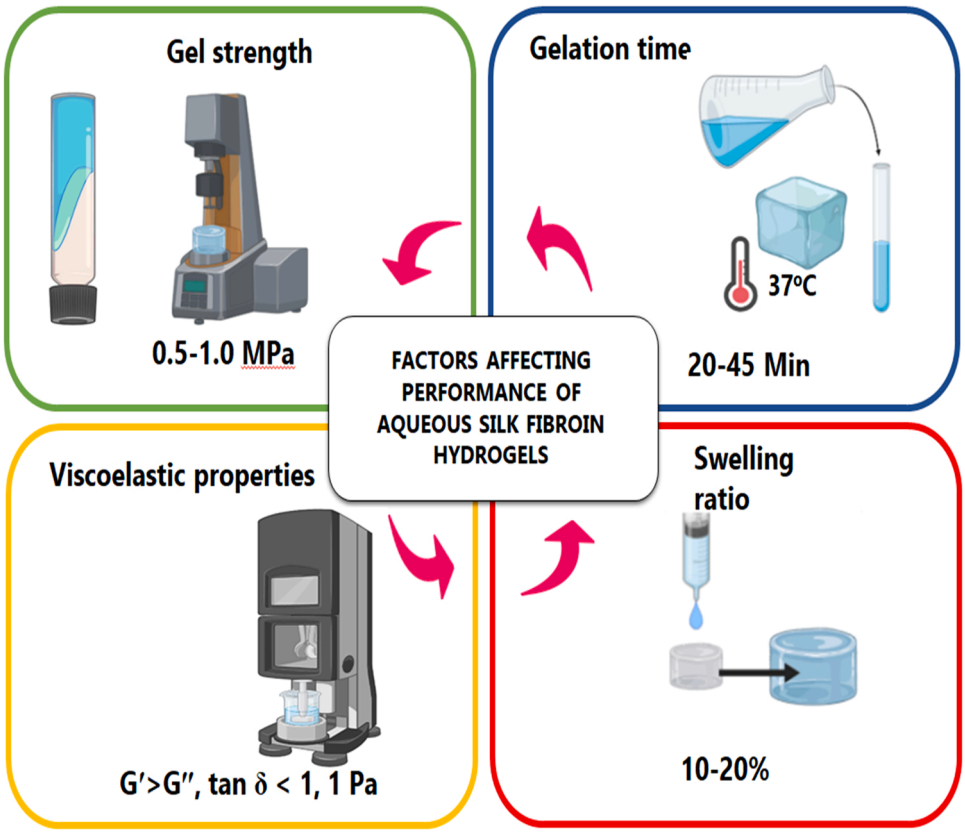

Fig. 3. A summary of the factors affecting the performance and biological response of aqueous based Silk Fibroin Hydrogels.

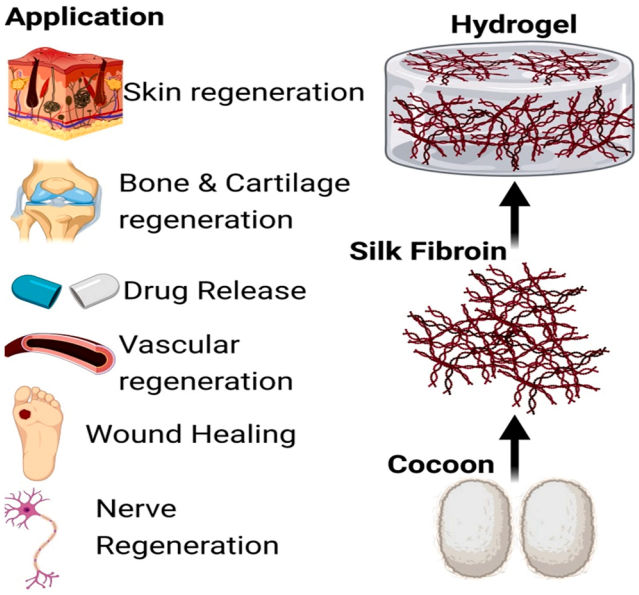

Fig. 4. Schematic representation of hydrogel formation from cocoon-derived silk worm and its potential application.

modulate their biological response, cell adhesion, cytotoxicity, cell proliferation and differentiation, degradation rate among others. Likewise, the rheological properties can be readily affected by several factors such as temperature, pH, particle content, molecular weight, and ionic strength, among others, limiting not only their biological response but also their functionality such as the drug delivery and degradation rate. Indeed, the visco-elastic properties will also govern its structural morphology by the size/shape of the particle's aggregates, the porosity, etc. Thus, the ideal silk fibroin hydrogels for skin, cosmetic and wound healing purposes should exhibit tailored mechanical, and viscoelastic properties accompanied by adequate tissue regeneration ability, and biocompatibility.

3. Conclusion

The present review discussed the relationship between storage/loss modulus, viscosity, gel strength, gelation time and swelling properties of silk fibroin hydrogel for biomedical applications. All these properties are potentially affected by various physical and chemical factors such as temperature, pH, ionic point, salts, plasticizers, and protein concentration. The ideal silk fibroin hydrogels for skin, cosmetic and wound healing purposes should exhibit enhanced biological response which is regulated by its tailored mechanical, rheological, viscoelastic properties, effective tissue regeneration ability, controllable swelling, hemostasis and biocompatibility. The viscoelastic and rheological properties also dominate the crystallinity and structure. The association of a specific set of physico-mechanical-chemical properties with a concomitant biological response is still controversial. i.e stiffer hydrogel with higher elastic modulus had shown improved degradation rate and cellular adhesion, cytotoxicity and proliferation. Nevertheless, similar biofunctional material with lower elastic modulus and more fluid features also shown to be suitable for tissue regeneration. Overall, the functional properties of hydrogel in biological and pharmaceutical applications are controlled by twisting the rheological properties of silk fibroin. Therefore, future research in this field, aiming to understand the chemical mechanism, predict and modulate the final biological performance by tailoring viscoelastic, rheological and physicochemical properties, is very attractive.

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CRediT authorship contribution statement

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Conflict of interest

We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. All authors have approved the manuscript and agree with its submission to Process Biochemistry.

Data Availability

No data was used for the research described in the article.

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