Biodegradable hydrogels from spider silk proteins: Synthesis, Characterization and Applications - A review

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Abstract: Spidroin is a protein biopolymer that is usually found in spider silk. Spider silks are gaining high interest as they act as a unique biomaterial with excellent mechanical properties. Spider skills shows good biocompatibility as they do not contain sericin, a protein which is present in the silk obtained from silk worm which might cause serious hypertensives. The spidroin is mainly composed regular repeats of amino acids namely alanine, glycine, and serine. Alanine gives strength to the spider silk whereas glycine provides elasticity. Spider silks has been employed in various biomaterials in recent times. Silk protein has been engineered to synthesis specific structures, including fibres, hydrogels, films, sponges, etc. Specifically, Hydrogels that are three-dimensional crosslinked polymers which have the potential to absorb high amount of water and its extremely tunable mechanical properties helps it to be compatible with different functional molecules. Hydrogels can be synthesized from both natural and synthetic polymer. Hydrogels made out of natural polymers reveals that hydrogels can gain extremely stable mechanical, physical and chemical properties when they are swollen, urging researchers to create and employ novel hydrogels for unique applications. This review focuses on different spidroin extraction methods and hydrogel synthesis out of the extracted spider silk.

Keywords: biocompatible, drug carrier, hydrogel, Spidroin

INTRODUCTION

Spider silk is a natural filamentous protein and a magnificent biomaterial (Romer, 2008) with unique physical, chemical and biological properties. The spider silk has gained more interest in past years due to its notable mechanical properties (Lin et al., 2015) such as incredible strength, flexibility, resilience, hypoallergic and antimicrobial properties. In recent times, spider silk has been widely used in a various bioresources such as matrices, hydrogels and thin films. Many biomedical applications (Withanage et al., 2021) utilize materials made of silk.

Naturally, spiders make varieties of silk fibers from different glands for various uses such as weaving webs, encasing their eggs, and making shields to ward off predators. Spiders possess upto seven silk producing glands, and they release proteins through spinnerets to create spider silk. They include major ampullate silk (dragline silk), minor ampullate silk, flagelliform silk, tubuliform silk, aciniform silk, aggregate silk, and piriform silk. Each types of silk fibers have peculiar mechanical properties, chemical composition and internal structure on its function. Due of its exceptional all- encompassing mechanical qualities, the majorampullate silk or dragline silk has been the subject of extensive research (Zhang et al., 2021).

Table 1. Mechanical properties of spider silk compared to other materials

Material	Strength (N m^2)	Elasticity (%)	Energy to break (J kg^{-1})	Thread diameter (nm) 10
Dragline silk (Major ampullate)	$4 \cdot 10^9$	35	$1 \cdot 10^5$	3-4
Fiber silk	$6 \cdot 10^8$	18	-	8-15
Kevlar	$4 \cdot 10^9$	5	$3 \cdot 10^4$	14
Nylon, type 6	$7 \cdot 10^7$	200	$6 \cdot 10^4$	16
Tendon	$1 \cdot 10^9$	5	$5 \cdot 10^3$	-
Steel	$1.5 \cdot 10^9$	0.8	-	-

Spider silk is composed of two main proteins spidroin I and spidroin II. These proteins are mostly made up of glycine- and polyalanine-rich amino acid motifs that are extremely repetitive. Silk gains incredible strength from alanine, and flexibility from glycine (Agapov et al., 2009).

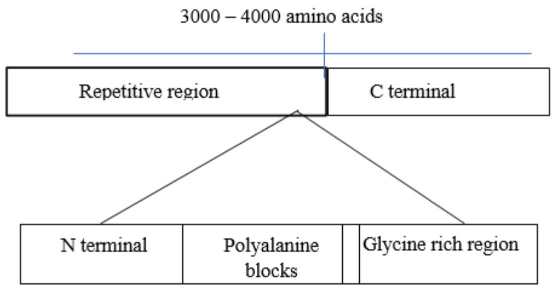

Figure 1 Spidroin structure

The key properties like biocompatibility, biodegradability, hydrophilicity and non-toxicity makes hydrogels a promising biomaterial and make them more suitable in medical and pharmaceutical field. Hydrogels are hydrophilic, three-dimensional crosslinked polymer network which can absorb and retain much amount of water. These biomaterials can integrate large quantum of biological fluid and swell. Hydrophilic functional groups connected to the polymeric backbone provide hydrogels the ability to absorb water, whereas cross-links between network chains give them their capacity to resist dissolution. Various polymers of natural and synthetic origin are used to make hydrogels. Hydrogels can be made out of natural, synthetic or combination of both natural and synthetic polymers.

Classification of hydrogels (Kaith et al., 2021) polymeric structure is useful for physical, chemical and biological applications. Hydrogels begun to play vital role in major developmental breakthroughs as technological development has progressed and novel aspects of science have been identified. Hydrogels are now widely used in important biomedical applications such as drug delivery, cell visualization, hygiene products, 3D printing, and so on.

2. DIFFERENT METHODS OF SPIDROIN EXTRACTION

2.1 Modified NaOH method

0.1g of spider web is weighted and dipped in 10ml of 0.1M NaOH for 1 hour at 90°C. The hydrolyzed web is allowed to cool and then centrifuged for 30 minutes at 4000rpm. The obtained supernatant is designated as spider silk extract and its stored at refrigerator for further use(Lateef et al., 2016).

2.2 Extraction using Ajisawa's reagent

Spider silk was collected, 10mg of the silk was weighted and dispersed in 10ml of Ajisawa's reagent (1:2:8 molar ratio; calcium

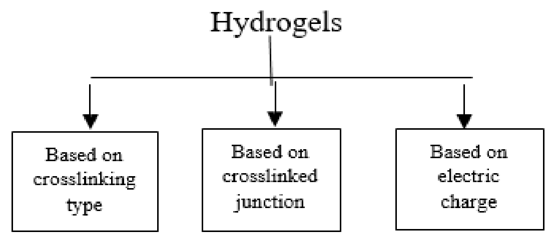

Figure 2 hydrogel classification

Hydrogels are usually synthesized by crosslinking these polymers, the crosslinking can be done either physically or chemically (Bashir et al., 2020). Physical hydrogels are formed by ionic, hydrogen bonding or hydrophobic forces and/or by molecular entanglements. Chemical hydrogels are formed by covalent cross linking. The hydrogels formed by physical crosslinking are considered as reversible gels because the bonds between them are weak. Whereas the hydrogels formed by chemical crosslinking are irreversible or permanent gels as covalent bonds are strong (Parhi, 2017). Covalent crosslinking method is mostly preferred because the chloride:ethanol:water) and the mixture was continuously stirred at 250rpm for 2.5 hours at 70°C. The protein solution was dialyzed using dialysis tube with molecular cutoff of 12-14 kDa against deionized water with six buffer changes in a row for three days. After dialysis the obtained protein solution was centrifuged at 4000rpm for 20 minutes at 4°C. The protein solution was then concentrated in vivaspin tube with molecular cutoff of 30kDa at 1500rpm for 20 minutes at 4°C and brought to the volume of 2ml. The solution was stored at -20°C for further use (Mohtar et al., 2018).

2.3 Extraction using SDS- ethanol mixture:

20mg of silk fibers were dissolved in 20ml of SDS-ethanol extraction mixture (20 wt%) where the ratio of SDS and ethanol is 10:3. The extraction process was done at fixed condition of 200rpm at 70°C for 2.5 hours. The extracted solution was then filtered to remove the silk fibre from the silk protein solution.

The extraction solution was removed by adding potassium dihydrogen phosphate. Further the protein solution was centrifuged at 4000rpm for 4 minutes and the supernatant containing protein was passed on to separate centrifuge tubes and stored at -20°C(Shing & Johan., 2018).

2.4 Extraction using Trifluoroacetic acid:

Spider silk was collected, made into small pieces and weighted. 20mg of spider silk was dispersed in 1ml of TFA and the solution was mixed constantly at 500rpm. The solution was checked for every 15min for complete solubilization. After which the spider silk solution was transferred into a dialysis membrane of pore diameter 12-14kDa. The sample was placed in a buffer solution and the dialysis was carried out at room temperature for 5 days. The pH of the solution was checked for every 6 hours and the buffer system is refreshed for every 6 hours until day 5. The final protein solution was obtained and refrigerated for future use (Withanage et al., 2021).

3.PROTEIN ESTIMATION

Protein estimation for the extracted silk protein is done using Bradford assay. The equilibrium between three forms of Coomassie blue G250 dye serves as the foundation for Bradford assay. The dye is more stable under highly acidic conditions as doubly protonated red form. When it binds to the protein it becomes more stable as an unprotonated blue form.

The quantity of protein can be estimated by determining the amount of dye in the blue ionic form followed by measuring the absorbance at 595nm(Kruger 2009)).

Protein + Coomassie brilliant blue G 250 dye
protein-dye complex (blue colored complex)

4.METHODS FOR SPIDROIN BASED HYDROGEL FORMULATION

4.1 Hyaluronic acid/ spider silk-based hydrogels:

Hyaluronic acid (HA) is one of the important constituents of the extracellular matrix of human tissues and its usually found in neural, epithelial and connective tissues. It is a versatile biological material with unique properties. Hyaluronic acid is a glycosaminoglycan having no sulfate bonds. It is observed that there is an increased amount of hyaluronic acid present at the site of wound. It also serves as a temporary structure during the early stages of wound healing due to its large molecular size (Dovedytis et al., 2020)

Hyaluronic acid solution was made by dissolving HA powder in deionised water. The silk protein and HA solution were mixed together with final weight ratios of 100/0, 80/20, 60/40, 50/50,40/60 and 0/100, respectively. The solution is prepared with a concentration of 2.5wt%. The 1- ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was added as a crosslinking agent and N-hydroxysuccinimide, 2- morpholinoethanesulfonic acid as assistant agent to the blended solution and the solution was mixed thoroughly. The bubbles aroused during stirring was eliminated and then it was observed in the oven at 25°C, 37°C, 45°C, 55°C. The gelation occurred at 45°C after which the formed gel was freeze dried and stored for further use(Yan et al., 2018).

4.2 Spidroin-containing Carbopol 934(CP934) gel formulation:

Carbopol is a high molecular weight, cross-linked, acrylic acid-based polymer. It readily absorbs water, gets hydrated and swell. Carbopol's hydrophilic properties, cross-linked structure, and near insolubility in water make it a promising option for use in controlled release drug delivery systems (Panzade & Puranik, 2010).

mM molar ratio was added as a crosslinking agent. Disodium hydrogen phosphate is then added to the obtained gel to deactivate the reactions of unreacted EDC. Then the sample is washed thoroughly with distilled water and air dried for 24 hours (Kulkarni et al., 2020).

5. CHARACTERIZATION STUDIES OF HYDROGEL

5.1 Determination of swelling ability

Swelling ability of the hydrogel can be determined by using various methods two of them are:

The spidroin extract was diluted with distilled water. 10ml of the diluted solution is taken and CP934 was sprinkled over it and the solution is kept in refrigerator overnight to get completely hydrated. The hydrated solution is then stirred using magnetic stirrer to get a homogeneous solution.

0.1M NaOH was added drop wise to increase the pH of the solution and to convert it into gel (Abd Ellah et al., 2019).

4.3 Gelatin based hydrogel formation:

Gelatin is a natural polymer which is derivative of collagen and it can be obtained from various parts of animal body including bones, connective tissues, skin, fish scales and insects. The various properties of gelatin like non-toxicity, non- immunogenicity, high availability and low cost made it more applicable in medical field such as wound healing, drug delivery, transdermal therapy and tissue repair (Petros et al., 2020).

The Gelatin- silk protein-based hydrogels are prepared by mixing 15wt% gelatin and 2wt% silk together. The gelatin solution was prepared by dissolving 1.5g of gelatin in 10ml of distilled water with continuous stirring at 55^{o}C for 4 hours. 3ml of gelatin and 1ml of silk protein was blended well for further gel formation. EDC/ NHS of 200 mM/50

5.1.1 Method: 1

As per the Japanese Industrial standard K8150 method, first the hydrogel is allowed to dry and then the dried hydrogel is immersed in water for 48 hours at room temperature. Once the hydrogel attained the swollen state, the swelling is calculated by using the below formula

Swelling = $(W_s - W_d) \backslash W_d$

Where, W_s – weight of hydrogel in swollen state

W_d – weight of hydrogel in dried state

5.1.2 Method: 2

As per the Japanese Industrial standard K7223, the dried hydrogel is placed inside the deionized water for 16 hours at room temperature and the swelling is calculated as follows:

Swelling = ‒ * 100

Where, W- Weight of the dried hydrogel

Y- Weight of the insoluble portion of the hydrogel in water after extraction (Azeera et al., 2019)

5.2 UV- Vis Spectroscopy

An efficient spectroscopic technique for identifying specific chemical bonds in small compounds and polymers is the absorption of light in the UV-Vis spectrum. UV spectroscopy is widely used to detect the presence and determine the quantity of polymers (Venkatachalam, 2016).

5.3 NMR spectroscopy

The examination of polymers and hydrogels is frequently done using nuclear magnetic resonance (NMR). Different NMR techniques, including pulsed field gradient NMR, C-NMR, and H-NMR, were used to study the hydrogels. In order to determine the final double-bond transition at the end of the polymerization, functional groups of the monomer and copolymer composition were identified using H-NMR studies. H-NMR spectroscopy can also confirm the end of the polymerization process and its mechanism. The proton NMR is sensitive to environments around hydrogen atoms and provides information about the exchange of water molecules between the free and bound states. On the other hand, a useful tool for characterising hydrogel-based drug delivery systems is pulsed field gradient NMR spectroscopy (Brandl et al., 2010).

5.4 Scanning Electron Microscope (SEM)

The information on morphology, topography and composition of the hydrogel can be obtained using SEM. For the SEM analysis, the swollen hydrogel is made into small pieces and freeze dried for 24 hours at -55^{o}C. The effects of pH on the swelling behaviour of hydrogel can also be investigated. SEM is considered as a powerful tool in capturing the ideal network structure of hydrogels (Aouada et al., 2005).

5.5 Fourier-Transform Infrared Spectroscopy

FTIR is a vital characterization tool to determine the structure of particles at molecular level. It can be used to determine the chemical composition and bonding patterns of the components in homopolymers, copolymers, polymer composites, and polymeric materials. The hydrogels are freeze dried prior to FTIR analysis (Barrios et al., 2012).

6. APPLICATIONS OF SILK-BASED HYDROGELS

6.1 Tissue Engineering

Tissue engineering is an emerging field that has been developed to meet immense requirement for tissues and organs. It involves engineering tissues by employing biocompatible scaffolds(Hu et al., 2012). Hydrogels are widely used in tissue engineering to replace or repair a damaged tissue or organ due to its properties such as high-water holding capacity, mechanical strength, biocompatibility, diffusivity, cell adhesion and cell signalling can be used to mimic various natural body tissues. The structural and functional similarity of hydrogels to natural tissues like maintaining structural integrity, transportation of biological molecules makes them more attractive in the field of tissue engineering. A notable advantage of the hydrogels is their ability to entrap cells during fabrication, because hydrogels are made in mild conditions which helps in maintaining cell viability (Kapoor & Kundu, 2016).

6.2 Wound dressing

A defect or break in skin due to some medical or physiological conditions is known as wound. Wounds are healed naturally by the body's immune system but sometimes the wound healing process becomes slower than usual or hindered. This is because of the presence of some abnormal bacterial infection, trauma, pressure, etc. An effective wound healing material should be capable of holding much amount of water thus retaining the moisture content in the damaged regions, preventing the external sources of infection, having good gas permeability, preventing the wound from heat and provide sterile condition.

Hydrogels are greatly suited for wound dressing as they can absorb and retain the contaminated exudates by the extension of crosslinked polymer chains within the gel mass, which results in the isolation of bacteria, debris, and odour molecules in the liquid. They play a vital role in the emergency treatment of burns because of their hydrating and cooling effect (Caló & Khutoryanskiy, 2015).

6.3 Drug delivery:

Hydrogels act as a potential smart drug delivery tool that meet the requirements for directing medications to specific areas and regulating drug release. The fascinating characteristics of hydrogels offers enormous applications in drug delivery. Especially, one of the most important properties of hydrogel is porosity that makes them most suitable for sustained release of the loaded drug. The active pharmaceutical ingredient is retained for a long period of time and the drug is released through the mesh or pores in the hydrogels filled with water by diffusion mechanism (Narayanaswamy & Torchilin, 2019).

Hydrogels have found to have widespread clinical usage and significantly improve the therapeutic success of medication delivery. With the use of hydrogels for drug administration, the temporal and spatial delivery of macromolecular medicines, small molecules, and cells has significantly improved(Chen et al., 2017).

6.4 Gene delivery system

Even though adenoviruses are well known for their transfection efficiency they have not yet reached clinical use because of toxicity issues that have hindered their use as gene delivery vectors. Such applications have made use of the family of silk-elastin-like protein polymers (SELPs), one class of hydrogels (Price et al., 2012).

6.5 Artificial skin

A human skin substitute called artificial skin is developed in a laboratory to fix or replace damaged skin tissue. Usually the artificial skin is expensive and its necessary to find an alternative method for the synthesis of artificial skin. Silk based hydrogels are considered as a suitable alternative because of its favourable properties and its minimal effects on the immune system (Y. Wang et al., 2006).

The healing time upon the application of silk-based hydrogel was noted to be less when compared to Cutinova hydro dressing. The wounds treated with a silk hydrogel instead of a Cutinova hydro dressing showed less inflammation and more progressed granulation, according to the histological findings, at all stages. The outcomes made it abundantly evident that the wound dressing using silk hydrogel promoted faster dermal and epidermal regeneration than a Cutinova hydro dressing (H. Y. Wang & Zhang, 2015).

6.6 Adhesives

By using medical adhesives, the second damnation brought on by the suturing of the wound can be avoided. In addition to serving as hard tissue adhesives in the restoration of teeth and bones, hydrogels can also serve as soft tissue adhesives in minimally invasive surgery, such as for the liver and kidneys. The biocompatibility studies showed that the hydrogels were not harmful to the Caco-2 cells. The silk-based hydrogel has a lot of potential to be used as tissue adhesives (Yuan et al., 2019).

6.7 Contact lenses

Contact lenses are thin lenses that are placed on the surface of the eyes for vision correction. Contact lenses should have good biocompatibility. Silk-based contact lenses are considered non-toxic and a better alternative for glass and plastics. This is because of its extraordinary mechanical properties, slow degradability and good biocompatibility (Tsukada et al.,1999).

7. CONCLUSION

Biodegradability, good biocompatibility, non-immunogenicity and other excellent mechanical properties of spidroin makes it a good material with great potential in tissue engineering (Zhang et al., 2021), targeted delivery of drug and other medical applications. Hydrogels with the ability to retain exudate in the wound accelerate the wound healing process, which promotes its active application in wound dressings. Another great advantage of hydrogel is its structure into which antibiotics and other pharmaceuticals can be introduced (Bessonov et al., 2018). The spider silk- based hydrogels can be a favourable tool for external drug delivery.

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated

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