Composite Hydrogel Preparation and Physico Chemical Characterization from Silk Fibroin

Manonmani S[1], Vidhya K[2], Sandhiya L S[3], Yuvetha C[4]

[1,2,3,4]Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode, India

[1]manosbiotech@gmail.com

ABSTRACT

Hydrogels are effective in wound healing and other tissue engineering applications. In our study hydrogel was prepared using Silk fibroin, Chitosan and Gelatin. A simple mixing of chitosan, gelatin and silk fibroin solutions was done to get a new composite silk fibroin based hydrogel (SFH) type. The hydrogels based on silk fibroin, chitosan and gelatin provide a moist wound environment which intern accelerate the process of wound healing and tissue regeneration. The applications silk proteins in biomedical products has been increased due to their mechanical properties and biocompatibility. The effectiveness of wound healing process was increased when using chitosan- based hydrogels or other combinations of hydrogels. Further characterization of silk based hydrogel is done using various tests to check the swell ability, biodegradability and biocompatibility of silk hydrogel. The results show that silk based hydrogel play a promising role in wound healing

Keywords

Silk fibroin; Chitosan; Gelatin; Hydrogel; Biocompatibility

Article Received: 10 August 2020, Revised: 25 October 2020, Accepted: 18 November 2020

Introduction

Hydro gels and scaffolds are considered one the significant achievements in tissue engineering. Unique qualities of hydrogels includes, high water content, 3-dimensional structure and versatility. They mimic the extra cellular matrix in vivo, aiding the cellular communications. There is also few limitations in using conventional irreversible hydrogels because of bulk material degradation, disintegration and lack of homogeneous mechanical properties. In order to overcome these difficulties, hydrogels with reversible gel– sol advances came in practice. Sol-gel process is a wet chemical method involving an inorganic colloidal suspension and gelation to form 3D structure. This is one of the methods to prepare a biocompatible hydrogels. Although these hydrogels are biocompatible and reversible, they require triggers like a change in temperature, pH or UV light exposure [1]. Nano fibers are used to make hydrogels and the triggers are used to assemble those fibers in hydrogel. At the same point, these triggers may disturb cell structure and tissue regeneration [2]. When hydrogels are used for cell encapsulation, the three-dimensional structure of cell culture needs to be preserved. These reversible sol-gel can be designed to simple mechanical properties rather than physical triggers [3].These hydrogels can change into a negligible viscoelastic liquid through a basic simple triggers. The silk fibroin (SF) can be effectively prepared into an assortment of material organizations [4].The SF hydrogel has attracted expanding tissue repair and medical applications because of its extraordinary biocompatibility and viscoelastic properties [3]. Many physical as well as blending techniques have been utilized in order to improve the gelation of SF. The physical techniques includes ultrasonic method, pH change, mixing with ionic surfactants. Mostly these gels are irreversible. A class of silk-like polypeptides utilizing quality-building methods have been delivered by researchers. The polypeptides can self-collect into hydrogel when physical triggers applied. A reversible hydrogel can be prepared with using any of the triggers such as temperature [5]. Chemical triggers like sodium dodecyl sulfate is also effective [6]. With a concentration– weakening technique and hatching temperature at 60 °C, the gel can be broken into short nanofibers and by means of ultrasonic treatment, they self-collect into bigger molecular accumulations without any change in β-sheets[7]. The light chain and heavy chain of mulberry silk fibroin are cross-linked by a disulphide bond, including hydrophobic, crystalline groupings and sporadic hydrophilic domains [8]. The silk fibroin have the potential to mimic the extra cellular matrix environment by providing strength and flexibility [9]. Some SF hydrogels are produced as an injectable hydrogels and they undergo conformational changes from random coil to β-sheets under physiological conditions [10]. SF side-chains also plays a vital role in polypeptides conformers in water [11]. It depends on protein concentration, pH or temperature, and by the addition of salts, metal ions and other components [12]. Crosslinking agents have a direct effect on the optical properties of SF hydrogel [13]. When these cross-linkers used in chemical technique they have much less biocompatibility [14]. The engineered SF is been used for soft tissue regeneration [15]. SF hydrogels can also be used as a drug carrier for the sustained release of photosensitizers [16]. The current project explains the physic chemical properties of composite hydrogel produced using silk fibroin.

Materials and Methods

Degummed silk bought from local shop at Sathyamangalam, Erode district. Gelatin and chitosan from HIMEDIA pvt. Ltd., were used for hydrogel preparation.

A. Preparation of silk fibroin

Degummed silk was used to produce fibroin solution. Different concentrations of (0.5%, 1%, 1.5% and 2%)

fibroin solutions were made using silk fibres. For 60 ml of silk fibroin solution with 0.3g, 0.6g, 0.9g and 1.2g of silk fibres are taken respectively. The silk fibres were dissolved in NaOH solution (10g of NaOH in 20 ml of water) by boiling in water bath at 55ºC for 30 mins by continuous stirring.

B. Dialysis of Silk fibroin solution

The silk fibroin solution derived after dissolving contains NaOH, which is basic in nature. Our skin pH is slightly acidic (5.5). In order to neutralize the solution, dialysis is done to remove NaOH from silk fibroin solution. It is dialyzed using dialysis membrane of 10kDa. Water is used as a solvent and it is changed for every 12 hrs for 3 consecutive days and the pH is checked before changing the water

C. Lyophilized composite hydrogel

Gelatine solution is prepared by dissolving 0.3g of gelatine in 100ml of distilled water. 0.3g of chitosan is dissolved in 10 ml of acetic acid and made up to 100 ml with distilled water. The silk fibroin solution is mixed with chitosan and gelatine. The mixed solution is then centrifuged at 9000 rpm for 20 mins at 4ºC. The supernatant is removed and the pellet (hydrogel) is stored at 4ºC for 24 hrs and lyophilized in freeze dryer for 12 hrs.

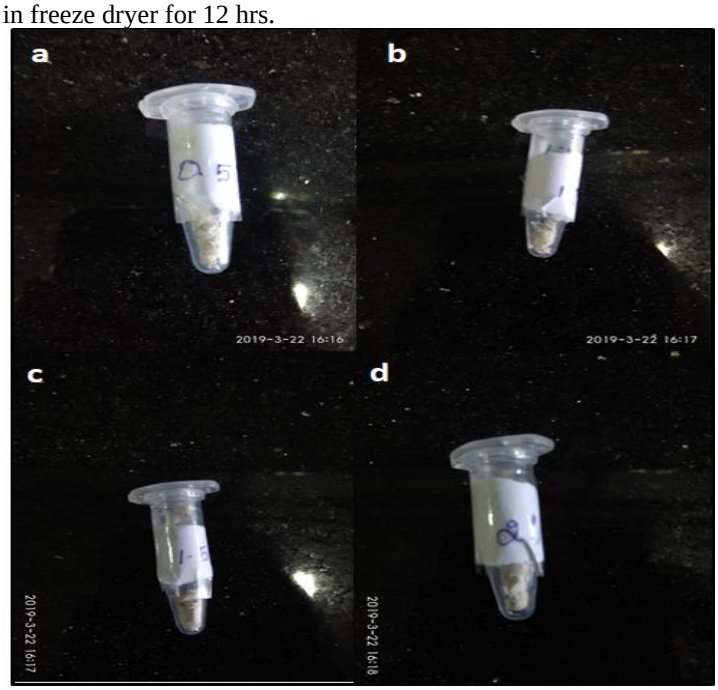

Fig 2.1 Lyophilized hydrogels a-0.5% b-1.0 % c-1.5 % and d-2.0% concentration respectively

Results and Discussion

A. Swellability Analysis

Swellability test is used to check the swelling property of hydrogel. The silk hydrogel of different concentrations are taken in different centrifuge tubes and water is added to check the swelling ability. Dry weight of pellet is noted. Then distilled water is added and the solution is allowed to absorb for 48hrs. Supernatant is removed. The wet weight of the pellet is noted using weighing machine.

Formula to calculate percentage of swell ability is

% Swell ability = ((Wet weight –Dry weight) / Dry weight)*100

Table 3.1. Swellability analysis

Concentration (mg/ml)	Dry Weight (g)	Wet weight (g)	% of Swelling
0.5	0.12	0.12	0
1.0	0.19	0.20	5.26
1.5	0.27	0.30	11.11
2.0	0.32	0.37	15.62

The water uptake capacity of hydrogel shows the physiological activity of fluid absorption and stabilization of silk hydrogel. The percentage of swell ability increases with increasing the concentration. The percentage of swellability is 0%, 5.26%, 11.11%, and 15.62% for 0.5%, 1%, 1.5%, and 2% hydrogel concentration.

B. FTIR Analysis

The signal at the FTIR detector is represented as a spectrum from 4000cm-1 to 400cm-1, which is the molecular fingerprint of the sample. Each unique fingerprint is used to analyze different samples.

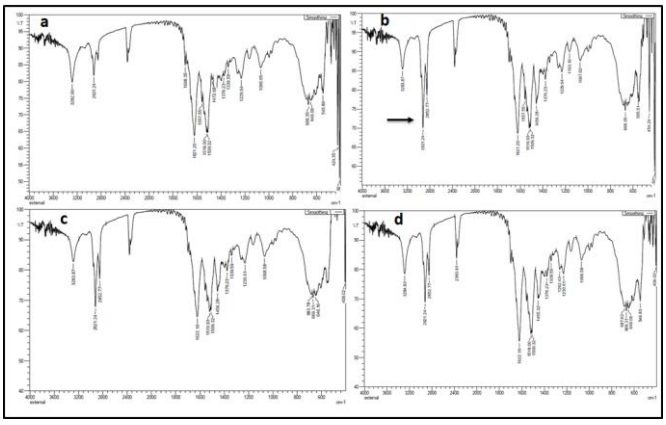

Fig3.1 FTIR figures for a-0.5% b-1.0 % c-1.5 % and d-2.0% concentration respectively

The silk hydrogel was obtained in ATR mode. The IR spectrum of chitosan confirms the presence of O-H and C-H stretching vibration at 3442cm-1.The band at 1641cm-1corresponds to N-H vibration of secondary amide. The C-O-C, C-O,COH form band at 1173cm-1.The C-H band was formed at 1378cm-1.The band at 900cm1 corresponds to the saccharide structure of chitosan. The IR spectrum of gelatin showed N-H stretching vibration at 3443cm-1,C=O vibration at 1639cm-1 and C-H stretching band were between 1078cm-1 and 1240cm-1.The IR spectrum of silk fibroin show band at around 1625cm-1 to 1650cm-1 for amide I(C=O) group, 1524cm-1 for amide II, 1235cm-1 for amide III and 665cm-1 for amide V. The silk is usually characterized by β sheet absorption peak.

Conclusion

Composite silk hydrogel was produced using different concentration of silk fibre (0.5%, 1%, 1.5%, and 2%), gelatin and chitosan. This hydrogel is used for further characterization which includes swell ability, FTIR analysis etc. The swell ability result shows that increasing the concentration of silk fibre will increase the swelling property of hydrogel, which indicates the hydrophilic nature of hydrogel. Gelatin is a mixture of peptide and proteins produced by partial hydrolysis of collagen, which helps in increasing the tensile strength of hydrogel. The C-H bond, C-O bond and N-H bond present in gelatin and chitosan helps in increasing the tensile strength and rigidity. Chitosan is insoluble in water. It also contains some antimicrobial activity. Due this property, the produced hydrogel may be used in wound healing as well as several tissue culture applications.

References

[1] X. Qiu, Y. Yang, L. Wang, S. Lu, Z. Shao and X. Chen, "Synergistic interactions during thermosensitive chitosan-β-glycerophosphate hydrogel formation", RSC Adv., 2011, 2, 282– 289 .

[2] R. N. Shah, N. A. Shah, M. M. D. R. Lim, C. Hsieh, G. Nuber and S. I. Stupp, "Supramolecular design of self-assembling nanofibers for cartilage regeneration", Proc. Natl. Acad. Sci. U. S. A., 2010, 107, 3293–3298 .

[3] C. T. S. W. P. Foo, J. S. Lee, W. Mulyasasmita, A. Parisi-Amon and S. C. Heilshorn, "Twocomponent protein-engineered physical hydrogels for cell encapsulation", Proc. Natl. Acad. Sci. U. S. A., 2009, 106, 22067–22072.

[4] D. Kaplan, W. W. Adams, B. Farmer and C. Viney, Silk polymers: materials science and biotechnology, American Chemical Society, Washington, DC, 1994 .

[5] W. H. Rombouts, D. W. de Kort, T. T. H. Pham, C. P. van Mierlo, M. W. Werten, F. A. de Wolf and J. van der Gucht, "Reversible Temperature-Switching of Hydrogel Stiffness of Coassembled, Silk-Collagen-Like Hydrogels", Biomacromolecules, 2015,16, 2506–2513 .

[6] X. Wu, J. Hou, M. Li, J. Wang, D. L. Kaplan and S. Lu, "Sodium dodecyl sulfate-induced rapid gelation of silk fibroin", Acta Biomater., 2012, 8, 2185–2192.

[7] S. Bai, X. Zhang, Q. Lu, W. Sheng, L. Liu, B. Dong, D. L. Kaplan and H. Zhu, "Reversible hydrogel-solution system of silk with high beta-sheet content", Biomacromolecules, 2014, 15, 3044–3051.

[8] C. Vepari and D. L. Kaplan, "Silk as a biomaterial", Prog. Polym. Sci., 2007, 32, 991–1007.

[9] Y. Liu, S. Ling, S. Wang, X. Chen and Z. Shao, "Thixotropic silk nanofibril-based hydrogel with extracellular matrix-like structure", Biomater. Sci., 2014, 2, 1338–1342.

[10] Viviana P .Ribeiro, Joana Silva-Correia, Cristiana Goncalves, Sandra Pina, "Rapidly responsive Silk Fibroin hydrogels as an artificial matrix for the programmed tumour cells death", PLoS One., 2018.

[11] Zhuping Yin, Feng Wu, Tieling Xing, Vamsi K. Yadavalli, "A Silk fibroin hydrogel with reversible sol-gel transition", Royal Society Of Chemistry, 2017.

[12] Agostini de Moraes, Mariana and Beppu, M.M, "Silk fibroin hydrogel: A review under biomaterial perspective". Hydrogels: Synthesis, Characterisation and Application. 209-226, 2012.

[13] Rustam zhon Melikov, Daniel Aaron Press, Baskaran Ganesh Kumar, Itir Bakis Dogru,"Silk Hydrogel lenses for light emitting Diodes", Scientific report 7,Art.no. 7258, 2017.

[14] Fang Zhang, Jiao Jiao Li, Rui Jiang, Shan Zhang, "Excellent Cell Compatibility in time controlled silk fibroin hydrogel", Material Science Forum, Vol. 815, 407-411, 2015

[15] Alexander N Mitropoulous, Benedetto Marelli, Chiara E.Ghezzi, Matthew B. Applegate, Transparent, "Nanostructured Silk Fibroin Hydrogel with Tunable Mechanical Properties", ACS Biomater. Sci. Eng., 2015, 10,964-970.

[16] Jose Eduardo U. Rojas, Barbara B Gerbelli, Anderson O. Ribeiro, “Silk Fibroin hydrogels for potential applications in photodynamic therapy”, Biopolymers vol. 110,2018.