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Enzymatic Degradation Comparison of Silk Fibroin Hydrogel (SFH), Silk Xanthan Hydrogel (SXH) and Silk Silver-Nanoparticles Hydrogel (SSH) for Drug Delivery Systems (DDS) Applications

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Abstract

Drug delivery systems (DDS) reduces side effects as oppose to conventional by targeting and releasing its dosage at specific site in the body. Successful DDS application requires a stable drug delivery mechanism of the drug carrier. Hydrogel based biomaterial has been used over the years to demonstrate successful biomedical application *in vitro*. This is due to their high biocompatibility and less cytotoxicity. Silk fibroin (SF) has proven to be an excellent choice of hydrogel based biomedical application, its three-dimensional stable network of polymers allows its swelling in large amount of water. In addition, it also degrades continuously overtime which makes it highly suitable as a smart drug vehicle. In an attempt to optimize the properties of silk fibroin, several methodological approaches to silk fibroin hydrogel preparation was adopted and evaluated. The approach here was to regenerate silk fibroin solution, process it by crosslinking with silver nanoparticles (AgNPs) and xanthan solution respectively. Silk Fibroin solution stored at 4°C was dried at room temperature for 3 days to obtain Silk Fibroin Hydrogel (SFH). Silk fibroin was cross-linked with Xanthan to obtain a stable hydrogel (SXH) at its optimum pH and temperature. Lastly, AgNPs was crosslinked with SF to produce SSN. All Hydrogels samples were freeze-dried into scaffolds, Essential Biomaterial Characterization and Enzymatic degradation analysis was carried out on all hydrogels at α=0.05 level.

Keywords: Hydrogel; Drug delivery system; Enzyme degradation; Nanoparticles

Introduction

Drugs have been used extensively to improve health care. Health care technology has shift to the practice of drug delivery systems (DDS) in the past few decades to directly deliver treatment at specific targeted while also minimizing side effect as oppose the conventional. This great advancement has been implemented to several treatment therapy and even greater changes are anticipated in the near future. Biomedical engineers have contributed greatly to the understanding of the biochemical mechanism of invaded tissue cells with its corresponding physiological barriers to efficiently deliver drug at this site. The biosynthesis of smart drug delivery using different biosynthetic techniques, polymer manipulation to form a stable hydrogel has picked the interest of several researchers in the field. DDS monitors and controls drug release dosage at specific site location. Silk fibroin obtained from Bombyx mori has been studied over the decades for hydrogel due to its excellent biocompatibility *in vitro* biomedical applications [1,2]. Its protein polymeric structures allows for its excellent biocompatibility with tissue cells. This polymeric network known as SILK I or SILK II possesses great mechanical quality excellent for bearing load which makes silk adequate for as a drug carrier, this diverse range of properties allows are broader span of silk fibroin as potential biomaterial. Xanthan is inhumane to a wide scope of temperature, pH and electrolyte fixations [3,4]. It likewise shows a high shear steadiness across this physiological conditions. The presence of silver nanoparticles in silk fibroin hydrogel has been demonstrated in vivo to reduce bacterial contamination. The study therefore aims to determine the optimum processing condition for a silk-induced hydrogel for drug delivery system.

Materials and Methods

Silk fibroin preparation

Silk was degummed by carefully cutting its cocoons into pieces (Figure 1). These pieces were put inside an aqueous solution of sodium carbonate (Na_2CO_3) under heating at 100°C and agitation for 75 minutes. The gotten silk was washed with distilled and dried for 24 hours. The dried silk was dissolved in a mixture of solvents known as ternary solvent consisting of 1 mole of Calcium Chloride, 2 mole of Ethanol and 8 mole of water (1:2:8 molar ratio) at 75°C for 90 minutes. Dialysis and filtration was carried out using purified water and dialysis bag (purchased from sigma) respectively for 3 consecutive days at 25°C [5].

Figure 1: Silk Fibroin Preparation (Silk cocoon-Left, degumming process-Right).

Xanthan solution preparation

A predetermined amount of powered xanthan gum was dissolved in distilled water under heating and agitation [6].

Green synthesis of silver nanoparticles

For purification, all glasswares were sterilized with 70% Ethanol solution. Olive leaves were air-dried in a shaded environment overnight. 20 g of dried olive leaf was grounded into powder form, and immersed in 20 mL Ethanol to begin extract. The solution was immersed in water bath at 30°C, 100 rpm for 3 days allowing the extraction of the olive oil [7]. The solution was later filtered using filter paper and dehumidify at room temperature. Aqueous solution of $AgNO_3$ of 0.034 gram was added to about 5 ml of Olive leaf extract to fix up the solution up to 1 x 10^{-3} M of silver nanoparticles [8].

Preparation of hydrogels

Silk Fibroin Hydrogel (SFH)	Silk Xanthan Hydrogel (SXH)	Silk Silver Nanoparticle Hydrogel (SSH)
The silk fibroin solution was stored at 4°C and was dried for 3 days to produce Silk Fibroin Hydrogel (SFH)	Crosslinking silk fibroin with xanthan to form Silk Xanthan Hydrogel (SXH).	Crosslinking silk fibroin with silver nanoparticles to form Silk Silver Nanoparticle Hydrogel (SSH).

Table 1: Preparation of Hydrogels.

FTIR spectroscopy

This imperative strategy is utilized to examine the functional groups present in sample, as each functional group absorbs different IR. In this investigation IRPrestige-21 (Shimadzu) machine is utilized to break down the synthesized polyelectrolyte complex gel at 4 cm^{-1} window determination in the scope of 600-4000 cm^{-1}.

Mechanical properties

A universal testing machine (Instron 5848 MicroTester, USA) equipped with a 100 N load cell at room temperature, was used to measure the mechanical properties of the samples.

In Vitro enzymatic degradation

The samples with known dried weight (Wo) were drenched with PBS pH 7.4 containing 10 µg/mL lysozyme at 37°C for 3 weeks and refreshed weekly. At different time intervals, the samples were expelled and dried at 65°C overnight. The dried samples were weighed and decided as dry weight after degradation (Wt) and the percentage of the remaining weight was calculated [9].

% Weight remained=Wt/Wo × 100.

Results

Silk fibroin hydrogel (SFH)

Silk fibroin was set up to get a cloudy aqueous solution without crosslinker. The gel was oven dried in the 37°C to obtain sticky adhesive hydrogel.

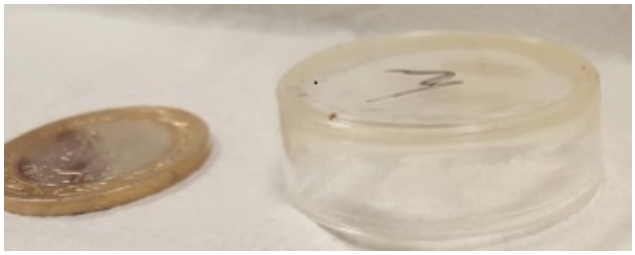

Figure 2: SFH turned upside down.

The adhesive (non-spilling) property of the hydrogel is confirmed by turning the formed hydrogel upside down without spillage (Figure 2).

Silk xanthan hydrogel (SXH)

Silk Fibroin solution was adjusted to desire concentration and xanthan dissolved in distilled water were mixed at a 3:1 (Xanthan: Silk) concentration ratio. The pH of the solution was concurrently monitored and adjusted. The mixture was stirred for 1 hour and casted on petri dishes at room temperature (Figure 3).

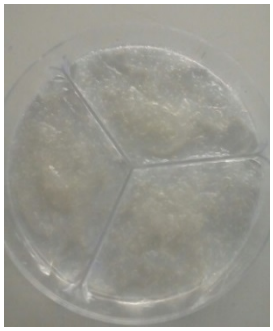

Figure 3: SXH casted on petri dish.

Silk silver nanoparticles hydrogel (SSH)

The crosslinking of silk fibroin with green synthesized silver nanoparticles (AgNPs) was visible using optical microscopy by allowing a more porous structure of the gel when compared with the optical image of SFH (Figure 4). 2.5 ml Silk Fibroin Hydrogel was cross-linked with 1.5 ml silver nanoparticles to allow porosity of the

hydrogel. The diffuse nature of hydrogel is linked to its loaded drug diffusion kinetics. The wider the polymeric mesh of hydrogel becomes, the easier drug diffusion becomes (Table 1) [10].

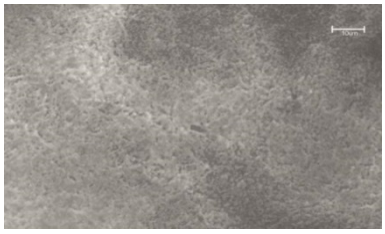

Figure 4: SSH x40 Mg.

Swelling ratio of SFH in distilled water

The swelling properties of the hydrogels can be affected by numerous parameters such as temperature, pH, crosslinking ratio etc. In an attempt to select the optimum condition of drug delivery using silk fibroin based biosynthesis, swelling studies of gels were performed at different conditions for the gels (Figure 5).

Figure 5: Swollen SFH.

The swelling ratio of Silk Fibroin Hydrogel (SFH) demonstrated a steady increase in distilled water up till the third day where a saturated equilibrium was attained (Figure 6).

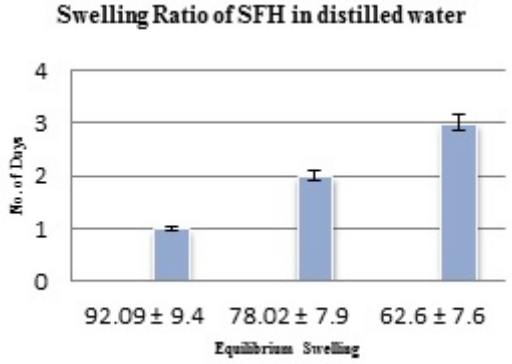

Figure 6: Swelling ratio of SFH in distilled water.

Swelling ratio of SXH at pH 10

Powdered xanthan was processed to be cross-linked with silk fibroin at a concentration ratio of 3:1 (Xanthan: Silk) to achieve silk-xanthan-hydrogel. Xanthan based Hydrogel has been shown to be specifically responsive to various physiological conditions such as temperature, pH, etc. [11]. This complex hydrogel due to its nature was tested against alkaline solution of pH 10 for swelling analysis (Figure 7). Experimentally, there is no significant change in swelling of silk fibroin hydrogel at pH 2 and pH 6.2, pH 10 gave highest significant swelling changes [12].

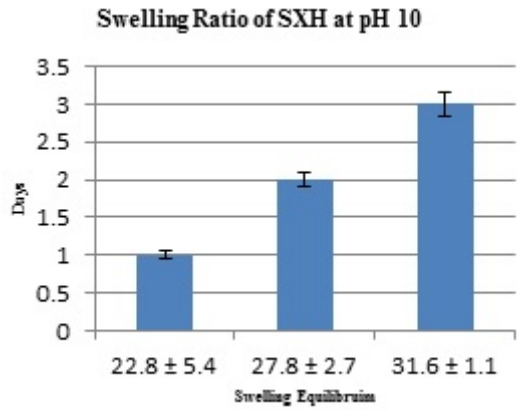

Figure 7: Swelling ratio of SXH at pH 10.

Swelling ratio of SSH in distilled waters

The swelling ratio of Silk Fibroin in Silver Nanoparticles (SF +AgNPs) in distilled water gave similar results to that of SFH which suggests that the presence of silver nanoparticles may not inhibit the swelling of silk hydrogel (Figure 8).

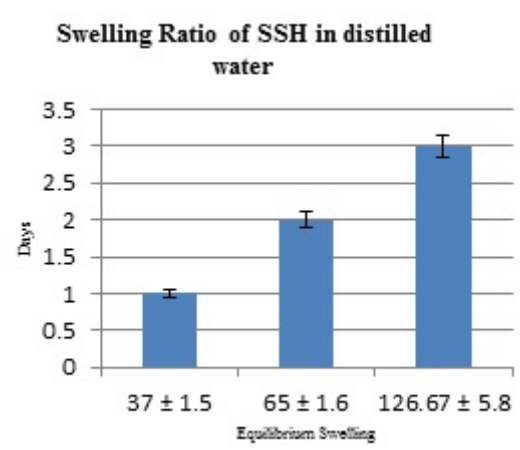

Figure 8: Swelling ratio of SSH in distilled water.

FTIR spectroscopy

FTIR spectroscopy uses absorbance peaks to confirms chemical compounds by recognizing bonding peaks. The absorbance peaks of FTIR analysis reveal standard peak position for specific elements and compound. Due to the crystallization behaviour of silk protein, its peak positions of amide I, II and III are known as C=O, N-H and C-N stretching modes. Observed absorbance peak of Silk Fibroin hydrogel (SFH) in amide I, II and III are 1639, 1524 and 1235 cm^{-1} respectively which is known as β-sheet structure. The FTIR of Silk Xanthan Hydrogel (SXH) in amide I, II and III are 1651, 1534 and 1233 cm^{-1} respectively. The FTIR of Silk Silver-nanoparticle Hydrogel (SSH) in amide I, II and III are 1636, 3454 and 2083 cm^{-1}. The broad band between 3454 cm^{-1} was use to confirm the presence of silver nanoparticles was confirmed using is due to the N-H stretching vibration of group NH_2 and OH which overlaps from the stretching vibration of attributed for water and A. indica leaf extract molecules from the green synthesized olive leaves. According to Zhang YQ et al. [13], to further confirm the presence of silk I was identified by the following region characteristic: amide I (-CN- and -CO-bonding) around the region of 1655-660 cm^{-1}, amide II (-NH-bending) at the 1531-1542 cm^{-1}, amide III (-CN-stretching) 1230 cm^{-1} region. Random bends were observed at 1640-1648, and 1235 cm^{-1}. The β-sheets silk II were confirmed for amides I, II, and III at the regions of 1620-1630, 1515-1530 and 1240 cm^{-1} respectively (Table 2) [14].

Hydrogels	Functional group	Peak (cm-1)
SFH	Amide I	1639
	Amide II	1524
	Amide III	1235
SXH	Amide I	1651
	Amide II	1534
	Amide III	1233
SSH	Amide I	1636
	Amide II	3454
	Amide III	2083

Table 2: FTIR analysis of hydrogels.

Mechanical test

Mechanical behaviour of hydrogel samples is expresses as Young's Modulus as obtained from the stress to strain curve. Poor mechanical behaviour of hydrogel may reduce its biocompatibility for biomedical application. Young's modulus of hydrogels samples increases with increasing silver nanoparticle concentration (Table 3). For a suitable biomedical application, hydrogel should be strong but not too flexible [15].

Hydrogel	Young's Modulus (MPa)
SFH	10 ± 0.42
SXH	11 ± 0.32
SSH	12 ± 0.21

Table 3: Tensile strength of Hydrogels.

In Vitro enzymatic degradation

In Vitro enzymatic degradation on Hydrogels samples SFH (blue diamond), SXH (grey triangle) and SSH (orange square) shows degradation kinetics of hydrogels with reproducible results (n=3). Degradation of hydrogel *in vitro* represents its behaviour in DDS application. Here we observe SFH degrading fast as oppose SXH and SSH which shows a steady degradation rate over time (Figure 9).

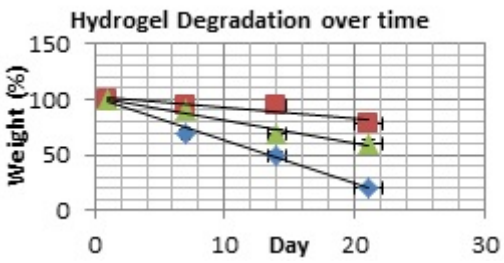

Figure 9: *In Vitro* Enzymatic Degradation of Hydrogels.

Discussion

Suitable biomaterial must possess high biocompatibility, adequate swelling, and certain degree of degradation over time for drug delivery system (DDS) applications. SFH, SXH, and SSH reported excellent swelling during the study. The study shows that the presence of green synthesized silver nanoparticles (AgNPs) had no visible effect on hydrogel's swelling as Xanthan did on silk fibroin. The swelling retentive ability of the hydrogels allows for controlled DDS application as there is a direct relationship between water absorption and increase in the polymeric mesh responsible for drug release [16]. pH sensitive SXH was found suitable for controlled pH/Temperature "triggered" DDS as its swelling is only responsive at a certain pH level (pH=10). All hydrogels degraded continuously overtime which makes them a potential smart drug vehicle. Hydrogel polymer solubility of loaded drug controls the release of drug in vivo, therefore, the diffuse porous nature of the hydrogels allows easier drug diffusion *in vitro*. Optical images of hydrogels revealed all hydrogels samples are porous in nature with increasing porosity as AgNPs concentration increases. The degradation mechanism of drugs differs significantly as affected by several factors ranging from the polymeric diffusion nature of the hydrogel carrier, the mode of loading of drug, pH, temperature and other external factors.

Conclusion

Drug degradation rate predetermines the suitable drug for DDS. The continuous degradation of hydrogel allows for steady increase in mesh size of hydrogel's polymer. The perforation of hydrogel's mesh allows loaded drugs to diffuse through. A steady degradation rate over time is desired mostly for DDS. Asides successful degradation, hydrogel swelling and mechanical properties is equally important. SFH, SXH, SSH was evaluated to determine the most fit for successful DDS application. Although both SXH and SSH displayed similar

steady degradation curve, we recommend SSH for containing AgNPs which provides anti-bacterial activities alongside other stable properties.

Test statistic

Statistical significance of bacterial inhibition between the ciproflaxin drug, SSH, and SSH* ($p<0.05$). There was no observed significant between the CONTROL and SFH ($p<0.05$) suggesting SFH shows no significant antibacterial activities in the study.

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