ORIGINAL ARTICLE

Development of Spider Silk-based Substrate for the Attachment and Spreading of Human Wharton's Jelly-derived Mesenchymal Stem Cells (hWJ-MSCs)

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ABSTRACT

Introduction: Cartilage tissue engineering needs certain surface criteria that meet the requirement to support the attachment, spreading and proliferation of cells. Spider silk (silk spidroin) is a versatile polymer because it is biodegradable, biocompatible, and has superior mechanical properties in terms of tensile strength. Meanwhile, gelatin has properties to facilitate conformational protein transfer and create strength, while lactose as a crosslinker provides biomaterial stability and glycerol as a plasticizer. The purpose of this work is to prepare lactose-mediated crosslinking gelatin - silk spidroin films for human Wharton's Jelly-derived Mesenchymal Stem Cell (hWJ-MSC) culture as a novel substrate for cartilage tissue engineering. **Material and Methods:** Several lactose-mediated crosslinking gelatin - silk spidroin were prepared as films. Films biocompatibility using MTT Assay and the films surface characteristics were determined by water contact angle (WCA) and Scanning Electron Microscope (SEM) was performed to observe cell adhesion. The hWJ-MSCs seeded onto films and after 24 hours, cell attachment, spreading, and morphology was evaluated. **Results:** The addition of 10%wt lactose was not toxic to the cell and when it blended into the gelatin-25µg/mL silk spidroin films its improved the hydrophilicity (GL10-25 group, $p \leq 0.05$). Cells successfully attached and spread on the silk spidroin-based substrate and showed a fibroblast-like morphology. **Conclusion**: This study reveals that the lactose-mediated crosslinking gelatin-silk spidroin films facilitate cell attachment and spreading on the film surface and confirm that these novel films were promising new material for cartilage tissue engineering.

Keywords: Silk spidroin, Gelatin, Films, Cell attachment, Cell spreading

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INTRODUCTION

Cartilage is a specialized connective tissue that absorbs load and reduces stress in synovial joints. It's a well-known avascular tissue and has very low cellularity. These distinctive cartilage features enable cartilage regeneration to be challenging, particularly in the field of orthopedics (1). To improve the outcome of cartilage remodeling, in vitro and in vivo tissue engineering has been thoroughly investigated (2). Cartilage tissue engineering requires some surface requirements to help cell attachment, spread, and proliferation. Modifying cell substrates to imitate extracellular matrix (ECM) is an up-and-coming technology to address inappropriate mechanical properties and shortage of biomaterial compatibility.

Due to its mechanical strength and nontoxicity properties, silk spidroin showed potential as biomaterials for cartilage tissue engineering. Silk spidroin from spider Argioppe apensa has an RGD sequence and suggests having a similar role as fibronectin as a cell substratum, albeit at an inexpensive price (3,4). However, silk spidroin cannot be used only as a film, since it is difficult to form the layers, because its brittleness and hydrophobicity hinder its further uses. To solve this challenge, silk spidroin must mix with another natural polymer to enhance its properties. Blending is an important way to enhance film production. Generally,

where the two materials used to blend are compatible, blend films may shape a homogeneous structure and perform better than individual components.

Gelatin, as a natural protein, can be deemed an ideal film-forming candidate and a useful material for tissue engineering applications due to its many potential benefits. First, it's relatively affordable, with excellent film-forming capabilities. Second, the FDA accepted it as "generally recognized as safe" (GRAS) material with impressive biodegradability and biocompatibility. Third, gelatin is much less antigenic than collagen and is widely used in cartilage tissue engineering (5,6). However, gelatin-based films demonstrate several disadvantages owing to their hydrophilic composition and brittleness. In particular, when submerged in aqueous solutions, films can absorb water, partially degrade, or break down, allowing crosslinkers to be necessary for sustaining their forms as films and enhancing their mechanical properties . Moreover, the addition of plasticizer is essential to decrease films' brittleness. Glycerol 10% was added to the mixture to increase biomaterial stability and plasticity (7). Selecting a non-toxic crosslinker is important since the chemical crosslinker causes adverse side effects. Several sugars (e.g. fructose, lactose, ribose) were recently used as crosslinkers to bond gelatin through a process called Maillard reaction (8).

Blended lactose-mediated crosslinking gelatin and silk spidroin generated by spider Argioppe appensa as films have never been reported. This research focused on developing and characterizing the silk spidroin-based films and lactose as a substitute to the frequently used chemical-crosslinking.. In this research, blended lactose-mediated crosslinking gelatin and silk spidroin were prepared and characterized its film surface using contact angle and scanning electron microscopy (SEM) to observe hWJ-MSCs attachment.

MATERIAL AND METHODS

Experimental Design

Blended silk spidroin and gelatin were made by adding 10 % glycerol as a plasticizer and lactose as a natural crosslinker. Serial concentration on lactose, 10%, 20%, and 30%, were used in this experiment. We evaluated the biocompatibility tests using MTT assay, the surface properties, film composition, and biological behavior in the attachment and spreading of hWJ-MSCs.

Materials

All chemicals and reagents used in this study were purchased from Thermo Scientific (Waltham, US), Invitrogen (Carlsbad, US) and Sigma-Aldrich (St. Louis, US), unless otherwise stated.

hWJ-MSCs Primary Cell Culture and Isolation

This research has been approved by the Medical and Health Research Ethics Committee (MHREC) of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia (Reference No: KE/FK/0868/EC/2020). The umbilical cords (UC) were obtained from healthy donors with informed written consent in the RSKIA Kota Bandung, Jawa Barat, Indonesia. Briefly, the UC was dipped and washed with phosphate buffer saline (PBS) three times and transferred to the transport medium containing PBS and 1% Antibiotic-Antimycotic. Upon arrival at the laboratory, the UC was dipped in 70% ethanol for 30 seconds, 10% povidone iodine for 30 seconds and washed again by PBS supplemented with 1% Antibiotic-Antimycotic three times. The UC was dissected longitudinally and the vessel was separated from the UC. After removing the vessel, Wharton's jelly was sliced into tiny segments (3-5 mm) and then minced using a sterile blade attached to the scalpel. The minced tissues were then transferred into a 50 ml tube containing a combined enzyme solution containing 2 mg/mL Collagenase Type I and Trypsin 0.25%. Then, the tube cap closes tightly and is placed in an incubator for 30 minutes at 37°C, 5% CO2. The enzymatic dissociation resulted in jelly-like suspension, and this suspension undergo prolonged incubation for 16-18 hours in 0.2 mg/mL Collagenase Type I. Suspension was neutralized using complete medium containing Dulbecco's Modified Eagle Medium High Glucose (DMEM HG) supplemented with 10% FBS and 1% Antibiotic-Antimycotic and filtered using 70 µm cell strainer (Biologix, Changzhou). Filtered suspension were added 10 mL complete medium cultured in T-75 flask (Biologix, Changzhou) in 37°C, 5% CO_2. The medium was changed every 2 days. As the primary cell (P0) approached 80% confluence, the cells were subcultured for cell expansion by 0.25% enzymatic treatment with Trypsin. Cell harvesting was conducted at passage 8 hWJ-MSC for analysis testing.

Analysis of surface specific antigen expression on hWJ-MSCs

The hWJ-MSCs passage 8 (1 x 10^6 cells) were suspended in 1 mL PBS sterile solution and transferred into five different tubes for further analysis by the protocol of the Human MSC Analysis Kit (BD Stemflow™, US). The tubes were incubated for 30 minutes at room temperature in the dark condition, then they were washed twice with PBS. The cells were resuspended in 500 µL PBS and analyzed with a flow cytometer (BD FACSLyric™). The cells had to fulfill the criteria of the International Society for Cellular Therapy (ISCT): the mesenchymal stem cells had to have cell surface markers (>95%) CD105, CD 90, and CD 73, and should not express (<2%) CD 45, CD 34, CD 14, CD 74 or CD 19, HLA class II.

Analysis of multipotential differentiation

The hWJ-MSCs passage 8 were cultured in 24 well plates at 1 x 10^4 cells/well in complete medium. After reach 80% confluence, the medium was replaced with differentiation medium, consist of adipogenic (Stempro™

Adipogenesis Differentiation kit, Gibco), chondrogenic (Stempro™ Chondrogenesis Differentiation kit, Gibco), and osteogenic (Stempro™ Osteogenesis Differentiation kit, Gibco) induction media. After 21 days, cell staining was performed by fixation using 4% paraformaldehyde. Oil Red O staining for adipocyte marker, Alcian Blue staining for glycosaminoglycan marker, as components of extracellular matrix in chondrocytes and Alizarin Red staining for osteocyte marker. Differentiation of hWJ-MSCs into adipocyte, chondrocyte and osteocyte was observed under the phase-contrast inverted microscope.

Synthesis of Lactose-mediated crosslinking Gelatin - Silk Spidroin Films

Gelatin solution was prepared by using the previously published method with slight modification (8). Briefly, 5 g of type B gelatin was dissolved in 100 ml of distilled water at 80 °C for 30 minutes under continuous stirring to obtain a homogeneous blend and followed by cooling at room temperature for 30 min. After that, 10%wt glycerol, which was based on dry gelatin, was added to the solution and the pH was adjusted to 10 with NaOH (1 M). Then, lactose as the crosslinking agent was added to the solution to obtain a final concentration of 10%wt, 20%wt, and 30%wt, also based on dry gelatin and was kept stirring for 5 min at 37 °C until it dissolved homogeneously.

Silk spidroin was collected from spider Argiope appensa. The spiders were put in a box with a lid covered by insect net and fed daily. Dragline silk was collected manually using a clean stirring glass rod without harming the spiders. The silk spidroin was then dissolved in formic acid solution (Merck, US) at 50°C for 3 hours with continuous stirring. The solution was then purified by dialyzing against Tris-HCl 5mM pH 8.00 in a cellulose membrane-based dialysis at 4°C. The Tris-HCl were changed every 2 hours for 2 days consecutive. Then, the solution was centrifuged at 7000 rpm for 10 minutes and the supernatant was collected and transferred in a clean 50 mL tube. Then, the supernatant was freeze-dried to a white powder. 25 µg/mL and 50 µg/mL silk spidroin powder were used in this experiment.

Blended lactose-mediated crosslinking gelatin and silk spidroin films were prepared according to the solvent casting method. The gel was pipetted into a custom silicon mold and vacuumed to eliminate the bubble followed by air-drying under the fume hood for 24 hours. All films stored in a desiccator at room temperature. By changing the percent content of lactose (10, 20, and 30%wt) and added spidroin, a series of blend films were coded as G (pure gelatin), GG (Gelatin-Glycerol), GGS (Gelatin-Glycerol-Spidroin), GL-10 (Gelatin-Glycerol-Lactose 10%wt), GL-10-25 (Gelatin-Glycerol-Lactose-Spidroin 25 µg/mL), GL-10-50 (Gelatin-Glycerol-Lactose 10%wt-Spidroin 50 µg/mL), GL-20 (Gelatin-Glycerol-Lactose 20%wt), GL-20-25 (Gelatin-Glycerol-Lactose 20% wt-Spidroin 25 µg/mL), GL-20-50 (Gelatin-Glycerol-Lactose 20%wt-Spidroin 50 µg/mL), GL-30 (Gelatin-Glycerol-Lactose 30%wt), GL-30-25 (Gelatin-Glycerol-Lactose 30%wt-Spidroin 25 µg/mL), GL-30-50 (Gelatin-Glycerol-Lactose 30%wt-Spidroin 50 µg/mL), respectively. The composition of the films was presented in Table I.

Table I. Labels used for different samples as a function of their composition.

Labels for Film Composition	Gelatin (%wt)	Glycerol (%wt)	Lactose (%wt)	Spidroin (µg/mL)
GL10	5%	10%	10%	-
GL10-25	5%	10%	10%	25 µg/mL
GL10-50	5%	10%	10%	50 µg/mL
GL20	5%	10%	20%	-
GL20-25	5%	10%	20%	25 µg/mL
GL20-50	5%	10%	20%	50 µg/mL
GL30	5%	10%	30%	-
GL30-25	5%	10%	30%	25 µg/mL
GL30-50	5%	10%	30%	50 µg/mL
G	5%	-	-	-
GG	5%	10%	-	-
GGS	5%	10%	-	50 µg/mL

Biocompatibility test of the Lactose-mediated crosslinking Gelatin - Silk Spidroin Films

The films were sterilized using 75% ethanol and UV for 30 minutes and washed in PBS five times before hWJ-MSCs seeding. The hWJ-MSCs passage 8 were cultured on sterile films at $1x10^5$ cells in 24 well plates. The cells were grown in a complete medium and put in an incubator at 37°C, 5% CO_2. After 72 hours of culture, the biocompatibilities of the films with the cells were analyzed using the MTT cytotoxicity assay. The complete medium was replaced and cells were washed with PBS. MTT reagent (Methylthiazolyldiphenyl-tetrazolium bromide) was added with a final concentration of 5 mg/mL and incubated at 37°C for 4 hours in dark conditions. The MTT reagent was then removed and 100 µL/well Dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystal. The absorbance was read using a microplate reader at 570 nm (Bio-Rad, US).

Lactose-mediated crosslinking Gelatin - Silk Spidroin Films Characterization

All films were cut into round shapes using a punch diameter of 60 mm. Films' visual aspects were captured using a digital camera. The film specimens were stored in a desiccator chamber at 25 °C and 50% relative humidity before the experiment. The water contact angle (WCA) was measured by dropping 5 µl of distilled water onto the surface of the films. The angle formed was captured using a portable digital microscope after settled and measured using software DinoCapture 2.0 (Dino-Lite, Taiwan). Films without hWJ-MSCs were

coated with gold by sputter coating, then were observed using the Scanning Electron Microscope (SEM, SU3500 Hitachi High Technologies, US).

Adhesion of hWJ-MSCs on the Lactose-mediated crosslinking Gelatin - Silk Spidroin Films

The hWJ-MSC passage 8 ($1x10^5$) cells were seeded on sterile films in 24 well plates and incubate at 37 °C, 5% CO_2. Cells adhesion and spreading were observed under an inverted microscope. After 24 hours, the culture media was replaced and washed with PBS three times. The films were fixed with 100 µL 2.5%v/v glutaraldehyde in 0.1 M cacodylate buffer and incubated overnight at 4°C. Then, films dehydration was performed by dipping them in a series of elevated concentration of ethanol (30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%), each for 15 minutes. The films were equilibrated in a 1:1 mix solution consist of 100% ethanol and 100% Hexamethyldisilazane (HMDS, Electron Microscopy Science, US). All films were immersed in 100 µL 100% HMDS and air-dried overnight. The dried films and cells were coated with gold by sputter coating, then were observed using the Scanning Electron Microscope (SEM, SU3500 Hitachi High Technologies, US).

Statistical Analysis

Evaluation of hWJ-MSCs cell cytotoxicity, film biocompatibility, and contact angle was analyzed using analysis of variance (ANOVA). All values are represented as mean ± SEM and replicate at least three times in experiments. The analysis was conducted using GraphPad Prism version 8.4.1 for Windows (GraphPad Software, US).

RESULT

hWJ MSC Isolation and Characterization

hWJ-MSCs were isolated from the umbilical cords from donors with cesarean sections using the enzymatic methods. The flow cytometry analysis showed that the isolated hWJ-MSCs were positive for these MSC markers: CD90 (98.54%) and CD73 (99.13%), at percentages higher than 95% and CD 105 (23.77%) and negative for CD34, CD45, CD11b, and CD19 (0.09%) at percentages lower than 2% (Fig. 1). CD105 was surprisingly lower than standards, probably because of higher passage cells that use in this experiment (passage 8). Although CD105 was relatively lower than the MSC characteristics standard, CD90 and CD73 are still meet the criteria as MSC following MSC characteristics standard as published by the International Society for Cellular Therapy (9).

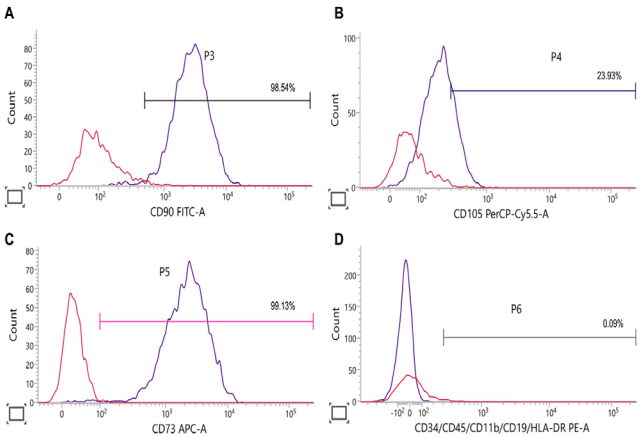

Fig 1: Flow cytometry result of specific hWJ-MSC cell surface markers. Cell expressed positive markers for (A) CD90, (B) CD73, (C) CD105, and (D) negative marker for CD34, CD45, CD11b, CD19, and HLA-DR.

Multipotency of the hWJ-MSCs was confirmed by induction with differentiation medium into adipogenic, chondrogenic, and osteogenic for 21 days in vitro cultured. The formation of lipid droplet was confirmed with Oil Red O staining (Fig. 2a), likewise calcium accumulation with Alizarin Red staining (Fig. 2b), and glycosaminoglycans (GAGs) masker with Alcian Blue staining (Fig. 2c). The morphology of the hWJ-MSCs passage 8 without differentiation medium was fibroblast-like and still adhere to the culture flask surface (Fig. 2d).

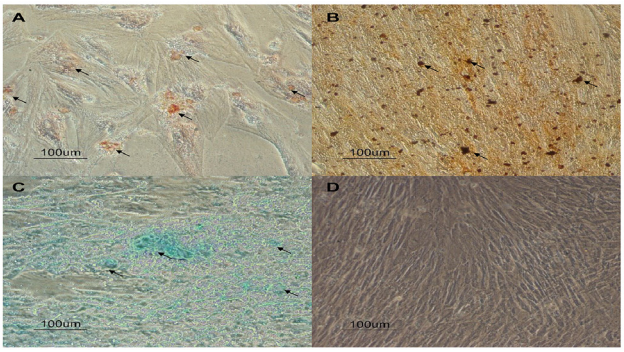

Fig 2: Multipotency characterization of hWJ-MSCs. (A) Oil Red O for adipocytes, (B) Alizarin Red for osteocytes and (C) Alcian Blue staining for GAGs in hWJ-MSC passage 8 induced by differentiation medium, and (D) non-staining group. Black arrow showed positive result from each staining group.

Cell cytotoxicity of Lactose-mediated crosslinking Gelatin - Silk Spidroin Films

The effect of the blended mixture films on cell cytotoxicity was observed through MTT assay (Fig. 3). Cells were grown for 72 hours on a complete medium. The results obtained from the cytotoxicity tests showed that the 10-20%wt lactose groups showed better cell viability when compared to the 30%wt lactose group ($p < 0.001$). It is interesting since the blend of lactose into gelatin-silk spidroin film increases the cell viability in GL-10-25 groups (84.73%). This condition indicating that films with silk spidroin were able to increase cell viability and have less toxicity compared to other films with higher lactose percentages ($p \leq 0.05$). This result was likely caused by direct cell adaptation and attachment on the surface of the films. A previous study showed that a film with a blend of recombinant spidroin had a similar capability to promote cell attachment to the film with higher spidroin composition (10). Based on these results, films with the compositions G, GL-10, GL10-25, GL10-50, GGS, and GG would be further tested in the following experiment.

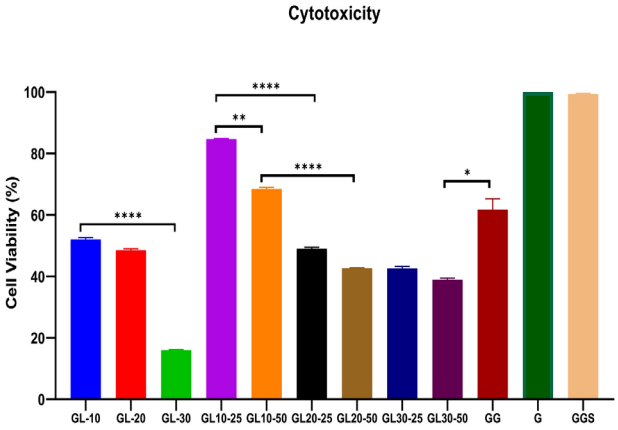

Fig 3: Cell Cytotoxicity Assay of Lactose-mediated crosslinking Gelatin-Silk spidroin Films.

Biomaterials Characterization of the Films

Macroscopically appearance and contact angle were conducted to determine the characteristics of gelatin-silk spidroin films. The films are colored yellowish (Fig.4a), transparent and homogeneous. The color presence in gelatin-silk spidroin films could be referred a crosslinking indicator. In tissue engineering, biomaterial hydrophilicity-hydrophobicity was previously suggested as an essential protein and cell adhesion regulator. Water contact angle (WCA) is used to measure film surface wettability. Overall, the films showed a broad variety of WCAs – from 51.82° for pure gelatin to 102.7° for the GL10-50 group (Fig. 4b). The graph shows that adding 10% lactose resulted in a hydrophilic surface and that films with a higher content of silk spidroin are more hydrophobic.

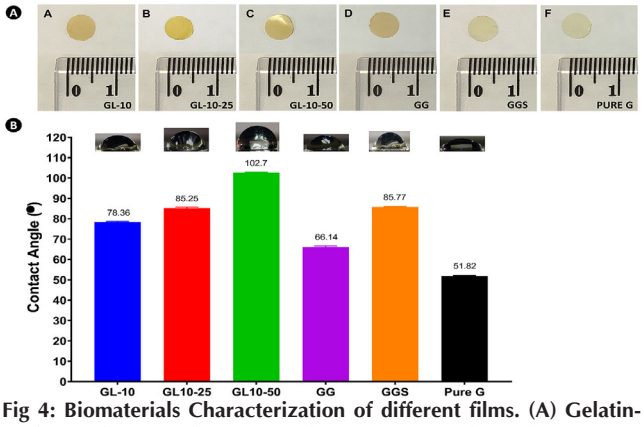

Fig 4: Biomaterials Characterization of different films. (A) Gelatin-Spidroin Films Visual Aspects and (B) Contact Angle Analysis.

Morphology of hWJ MSC Seeded on Gelatin-Silk spidroin Films

An optical micrograph using a Phase-Contrast Inverted Microscopy was used to observe the morphology of the films hWJ-MSC grew on the film for 24 hours, whereas Scanning Electron Microscopy (SEM) was used not only to observe the surface of the films without cells but also the cell adhesion and spreading after seeded the hWJ-MSC onto the films (Fig. 5). The cell surface observation using SEM revealed that the film appeared homogeneous and smooth (Fig. 5a). Figure 5b demonstrated hWJ-MSCs adhering and spreading onto the film for 24 hours. HWJ-MCSs can be seen as fibroblasts-like as they have an elongated cytoplasm with a cell nucleus in the center. This condition shows hWJ-MSC cells are alive. Meanwhile, figure 5c shows the cell attachment of hWJ-MSC to gelatin-silk spidroin film. As we can see, HWJ cells bind to all film surfaces during 24-hour in vitro culture. A yellow arrow marks the hWJ MSC cells, indicating cell adhesion.

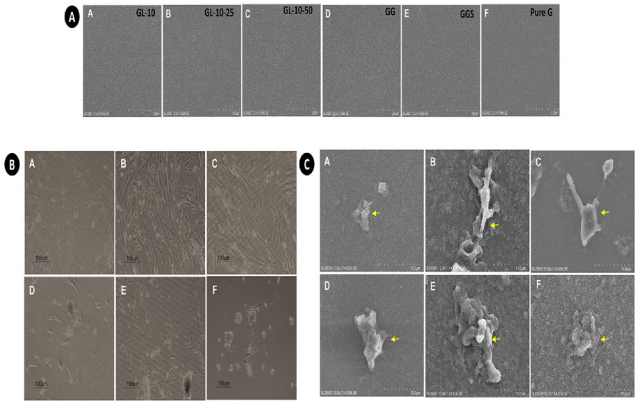

Fig 5: Optical micrograph and SEM images of the six different films. (A) Optical micrograph of cell adhesion and migration on films 24 hours after seeding. (a) GL-10, (b)

DISCUSSION

Spider silk (silk spidroin) is known for having great mechanical properties for cartilage tissue engineering but lacks in shape processing and ductility. These weaknesses are compensated by mixing it with gelatin to increase its processability and glycerol to increase its ductility. Lactose was chosen as a natural crosslinking agent and used to promote gelatin-silk spidroin films crosslinking. Even though mechanical properties have an important part, however, any biomaterial's ultimate necessity is biocompatibility. Accordingly, cytotoxicity of lactose-mediated spidroin gelatin-silk films was studied. The findings obtained from the cytotoxicity experiments revealed that the mixture of lactose into gelatin-silk spidroin film increases cell viability in GL-10-25 groups (84.73%), which was above 70%, which excludes any potential cytotoxic impact and indicates excellent biocompatibility following the guideline published by ISO 10993-5:2009 for the biological evaluation of medical devices (9).

The physical appearance of the films was translucent and yellowish, which can be described as a crosslinking marker. Possible explanations for the color change of the films were the interactions between proteins and protein conformation, contributing to non-enzymatic glycation, known as the Maillard reaction (8). The initial stage of the Maillard reaction would be between the reduction sugar carbonyl group, which can occur in

open-chain aldehyde form, and the gelatin amino group of lysine residues, creating an unstable Schiff base. The Amadori rearrangement transforms the Schiff base into the protein-bound Amadori substance (7,8,10). The initial type of the Amadori product is degraded and antioxidant dark yellow pigments, known as Maillard reaction products (MRPs) or melanoidins are developed (7,8,11). Cell adhesion and spreading are induced by the development of unique and non-specific interactions, based on hydrophilicity and substrate surface chemistry. The more hydrophilic a biomaterial is, the more nutrients the biomaterial can absorb, which can be used to provide food for cell growth. On the one side, cells are hydrophilic, so they would potentially have more preference for hydrophilic compounds. Hydrophilicity of the film surface was detected using the WCA test. Previous research on gelatin thin film with solvent casting technique found that the contact angle of gelatin films (3% wt) was 50.3 ± 6.4° (12), which was slightly around the result obtained in this study at 51.82±0.19°. . Interestingly, the addition of 10% lactose into silk spidroin, glycerol and gelatin films resulted in a WCA of around 85.25° (GL-10-25 group) and it seems ideal for cell spreading. The increase in WCA is caused by adding non-polar substances, silk spidroin and glycerol, into gelatin, increasing the hydrophobicity of the films. In terms of adding lactose, even though it is more polar than gelatin, lactose is observed to increase the hydrophobicity of gelatin films. This perhaps is part of the crosslinking process between lactose hydroxyl groups and gelatin carbonyl groups which decrease the films' polar region and increase its hydrophobicity.

On the other hand, hydrophobic surfaces are usually deemed more protein-adsorbent, but strongly hydrophobic materials replace interfacial surface water with hydrophobic domains of the proteins, allowing them to denature (13).

The optical observation and SEM result showed that hWJ-MSC successfully attached and spread better on gelatin films containing silk spidroin rather than gelatin films. This condition indicates that the hWJ-MSC cells are alive because they can only grow if they can protrude lamelapodia on the dish's bottom surface or growing media due to its anchorage-dependent cell properties (14). The observed cell shapes varied; some were round and elongated. The round shape indicates the cell is in a passive activity; that is, the cell has just made initial contact with the film (early spreading). The elongated shape shows an active movement. Cells will protrude their cytoplasm on the film and change its shape to become flattened. The condition is an advanced stage of initial cell contact called cell spreading (15). The attachment of cells onto the film is mediated through focal contact between actin in the cell and the amino acid Arginine-Glycine-Aspartic Acid (RDG sequences) (16). These amino acid compounds are present in the gelatin and silk-spidroin used in this study. Gelatin and silk spidroin have (RGD)-like sequences that can recognize cell surface receptors, the integrin. These motifs eventually promote cell adhesion and cytoskeletal realignment stability on films via cell-ECM interactions called focal adhesions (FA) (7,17). It seems that both cell adhesion and spreading were relatively high on the mixed gelatin-silk spidroin films owing to their potent hydrophilicity and free adhesion sequences.. Prior studies have indicated that the usage of silk spidroin, an extracellular matrix protein utilized in 3D scaffolds, has a positive influence on cell proliferation and chondrogenic differentiation of HWJ-MSCs (4). The results of measuring the hydrophilicity of the films and observations of cell interactions in these films show that the spidroin films can support cell adhesion and cell spreading faster than spidroin-free films, which means it also supports the activity and growth of HWJ-MSC cells. However, to determine the number of cells that grow and differentiate into chondrocytes on the film quantitatively, further research is needed with the cell proliferation and gene expression assay

CONCLUSION

Lactose-mediated crosslinking gelatin-glycerol-silk spidroin has been successfully prepared with good biocompatibility.. The supplementation of 10% lactose into gelatin, glycerol, and25 µg/mL silk spidroin film resulted in awater contact angle of 85.25°and facilitated the proper attachment and spreading of hWJ-MSCs with higher cell viability. These results indicate that lactose-mediated crosslinking gelatin-glycerol-silk spidroin films are compatible with hWJ-MSCs and can be a promising candidate for the development of bio-based films that may lead to additional improvement in cartilage tissue engineering.

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REFERENCES

1. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health [Internet]. 2009 Nov;1(6):461–8. Available from: https://pubmed.ncbi.nlm.nih.gov/23015907
2. Liu Y, Zhou G, Cao Y. Recent Progress in Cartilage Tissue Engineering—Our Experience and Future Directions. Engineering [Internet]. 2017;3(1):28–35. Available from: https://www.sciencedirect.com/science/article/pii/S2095809917301509
3. Wibowo UA, Judawisastra H, Giovanni R, Barlian A. Sutera Laba-laba dan Ulat Sutera sebagai Material Scaffold untuk Aplikasi Sutera Laba-Laba dan Ulat

Sutera sebagai Material Scaffold untuk Aplikasi Rekayasa Jaringan Kulit. Pros Semin Nas Mater dan Metal (SENAMM VIII). 2015;(August):246–50.
4. Barlian A, Judawisastra H, Ridwan A, Wahyuni AR, Lingga ME. Chondrogenic differentiation of Wharton's Jelly mesenchymal stem cells on silk spidroin-fibroin mix scaffold supplemented with L-ascorbic acid and platelet rich plasma. Sci Rep [Internet]. 2020;10(1):19449. Available from: https://doi.org/10.1038/s41598-020-76466-8
5. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev. 2007 May;59(4–5):207–33.
6. Santoro M, Tatara AM, Mikos AG. Gelatin carriers for drug and cell delivery in tissue engineering. J Control Release [Internet]. 2014/04/16. 2014 Sep 28;190:210–8. Available from: https://pubmed.ncbi.nlm.nih.gov/24746627
7. Etxabide A, Vairo C, Santos-Vizcaino E, Guerrero P, Pedraz JL, Igartua M, et al. Ultra thin hydrofilms based on lactose-crosslinked fish gelatin for wound healing applications. Int J Pharm [Internet]. 2017;530(1–2):455–67. Available from: http://dx.doi.org/10.1016/j.ijpharm.2017.08.001
8. Etxabide A, Urdanpilleta M, Guerrero P, de la Caba K. Effects of cross-linking in nanostructure and physicochemical properties of fish gelatins for bio-applications. React Funct Polym [Internet]. 2015;94:55–62. Available from: https://www.sciencedirect.com/science/article/pii/S1381514815300250
9. Standardization IO for. ISO 10993-5:2009 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity. Switzerland: International Organization for Standardization; 2009.
10. Oliver CM, Melton LD, Stanley RA. Creating Proteins with Novel Functionality via the Maillard Reaction: A Review. Crit Rev Food Sci Nutr [Internet]. 2006 Jul 1;46(4):337–50. Available from: https://doi.org/10.1080/10408690590957250
11. Echavarrна AP, Pagбn J, Ibarz A. Melanoidins Formed by Maillard Reaction in Food and Their Biological Activity. Food Eng Rev [Internet]. 2012;4(4):203–23. Available from: https://doi.org/10.1007/s12393-012-9057-9
12. Figueroa-Lopez KJ, Castro-Mayorga JL, Andrade-Mahecha MM, Cabedo L, Lagaron JM. Antibacterial and Barrier Properties of Gelatin Coated by Electrospun Polycaprolactone Ultrathin Fibers Containing Black Pepper Oleoresin of Interest in Active Food Biopackaging Applications. Nanomater (Basel, Switzerland) [Internet]. 2018 Mar 28;8(4):199. Available from: https://pubmed.ncbi.nlm.nih.gov/29597268
13. Gray JJ. The interaction of proteins with solid surfaces. Curr Opin Struct Biol. 2004 Feb;14(1):110–5.
14. Zhao G, Liu F, Lan S, Li P, Wang L, Kou J, et al. Large-scale expansion of Wharton's jelly-derived mesenchymal stem cells on gelatin microbeads, with retention of self-renewal and multipotency characteristics and the capacity for enhancing skin wound healing. Stem Cell Res Ther [Internet]. 2015;6(1):38. Available from: https://doi.org/10.1186/s13287-015-0031-3
15. McGrath JL. Cell spreading: the power to simplify. Curr Biol. 2007 May;17(10):R357-8.
16. Bini E, Foo CWP, Huang J, Karageorgiou V, Kitchel B, Kaplan DL. RGD-functionalized bioengineered spider dragline silk biomaterial. Biomacromolecules. 2006 Nov;7(11):3139–45.
17. Kurihara H, Nagamune T. Cell adhesion ability of artificial extracellular matrix proteins containing a long repetitive Arg-Gly-Asp sequence. J Biosci Bioeng [Internet]. 2005;100(1):82–7. Available from: https://www.sciencedirect.com/science/article/pii/S1389172305704321