SURFACE ENTRAPMENT OF COLLAGEN ON SINGLE LAYER POLYLACTIC ACID FILM CONTAINING SUSTAINABLE DRUG DELIVERY PROPERTIES FOR CORNEAL TISSUE ENGINEERING APPLICATION

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Abstract: Tissue engineering is a promising tool in repairing and treating corneal disease by developing new functionalized biological constructs using tissue scaffold. In this study, the inert surface of the polylactic acid (PLA) film was modified by using surface adsorption of collagen at different concentrations. The films were characterized for their tensile, swelling, water contact angle, in-vitro degradation, and light transmittance. Based on the mechanical and physical evaluations, the film was suggested to be optimum at 5wt% of collagen entrapment on the neat PLA film. Topographic analysis of the modified PLA film revealed that the inclusion of collagen induced a rougher surface, which is suitable for drug loading, biomolecule entrapment, and cell attachment. Fourier transform infrared (FTIR) confirmed the attachment of the collagen molecule at the PLA backbone by the presence of amino group's spectra. Additionally, drug release studies showed that the PLA/5%Col film has a controllable release profile and followed Fickian's diffusion kinetics release. In-vitro cytotoxicity studied using MTT assay revealed good biocompatibility of the human fibroblast cell (HSF1189), resulting in 93±0.13% cell viability after 48 hours of incubation. This new modified corneal film material could reduce the dependency on the corneal donor for corneal transplants in the future.

Keywords: Polylactic acid, collagen, surface modification, corneal tissue engineering, controlled drug release.

Introduction

The human eyes are covered with a transparent, elastic and clear tissue called the cornea that plays an important role in vision. Over the years, corneal diseases such as trachoma, keratoconus, and corneal ulceration have affected around 10 million people which have led to loss of vision and blindness (Liu *et al.,* 2013; Goodarzi *et al.,* 2019). Keratoplasty, known as corneal transplantation, is the dominant technique worldwide that is used to treat corneal diseases and which involves the replacement of full corneal thickness (Lee *et al.,* 2016; Liu *et al.,* 2016). However, the availability of allogeneic cornea tissue donors has failed to meet the demand in many regions and countries, in which about 53% of the world's population had

no access to corneal transplantation (Long *et al.,* 2015; Mansoor *et al.,* 2019). Due to some other reasons, allografts also have limitations on tissue rejection and neovascularization due to the stimulation of host immune responses. Therefore, corneal tissue engineering involving natural biomaterials seems to be the best alternative in repairing and regenerating new cornea tissue (Liu *et al.,* 2014a; Liu *et al,* 2016).

Polylactic acid (PLA) is a synthetic polymer that is commonly used in the biomedical field that possesses good biocompatibility, biodegradability, physical (transparent) and mechanical properties (Hu *et al.,* 2016; Razak *et al.,* 2016). PLA also showed excellent potential as a drug carrier due to its ability to protect drugs against degradation in a long period and

prolonged drug release process. However, PLA has poor functional sites for biological activities that can cause inflammation towards the host tissue (Yang *et al.,* 2002; Wang *et al.,* 2019). Surface modification is a feasible approach that involves the modification of bonds between the PLA with other biomolecules, and there are several types of modifications such as plasma treatment, surface hydrolysis, chemical grafting modification, physical adsorption and biomimetic self-assembly technology (Nakagwa *et al.,* 2006; Ospina-Orejarena *et al.,* 2016; Alippilakkotte & Sreejith, 2018; de la Mata *et al.,* 2019; Wang *et al.,* 2019).

A physical adsorption is a simple approach applied in this study which attaches the targeted filler on a polymer substrate using hydrophilic interaction, van der Waals forces, hydrogen bonding or even electrostatic forces, resulting from the miscible mixture of solvent and non-solvent (Childers *et al.,* 2015; Tallawi *et al,* 2015). The filler materials include the polyethylene glycol (PEG), chitosan; polydopamine and collagen are widely used in the modification of PLA composites (Meng *et al.,* 2011; Haaparanta *et al.,* 2014; Serra *et al.,* 2014; Kao *et al.,* 2015). Though previous reports have shown the feasibility of this technique on biomedical applications, there is still a lack of studies on the physical and drug delivery properties of the modified composite in the corneal tissue healing process. Collagen is one of the structures that makes the stromal fibrils of the cornea to have perfect biocompatibility and biodegradability, and is conducive to active progenitor cells that promote regeneration (Liu *et al.,* 2016). Type I collagen functions as the most favourable reconstructive material for tissue engineering applications, especially in corneal regeneration for the purpose of healing the corneal scars or keratoconus (Goodarzi *et al.,* 2019; Yousaf *et al.,* 2019).

To date, there are no studies on the surface modification of PLA film with collagen for the regeneration of corneal tissue have been reported. Therefore, this study aims to investigate the potential of the modified PLA film using collagen for corneal tissue engineering by evaluating its physical, mechanical, surface functional group, drug release profile and its cytotoxicity. Benzalkonium chloride (BAC), a type of drug widely used in ophthalmology whose main functions are to kill microorganisms and act as an antibacterial material was used in the drug model (Chen *et al.,* 2012). BAC is usually non-irritating, non-sensitizing and is well tolerated in dilutions normally employed on the skin and mucous membranes (Awwad *et al.,* 2017).

Materials and Methods

Preparation of PLA Film

The PLA film was prepared using 10% w/v of PLA granules (Sigma Aldrich) in 100 mL of 1,4-dioxane by continuously stirring until a clear polymer solution forms in 8 hours (Razak *et al.,* 2016). The polymer solutions were poured into a mould and were air-dried and rinsed three times with deionized water to remove excess solvent.

Surface Modification of PLA Film

The PLA undergoes surface modification via the physical adsorption method using collagen as the biomolecule fillers. Collagen powder (Argin Chemicals) of 1, 3, 5 and 7 wt% was dissolved in 100 mL acetic acid (Sigma Aldrich), forming a clear collagen solution. Later, the PLA film was immersed in acetone containing a solution with a ratio of 70:30 for 24 hours and rinsed 3 times using distilled water to remove any excess solvent before drying for 24 hours at room temperature. The PLA films were soaked into a collagen solution for 24 hours. The film was then removed and rinsed 3 times in distilled water to remove any excess of free biomolecules. The films were then dried at room temperature for 24 hours. In this study, the different modified films are called as neat PLA, PLA/1%Col, PLA/3%Col, PLA/5%Col and PLA/7%Col films.

Mechanical Testing

The tensile test was performed using the Instron mechanical testing machine (Model 4301, Instron 0.01±0.001 mm thickness), which were

immersed in a phosphate buffer saline solution (PBS) for 2 hours before the test, and were then clamped for tensile testing (n=5). The load used in the testing was 5 kN with a crosshead speed of 5 mm/min at atmospheric ambient temperature.

Degree of Swelling

Swelling or water absorption test was performed by immersing the PLA/Col films in PBS (pH 7.4) at 35 $^{\circ}$C for 24 hours. The wet scaffolds were blotted with the filter paper to rid of the excess solution and were weighed immediately. The thickness and surface area dimension changes were measured using a ruler and micrometer caliper. The percentages of solution adsorption or degree of swelling, surface area, and thickness increase were calculated using the following equations (Liu *et al.,* 2013):

$$Degree\ of\ swelling\quad = \frac{w_t - w_o}{w_t} \ x \ 100\% \qquad (1)$$

$$Surface\ area\ increase\quad = \frac{s_t}{s_o} \qquad (2)$$

$$Thickness\ increase\quad = \frac{H_t}{H_o} \qquad (3)$$

where W_t represents the wet weight of the films and W_o is the initial dry weight of the samples. H_t and S_t are the thickness and surface area of the wet samples at target times, respectively. H_0 and S_0 are the initial thickness and surface area of the dry membranes, respectively.

Water Contact Angle

The water contact angle was done by using the sessile drop method to measure the hydrophobicity and/or hydrophilicity of films. One drop of deionized water was placed on the sample and the contact angles were calculated from the image captured by the measuring system, VCA Optima, AST Products, Inc.

Optical Measurements-Light Transmittance

The composite films were immersed in PBS for 2 hours and fixed directly into 1.5 ml specimen chamber of the UV-visible spectrophotometer (Thermo Evolution 200 Series). The spectrophotometer photographed the optical clarity and transparency in the range of 400 to 800 nm to determine the light transmittance trend.

In-vitro Biodegradation

The in-vitro biodegradation property of the films was performed using phosphate buffer saline (PBS) at the pH 7.4 and 37 $^{\circ}$C. The neat PLA and PLA/Col films were placed in 10 mM PBS with 10 µ/ml collagenase for 7 days (Goodarzi *et al.,* 2019). At the time intervals, the films were weighed carefully and the degradation percentage was determined according to the following equation:

$$Degradation/weight\ loss\ (\%) = \frac{w_o - w_d}{w_o} \ x \ 100 \quad (4)$$

where, W_o represents the weight of scaffold before incubating in PBS solution, (g) and W_d is the weight of scaffold after incubating in PBS solution, (g).

Scanning Electron Microscope (SEM)

The surface morphology of the neat PLA and PLA/Col composite films was analyzed using a scanning electron microscope (SEM, Hitachi SU8010) at 1000 to 3000 magnifications.

Atomic Force Microscopy (AFM)

The topographic feature of neat PLA and PLA/ Col films was evaluated using AFM (Park System XE-100). The testing was performed under ambient conditions (dry film) with a sample size of 1 cm x 1 cm.

Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of PLA and PLA/Col composites film was measured using the FTIR (PerkinElmer Frontier and Spectrum Two, Perkin Elmer). FTIR testing is known as a powerful tool to identify the type of chemical bonds and functional groups in a molecule by producing an infrared absorption spectrum. The neat PLA and PLA/Collagen film with a size of

1 cm x 1 cm was prepared for testing by using a wave range from 400 to 4000 cm^{-1}.

Drug Loading and Release

To test the loading capacity towards the modified film, the dry film was weighed first before being immersed in 50 ml of 0.03, 0.05, 0.07, 0.09, 0.11 and 0.13% of benzalkonium chloride (BAC) solution for 24 hours. Then, the sample was wiped using filter paper to remove excess drug solution and the weight of the soaked film was measured. The loading capacity was calculated by using the formula:

$$Drug\ load\ capacity\ (mg/cm^2) = \frac{w_f - w_i}{Area\ of\ the\ dry\ film} \quad (5)$$

where W_i is the initial and W_f is the final weight of the film after being immersed in the BAC solution. The release profile of the neat PLA and PLA/Col films was investigated by placing it in a beaker of the simulated tear (ST) and the solution was analyzed every 2 hours by using ultraviolet-visible (UV-Vis) spectroscopy, Thermo Evolution 200 Series. The ST concentration was determined spectrophotometrically by measuring the absorbance value at 272 nm respectively. The mechanism was also studied with several kinetic models (Dash *et al.*, 2010; Singhvi & Singh, 2011):

Zero-Order Kinetic Model

$$Q_t = Q_0 + K_0 t \quad (6)$$

where Q_t is the amount of drug dissolved in time, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero-order release constant expressed in units of concentration/time

First-Order Kinetic Model

$$log\ C = log\ C_0 - \frac{K_t}{2.303} \quad (7)$$

where C_0 is the initial concentration of the drug, k is the first-order rate constant, and t is the time.

Higuchi Model

$$Q = K_H \times t^{1/2} \quad (8)$$

where Q is the amount of drug released in time, K_H is the Higuchi dissolution constant and $t^{1/2}$ is the square root of time.

Korsmeyer-Peppas Model

$$\frac{M_t}{M_o} = Kt^n \quad (9)$$

where M_t is the amount of drugs dissolved as a function of time. M_0 is the total amount of drugs being released and t is an account for the lag time measured as a result of the dissolution process.

Cell Seeding on Scaffold Sample

Human fibroblasts cells used in this study were seeded in T-flasks containing DMEM supplemented with penicillin, streptomycin, and FBS for complete growth medium. The fibroblast cells were incubated at 37°C with 5% CO_2 at 95% humidity and the medium was changed every 3 days. In this study, the 5th to 15th passage fibroblast cells was used for cytotoxicity and cell proliferation study. The film samples were immersed in 75% ethanol for 12 hours for sterilization, followed by solvent exchange by phosphate buffer saline solution (PBS) for 6 times. Then, the films were placed on the 6-well polystyrene plate and seeded with 20 µl of human fibroblast cell suspension at a density of 1 x 10^6 cells/ml. The cells were allowed to attach for 1 hour before the medium was refreshed with new culture media and incubated for 1 to 7 days according to the assay requirement (Ma *et al.,* 2003; Sangsen *et al.,* 2012).

MTT assay - Cell cytotoxicity

MTT assay is a colorimetric assay used to assess cell metabolic activity. The reduction of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), a yellow tetrazole turned into purple formazan crystals, only took place when the mitochondrial reductase enzymes were active whereby the conversion correlates to the number of cells that survived. The cytocompatibility was examined after 24 and 48 hours of the incubation period of the cells on the films. The culture medium was discarded and MTT reagent (5 mg/ml) was

added into each well and incubated again for 4 hours to allow peak formazon production. Then, the undissolved MTT was aspirated without disturbing the formazan crystals formed and 10mM HCL solution in isopropanol was added to dissolve the formazon (Bohari *et al.,* 2011). Later, the purple solution was transferred into a 96-well polystyrene plate to measure the absorbance at 570 nm using a microplate reader.

Results and Discussion

Mechanical Characterization

In corneal tissue engineering, the scaffolds must be able to withstand the tension induced from the eye movements and intraocular pressure which will help to prevent rapid in vivo degradation after being transplanted into the human body (Aldana & Abraham, 2017). Besides that, the corneal repair scaffold must be able to tolerate the surgery suture procedure in keratoplasty treatment. The mechanical properties for different film formulations are presented in Table 1. Tensile strength and the Young modulus of the neat PLA film were at 3.56 MPa and 8.30 MPa respectively. Inclusions of collagen from 1% to 7% have increased the strength and modulus of the neat PLA film significantly. The same trend was reported by other researchers in the capability of the collagen acting as a reinforcement filler and the surface attachment that resulted in high surface area to volume ratios of the collagen (Park *et al.,* 2006). The addition of 3 to 7% collagen exhibits suitable mechanical properties where the tensile strength was close to the native corneal tissue (11.0±0.1 MPa) (Liu *et al.,* 2016). However, a high amount of collagen will simultaneously reduce the strength and modulus due to the loss of structural integrity resulting from the highly collagen molecule on the film surface that disrupts the PLA backbone structure (Kuorwel *et al.,* 2013). Elongation at break of the films with collagen was increased with the addition of collagen percentage that helps with better suture retention strength during surgery (Long *et al.,* 2014). Overall results indicated that the toughness of the PLA/Col film is higher than the neat PLA film. The surface entrapment process did not reduce the mechanical strength of the PLA but increased the toughness of the material to a certain degree depending on the filler amount.

Degree of Swelling

The degrees of swelling results are shown in Figure 1. It was reported that as the cornea is a wet tissue, therefore, the water content and hydrophilicity therefore play major roles in corneal repair because the properties are related to cell adhesion and proliferation. Swelling capacity will increase the pore size diameter, which not only allows the cell to attach, but also migrate inside the pore to grow in the 3D pattern during the healing process (Lasprilla *et al.,* 2011; Long *et al.,* 2014; Kiaee *et al.,* 2016). Figure 1(a) shows that after 10 min the water absorption of all samples was steady. The water absorption of all samples increased and constantly reached the saturation level after 20 min. It may be convenient to apply it in the clinic, as the material can be stored in dry conditions and just needs to be hydrated for a few minutes before the corneal transplant operation (Long *et al.,*

Table 1: Tensile properties of neat PLA and PLA/Col films after immersing in PBS

Sample	Tensile Strength (MPa)	Young Modulus (MPa)	Elongation at break (%)
Neat PLA	3.56±1.3	8.3±0.3	20.06±0.06
PLA/1% Collagen	6.63±0.5	12.13±0.2	32.43±0.04
PLA/3% Collagen	8.88±1.2	18.37±0.7	45.00±0.01
PLA/5% Collagen	9.60±2.3	24.66±0.8	55.65±0.02
PLA/7% Collagen	8.69±0.8	18.11±0.5	42.92±0..09

2014). The inclusion of the collagen on the PLA film surface improved the equilibrium water content as indicated in Figure 1(b) where the water equilibrium after neat PLA, PLA/1%Col, PLA/3%Col, PLA/5%Col and PLA/7%Col was 58.84±1.3 %, 65.78±1.2 %, 84.88±1.5 %, 82.74±1.3 %, and 81.69±1.6 % respectively. Studies have reported that the human cornea has a water uptake capability of about 78.0±3.0 % (Wang *et al.,* 2009; Liu *et al.,* 2014b). Very

clearly, the water absorption of 3 to 7% collagen inclusion is closer to the native cornea due to the introduction of collagen hydrophilic groups in the PLA backbone. Figures 1 (c) and (d) show the variation of the films' surface area and thickness versus time in PBS, indicating that the dimensions of the samples tended to be constant, controlled and repeatable. This gives an advantage for the films to be prepared with various dimensions according to the patient's need.
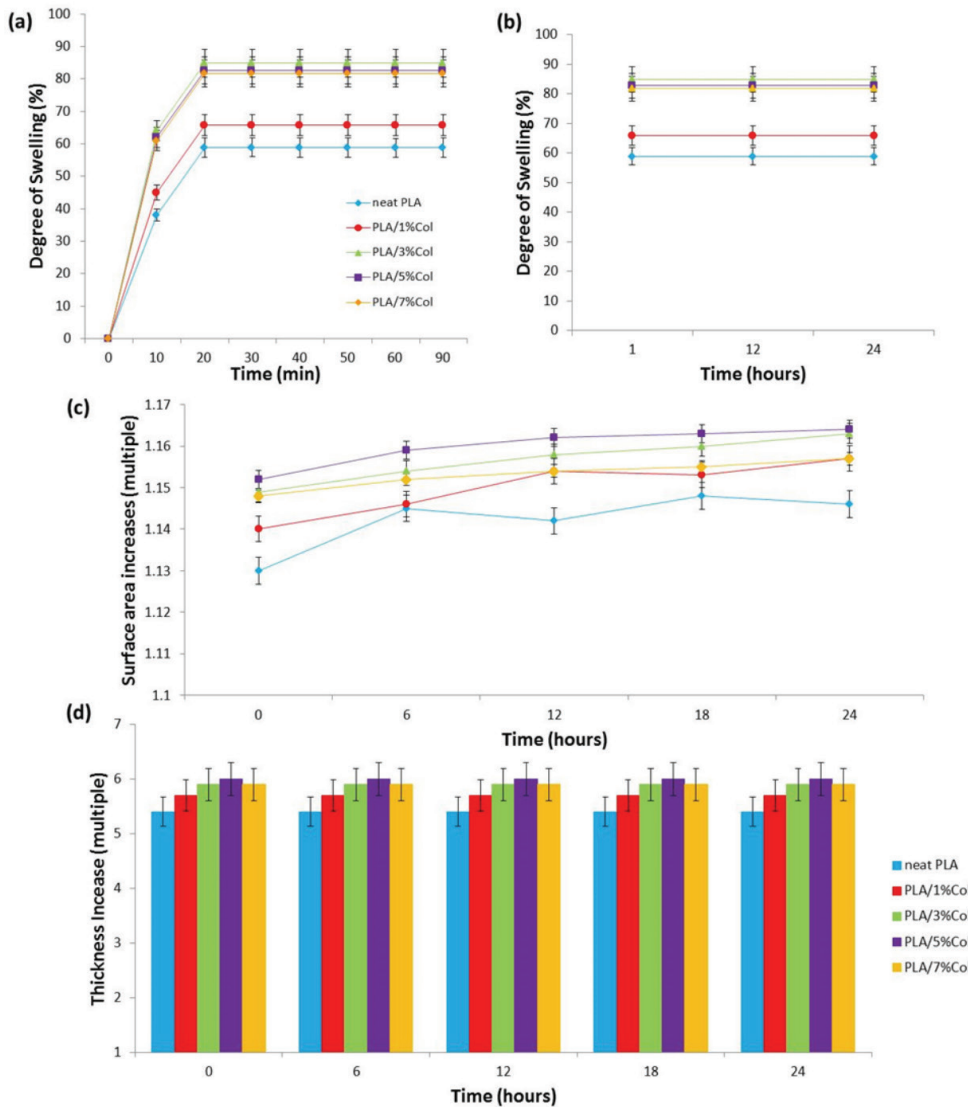

Figure 1**:** Degree of swelling of the films (a) for 1-hour condition (b) 24 hour-condition. Variation of the films' thickness (c) and surface area (d) increase over time

Surface Contact Angle

The performance of a material is often dictated by surface and interfacial properties, such as wettability, friction, and adhesion. The water contact angle is one of the methods used to study the wettability of the film. Figure 2 presents the water contact of the neat PLA and its collagen-containing films. The neat PLA film was found to be a hydrophobic surface at 108.8° compared to other films. As the collagen was introduced on the surface of the films, the result reduced significantly to 63.1° for PLA/7%Col which correlated with swelling and water absorption properties that have been discussed previously. This suggests that the surface of neat PLA film has been incorporated with the hydrophilic group (NH_2, COOH, OH, C=O) of collagen (Sizeland *et al.,* 2015; Goodarzi *et al.,* 2019). Besides, the surface modification led to changes in the surface roughness that contributes to the penetration of wetting medium (Umoren *et al,* 2015). Based on the mechanical evalution, degree of swelling and water contact angle analysis, PLA/5%Col was suggested for further testing and characterization due to its tensile strength that is relatively near to the native cornea compared to other formulations and excellent hydrophilic improvements.

Optical Performance: Light Transmittance

The light transmittance curve of neat PLA and PLA/5%Col films is presented in Figure 4. Overall, the transmittance of both films increased as the wavelength increased. However, as the collagen was introduced onto the surface of the PVA film, the transmittance reached 90 % in the visible range and tended to be constant. The PVA/5%Col film showed better optical characteristics and the luminousness is similar to the native cornea (Liu *et al.,* 2013). It is reported that a cellular human corneal stromal has between 88 % and 90 % transmittance at 400 nm - 600 nm wavelengths, respectively (Goodarzi *et al.,* 2019).

In-vitro Biodegradation

The in-vitro degradation study uses enzymatic degradation simulation which easily mimics in-vivo or clinical conditions. The degradation profile of neat PLA and PLA/5%Col films shown in Figure 4 was characterized by showing a residual mass percentage as a function of time. Over time, the biodegradation rate of the films increased. The incorporation of the collagen molecules on the PLA surface shifts the slower degradation rate which indicates the film could

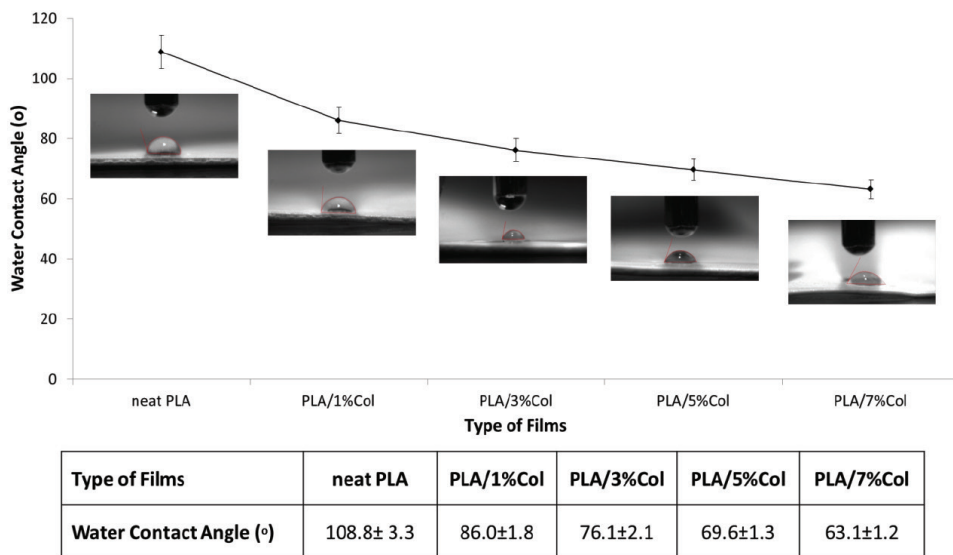| Type of Films	neat PLA	PLA/1%Col	PLA/3%Col	PLA/5%Col	PLA/7%Col
Water Contact Angle (°)	108.8± 3.3	86.0±1.8	76.1±2.1	69.6±1.3	63.1±1.2

Figure 2: The water contact angle of neat PLA and PLA/Col films
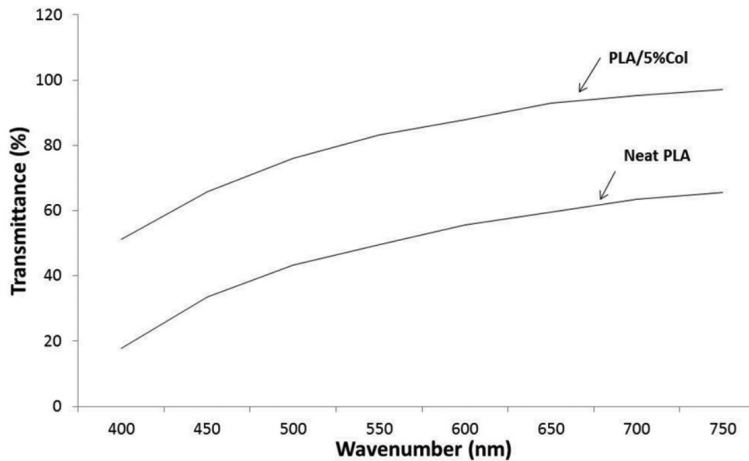
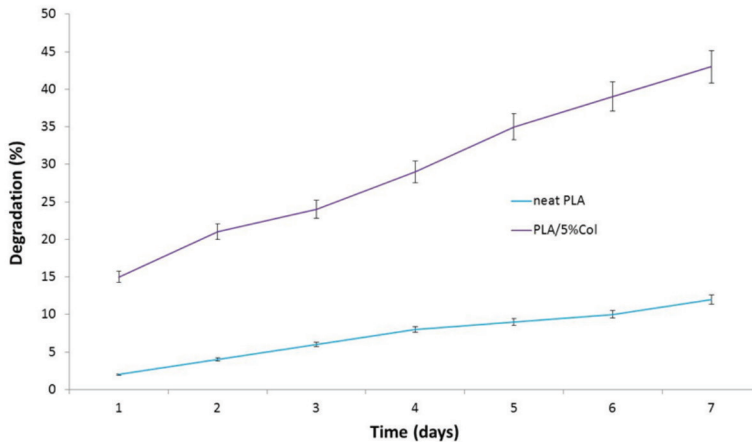Figure 3: The light transmittance of neat PLA and PLA/5%Col films

Figure 4: Enzymatic degradation profile of neat PLA and PLA/5%Col films in 10U/ml collagenase enzyme

be degraded or remodeled by the host tissue (Liu *et al.,* 2013). The data strongly showed the influence of collagen to biodegradation behavior by improving the enzymatic and hydrolytic degradation process. The adjustable degradation rates are useful for the development of the corneal tissue, as the tissue growth on the specific direction (channel by film scaffold) of the film will dissolve in the human body without causing an inflammatory response (Naahidi *et al.,* 2017).

Topographical Analysis

Figure 5 shows the AFM images of changes in the topography of the film after surface modification of PLA with collagen. 3D images of the film revealed rough surfaces on the film especially with the presence of the collagen. The higher Rq values represent the rougher surfaces of the materials (Jaiswal *et al.,* 2016). PLA/5%Col film has higher surface roughness compared to the neat PLA film due to the change of surface texture resulting from collagen entrapment as proposed in Figure 5. This AFM result corroborates the findings of the water contact angle.
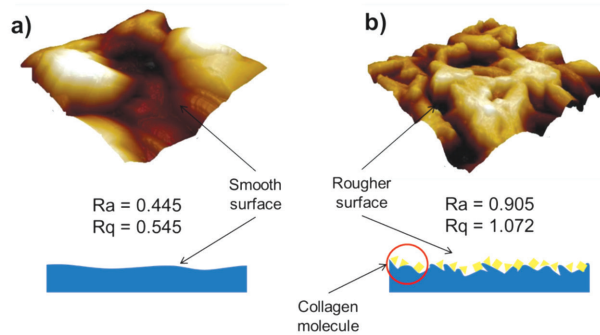
Figure 5: Atomic force microscopy images and proposed surface illustration of (a) neat PLA and (b) PLA/5%Col films

FTIR Spectra Analysis

To identify and examine the chemical interactions in the compound, FTIR analysis was performed and the results of neat PLA collagen and PLA/5%Col were compared as shown in Figure 6. PLA regions of interest were focused on the peak of between 1780 and 1680 cm^{-1} for the C=O stretch and at about 3100 to 3000 cm^{-1} exhibit O-H stretch. The peaks at about 1750 and 1180 cm^{-1}, which belong to C=O as mentioned before were stretching and the C-O-C stretching of PLA was clearly shown in the graph of spectra in Figure 6. In collagen, the transmittance of the IR Spectroscopy exhibited absorptions at 1082 cm^{-1} which arose from C-O-C. Several peaks also exhibited bands at 1398, 1450, 1535 and 1242 cm^{-1}, which may be contributed by CH_2, CH_3, C-N, and N-H bonding. The modified PLA film with 5% of collagen seemed to exhibit the intermolecular forces between PLA and collagen based on the analysis on the spectra labeled (c) in Figure 6 and a new peak exists indicating that the stretching bond is between 1140 - 1080 cm^{-1}. The possible bond that exists between the materials is the amide linkage, which was generated from carboxylic groups of PLA and primary amine of collagen (Mhlanga & Ray, 2015). Collagen contains several amino groups that may exist at peaks between 1650 to 1630 cm^{-1} that allow the carboxyl groups of PLAs to undergo hydrolysis and crosslink with an amine of collagen. The proposed structure and interaction between both materials are shown in Figure 6 (d).

Loading and Release of Drug

The drug loading capacity of neat PLA and PLA/5%Col under different concentrations of BAC are shown in Figure 7. Overall, the results showed that the drug loading capacity of the film increased as the concentration of drug increased. PLA/5%Col loading capacity is higher than the neat PLA. This indicates that the treated PLA surface enhanced the specific drug binding interaction area by altering the surface, forming a rougher and sponge-porous like structure (Walker, 2015; Mamat *et al.,* 2019). These loading properties corroborated with the AFM image of Figure 5 (b).

The investigation of BAC release from the PLA/5%Col film is shown in Figure 8. The ultimate goal of tissue engineering scaffold is to allow the growth of cell/tissue in the wounded or damaged area. Thus, to ensure the scaffold can maintain its function, a sustainable or controlled drug/antibacterial release is important to protect the new cell/tissue from infection (Biswas & Lopez-Collazo; 2009; Foong *et al.,* 2018). The burst releases of BAC occurring in the first 6 hours will provide immediate relief towards patient comfort and prevent the onset of infection. Then, the healing process will be promoted by prolonging the release of BAC after the burst phases until no remaining drug exists. Throughout the process, the physical structure of the film will be degraded through enzymatic or hydrolytic processes which will produce a new, healthy and matured biological structure (Shah *et al.,* 2008; Farah *et al.,* 2016).
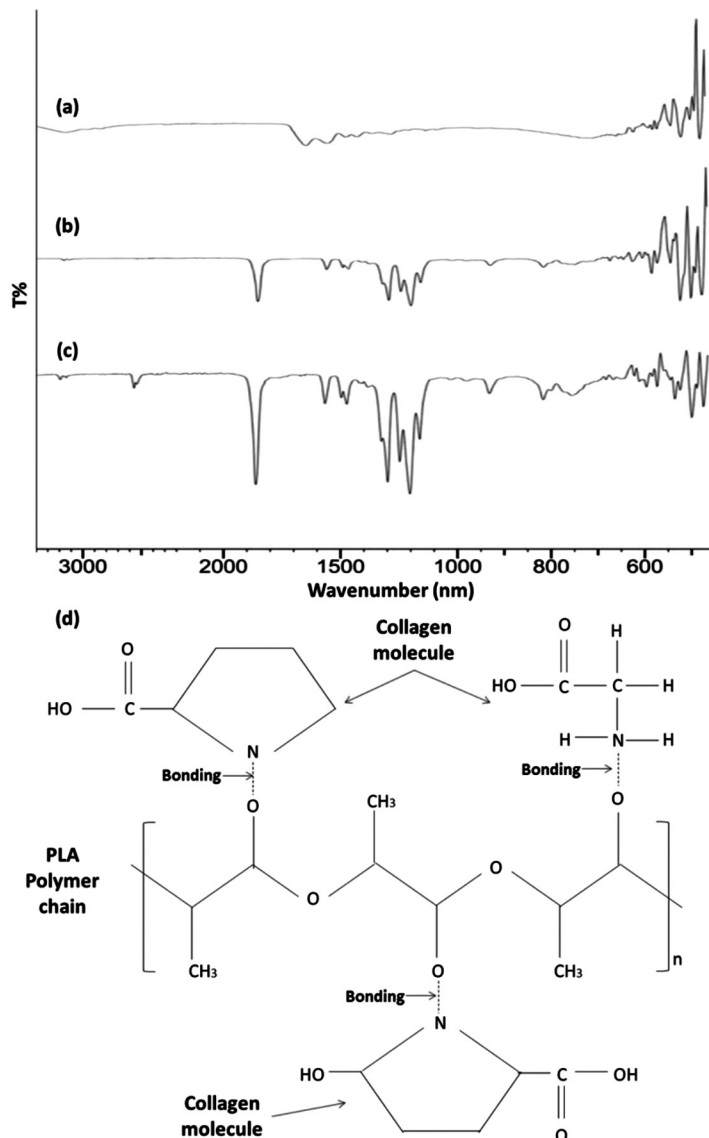
Figure 6: The FTIR spectra of collagen (a), neat PLA (b) and PLA/5%Col (c) films. The proposed interaction between PLA and collagen (d)

Kinetic Model of Drug Release

The drug release profile of PLA/5%Col film was evaluated mathematically using Zero-order, First-order, Higuchi, and Koshmeyer-Peppas models as tabulated in Table 2. The PLA/5%Col film is fitted most on the Higuchi model compared to Zero-order and First-order model. The drug release increased over time as shown in Figure 8 can hypothesize that the release of BAC is dominated by diffusion, which was confirmed from the kinetic analysis. In detail, Korsmeyer-Peppas models were used. The correlation showed by PLA/5%Col film is higher with these models ($R_2 > 0.93$), indicating a significant analysis. According to the Korsmeyer-Peppas, $n < 0.45$ suggested that the drug release followed the Fickian diffusion mechanism (Kong *et al.,* 2016). Thus, the release
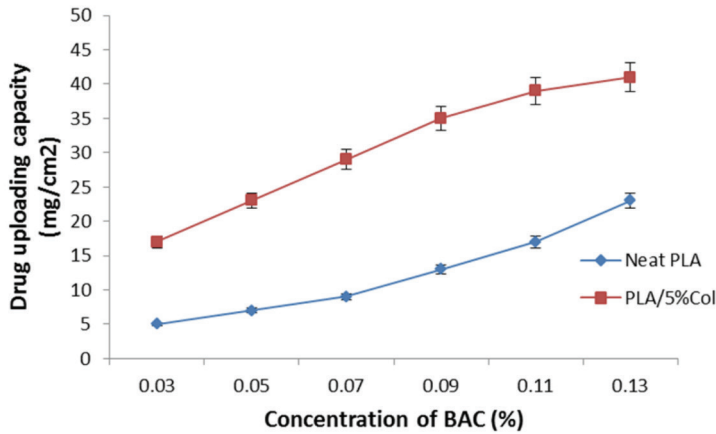
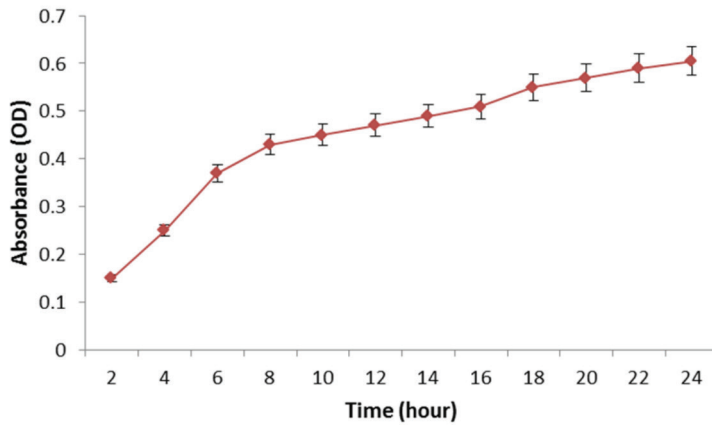Figure 7: Drug loading capacity of neat PLA and PLA/5%Col under different concentrations

Figure 8: The absorbance of BAC release at 0.11 mg/cm2 from PLA/5%Col film

Table 2: Zero-order, First-order, Higuchi and Korsmeyer-Peppas equation release profile

Film	Zero-order	First-order	Higuchi		Koshmeyer-Peppas
	R^2	R^2	r^2	K_H	N
PLA/5%Col	0.7452	0.7334	0.8759	0.1324	0.2333

of BAC is directed by the diffusion process and the entrapment of collagen provides an optimal structure for the film to control the release rate.

Cytotoxicity

The films were subjected to MTT assay to evaluate cytotoxicity potential in comparison with control positive (Triton-X) and control negative (normal culturing). The cell viability of human fibroblast cell after 24 hours and 48 hours incubation time is shown in Figure 9. The seeded cells were proliferated in all film samples. The PLA/collagen film demonstrated higher cell growth compared to neat PLA scaffold. The high absorbance of PLA/5pectin sample indicated the high viability of cells (> 90 %) on the film. This essentially proved that PLA surface modified with 5 wt% collagen is
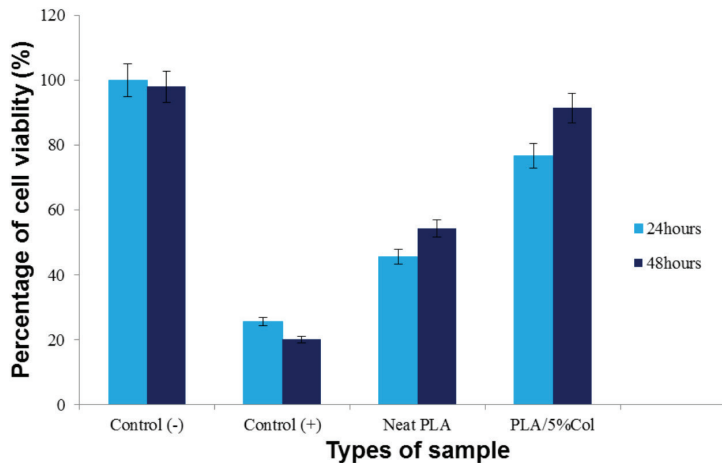
Figure 9: The differences on percentage of cell viability

biocompatible. However, when compared with a negative control, there are possibilities of cell death caused by the chances of the influx of Ca^{2+} ions in the culture medium (Jackson *et al,* 2007).

Conclusion

Herein, we have presented and optimize the fabrication process of biocompatible PLA film modified with collagen using surface adsorption method containing sustainable drug release properties for corneal tissue replacement. PLA/5%Col exhibits optimal formulation to fabricate the film. The results show that this modified film exhibits similar mechanical, hydrophilic, and light transmittance to the human cornea tissue. Besides, this film shows good biodegradation behaviour through the enzymatic and hydrolytic process. The inclusion of collagen improves the surface characteristic of smooth PLA film in addition to the FTIR spectra which confirmed the well-developed interaction between the PLA chain and collagen molecule. Besides, the kinetic release of BAC drugs is expected for the controllable release which can reduce the risk of infection that contributes to tissue growth success rate within corneal defective patients. In-vitro cell culture studies with human fibroblast cell determined the non-toxicity of the film as well as the feasibility of PLA/5%Col film for human tissue regeneration. In spite of that, future works need to be conducted especially to know the capability of scaffold for in-vivo transplantation which reassures the potential of the film for corneal tissue engineering.

Acknowledgements

References

Aldana, A. A., & Abraham, G. A. (2017). Current advances in electrospun gelatin-based scaffolds for tissue engineering applications. *International Journal of Pharmaceutics*, *523*(2), 441-453.

Alippilakkotte, S., & Sreejith, L. (2018). Benign route for the modification and characterization of poly lactic acid (PLA) scaffolds for medicinal application. *Journal*

of Applied Polymer Science, *135*(13), 46056.

Awwad, S., Mohamed Ahmed, A. H., Sharma, G., Heng, J. S., Khaw, P.T., Brocchini, S., & Lockwood, A. (2017). Principles of pharmacology in the eye. *British Journal of Pharmacology*, *174*(23), 4205-4223.

Biswas, S. K., & Lopez-Collazo, E. (2009). Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends in Immunology*, *30*(10), 475-487.

Bohari, S. P., Hukins, D. W., & Grover, L.M. (2011). Effect of calcium alginate concentration on viability and proliferation of encapsulated fibroblasts. *Bio-Medical Materials and Engineering*, *21*(3), 159-170.

Chen, W., Hu, J., Zhang, Z., Chen, L., Xie, H., Dong, N., Chen, Y., & Liu, Z. (2012). Localization and expression of zonula occludins-1 in the rabbit corneal epithelium following exposure to benzalkonium chloride. *PLoS One*, *7*(7), e40893.

Childers, E. P., Wang, M. O., Becker, M. L., Fisher, J.P., & Dean, D. (2015). 3D printing of resorbable poly (propylene fumarate) tissue engineering scaffolds. *Mrs Bulletin*, *40*(2), 119-126.

Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica*, *67*(3), 217-23.

de la Mata, A., Mateos-Timoneda, M. A., Nieto-Miguel, T., Galindo, S., López-Paniagua, M., Planell, J. A., Engel, E., & Calonge, M. (2019). Poly-l/dl-lactic acid films functionalized with collagen IV as carrier substrata for corneal epithelial stem cells. *Colloids and Surfaces B: Biointerfaces*, *177*, 121-129.

Farah, S., Anderson, D. G., & Langer, R. (2016). Physical and mechanical properties of PLA, and their functions in widespread applications—A comprehensive review.

Advanced Drug Delivery Reviews, *107*, 367-392.

Foong, C. Y., Hamzah, M. S. A., Razak, S. I. A., Saidin, S., & Nayan, N. H. M. (2018). Influence of poly (lactic acid) layer on the physical and antibacterial properties of dry bacterial cellulose sheet for potential acute wound healing materials. *Fibers and Polymers*, *19*(2), 263-271.

Goodarzi, H., Jadidi, K., Pourmotabed, S., Sharifi, E., & Aghamollaei, H. (2019). Preparation and in vitro characterization of cross-linked collagen–gelatin hydrogel using EDC/NHS for corneal tissue engineering applications. *International Journal of Biological Macromolecules*, *126*, 620-632.

Haaparanta, A. M., Järvinen, E., Cengiz, I. F., Ellä, V., Kokkonen, H. T., Kiviranta, I., & Kellomäki, M. (2014). Preparation and characterization of collagen/PLA, chitosan/PLA, and collagen/chitosan/ PLA hybrid scaffolds for cartilage tissue engineering. *Journal of Materials Science: Materials in Medicine*, *25*(4), 1129-1136.

Hu, Y., Daoud, W., Cheuk, K., & Lin, C. (2016). Newly developed techniques on polycondensation, ring-opening polymerization and polymer modification: Focus on poly (lactic acid). *Materials*, *9*(3), 133.

Jackson, C. L., Dreaden, T. M., Theobald, L. K., Tran, N. M., Beal, T. L., Eid, M., Gao, M. Y., Shirley, R. B., Stoffel, M. T., Kumar, M. V., & Mohnen, D. (2007). Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. *Glycobiology*, *17*(8), 805-819.

Jaiswal, A. (2016). Nanofibrous scaffolds for tissue engineering applications. *Brazilian Archives of Biology and Technology*, 59.

Kao, C. T., Lin, C. C., Chen, Y. W., Yeh, C. H., Fang, H. Y., & Shie, M. Y. (2015). Poly (dopamine) coating of 3D printed poly (lactic acid) scaffolds for bone

tissue engineering. *Materials Science and Engineering, C*, *56*, 165-173.

Kiaee, G., Etaat, M., Kiaee, B., Kiaei, S., & Javar, H. A. (2016). Multilayered controlled released topical patch containing tetracycline for wound dressing. *Journal of In Silico & In Vitro Pharmacology*, *2*, 2.

Kong, B. J., Kim, A., & Park, S. N. (2016). Properties and in vitro drug release of hyaluronic acid-hydroxyethyl cellulose hydrogels for transdermal delivery of isoliquiritigenin. *Carbohydrate Polymers*, *147*, 473-481.

Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J., & Bigger, S. W. (2013). Water sorption and physicomechanical properties of corn starch-based films. *Journal of Applied Polymer Science*, *128*(1), 530-536.

Lasprilla, A. J. R., Martinez, G. A. R., & Hoss, B. (2011). Synthesis and characterization of poly (lactic acid) for use in biomedical field. *Chemical Engineering*, *24*, 985-990.

Lee, M. C., Kim, D. K., Lee, O. J., Kim, J. H., Ju, H. W., Lee, J. M., Moon, B. M., Park, H. J., Kim, D. W., Kim, S. H., & Park, C. H. (2016). Fabrication of silk fibroin film using centrifugal casting technique for corneal tissue engineering. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *104*(3), 508-514.

Liu, Y., Lv, H., Ren, L., Xue, G., & Wang, Y. (2016). Improving the moisturizing properties of collagen film by surface grafting of chondroitin sulfate for corneal tissue engineering. *Journal of Biomaterials Science, Polymer Edition*, *27*(8), 758-772.

Liu, Y., Ren, L., & Wang, Y. (2013). Crosslinked collagen–gelatin–hyaluronic acid biomimetic film for cornea tissue engineering applications. *Materials Science and Engineering: C*, *33*(1), 196-201.

Liu, Y., Ren, L., & Wang, Y. (2014a). A novel collagen film with micro-rough surface structure for corneal epithelial repair fabricated by freeze drying technique. *Applied Surface Science*, *301*, 396-400.

Liu, Y., Ren, L., Long, K., Wang, L., & Wang, Y. (2014b). Preparation and characterization of a novel tobramycin-containing antibacterial collagen film for corneal tissue engineering. *Acta Biomaterialia*, *10*(1), 289-299.

Long, K., Liu, Y., Li, W., Wang, L., Liu, S., Wang, Y., Wang, Z., & Ren, L. (2015). Improving the mechanical properties of collagen-based membranes using silk fibroin for corneal tissue engineering. *Journal of Biomedical Materials Research Part A*, *103*(3), 1159-1168.

Ma, L., Changyou, G., Zhengwei, M., Jie Z., Jiacong, S., Xueqing, H., & Chunmao, H. (2003). Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. *Biomaterials, 24*(26), 4833-4841.

Mamat, N., Jaafar, M., & Hamid, Z. A. A. (2019). Surface modification of gentamicin-loaded polylactic acid (PLA) microsphere using double emulsion and solvent evaporation: Effect on protein adsorption and drug release behaviour. *Journal of Physical Science*, *30*, 109-124.

Mansoor, H., Ong, H. S., Riau, A. K., Stanzel, T. P., Mehta, J. S., & Yam, G. H. F. (2019). Current trends and future perspective of mesenchymal stem cells and exosomes in corneal diseases. *International Journal of Molecular Sciences*, *20*(12), 2853.

Meng, Z. X., Zheng, W., Li, L., & Zheng, Y. F. (2011). Fabrication, characterization and in vitro drug release behavior of electrospun PLGA/chitosan nanofibrous scaffold. *Materials Chemistry and Physics, 125*(3), 606-611.

Mhlanga, N., & Ray, S. S. (2015). Kinetic models for the release of the anticancer drug doxorubicin from biodegradable polylactide/metal oxide-based hybrids. *International Journal of Biological Macromolecules*, *72*, 1301-1307.

Naahidi, S., Jafari, M., Logan, M., Wang, Y., Yuan, Y., Bae, H., Dixon, B., & Chen, P. (2017). Biocompatibility of hydrogel-based scaffolds for tissue engineering applications. *Biotechnology Advances*, *35*(5), 530-544.

Nakagawa, M., Teraoka, F., Fujimoto, S., Hamada, Y., Kibayashi, H., & Takahashi, J. (2006). Improvement of cell adhesion on poly (L-lactide) by atmospheric plasma treatment. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 77*(1), 112-118.

Ospina-Orejarena, A., Vera-Graziano, R., Castillo-Ortega, M. M., Hinestroza, J. P., Rodriguez-Gonzalez, M., Palomares-Aguilera, L., Morales-Moctezuma, M., & Maciel-Cerda, A. (2016). Grafting collagen on poly (lactic acid) by a simple route to produce electrospun scaffolds, and their cell adhesion evaluation. *Tissue Engineering and Regenerative Medicine, 13*(4), 375-387.

Park, S. D., Todo, M., Arakawa, K., & Koganemaru, M. (2006). Effect of crystallinity and loading-rate on mode I fracture behavior of poly (lactic acid). *Polymer*, *47*(4), 1357-1363.

Razak, S. I. A., Dahli, F. N., Wahab, I. F., Abdul Kadir, M. R., Muhamad, I.I., Yusof, A. H. M., & Adeli, H. (2016). A conductive polylactic acid/polyaniline porous scaffold via freeze extraction for potential biomedical applications. *Soft Materials, 14*(2*),* 78-86.

Sangsen, Y., Benjakul, S., & Oungbho, K. (2012). Fabrication of novel shark collagen-pectin scaffolds for tissue engineering. In *The 4th 2011 Biomedical Engineering International Conference* (pp. 273-278). IEEE.

Serra, T., Ortiz-Hernandez, M., Engel, E., Planell, J. A., & Navarro, M. (2014). Relevance of PEG in PLA-based blends for tissue engineering 3D-printed scaffolds. *Materials Science and Engineering: C, 38,* 55-62.

Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: a comprehensive review. *Biotechnology Advances*, *26*(3), 246-265.

Singhvi, G., & Singh, M. (2011). In-vitro drug release characterization models. *International Journal of Pharmaceutical Studies and Research*, *2*(1), 77-84.

Sizeland, K. H., Edmonds, R. L., Basil-Jones, M. M., Kirby, N., Hawley, A., Mudie, S., & Haverkamp, R. G. (2015). Changes to collagen structure during leather processing. *Journal of Agricultural and Food Chemistry*, *63*(9), 2499-2505.

Tallawi, M., Rosellini, E., Barbani, N., Cascone, M. G., Rai, R., Saint-Pierre, G., & Boccaccini, A. R. (2015). Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering: a review. *Journal of the Royal Society Interface*, *12*(108), 20150254.

Umoren, S. A., Obot, I. B., Madhankumar, A., & Gasem, Z. M. (2015). Performance evaluation of pectin as ecofriendly corrosion inhibitor for X60 pipeline steel in acid medium: experimental and theoretical approaches. *Carbohydrate Polymers*, *124,* 280-291.

Walker, E. K. (2015). Surface modification of traditional and bioresorbable metallic implant materials for improved biocompatibility (Doctoral dissertation), Purdue University. https://docs.lib.purdue.edu/open_access_dissertations/582.

Wang, J., Nor Hidayah, Z., Razak, S. I. A., Kadir, M. R. A., Nayan, N. H. M., Li, Y., & Amin, K. A. M. (2019). Surface entrapment

of chitosan on 3D printed polylactic acid scaffold and its biomimetic growth of hydroxyapatite. *Composite Interfaces, 26*(5), 465-478.

Wang, S., Liu, W., Han, B., & Yang, L. (2009). Study on a hydroxypropyl chitosan–gelatin based scaffold for corneal stroma tissue engineering. *Applied Surface Science*, *255(*20), 8701-8705.

Yang, J., Bei, J., & Wang, S. (2002), Enhances cell affinity of poly(d, l-lactide) by combining plasma treatment with collagen anchorage. *Biomaterials, 23*, 2607-2614.

Yousaf, S., Keshel, S. H., Farzi, G. A., Momeni-Moghadam, M., Ahmadi, E. D., Asencio, I. O., Mozafari, M., & Sefat, F. (2019). Sacffolds for corneal tissue engineering. *In Handbook of Tissue Engineering Scaffolds: Volume Two,* 649-672, Woodhead Publishing.