

ORIGINAL ARTICLE

Synthesis and Characterization Scaffold Chitosan / Poly (ϵ -caprolactone) as Candidate for Skin Tissue Engineering in Burns

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ABSTRACT

Introduction: The development of skin tissue engineering as an alternative for burn therapy has advantages over biological dressing. Using Polycaprolactone (PCL) with chitosan in skin tissue engineering is expected to produce materials that meet the standards in skin tissue engineering. This research aims to study the variations of PCL in chitosan/PCL skin tissue engineering. **Methods:** From the Fourier-transform infrared spectroscopy (FTIR) results, there were no difference in functional groups between four chitosan/PCL samples with variations in PCL concentration. They were all non toxic due to cytotoxicity assay. **Results:** The addition of PCL concentration also decreases the samples degradation rate but increases the sample contact angle. **Conclusion:** Based on these results, chitosan/PCL composites can be product innovation for skin tissue engineering based on the characteristics of fiber size, tensile strength, degradation rate, and biocompatibility.

Keywords: Scaffold, PCL, Chitosan, product innovation, burn

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INTRODUCTION

Burns are damage to body tissue on the skin or other tissues caused by heat, radiation, radiocytes, electricity, or chemical (1). Skin damage due to burn trauma can disrupt the thermoregulatory, sensory, protective and metabolic functions of the skin (2). Damage to all skin tissue in cases of severe burns can result in infection due to the entry of microorganisms or bacteria that not only affect the skin, but also organs. Not only all type of burn can be heal by previous burn therapy especially in severe, deep and wide burn lesion. The recovery speed of the host cell can not compensate for the extent, depth and severity of the burn. In severe burns, it is necessary to use tissue engineering. Tissue engineering is a multidisciplinary field that utilizes biomaterials for the development of artificial tissues and organs to add, repair, and / or replace damaged and / or

diseased tissue (3). The utilization of tissue engineering for skin (epidermis and dermis) are more than for skin appendages/ adnexa (hair, nails, and glands) (4). In tissue engineering, one of the important factors is the structure of the scaffold, whose role is to fill the void of tissue, provide structural support to provide growth factors and provide cells with ability to form tissue in the body.

One of the scaffold fabrication methods is to use the electrospinning method which will produce fibers in micro or nano sizes. The advantage of using this method is the resulting scaffold with a structure that resembles an extracellular matrix (5). With this method, the fiber is very good and the ratio of surface area to volume is large to accommodate protein absorption thereby encouraging cell-matrix interaction. In addition, the diameter of each fiber, mechanical properties and scaffold porosity can be adjusted by setting electrospinning parameters (6). These characteristics support better cell growth in cell adhesion, cell expression, and nutrient transportation to cells so as to provide an optimal environment for new tissue growth.

Polycaprolactone-based nanofiber is often used in tissue engineering. Polycaprolactone is promptly refined into nanofibers using electrospinning, and their resulting web-like structures (i.e., the formation of electrospun nanofibers) makes them the excellent candidate for resembling the host extracellular matrix. Commonly, as-spun PCL nanofibers can possibly enlarge cell attachment, proliferation, and can advance cell penetration due to pore sizes, which are appropriate appearance that aid the growth of soft tissues (7). PCL has good mechanical properties, biodegradable, bioresorbable, and biocompatible for its application to the body making it one of the suitable materials in tissue engineering. However, in its application as a scaffold, PCL has a long degradation time and is less biocompatible when compared to natural polymers. For this reason, combination PCL and chitosan which has characteristic such as non-toxic, anti-bacterial, biocompatible, and biodegradable properties has often performed. Synthesis of PCL/ Chitosan scaffold can be performed using the electrospinning method to yield nanofiber. In some cases many beads were found in nanofiber based on PCL/Chitosan, so further research is needed to optimize the ratio composition of PCL / chitosan (8).

MATERIALS AND METHODS

Synthesis of Samples

Polycaprolactone (PCL) (Mn 80.000) was purchased from Sigma Aldrich, Singapore. Chitosan (DA 85%) was purchased from Bio Chitosan Indonesia. Chloroform, methanol, acetic acid glacial, and syringe purchased from Sumber Ilmiah Persada, Indonesia. All chemical were used as received. PCL solution of 8%, 8.5%, 9%, and 9.5% were prepared by dissolving 0.8 g, 0.85 g, 0.9 g, 0.95 g of PCL pellet in ten ml methanol/ chloroform mixture (70:30). For chitosan solution of 2%, dissolve 0.1 g of chitosan powder in 5 ml acetic acid 90%. Both solutions were stirred for three hour. After mixed well, blended PCL and chitosan into 8:2. All of the concentrations of Chitosan/PCL were electrospun at room temperature. The solution was delivered through a ten ml syringes with blunt ended 22-gauge needle. A DC of 27 kV and a distance of 18 cm was applied spinneret also all the sample solutions were spun at a rate of 0.3 ml/h.

Fourier-transform infrared spectroscopy (FTIR)

Chitosan and PCL functional groups in the Chitosan/PCL samples produced were tested using infrared spectroscopy using FTIR (Bruker Alpha II) with a range between 4000 - 400 cm^{-1} .

SEM Test

The morphology of Chitosan/PCL fiber produced was evaluated using SEM (Phenom Pro X Desktop). Samples attached to the aluminum foil are cut to the required

size and coated with gold and observed with various magnifications.

Tensile Strength Test

The tensile strength of Chitosan/PCL samples was tested using (Shimadzu AG-10TE) where the analyzed results were the results of ultimate tensile strength (UTS) produced by each sample.

Degradation Rate Test

Degradation that occurred in the sample was carried out using a Phosphate Buffer Saline (PBS) solution. Mass measurements were carried out periodically every four days, namely the 4th day, 8th day, 12th day, and 16th day. Alteration that can be observed are the gradual loss of material or thinning due to interactions with the environment.

MTT Assay for Toxicity

Chitosan/PCL samples were incubated for 72 hours after being given MTT reagents and then incubated for two hours. Then the measurement of OD (optical density) on the sample by using a wavelength of 550-600 nm.

Contact Angle Test

The contact angle of the Chitosan/PCL samples were observed by dripping water on the sample and the results of the photo were analyzed using ImageJ software.

Cell Adhesion Test

Cell adhesion of Chitosan/PCL samples were observed through culture cells three times at 24 hours, 48 hours, and 72 hours.

RESULT

Fourier-transform infrared spectroscopy (FTIR)

The FTIR Chitosan/PCL sample results have the same functional groups as existing theories where the spectra produced can be seen in Figure 1. Typical PCL function groups can be found at the peak of 1728 cm^{-1} which is a carbonyl group (C = O) as well as at the peaks of 2869 cm^{-1} and 2917 cm^{-1} representing symmetrical and asymmetrical C-O-C bonds (9). In chitosan, O-H bonds were found at the peak of 1260 cm^{-1} and N-H II bonds and N-H I bonds at 1585 cm^{-1} and 1639 cm^{-1} (10). The Chitosan/PCL scaffold is dominated by PCL functional groups because the composition of the scaffold is dominated by PCL. N-H groups were not found in samples with PCL composition 8% and 8.5% while for 9% composition only found N-H II bonds at 1578 cm^{-1} and N-H I bonds at 1618 cm^{-1} for 9.5% compositions.

Morphology SEM

SEM image results from Chitosan/PCL samples can be seen in Figure. 2. It appears that the fiber of the four samples formed is still in nano size (1-1000 nm) because the average diameter size is produced $<1\mu\text{m}$ (11). Pores

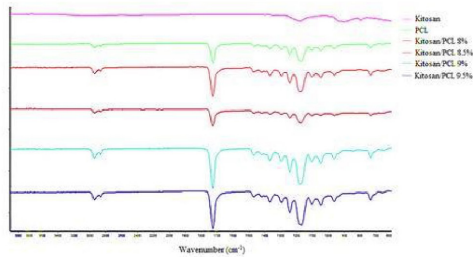


Figure 1: FTIR Spectra of (a) Chitosan (b) PCL (c) Chi/PCL 8% (d) Chi/PCL 8.5% (e) Chi/PCL 9% (f) Chi/PCL 9.5%

produced in Chitosan/PCL samples have a range of 9-16 μm .

Tensile Strength Test

The results of UTS for each Chitosan/PCL sample can be seen in Table I. The additional concentration of PCL in the sample is proportional to the UTS value produced by the Chitosan/PCL sample. The UTS results for each sample of the combination of chitosan and PCL have succeeded in providing a UTS value that has entered into the range of UTS values in human skin.

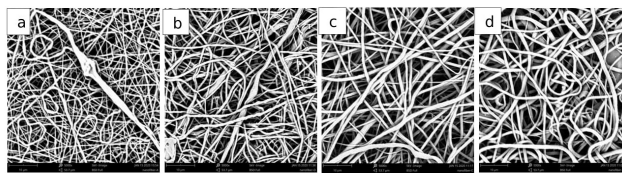


Figure 2: SEM Observation Sample (a) Chi/PCL 8% (b) Chi/PCL 8.5% (c) Chi/PCL 9% (d) Chi / PCL 9.5%

Table I. UTS Results of Chitosan/PCL Samples with Variation in PCL Concentration

Sample		Force (N)	Thick-ness (mm)	Wide (mm)	Sur-face Area (mm ²)	UTS (MPa)
Chi-tosan (b/v)	PCL (b/v)					
2%	8%	1	0.02	10	0.2	5
2%	8.5%	2	0.03	10	0.3	6.67
2%	9%	2	0.02	10	0.2	10
2%	9.5%	3	0.02	10	0.2	15

Degradation Rate Test

The degradation rate of the Chitosan/PCL sample can be seen in Figure 3. The more PCL concentration in the sample, the lower the percentage of mass lost. This is because PCL has a long degradation time, which is around 2-3 years (12). Extracellular Matrix (ECM) on the skin takes approximately 1 month to repair damaged tissue. The new skin tissue formed reaches 70-80% of good original tissue strength at the end of the third month and will continue to strengthen within a period of 12 months (13). Based on this, the results of Chitosan/PCL samples obtained were showed degradation rate

within the range of repairing damaged tissue, but still has not fulfilled its role in supporting the maturation of skin tissue until it has same strength as real skin tissue because the sample has been completely degraded within 1-5 months.

Biocompatibility Test

The percentage of cell Viability in Chitosan/PCL samples can be seen in Figure 4. Chitosan/PCL samples produced have nontoxic properties, this is indicated by the percentage of living cells that are above 60% (14).

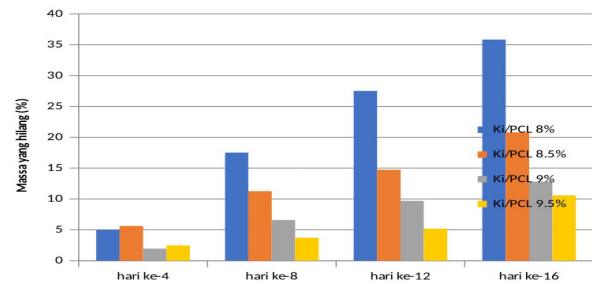


Figure 3: Graph of Percentage of Degraded Sample Mass

Contact Angle Test

The results of the contact angle analysis of Chitosan/PCL samples can be seen in Table II. It can be seen that the contact angles of the four samples have values above 90° which means the sample has hydrophobic properties. Even though the sample contained hydrophilic chitosan

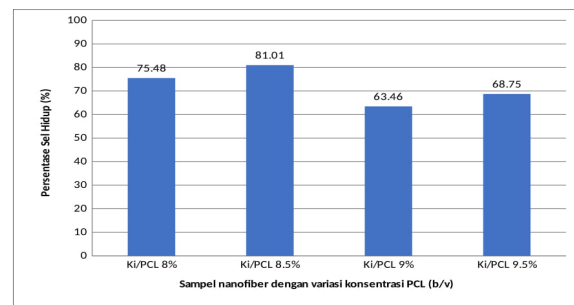


Figure 4: Graph Cell Viability Percentage of Chitosan/PCL Samples with Variation in PCL Concentration

content, the resulting characteristic was hydrophobic because the sample had a greater PCL concentration ratio than the chitosan concentration. Based on the results it can be seen that the contact angle produced by Chitosan/PCL samples is not optimal for skin tissue engineering applications where a good contact angle for the membrane surface is between 20 - 60° (15).

Cell Attachment Test

Adhesion of cells to Chitosan/PCL samples can be seen in Figure 5. From the figure it can be seen that the longer the time of observation, the more dead cells are marked, the more cells are black. Cell attachment due to the dominant sample will be PCL composition. In addition,

Table II. Contact Angle of Chitosan / PCL Sample with PCL Concentration Variations

Sample	Contact Angle (°)
Chitosan/PCL 8%	117.655
Chitosan/PCL 8.5%	120.646
Chitosan/PCL 9%	125.344
Chitosan/PCL 9.5%	127.977

the presence of methyl groups in the samples showed low cell attachment and cell distribution (16).

DISCUSSION

FTIR test is showed the chitosan/PCL sample has a functional group resulting from a combination of chitosan and PCL ingredients and does not make the tendency of

PCL samples have met the UTS range of human skin 5–30 MPa, which in accordance with Tran’s research (17). The chitosan/PCL samples are non-toxic because the percentage value of cell viability is >60% (14). Meanwhile, in the result of degradation test, they were shown that the decay time in the range of 1–5 months, and according research by Urbanek (13) have enough time to repair the injured tissue, but still not enough to strengthen the tissue back to its initial strength. PCL is an aliphatic polyester that has ability to be easily fabricated into various shapes, has good thermal stability and mechanical properties, biodegradable, bioresorbable, and biocompatible (8).The non-toxic, anti-bacterial, biocompatible, and biodegradable properties of chitosan make chitosan widely applied in medical biomaterial. Chitosan is easily processed and has a high potential for applications in tissue engineering (18). In its application as a scaffold, PCL has a long degradation time when compared to natural polymers. For this reason, mixing PCL with natural polymers such as chitosan has been carried out to make ideal scaffolds.

The chitosan /PCL samples produced were dominated by PCL characteristics rather than chitosan characteristics. This is because the PCL concentration in the sample is greater than chitosan, in addition, the PCL composition is also greater than chitosan in the sample solution, which is 8: 2. The addition of PCL concentration did not make the resulting absorption wave tendency to shift up or down. The PCL/chitosan scaffold is showed confirmation functional groups from both materials and did not result new functional groups.

The greater addition of PCL concentration, the greater the diameter and the pore of the fiber is obtained because it will increase the chain of bonds in the solution so that it will increase the viscosity of the solution (19). The pores produced in chitosan/PCL samples with various PCL concentrations ranged from 9-16 m. The resulting pores in this research are increased with the addition of PCL concentration. This research is in accordance with Zhang research at 2017 that showed the fiber diameter becomes larger due to the addition of PCL concentration which causes a widening of the distance between the fibers so that the resulting pore size will increase. The pore size of human skin is 25-120 μm (6). Pore size is related to the diameter of the fiber (20). This is due to the widening of interfiber space with the increasing diameter of the fiber produced (21). The UTS values are increases along with increasing PCL composition in the sample. This is because PCL has many methyl groups, where the methyl group has a role in providing a high UTS value and density of a sample (22). The interaction between PCL and chitosan is showed rise to a hydrogen bond between the carbonyl group PCL and chitosan, where this bond serves to increase the UTS sample from the combination of the two materials (23). Referring to the research (17) where the UTS value possessed in human skin is 5-30 MPa, the UTS results for all samples

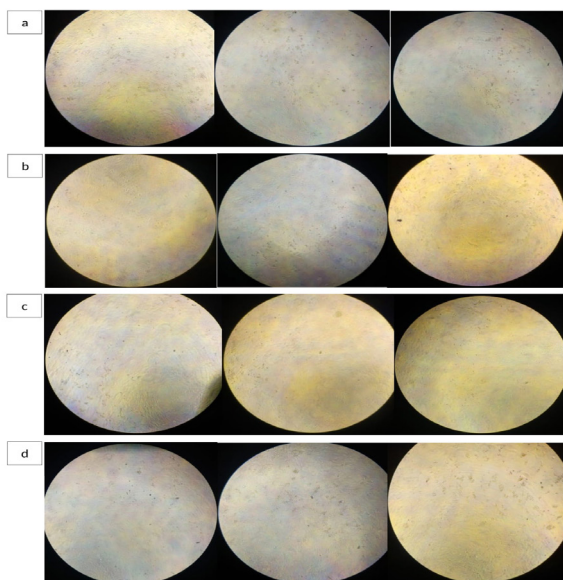


Figure 5: Observation of cells attachment for 24 hours, 48 hours, & 72 hours of samples (a) Ki/PCL 8% Ki/PCL 8.5 % Ki/PCL 9% (d) Ki/PCL 9.5%

the resulting absorption wave to shift up or down. This is in accordance with research conducted by Jana which states that the combination of these two constituent materials does not cause the emergence of new groups in the chitosan/PCL sample (10). No amino and carboxyl groups were found that could promote cell attachment to the resulting sample. This is also comparable to the results of the contact angle test where the contact angles of all chitosan/PCL samples were more than 90°, while from Ken research (15), the optimal contact angles for cells could adhere to samples in the range of 20–60°. The morphology produced by the four samples of chitosan / PCL had pore size in the 9–17 μm range where the size still did not resemble the pore size of human skin which had a 25–120 μm range (6). However, if it is seen from the tensile strength results, the chitosan/

composition of PCL/chitosan scaffold have succeeded in providing a UTS value that has entered into the range of UTS values in human skin.

In its application as a scaffold, chitosan / PCL samples must have a degradation rate that is not too fast to carry out its function so that cells can proliferate and not be too long to interfere with biological functions in the tissue. The degradation rate that is too long than the tissue regeneration can trigger a Foreign Body Reaction due to the presence of scaffold residues and cause unwanted reactions. The more PCL concentration in the sample, the lower the percentage of mass lost. The length of time of PCL degradation is due to the content of five methylene groups which provide hydrophobic characteristics so that liquid is difficult to enter the space in PCL (24). The PBS liquid can only stick to the surface of the sample to cause the cutting of polymer chains only occurs on the surface of the sample. The sample appears to be thinning slowly (surface degradation) instead of being degraded in bulk (bulk degradation) (8).

Both PCL and chitosan are non-toxic materials (25, 26). Fluctuations in the toxicity results because chitosan/PCL samples cannot dissolve with culture media, remarkably it must be washed repeatedly which results in the reduction in number of cells. The PCL has a five-carbon chain between its ester (27). Carbon has non-polar properties and the greater the amount of carbon, the more non-polar a molecule will be. This longer five-carbon unit gives PCL its hydrophobic properties. This is comparable to the results of the chitosan / PCL sample, the higher the PCL concentration in the sample, the greater the value of the contact angle or in other words the smaller the wettability (28). This contact angle result affecting the low cell adherence to the chitosan / PCL samples. Due to the hydrophobic nature of PCL, it difficult for cells to adhere to the sample surface (29). It was found that a good contact angle for adhering cells was in the range of 20-60° (30). If you look at contact angle results of chitosan / PCL sample which produces an angle of > 90°, this means that the contact angle of the chitosan / PCL sample is not suitable for cells to stick to the sample. Cell attachment is the initial step in a cascade of cell-biomaterial interactions, and is important to cellular processes such as cell guidance, proliferation, and differentiation (31). The proliferation and differentiation is the sequence of burn healing (32). Morphology with the parameter pore size has correlation with the space for cell infiltration which support healing. Mechanical property (tensile strength) has correlation with the integrity of scaffold to support healing, the biocompatibility test (cell viability) as the confirmatory test / requirement for clinical application, degradation test has function to ensure that the material must be dissolved after running its function in the host and its residue are not harmful for the host (and do not stimulate foreign body reaction).

CONCLUSION

The chitosan/PCL samples are yielded good fibers with smooth surface, no beads, diameters and pore sizes in nano size. All samples are had Ultimate Tensile Strength (UTS) value in the range of human skin UTS standard. The UTS values are increased along with the enhancement of PCL composition in samples. The chitosan / PCL samples were non toxic and were estimated to be completely degraded in the monthly range due to hydrophilic properties of chitosan which can increase the wettability and permeability of the sample, can accelerate the hydrolytic degradation of samples with PCL content. The composition of PCL and chitosan need to be optimized in order to improve the hydrophilicity and the value of cell attachment. Finding an valid ratio composition of PCL/Chitosan is still a challenge and we still fight from obtaining the ideal physical, mechanical, chemical, biological properties. Doing further research and performed optimization in the formulation of material is important. A wide range of experiments and examinations should be done to verify the quality of skin scaffold.

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