**EVALUATION OF SURFACE MODIFIED PLLA-COLLAGEN HOLLOW FIBER AS VASCULAR GRAFT**

**ABSTRACT**

*Cardiovascular disease as the first silent killer in the world is related with atherosclerosis plaque. Coronary artery bypass grafting as a gold standard for the severe atherosclerosis need alternative materials for replace the narrowed artery due to plaque. PLLA is one of biodegradable polymer which is suitable for the vascular graft but some of its weakness should be addressed. The aim of this research is to improving PLLA characteristics for a vascular graft candidate by using collagen and chitosan. PLLA was blended with collagen and formed into hollow fiber using spinneret apparatus. The hollow fiber was then coated with chitosan. The concentration of chitosan was varied between 0.05%-0.15% (wt/v). The result showed that chitosan can improve the biological characteristics of hollow fiber PLLA-collagen, non-toxic and non-hemolytic.*

**Keywords:** *Vascular Graft; PLLA; Collagen, Chitosan, Hollow Fiber*

**ABSTRAK**

*Penyakit kardiovaskuler sebagai pembunuh pertama di dunia dikaitkan dengan plak aterosklerosis. Prosedur operasi bypass arteri koroner sebagai standard solusi untuk aterosklerosis yang parah memerlukan material alternatif untuk menggantikan pembuluh darah yang menyempit. PLLA sebagai salah satu polimer dengan sifat biodegradasi sangat cocok untuk cangkok arteri, namun beberapa kekurangan dari PLLA perlu diatasi. Penelitian ini bertujuan untuk meningkatkan karakteristik PLLA untuk kandidat cangkok arteri dengan menggunakan kolagen dan kitosan. PLLA dicampurkan dengan kolagen dan dibentuk menjadi hollow fiber menggunakan spinneret. Hollow fiber yang terbentuk dilapisi dengan kitosan. Konsentrasi kitosan yang digunakan bervariasi antara 0.05%-0.15% (wt/v). hasil penelitian ini menunjukkan bahwa adanya kitosan dapat meningkatkan karakteristik biologi dari hollow fiber PLLA-kolagen, tidak toksik dan tidak hemolitik.*

***Keywords:*** *Vaskuler; PLLA; Kolagen; Kitosan; Hollow Fiber*

## INTRODUCTION

Cardiovascular disease (CVD) is the first silent killer in the worldwide. Even though it has declined on the last 25 years but it is still being the first killer [1,2]. It has been predicted that 45% adult population in US will be CVD sufferer in 2035 [3]. CVD is related with coronary atherosclerosis plaque. Atherosclerosis is a condition where the blood vessel narrowing due to plaque [4]. The most common effective treatment for severe atherosclerosis is coronary artery bypass grafting (CABG) [5]. CABG as gold standard is a surgery for replacing narrowed artery with new vessels. The new vessels for CABG usually comes from autologous vessels, such as saphenous vein and internal thoracic artery. Unfortunately, the viability of the source is limited, require invasive harvest and often unsuitable for use [6, 7]. Due to some limitations for the autologous vessels, synthetic vascular graft is needed as an alternative [6].

Synthetic vascular graft should be made of materials which has good characteristics, such as, hemocompatible, non-toxic, non-thrombogenic and good mechanical properties. Polymer is one of materials that has been used for vascular graft. One kind of polymers that suitable for vascular graft is poly L-lactic acid (PLLA). PLLA is a common biodegradable polymer for synthetic vascular graft [8]. PLLA has degradation times around 6-12 months [9]. Porous PLLA nano-fiber scaffold is very potential for vascular graft tissue engineering [10]. PLLA can be accepted on human body due to the presence of lactic acid. The degradation product of PLLA is able to enter the Kreb’s cycle. Degradation process of PLLA due to hydrolytic mechanism. It is started with diffusion process of body fluid into materials and cut the chain off on the oligomer [11]. Unfortunately, PLLA has hydrophobic characteristics which leads to thrombogenecity. Therefore, modification is needed to improve the characteristics of PLLA.

The aim of this research is improving the characteristics of PLLA using chitosan and collagen. Collagen as the main component on extracellular matrix will enhance the cell adhesion and also increase the elasticity [12]. Collagen also has a play role on promoting the formation of endothelial cell [13]. Collagen has good tensile strength therefor important component for ligament, tendon and also responsible for skin elasticity. Degradation rate of collagen can be controlled using enzymatic pre-treatment or crosslinking.

Chitosan were added on the surface to cover the hydrophobic surface of the PLLA. Chitosan will induced the cell proliferation and minimized the foreign body reaction. Chitosan as natural cationic can act as a buffer to maintain the PLLA while degradation process [14]. The structure of chitosan is similar with glycosaminoglycans (GAGs) in mamalia which can be found many on the cell surface and matrix extracellular [15]. Chitosan on the surface of materials will support cell attachment and proliferation [16, 17]. Chitosan is potential for tissue regeneration due to its biocompatible, biodegradable and bioabsorbable. Chitosan also increase the production of transforming-beta 1 (TGF 1) and platelet-derived growth factor (PDGF) [18].

# METHODS

## Hollow Fiber Fabrication

Hollow fiber was made of PLLA (Mw 180kDa) and collagen. PLLA was dissolved on chloroform-toluene (5:1). 20% PLLA solution was blended with 1% collagen solution (2:1) using magnetic stirrer in room temperature. It was formed into hollow fiber using spinneret apparatus (figure 1). Alcohol was used as coagulant.

The next process was coating hollow fiber with chitosan. Chitosan was dissolved on 1% acetate acid and added 1N NaOH to get 6 of pH. There were five chitosan concentration, 0.05%; 0.075%; 0.1%; 0.125% and 0.15% (wt/v). Hollow fiber was dipped into chitosan solution for 30 minutes. The last process of the coated hollow fiber was freeze drying.



Figure 1. Spinneret apparatus scheme

## Cytotoxicity Test

Cytotoxicity testing is a mandatory test for biomaterials. MTT assay was used on this research to assess the cytotoxicity characteristics of hollow fiber. The confluent BHK-21 cell culture from cell line was harvested by using 0.25% trypsine versene solution. Afterwards, those cells were moved to 96-microwell plate. The sterilized samples were put into well plates. Those well plates were incubated for 4 hours with temperature at 37oC. The next step was removing samples from the well plates and added 50μL on each well. At the end, the optical density of formazan crystals on 96-microwell plate was monitored by ELISA reader with 630λ [20]. The living cells percentage can be known by following equation:

$$\% Living Cells= \frac{OD\_{treatment}+OD\_{media}}{OD\_{cell}+OD\_{media}}×100\%$$

## Hemolytic Test

Hemolytic testing is a mandatory test for biomaterials which contact with blood. This test was using anti coagulated human blood. 200μL blood was diluted on 10mL of 0.9% saline as a negative control and 200μL blood was diluted on 10mL aquades as a positive control. Samples were put into microtube with 200μL of blood-saline and incubated for 2 hours on waterbath with temperature at 37oC. Afterwards, samples were removed and the blood was centrifuged with 3000 rpm for 10 minutes. The supernatant was taken from microtube to assess the absorbance using UV-Vis spectrophotometry with 490nm of wavelength. This absorbance can be used for calculate the hemolytic percentage by using following equation (21):

$$\%Hemolytic= \frac{A\_{sample}-A\_{negative control}}{A\_{positive control}}×100\%$$

# RESULTS AND DISCUSSION

 Hollow fiber was successfully formed from the mixture of PLLA-Collagen using spinneret apparatus (figure 2). These hollow fiber has smooth white surface with diameter around 2.225-2.955 mm. Based on the size of diameter, the vascular graft suitable for arterial coronary replacement [22]. The thickness of vascular graft also measure using scanning electron microscope (SEM). The result showed that the thickness is between 90.65 – 236.26μm (figure 3). This wall was flimsy, far from the vascular wall thickness that should be around 1.52-1.89mm. The thickness of vascular graft wall was determined by the diameter of the outlet of the spinneret and the air gap between the spinneret hole and the coagulant pool [21].



Figure 2. Hollow fiber PLLA-Collagen



Figure 3. Cross section of hollow fiber PLLA-Collagen from Scanning Electron Microscope (SEM) with 80 magnitude.

 The pore size of vascular graft was between 4.525-18.06μm. The porous on hollow fiber is affected by the composition of the solution, hydrogen bond and temperature [23]. The increase of chitosan concentration would fill the macro-porous on PLLA with low density. Pore size has a pivotal role on vascular graft. It is able to affect the cell adhesion which is a key role on the vascularization [24]. The best size of pore on vascular graft is 5-45μm. By this size, the endothelial cell can grow better [25].

 Cytotoxicity of vascular graft with different concentration of chitosan coating was evaluated on BHK-21 cell culture. The optical density (OD) of formazan crystal and cell viability can be seen on table 1. The percentage of the living cell for all groups was more than 50%. It means that the vascular graft from all groups are non-toxic. High optical density value indicates high cell viability in case of MTT Assay. This OD show the concentration of formazan crystal. Living cell absorbed MTT and broke it down through reduction reaction using reductase enzyme on mitochondria respiration chain [26].

Table 1. The Result of Cytotoxicity Test

|  |  |  |
| --- | --- | --- |
| Group | Optical Density Value | Cell Viability (%) |
| Control | 0.383+0.027 | 96.03% |
| Sample 1 | 0.344+0.025 | 88.10% |
| Sample 2 | 0.361+0.047 | 91.56% |
| Sample 3 | 0.387+0.012 | 96.85% |
| Sample 4 | 0.342+0.037 | 87.69% |
| Sample 5 | 0.329+0.042 | 85.05% |

 Hemocompatibility is one of the crucial characteristics for blood contacted materials. It is determined by using hemolytic test. Hemolytic is a condition where erythrocytes damage due to the disruption of membrane cell integrity. The principle of hemolytic test is measuring hemoglobin level due to the destruction of red blood cell when contact with foreign materials [27].

Table 2. The Result of Hemolytic Test

|  |  |  |
| --- | --- | --- |
| Group | Absorbance Average | Hemolytic Percentage (%) |
| Control | 0.0503+0.004 | 4.24% |
| Sample 1 | 0.0453+0.003 | 3.29% |
| Sample 2 | 0.0447+0.002 | 3.15% |
| Sample 3 | 0.0420+0.001 | 2.61% |
| Sample 4 | 0.0413+0.002 | 2.45% |
| Sample 5 | 0.0380+0.001 | 1.57% |

The results showed that hemolytic percentage from all groups were under 5%. It means that all of the vascular graft were safe to contact with blood [28]. The highest hemolytic percentage was sample 1 and the lowest was sample 5. The percentage of hemolytic was getting smaller along the increase of chitosan concentration. It proves that chitosan is able to improve the hemocompatibility of the PLLA-collagen hollow fiber. Chitosan on the surface of hollow fiber will decrease the shear stress between blood and surface therefore suppress the hemolytic [29].

**CONCLUSION**

This research was successfully made hollow fiber PLLA-collagen using spinneret apparatus and covered surface with chitosan. It was showed that all samples from all groups has good biological characteristics based on the result of cytotoxicity and hemolytic test. The hemolytic percentage was decrease as an increase of chitosan concentration. It can be concluded that chitosan can enhance the hemocompatibility of the materials which is pivotal for vascular graft candidate. Further research is still needed to evaluate the effect of chitosan concentration against endothelialization process.

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