

Electrospun Collagen-based Scaffold as Therapeutic Agent for Ocular Chemical Injury

N A F Hasbiyani¹, D Hikmawati² and Siswanto²

¹Biomedical Engineering, Physic Department, Airlangga University

²Physics Department, Airlangga University

Email: nurulawali14@gmail.com

Abstract: Ocular chemical injury hit on cornea and conjunctiva due to chemical contact. This injury damages to the epithelial surface of the eye, cornea, anterior segment and permanent unilateral or bilateral permanent viscous damage). Nowadays, human amniotic membrane, allograft biomaterials is used as the treatment for the injury. However, this method has many obstacles. Collagen-based Scaffold is a new study of electrospinning membrane made of collagen, hyaluronic acid, and polyethylene oxide. This research is aimed to obtain the optimum concentration of polymer solution that produces the best morphology in the formation of electrospun collagen-based scaffold. The result show that the fiber had mean diameter of 212,63 nm to 500,8 nm and mean pore diameter 498,56 until 2788 nm. FTIR test shows the loss of functional groups Amide A and Amide II indicate successful crosslinking process with glutaraldehyde. The cytotoxicity test showed electrospun collagen-based scaffold was nontoxic.

Keywords: collagen, corneal injury, electrospinning, PEO, scaffold

1. Introduction

Chemical substances both acids and bases that contact the eyeball can cause injury called ocular chemical injury. This injury is one of the emergency ophthalmology conditions because it can damage the limbal, and causing deficiency of limbal stem cells. Furthermore, corneal epithelial cells have difficulty regenerating and causing corneal brushing which decreases the eye's ability to see [1]. In certain cases, chemical burned cornea induces eye blindness due to complete eyeball damage [2].

Nowadays, human amniotic membrane, allograft biomaterials is used as the treatment for the injury. It can repair epithelial layer and decrease scar formation [3]. However, this method has many obstacles such as: risk of contamination, transmission of infectious diseases, and biological variables between donor tissues [4]. In addition, the availability of amniotic membrane donors is relatively limited and only provided by certain hospitals. Current technological developments have produced



collagen-based three-dimensional corneal tissue engineering that able to help corneal epithelialization in the second week after the eventual phase [5].

There are many methods in manufacturing three-dimensional corneal tissue engineering. Biomimetic membrane is a matrix for the construction of corneal tissue. The membrane can be manufactured of nanofiber matrices and micro fibrous scaffolds [6]. Thus, nanofiber scaffold can be produced by electrospinning technique.

Ye [7] in their research have succeeded in synthesizing Collagen / HA / PEO scaffold by electrospinning method. In that study, Collagen / HA / PEO scaffold was crosslinked with glutaraldehyde has nanofiber diameter ranging from 79 nm produced from a solution of 10 wt%. Research on variations in solution concentration needs to be done to determine the effect on the diameter of fiber electrospun collagen-based scaffold. This is an attempt to obtain scaffold with the best morphological characteristics in the form of fiber diameter and pore size, as a condition for attaching corneal epithelial cells.

In this study a variation of the concentration of polymer solution was 8 wt%, 9 wt%, 10 wt%, 11 wt%, and 12 wt%. Characterization was carried out to determine the physical-chemical properties of the material including SEM observation, FTIR assay, and cytotoxicity assay.

2. Experimental Method

2.1. Materials

The materials involved in this study were collagen type 1 (from fish), hyaluronic acid (HA), Polyethylene Oxide (PEO) with molecular weight 600 kDa, methanol, DI water, aquades, and glutaraldehyde.

2.2. Methods

Collagen, HA and PEO were dissolved in 80% methanol with different blend ratios to optimize the electrospinning conditions. A blend mixture of collagen, HA and PEO (80/5/15 weight ratio, 100 mg) was dissolved in acetic acid (80% v/v) at a concentration of 8, 9, 10, 11, and 12 wt%. The solution was gently shaken for 3 h by magnetic stirrer at room temperature. The solution used for electrospinning by the parameters as follow: distance between the syringe and the collector was 17 cm, the voltage was 8.5 kV, the fluid flow velocity was 20 μ l / minute, and the size of the syringe needle is 18 gauge.

The electrospun scaffold then crosslinked with 25% glutaraldehyde steam in a closed beaker glass. The crosslinking process was carried out for 24 hours at room temperature.

3. Results and Discussion

3.1. SEM Observation

The microstructure of electrospun collagen-based scaffold was characterized using Scanning Electron Microscope (SEM) Phenom Pro X Dextop with EDX. Figure 1 shows the fiber of uncrosslink electrospun collagen-based scaffold has good morphological appearance: uniform shape, smooth surface, and did not form any beads. The image processing software Image J was used to determine the average diameter of the fibers and at least 100 fibers were assessed.

Based on SEM observation data and graphic of the fiber distribution in various concentration of the solution of 8%, 9%, 10%, 11%, and 12%, an increase in solution concentration has an impact on increasing the average diameter of fiber electrospun collagen-based scaffold. The increase in the average diameter of fiber electrospun collagen-based scaffold is listed in Figure 2.

Increasing fiber diameter and uniformity of diameter are associated with an increase in solution concentration (other electrospinning parameters are constant) and the viscosity of the solution. The solution's viscosity is related to the polymer chain molecules that are bound in a solution. Increasing polymer chain bonds due to an increase in the number of polymer molecules induce viscosity increasing [8]. Thus, chain entanglement between the polymers chains increase too. The chain attachment overcomes surface tension and allows solvent molecules to be distributed over polymer molecules bound and ultimately results in producing smooth and uniform nano electrospun fibers [9].

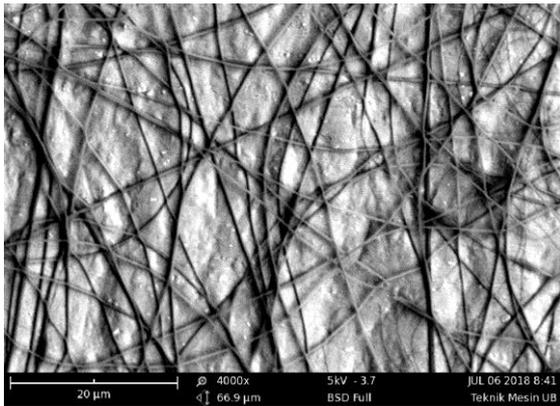


Figure 1. SEM observation of sample 8%

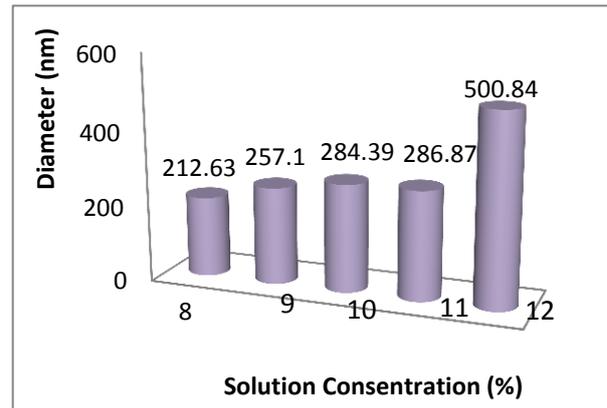


Figure 2. Fiber diameter graphic

Furthermore, Figure 3, 5, 7, 9, and 11 show successfully crosslinked electrospun collagen-based scaffold solution concentration 8, 9, 10, 11, and 12%. There are no beads or fibers because all the fibers have merged and formed pores. Then an analysis was carried out by ImageJ software as seen on Figure 4, 6, 8, 10, and 12 shows the histogram of pore diameter electrospun collagen-based scaffold solution concentration 8, 9, 10, 11, and 12%.

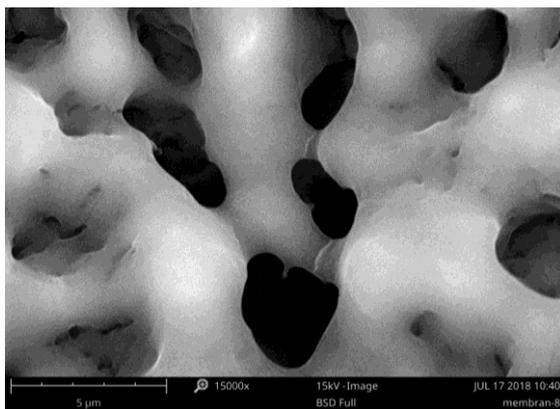


Figure 3. SEM observation of crosslinked sample 8%

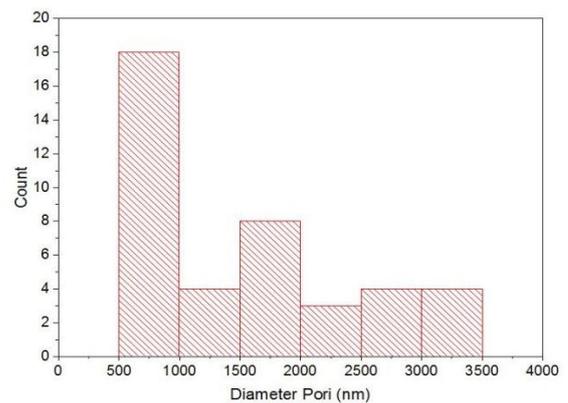


Figure 4. Graphic of pore diameter sample 8%

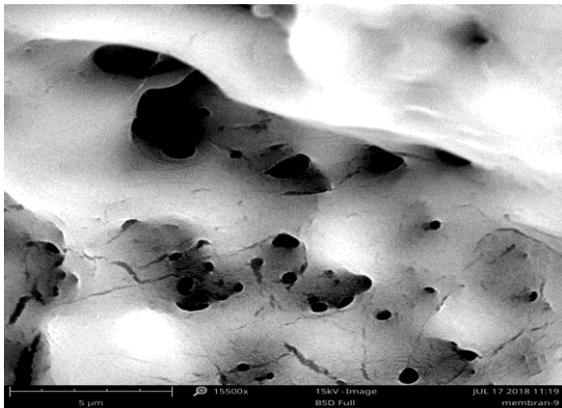


Figure 5. SEM observation of crosslinked sample 9%

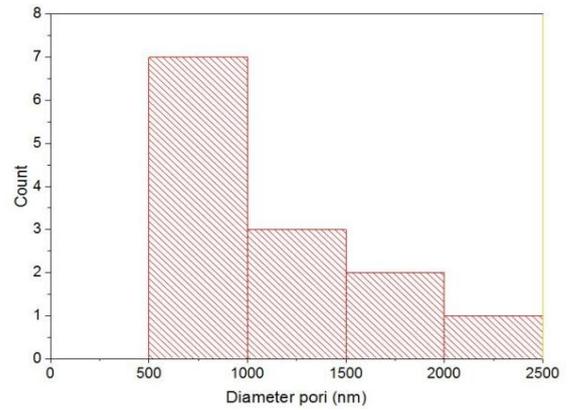


Figure 6. Graphic of pore diameter sample 9%

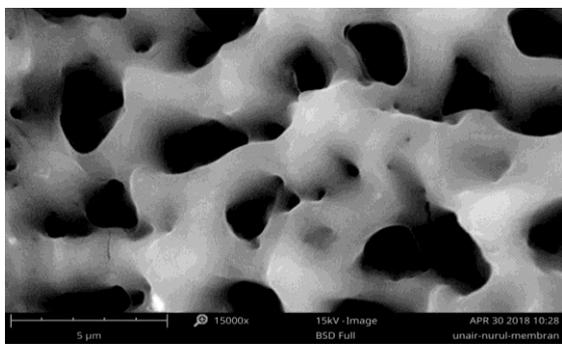


Figure 7. SEM observation of crosslinked sample 10%

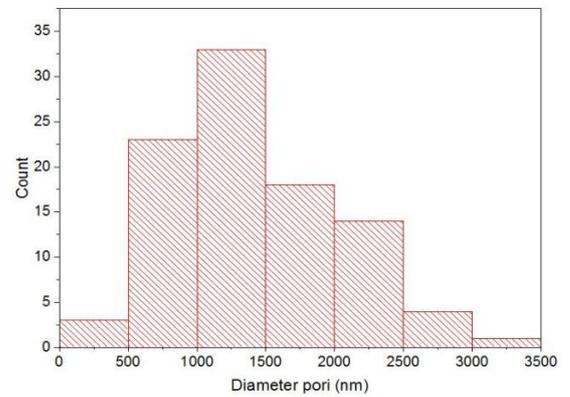


Figure 8. Graphic of pore diameter sample 10%

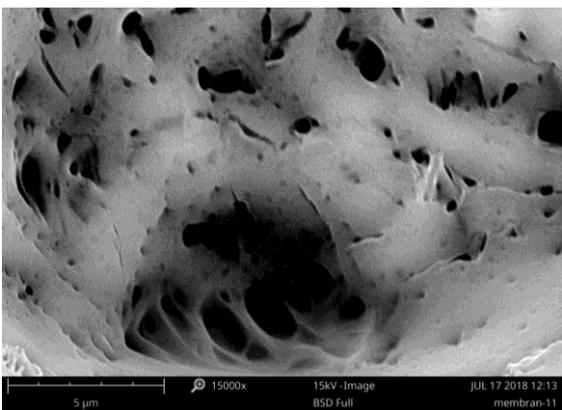


Figure 9. SEM observation of crosslinked sample 11%

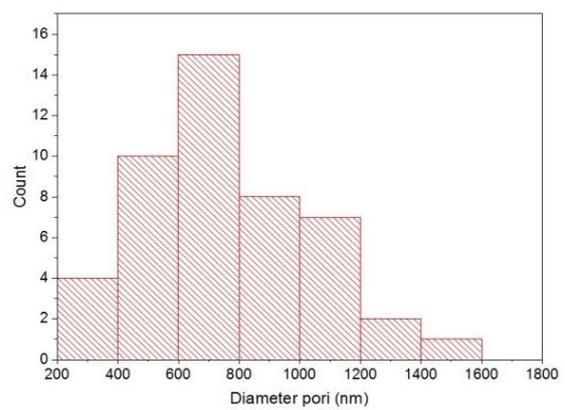


Figure 10. Graphic of pore diameter sample 11%

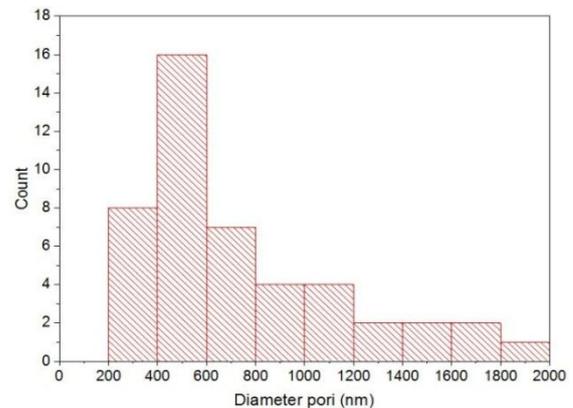
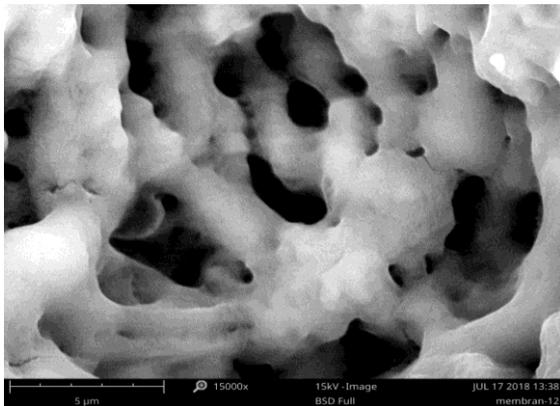


Figure 11. SEM observation of crosslinked sample 12%

Figure 12. Graphic of pore diameter sample 12%

Based on the data above, we analyzed the mean diameter pore and porosity of electrospun collagen-based scaffold as seen in table 1 below.

Table 1. Image J analysis result

Solution Concentration (%)	Mean Diameter (nm)	Porosity (%)
8	2.788,73 ± 343	26,97
9	989 ± 8,46	19,334
10	1.266 ± 97,8	44,11
11	707.42 ± 38.33	17,64
12	498,56 ± 20,22	26,71%

Electrospun collagen-based scaffold has a trabecular pore shape with a mean pore ranging from 498.56 nm to 2,788 nm. It is included in the macro pore range (pore diameter > 50 nm) [10]. Certain pore diameter sizes are needed by corneal epithelial cells to grow optimally.

According to Fitton at all [11] corneal epithelial tissue can grow in scaffold which has pore diameter size of 0.45 - 0.8 μm, but optimal at a size of 0.22 μm. Corneal epithelial tissue cannot grow on scaffold with a pore diameter above 0.8 μm. Karuri's research [12] showed more attachment of corneal epithelial cells on a pore diameter 400 nm compared to bigger pore diameter. Increasing scaffold pore diameter inhibits corneal epithelial cell growth. This is occurring due to cell growth does not spread, but the cell moves downward and is entangled in the pore. Fitton also stated that other factors that support corneal epithelial cell growth are porosity. Scaffold with porosity of 26-35% can support corneal epithelial cell growth.

3.2. IR Spectra Analysis by Fourier Transform Infra-Red

Infrared (IR) spectra for the electrospun collagen-based scaffold measured on Fourier *Transform Infra-Red* (FTIR) Spektrofotometer IR Perkin Elmer. Those of pure collagen, HA, PEO and glutaraldehyde were also tested (data not shown). The assessment by FTIR spectroscopy is presented in Figure 13. The IR spectra of electrospun collagen-based scaffold data show a reduction on functional group Amide A and Amide II. This indicates successful of crosslinking process with glutaraldehyde. NH stretching groups in Amide A and Amide II were involved in hydrogen bond formation during the crosslinking process [13].

Typical collagen functional groups are Amide B, Amide I, and Amide III. Amide B has wave number absorption in 2923.68 cm^{-1} which is CH₂ stretching. Amide I have wave number absorption in 1642.74 cm^{-1} C=O stretching and hydrogen bonding. Amide III absorbs wave numbers 1242.65 cm^{-1} C-N stretching and N-H deformation [14].

Electrospun collagen-based scaffold sample show some functional groups which indicates the presence of hyaluronic acid (HA), i.e. wave number absorption of 3464.56 cm^{-1} which is a hydroxyl O-H functional group. At the wave number 2923.56 cm^{-1} a CH₂ stretching functional group was found which overlapped with Amide B collagen. Absorption of wave number 1148.67 cm^{-1} is a group of C-N stretching amine functional groups [15].

The tested sample also found eight functional groups that indicate polyethylene oxide (PEO) material. On wave number absorption 2891.57 cm^{-1} , the C-H stretching functional group was found. And on wave number absorption 1453.60 cm^{-1} which is a CH₂ scissoring function group. Wave numbers at wavenumbers of 1359.68 cm^{-1} were also found to be CH₂ wagging functional groups. The absorption peak of the CH₂ twisting group is at wave number absorption 1280.66 cm^{-1} . Absorption of wave number 1148.67 cm^{-1} indicates the presence of a stretching C-O-C functional group. In addition, wave number absorption 961.71 cm^{-1} also appears, namely the C-O-C rocking functional group. The CH₂ rocking function group is shown by wave number absorption peak 843.73 cm^{-1} . On wave number absorption 564.64 cm^{-1} , a C-O-C bending functional group was found.

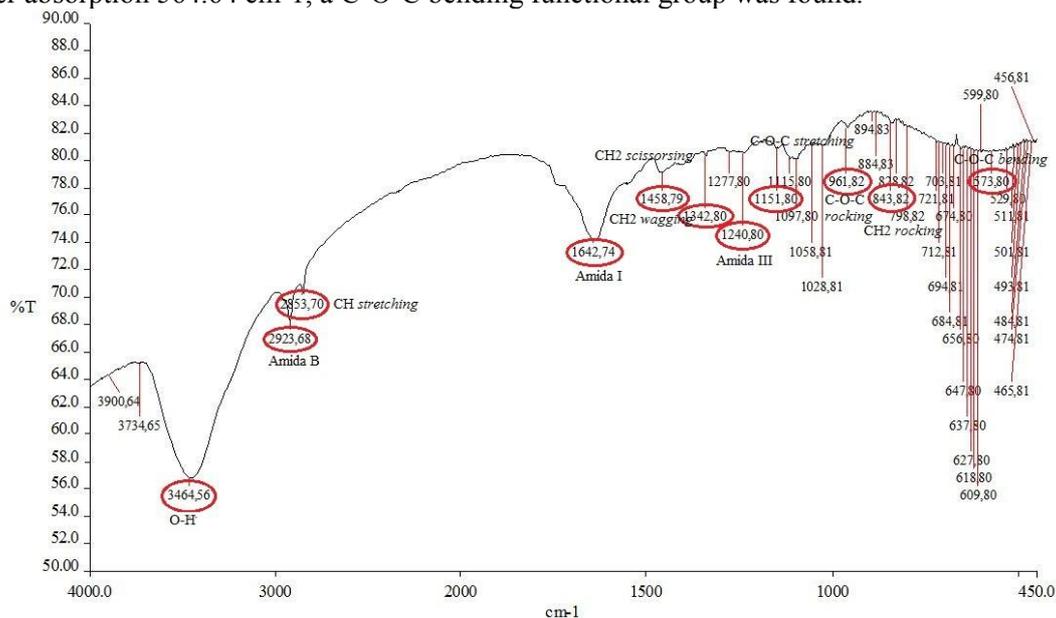


Figure 13. Electrospun collagen-based scaffold IR spectrum

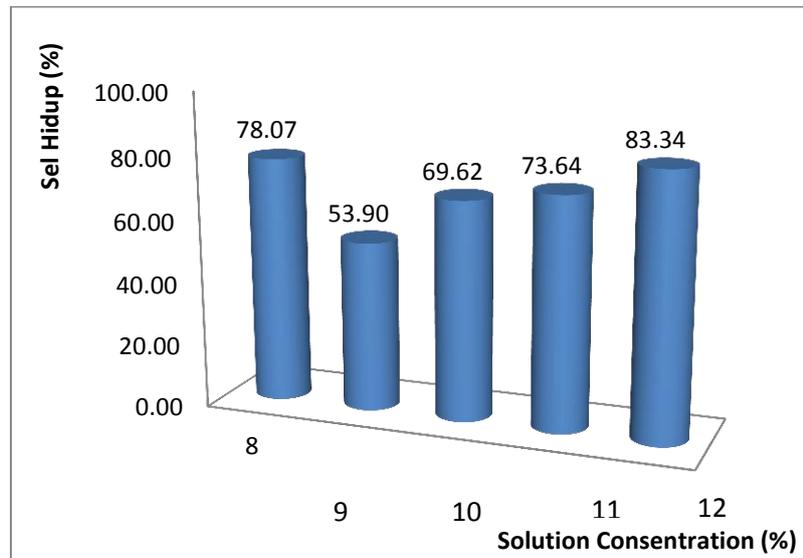


Figure 14. Cytotoxicity test graphic

3.3. Cytotoxicity Assay

Cytotoxicity tests were carried out to determine toxicity of a material that can be seen from the growth rate, proliferation and differentiation of cells. Baby Hamster Kidney fibroblast cells (BHK-21) were given MTT reagents to observe live cell presentations in the sample.

Percentage of viability of living cells in each sample has ununiformed score. Electrospun collagen-based scaffold which has the biggest percentage of living cells has the smallest average pore diameter and the distribution of pores is evenly distributed, so the scaffold has a growing space for cells the most extensive. This causes more cells to live on surface scaffold.

The five samples show values that are above the toxicity standard sample. This is indicated by the percentage of living cells from the five samples shows values above 50% with a range of percentage of living cells, namely between 53.90% - 83.34%. According to the literature the value is in accordance with what is expected, because the sample is said to be non-toxic when the percentage of living cells is above 50% [16]. These results indicate the value of cell viability at above 50% because the material used is fish collagen which is the main constituent is a fibril protein found in all organisms multicellular. Collagen is biocompatible and does not cause a reaction humoral immunity. The second ingredient is hyaluronic acid which is carbohydrate the type of mucopolysaccharide that is naturally found in all living things. The third ingredient, polyethylene oxide (PEO), is a polymer biocompatible because it is non-toxic and non-irritant. Addition Glutaraldehyde 25% as a crosslinker has been shown not to cause toxic properties because the sample is washed with deionized water to remove the residue glutaraldehyde which is not crossed. So that it can be stated that electrospun collagen-based scaffold is safe to use as candidate therapeutic agent for ocular chemical injury.

4. Conclusion

The electrospun collagen-based scaffold was successfully synthesized in 80% methanol. The microstructure of electrospun collagen-based scaffold show that it has pore diameter of 498.56 to 2788 nm. IR spectra analysis shows losing of functional groups Amide A and Amide II. This indicates the succeed of crosslinking with glutaraldehyde. Cytotoxicity assay shows that electrospun collagen-based scaffold was not toxic by the percentage of living cells above 50%. Optimal concentration of polymer solutions that produce the best morphology in the formation of electrospun collagen-based scaffold is 12%.

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