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Nonporous Chitosan/Collagen Scaffold for Skin Tissue Engineering

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Abstract. This study aimed to determine film characteristics of chitosan/collagen scaffold for tissue engineering applications. Scaffold prepared using freeze drying method. Surface structure and biological testing chitosan/collagen scaffold crosslinking reagent addition Glutaraldehide studied using Scanning Electron Microscopy test (SEM) and Microscope inverted. Variations in the ratio of chitosan/collagen (10:0, 8:2, 7:3, 6:4, 5:5 4:6, 3:7, 2:8, 0:10), and treated with a crosslinking reagent 0.25% of Glutaraldehyde (GA) of the total weight of the polymer. The next process dissolving and mixing, followed by printing in glass moulds (7,5x7,5) with a thickness of 5 mm. This was followed by the freezing and drying with a freeze dryer. Scaffold chitosan/collagen ratio of 80:20 and a concentration of 0.25% GA showed growth of human skin fibroblast cells most and nonporous surface structure. This study is part of a study of the processing of chitosan/collagen scaffold for applications in tissue engineering.
Keywords: Scaffold, chitosan, collagen, crosslinking, tissue engineering, skin

Introduction

One important factor in skin tissue engineering is the structure of the scaffold, which is a necessary or a matrix for cell infiltration and physical support of cells that lead to cell proliferation and differentiation into functional tissues or organs of human. A three-dimensional scaffold or an analogue called Extra Cellular Matrix (ECM), serves as an ideal scaffold used for tissue engineering, and must have excellent biocompatibility characteristics, the corresponding microstructure, such as 100-200 μm (pore size pores and porosity above 90%), control of biodegradability and suitable mechanical properties.

Chitosan, an amino polysaccharide (poly-1,4-D-glucosamine), which is derived from the deacetylation of chitin which has been applied widely in biomedical applications, due to their nature as non-toxic and biocompatible. Chitosan containing two reactive groups (amino and hydroxyl), which is chemically or physically modified, so that the chitosan has a high potential in tissue engineering applications. One of the most interesting effect of chitosan on wound healing is the formation of granulation tissue with angiogenesis. It has been observed that chitosan can induce fibroblasts to release interleukin, which involves the migration and proliferation of fibroblasts. Therefore, chitosan, a biomaterial, the potential for fabricating scaffold of collagen/chitosan. However, the mix ratio between collagen and chitosan, the effects on the physical properties and biological scaffold of collagen/chitosan remains unclear reported.

Collagen is known as the most promising materials with applications in tissue engineering, biocompatibility and biodegradability properties is excellent. However, due to rapid biodegradation rate and low mechanical strength of the scaffold, causing collagen to limited use.

Cross-linking of chitosan-based scaffold is an effective method for modifying the level of biodegradation and to optimize the mechanical properties. Currently, there are two kinds of cross-tie method is used to improve the properties of collagen-based scaffold: methods of chemical and physical methods. The latter method includes the use of photo oxidation, processing dehydrothermal (DHT) and ultraviolet irradiation, which can avoid

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potential cytotoxic chemical residues and retaining excellent biocompatibility of collagen material. Most of the physical processing cannot produce the degree of crosslinking high enough to meet the demand of collagen in tissue engineering of skin. Therefore, treatment with chemical methods are required in almost all cases. Use Glutaraldehydes, GA as a cross-linking bifunctional reagents that can be connecting two polypeptide amino groups of adjacent chains, the main option in skin tissue engineering, due to their solubility in water, the efficiency of cross-tie and low cost.

Materials and Methods

Collagen (0.5% w/w) and chitosan (0.5% w/w) of each perfectly dissolved in a solution of 1% glacial acetic acid, to make a solution of 0.5% (w/v). This mixture was stirred until homogeneous with variations weight ratio (10:0, 8:2, 7:3, 6:4, 5:5 4:6, 3:7, 2:8, 0:10), for 1 hour. Then the bubbles of water formed are removed from the polymer solution. Polymer mixture centrifuged for 15 min at 3000 xg, to remove insoluble impurities. Solution of collagen/chitosan was poured into glass moulds (7,5x7,5) with a thickness of 5 mm, were frozen at room temperature for 24 hours, and then freeze-drying at a temperature of-40^{o}C and pressure of 10^{5} atm for 24 hours, to get the collagen/chitosan scaffold. To improve the bio stability, collagen/chitosan cross-linked scaffold in a solution of GA (double distilled water, pH 5.6) with a concentration of 0.25%, at 4^{o}C for 24 hours. After washing with double distilled water (every 10 minutes, 5 times), freeze-dried scaffold dryer back to get a scaffold of collagen/chitosan treated with GA. Stages of processing chitosan/collagen scaffold can be seen in the following scheme:

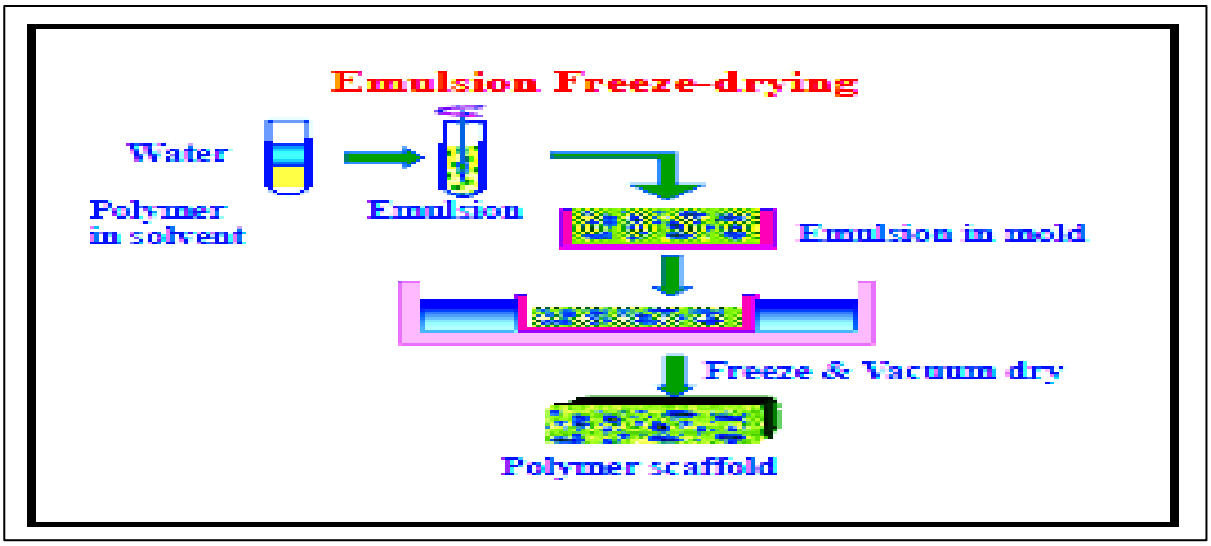

Figure 1 The processing of scaffold by freeze-drying method.

Results and Discussion

Microstructure Test

Scaffold microstructure observed under Scanning Electron Microscopy (SEM, Cambridge stereoscan 260). After experiencing the process of drying, the surface and cross section of footage was observed using scanning electron microscope (SEM, Model JEOL JSM-651OLA, Cambridge). All test films coated with an ultra-thin layer of gold and observed in surface morphology from 250-1000x magnification.

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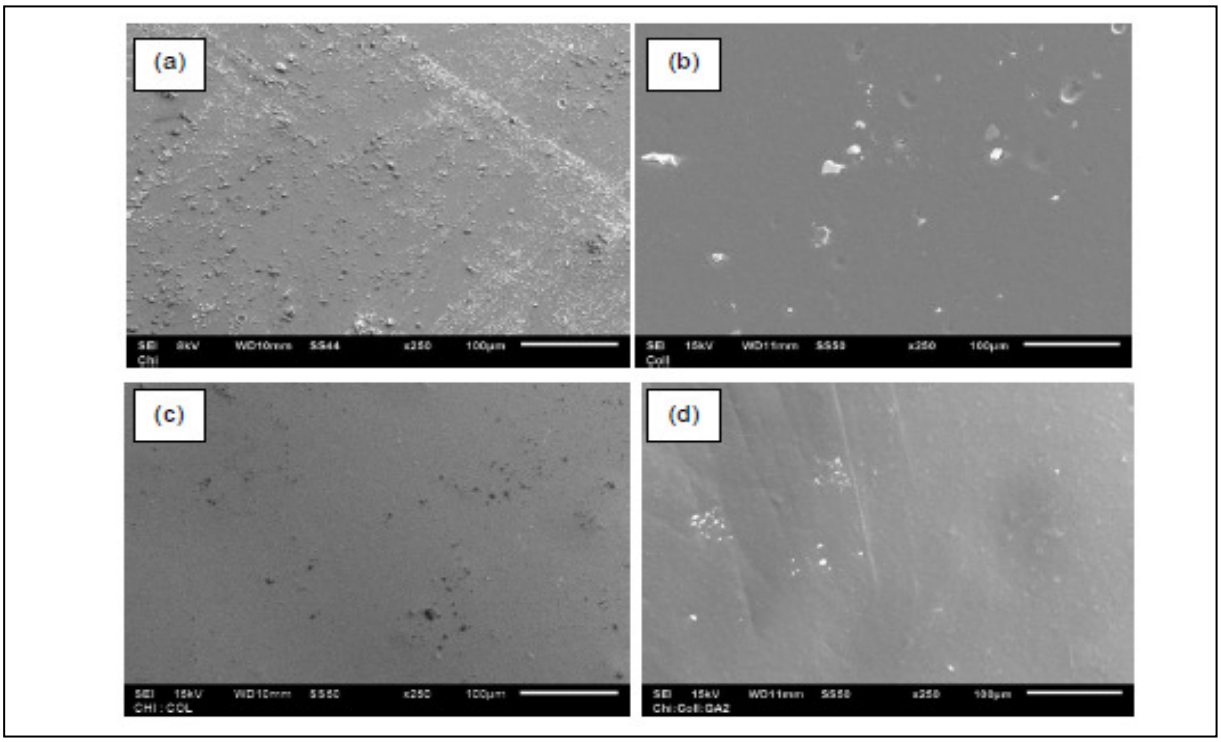

Figure 2. Picture the surface of chitosan, collagen and chitosan/collagen/GA films scaffold with a magnification of 250X. (a) Chitosan in 1% acetic acid film, (b) Collagen in 1% acetic acid film (c) Chitosan/Collagen film (1:1), (d) hitosan/collagen/GA film.

The results of the SEM image showing the surface of the scaffold is made of non-porous structure in the form of sheet or film (Figure 1). In this study, solvent casting and printing techniques with freeze dryer drying method used to prepare the film, because the process is simple and inexpensive. The difference in preparation techniques causing differences in the scaffold morphology.

There are several factors that affect the structure of the scaffold morphology, the process of dissolving the polymer material, solvent concentration, agitation method, the printing process, the rate of freezing and drying scaffold. The concentration of acetic acid is too thick causing the film cannot be formed or broken during the dry film is removed from the mould (data not shown). It has been tested several variations of concentrations of solvents that can form a layer of film and easily removed from the mould. The concentration of acetic acid used in dissolving the polymer is approximately 1-2%. In Figure 1 (a-d) can be seen of the film surface is not perfect. There are still parts of the insoluble material of each component used. Methods of stirring and mixing the polymer film surface are critical condition after printing. Air bubbles are formed is also shown in Figure 1 (a-d). Printing techniques also affect the morphology of the polymer solution film surface scaffold. Freeze drying conditions at temperature -40^{o}C and 10^{5} atm pressures for 24 hours. Pore structure cannot be formed on the scaffold. This is due to the absence of temperature variations during the freezing process. Pore morphology can be formed by varying the freezing temperature during the drying process scaffold. The addition of GA to the scaffold did not show a significant difference to the film surface scaffold that is not treated with GA (Figure 1c and 1d)

From the research Faikrua A (2009), explained that the strength of the non-porous scaffold is very high but the nature of the flexibility of the material is generally low. Instead, a porous scaffold generally has low strength and low elongation properties as a function of the orientation of the pores and interconnections. Therefore, the scaffold should have sufficient strength to overcome the divisions during implantation and maintain

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integrity during testing in vivo and in cell growth in vitro. However, it should be considered that the mechanical properties, including tensile strength and elongation percentage of the value of the film can be changed after the sterilization process. Further studies are needed to clarify the effect of sterilization on the mechanical properties of the scaffold were developed.

Cell Growth on the scaffold Test

In Figure 2a it can be seen that the fibroblast cells can be attached on the surface of chitosan/collagen ratio scaffold of 20:80, and cell shape extends the inherent increased, but the number of cells did not increase much.

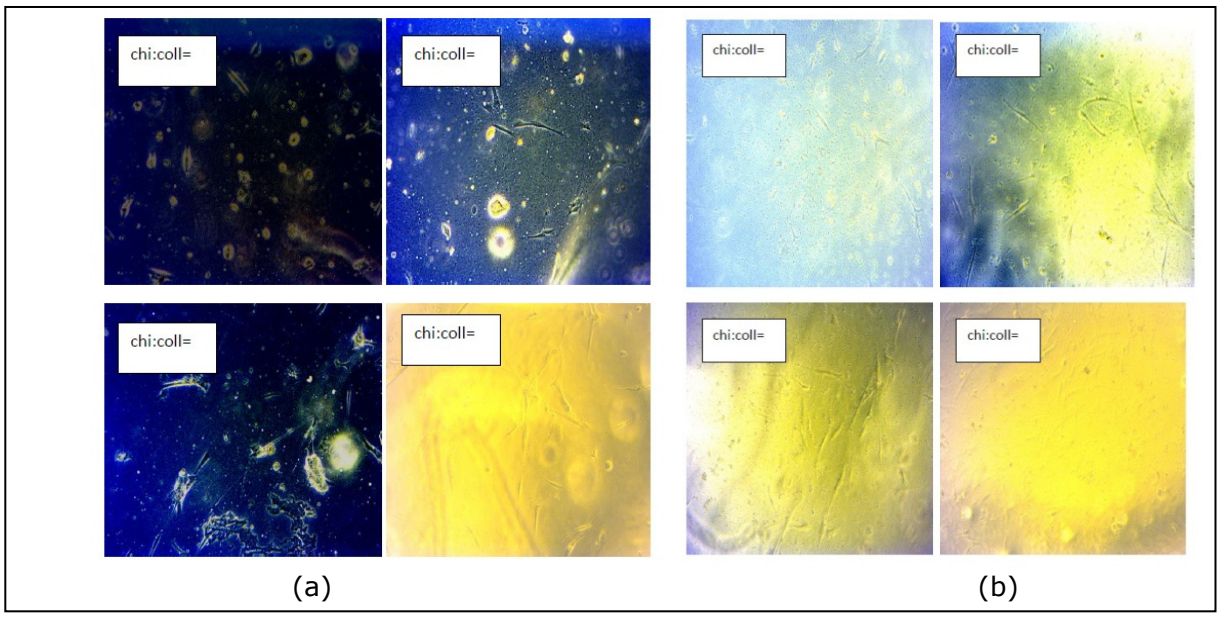

Figure 3. Photos on cell growth scaffold chitosan / collagen ratio: (a) 20:80 and (b) 80:20, magnification 10x.

This could be due to: the structure of the surface that is not porous scaffold, which has not been proper sterilization methods and composition of the scaffold, which causes the cells cannot reproduce them on the scaffold, compared to the number of cells attached to the bottom dish (the medium at the edge of the scaffold). In Figure 2b it can be seen that the fibroblast cells can be attached on the surface of chitosan/collagen ratio scaffold of 80:20, and cell shape are attached increasing widespread. The number of cells with a scaffold more than a ratio of 20:80. This could be due to chitosan scaffold with more content more appropriate for cell growth and development.

In Figure 3a it can be seen that the fibroblast cells can be attached on the surface of chitosan/collagen scaffold ratio of 40:60, and the number of cells develop abnormally, although many cell numbers. The number of cells with a scaffold more than the ratio of 60:40, although cell shape was not rapid growth occurs.

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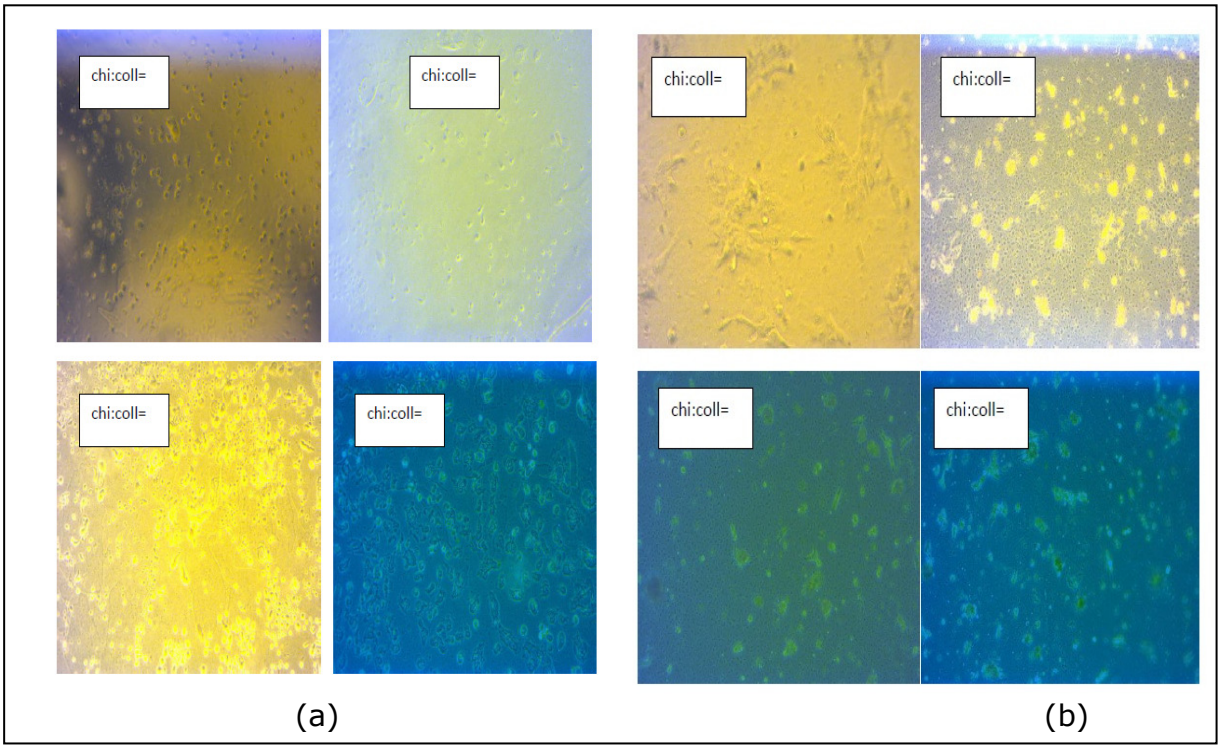

Figure 4 Photos on cell growth chitosan/collagen scaffold ratio: (a) 60:40 and (b) 40:60, magnification 10x

In Figure 3b it can be seen that the fibroblast cells can be attached on the surface of chitosan/collagen scaffold ratio of 60:40, and the number of cells growing very well, but the rapid growth of cell shape. The number of cells is less than the collagen scaffold with chitosan ratio of 40:60, although the growth of the same cell lines (not quickly occur). In Figure fibroblast cell growth on the scaffold of chitosan/collagen (Figure 2 to Figure 3), demonstrating the ability and biocopatibilitas of scaffold for tissue engineering. Nonporous scaffold surface, fibroblast cells can attach and grow on the scaffold. Scaffold sterilization methods and properties of human fibroblast cell adhesion on the scaffold needs to be further investigated. It deals with the fact that although the amino groups of the polymer interacts with the aldehyde group of glutaraldehyde, which led to free amino groups of the polymer chains interacting electrostatically with the negative charge of the surface of the cell membrane. This means also that glutaraldehyde does not like to interact with the polymer leading to cell proliferation and adhesion properties. Another possibility is the interaction between the recipient cell bio specific with chitosan or collagen molecule from given adhesion and proliferation of cells strongly depends on the surface of specific cell receiver used by cells to interact with the scaffold, causing adhesion of the cells on the scaffold.

Conclusions

The results of this study show that chitosan has potential as a structural material for tissue engineering (film scaffold). Conditions processes determine the quality of the resulting scaffold movie. Biopolymer chitosan and collagen cross-linked with Glutaraldehide, using freeze drying process produces thin film nonporous shaped scaffold. Physical interactions between chitosan and collagen influence on physical and biological properties of the scaffold. Scaffold chitosan/collagen is sitocompability and testing using fibroblast cells indicate a potential scaffold for tissue engineering applications. Scaffold chitosan/collagen ratio of 80:20 and a concentration of 0.25% GA showed growth of human skin fibroblast cells of the nicest.

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