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Degradation Profile and Fibroblast Proliferation on Synthetic Coral Scaffold for Bone Regeneration

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Abstract. For bone regeneration, one of the important key factors is the ability of the scaffold to be able to be degraded gradually at the implant side and the ability to provide a microenvironment for cells. No information available yet regarding our new synthetic coral scaffold. The purpose of this study was to observe the degradation profile and the human fibroblast gingiva proliferation on the synthetic coral scaffolds prepared from gelatin and calcium carbonate ($CaCO_3$) in a various concentration of weight% wherein gelatin only (G) was used as a control. Scaffolds were incubated in non-phenol red DMEM for 1, 3, 6, 24, 48, and 72 h, then the supernatant was analyzed by spectrophotometer in 280 nm wavelength**.** After 72 hours, the degradation rate of the scaffold was accelerated using 1N HCl.The proliferation of human fibroblast gingiva was observed at 24, 48 and 72 hours after seeding 2.10^4 cells/well. A plate of 96 well and DMEM were used in this study. The degradation percentage of the scaffolds within 24 hours was considered small, ranging between 4.23 - 5.15%. Meanwhile, the degradation between 24 to 72 hours incubation was confirmed at 38.35, 62.16, 52.86, 47.86, 48.42, and 53.17% for G, 7/3, 6/4, 5/5, 4/6 and 3/7 respectively. In the accelerated condition, the fastest degradation was found in G.Fibroblast proliferation increased following the incubation time except in control group and 3:7, but no significantly different between the various concentration. The fastest degradation rate was found in the scaffold without $CaCO_3$ addition, confirming that $CaCO_3$ in certain concentration prolonged the half-life of the scaffold. The proliferation of fibroblast is increased for all incubation time.

INTRODUCTION

Tissue engineering is developed based on the idea to reconstruct the damaged tissue by the application of scaffold, signaling molecules and cells, either alone or in combination. Thus, scaffold, signaling molecules and cells are considered three main components in tissue engineering [1,2]. Scaffold guides cells to attach, grow and synthesize extracellular matrix (ECM) and biological molecules [3]. The biomaterials of scaffold should be designed to have properties for not inducing inflammation and biocompatible to the body. The scaffold is designed and prepared for the living system. Therefore, the scaffold has a function to provide a microenvironment for cells attachment, proliferation and tissue regeneration [1,3]. Factually coral has been used as a scaffold for bone regeneration. Coral have design and ability to serve microenvironment for living cell [4-6].

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A biodegradable scaffold is a scaffold which is able to dissolve when exposed to organism body fluid [2]. For bone regeneration, the availability of biodegradable scaffold is required. To fulfill the requirement, a new developed biodegradable scaffold mimicking sea coral composition was fabricated in this study. In in vitro model, fibroblast cells are seeded and cultured in a certain growing medium, such as Dulbecco's Modified Eagle Medium (DMEM). The scaffold immersed in cell culture medium is exposed to the culture medium and gradually degraded. On the other hand, during there generation process, cells generate their own natural ECM to regenerate tissue. The degradation rate of the scaffolds should be in balance with new tissue formation [7]. In this point, the information on the degradation rate of synthetic scaffold for tissue engineering application is needed. For that purpose, this study was directed to investigate the degradation profile of the newly developed synthetic coral scaffold in non-phenol red DMEM (NPR-DMEM).

MATERIALS AND METHODS

Scaffold preparation

The synthetic coral scaffolds were prepared in a thick film/membrane (**Fig. 1**) from bovine isolated gelatin produced by Nitta Co. (Osaka, Japan) and calcium carbonate ($CaCO_3$) from Wako (Osaka, Japan) in a various concentration of 7/3, 6/4, 5/5, 4/6 and 3/7 weight % then cross-linked physically. A gelatin membrane without the addition of $CaCO_3$was also prepared as a control.

Sodium citrate (Sigma-Aldrich, Germany) was used as the dispersant. $CaCO_3$was added and stirred in a gelatin system until a homogenous solution was achieved. The solution was then molded to prepare thick film-like scaffold with 0.3 mm thickness. Scaffolds were frozen at -20˚C for 24 hours, freeze drying for 24 hours, and then cross-linked by dehydrothermal method for 72 hours.

FIGURE 1. The fabricated scaffolds were formed in thethick film.

Degradation experiment in NPR-DMEM

Degradation experiment done in this study was referred to some previous studies [8,9,10]. Scaffolds were incubated in NPR-DMEM for 1, 3, 6, 24, 48 and 72 hours. The supernatant collected from each time point was analyzed by UV-Vis 1800 Spectrophotometer (Shimadzu, Tokyo, Japan) at 280 nm wavelength. After 72 hours, 1N HCl was used to immerse the scaffolds to allow the scaffolds to be degraded in an accelerated condition. Degradation percentage was calculated based on the following formula:

$$\text{Degradation}\,(\%) = \frac{\text{Absorbance}_{\,(1, 3, 6, 24, 48 \text{ or } 72 \text{ hours})}}{\text{Absorbance}_{\,(\text{total})}} \times 100\% \qquad (1)$$

Proliferation human fibroblast gingiva on synthetic coral scaffold

The ethical clearance for this study was approved by Health and Medical Research Ethics Committee of Universitas Gadjah Mada (EC No. 694/KKEP/FKG-UGM/2014). Fibroblast cells used in the study were obtained

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from a primary culture of human gingival. The complete medium containing DMEM (Gibco, USA), FBS (Gibco, USA), pen strep (Sigma, USA) and fungizone (Gibco, USA) were used for the fibroblast culture. Immediately after 80% confluency, fibroblast was seeded on the surface of the scaffold placed on the bottom of 96 well plate, with cell density of 2x104 cells per well. The experiment was done in triplicate for each different parameters. Dye exclusion method was used to investigate cell viability for 24, 48 and 72 hours by counting fibroblast using Neubauer improved haemocytometer (Sigma, USA).

Data analysis

The data were presented descriptively. The absorbance read from the spectrophotometer was used to express the degradation rate in percentage. Multiway analysis of variance (ANOVA) and LSD was performed to analyze the data of fibroblast proliferation. The significance of the study was determined at p=0.05 value.

RESULTS AND DISCUSSION

Degradation in NPR-DMEM

Fig.2. shows the resulted scaffold formed in the thick film (membrane). Within 24 hours, regardless the concentration of the $CaCO_3$ in the scaffold, the degradation rate was considered very low, less than 5%. The information related to the slow degradation rate of the scaffold prepared in various concentrations within 24 hours is considered positive aspect for the next development. After 24 hours, the degradation rate increased rapidly, ranging between 23.8 – 31.5% in 48 hours and 38.3 – 62.1% in 72 hours.

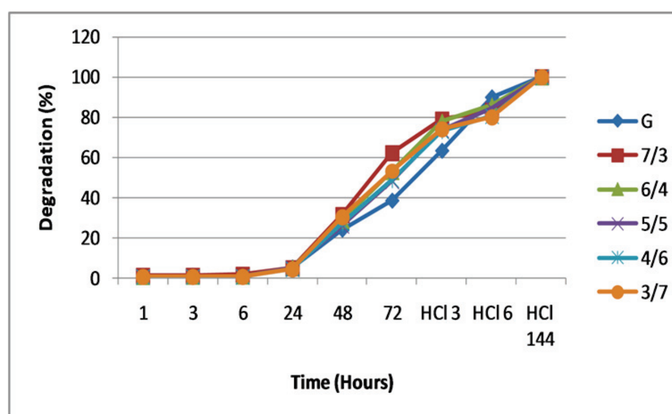

FIGURE 2. Degradation profile of synthetic coral scaffolds in NPR-DMEM and were followed the accelerated degradation in 1N HCl.

In the accelerated phase, the degradation percentage increased from 63.2 – 78.6% within 3 hours to 83.1 – 89.9% within 6 hours, as shown in **Fig. 3-4**. From the figures, it was observed that the degradation rate of the gelatin was very low, less than 1.2% but slightly increased to 5.1% in 24 hours. The rate of the gelatin degradation increased four times within 48 hours and five times within 72 hours approximately.

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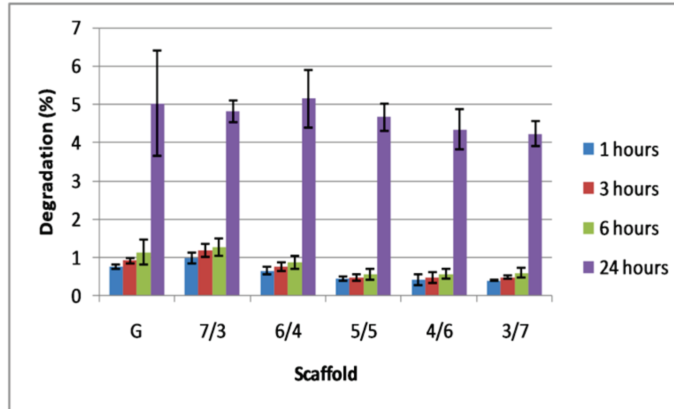

FIGURE 3. Degradation profile of the scaffold within 24 hours.

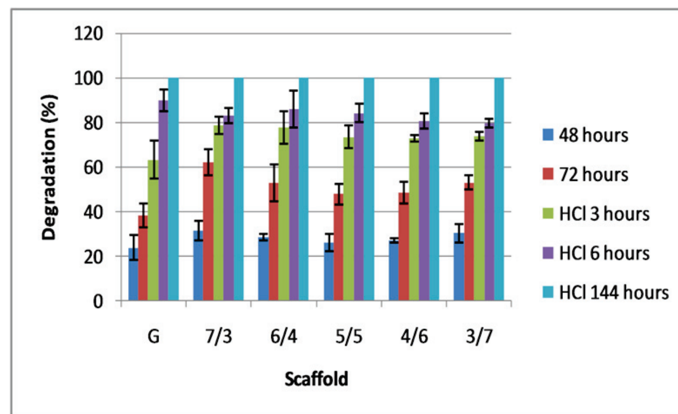

FIGURE 4. Degradation profile of the scaffold after 24 hours.

Biodegradable scaffolds have a significant influence in engineering application [11]. Significant efforts have been devoted to preparing biodegradable polymers and use them in medical applications as implants, drug delivery devices, and tissue engineering scaffold. In this study, gelatin resulted from thermally denaturalized collagen was used to prepare the scaffold. Degradation occurs by dissolution of scaffold when exposed to water or organism body fluid [12]. Firstly hydrogel swell when exposed to water then gradually degraded [13]. The polymer degradation reactions can occur because of the decrease of its molecular weight through scission of the main chain. Previous degradation studies in PBS reported that non-crosslinked scaffold has higher degradation than crosslinked scaffold. This is because crosslinking has functioned to maintain the structural integrity of the scaffold. The degree of crosslinked part is possible to influence the degradation [7].

It was confirmed that the degradation profile after 24 hours was fastest in the scaffold without the addition of $CaCO_3$. It can be predicted that the addition of $CaCO_3$ in a certain concentration influenced the degradation rate of the scaffold. In view of this, it was predicted that physicochemical degradation or dissolution of $CaCO_3$ plays an important role at the exchanges of a solid-liquid interface. Since the addition of minerals ($CaCO_3$) into the scaffold system may change the dynamic process in the non-phenol red DMEM, further studies are needed to confirm the phenomena. Since the diffusion process, pore size and the velocity of mass transport throughout the scaffold influenced the degradation rate [13], it was confirmed from this study that the final composition of the scaffold is the most important aspect to be investigated in vitro and in vivo.

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Fibroblast proliferation

Fibroblast proliferation increased following the incubation time except in control group and 7/3as shown in **Fig. 5**. I within 48 h. Meanwhile, for scaffold 7/3 there was no cell attached an the control group, proliferation slightly decreases nd proliferated on 24 hours, because the difficulty in handling, it tended to scroll when was given medium due to the thick film was very thin for this concentration. Between the concentration, the statistical analysis showed no significant different ($p>0.05$) in all scaffold.

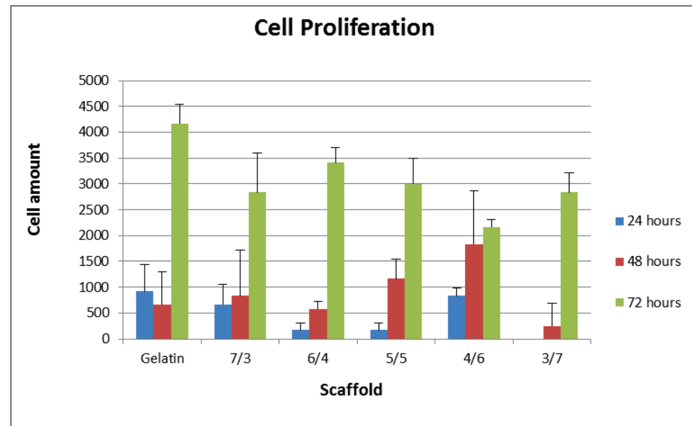

FIGURE 5. Fibroblast proliferation on scaffolds.

The biocompatibility of synthetic coral scaffold has been confirmed by the ability of the scaffold to provide a microenvironment for cell living. The scaffold was made from gelatin and $CaCO_3$. Gelatin is denatured of collagen so it is free of immunogenic concern, and contains integrin binding protein for cell attachment [14].

The result showed that the human fibroblast cell attached and proliferated in the scaffold. In all concentration except 7/3 presented that cells adhered in 24 hours, increased in 48 and 72 hours. For this point it can be assumed that human fibroblast cell can attach, grow and proliferate in the synthetic coral scaffold. In gelatin as a control group, cells proliferation decreased on the 48 hours and increased on 72 hours. This phenomenon may be caused by degradation characteristic of the scaffold. Degradation of gelatin was faster compared to the other concentrations, therefore it disturbed the activity of cells proliferation. Since almost all gelatin degraded, the cells proliferation increased because cells grew on the floor of the culture well.

It was confirmed that design of degradation scaffold plays an important role due to appropriate environment for the cell to adhere, proliferate, differentiate and generate an extracellular matrix for tissue regeneration [15].

CONCLUSIONS

It was found that the degradation of synthetic coral scaffolds in NPR-DMEM in various concentrations was similar within 24 h, meanwhile after 24 h scaffold without the addition of $CaCO_3$ had faster degradation. The synthetic coral scaffold is a biocompatible biomaterial that can provide a microenvironment for cell living. This study showed that design of degradation scaffold is important to reach the success of tissue regeneration.

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