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Biodegradation and Compressive Strength Test of Scaffold with Different Ratio as Bone Tissue Engineering Biomaterial

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Abstract

Bone destruction in oral cavity due to trauma, chronic infection, congenital malformations, or surgical procedure have most often been treated using autologous bone-grafting procedure. Another solution emerged in tissue engineering is the use of scaffold. Calcium carbonate is one of potential ceramic materials that have osteoconductive properties that can be used as scaffold constituents.

To investigate the comparative bio-degradation value and compressive strength of chitosan, gelatin, and calcium carbonate scaffold with various ratio.

The scaffold were made using freeze-drying method. Degradation test was done by dissolving scaffold in PBS containing 1.6 µg/ml of the lysozyme. Compressive strength test is done by using autograft tool with load cell compress machine 100 kN.

Degradation rate of scaffolds with ratio 40:60 was lower than scaffolds with 30:70 ratio. Compressive strength of scaffold with ratio 40:60 was higher than 30:70 ratio scaffold. The data were analyzed using T-test and showed significant difference in degradation rate and compressive strength test.

The scaffolds with ratio 40:60 have better bio-degradation rate and compressive strength properties than scaffolds with ratio 30:70. The chitosan, gelatin and calcium carbonate scaffolds with ratio 40:60 have potency as an alternative biomaterial in bone tissue engineering.

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Introduction

Bone destruction in oral cavity caused by trauma, chronic infections, congenital malformations, or resection surgery, have most often been treated using autologous bone-grafting techniques. More than 2.2 million patients in the world suffers from bone damage were treated with bone tissue engineering techniques. The limitations of this technique are the limited donors and suppliers, require a high cost, the potency of undesired immune response by the host as it accepts the foreign tissue and the possibility of disease transmission [1-2].

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Alternative ways emerge in tissue engineering to treat bone loss and increase intra oral bone regeneration is using scaffolds combined with growth factors, cells or genes [3-4]. Scaffolds should be biocompatible, have good biodegradation and biomechanical properties and have good porosity and interconnectivity. As a material for tissue regeneration, scaffolds should have good mechanical strength, high porosity, high swelling ratio and low degradation rate. The rate of scaffold degradation is required to support cell culture during bone regeneration and new bone formation [5].

Constituent materials for scaffold in this study were chitosan, gelatin and calcium carbonate. Chitosan has been studied and used in tissue engineering as an ingredient for bone, skin, and nerve tissue regeneration. Scaffolds made of chitosan has been used in tissue engineering and regenerative therapy [6]. Gelatin was added to chitosan scaffold in order to get scaffolds with

better hydrophilicity and biocompatibility[7]. Calcium carbonate has better natural biodegradation properties than calcium phosphate and hydroxyapatite, and has been used in craniofacial reconstruction in pediatriy, a field that require rapid scaffold resorption in skeletal regeneration [8].

The mixture of chitosan, gelatin, and calcium carbonate materials is expected to boost the properties of each ingredient, potentially producing a scaffold with better properties suitable for bone regeneration. The purpose of this research is to find the comparative value of biodegradation and compressive strength of chitosan, gelatin and calcium carbonate scaffold with the different ratio.

Methodology

The material used was calcium carbonate (Made from the shell of blood shells processed at Bank Network, Dr. Soetomo Hospital, Surabaya), gelatin (Cat 076-02765, Work Pure Chemical Industries, Richmond, USA), chitosan (Sigma No. 93646, St. Louis, USA), 10% NaOH with deacetylation degree of more than 81%, 2% acetic acid and sterile distilled water (PT Duta Farma).

Scaffolds making procedure was referred from the previous study with modification. Scaffolds with 30:70 ratio were composed of 1.75 gram calcium carbonate, 0.375 gram gelatin, and 0.375 gram chitosan. Scaffolds with ratio 40:60 consisted of 0.5 gram chitosan powder, 0.5 g of gelatin powder, and 1.5 gram of calcium carbonate powder. All types of scaffolds undergone the same procedure for the next process. Weighed calcium carbonate was mixed with 98 ml of deionized distilled water. Stirring was carried out at room temperature for 30 minutes until the powder of calcium carbonate dissolved in water, then premixed chitosan with 2 ml of 0.1 M acetic acid was added and stirred. After stirring is complete, the mixture was moved to a water bath in 40oC temperature and gelatin was added. The mixture then stirred until homogeneous and added with 2 ml of 0.1 M NaOH to neutralize the acidity. After homogeneous, the scaffold was washed with deionized distilled water up to pH 7. The mixture was then was frozen at -40 ° C for 2 hours and was put to a freeze-drying process for 24 hours [5-9-10].

The measurement of scaffold degradation rate, initially the scaffolds were weighed to determine the initial weight (Wi). Then the scaffold was dissolved with PBS containing 1.6 µg / ml (112 units/ml) of the lysozyme enzyme. The concentration was equated to the concentration of lysozyme enzymes that present in human serum. The lysozyme solution was replaced daily to ensure enzyme activity continues. After the 3rd and 7th days, the sample is taken from the medium, washed with distilled water, dried, then the freeze-dried again. The degradation rate is calculated based on the following formula: [11]

$$\text{Degradation rate } (\%) = \frac{(Wf - Wi)}{Wi} \times 100$$

The compressive strength test was performed using a scaffold with a diameter of 8 mm and a height of 10 cm. The size of the scaffold used to adjust the specifications of the tools used. The scaffolds' surface area was measured formerly. The scaffold was placed in the center of the table with the vertical axis position of the sample perpendicular to the flat plane. The autograft was turned on and then the sample was pressed at 10 mm / min and load cell compress machine 100 kN until the scaffold is distorted. The tool will stop automatically and the outgoing number is recorded. The compressive strength value is calculated using the following formula: 12

$$\text{Compressive strength } (N/mm^2) = \frac{\text{Force (Newton)}}{\text{Surface area (mm2)}}$$

Results

Data analysis was done using T-test and showed significant difference in degradation rate on day 3 ($p=0.005$) and day 7 ($p=0.003$); and compressive strength ($p=0.000$) scaffold chitosan, gelatin and calcium carbonate at 30:70 and 40:60 ratio with signification $p < 0.05$. The results of degradation rate test of chitosan, gelatin and calcium carbonate degradation are shown in Figure 1.

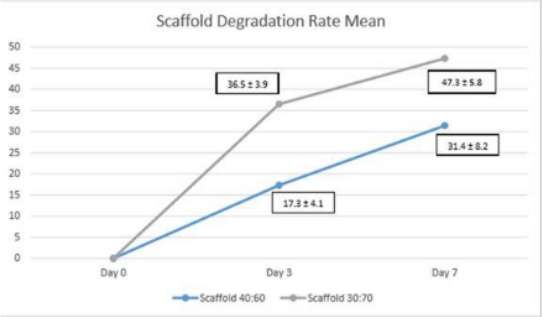

Figure 1. The degradation rate mean of scaffold chitosan, gelatin and calcium carbonat ratio 40:60 and 30:70 at day 3 and 7.

Scaffold ratio 40:60 on day 3 with mean value of 17.3% ± 4.1 and day 7 with mean value of 31.4% ± 8.2 had a lower degradation rate compared to the scaffold ratio 30:70, observed at days 3 and 7. The result of compressive strength test of chitosan, gelatin and calcium carbonate scaffold ratio 30:70 and 40:60 can be seen in figure 2. Scaffold ratio 40:60 with mean value 3.2 mpa ± 0.7 had higher mean value compared with scaffold ratio 30:70.

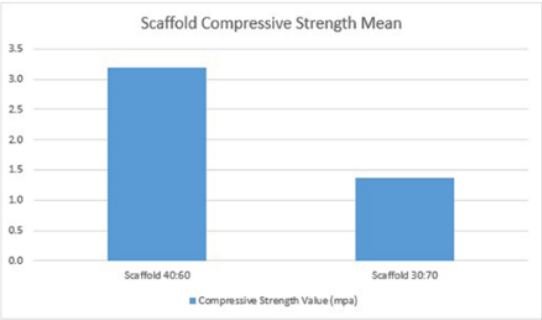

Figure 2. The compressive strength mean of scaffold chitosan, gelatin and calcium carbonat ratio 40:60 and 30:70.

Discussion

The most important thing in tissue engineering is the selection of optimal biomaterials for scaffold manufacturing. The biomaterials used must have good biocompatibility, biodegradation and bioactivity properties. The ideal scaffold serves as a template for tissue regeneration and has a major effect on the formation of the final structure in tissue engineering [5].

The scaffolds used in this study consisted of materials from chitosan, gelatin and calcium carbonate. The addition of chitosan and gelatin to the scaffold is expected to match the main composition of bone that is composed of inorganic components of collagen and non-collagen inorganic components. Collagen inorganic components are represented by gelatin while non-collagen inorganic components are represented by chitosan [10]. Calcium carbonate consists of calcite, the most stable carbonate, and polymorphic minerals. Other polymorphs are aragonite and vaterite minerals. Aragonite will change to calcite at a temperature of 380-470°C, and vaterite is less stable. Aragonite is a carbonate mineral, which occurs naturally, is formed from calcium carbonate crystals [13]. Calcium carbonate, the natural component of coral, is more soluble than hydroxyapatite by controlled conversion of the proportion [14]. The mechanical and biological properties of the scaffold can be improved in tissue engineering applications. Therefore, in this study carried out the incorporation of materials such as chitosan, gelatin and bioactive ceramic such as calcium carbonate [15].

The degradation rate test conducted in this study compares the two ratios, the ratio of 30:70 and 40:60. From the observation, it is found that the lowest percentage rate of degradation rate is at 40:60 ratio. The ideal bone replacement material should have the capability to be resorbed in new bone formation and degraded for some time according to the natural cycle of new bone formation. The resorption and degradation of bone replacement materials should not be too fast or slow. Calcium carbonate is a relatively rapid resorbed ingredient in the body [16]. Therefore, the highest percentage rate of degradation rate is obtained at a ratio of 30:70 where the calcium carbonate content is higher than the ratio of 40:60. While at a ratio of 40:60, the average percentage of degradation rate is low because in addition to less calcium carbonate content, it is likely due to the influence of more chitosan and gelatin content than the ratio of 30:70.

The mechanical properties of the scaffold are an important factor in the process of making the scaffold. The scaffold must be strong enough to be under mechanical pressure from the surrounding tissue. Low mechanical strength in the scaffold can affect in dimensional shape

changes in the scaffold. The highest compressive strength test result in this research is obtained at 40:60 ratio with mean of 3.2 MPa. This is in accordance with the criteria of compressive strength on the cancellous bone between 2-12 MPa [17]. As for the 30:70 ratio scaffold, the compressive strength value is below the compressive strength value of the cancellous bone.

In scaffold ratio 40:60, the calcium carbonate content is lower than the scaffold ratio 30:70. In the previous research, we have investigated the effect of calcium carbonate with various ratios on a mixture of materials. The compressive strength value was increased maximal on a percentage of calcium carbonate content as much as 15%, while the content above 15% obtained compressive strength value decreased or low [18].

Conclusion

Based on the degradation rate test, chitosan, gelatin and calcium carbonate scaffold ratio 40:60 have a lower percentage of degradation rate than the 30:70 ratio scaffold. Besides, compressive strength test of chitosan, gelatin and calcium carbonate scaffold ratio 40:60 have a higher value than the 30:70 ratio scaffold. The chitosan, gelatin and calcium carbonate scaffold ratio 40:60 has better degradation rate and compressive strength than the scaffold ratio 30:70. The chitosan, gelatin and calcium carbonate scaffold ratio 40:60 considered to have potential as a replacement biomaterial in bone tissue engineering.

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