Effect of drying on the porosity of the hydroxyapatite and cellulose nata de coco composite as bone graft candidate

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Abstract. Bone graft is used to replace bone parts damaged by illness and accident. As a bone replacement material, the bone graft should be able to stimulate the process of the osteogenesis. The process of osteogenesis is influenced by the osteoconductive properties of a biomaterial, that porosity affects this process. The shells of blood scallop (Anadaragranosa) are producing hydroxyapatite (HAp), having high compressive strength, biocompatibility and osteoconductive properties, but low porosity while cellulose nata de coco (Cnc) have low compressive strength but high porosity. Therefore, the combination of two biomaterials are expected to produce composite that have high osteoconductive properties. The purpose of this research was knowing the porosity of HAp/Cnc composite which was being precipitated for 5 hours, 15 hours, 25 hours and was dried for 24 hours, 48 hours and 72 hours. This research used wise drop technique to synthesis HAp powder and cellulose immersion technique for synthesis of HAp/Cnc. Results of this research, there was difference in porosity between HAp/Cnc that was precipitated for 5 hours, 15 hours and 25 hours, as well as was dried for 1 day, 2 days and 3 days. The conclusion, the synthesis of HAp/Cnc was useful as bone graft candidate.

1. Introduction
Periodontal disease is an irreversible periodontal attachment loss, destruction of periodontal ligament and alveolar bone. In this situation bone repair and augmentation are needed [1]. Bone repair and augmentation involve the use of biomaterial and can be transplanted with biomaterial-based on scaffold for tissue regeneration. For this, biomaterial is used as a scaffold which should have specific features such as chemical, biological and mechanical properties [2].
The biological properties of scaffold must make osteoblast can be seeded, cultured and then implanted to the bone to induce the growth of new bone. The scaffold must be osteoconductive and preferably, osteoinductive which it can be induce efficiently osteogenesis process[3]. The ability of osteoblast attached to the material is influenced by the porosity, pore size and interconnectivity. One of several materials which use as bone graft is hydroxyapatite (HAp) that combine with synthetic polymer like cellulose nata de coco[1,4].

The hydroxyapatite from Anadara granosa is a kind of calcium phosphate compound and it has been used as a ceramic biomaterial because of its excellent bioactive, compressive strength, biocompatibility and osteoconductive ability, but HAp undesirable material for augmentation[2,5,6,7].

The nata de coco is bacterial cellulose (BC) that is produced from Acetobacter xylinum with excellent porosity but low compressive strength. Morphologically, the fibrous structure of cellulose nata de coco (Cnc) is similar to the collagenous fibrils of bone. That morphology support Cnc as a scaffolding material to supporting the growth and differentiation of human bone. The cellulose nata de coco is desirable scaffold for tissue engineering because it is biocompatibility and high porosity and accessible surface area/volume ratios [8-11]

However, study of HAp/Cnc composite material as a bone regeneration are limited. The purpose of this study was a comparison time of precipitation for 5 hours, 15 hours and 25 hours and time of drying for 24 hours (1 days), 48 hours (2 days) and 72 hours (3 days) as a basis to development a new biomaterial that can be a new bone graft candidate.

2. Materials and methods

2.1. Material sampling
The study conducted at October 2017. The place of sampling of material conducted at Samarinda, East Kalimantan. Processing of material sample from blood scallop shell and cellulose nata de coco conducted at the Research Laboratory, Faculty of Medicine, Universitas Mulawarman. The study protocol was approved by the Research Ethics Committee on Faculty of Medicine Universitas Mulawarman.

2.2. Calcination of blood scallop shell (A. granosa)
The preparation of blood scallop shells included the process of collection, cleaning, drying, and calcination. Preparation began with the process of cleaning shells from the shells of dirt and then was dried on the air. After cleaning was continued with calcination and it was done twice time, at the temperature 800 °C for 18 hours to get calcium [12].

2.3. Hydroxyapatite synthesis
The manufacture of 1 M Ca(OH)$_2$ solution by inserting 24.81 grams of CaO into a beaker glass and added with 600 ml of distilled water while stirring at 150 rpm. Then, it was added 300 ml of phosphoric acid 1.8M and was heated above hot plate with a temperature of 40°C and was stirring using a magnetizer with a speed of 300 rpm for 60 minutes. The solution was aging for 24 hours. The result was filtered by whatman membrane and was dried at 100°C for 3 hours. The sintering process was carried out at 900°C for 5 hours with a temperature rise rate of 50°C / min [12].

2.4. Synthesis of HAp/Cnc
The synthesis of HAp/Cnc was performed by cellulose immersion technique. First step, purifying Cnc and making HAp suspension distilled water. Cnc was purified in distilled water for 2 days and it replaced the distilled water every 5 hours. We immersed the cellulose with 1 M NaOH solution for 2 hours. The result was a product of salt deposited in the pellicle, it was removed the salt on Cnc by distilled water until the pH is neutral and odorless.
Making HAp suspension from 1 gram of HAp powder added with 30 ml distilled water. The suspension was being sonication for 30 minutes. The purified Cnc was immersed in suspension while stirring using a magnetizer with a speed of 200 rpm for 5 hours, 15 hours and 25 hours. After completion of immersion, the pellicle was removed and was dried into remove its water content with a 50°C incubator temperature [12].

2.5. Porosity test
Porosity test is given by the following equation [13]

\[ \text{Porosity(\%)} = \frac{W_w - W_o}{V_f - V_o} \times 100\% \]  

(1)

Five samples off all composites were equally dry weighed (Wo) and initial volume (Vo) and then immersed in distilled water, these were final volume (Vf). After 24 hours, each sample was taken out from the tube and this was weighed of wet (Ww).

2.6. Statistical analysis
Data are expressed as the mean ± standard deviation for five independent samples. Statistical comparisons between groups were performed with one-way ANOVA and Kruskal Wallis according to SPSS 23.00 software, with significant being p<0.05 or less.

3. Results
The Time of precipitation for 25 hours and the time of drying for 24 hours (1 day) showed lowest average porosity (0.17 ±0.05%) compare time precipitation for 5 hours (0.234± 0.03%) and 15 hours (0.315 ±0.11%) as were illustrated figure 1a. Analyse of Kruskal Wallis statistical showed that the average porosity of the HAp/Cnc composite which time of precipitation for 5 hours, 15 hours and 25 hours and were dried for 24 hours were significant different (p<0.05).

But, there were difference of result on drying of HAp/Cnc for 48 hours. There was not difference between average porosity which time of precipitation for 5 hours, 15 hours and 25 hours as were shown in figure 1b. Based on one-way ANOVA statistical showed that were not significant different between average porosity of the HAP/Cnc composite which time of precipitation for 5 hours, 15 hours and 35 hours and were dried for 48 hours.

The best results were found for 72 hours drying at illustrated in figure 1c. The average of porosity of HAp/Cnc was precipitated for 25 hours had average porosity (0.09 ± 0.02%) approaching average porosity of bone (0.03± 0.01%). Analyse by one-way ANOVA statistical showed that were significant different between time of precipitation for 5 hours, 15 hours and 25 hours (p<0.05) and post hoc analyses by Tukey’s HSD showed that precipitation time for 25 hours had the best average porosity.

The time of precipitation for 5 hours and the time of drying 24 hours, 48 hours and 72 hours showed that were not difference average porosity of time of drying for 24 hours (0.234± 0.03%), 48 hours (0.205 ± 0.05%) and 72 hours (0.20 ± 0.08%) as illustrated in figure 2a. One-way ANOVA statistical showed that was not significant different (p<0.05).

The average porosity HAp/Cnc was precipitated for 15 hours and was drying 72 hours (0.15 ± 0.02) % had lowest compare with time of drying 24 hours (0.315 ±0.11)% and 48 hours(0.194 ± 0.06) % (Figure 2b). Based on Kruskal Wallis statistical analysis showed that were significant different (p<0.05).

The samples were precipitated for 25 hours and were drying for 72 hours had average porosity (0.09 ± 0.02%) highest compare with a drying for 24 hour (0.17 ±0.05%) and 48 hours (0.207 ± 0.03%) as illustrated in figure 2c. Analysis data for all samples which time of precipitation were 5 hours, 15 hour and 25 hour and were compare with time of drying for 24 hours, 48 hours and 72 hours, the best average porosity was 25 hours precipitation and was drying for 72 hours (Table 1). Analyse by one-way ANOVA statistical, there were significant different (p<0.05) between the drying for 24
hours, 48 hours and 72 hours. Post-Hoc analyses by Tukey’s HSD, the best average porosity were precipitation time 25 hours and drying for 72 hours.

![Graph showing porosity vs time of precipitation](image)

**Figure 1.** The average porosity of HAp/cellulose nata de coco with different time of precipitation. Data are presented as mean ±SD (n=5)

### 4. Discussions

Among various synthetic polymers, Bacterial Cellulose (BC) has gained attention due to its purity and abundance. Character of BC was biocompatibility, non-toxicity, non-allergic and high swelling ability make BC a potential scaffold for tissue engineering [10]. The BC scaffold have been shown to support the ingrowth of human chondrocytes and human smooth muscles cells in vitro because morphology structure of BC is similar to the collagen fibrils of human bone, but BC has too small pore size to allow the ingrowth of cells into the scaffold after in vivo implantation [5,10]. Many BC composites such as BC/polyacrylamide, BC/polyester and BC/collagen has been used as biomaterial in dentistry. One of exciting BC is cellulose nata de coco derived from coconut water fermentation and *A. xylinum*. This cellulose was used in this study besides hydroxyapatite from *A. granosa*.

### Table 1. The average porosity of HAp/cellulose nata de coco with different time of precipitation and drying process. Data are presented as the mean ±SD (n=5)

| Precipitation Time (Hours) | Drying for 24 hours | Drying for 48 hours | Drying for 72 hours |
|---------------------------|---------------------|---------------------|---------------------|
|                           | Mean ± SD (%)       | Mean ± SD (%)       | Mean ±SD (%)        |
| 5                         | 0.23± 0.03          | 0.205 ± 0.05        | 0.20 ± 0.08         |
| 15                        | 0.315 ±0.11         | 0.194 ± 0.06        | 0.15 ± 0.02         |
| 25                        | 0.17 ±0.05          | 0.207 ± 0.03        | 0.09 ± 0.02         |
| C+                        | 0.03± 0.01          | 0.03 ± 0.01         | 0.03± 0.01          |
| C-                        | 0.88 ± 0.22         | 0.60 ± 0.15         | 0.60 ± 0.07         |
Figure 2. The average porosity of HAp/cellulose nata de coco with different time of drying. Data are presented as the mean±SD (n=5)

The Hydroxyapatite (HAp) enhances osteoblast cell growth, osteoprogenitor attachment and differentiation to promote a new bone [10]. HAp/BC has shown better biocompatibility and osteoconductivity compared combination HAp/Polyester and HAp/Collagen [10,14]

In this study, manufacturing HAP/ BC from cellulose nata de coco (Cnc) composites with different time of precipitation and time of drying influence the result of porosity. The comparison of manufacture based on time of precipitation shows that time of precipitation for 25 hours has lowest porosity due to it produces more HAp deposits on the surface and penetrate into Cnc fibrils.

As we know, the Cnc is hydrophobic material that has high porosity which causes ability to absorption the water was very high. While HAp is hydrophilic material with low porosity but big pore size. The combination HAp/Cnc is expected have the ability of water absorption as well as human bone [15,16]. If HAp/Cnc has ability as well as human bone, this material can make osteoblast, chondrocytes, endothelial cells, and smooth muscle cells migration and attached on the scaffold [17].

Besides, result of precipitate influences porosity composite, drying which causes shrinkage and crack in pore size, damaging interconnected porous network and porosity then makes decreasing the porosity. In this study, the composites of HAp/Cnc was dried for 72 hours had lowest porosity because it made the pore of composites was damaged and it made porosity composite decrease approaching porosity human bone. The porosity composite that approaching with porosity human bone are good candidate for new bone graft due scaffold that result from this material can attached by osteoblast which can trigger osteogenesis process and process of making new capillary [18].

5. Conclusions
The good porosity of biomaterial HAp/Cnc was precipitated of 25 hours and drying for 72 hours (3 days) because it had porosity near the bone porosity so it was feasible as bone graft candidate
Acknowledgements

Financial support provided by Faculty of Medicine Universitas Mulawarman is gratefully appreciated.

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