

## Evaluation of chitosan-hydroxyapatite-collagen composite strength as scaffold material by immersion in simulated body fluid

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**Abstract.** The reconstruction of bone tissue defects is a major challenge facing oral and maxillofacial surgeons. The essential elements needed for tissue engineering are cells, scaffolds (matrix), and stimulant molecules (growth factors). The mechanical properties of chitosan-hydroxyapatite-collagen scaffolds produced by BATAN, Jakarta, have not yet been studied. This study therefore analyzed the mechanical properties of chitosan-hydroxyapatite-collagen composite scaffolds prepared by BATAN, Jakarta, before and after immersion in simulated body fluid (SBF) for eight days. The compressive and tensile strengths of the chitosan-hydroxyapatite-collagen composite scaffolds were analyzed after immersion in SBF at 37°C for eight days. Each scaffold was removed and dried at room temperature on days 0, 2, 4, 6, and 8. The data obtained were processed and analyzed. Variations in the compressive strength and tensile strength were attributed to several aspects, such as the specimen size, which was not uniform, the scaffold composition, scaffold pore size, which was also not uniform, and the degradation of the polymer. The chitosan-hydroxyapatite-collagen composite scaffold does not exhibit differences in the tensile strength and compressive strength before and after immersion in SBF.

### 1. Introduction

Reconstruction of bone tissue defects is a major challenge facing oral and maxillofacial surgeons. Some circumstances such as surgery, trauma, congenital abnormalities and progressive disease of bone, and bone structure can cause damage that leads to facial deformities [1]. Apart from these, defects are also caused by widespread infection in the jaw, tumors of the jaw, jaw fracture, etc. In these cases, tissue defect reconstruction is needed to restore normal functioning of the craniofacial unit [2]. Currently, the standard method for reconstruction of tissue defects is autografts with materials that are biocompatible, do not trigger an immune response, and carry no risk of disease transmission. However, this method has its drawbacks: requirement of a second surgery in the donor location, increase in morbidity, and infection in the donor bone, which can cause neurosensory deficit [3].

The essential elements needed in tissue engineering are cells, scaffolds (matrix), and stimulant molecules (growth factors). The scaffold plays an important role in cell growth, as a support structure for the cell attachment point of the growth of cells into bone tissues. The combination of materials



used as scaffolds include composites of hydroxyapatite, chitosan, and collagen. The scaffold must have adequate mechanical properties [4]. The mechanical properties of chitosan-hydroxyapatite-collagen scaffolds have become an important factor in determining the success of surgery and bone healing phase. The strength of the scaffold may also be associated with the level of porosity and high content of hydroxyapatite [5]. Soeroso et al. (2012) found that Chitosan concentration of 0.25 % has a potential effect in increasing osteogenesis process in periodontal ligament cells for regeneration of periodontal tissues [6]. Testing of these requirements is first performed in vitro in the laboratory under conditions that resemble the human body. Scaffolds increasingly resemble bone after immersion in simulated body fluid, wherein the surface layer of the mineral layer is filled with apatite. Previous research stated that the addition of chitosan on a scaffold material soaked in SBF for 21 days yielded a mechanically stable scaffold, with increased strength and strain [7]. The maximum tensile strength of the chitosan-hydroxyapatite-collagen scaffold without soaking in SBF is 18.93 MPa [8]. A 2014 study involved a pressure test on a chitosan-hydroxyapatite-collagen scaffold soaked in SBF for seven days, and the results showed that the compressive strength of the scaffold reached 1.75 MPa [9]. Basically, the overall results of mechanical tests show that scaffolds soaked in SBF are more resistant than materials not soaked in SBF [10].

## 2. Materials and Methods

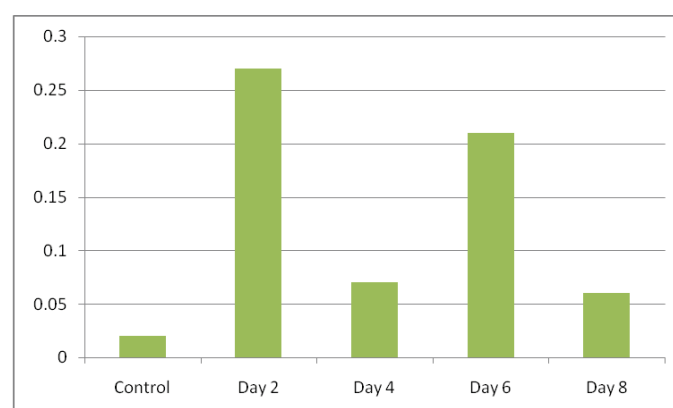
National Nuclear Energy Agency of Indonesia, Jakarta, produced the scaffolds by incorporating chitosan gel from crab shell, collagen gel from bovine tendon, and hydroxyapatite powder from bovine bone. The ratio of chitosan: hydroxyapatite: collagen is 2:3:3. The mixture was molded into certain shapes and sizes. It was then freeze-dried for 2 x 24 h before white sponge-like scaffolds were produced.

The Kokubo method was then applied to make SBF for the scaffold immersion. In the next stage, the scaffold specimens were immersed for 2, 4, 6, and 8 days in 37 °C SBF. The samples were removed from SBF in time groups and air-dried at room temperature for 24 h. Finally, compressive and tensile tests were performed. The compressive and tensile strength of a sample was tested up to 50 N with a speed of 2.2 mm/min.

## 3. Results and Discussion

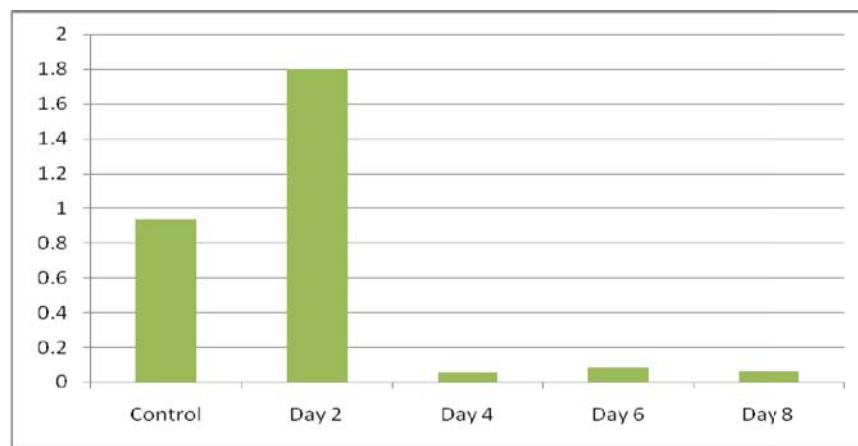
### 3.1 Results

The compressive strength of the chitosan-hydroxyapatite-collagen scaffold on day 0 is 0.02 MPa. After soaking, the increases in the compressive strength on days 2, 4, 6, and 8 are 0.27, 0.07, 0.21, and 0.06 MPa, respectively. Figure 1 shows that the compressive strength of the scaffold generally increased after soaking, when compared to the scaffold without soaking.



**Figure 1.** Average value of compressive strength from Universal Testing Machine of the chitosan-hydroxyapatite-collagen scaffold before and after immersion in simulated body fluid.

The tensile strength of the chitosan-hydroxyapatite-collagen scaffold on day 0 (prior to immersion/control) was 0.934 MPa. After soaking, the variations in the tensile strength on days 2, 4, 6, and 8 are 1.8, 0.053, 0.083, and 0.06 MPa, respectively. The figure shows that the tensile strength of the scaffold generally varied after immersion.



**Figure 2.** Average value of the tensile strength from Universal Testing Machine of the chitosan-hydroxyapatite-collagen scaffold before and after immersion in simulated body fluid

### 3.2 Discussion

The compressive forces measured in this study are lower than those reported previously. Though not significant, there is an increase in the compressive strength and tensile strength in this study on day 2 when compared with the scaffold with no immersion (control). This may be due to the immersion in the SBF leading to the formation of a mineral layer, as reported in other studies. A layer of calcium and phosphorus is formed on the surface of the polymer and hydroxyapatite composite scaffold after being soaked for 12 h in SBF [11].

Similarly, the compressive and tensile strengths of the soaked scaffolds were higher than those of the control. Variation in the compressive and tensile strengths can also be linked to the possibility of degradation of the scaffold. The scaffold used in this study is a structured composite of hydroxyapatite as a filler that is embedded in a matrix of chitosan and collagen. Chitosan and collagen in this study are polymers that undergo degradation in SBF. It seems that immersion for days 4 and 8 induces a higher effect of scaffold degradation than the effect of the surface coating minerals from SBF.

Changes in the value of the compressive and tensile strengths can be linked to several aspects, such as the non-uniform specimen size in the study, because the samples were obtained by cutting the scaffold into smaller sizes. Scaffolds with a larger face area had a greater exposure to the SBF solution and may thus cause more degradation of the matrix scaffold, when compared with scaffolds with a smaller surface area [12]. Furthermore, variations in the compressive strength can be attributed to the use of different scaffolds for each immersion time. Different scaffolds have different pore sizes and porosity, which leads to variation in the compressive and tensile strengths [2].

### 4. Conclusion

In this study, chitosan-hydroxyapatite-collagen scaffolds did not exhibit significant differences in the compressive and tensile strengths between before and after soaking in SBF. This study is preliminary

research on the mechanical properties of chitosan-hydroxyapatite-collagen scaffolds. Future research can also focus on the selection of specimens and changing the method of scaffold preparation. Although the results of this study indicate a variation in the mechanical properties of the scaffold after immersion in SBF, further research is still needed both in vivo and in vitro until this scaffold is developed into a product that can be applied clinically in the field of oral and maxillofacial surgery.

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