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Original Article

EVALUATION OF COMPRESSIVE AND TENSILE STRENGTH OF HYDROXYAPATITE NANOPARTICLES FROM SPIRAL *BABYLON* SHELLS *(BABYLONIA SPIRATA)* AS ALVEOLAR BONE GRAFT MATERIAL CANDIDATES

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ABSTRACT

Objective: This research is a pilot study that aims to determine the compressive and tensile strength values of nanoparticles hydroxyapatite (nHAp) from Spiral *Babylon* shells (*Babylonia spirata*).

Methods: The materials used are nHAp made from Spiral *Babylon* shells using the sol-gel method. The process of hydroxyapatite casting was carried out by mixing hydroxyapatite powder with a binding agent in a ratio of 70% (nHAp) to 30% (Epoxy resin). The specimens were then tested with the Universal Testing Machine to obtain the values of the compressive and tensile strength. Each test was performed in triplicate (n = 3).

Results: The values mean of compressive strength obtained was 45.7±3.86 MPa and tensile strength was 6.69±0.53 MPa.

Conclusion: Spiral *Babylon* shells show potential as a candidate material for alveolar bone grafts.

Keywords: Compressive strength, Tensile strength, Spiral *Babylon*, Hydroxyapatite

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INTRODUCTION

The definition of periodontitis is an inflammatory disease of the tissues supporting the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the alveolar bone and periodontal ligament with pocket formation, recession, or both, is the definition of periodontitis [1]. The main objective of periodontal therapy is to halt the progression of periodontal disease and preserve the natural health and function of the teeth. For mild to moderate periodontitis, this can often be accomplished through non-surgical treatments. However, in more advanced cases, particularly those involving bone loss, surgical interventions may be required to regenerate the affected periodontal tissues [2].

Various surgical techniques can be employed to achieve reliable periodontal regeneration, such as using different types of bone grafts or substitutes, root surface demineralization, Guided Tissue Regeneration (GTR), growth and differentiation factors, enamel matrix proteins, or a combination of these methods [3].

One successful method for reconstructing bone defects is through grafting bone substitute biomaterials [4]. Bone grafting, commonly used in reconstructive periodontal surgery, is a technique for filling periodontal defects and allowing for the regeneration of periodontal tissue [5]. Based on their source, bone grafts used for periodontal regeneration are classified into natural transplants (autografts, allografts, xenografts) and synthetic materials (alloplasts) [5, 6]. Alloplastic materials proven to have great potential as bone graft materials include hydroxyapatite (HAp) [7].

Recent years have seen a rise in interest in the subject of biomaterials from researchers studying hydroxyapatite (HAp), also known as Ca10(PO4)6(OH)2. Because of its structural and chemical resemblance to the mineral phase of teeth and bone, hydroxyapatite is frequently utilized in hard tissue repair procedures [8]. The material used to replace or repair bone needs to be able to form a connection with bone. High bioaffinity and bioactivity characterize hydroxyapatite as a ceramic material. It is also known to be biocompatible, non-toxic, non-immunogenic, and to have osteoconductive qualities. Furthermore, it bears a striking resemblance to bone apatite [9].

Synthetic hydroxyapatite can be made using calcium sources such as chemicals Ca(NO3)2, Ca(OH)2, and CaCO3, natural materials like limestone, or bio-inorganic materials such as conch shells, bones, seashells, coral, or eggshells [10-12]. The Spiral *Babylon* (*Babylonia spirata*), originally named *Buccinum spiratum* by Linnaeus, is a type of mollusk from the marine gastropod class that holds great potential. It's have high economic value and have considerable potential to be cultivated. This conch can be utilized from the meat to the shell, the meat has been a food source in the Indo-Pacific region for many years, the operculum is utilized for medicinal purposes, while the shell is hard and can be used for industrial purposes and for decoration in the form of ornaments. Thus, this conch are commonly consumed as food in Indonesia, and its shell will cause waste. Compared to synthetic or commercial calcium sources, biowaste-derived calcium is more eco-friendly and cost-effective, though it may require more processing to ensure purity [13].

The sol-gel method has attracted considerable attention due to its advantages, including producing hydroxyapatite powder with relatively uniform grain size, high crystallinity, low processing temperatures, and the capability to generate nano-sized particles [14-16]. Nano-sized hydroxyapatite powder has a high specific surface area, leading to increased activity in chemical and biological interactions within the human body [17]. Hydroxyapatite nanoparticles (nHAp) are nano-sized hydroxyapatite synthesized from spiral *Babylon* shells using the sol-gel method.

One of the fundamental requirements related to bioceramics, in addition to its biotolerance and controlled porosity, is its mechanical properties. The mechanical properties of bone graft materials are expected to be similar to those of natural bone [18]. Mechanical properties can be measured by several parameters. The most commonly used parameters are compressive strength and tensile strength [19–21].

The objective of this study is to determine the compressive and tensile strength values of hydroxyapatite nanoparticles from Spiral Babylon shells (Babylonia spirata) synthesized using the sol-gel method.

MATERIALS AND METHODS

Materials used are nHAp made from Spiral *Babylon* shells using the sol-gel method. A total of 8.52 gs of disodium hydrogen phosphate powder (Na_2HPO_4 98%) was dissolved in 100 ml of aquabides until it reached 0.6 M. Then add 100 ml of $Ca(NO3)2$ solution little by little using magnetic stirring for 30 min until the Ca/P ratio was reached 1.67. After the mixture was formed, the mixture was stirred again for 15 min to trigger precursor reactivity. The solution was left for 24 h at room temperature, then refluxed in an oil bath for 16 h at 70 °C and evaporated into a water bath for 15 h at 100 °C. The resulting gel was dried in an oven and smoothed using a mortar and pestle. The powder that has been ground is then sintered at a temperature of 800 °C. Hydroxyapatite powder was then put into a ball mill at 250 rpm for 30 min. The resulting nHAp powder weighs 2.6 gs with a particle size of 51 nm. The powders were characterized using Scanning Electron Microscopy (SEM), Transmission Electron Microscope (TEM) and X-ray diffraction (XRD).

The dimensions of the specimen were aligned with the specifications of the equipment used. Compressive strength testing was performed on nHAp specimens by molding them using a cylinder mold with an inner diameter of 10 mm and a height of 20 mm. Meanwhile, for the tensile strength testing, the specimen molding was carried out using a polyethylene mold with dimensions according to ASTM E8 standards.

The process of hydroxyapatite casting was carried out by mixing hydroxyapatite powder with a binding agent in a ratio of 70% (nHAp) to 30% (Epoxy resin), 70 gs of hydroxyapatite powder were weighed, and 30 gs of binding agent (20% resin A and 10% resin B). The hydroxyapatite was added to the binding agent and then stirred using a motorized stirrer until the mixture was homogeneous and poured into the mold. The specimen was left for 7 h until it hardened, then removed from the mold. After casting, the specimen underwent a finishing process to ensure all surfaces were even and clean (fig. 1a and b).

a b

Fig. 1: Finished specimen, a) Specimen for compressive srength testing, b) Specimen for tensile strength testing

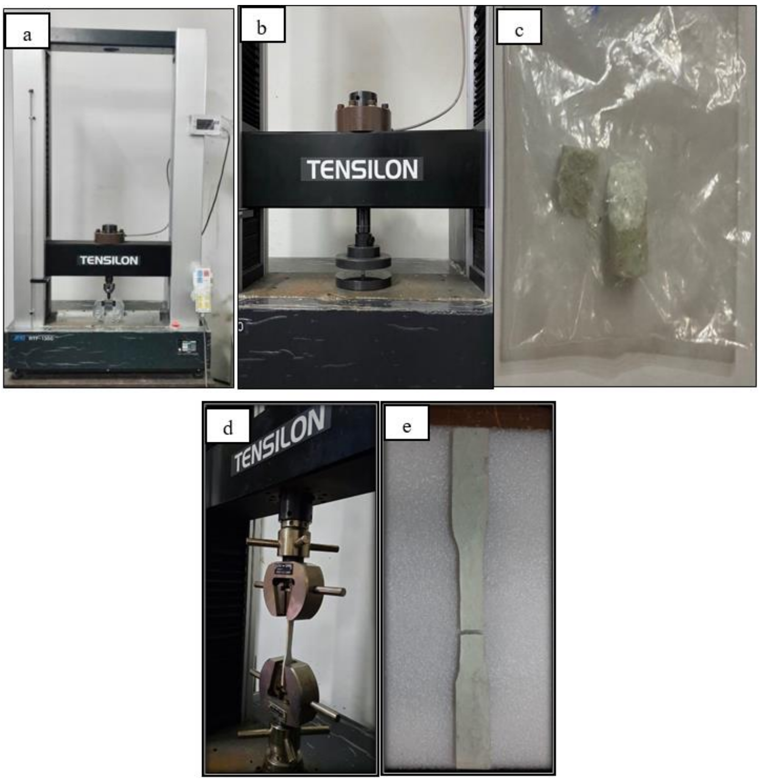

Fig. 2. a) Universal testing machine tensilon RTF-1350, b) The specimen during compressive strength testing, c) Specimen after compressive strength testing, d) The specimen during tensile strength testing, e) Specimen after tensile strength testing

The compressive and tensile strength test was conducted using a universal testing machine, tensilon RTF-1350 type (fig. 2a), with three samples (n=3) for each test. For the compressive strength testing, after the machine was prepared for the compressive test, the sample was placed in the testing area where pressure would be applied with a maximum load of 5 kN (fig. 2b). After that, the UTM was turned on, and pressure was applied at a crosshead speed of 0.5 mm/min. The pressure increased gradually until a fracture occurred (fig. 2c). Once the fracture happened, the pressure automatically decreased, and the values in the form of numbers and curves were displayed on the computer connected to the machine.

For the tensile strength test, after the specimen was put on the UTM, a pull was applied at a crosshead speed of 0.1 mm/min (fig. 2d). The applied force increased gradually until necking occurred (fig. 2e). The force automatically decreased after necking, and the recorded value was the maximum stress when necking occurred. The UTM then automatically calculated values indicating the tensile strength of the material being tested.

RESULTS

Hydroxyapatite nanoparticles obtained Spiral *Babylon* shells were characterized using XRD. The analysis results were then compared with data from the Joint Committee on Powder Diffraction Standards (JCPDS) for interpretation. Spiral *Babylon* shells are characterized by peak intensities at 2θ values of 25.781; 31.900; 32.080; 32.970; 34.020. The analysis results show that the diffraction patterns of the nHAp from Spiral *Babylon* shells have almost the same 2θ values and are close to the values of standard hydroxyapatite. This proves that Spiral *Babylon* shells do indeed contain hydroxyapatite.

Hydroxyapatite nanoparticles powders were then analyzed using SEM and images were taken at 20x magnification. Images of the nHAp surface were taken from several surfaces to observe the range of pore sizes and porosity. The pore size of nHAp Spiral *Babylon* shells has micropores with a diameter range of 23–97 nm, with an average micropore size of 61 nm and porosity ranging from 62–68%, with an average porosity of 65%.

TEM was also used to analyze hydroxyapatite nanoparticles produced from spiral *Babylon* shells, images were captured at magnifications of 5,000, 10,000, 30,000, 50,000, and 100,000 to observe the morphology of the particles and their surfaces. Hydroxyapatite nanoparticles have varying shapes (irregular). The edges of the particles tend to be curved and spherical in shape, with particles of varying sizes, average diameter of 41 nm and a size range of 11 nm to 93 nm.

Results from testing the compressive and tensile strength with The Universal Testing Machine are shown with a curve displayed in the machine.

For the compressive strength, mean of the maximum pressure that can be withstood by the Spiral *Babylon* specimen is 45.73±3.86 MPa. The values ranged from 41.35 to 48.62, indicating relatively homogeneous distribution. As for the tensile strength, it can be seen that the mean of maximum point stress that can be withstood by the specimen was 6.69±0.53 Mpa, with the valued ranged from 6.14 to 7.19 as shown on fig. 3.

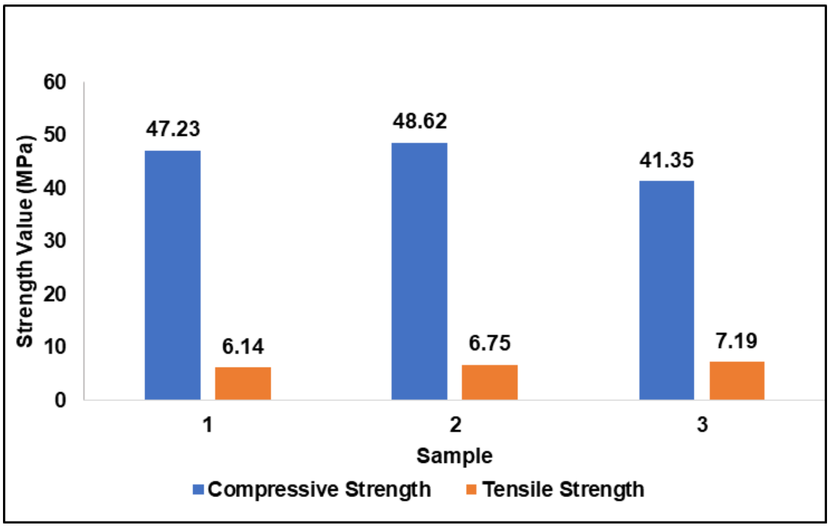

Fig. 3: Graph showing compressive strength and tensile strength values of each sample

DISCUSSION

The illness known as periodontitis inflames the tissues that support teeth, causing the periodontal ligaments and the alveolar bone around them to gradually deteriorate [1]. Using the patient's own tissue or artificial, synthetic, or natural replacements, bone grafting is a surgical procedure that replaces lost bone [22]. Synthetic hydroxyapatite can be produced from basic calcium compounds or natural sources, offering osteoconductive properties. It is non-toxic, non-immunogenic, biocompatible, and closely resembles the apatite found in bone [23]. Hydroxyapatite [Ca10(PO4)6(OH)2] is a biomaterial that shares chemical composition and structural characteristics with the inorganic phases of bone and tooth minerals [24].

This research is a pilot study that aims to determine whether the compressive and tensile strength values of hydroxyapatite nanoparticles (nHAp) from Spiral *Babylon* shells (*Babylonia spirata*), synthesized using the sol-gel method, have the same values as those of the jawbone, either cortical or cancellous. Various methods have been used for the synthesis of HAp powder. One of the most promising technologies for creating multifunctional monoliths, nanocomposites, nanostructured coatings, and nanostructured materials is the sol-gel process [25]. It is chosen because it has the advantage of producing nano-sized HAp particles that are more homogeneous [26].

Nano-sized particles will make bone grafts denser and stronger [27]. Nanoparticles can enhance osseointegration, osteoconduction, and osteoinduction. Nano-tricalcium phosphate (nTCP) and nano-hydroxyapatite have been the most commonly applied materials for bone tissue regeneration [28]. The use of nano-sized particles is also known to enhance the bioactivity of ceramic materials. Additionally, the inorganic phase can act as a reinforcing agent to improve the material's mechanical properties [29].

The perfect synthetic bone substitute should undergo remodeling, support the production of new bone, have minimal fibrotic reactivity, and be biocompatible. Mechanically speaking, the artificial bone graft replacement ought to be as strong as the cortical or cancellous bone that is being substituted [30]. The ability of a material or structure to bear loads that cause them to contract in size is known as its compressive strength. A specific degree of distortion might be thought of as the limit for compressive strength since certain materials fracture at their compressive strength limit while others deform permanently [31].

The stress-strain curve from the compressive strength test of hydroxyapatite nanoparticles from the spiral *Babylon* (*Babylonia spirata*) shows the deformation process of the spiral *Babylon* shells. This process begins with elastic deformation, followed by plastic deformation, eventually leading to fracture [19]. Since these materials are intended to replace real bone, it's critical to keep in mind that cancellous bone has a compressive strength of 2 to 45 MPa, while human cortical bone has a range of 90 to 230 MPa [30].

Hannora and Ataya's research using synthetic nano-sized hydroxyapatite (<100 nm) got a compressive strength value of 40 MPa. When compared to these studies, it can be said that the compressive strength value in this study is not much different [32]. Another study by Siregar and Sulistyowati who used freshwater snail shells as a source of calcium produced hydroxyapatite measuring 0.9135 µm, has a compressive strength value of 9.807-19.61 MPa [33]. Compressive strength variation can be caused due to the presence of pores or gaps in the hydroxyapatite material so that it causes lower mechanical properties [8]. The compressive strength analysis was also conducted by Iis Sopyan *et al.* using synthetic hydroxyapatite measuring 50-200 nm and got a compressive strength value of 1.96 MPa. This value is much lower than the results of this study [34].

The compressive strength of hydroxyapatite nanoparticles (nHAp) varies depending on factors like particle size, composition, and manufacturing process. Badea *et al.* discovered that compressive strength of nHAp is governed by its mechanical properties due to sintering temperature effects, titania addition effects and strength of coarsening suppression [8]. Research by Oktar *et al.* on compressive strength of bovine hydroxyapatite showed that the highest compressive strength was obtained when hydroxyapatite was added with mgo, which amounted to 121Mpa [35]. Clinically, hydroxyapatite with compressive strength values in the range of 20–40 MPa is considered suitable for non-load-bearing bone grafts, such as alveolar ridge augmentation and periodontal defects. The comparable strength values observed in this study suggest that the material could be a viable candidate for such applications, especially where moderate mechanical support and high osteoconductivity are required [36, 37].

Tensile strength is the maximum stress that a material structure can withstand under tension conditions. Testing can be conducted to determine a material's tensile strength. The greatest tensile stress that can be tolerated results from external loading without significant deformation [20]. In other words, compressive strength resists compression (pushing), while tensile strength resists tension (pulling).

The universal tester is the most commonly used machine for testing compressive and tensile strength. In this study, the machine used is from the brand Tensilon. The universal tester can also be used to test bending strength. Its main function is to create a stress-strain curve [36]. For this study, the specimen standard used is the international standard ASTM-E8, by American standards [26]. The analysis method involves calculating the compressive and tensile strength, defined as the force per unit area required to break the specimen. In this study, manual calculations were not performed because the universal testing machine used is computer-based. This allows for paperless curve displays and the ability to electronically store measurements related to the curve [38].

Bones are generally classified into two types: cortical and cancellous. Cortical bone, also known as compact bone, has a higher bone density than cancellous bone [39]. The results of this study indicated that the Spiral *Babylon* Shells' compressive strength value was 45,773 MPa, which is within the category of the compressive strength values for human cancellous bone, which is between 2 and 45 MPa [30]. Meanwhile, the tensile strength value was 6,69 MPa. Carter discovered that the tensile strength of cancellous bone ranges from 3 to 15 MPa [40]. Hence, the tensile strength value of nHAp derived from Spiral *Babylon* shells in this study falls within the range typical for human cancellous bone.

CONCLUSION

Based on this study's findings and considering the compressive and tensile strength values obtained, Spiral *Babylon* shells show potential as a candidate material for alveolar bone grafts. However, further *in vitro* and *in vivo* studies and long-term biocompatibility assessments are needed to fully validate their clinical applicability.

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Nil

AUTHORS CONTRIBUTIONS

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CONFLICT OF INTERESTS

Declared none

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