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Influence of Composition and Lyophilization Time on Physical Properties of HA/Cs/Coll/ Hydroxypropyl Methylcellulose Biocomposites for Bone Scaffolds

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Abstract

Biomaterial implants are one of the alternatives to replace damaged organs in the body system temporarily (scaffolding) or permanently. Bone biomaterial implants can be obtained through the manufacture of HA/Cs/Coll (HA/Cs/Coll) biocompositeses with the addition of Hydroxypropyl Methylcellulose (HPMC) matrix. The objective is to evaluate how variations in material composition and lyophilization time affect the physical properties of the biocomposites, including density, compressive strength, Young's modulus, and surface morphology. The manufacture of biocomposites uses the mechanical thermal method for mixing materials and the freeze drying method for the biocomposites drying process. Composition ratios of HA:Cs/Coll were varied at 3:7, 5:5, and 7:3, while lyophilization durations were set at 24, 48, and 72 hours. Characterizations were performed through density measurements, mechanical testing using a Tensilon machine, and surface morphology analysis using a digital microscope. The results showed that the comparison of biocomposites with a ratio of 7:3 had the highest density of 0.150 gr/cm³, compressive strength of 0.046 MPa, and young modulus of 0.3 MPa. Meanwhile, the biocomposites that was lyophilized for 48 hours showed the best balance between a density of 0.145 gr/cm³, a compressive strength of 0.08 MPa, and a young modulus of 0.17 MPa. Morphological analysis revealed improved porosity and surface uniformity with longer freeze-drying times. Based on this, the resulting HA/Cs/Coll biocompositese has potential as an implant material but further research is needed to improve its mechanical properties by increasing the concentration of the binder, namely HPMC.

Introduction

The use of biomaterials in the health field is growing along with the increasing cases of bone fractures. According to the 2018 Basic Health Research in Indonesia, there were around 5.5 million fractures, with the majority - 67.9% - occurring in the lower extremities. This condition

is most commonly experienced by the elderly, at around 14.5%. Most fracture cases were caused by traffic accidents, with 45,987 recorded. Of these, motorcyclists made up the largest group involved, at 72.2%, followed by motorcycle passengers at 19.2%, pedestrians at 4.3%, non-motorized vehicle riders at 2.7%, car passengers at 1.3%, and car drivers at 1.2% [1]. According to Basic Health Research conducted by the Health Research and Development Agency in 2018, the incidence of fractures in Indonesia was recorded at 5.5% [2]. The fracture condition can cause prolonged pain that radiates to the bones, muscles, and joints around the fracture area. It also has an impact on impaired mobility, sleep quality, and mood, which can sometimes lead to depression in sufferers [3]. One solution to deal with fractures is through bone grafting.

Bone graft is a surgical procedure that involves transplanting bone tissue and implants to repair and reconstruct bone damaged by injury or disease. This process is carried out by transferring bone cells from the donor to the recipient. Bone grafts can come from the patient's own body, artificial substitutes, or natural sources [4]. This procedure involves surgery by transplanting bone tissue or implants to repair and reconstruct bone damaged by injury or disease. Bone transplants can come from the patient's own body (autograft), bone grafts from the same species but genetically different, (allograft) and bone grafts from a species other than humans (xenograft) [5]. Although it has advantages in repairing bone damage, bone grafts also have weaknesses, namely the risk of infection and allergic reactions to graft materials, so bone grafts from biomaterials are an alternative to overcome this [6]. One of the commonly used types of bone grafts is scaffolds. Scaffolds are three-dimensional biomaterial structures that function for the reconstruction of bone damage [7]. Scaffolds are designed to support the formation of specific tissues with the shape, size, and function as needed, and can stimulate the attachment and proliferation of osteoinductive cells on their surface [8]. There are various methods of making scaffolds, such as solvent casting/particulate leaching, freeze drying, electrospinning, gas foaming, thermal-induced phase separation, and other techniques [9].

Freeze-drying is particularly well-suited for bone scaffold fabrication due to its ability to preserve the bioactivity of heat-sensitive polymers and ceramics by avoiding the use of toxic organic solvents. This method allows precise control over pore structure—size, shape, and interconnectivity—through tailored molds and freezing parameters, which are essential for supporting cell adhesion, proliferation, differentiation, vascularization, and biofluid transport [10]. Additionally, freeze-drying minimizes shrinkage and structural deformation, maintaining mechanical stability while producing highly porous scaffolds with superior interconnectivity. These characteristics enhance the scaffold's biological performance and make the method more environmentally friendly and biocompatible. Although the process has high operational costs, it remains advantageous for water-soluble polymers and ceramic-based materials due to its efficiency in water removal, nutrient preservation, and maintaining the material's structural integrity [11]. To further enhance the biological functionality of scaffolds produced through freeze-drying, incorporating bioactive components into the biocomposite formulation is essential.

It has been recognized that an appropriate solution to improve the biological properties of scaffolds is to add other bioactive components to the scaffold biocompositesion.

Hydroxyapatite (HA) ($Ca_{10}(PO_4)_6(OH)_2$) is a material that is often used as a synthetic bone graft because it meets the criteria for ideal structure and biocompositesion. The material has a hexagonal crystal structure with a calcium (Ca) and phosphorus (P) biocompositesion of 39.9% and phosphorus (P) of 18%, as well as a calcium-phosphate (Ca/P) ratio of 1.67, according to the specifications required for bone implant applications [12]. HA is also known for its osteoconductive and osteoinductive properties, as well as its good biocompatibility and bioresorption, so it is able to support the bone regeneration process. However, HA has a major drawback, namely its fragile nature [13], thus limiting its use as a bone implant material if it is not combined with other materials that can increase its strength.

To overcome these weaknesses, Chitosan (Cs) is present as a supporting material that is able to increase the flexibility and strength of HA, while providing important antibacterial and biocompatibility properties in bone implant applications. Cs is an abundant natural polymer and is the only cationic linear polysaccharide [14]. This polymer is the result of a chitin deacetylation process that can be extracted from the exoskeleton of shrimp, lobster, crab, and similar sources [15]. This material is known for its superior properties, such as biocompatibility, biodegradable ability, antibacterial properties, non-antigenicity, anti-inflammatory response, as well as its high antimicrobial ability. Due to these characteristics, Cs is one of the most attractive materials for various biomedical applications, especially in supporting tissue regeneration and improving the weaknesses of implantable materials such as HA.

In addition, the incorporation of collagen (Coll) into biocomposite materials significantly enhances their bioactivity and biocompatibility, with the synergistic interaction between Cs and Coll supporting more effective tissue regeneration and addressing the mechanical limitations of HA. Coll, accounting for approximately 30% of total human protein, exhibits excellent compatibility with human tissues and plays a crucial role in bone healing and maintaining structural integrity [16]. In this study, HA and Cs were synthesized from pearl shells, yielding HA with a Ca/P ratio of 1.67—ideal for scaffold applications. The present research introduces a novel approach by combining naturally derived HA and Cs with Coll and hydroxypropyl methylcellulose (HPMC), using the freeze-drying method to fabricate scaffolds. Research conducted by [17] reported that chitosan/collagen/hydroxyapatite composites with ratios (1:4, 2:3, 3:2, 4:1) produced porosity that decreased as the concentration of chitosan and collagen decreased. However, the compressive strength increased with increasing HA concentration but the durability was lower. The study aims to evaluate the effects of material composition ratios and lyophilization durations on the physical properties—namely density, compressive strength, Young's modulus, and morphology—of HA/Cs/Coll/HPMC biocomposites for potential bone scaffold applications.

Experimental Method

Tools and materials

This experiment used several equipment including a stirring rod, petri cup, beaker glass, measuring cup, *freezer*, *lyophilizer*, magnetic bar, hot plate (DIAB MS-H280-Pro), analytical balance, thermometer, droppipette, volume pipette, wellplate, digital microscope, and RTG Compression test. HA and Cs were synthesized from Pinctada maxima (pearl oyster) shell

powder using a calcination and deacetylation process, respectively. The Cs had a degree of deacetylation (DD) of approximately 85% and a molecular weight of ~300 kDa [18]. Commercial Coll was sourced from Maxfood (Turkey), classified as Type I Coll derived from bovine tendon. Hydroxypropyl methylcellulose (HPMC) was obtained in powder form (viscosity: 4000 cps, Shin-Etsu, Japan). Acetic acid (CH_3COOH, pro analyst grade) was supplied by Mallinckrodt (USA), and distilled water (aquadest) was used throughout the study.

Research Procedure

This biocomposite fabrication experiment was divided into two main stages: (1) the synthesis of biocomposites using a mechanical–thermal method, and (2) the drying process using freeze-drying. Prior to these stages, raw materials were prepared through Cs isolation and HA (HAp) synthesis from Pinctada maxima (pearl) shells. Cs isolation was conducted via sequential steps: demineralization using 1 M HCl to remove calcium carbonate, deproteination with 4% NaOH to eliminate proteins, and decolorization using 0.5% NaOCl to remove pigments that may interfere with the purity and biocompatibility of the final product. The deacetylation process was carried out as a modified version of the method reported by Alaydrus et al. [19], wherein chitin was treated with 40% (w/v) NaOH at 60°C for 2 hours under constant stirring to cleave acetyl groups and obtain Cs with a high degree of deacetylation. This modification enhances the biocompatibility of Cs, ensuring that it is more readily accepted by body tissues and does not disrupt the balance of healthy microbiota. The decolorization step is critical in improving the purity, appearance, and potentially the surface chemistry of Cs, making it more suitable for biomedical scaffold applications.

Furthermore, HA powder was obtained from pearl shells, where the shell powder was calcined at 900^{o}C for 6 hours to obtain CaO powder. The temperature of 900 ^{o}C was high enough to ensure that the reaction was efficient and complete. The resulting CaO powder, 5.6 grams, was mixed with 200 mL of distilled water to produce a 0.5 M $Ca(OH)_2$ solution. Then the solution was added with H_3PO_4 0.3 M by titration method and homogenized for 1 hour at 80^{o}C. After titration, the pH of the solution was maintained in an alkaline state (pH = 10). If the pH was less than 10, 25% PA ammonium hydroxide (NH_4OH) was added. The resulting solution was allowed to stand for 24 hours at room temperature. The resulting precipitate was then dried and calcined at 900^{o}C for 3 hours to produce HAp.

The initial step in the preparation of biocomposites HA/Cs/Coll/HPMC was the dilution of Cs and coll biopolymers were prepared using 2% acetic acid solvent (1.8 grams of Cs and Coll, respectively, were dissolved in 90 mL of 2% acetic acid). The resulting Coll solution was then mixed dropwise with the Cs solution in a 1:1 ratio and homogenized for 2 hours at 60^{o}C. The next step was to make 4% HA solution (8 grams dissolved in 200 mL of distilled water) and then homogenized for 2 hours at 60^{o}C. After that, HAp solution was added drop by drop with the ratio of HA solution: Cs/Coll solution 7:3 and homogenized using a magnetic stirrer at 700 rpm with a temperature of 60 ^{o}C for 2 hours, until a HA/Cs/Coll hybrid solution was obtained. This ratio was used because bone requires more calcium for growth. HA with a higher amount, will provide bone healing stability [19]. Next, 3.3% HPMC was poured little by little into the Cs/Coll/Ha solution and homogenized using a mini hand mixer at 60^{o}C for 1 hour. After the sample was in paste form, freeze drying was carried out at -50 ^{o}C. In the manufacture of HA/Cs/Coll biocompositese, 3 variations of biocompositesion ratio were carried out, namely (A) HA:Cs/Coll ratio of 3:7 with 72 hours of freeze drying, (B)

HA:Cs/Coll ratio of 5:5 with 72 hours of freeze drying, (C) HA:Cs/Coll ratio of 7:3 with 72 hours of freeze drying, and variations in freeze-drying duration at a fixed HA:Cs/Coll ratio of 7:3, including (D) 24 hours, (E) 48 hours, and (F) 72 hours.

Biocomposites Characterization

The resulting scaffolds (biocomposites) were evaluated for organoleptic properties, morphology, density, and mechanical performance. Organoleptic testing was conducted to assess surface characteristics such as color, odor, and physical form. Morphological analysis using a digital microscope was performed to observe surface topology and porosity, while density and mechanical properties were measured to support the morphological findings and assess their relevance to bone graft applications.

Mechanical properties were assessed using a Tensilon-type RTG compression testing machine, operated at a loading rate of 2.5 mm/min with cylindrical samples of 10 mm in height and 5 mm in diameter. All tests were performed in triplicate to ensure reproducibility. The measured parameters included compressive strength, Young's modulus, and elongation. A scaffold material is considered suitable for cancellous bone grafting if it exhibits compressive strength in the range of 2–12 MPa, a Young's modulus between 0.05–0.5 GPa, and a density of 0.05–1.1 g/cm³ [20]. When testing the mechanical properties of the modulus of elasticity (E) can be determined statically by pulling the load at both ends where the applied tensile force is F (Newton) then the material will experience an increase in Length (Δl). The comparison between the increase in Length and Length is first called the stretch and is expressed in the equation:

$$\varepsilon = \frac{\Delta l}{l_0} \tag{1}$$

With ε is a stretch, Δl is an increase in Length (m), l is the initial length (m) and l_0 is the final length (m). The ratio of force to the cross-sectional area at the time of force application is called stress (stress). The maximum tensile stress is the tensile strength a material is defined by dividing the maximum tensile force by the initial cross-sectional area, expressed in the following equation:

$$\sigma = \frac{F_m}{A_0} \tag{2}$$

With is the maximum tensile stress σ (N/m²), A_0 is the cross-sectional area (m²), dan F_m is the maximum tensile force (N). Meanwhile, the amount of Young's modulus is determined by the equation:

$$E = \frac{\sigma}{\varepsilon} \tag{3}$$

With E is the modulus of elasticity or the modulus of young (N/m²) [21]. These mechanical characteristics are critical in evaluating scaffold performance, as mechanical compatibility with host bone ensures structural support and minimizes the risk of implant failure. A scaffold that mimics the mechanical properties of native bone can better facilitate tissue ingrowth and remodeling, supporting the regenerative process effectively.

Result and Discussion

HA/Cs/Coll/HPMC biocomposites have been successfully made using the freeze drying method (figure 1). Based on organoleptic analysis, the resulting biocompositese has a fiber texture and a sharp acetic acid aroma. Variations in biocompositesion affect the color yield of biocomposites. In biocomposites, the ratio of 3:7 and 5:5 (samples A and B) has almost the same color, namely yellowish-white. Meanwhile, the white biocompositese color in the ratio of 7 : 3 (sample C) has a higher HA content than samples A and B.

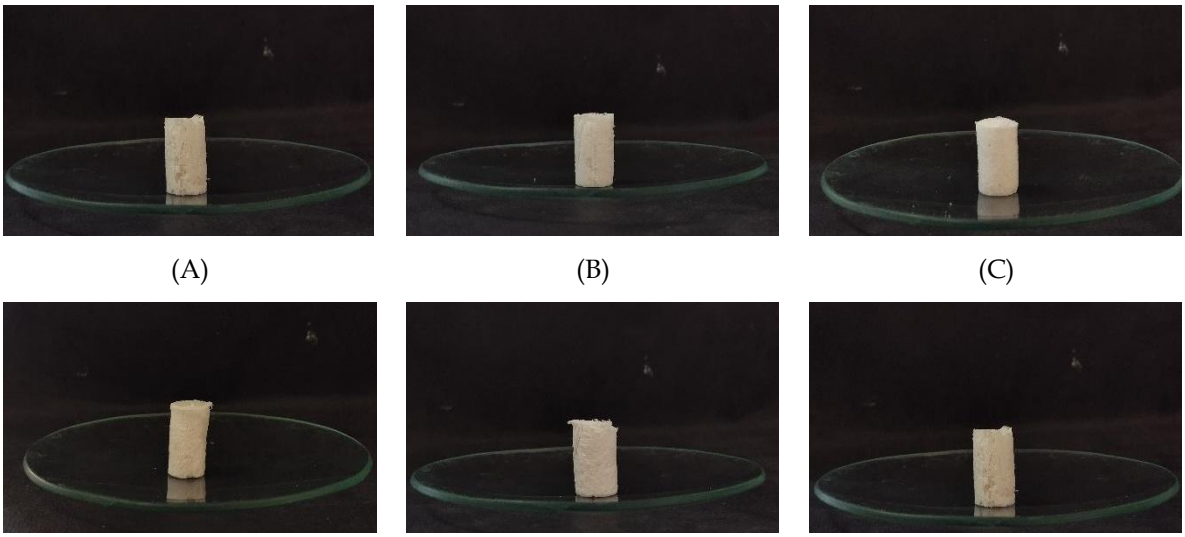


Figure 1. Analysis was conducted on HA/Cs/Coll/HPMC biocomposites prepared with different HA to Cs/Coll ratios and freeze-drying durations, including: (A) HA:Cs/Coll ratio of 3:7 with 72 hours of freeze drying, (B) HA:Cs/Coll ratio of 5:5 with 72 hours of freeze drying, (C) HA:Cs/Coll ratio of 7:3 with 72 hours of freeze drying, and variations in freeze-drying duration at a fixed HA:Cs/Coll ratio of 7:3, including (D) 24 hours, (E) 48 hours, and (F) 72 hours.

According to Cahyaningrum et al, 2017 [22] that an increase in Cs and coll can give it a darker or creamy yellow color. In addition to having a yellowish-white color, samples A and B also have the most hollow structure and many fibers. This is due to the Cs/coll biocompositesion which is more dominant than the HA biocompositesion. Increased Coll concentrations in scaffold manufacturing can increase pore formation thereby providing space for osteoblast cells that serve as new bone formation [23]. However, in sample C, it was seen that the biocompositese structure was denser and had the least fibers and cavities. This suggests that the biocompositesion of HA, Cs and coll can be used to control the physical properties of biocomposites as per the needs of clinical applications. When viewed at the variation of freeze drying time, the resulting color is almost similar and has a drier and coarser fiber structure.

Physical analysis was performed by observing the microstructure of the surface of the resulting biocomposites using a microscope connected to a digital camera at 40x magnification. The morphology of the scaffold bone graft was obtained from the integration of the microscope into the computer. (figure 2).

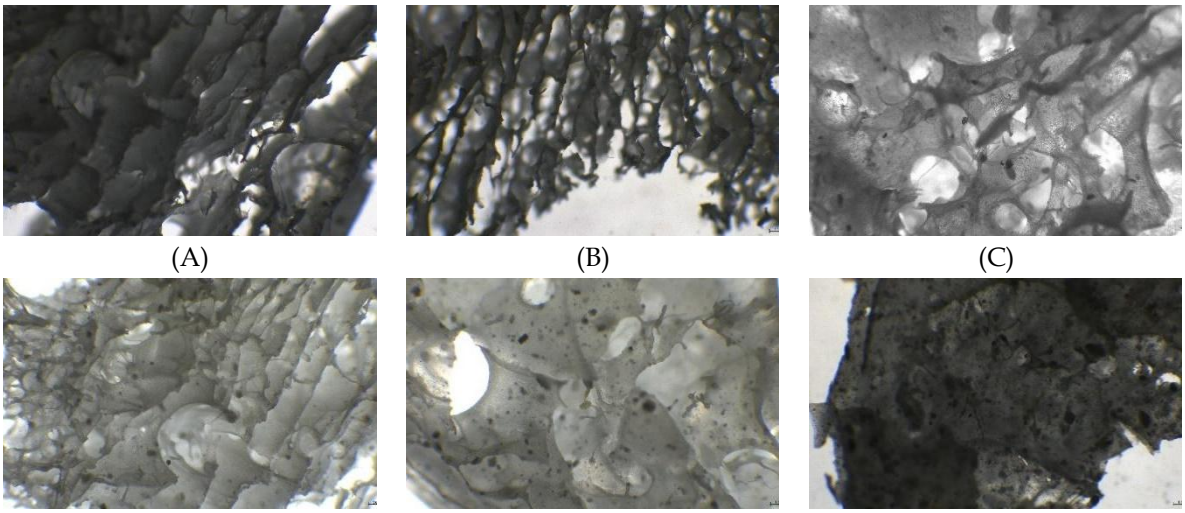


Figure 2. Biocomposites morphology of HA/Cs/Coll/HPMC with (A) HA Ratio: Cs/Coll 3: 7 (B) HA Ratio: Cs/Coll 5: 5 (C) HA Ratio: Cs/Coll 7:3 (D) Freeze Drying Time 24 hours (E) Freeze Drying Time 48 hours, and (F) Freeze Drying Time 72 hours.

The morphological differences of the three samples A, B, and C were identified with the formation of different pores (Figure 2). The difference is due to a greater difference in the biocompositesion of Cs and coll polymers than HA. Sample A has more dominant fibers due to the presence of more Cs/coll compounds so that HA particles are only as fillers that are evenly dispersed when the Cs/coll biocompositesion is higher. Biocompositeses with the addition of Cs/coll that increasingly form larger and more even pores are caused by Cs/coll which has the property of being able to form pores [24]. Then in sample B the comparison of the same biocompositesion between Cs/coll and HA showed that the pores were formed slightly, the C sample of biocomposites with a higher HA biocompositesion had very few pores formed and the size was very small so that it was not visible. This is because the Cs phase is fused as an adhesive between particles and HA as a filler. This phenomenon is further influenced by the presence of coll, which acts as a structural matrix binding HA particles, thereby contributing to the overall density and the low porosity observed in sample C. Coll is effective in forming pores because, during the freeze-drying process, water molecules that freeze into ice crystals push coll molecules toward the edges of the crystals. Upon sublimation, these ice crystals disappear, forming a porous structure that replicates the original crystal morphology, resulting in well-interconnected pores [25], [26]. The pore size can be controlled by adjusting the freezing rate and temperature [26] [27]. In addition to enhancing biocompatibility and osteointegration, coll also plays a role in the mechanical properties of the scaffold, particularly through pore distribution, which affects the material's density and flexibility. Smaller pore sizes, as seen in sample C, tend to increase structural stiffness while reducing flexibility due to a denser and less elastic scaffold structure [28][29]. These findings

suggest that Coll contributes not only biologically but also mechanically, by influencing the microstructural configuration.

The formation of pores in biocomposites is mainly caused by the loss of water molecules in the biocompositese due to the freeze driying (lyophilization) process. Technically, lyophilization causes water molecules to lift up and form pores in the biocomposites. These pores have a function as a space for tissue absorption into the implant so that the regeneration process can take place [30]. The difference in freeze drying time results in a ifferent pore structure for each time variation (Figure 2). Sample D formed an uneven pore structure due to the short drying process so that the water content in the biocomposites has not been thoroughly sublimated. Then in sample E a uniform pore structure and pore size are formed due to the balance of drying time. Meanwhile, in sample F, it shows a larger and uneven pore structure. Therefore, differences in biocompositesion and freeze drying time greatly affect the morphology and structure of the pores formed. Freeze drying time significantly influences scaffold morphology and pore structure through factors like ice crystal growth, annealing time, and sublimation rate [31][25]. Longer freezing durations promote larger ice crystals and more effective annealing, resulting in larger pores and thicker walls, which affect scaffold mechanics and function. In contrast, shorter freeze times yield finer pores with denser microstructures, enhancing strength but possibly reducing cell infiltration and permeability. Sublimation rate also plays a role, altering interconnectivity and wall thickness, which are crucial for scaffold strength, nutrient transport, and cellular support [32][33]

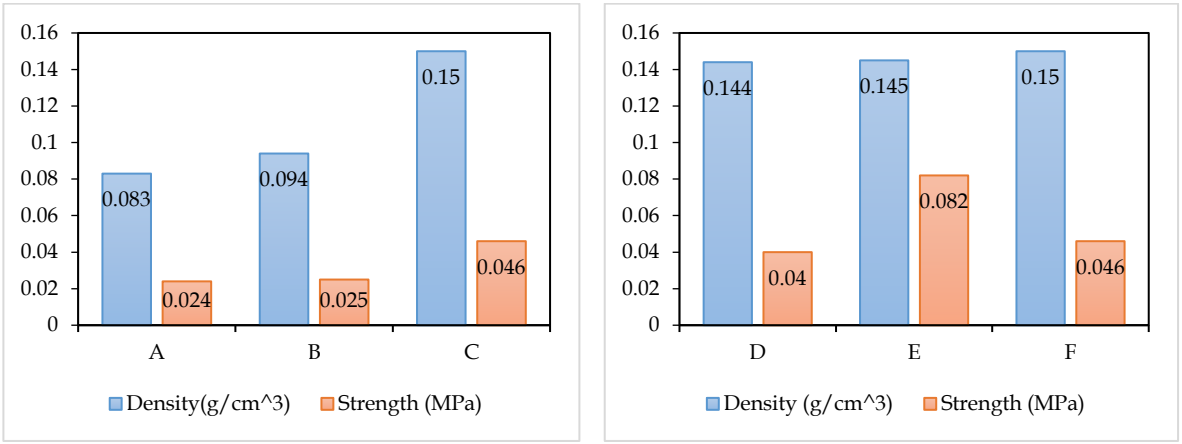
The pore structure in biocompositese is closely related to the quality of biocomposites, especially their mechanical properties and density (table 1). Theoretically, the larger and more pores, the smaller the density and mechanical properties. Sample F exhibited larger pores but had the highest density compared to samples D and E, despite undergoing the longest freeze-drying duration. This may be due to partial pore collapse and microstructural reorganization during prolonged sublimation. Additionally, the uneven pore distribution likely caused most areas to remain dense, thereby increasing the overall density.

Table 1. Density Analysis and Mechanical Properties of HA/Cs/Coll/HPMC Biocomposites

Sample	Density (gr/cm^3)	Strength (MPa)	Young Modulus (MPa)	*Elongation* (mm)	*Strain (%)*
A	0.083 ± 0	0.024 ± 0.006	0.04 ± 0.02	8.19 ± 1.6	48.2 ± 9.04
B	0.094 ± 0.006	0.025 ± 0.009	0.07 ± 0.03	7.33 ± 1.2	43.26 ± 7.04
C	0.150 ± 0.009	0.046 ± 0.007	0.3 ± 0.1	6.8 ± 0.7	40.02 ± 3.86
D	0.144 ± 0.005	0.04 ± 0.01	0.16 ± 0.09	6.64 ±1.3	39.12 ± 7.41
E	0.145 ± 0.004	0.082 ± 0.056	0.17 ± 0.07	8.69 ±3.8	53.84 ± 17.09
F	0.150 ± 0.009	0.046 ± 0.007	0.3 ± 0.1	6.8 ± 0.7	40.02 ± 3.86

In addition to density, the analysis of mechanical properties was also identified, including compressive strength, young modulus, elongation, and strain. The results of the analysis showed that the density of the tendencies was directly proportional to the compressive strength value (figure 3). However, in the HA/Cs/Coll 7:3 biocompositese with variation of freeze drying time,

the most optimal value was analyzed in sample E. This condition occurs because at a drying time of 48 hours the most optimal sublimation occurs (Figure 4).



(a) (b)

Figure 3. Analysis of Mechanical Properties of HA/Cs/Coll/HMPC Biocomposites (a) Variation in Biocompositesion (b) Variation in Freeze Drying Time

Based on data analysis, it was also produced that the young modulus was inversely proportional to the elongation and strain values (table 1). The values of density, compressive strength, and young modulus tend to increase with increasing concentrations of HA because they have a stable and rigid crystalline structure, resulting in denser biocomposites. The formed density can increase the density of the material and improve mechanical properties such as compressive strength and Young's modulus, but biocomposites are less flexible which is reflected in decreased elongation and strain.

Analysis of the effect of freeze drying time on the density and mechanical properties of the biocomposites is also very important to determine the most effective time to obtain the best characteristics. Based on the results obtained, biocomposites E shows the most optimal density and compressive strength values compared to other biocomposites (Figure 4).

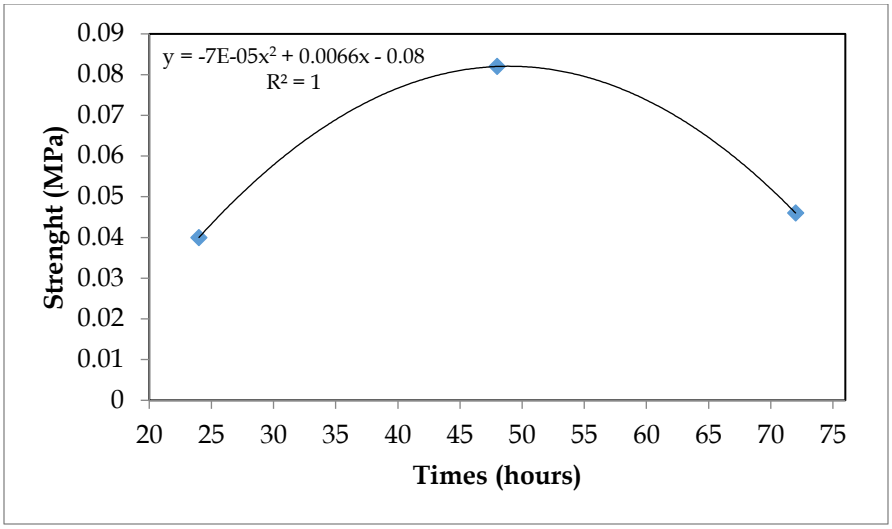


Figure 4. Analysis of the optimum freeze drying time of H/Cs/Coll/HPMC biocomposite

Longer freeze drying times are likely to increase the compactness of biocompositese structures, which will have an impact on increasing density and mechanical strength. Conversely, a shorter time is likely to result in a more fragile or less dense structure. Meanwhile, in the E sample which has the highest compressive strength value (0.082 ± 0.056 MPa) and the largest elongation (8.69 ± 3.8 mm), it indicates a balance between strength and flexibility. This can be attributed to the freeze drying process which allows the formation of optimal pore structures, resulting in biocomposites with good mechanical properties. Therefore, the freeze drying time can affect the pore structure and mechanical properties of the biocomposites. A more regular and controlled pore structure at a given duration increases density and compressive strength. However, a duration that is too long or too short can result in a change in pore distribution that negatively impacts the flexibility of the material. In addition, higher densities do not always result in optimal mechanical properties due to the balance between porosity and material distribution [34]. Thus, the freeze-drying time resulting in sample E can be considered as the optimal condition for achieving balanced mechanical properties and flexibility in this biocomposites. The results of the HA/Cs/Coll biocomposites research obtained when compared with the characteristics of cancellous bone, the HA/Cs/Coll biocomposites meets the density standards of human cancellous bone in the range of 0.05-1.1 gr/cm3 but the compressive strength and young modulus values are lower than those of cancellous bone, namely 2-12 MPa, and 0.05-0.5 GPa. So further research is needed to examine the arrangement of material biocompositesion in the manufacture of scaffolds so that it can produce mechanical properties that resemble the mechanical properties of concelleus bone to be effective as a biomaterial for bone scaffolds.

Conclusion

Biomaterial as a *bone graft* was successfully fabricated from biocomposites of HA, Cs, and Coll with HPMC reinforcement. The resulting biomaterial has a hollow, fibrous structure and a yellowish-white to white color, depending on the HA content. Biocomposites with higher HA content have an effect on their density and mechanical properties. The difference in the biocompositesion of biocomposites formation greatly affects the color, morphology, density, and mechanical properties of HA/Cs/Coll/HPMC biocomposites. Biocomposites with a

ratio of HA:Cs/Coll 7:3 produce the best characteristics, namely porous morphology with few pores, density 0.150 ± 0.009 g/cm3, compressive strength 0.046 ± 0.007 MPa, and young modulus 0.3 ± 0.1 MPa. Because HA is the main inorganic phase in human bone, it is able to stimulate osteoblast differentiation and support the formation of new bone tissue. Therefore, scaffolds with this ratio are suitable for application to bone tissues that require moderate mechanical support, such as maxillofacial bone or trabecular bone. However, the high HA content may reduce flexibility and inhibit the natural degradation of the scaffold in the body, which may slow down tissue integration. Even the freeze drying time also greatly affects the characteristics of HA/Cs/Coll/HPMC biocomposites with optimal conditions at 48 hours of treatment. This biocomposites produces a porous morphology with uniform pore size, density 0.145 ± 0.004 g/cm3, compressive strength 0.082 ± 0.056 MPa, and young modulus 0.17 ± 0.07 MPa. Therefore, these parameters can be used as a starting reference in making bone grafts that are safe for the human body. However, these results are still below the set medical standards of 2-12 MPa for compressive strength, 0.05-0.5 GPa young modulus value and 0.05-1.1 g/cm^3 for density, so further research related to mechanical properties is needed even to in vitro and in vivo testing to produce the ideal bone graft.

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