Journal of Dentistry and Dental Practices
ISSN: 2689-5994

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*Research Article* *Volume 6 Issue 2*

# Characteristics of Type II Dental Gypsum Hydroxyapatite (DGHA II)-Gelatin Scaffold Using Freeze-Drying Method in Different Composition Ratios

**Yustisia Y[1]*, Noviyanti NW[2], Sekarini GA[3], Ardhiyanto HB[4] and Soesetijo FXA[5]**

[1]Department of Oral Biology, Faculty of Dentistry, Universitas Jember, Indonesia
[2]Faculty of Dentistry, Universitas Jember, Indonesia
[3]Faculty of Dentistry, Universitas Jember, Indonesia
[4]Department of Biomedic, Faculty of Dentistry, Universitas Jember, Indonesia
[5]Department of Prosthodontic, Faculty of Dentistry, Universitas Jember, Indonesia

***Corresponding author:** Yenny Yustisia, Department of Oral Biology, Universitas Jember, Kalimantan I/37 Jember, Indonesia, Email: yennyyustisia.fkg@unej.ac.id

**Received Date:** December 12, 2024; **Published Date:** December 18, 2024

## Abstract

Hydroxyapatite (HA) is commonly used as a scaffold material for bone substitutes. It can be synthesized from type II dental gypsum to produce Dental Gypsum-Hydroxyapatite II (DGHA II) through a hydrothermal process. DGHA II can be combined with gelatin (GEL) to create a porous HA scaffold with suitable mechanical properties, as both materials are expected to mimic the structure and function of natural bone. This study prepared porous scaffolds by mixing varying amounts of DGHA II with GEL solution in ratios of 2:3, 3:3, and 4:3, followed by processing in a freeze dryer. The characteristics of the scaffolds, including morphology, porosity, and mechanical properties, were analyzed. Scanning Electron Microscope (SEM) micrographs revealed that the morphology in all groups exhibited irregular pore edges, a connected pore structure, and a non-homogeneous pore size distribution. The DGHA II-GEL (2:3) scaffolds displayed the largest pore diameter (146.23 – 515.44 μm), pore area (5112.53 $\mu m^2$), and porosity (90.44% ± 1.65) compared to the other compositions. Reversely, the DGHA II-GEL (4:3) scaffolds exhibited the highest compressive strength (2.48 MPa) with an elastic modulus of 5377.82 MPa.

**Keywords:** Characterization; Dental Gypsum; Gelatin; Hydroxyapatite; Scaffold1

## Introduction

In dentistry, bone graft is one of the most commonly used reconstruction materials for bone defects involving massive bone loss, such as cleft palate, mandibular resection, alveolar cleft, and periodontitis [1-3]. Autograft and allograft are still considered the ideal treatment. Still, some disadvantages have been reported for these methods, such as increased patient morbidity, immune rejection, high cost, and limited availability. To overcome those disadvantages, synthetic materials need to be developed to meet the clinical demands [4].

Hydroxyapatite (HA) ($Ca_{10}(PO4)_6(OH)_2$) is one of the most popular synthetic materials that can be used as bone graft due to its mineral structure, which is similar to the mineral phase in bones and teeth. HA has good biocompatibility with hard tissues and high osteoconductivity and bioactivity [4,5]. HA can be synthesized from many sources, including gypsum [6]. In a previous study at the Faculty of Dentistry, Universitas Jember, hydroxyapatite materials were developed from used-type II dental gypsum using the hydrothermal method (DGHA II). Despite their different morphology and size, DGHA II showed characteristics like commercial hydroxyapatite [7]. It made DGHA II one of the hydroxyapatite alternatives that can be used in the field of bone regeneration.

As a potential bone graft material, hydroxyapatite needs to be developed to become a three-dimensional scaffold that functions as a synthetic framework implanted in tissues, a place for cell attachment, proliferation, and differentiation to form the extracellular matrix. The scaffold should meet specific requirements, which are biocompatible, support osteoconduction, have sufficient degradation time, have an interconnected porous structure, and exhibit appropriate mechanical support. Scaffold porosity is more than 90 percent, and pore diameter ranging between 200 and 400 µm is considered adequate, where the porosity, pore size, and interconnection play an essential role in increasing cell integration and nutrient exchange and waste exchange in tissue engineering. Well-suited mechanical strength is also necessary to provide structural support for load-bearing applications [8-10].

As a single material, the HA scaffold has low mechanical strength. To improve the properties, HA can be combined with other materials, such as gelatin [11]. Gelatin (GEL) is a type of protein produced by partial hydrolysis of collagen-containing from animal skin, tendons, cartilage, and bones. Gelatin is a partially derived product of collagen comprised of Arg-Gly-Asp (RGD) sequences, typically found in the extracellular matrix. Therefore, it allows for cell adhesion, attachment, and cell spreading more easily in a biocompatible manner [12]. The addition of elastic gelatin can increase tensile strength, compressive strength, and resistance to high fractures, shorten the degradation time, and increase its bioactivity. GEL homogenizes well with HA in aqueous solution and has a strong affinity due to its hydrophilicity [13]. A scaffold composite of HA and GEL is expected to have similar structures as natural bone and show increased osteoconductivity and biodegradation together with sufficient mechanical strength where the composition ratio can affect the characteristics of the scaffold.

Although hydroxyapatite developed from used-type II dental gypsum has characteristics similar to commercial ones, the scaffold made from DGHA and gelatin has not yet been evaluated. One of the essential parameters in scaffold fabrication is the composition ratio since it will determine the characteristics of the scaffold. Therefore, in this study, we fabricated a scaffold from DGHA II and gelatin using the freeze-drying method and observed the scaffold's mechanical properties and porosity.

## Material and Method

### Preparations of DGHA II-Gelatin Scaffold

In this experiment, three composites of DGHA II-GEL scaffold were prepared by mixing fixed amounts of gelatin (SIGMA) 375 mg with three different amounts of DGHA II (250 mg, 375 mg, 500 mg). DGHA was synthesized as in our previous study7. GEL powder was dissolved in 5 mL water (45ºC) using a beaker glass on a magnetic stirrer and mixed for 10 min until the GEL solution was homogenous. The respective amount of DGHA II powder (250 mg, 375 mg, and 500 mg) was added to the GEL solution and stirred with a sonic homogenizer for 6 min. The mixtures were then injected into a teflon mold with diameters of 5 mm and 8 mm with heights of 10 mm. The mixtures were frozen at -60$^0$C for two hours and freeze-dried for 24 hours at −70°C.

### SEM Characterization

The scaffolds were cut transversely and longitudinally by razor and then were gold-sputtered. The morphology and microstructure of the scaffolds were examined using SEM FEI Inspect-S50 at 20 kV in 250x and 500x magnifications. The pore diameter and pore area of scaffolds were measured using ImageJ software.

### Porosity Measurement

The porosity of the scaffolds was measured using a liquid displacement method. A scaffold (diameter of 8 mm and height of 10 mm) was immersed in a graded cylinder containing a known volume (V1) of ethanol. The cylinder was placed in a vacuum chamber to force the ethanol into the pores. The scaffolds were immersed until no air bubbles emerged. The total volume of the ethanol and scaffold was then recorded as V2. The volume difference (V2 - V1) was the volume of the skeleton of the scaffold. The scaffold was removed from the ethanol, and the residual ethanol volume was measured as V3. The porosity of the scaffold (ε) was evaluated using equation:

$$\varepsilon = \frac{V1 - V3}{V2 - V3}$$

### Mechanical Properties

Each sample was cylindrical, with a diameter of 5 mm and a height of 10 mm. All were tested using the Shimadzu Universal Testing Machine with a 1 mm/min cross-head to determine their compressive strength and modulus elasticity. The load

was applied until the scaffold was cracked. The results of this test were then converted to the Megapascal formula:

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

## Results

In this study, DGHA II-GEL scaffold was prepared with a ratio of 2:3, 3:3, and 4:3 using a freeze-drying method. The Scaffolds have a white cylindrical appearance with a height of 10 mm and diameter of 8 mm and 5 mm (Figure 1). SEM micrographs of the transverse cross-section scaffold showed pores with non-homogenous size and irregular shapes (Figure 2). It can also be seen from the longitudinal cross-section (Figure 3) that the pores were formed in the same direction as the vacuum direction in the freeze-drying process. The interconnectivity of the pores was shown but at a low level.

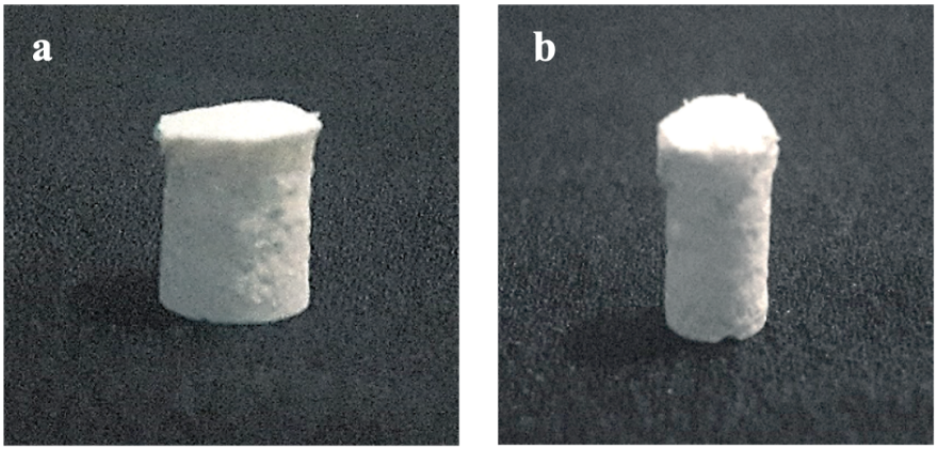


**Figure 1:** DGHA II-gelatin scaffold with a cylindrical shape A: diameter of 8 mm and height of 10 mm; B: diameter of 5 mm and height of 10 mm.

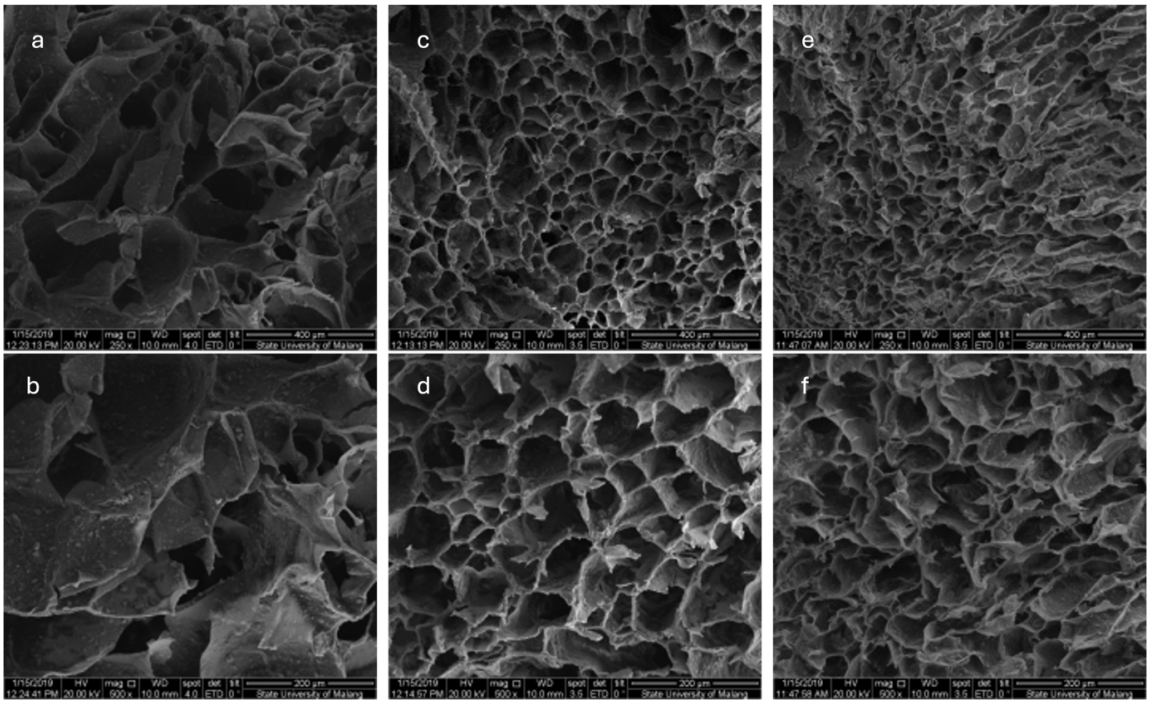


**Figure 2:** SEM micrographs of scaffold (transverse cross-section) a. DGHA II-GEL (2:3) 250x; b. DGHA II-GEL (2:3) 500x; c. DGHA II-GEL (3:3) 250x; d. DGHA II-GEL (3:3) 500x; e. DGHA II-GEL (4:3) 250x; f. DGHA II-GEL (4:3)500x.

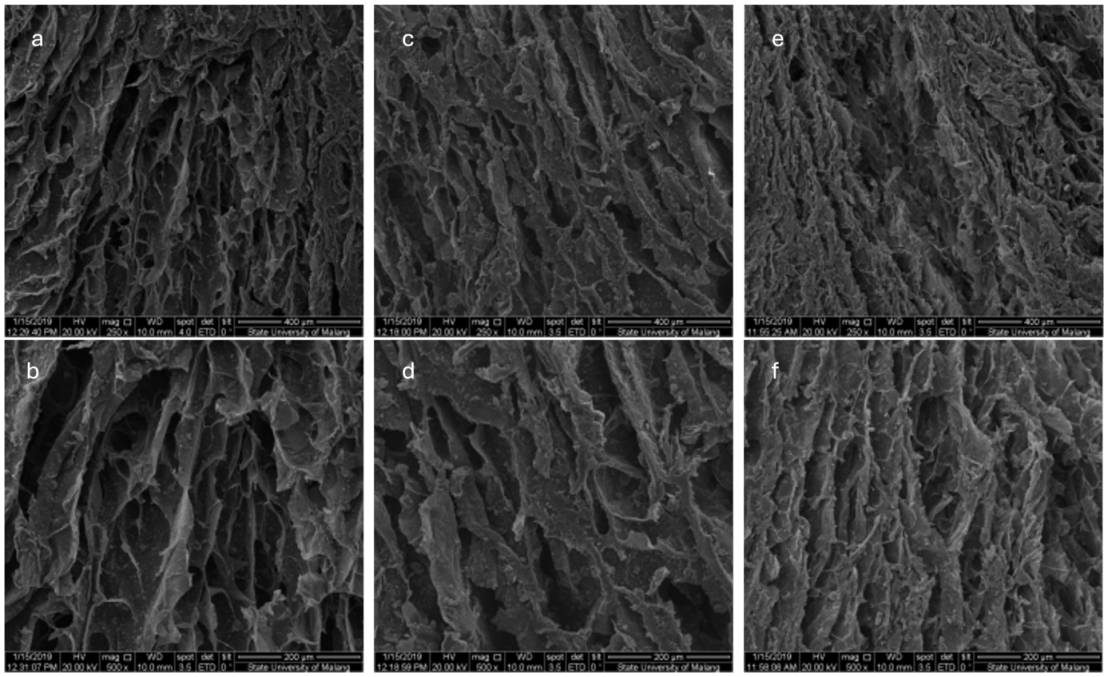


**Figure 3:** SEM micrographs of scaffold (longitudinal cross-section) a. DGHA II-GEL (2:3) 250x; b. DGHA II-GEL (2:3) 500x; c. DGHA II-GEL (3:3) 250x; d. DGHA II-GEL (3:3) 500x; e. DGHA II-GEL (4:3) 250x; f. DGHA II-GEL (4:3)500x.

| | DGHA II-GEL (2:3) | DGHA II-GEL (3:3) | DGHA II-GEL (4:3) |
|---|---|---|---|
| Pore Diameter | 146.23 – 515.44 µm | 93.86 – 262.87 µm | 78.81 – 141.22 µm |
| Pore area | 5112.09-49379.53 $\mu m^2$ | 3418.85-10619.37 $\mu m^2$ | 2161.56-8998.44 $\mu m^2$ |

**Table 1:** Pore diameter and Pore area of DGHA II-gelatin scaffold in different composition ratio.

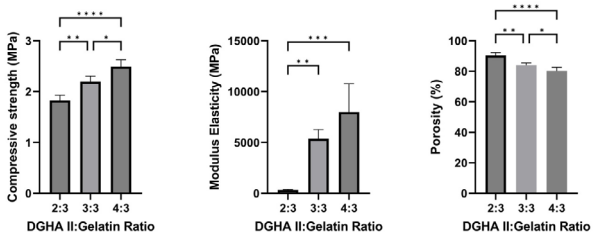


**Figure 4:** Compressive strength, Modulus Elasticity, and Porosity of DGHA II-Gelatin Scaffold in different composition ratio.

Using the software ImageJ, the pore size and area measurement showed a wide range, especially in low HA groups (Table 1). DGHA II-GEL (2:3) scaffold showed the highest pore diameter (146.23 – 515.44 µm) and pore area (5112.09-49379.53 $\mu m^2$). The low HA ratio group showed a higher porosity degree compared to the high HA ratio group. DGHA II-GEL (2:3) scaffold has 90.41 % ± 1.84 of porosity, while DGHA II-GEL (3:3) has 84.08 % ± 1.44 of porosity and DGHA II-GEL (4:3) has 80.21 % ± 2.29 of porosity.

The compressive strength was highest in the group with the high HA ratio, specifically the DGHA II-GEL (4:3) scaffold, which measured 2.48 MPa. This was followed by the DGHA II-GEL (3:3) at 2.19 MPa and the DGHA II-GEL (2:3) at 1.82 MPa. The elastic modulus across all groups ranged from 348.83 to 7994.41 MPa, with the highest HA ratio group exhibiting the most significant elastic modulus (Figure 4).

## Discussion

The different composition ratio of DGHA II-GEL scaffold produces different characteristics. In the SEM micrographs, the entire scaffold group showed morphology: irregular pore edge shape, non-homogeneous pore size, and connected pore structure. Non-homogeneity of the pores was also indicated by the wide range of pore size and pore area measured by ImageJ software. Irregular edge conditions can be assumed due to the freezing temperature used in this study. At a temperature of -60°C for two hours, ice crystal dendrites formed, which will cause irregular shapes of the edges. This condition follows Libbrecht [14], where the freezing

phase formed an ice crystal nucleus in the scaffold, and the hot temperature and particles in the crystal moved toward the edges, forming small lumps that would extend towards the nucleus so that the edges of the ice crystals appeared irregular.

Non-homogeneous pore size can be assumed because, during freezing for two hours with a temperature of -60°C, the temperature on the scaffold will drop, causing irregular shape of ice crystal dendrites. In addition, according to Hariyadi [15], the cooling process first occurs on the side and then continues to the middle. This condition may affect the homogeneity of the ice crystal size. During freeze-drying, the ice crystals will sublime and leave the cavities, forming the interconnecting pores.

Another factor that might cause different pore sizes was the DGHA II properties, which have non-homogeneous particle size [7]. DGHA II particles, whose molecular weight is heavier than that of the GEL solution, will settle at the base of the Teflon mold. DGHA II particles, whose molecular weight is heavier than that of the GEL solution, will settle at the base of the Teflon mold. So, the most binding DGHA II is the bottom of the scaffold; therefore, in the future, homogeneous shaking is needed when placing the scaffold in the freezing phase so that the resulting pore size can be more homogeneous.

The pore diameter average and area of pore scaffold DGHA II-GEL (2:3) were higher than scaffold DGHA II-GEL (3:3) and DGHA II-GEL (4:3). These results showed that less HA ratio would cause the GEL matrix did not bind DGHA II as filler material and failed to construct, resulting in large pore size. In this study, DGHA II particles act as filler material that will spread and be bound by the GEL matrix into a pore wall. If the amount of DGHA II is low, then the bond between filler and matrix is also less, and filler density is low, causing the size of the wall not to be thick and the pore to become large [16]. Razali, et al. [17] support the idea that the addition of HA will affect the pore size; more HA will produce a smaller pore size.

The ideal pore size of the scaffold for bone regeneration is 100-300 μm. The pore size of the scaffold greatly influences the continuity of proliferation and angiogenesis in tissues for bone regeneration. Pore size is expected to be following the physiological size of cells and blood vessels so that it can be an ideal place for proliferation. Small pore diameter will cause hypoxic conditions and induce osteochondral formation before osteogenesis occurs. Large pore size will be a place of vascularization that will induce osteogenesis directly [18]. In this study, the pore size produced by the whole group is in the range of 78.81-515.44 μm, so it shows characteristics that can potentially be used as bone graft material because the size of the scaffold pores must be able to support the angiogenesis process to cause vascularization for the recovery process network. The group closest to the ideal pore size of the scaffold is the DGHA II-GEL (3:3). However, there are still weaknesses, namely, the pore diameter is still not homogeneous and wide variation, so further research is needed to obtain the appropriate pore diameter.

In the porosity test, the DGHA II-GEL (2:3) scaffold has the most significant percentage of porosity compared to other groups. This condition can be assumed due to the composition ratio of DGHA II and GEL. Less DGHA II will cause higher porosity because the filler particle density in gelatin as a matrix becomes low. As the function of the filler itself, it acts as a matrix-filling material that adds dimensional stability. The more DGHA II is added, the spread on the matrix will be evenly distributed, and the density will be higher, causing the pore wall to be thicker, resulting in lower porosity. Also, the nature of gelatin, which absorbs water during cold freezing, causes the water in the gelatin to sublime so that a higher gelatin ratio impacts the higher porosity [19]. The ideal porosity is around 90%, with interconnected pores that discharge metabolic waste and transport nutrients. In this study, all groups had the ideal porosity percentage, ranging from 81.57 to 90.44%. This also corresponds to cancellous bone porosity of 30-90% [20].

The scaffold strength of DGHA II-GEL (2:3) has the most minor results compared to other groups. HA at a low ratio (2:3) showed lower compressive strength than a higher HA ratio. This result might occur due to low HA particles that act as fillers and cannot spread evenly on the pore wall, affecting the scaffold's dimensional stability and compressive strength. Otherwise, a higher ratio of HA has a greater compressive strength. HA was more evenly distributed, binding complexly with the Gelatin matrix and increasing compressive strength. The compressive strength might also be related to the scaffold's pore size and porosity degree. Low scaffold porosity can increase its compressive strength because the structure of the scaffold becomes denser due to its composition ratio. The compressive strength of cancellous bone is 2-12 MPa, and cortical bone is 30-160 MPa [20]. All groups still showed compressive strength below the natural bone.

The modulus of elasticity of DGHA II-GEL (2:3) is smaller than DGHA II-GEL (3:3). This can be assumed because the increasing number of DGHA II will cause a high modulus of elasticity. The filler function is a strength-support for the composite because the voltage applied to the composite will be received first by the matrix and will transfer the load to the filler, which will hold the pressure up to the maximum load. In this study, DGHA II has a higher modulus of elasticity than gelatin, so the modulus of elasticity in composites with more DGHA II composition is also higher. The modulus of elasticity of DGHA II-GEL (3:3) is higher than DGHA II-GEL

(2:3) because the amount of DGHA II used is more, according to Razali et al. [17] more use of hydroxyapatite will cause a higher modulus of elasticity. However, in this study, the modulus elasticity of the DGHA II-GEL (4:3) scaffold is smaller than the DGHA II-GEL (3:3) scaffold; this is not suitable for the theory. These results are likely due to the inhomogeneity of the scaffold pores. The elastic modulus obtained in all groups ranged from 348.83 to 7994.41 MPa, which was still too high when compared to the modulus elasticity of compact bone (17-18.9 MPa) and trabecular bone (5-150 MPa) [21]. Therefore, further research is needed to achieve mechanical properties ideal to meet the requirements as a scaffold for bone substitutes. The additional parameters that can increase the mechanical properties can also be considered.

## Conclusion

Scaffold DGHA II-gelatin made by the freeze-drying method in different composition ratios has distinct characteristics. Scaffolds showed pores with non-homogenous size and irregular shapes, whereas the low DGHA ratio group showed higher pore diameter, pore area, and porosity degree but lower mechanical properties compared to the high DGHA ratio group. Further experiments are needed regarding the scaffold composition ratio of DGHA II-GEL to produce scaffolds with porosity and mechanical properties that are more suitable for clinical purposes.

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