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# Development and Characterization of Hybrid Film Made of Hydroxyapatite, Poly Vinyl Alcohol and Gelatin for Biomedical Application

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## Abstract

In the recent research field of bone tissue engineering, polymeric materials play an implacable role in mimes the natural behavior of hard and soft tissues. In some medical conditions such as diabetics, osteoarthritis, burns, or joint replacement conditions, this polymeric materials implication enhances the internal mechanical activities which result in the early recovery of disease by facilitating the wound healing process. In this study, hybrid films have been synthesized based on polyvinyl alcohol (PVA), gelatin, and gelatin with glycerin incorporated with different concentrations of pre-prepared hydroxyapatite (HAP) by solution casting method at room temperature in biosafety cabinet. Glutaraldehyde has been added as a crosslinker in this whole procedure. The mechanical property, swelling, and porosity percentage have been conducted to characterize the structural stability of the synthesized hybrid films. Porosity and swelling of samples are also represented by proper biocompatibility (>90% porosity and swelling in DDW and PBF vary between 287%~72%). Tensile strength (TS), E modulus (Young's modulus), Elongation at maximum, and Elongation at break are observed to perceive the mechanical properties of hybrid film samples, which are compatible with mechanical properties of different tissue such as trabecular bone, articular cartilage, tendon, nerve and skin tissue. Though, biocompatibility tests both *in vivo* and *in vitro* are essential for clinical application in the future. However, the experiment carried out till now explains the true possibility of newly synthesized hybrid films for long-term drug delivery directly on wound sites for wound healing and burn dressing patients in head-neck surgery reconstruction, diabetic gangrene foot, as well as cartilage or joint replacement therapy.

**Keywords**

HAP, PVA, Gelatin, Swelling Test, Tensile Strength

## 1. Introduction

In today's advanced medical science, history is combined with multidisciplinary sectors such as tissue engineering, biomaterials, molecular biology, regenerative medicine, and so on. Due to the increasing life expectancy of humans as well as modern civilization's demands for more advancement in medical science, for more than half a decade, polymers have been used in the practice of preparation of biomaterials. There are two main types of polymers for biomaterial preparation: natural and synthetic. Synthetic polymers are more advantageous than natural as they cause less allergic reactions, and contamination. Less immunogenic, controlled biodegradability, and improved mechanical properties [1].

In various sectors of tissue engineering, for instance, bone, cartilage, tendon, skin tissue engineering, wound dressing, and drug delivery polymeric biomaterials are used significantly, among them polyvinyl alcohol, and gelatin is one of the majors [2]. PVA was first studied in medical applications in 1951 as plombage material for thoracic surgery [3]. Hydroxyapatite (HAP) is another major competent biomaterial used in tissue engineering for medical applications since 1950. According to different research articles, doping or modification with HAP was commenced in 1995 for the purpose of diverse tissue engineering application, and further research is still continued [4]. The vast application is included multiple tissue engineering techniques among them bone tissue, skin tissue, bone fixation, periodontal, cartilage, blood vessel, liver, tendon/ligament, and corneal regeneration can be mentioned. HAP is white colored mineral form of calcium apatite with general formula $Ca_{10}(PO_4)_6(OH)_2$ with a Ca/P ratio of 1.67 and contains a hydroxyl end member of the complex apatite group. The researcher has used a variety of methods to synthesize hydroxyapatite in laboratory among them wet-chemical precipitation method is a simple and commonly used method that has high scalability [4] [5]. Mechanical properties like tensile and compressive strength of HAP are relatively low, which is one of the major disadvantages of this competent biomaterial. On the other hand, Polyvinyl alcohol (PVA) has relatively higher mechanical strength than HAP with higher water solubility, and insulating behavior that are considered the limitation of the application of this polymer [6].

On the basis of polymer crystallinity, both crystalline and amorphous states of gelatin coexist. The crystalline form has a defined pattern, but the amorphous form follows no defined shape. Owing to these crystalline properties, when gelatin combines with other biomaterials brittleness has been observed [7].

In the field of tissue engineering, control drug delivery systems, and wound healing, porous biopolymers are necessary for proper biomedical application. There are various factors responsible for the design of scaffolds in tissue engineering and

repair application among them degree of porosity as well as interconnectivity of pores are essential. Diffusion of oxygen, nutrition, metabolites, growth of tissue, cell-cell interaction, proliferation, cell-carrier substance saturation into and through the scaffolds all rely on these important factors [8] [9]. Cartilage tissue formation depends on porosity, and on the basis of a study by Spiteri *et al*., about four times more cell attachment is provided by highly porous substrates than by less porous substrates [10]. Stiffer the scaffolds, whereas less porosity persists. Micro and nanofibers scaffolds provide 84% - 90% porosity, conversely, sponge scaffolds have porosity in the range of 48% - 95% [8]. A study on poly (chitosan-*g*-lactic acid) (PCLA) scaffold where porosity was measured by ethanol volume displacement method and results showed that the porosity of the PCLA scaffolds decreased with an increase in LA/CS (lactic acid/chitosan) feed ratio [9]. Glutaraldehyde as a crosslinker was used in chitosan scaffolds for the cultivation of human dermal fibroblast where the average porosity was found about 93% ± 12.57% [11]. Around 70% porosity was observed in the solvent-casting method combined with freeze-drying of HAP/gelatin composite scaffolds for bone tissue engineering [12]. For the purpose of wound healing, the high porosity of materials helps in wound exudate absorption and endorses the exchange of substances between cells. Gelatin/CS/Ag composite was developed to meet this purpose and a maximum of 85.13% ± 2% porosity was found with the rise of silver nanoparticles in the composite [13]. Chitosan/starch/halloysite nanotubes ternary nanocomposite films represented percentage porosity in the range between 89.22 ± 0.61 and 91.56 ± 0.75 and with increasing the number of halloysite nanotubes decreased the porosity of these nanocomposite films. Rapid wound healing in addition to nutrients, medium, and oxygen transfer was facilitated with highly porous structures of ternary nanocomposite films [14]. Nanohydroxyapatite-collagen-poly (L-lactide) and Hydroxyapatite/chitosan-gelatin network (HA-CS-Gel) both exhibited about 90% porosity was mentioned by the D. W. Hutmacher *et al*. in the review article of scaffold-based bone engineering [15].

In addition, measurement of swelling index or capacity is important to manage exudate adsorption and to prevent wounds from fluid accumulation that are aimed to be used in wound dressing [16]. The swelling ratio which is characterized by hydrophilicity would enhance cell viability and proliferation [5]. Polyvinyl alcohol (PVA) contains free hydroxyl groups in its polymeric matrix structure which makes it entirely dissolved in water and consider one of the major causes of higher water solubility or uptake of PVA-based composites/films. Therefore, swelling tests or water absorption capacity tests are one of the major design criteria to be discussed [17]. Gelatin is another chief material used in this study which also has effects on increasing water content. The self-diffusion of gelatin chains is interfered with by the presence of water content. In contrast, Hydroxyapatite (HAP) has the predisposition to reduce water uptake and swelling. Incorporating higher amounts of HAP is anticipated to decrease of water-uptake and swelling [18]. A study on Sodium Alginate/Poly (vinyl alcohol)-Based Hydrogels depicted that the

swelling index declined in PBS fluid than in water and the swelling capacity didn't reach above 300% for all samples. Since glycerin was used as a plasticizer along with gelatin it makes the film softer and more flexible. The presence of glycerin significantly reformed the propensity of the materials to swell, leading to rapid fluid uptake by the materials. In general, without glycerin samples are hard and inflexible and swell up more rapidly. Adding glycerin with other materials in the sample caused a reduction in the swelling trend as it increases the moisture content of the materials by binding water, which provides flexibility and also limits excess fluid absorption [19].

In the clinical medicine and biomaterials field, the necessity of tissues and biological materials characterization of mechanical properties are known no bound. Although, there is information scarcity concerning the role of tissue mechanics in disease, tissue repair, and remodeling mechanisms associated with medical treatments [20]. Depending on the strength, indentation depth, and type of media being used for hydration mechanical strength of articular cartilage is between 1 - 50 MPa [21]. For human young's moduli of 1.64 ± 0.34 and 1.57 ± 0.23 MPa and 0.28 ± 0.14 and 0.27 ± 0.15 MPa for bovine femoral cartilage were found at compression strains of 10% and 20%, respectively [22] The compressive stresses of articular cartilage in the hip reached up to 18 MPa in more stressful activities such as standing up and modulus cartilage of ranges from 0.08 to 2 MPa that differs by the depth in the tissue and location on the joint. Depending on the body's location tensile modulus of healthy human cartilage is in the range of approximately 5 - 25 MPa and the ultimate tensile stress is 15 - 35 MPa [23]. During normal physiological loading ranges applied peak force to knee cartilage corresponds to about 0.84 - 3 MPa for a 70 kg adult person [24]. As a replacement for artificial cartilage PVA hydrogel could be used when TS in the cartilage varies from 1 to 17 MPa and the compressive modulus range of 0.0012 - 0.85 MPa depending on the concentration of polymer. For the application of artificial cartilage researched on gelatin-PVA film observed the γ radiation effect where irradiated samples found better TS and Eb% that were 16% and 389% + 18% respectively [15]. In contrast, the compressive strengths of cortical and cancellous bone varied between 100 - 230 and 2 - 12 MPa, although Young's modulus was in the ranges of 7 - 30 and 0.5 - 0.005 GPa, respectively [25]. The compressive strength was stated to be eight times larger than the tensile strength. A summary of published data on the mechanical properties of human trabecular bone was presented as a function of anatomic location where strength and modulus were examined in fresh frozen storage method conditions and summarized according to a different region. The strength of the proximal tibia, distal femur, proximal femur, vertebral bodies, patella, distal tibia and talus, humerus, distal radius, and Iliac crest was 1.5 - 116.4 MPa, 0.98 - 66.2 MPa, 0.45 - 16.2 MPa, 0.3 - 15 MPa, 5 - 65 MPa, 0.03 - 6.3 MPa, 0.12 - 8.2 MPa whereas modulus was 4 - 552 MPa, 7.6 - 2942 MPa, 20.68 - 9800 MPa, 1.1 - 428 MPa, 121.3 - 580 MPa, 1.1 - 448 MPa, and 5 - 282 MPa respectively [26].

In tendon tissue engineering to promote physiological tendon regeneration and

reconstruction Mkx-bio-tendon improved tendon reconstruction biomechanically where the tensile strength and Young's modulus of Mkx bio-tendon was compared to that of the control normal tendon while TS denoted 0.45 ± 0.5 and Young's modulus was 10.5 MPa respectively [27]. On the basis of age, sex, and body region mechanical properties of human skin may vary for instance, tensile strength ranging between 2.5 - 30 MPa, elongation 10% - 115%, and elastic modulus 0.4 - 20 MPa correspondingly [16]. In 2017, Griffin *et al*. compared the mechanical properties of different skin sites for auricular and nasal reconstruction where tensile young's elastic modulus was determined among different skin sites such as submandibular, postauricular mastoid, temporoparietal skin, forehead, and forearm skin among the highest e modulus was found at submandibular region 1.28 ± 0.06 MPa, then forearm was 1.03 ± 0.06 MPa and lowest Young's modulus was obtained from forehead 0.33 ± 0.04 MPa. Despite these 0.86 ± 0.05 MPa and 0.65 ± 0.05 MPa were found in post auricular mastoid and the temporoparietal skin individually [28]. In 2020, polyvinyl alcohol (PVA)/sodium alginate (SA) hydrogel incorporating PCL microspheres containing a hybrid microsphere/hydrogel system was introduced as a skin scaffold to hasten wound healing where maximum tensile strength, young's modulus, elongation % was found 1.902 ± 0.141 MPa, 0.589 ± 0.056 MPa and 394% ± 24.457% respectively [29]. A. Saudi *et al*. reported for never tissue engineering HAP was used along with polycaprolactone/poly glycerol sebacate by electrospinning where the fibers containing 5 and 10 wt% HAP, Young's modulus or E modulus was 0.2 ± 0.02 MPa, and 0.23 ± 0.04 MPa, Tensile strength was 0.43 ± 0.08, MPa, and 0.56 ± 0.05 MPa, elongation of break was reported 28% ± 2.9%, and 26% ± 2.5%, respectively [30]. Research on Gelatin/HAP composite revealed that the combination of inorganic HAP with gelatin sharply decrease the tensile elongation and increased the tensile strength and plasticizing effect made the gelatin comparatively softer [31]. On the other hand, Interpenetrating Polymer Network PVA/gelatin hydrogels by freeze-thawing process also observed higher tensile strength and elastic modulus with the increased gelatin content. When the 2% and 7% w/v gelatin were into PVA blend, TS elevated from 0.66 ± 0.12 MPa to 0.75 ± 0.18 MPa and E modulus from 0.80 ± 1.50 MPa to 0.98 ± 2.45 MPa correspondingly which was considered to be applied as a possible candidate for scaffolds tissue engineering [32].

A few studies also suggested that elastic modulus and elongation at break were associated with the crystalline and amorphous regions of gels and the free water that flows in the network polymer [33] [34]. PVA/HAP composite hydrogel e modulus was found 0.1 - 2 MPa. It was also noted that stiffness would be a factor for lack of adherence at the interface between the cartilage and PVA/HA and for moderate daily activities joint could bear the pressure 1 to 6 MPa in contrast, if the condition became extreme, it would be reached upto 12 MPa. There was a deterioration of the mechanical properties of composite materials (PVA/HAP) for higher nano-HA contents (above a certain percentage) that caused easy agglomeration due to the presence of high surface energy [35].

In this research work, HAP/PVA/Gel films have been synthesized by the solution casting method. Total four samples have been experimented for structural and chemical analysis where two different percentages of HAP (5% and 10%) have been used and in two samples glycerin has been used along with increasing concentration of gelatin. The objective of this research study is to synthesize hybrid films made of HAP, PVA and gelatin and to characterize and compare the structural and chemical stability of the synthesized hybrid film by porosity, swelling and mechanical test. It is expected that these newly synthesized hybrid films could be a better opportunity for different tissue engineering sectors such as skin, bone, tendon, and cartilage due to the insufficiency and high expense of grafting materials for the replacement of tissue in burn injury, trauma patients, or various medical conditions such as diabetic foot ulcer, orthopedic surgery, and craniofacial surgery. Moreover, this novel hybrid film could be supportive of drug delivery in wound sites both in internal and external injury.

## 2. Materials & Methodology

### 2.1 Materials

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) was synthesized by chemical precipitation from Calcium nitrate tetra-hydrate ( $Ca(NO_3)_2 \cdot 4H_2O$ ) with Disodium hydrogen phosphate ( $(NH_4)_2 HPO_4$ )where Ca/P molar ratio of 1.67 and pH 10 - 11 was maintained by small amount of ammonium hydroxide ( $NH_4OH$ ) [36]. Calcium nitrate tetra hydrate, Di ammonium Hydrogen phosphate and Gelatin Purified were purchased from Merck, India, while Ammonium Solution 25%, Glutaraldehyde Solution 25%, Ethanol was purchased from Merck, Germany. Poly vinyl alcohol (PVA), molecular weight of 115000 kDa, with a polymerization degree of 1700 - 1800 and a hydrolysis degree of 98.99% was purchased from Qualikems Fine Chem Pvt. Ltd, India. Acetic Acid with 99.5% purity from BDH chemical, England and Glycerin pure from UNITAD Product, Bangladesh was used including Phosphate buffered solution (PBS) pH 7.4, purchased from New Mumbai for experiment. All the chemicals were used without further purification. Deionized water was used throughout the experiment.

### 2.2 Methodology to Prepare HAP/PVA/GEL Hybrid Film

HAP/PVA/GEL Hybrid film was made by two different methods, one is adding humectants and another is without humectants. In this experiments, Glycerin/Glycerol (Gly) was used as humectants which preserve the moisture of synthesized hybrid film. Two different amounts of synthesized HAP were chosen 5%, and 10% and this HAP solution was prepared individually by adding acetic acid that helped to dissolve HAP properly. At 70˚C with continuous stirring by magnetic stirrer for around 1 hour 5% PVA solution was prepared and add with individual HAP solution. On the other hand, Gelatin solution was prepared by two ways. In one solution only deionized water was used to produce 5% Gelatin solution and on another, Glycerin was used along with gelatin solution where Gelatin: Glycerin:

Deionized water ratio was maintained 2:1:2 and prepared 10% Gelatin solution. These two Gelatin solutions were mixed with HAP and PVA mixtures separately. Stirring and heating were continued during the whole procedure. After 30 minutes of continuous stirring and heating, 1 mL of 2.5% Glutaraldehyde was added for chemical crosslinking of the materials. During this procedure temperature was maintained 40˚C - 50˚C. After another half an hour of continuous heating and stirring, the final solution is transferred into petridish covering of thermal paper and keep it in room temperature for 5 - 6 days to obtain the desired film. A total of four samples were synthesized and used for further characterization and study.

Four samples and the composition ratio are mentioned accordingly (Table 1).

Table 1. Four samples and the composition ratio of synthesized hybrid film.

| Sample No. | Composition Ratio |
|---|---|
| S1/5 HPG | 5% HAP: 5% PVA: 5% Gelatin |
| S2/10 HPG | 10% HAP: 5% PVA: 5% Gelatin |
| S3/5 HPGGly | 5% HAP: 5% PVA: 10% Gelatin |
| S4/10 HPGGly | 10% HAP: 5% PVA: 10% Gelatin |

## 2.3. Characterization of Chemical and Structural Properties

### 2.3.1. Porosity Test

The liquid displacement method was used to see the porosity of the synthesized hybrid film. Ethanol was used in the procedure because of its easy penetrability to the pores of the matrices and major advantage is that it does not interfere with the shrinkage or swelling of the material. The measurement of the sample was 5 mm × 2 cm (Figure 1). Experiment was repeated three times individually for every sample. Each sample percentage was averaged and standard deviations were taken to calculate percentage of hybrid film porosity.

Figure 1. Sample (5 mm × 2 cm) used for porosity test.

The porosity ($\varepsilon$) of hybrid films was calculated using the following equations [37]:

$$\varepsilon = \frac{V_\rho}{V_\rho + V_d} = \frac{W_2 - W_3 - W_d}{W_1 - W_{3?}}$$

Here,

$\varepsilon$ = the porosity of the hybrid film was obtained by:

$\rho$ = The density of the ethanol;

$Vd$ = volume of the synthesized hybrid film, $V_d = \frac{W_1 - W_2 + W_d}{\rho}$;

$V_\rho$ = volume of the synthesized hybrid film, $V_\rho = \frac{W_2 - W_3 - W_d}{\rho}$;

$W_d$ = Weight of dry film;

$W_1$ = Weight of the weight of vacuum tube full of ethanol;

$W_2$ = Weight of total weight of ethanol and the ethanol-soaked hybrid film;

$W_3$ = Weight of residual ethanol and vacuum tube after removing ethanol-soaked hybrid film.

### 2.3.2. Swelling Test

Swelling tests of synthesized hybrid films were conducted by two media- distilled water and phosphate buffer saline (pH: 7.4). The swelling ratio of films in distilled water was also considered as both water absorption and water retention capacity. To evaluate the swelling rate of the samples, each rectangular shaped sample film (10 mm × 10 mm) was cut (**Figure 2**) and overnight dried in a dryer at 50˚C. Then the dried samples were immersed in deionized water and phosphate-buffered saline for 12 h at room temperature. After immersion for a certain time interval (> 12 hours), the weight of the films was measured immediately after removing extra liquid on the surface with filter paper [16] [19] [38].

**Figure 2**. Sample (10 mm × 10 mm) used for swelling test.

Each such experiment was repeated three times individually. The average value and standard deviation were taken to calculate percentage of films swelling. The swelling ratio of hybrid films was calculated according to the following equation [5].

Swelling Ratio $S(\%) = \frac{W_t - W_0}{W_0} \times 100\%$

Where, WA ($t$) is the water absorption at time $t$ = Swelling ratio $S$ (%);

$W_0$ is the initial weight of the sample;

$W_t$ is its weight at time $t$.

### 2.3.3. Mechanical Test

The mechanical test was done to identify the mechanical properties of the synthesized hybrid film such as tensile strength (TS), elastic modulus (Em), elongation to break % (Eb), elongation of maximum (Emax) were measured with a computer-controlled Hounsfield H10ks Machine (UTM; H10ks-0572, ASTM D882). The load range was 500 N with 100 mm/min crosshead speed and 25 mm gauge length. The dimensions of the test specimen were 60 mm × 5 mm (Figure 3). The films were secured at both ends using mechanical grips and their strength was determined. Mechanical properties for each type of insert were replicated for at least three samples, and average values are reported as the results.

Figure 3. Equipment and sample (60 mm × 5 mm) used for mechanical test.

## 3. Results and Discussions

### 3.1. Porosity Test

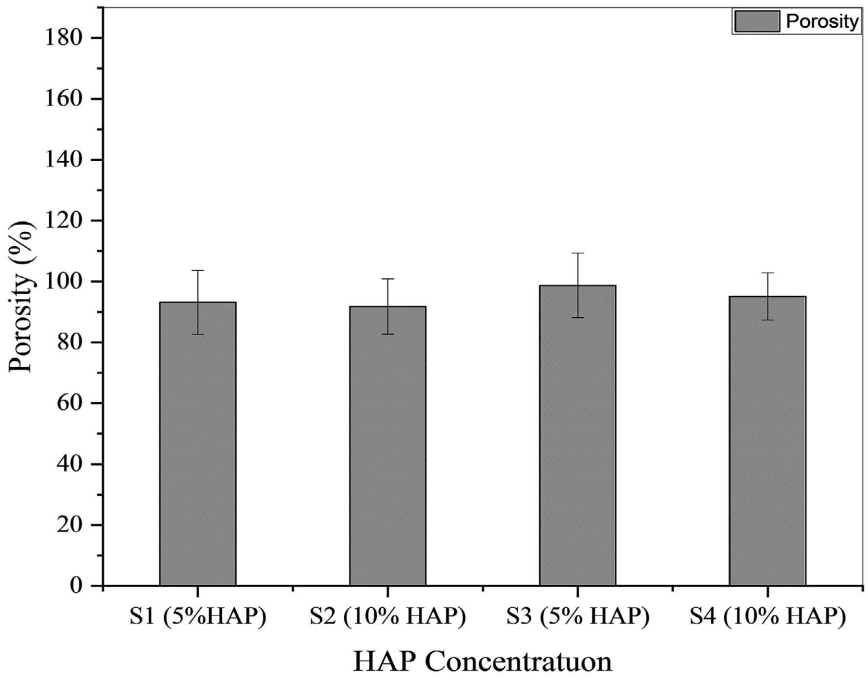


Figure 4. Porosity test of HAP/PVA/Gel (S1 and S2) and HAP/PVA/Gel/Glycerin film (S3 and S4).

Figure 4 clearly shows that there is more than 90% porosity represented by all newly synthesized HAP/PVA/Gel hybrid films. However, with the increment of HAP, the percentage of porosity slightly declined, which might be due to

increasing crystallinity or molecular interaction. Alternatively, S3 and S4 show more porosity 98.73% ± 10.60% and 95.08% ± 7.79% respectively which is more than the porosity of S1 (93.13% ± 10.50%) and S2 (91.77% ± 9.06%) (Table 2). As S3 and S4 have 10% gelatin, this could be the reason behind the higher porosity than the other two samples.

### 3.2. Swelling Test

Phosphate Buffer Solution (PBS, pH 7.4) and Double Distilled Water were chosen to perform the swelling test of synthesized samples and all the test was done at room temperature.

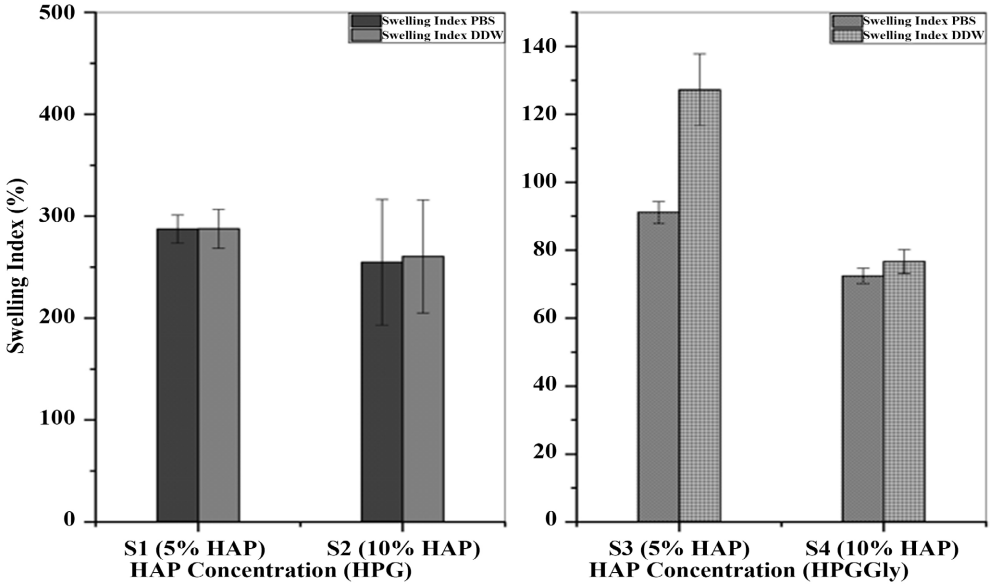


Figure 5. Swelling index of HAP/PVA/Gel (S1 and S2) and HAP/PVA/Gel/Glycerin film (S3 and S4) in PBS and DDW.

Figure 5 and Table 2 demonstrate clear evidence to decrease swelling with the addition of 10% gelatin with glycerin in S3 and S4 [19]. Moreover, after increasing HAP concentration in S2 and S4 the swelling index or water absorption was decreased, which also corresponds with the literature review [18]. The swelling index of DDW was relatively more than the swelling index of samples in PBS as there might be more crosslinking occurring [25].

Table 2. Swelling test and porosity results of synthesized hybrid film.

| Sample | Swelling index (DDW) % | Swelling index (PBS) % | Porosity % |
|---|---|---|---|
| S1/5 HPG | 287.72 ± 19.07 | 287.37 ± 13.72 | 93.13 ± 10.50 |
| S2/10 HPG | 260.55 ± 55.37 | 254.78 ± 61.65 | 91.77 ± 9.06 |
| S3/5 HPGGly | 127.27 ± 10.53 | 91.13 ± 3.23 | 98.73 ± 10.60 |
| S4/10 HPGGly | 76.65 ± 3.52 | 72.41 ± 2.27 | 95.08 ± 7.79 |

### 3.3. Mechanical Test

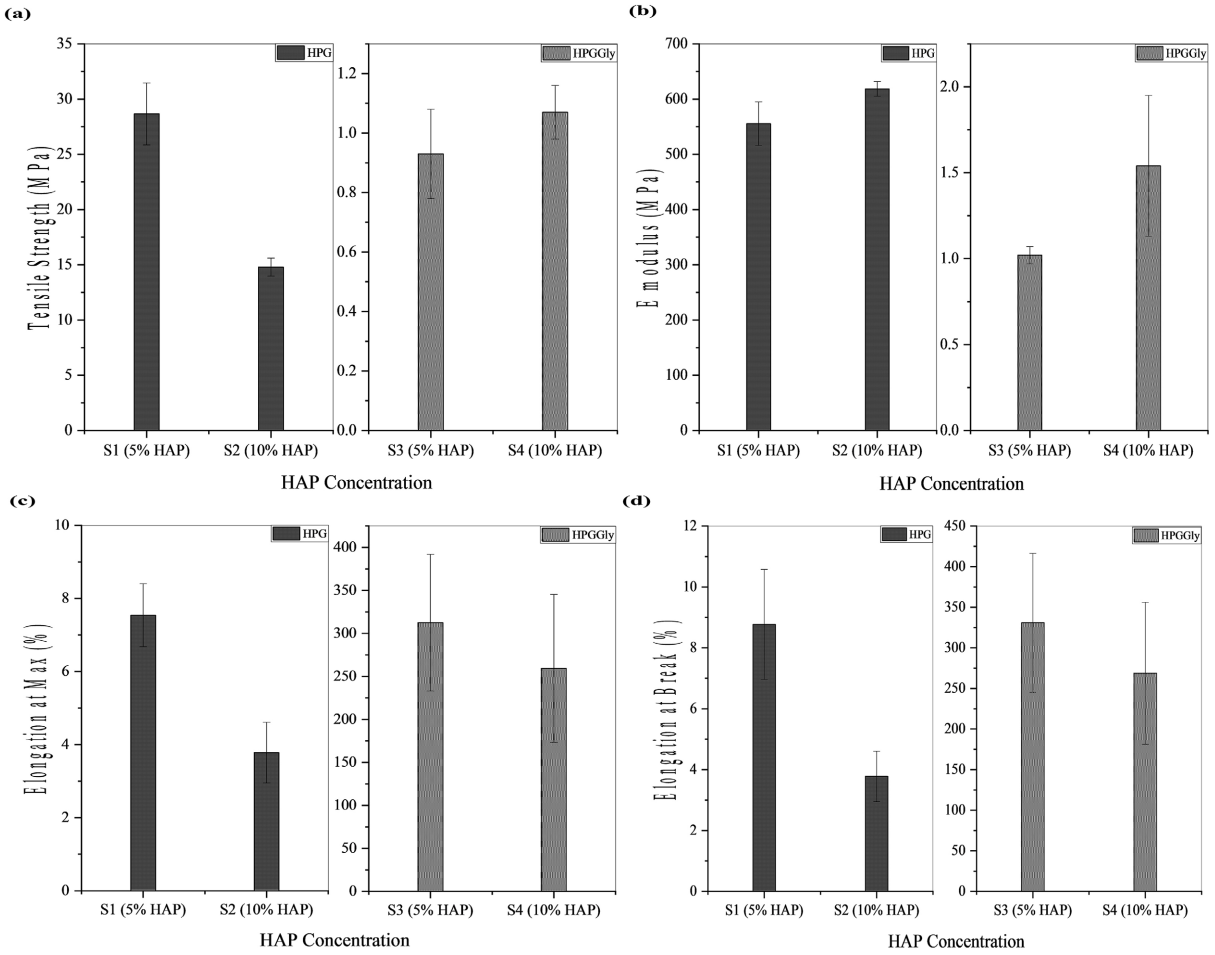


**Figure 6**. (a) Tensile strength; (b) E modulus of S1, S2, S3, S4; (c) Elongation at maximum; (d) Elongation at break of S1, S2, S3, S4.

**Figure 6** and **Table 3** depict that S1 and S2 tensile strength, elongation of maximum, and elongation of break were reduced but E-modulus increased with the increase of HAP concentration whereas S3 and S4 figure depicts that TS, E-modulus all were elevated but Emax and Eb were reduced with the increased of HAP concentration. Based on this experiment, HAP/PVA/Gel hybrid film TS, Emax, and Eb were decreased with an increment of HAP percentage concentration due to the reduced crosslinking density in the samples [29]. In contrast, TS and E modulus was increased with the increment HAP in HAP/PVA/Gel/Glycerin Hybrid film. Emax and Eb were relatively higher at S3 and S4 than at S1 and S2 because of adding 10% gelatin along with glycerin [39]. However, in both cases, when the HAP concentration was increased, Emax and Eb was decreased. The factor related to this might be the crystallinity of HAP. On the other hand, the incorporation of HAP and PVA in highly oriented gelatin structures significantly increases the tensile strength and E modulus of S3 and S4 [31]. Different research suggests that the higher the PVA concentration, the higher the tensile strength

and Eb, however in this study amount of PVA was constant for all samples was 5%, so the variation between the TS or Eb was all dependent on the other materials used such as HAP, Gelatin and glycerin [40]. TS deteriorated in S2 than in S1 due to the presence of high surface energy that causes easy agglomeration for the concentration of HAP in S2 but on S4 10% gelatin with glycerin with 10% HAP shows improved TS and E modulus then S3 [35].

**Table 3.** Mechanical test results of synthesized hybrid film.

| Sample | Tensile Strength MPa | Elongation of maximum % | Elongation of Break % | E-modulus MPa |
|---|---|---|---|---|
| S1/5 HPG | 28.66 ± 2.80 | 7.54 ± 0.86 | 8.77 ± 1.81 | 555.67 ± 39.31 |
| S2/10 HPG | 14.79 ± 0.82 | 3.78 ± 0.83 | 3.78 ± 0.83 | 618.53 ± 13.23 |
| S3/5 HPGGly | 0.93 ± 0.15 | 312.50 ± 79.49 | 330.90 ± 85.54 | 1.02 ± 0.05 |
| S4/10 HPGGly | 1.07 ± 0.09 | 259.30 ± 86.13 | 268.60 ± 87.41 | 1.54 ± 0.41 |

## 4. Statistical Analysis

Each experiment was performed in triplicate if without a particular explanation, and the results are expressed as means ± SDs. Statistical analyses were performed using the OriginPro 2018 68bit software package.

## 5. Conclusion

In the present research, HAP/PVA/Gel and HAP/PVA/Gel/Gly films containing different HAP percentages (5% and 10%) were developed by the solution casting method. All sample shows more than 90% of porosity in the fluid displacement method. Swelling index experimented on both PBS and DDW to perceive the compatibility of synthesized film during both internal and external wound healing which might be helpful for different tissue engineering applications. Though the increment of HAP, porosity, and swelling percentage was reduced in this study, the percentage is compatible with biomedical applications. The mechanical test reveals that the elastic modulus was raised with the increased HAP percentage but TS was decreased in HAP/PVA/Gel. In bone tissue engineering specially in trabecular bone repair, these synthesized films could be used as grafting in orthopedic surgery. In contrast, the addition of glycerin and gelatin reduced the tensile strength prominently and e modulus but the elongation of break and elongation of maximum was relatively higher than HAP/PVA/Gel film. This relatively low tensile strength and high elongation are essential for proper wound tissue coverage and healing for the grafting in skin, tendon, and cartilage tissue engineering in various medical conditions. However, it is recommended to do SEM for understanding of actual pore size and morphology of the synthesized films as well as biocompatibility tests are also suggested for future medical applications to see the cell proliferation and antibiotic resistance. Lastly, it could be said that in this study, newly developed HAP/PVA/Gel and HAP/PVA/Gel/Gly are demonstrated

as a new opportunity for drug delivery, wound healing, and grafting in different tissue engineering sectors. There is wide scope in the future to experiment with these synthesized hybrid films for the proper biomedical applications.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Kalirajan, C., Dukle, A., Nathanael, A.J., Oh, T. and Manivasagam, G. (2021) A Critical Review on Polymeric Biomaterials for Biomedical Applications. *Polymers*, **13**, Article 3015. https://doi.org/10.3390/polym13173015

[2] Xue, K., Teng, S., Niu, N. and Wang, P. (2018) Biomimetic Synthesis of Novel Polyvinyl Alcohol/Hydroxyapatite Composite Microspheres for Biomedical Applications. *Materials Research Express*, **5**, Article ID: 115401. https://doi.org/10.1088/2053-1591/aadc7d

[3] Jain, N., Singh, V.K. and Chauhan, S. (2017) A Review on Mechanical and Water Absorption Properties of Polyvinyl Alcohol Based Composites/Films. *Journal of the Mechanical Behavior of Materials*, **26**, 213-222. https://doi.org/10.1515/jmbm-2017-0027

[4] Bhat, S., Uthappa, U.T., Altalhi, T., Jung, H. and Kurkuri, M.D. (2021) Functionalized Porous Hydroxyapatite Scaffolds for Tissue Engineering Applications: A Focused Review. *ACS Biomaterials Science & Engineering*, **8**, 4039-4076. https://doi.org/10.1021/acsbiomaterials.1c00438

[5] Chaudhuri, B., Mondal, B., Ray, S.K. and Sarkar, S.C. (2016) A Novel Biocompatible Conducting Polyvinyl Alcohol (PVA)-Polyvinylpyrrolidone (PVP)-Hydroxyapatite (HAP) Composite Scaffolds for Probable Biological Application. *Colloids and Surfaces B*: *Biointerfaces*, **143**, 71-80. https://doi.org/10.1016/j.colsurfb.2016.03.027

[6] Cox, S.C., Walton, R.I. and Mallick, K.K. (2015) Comparison of Techniques for the Synthesis of Hydroxyapatite. *Bioinspired, Biomimetic and Nanobiomaterials*, **4**, 37-47. https://doi.org/10.1680/bbn.14.00010

[7] Kozlov, P.V. and Burdygina, G.I. (1983) The Structure and Properties of Solid Gelatin and the Principles of Their Modification. *Polymer*, **24**, 651-666. https://doi.org/10.1016/0032-3861(83)90001-0

[8] Izadifar, Z., Chen, X. and Kulyk, W. (2012) Strategic Design and Fabrication of Engineered Scaffolds for Articular Cartilage Repair. *Journal of Functional Biomaterials*, **3**, 799-838. https://doi.org/10.3390/jfb3040799

[9] Zhang, Z. and Cui, H. (2012) Biodegradability and Biocompatibility Study of Poly(Chitosan-G-Lactic Acid) Scaffolds. *Molecules*, **17**, 3243-3258. https://doi.org/10.3390/molecules17033243

[10] Spiteri, C.G., Pilliar, R.M. and Kandel, R.A. (2006) Substrate Porosity Enhances Chondrocyte Attachment, Spreading, and Cartilage Tissue Formation *in Vitro*. *Journal of Biomedical Materials Research Part A*, **78**, 676-683.

https://doi.org/10.1002/jbm.a.30746

[11] Mohd Hilmi, A.B., Halim, A.S., Hassan, A., Lim, C.K., Noorsal, K. and Zainol, I. (2013) *In Vitro* Characterization of a Chitosan Skin Regenerating Template as a Scaffold for Cells Cultivation. *SpringerPlus*, **2**, Article No. 79. https://doi.org/10.1186/2193-1801-2-79

[12] Kazemzadeh Narbat, M., Orang, F., Solati Hashtjin, M. and Goudarzi, A. (2006) Fabrication of Porous Hydroxyapatite-Gelatin Composite Scaffolds for Bone Tissue Engineering. *Iranian Biomedical Journal*, **10**, 215-223.

[13] Ye, H., Cheng, J. and Yu, K. (2019) *In Situ* Reduction of Silver Nanoparticles by Gelatin to Obtain Porous Silver Nanoparticle/Chitosan Composites with Enhanced Antimicrobial and Wound-Healing Activity. *International Journal of Biological Macromolecules*, **121**, 633-642. https://doi.org/10.1016/j.ijbiomac.2018.10.056

[14] Devi, N. and Dutta, J. (2019) Development and *in Vitro* Characterization of Chitosan/Starch/Halloysite Nanotubes Ternary Nanocomposite Films. *International Journal of Biological Macromolecules*, **127**, 222-231. https://doi.org/10.1016/j.ijbiomac.2019.01.047

[15] Hutmacher, D.W., Schantz, J.T., Lam, C.X.F., Tan, K.C. and Lim, T.C. (2007) State of the Art and Future Directions of Scaffold-Based Bone Engineering from a Biomaterials Perspective. *Journal of Tissue Engineering and Regenerative Medicine*, **1**, 245-260. https://doi.org/10.1002/term.24

[16] dos Santos, D.M., Souza Leite, I., de Lacerda Bukzem, A., de Oliveira Santos, R.P., Frollini, E., Inada, N.M. and Campana-Filho, S.P. (2018) Nanostructured Electrospun Nonwovens of Poly(ε-Caprolactone)/Quaternized Chitosan for Potential Biomedical Applications. *Carbohydrate Polymers*, **186**, 110-121.

[17] Mhatre, A., Bhagwat, A., Bangde, P., Jain, R. and Dandekar, P. (2021) Chitosan/Gelatin/PVA Membranes for Mammalian Cell Culture. *Carbohydrate Polymer Technologies and Applications*, **2**, Article ID: 100163. https://doi.org/10.1016/j.carpta.2021.100163

[18] Lendlein, A., von der Mark, K. and Multhaup, G. (2011) Physical Crosslinking of Gelatin: A Supramolecular Approach to Biomaterials. Institutional Repository of the University of Potsdam. http://opus.kobv.de/ubp/volltexte/2011/5288/

[19] Bialik-Wąs, K., Pluta, K., Malina, D., Barczewski, M., Malarz, K. and Mrozek-Wilczkiewicz, A. (2021) The Effect of Glycerin Content in Sodium Alginate/Poly(Vinyl Alcohol)-Based Hydrogels for Wound Dressing Application. *International Journal of Molecular Sciences*, **22**, Article 12022. https://doi.org/10.3390/ijms222112022

[20] Ebenstein, D.M. and Pruitt, L.A. (2006) Nanoindentation of Biological Materials. *Nano Today*, **1**, 26-33. https://doi.org/10.1016/s1748-0132(06)70077-9

[21] Franke, O., Durst, K., Maier, V., Göken, M., Birkholz, T., Schneider, H., *et al*. (2007) Mechanical Properties of Hyaline and Repair Cartilage Studied by Nanoindentation. *Acta Biomaterialia*, **3**, 873-881. https://doi.org/10.1016/j.actbio.2007.04.005

[22] Démarteau, O., Pillet, L., Inaebnit, A., Borens, O. and Quinn, T.M. (2006) Biomechanical Characterization and *in Vitro* Mechanical Injury of Elderly Human Femoral Head Cartilage: Comparison to Adult Bovine Humeral Head Cartilage. *Osteoarthritis and Cartilage*, **14**, 589-596. https://doi.org/10.1016/j.joca.2005.12.011

[23] Athanasiou, K.A., Darling, E.M. and Hu, J.C. (2009) Articular Cartilage Tissue Engineering. Springer. https://doi.org/10.2200/s00212ed1v01y200910tis003

[24] Natoli, R.M. and Athanasiou, K.A. (2008) P188 Reduces Cell Death and IGF-I Reduces GAG Release Following Single-Impact Loading of Articular Cartilage. *Journal of Biomechanical Engineering*, **130**, Article ID: 041012.

https://doi.org/10.1115/1.2939368

[25] Al-Mamun, A., Haque, P., Debnath, T., Rahman, M.F., Islam, J.M.M., Rahman, M.M., *et al*. (2018) γ-Irradiated Gelatin and Polyvinyl Alcohol Films as Artificial Articular Cartilage. *Journal of Thermoplastic Composite Materials*, **33**, 614-627. https://doi.org/10.1177/0892705718808555

[26] Goldstein, S.A. (1987) The Mechanical Properties of Trabecular Bone: Dependence on Anatomic Location and Function. *Journal of Biomechanics*, **20**, 1055-1061. https://doi.org/10.1016/0021-9290(87)90023-6

[27] Tsutsumi, H., Kurimoto, R., Nakamichi, R., Chiba, T., Matsushima, T., Fujii, Y., *et al*. (2022) Generation of a Tendon-Like Tissue from Human Ips Cells. *Journal of Tissue Engineering*, **13**, 1-13. https://doi.org/10.1177/20417314221074018

[28] Griffin, M.F., Leung, B.C., Premakumar, Y., Szarko, M. and Butler, P.E. (2017) Comparison of the Mechanical Properties of Different Skin Sites for Auricular and Nasal Reconstruction. *Journal of Otolaryngology—Head & Neck Surgery*, **46**, 1-6. https://doi.org/10.1186/s40463-017-0210-6

[29] Bahadoran, M., Shamloo, A. and Nokoorani, Y.D. (2020) Development of a Polyvinyl Alcohol/Sodium Alginate Hydrogel-Based Scaffold Incorporating bFGF-Encapsulated Microspheres for Accelerated Wound Healing. *Scientific Reports*, **10**, Article No. 7342. https://doi.org/10.1038/s41598-020-64480-9

[30] Saudi, A., Zebarjad, S.M., Salehi, H., Katoueizadeh, E. and Alizadeh, A. (2022) Assessing Physicochemical, Mechanical, and *in Vitro* Biological Properties of Polycaprolactone/Poly(Glycerol Sebacate)/hydroxyapatite Composite Scaffold for Nerve Tissue Engineering. *Materials Chemistry and Physics*, **275**, Article ID: 125224. https://doi.org/10.1016/j.matchemphys.2021.125224

[31] Hossan, M., Gafur, M., Karim, M. and Rana, A. (2015) Mechanical Properties of Gelatin Hydroxyapatite Composite for Bone Tissue Engineering. *Bangladesh Journal of Scientific and Industrial Research*, **50**, 15-20. https://doi.org/10.3329/bjsir.v50i1.23805

[32] Hago, E. and Li, X. (2013) Interpenetrating Polymer Network Hydrogels Based on Gelatin and PVA by Biocompatible Approaches: Synthesis and Characterization. *Advances in Materials Science and Engineering*, **2013**, Article ID: 328763. https://doi.org/10.1155/2013/328763

[33] Hassan, C.M., Ward, J.H. and Peppas, N.A. (2000) Modeling of Crystal Dissolution of Poly(Vinyl Alcohol) Gels Produced by Freezing/Thawing Processes. *Polymer*, **41**, 6729-6739. https://doi.org/10.1016/s0032-3861(00)00031-8

[34] Stammen, J.A., Williams, S., Ku, D.N. and Guldberg, R.E. (2001) Mechanical Properties of a Novel PVA Hydrogel in Shear and Unconfined Compression. *Biomaterials*, **22**, 799-806. https://doi.org/10.1016/s0142-9612(00)00242-8

[35] Gonzalez, J.S. and Alvarez, V.A. (2014) Mechanical Properties of Polyvinylalcohol/Hydroxyapatite Cryogel as Potential Artificial Cartilage. *Journal of the Mechanical Behavior of Biomedical Materials*, **34**, 47-56. https://doi.org/10.1016/j.jmbbm.2014.01.019

[36] Mobasherpour, I., Heshajin, M.S., Kazemzadeh, A. and Zakeri, M. (2007) Synthesis of Nanocrystalline Hydroxyapatite by Using Precipitation Method. *Journal of Alloys and Compounds*, **430**, 330-333. https://doi.org/10.1016/j.jallcom.2006.05.018

[37] Chang, Y.C., Ting, L.Y., Hsiu, C.P., Hao, H.C., Wei, W.Y. and Hsin, C. (2009) Characterizing Microporous PCL Matrices for Application of Tissue Engineering. *Journal of Medical and Biological Engineering*, **29**, 92-97.

[38] Peh, K.K. and Fun, C. (1999) Polymeric Films as Vehicle for Buccal Delivery:

Swelling, Mechanical, and Bioadhesive Properties. *Journal of Pharmaceutical Sciences*, **2**, 53-61.

[39] Jain, D., Carvalho, E., Banthia, A.K. and Banerjee, R. (2010) Development of Polyvinyl Alcohol-Gelatin Membranes for Antibiotic Delivery in the Eye. *Drug Development and Industrial Pharmacy*, **37**, 167-177. https://doi.org/10.3109/03639045.2010.502533

[40] El Bahy, G.S., El-Sayed, E.S.M., Mahmoud, A.A. and Gweily, N.M. (2012) Preparation and Characterization of Poly Vinyl Alcohol/Gelatin Blends. *Journal of Applied Sciences Research*, **8**, 3544-3551.