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Universitas Indonesia

THE 12th INTERNATIONAL CONFERENCE On QiR (QUALITY in RESEARCH)

in Conjuction with

ICSERA 2011

Program Book Volume 2

QiR

Bali,
4-7 July 2011

ISSN 114-1284

http://qir.eng.ui.ac.id

SYMPOSIUM E

SYMPOSIUM F

Effect of PVA Coating on Diametral Tensile Strength of a Porous Block of Gelatine-Hydroxyapatite Composites

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ABSTRACT

This paper reports an effect of 0.2% w/v PVA coating on a porous block of Gelatine (G)-Hydroxyapatite (HA) Composites. The porous composite was prepared by freezed drying of a mixing of G and HA. The ratio by weight of G/HA was 1/1, while concentration of the composite within the aquadest were 30, 40 and 50% w/v. Three HA's have been used i.e gHA, coHA and bHA. The first two were synthesized from natural gyspum and natural calcite using hydrothermal method, while bHA was prepared by calcination of natural bovine at 900 °C. The specimens were tested by tensile test and its morphology by SEM. It was found that coating of 0.2%w/v PVA on the porous block composites with high ratios and pores size improved their diametral tensile strength in the ranges of 19% to 85%.

Key words

Hydroxyapatite, gelatine, PVA, coating, tensile strength, composite

1. INTRODUCTION

Composites based on apatite crystals and natural polymers have received increasing attention in bone tissue engineering applications due to their ability to preserve the structural and biological functions of the damaged hard tissues in a biomimetic way. Bone regeneration employs three-dimensional porous materials with adequate mechanical properties to provide the necessary support for cells to proliferate and maintain their differential function. Pay attention has been made to address the problem in forming pure bioceramic into a certain geometry that mimic the application. Bioceramic such as hydroxyapatite (HA) is bioactive material which is able to provide osteogenesis environment for growing the bone. Naturally, human bone contains about 69% of HA [1]. However, in the form powder, sinthetic HA is difficult to be formed into a certain shape and geometry at room temperature as it is hard and brittle. Forming it into 3D part at high temperature (1250 °C) is possible by applying sintering process [2-4], but it is costly process and HA might change its phase into TCP. Efforts have been taken to develop biocomposite by mixing HA with other biocompatible materials which acts as an additional reinforcement material or as a matrix. HA can be derived from various natural resources such as gypsum [5-8], calcite [9], bovine bone [10,11], corals [12], seashell [13], eggshell [14,16], and cattlefish bone [17,18] with several methods like hydrothermal [5-8,19], sol-gel [20,21] and electrophoretic deposition [22]. In composing composite, as a matrix of HA, study on these materials have also been done. Particular attention has been on applying natural and synthetic polymers such as collagen type-I, gelatin, PCL, PLA, PLLA, PMMA and many others. Among those materials, gelatin is favorable material since gelatin can be prepared from acid-treated collagens has a biocompatibility that is similar to collagen but is much cheaper to produce [23]. Its structural protein of gelatin can be found in the skins, tendons, cartilage, bones and other connectives tissue of animals [23]. It has a high biocompatibility, biodegradability and bioactivity [24]. Thus, gelatin is widely used in medical products application, such as wound dressings [25], drug delivery systems [26], and nerves [27].

In developing biocomposite in which gelatin plays in role of flexibility and as a matrix of the composite, while bioactive HA component provides a favorable environment for cell to attach, proliferate and differentiate, this composite is possible to be prepared at room temperature. However, this composite without enhanching or cross-linking is still weak that is not match with application. The composite is easy to swell in contacting within biological environment such as in Simulated Body Fluid (SBF). Several additive materials as cross-linkers have been widely used such as genipin (GP) [27], enzym:mTGase [29] and glutaraldehyde (GA) [30,31]. In the case of gelatin, cross-linking with either GP or GA can improve their mechanical properties and swelling resistance. While additive material as a binder, polyvynil alcohol (PVA) [32] and chitosan [33-35] are the examples. PVA is accounted to be crystalline polymer. Crystallinity of PVA can be reduced by blending PVA with hydrophilics component such as cyclodextrin, chitosan or carbon nanotube [36]. Crystallite within PVA acts as chain crosslink, thus PVA can be a potential material for improving mechanical properties of composite. In case of composit nano-HA and collagen, its durability and mechanical properties can be improved by the use of polymeric binder such as PVA. In this, PVA facilitates strong adhesion between HA and collagen trough hydrogen bonding and by formation of [OH-]-Ca^{2+}-[-OH] linkage [37]. In the hydrogel form, PVA exhibits a high elastic modulus and biocompatibility, and it has been employed in several biomedical applications including drug delivery, contact lenses, artificial organs, wound healing and cartilage [38,39] as well as heart-valve implant application [40,41].

In developing composite using HA as a base material, there are several matrices and reinforcements have been applied with combination of two or three materials. For example HA/gelatine/bioactive glass [28], HA/gelatine [30,31,43], HA/chitosan [34], HA/collagen/PVA [42], HA/glass ionomer cement [44], HA/photopolymer [45], HA/Ni_3Al/Al_2O_3 [46], HA/ZrO_2/CaF_2 [47]. In the current work presented in this paper, the composite was developed by combination of HA and gelatin with PVA coating as reinforcement. Several HA were obtained from gypsum, calcite and bovine bone. Evaluation of the effects of 0.2% w/v PVA coating was carried out on the physico and mechanical properties of three-dimensional sponge-like scaffolds, based on a porous block of Gelatine (G)-Hydroxyapatite (HA).

2. MATERIALS AND METHOD

Two type of samples were prepared i.e. G/HA porous scaffold without and with PVA film coating prior to diamtral tensile strength (DTS) test and morphology images.

2.1. Preparation of G/HA Porous scaffold

Materials have been used in the experiment are Hydroxyapatite (HA), gelatine (G), polyvynil alcohol (PVA) and liquid nitrogen (N2). Three types of HA were used. Two of those were synthesized from gypsum (gHA) and calcite (coHA); and another one was calcinated bovine bone (bHA). G and PVA (Mw=13000-23000 was categorized as low molecule weight which has low viscosity) were obtained from Sigma Aldrich. The scaffolds of composites were prepared using three HA i.e G/gHA, G/coHA and G/bHA. Preparation was carried out by dispersing of each HA (gHA, coHA and bHA) powder and G powder with ratios of 1/1 in destilled water with various ratios (30%, 40% and 50% w/v) to become solution. This solution was then stirred under magnetic steering at 50 °C and 300 rpm for about 30 minutes. The GHA solution is then casted in mold kit with diameter of 12mm and 6mm depth to prepare samples for DTS test and morphology images. All the samples were then freezed overnight at -20 °C and followed by freezed drying at -20 °C and -0.25 Bar for 24 hours prior to PVA film coating.

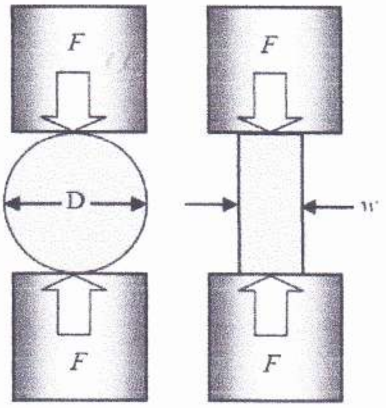
2.2. Preparation of Scaffold Samples G/HA with PVA Film Coating

Prior to PVA coating after having the scaffold of the result at section #2.1, solution of 0.2%w/v PVA was prepared. Preparation of the PVA solution was carried out by heating up 50 ml aquaeus water in baker at 80 °C, then it was added with PVA powder slowly while stirring at 300 rpm for about 20 minutes. Once the powder was fully dispersed and solubilized, stirring and heating were stop. The PVA solution was then cooled down at room temperature for few hours and the solution will be ready for dip coating. When the solution was ready, the samples of a porous scaffold obtained in the section #2.1 were then immersed in the PVA solution for few minutes allowing the solution completely infiltrates the pores and forms a thin film on the pores wall. To avoid the exessive solution blockage in the pores, the samples were gently rolled on paper. Next, the samples were freezed and freezed drying for the second time the same as the section #2.1 prior to quenching. Finally, the samples were quenched in liquid nitrogen to get cryogenic temperature (-192 °C) condition for 5 minutes.

2.3. Diametral Tensile Strength (DTS) Test and SEM

Diametral tensile strength (DTS) test of the samples were carried out using UTS machine. Samples in the form of disk, were prepared following the procedure described in section #2.1 and #2.2 above with diameter of 12mm and thickness of 6 mm. The test procedure was done by pressing the sample until it is broken. The test procedures is shown in Figure 1. To obtain the DTS value, it is calculated by applying equation 1 where the force (F), the diameter and the thickness of the sample (D and w), and the DTS value are in Newton (N), mm, and in MPa, respectively.

For SEM morphology, samples were prepared using the same samples for DTS tes by breaking it and keep the surface of the broken sample in natural appearance, then it was placed at the specimen holder. As the sample is non conductive material, therefore, the sample needs to be coated with the gold coating using sputter coater for at least one cycle coating. If using one cycle coating the image is not clear, coating can be done twice or more to get thicker coating.



$$\mathrm{DTS} = \frac{2F}{\pi D w} \text{ (MPa)} \qquad (1)$$

Figure 1. DTS test procedure

3. RESULTS AND DISCUSSION

3.1. Diametral Tensile Strength (DTS)

Results of DTS for G/gHA, G/coHA and G/bHA scaffold coating and uncoating with PVA are shown in Figure 2 and 3. In general, for both type of scaffold without and with PVA coating show that DTS increases proporsionally by the increase of G/HA concentration from 30%w/v to 50%w/v. Among those types of HA, for the composite without PVA coating shown in Figure 2, gHA that synthesized from gypsum showing contribution the highest DTS values compared to the others. Whilst coHA shows the lowest contribution in the effort of DTS improvement. In this condition, therefore, HA synthesized from gypsum gives better diametral tensile strength compared to other HA that were synthesized from calcite and calcinated of bovine. However, although composite with 50% w/v concentration of [G/gHA] in H_2O gives the highest DTS value i.e 4.2 MPa, this value is still lower than that of the requirement of bone graft. By PVA coating, the mechanical strength will improve.

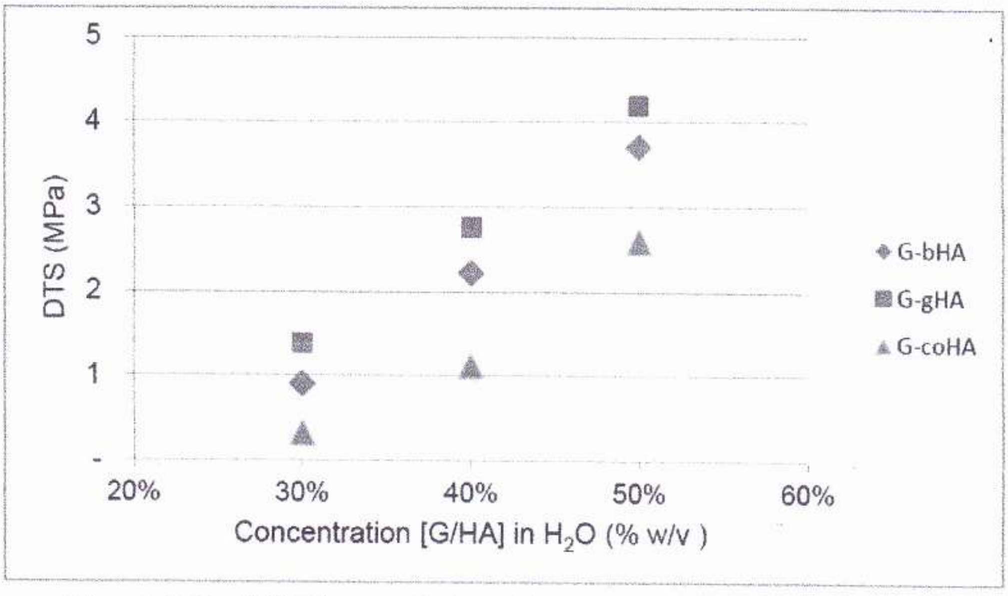


Figure 2. DTS of [G/HA] composite for various concenration without PVA coating

As the PVA coating was treated to the previous samples which the DTS result is shown in Figure 3, this coating gives improvement of its DTS. The improvements are in the range of 19.4 to 85.5% depending to the types of HA: gHA, coHA or bHA has been used in the composite. HA which was obtained by calcination of bovine at 900 °C for 2 hours gives improvement in range of 33.5 to 67.2%, while HA synthesized from gypsum contributes DTS improvement in the range of 19.4 to 36.5%, and HA synthesized from calcite increases DTS in the range of 33.9 to 85.5%. Among those composites, significant DTS improvement after PVA coating has been achived to the scaffold of [G/bHA] with concentration of 50% w/v. The DTS value of this composite is 6.219 MPa. This value, moreover, is in agreement to the ultimate stress of human cancelleus bone with age of about 62 years old as studied by McCalden, *et. al.* Shown in Figure 4 [49].

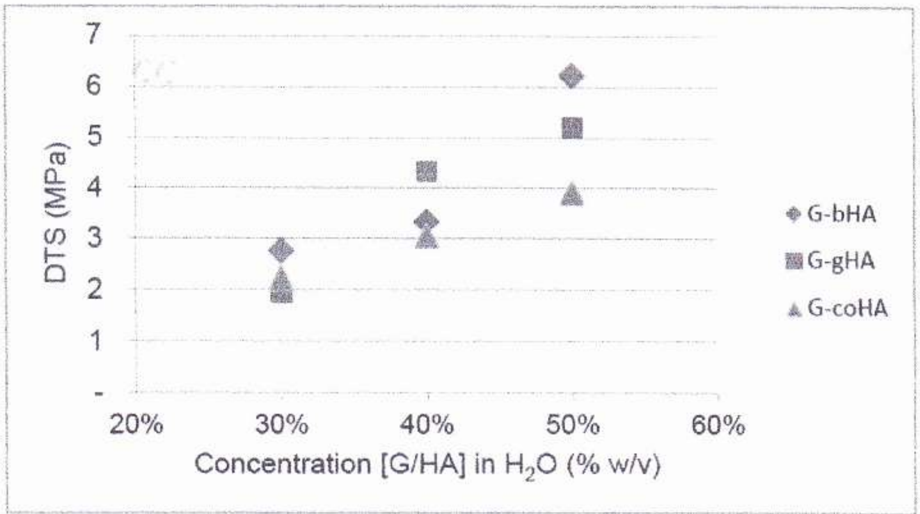


Figure 3. DTS of [G/HA] composite for various concenration with PVA coating

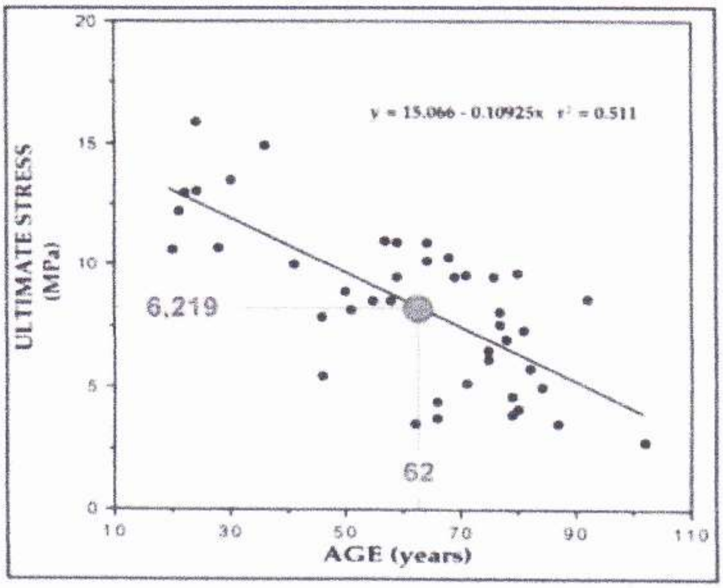


Figure 4. DTS value of [G/HA] composite with 50%w/v which is confirmed to McCalden *et.al.* [49]

3.2. Morphology Images

The morphology of composite scaffold after coating with 0.2% w/v PVA i.e. G/bHA. G/gHA and G/coHA are shown in Figure 5 to 7. In these images we can see that all three types scaffold still have pores with various sizes and shapes which are needed to acommmodate cell proliferation and biological function. The pores have been formed by the connected wall of composite of [G/bHA]. [G/gHA] and [G/coHA]. In these figures, the mixed of particles of HA and gelatine forms pores walls with PVA coating. However, although the thickness of the PVA film coating was not measured yet, from the DTS results shown in Figure 2 and 3 have given evident that the PVA film indicates the contribution in mechanical strength improvement. Theoretically, PVA with its [–OH] group helps to engage HA compound and gelatine to get better bonding which is in return increase the strength. In term of scaffold fabrication, among those SEM images, the composite of [G/bHA] with concentration of 50%w/v of H_2O shows better example of scaffold. Other composites such as [G/gHA] and [G/coHA] indicate to much concentration as a result to low percentage of porosity which is not suitable as a scaffold. In order to meet with requirement of scaffold in bone regeneration application, the porosity of the scaffold should be around 80 to 90% or the pore size of 100 to 300μm. For this, if we look at the pore size, it seems the size is less than 300μm which is still in the range of scaffold requirement. So, the operating procedure that we have used in forming pores i.e. freezing-freezed drying before PVA coating, is right. The walls of the pores which consists of HA particles and gelatine has shown as what we have expected, particularly [G/bHA] shown in Figure 5. The particle of HA plays a role as reinforcement, the gelatine acts as a matrix within the composite and PVA film as a skin reinforcement.

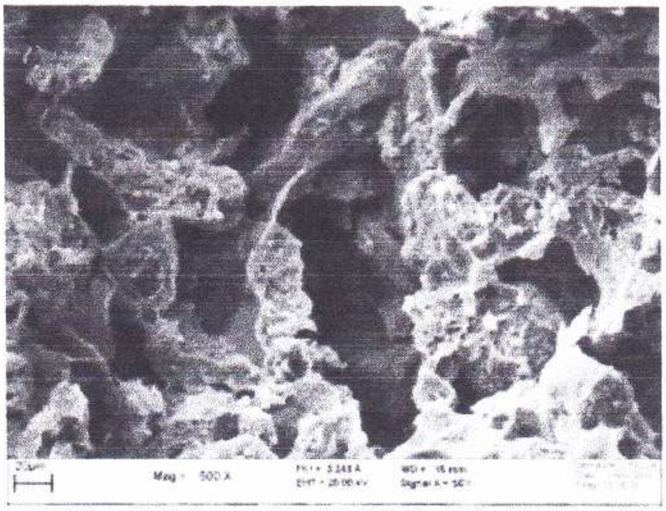


Figure 5. Morphology of [G/bHA] 50%w/v after coating with 0.2%w/v PVA.

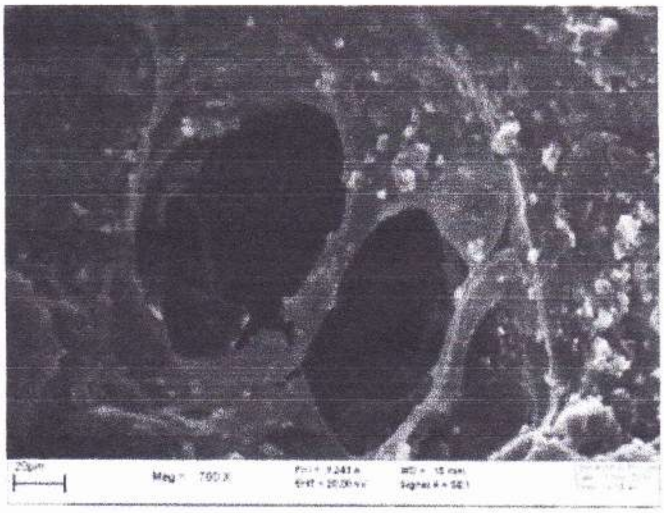


Figure 6. Morphology of [G/gHA] 50%w/v after coating with 0.2%w/v PVA.

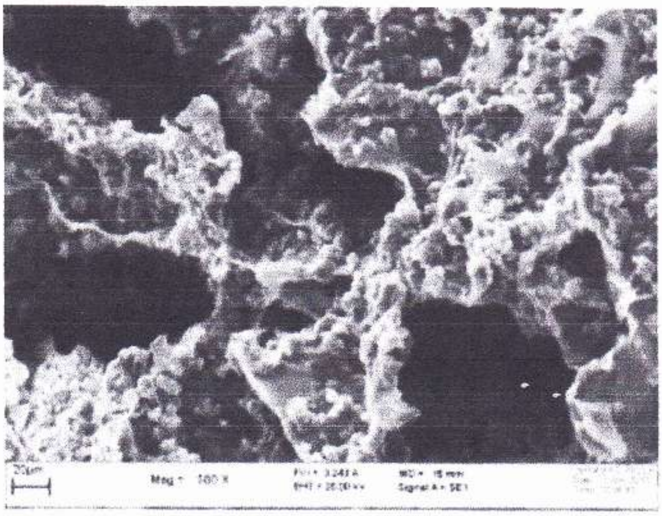


Figure 7. Morphology of [G/coHA] 50%w/v after coating with 0.2%w/v PVA.

Comparing to the composite of [G/gHA] and [G/coHA] with the same concentration (50%w/v), the pore wall thickness of [G/bHA] is higher than that of others. This gives us evident that why the DTS value of [G/bHA] is higher than that of others. In another word, the composite of [G/bHA] with 50%w/v concentration has better mechanical strength comparing to other composites.

4. CONCLUSIONS

In this study, a novel procedure in fabrication of [G/bHA], [G/gHA] and [G/coHA] porous scaffold with PVA film coating has been established. The scaffolds have been made by mixing HA which was synthesized from gypsum and calcite as well as calcinated bovine and gelatine solution with 1/1 ratio. The concentration of the composite to the destilled water were varied by 30, 40 and 50% w/v. In this work, to obtain the porosity of the scaffold before and after PVA coating, a procedure of twice freezing and freezed drying processes has been applied. The first has been used to the samples before PVA coating; and the second was applied after coating just before quenching in liquid nitrogen to keep its porosity. After following those several step processes, PVA coating improves the diametral tensile strength (DTS) of the scaffold composite in the range of 19.4 to 85.5% depending to the types of HA. Among those composites i.e. G/gHA, G/coHA and G/bHA, PVA coating to G/coHA gives the highest improvement, but its DTS value is still lower than that needed for scaffold. In this sense, coating of PVA onto G/bHA composite gives significant DTS value that suitable to be a scaffold. This composite after PVA coating has DTS value of 6.219 MPa which is confirmed to be applied for scaffold of human with age of 62 years old as the study of McCalden *et.al.*

ACKNOWLEDGEMENTS

A part of this research was supported by Graduate School research grant UGM 2010 and characterization work of the materials have been carried out at Politecnico di Torino, Italy as part of the program of the Erasmus Mundus External Cooperation Window 2010 in which the first author has worked for 3 months.

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