Preparation of bio-composite hydrogel of hydroxyapatite based using gamma irradiation for artificial bone

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Abstract. The aim of this study is to prepare bio nano-hydroxyapatite based biomaterial for artificial bone using gamma -γ. Preparation of composite hydrogel bio nano-hydroxyapatite based on artificial bone has been carried out via gamma irradiation technique. The PVA-HAp vis cose solutions were prepared using different ratios of HAp (15-30%) with a single PVA solution concentration (20 %) mixed strongly until homogenous. The prepared PVA-HA solution was molded, freeze-thaw to be composites and irradiated using gamma rays (0-20 kGy). The composites were measured its mechanical properties and biodegradation. Surface micrographs of composites were measured using Scanning Electron Microscopy (SEM). The results showed that at optimum conditions (20% PVA -30%, HAp, irradiation dose 20 kGy), the physical appearances of composite more flexible and strong with tensile strength were 1.003 MPa and the elongation at break was 372.33%. With increasing irradiation and HAp content up to 30%, the biodegradation of composite decreased. From SEM measurement the pore sizes of the composite were heterogeneous. PVA-HAp composites prepared using gamma irradiation can be considered as a candidate for biomaterial.

1. Introduction
Development and application of biomaterials in the biomedical field as bone implants are becoming important, especially in developing countries like Indonesia, because human life expectancy at the age of 80 years increases [1]. In general, the probability of a person suffering from osteoporosis and skeletal fragility increasing with age. In addition to aging, soft and hard tissue are damaged or cracked due to traumatic injury also increases. Therefore, the development of biomaterials that can be transplanted into the human body in order to replace or repair tissues damaged and can be accepted both physiologically and economically is very important now [2].

A biomaterial is defined as a material that has been engineered into a particular form, alone or as part of a complex system, used to direct, by controlling the interactions of components of body living systems following the treatment procedure any therapeutic or diagnostic in the animals or human [3]. Whereas Valleg-Reg[4] defines biomaterial is an implant material that can be function when in contact with living tissue. Depending on its functions, the biomaterial can be made from a variety of materials such as metal, polymers, ceramic material or composite forms.

Poly (vinyl) alcohol (PVA) is a potential hydrophilic polymer that can be used for biomedical applications especially in form injectable liquid gel [5]. At present, PVA has been widely used to
replace body tissue damaged or diseased due to its physicochemical properties, especially its excellent bio-tribological properties, which have a slippery surface, resistant to friction and wear [6-9]. In addition it can prevent erosion and corrosion and does not cause inflammation. In addition, the use of PVA is increasing as a substitute for body tissue damaged due to trauma or disease. This is because PVA is biocompatible, non-toxic, not carcinogenic, and shows good elasticity and compressive strength. However, PVA is a fragile homopolymer. Therefore, for its use as a biomaterial, it is generally blended with other polymers or ceramic materials to increase its mechanical strength (10-13). Hydroxyapatite (HAp) can be good candidates to be blended with PVA for biomaterial.

The use of calcium phosphate-based implants such as hydroxyapatite Ca (PO$_4$)$_2$(OH)$_2$-HAp for bone and dental reconstruction is well known. This is due to the fact that HAp is one of the inorganic mineral components of hard biological tissue with very high biocompatibility [14-16].

Natural HAp derived from biological material is essentially crystalline nano in the size range of hundreds of nanometers. The HAp implant also exhibits high bioactivity and osteoconductivity despite the low degradation rate and relatively good mechanical properties. HAp can be utilized in the form of solid or solid gels and can be made in the form of nano size.

However, HAp-based implants have limitations in their application because their solid form is fragile and unstable in the long-term and HAp is good used for regenerating bone defects in parts of the body that do not bear the burden. Therefore, HAp is only used in areas with relatively low mechanical strength, such as bone and tooth fillers, or coatings on implantable devices. In fact, human bones often experience fractures including tibia and fibula bones that support body weight when someone stands up. Thus, mechanical strength also plays an important role. In recent years, to overcome the limitations of using both PVA and HAp if used as implants, hence polymer-bioceramic composites have been developed.

Biocomposite materials should be easily made in certain forms to display specific functions, for example to improve the mechanical properties of polymers or the hardness of HAp. A number of research has been carried out on the preparation of polymer-ceramic biocomposites based on consideration of the fact that bone is an anisotropic composite consisting of organic polymers (collagen and fibrin fibers) and inorganic minerals (HAp carbonation or HAp derivatives). Biocomposite types include HAp-polyacrylamide, HAp-collagen, HAp-collagen-hyaluronic acid scaffold for tissue engineering [17,18]. The development of biocomposite is basically motivated by the fact that pure material cannot stand alone in meeting all the requirements needed to make it a biomedical implant. In general, a composite consists of at least two different chemical compounds which mutually coexist, one as a matrix and the other as a filler (filler) that is different and not dependent on the other. Balgova et al. [19] prepared PVA membrane composites blended with HAp at concentrations of up to 50% by drying techniques at 30 °C for 7 days. The weakness of the resulting composite does not have good tensile strength, because the process is carried out at low temperatures that do not produce strong crosslinked in the composite. The parameters tested include surface properties, IR spectrum and membrane bioactivity test in SBF solution.

Based on the description above, in this study bio-nano HAp particles obtained from fish scales extraction were used for composite hydrogels preparation. The HAp were blended with PVA through gamma irradiation techniques. PVA-HAp biocomposites were prepared by mixing reinforced 20% PVA solutions with different HAp concentrations 15%, 20%, 25% and 30%, casted, freeze-thawed and then irradiated at irradiation doses 10 and 20 kGy. The irradiated biocomposite PVA-HAp hydrogel was determined for its mechanical properties and biodegradation. As well as microphotograph characterization using a Scanning Electron Microscope (SEM).
2. Materials and methods

2.1. Materials

The materials used in this study were Poly (vinyl) alcohol (PVA) Kuraray Brand made in Japan, Bionano hydroxyapatite (HAp) made by PAIR BATAN obtained from the extraction of Baramundi scales with a purity level for around 98%. PBS solution (Phosphate buffer solution), made by Merck. Other chemicals of the quality p.a.

2.2. Methods

2.2.1. Bionano hydroxyapatite (HAp) extraction from barramundi fish scales. Fish scales obtained from the center of the fish auction, Muara Karang, washed clean with tap water to remove surface impurities. Then, fish scales soaked in a surfactant solution to remove fats on the surface of fish scales for 24 h. Furthermore, the fish scales were cleaned from the surfactants with tap water, then the fish scales were soaked in 1 N NaOH solution for 24 h to remove the fatty acids that were strongly attached to the fish scales. The clean fish scales were soaked again in 1 N NaOH solution and heated at 120 °C at a pressure of 1 bar for 2 h. The result of heating obtained white precipitate as bio-nano HAp and turbid yellow solution which was a solution of gelatin / collagen degradation. The white precipitate separated from the filtrate, and then the white precipitate rinsed with distilled water and dried in the oven for 24 h. The dried white powder was bio nano-hydroxyapatite.

2.2.2. Preparation of PVA-HAp biocomposite. A series of 20% PVA solutions were prepared by dissolving 20 g of PVA in 100 mL of distilled water using an autoclave at 121 °C for 2 h. Furthermore, PVA solutions were mixed with different concentration 15%, 20%, 25% and 30% (% wt) of HAp powder respectively, then the solution stirred quickly to prevent the precipitation of HAp from solution using a mixer with a high speed of 300 rpm until the HAp homogeneously mixed with the PVA solution. Then, 100 ml of the mixed solution cast in polypropylene (PP) plastic with a size of 10 x 15 x 0.5 cm³, the solution was immediately frozen in the freezer for 3 cycles at 4°C (freeze, 16 h) and room temperature 30 °C (thawing, 8 h). Finally, the freeze-thaw PVA-HAp composite was irradiated using gamma rays at doses 10 and 20 kGy in the Irpasena irradiator at a dose rate of 2.5 kGy / h.

2.2.3. Tensile strength and elongation strength testing. Tensile strength and elongation at break are important mechanical properties of the hydrogel biocomposite, representing the maximum tensile stress during the extended breakout process and the percentage increase in length (elasticity) of the test sample due to tensile stress, measured according to the ASTM (American Standard Testing Mechanical) standard method using the Instron machine. Composite dumbbell-shaped bell with standard size was used, both ends of the samples were clamped with one moving and the other end in a state of rest in the Instron machine. Clamping speed of 30 mm / min at room temperature. Measurement data recorded. The test was carried out with 5 replications. Elongation at break was calculated by the equation,

\[
\text{Elongation at break (EB)} = \frac{L_1 - L_0}{L_0} \times 100\%
\]

\( L_0 \) initial sample length; \( L_1 \) measures the length of the final sample

Tensile strength is calculated by the equation

\[
\text{Tensile strength (TS)} = \frac{F}{A}
\]

\( F \) = Load from tool to breaking material (kg), \( A \) = Material cross-sectional area (cm²)
2.2.4. Biodegradation test. The composites were cut to a size of 1 x 1 cm$^2$, then dried in an oven at 60°C for 24 h up to constant. Then, the composites were soaked into buffer solution (pH 7.4) for 1 day and in shaking incubator with a speed of 40 rpm at room temperature, then the composite was removed from the test container and then dried in a vacuum oven at 60°C for 2 h and dried composite weighed ($W_1$). Dry composite then soaked back into the original container containing PBS solution. The above treatment was repeated for biodegradation testing at intervals of 4, 6, 8, 10, 12, 24 days. The remaining composite weights were calculated by the following equation:

$$\text{Remaining composite weight} = \frac{W_1}{W_0} \times 100\%$$  \hspace{1cm} (3)

$W_1$ = dry composite weight after immersion (g), $W_0$ = initial dry composite weight

Degraded composite = 100% - % by weight remaining  \hspace{1cm} (4)

2.2.5 SEM measurement. The surface characteristics of the composite hydrogel were investigated using the Scanning Electron Microscope (SEM), Zeiss, made in Germany. Composite hydrogels in dry form, soaked in water to maximum swelling. Then the composite hydrogel has frozen in the freezer at -25°C for 48 h. Then, the composite hydrogel was lyophilized using freeze-drying at -40°C (24 h). Dry composite hydrogels were then observed for surface properties using SEM and recorded.

3. Results and discussion

3.1. Tensile strength of PVA-HAp composites

In this study the white HAp powder (Figure 1b) was obtained from the extraction of white barramundi fish scales (Figure 1a). White barramundi scales are one of the fisheries wastes with a relatively large amount. In Indonesia this waste has not been utilized maximally yet. Basically fish scales waste consists of 2 major components of potential biological compounds as important materials for biomaterials, including collagen / gelatin and HAp. Therefore, HAp extraction has been successfully carried out with mild processing of fish scales in our laboratory [20]. The characterization results show that HAp specific characters such as IR spectrum, XRD, are the same as commercial HAp characters. In addition, the TEM, HAp measurement results are nano-quality [21]. The results of in vitro studies conducted on mice, showed that HAp fish scales could induce the growth of rat tooth bones [22]. As is generally well-known methodologies for HAp synthesis can be produced by several methods, such as wet chemical precipitation, hydro-solvothermal, sol-gel, SPCS, solid-state reactions, emulsions and microemulsions [23]. The final HAp product is generally purified using a furnace and calcination. Process temperatures range from -85°C to 1250°C, the resulting particle size ranges from 0.1 µm up to 1.0 µm. In recent years research in Indonesia has been carried out for the manufacture of HAp using natural raw materials such as limestone, rice conch, and cow bones with calcination and kiln processes [24-26].

![Figure 1. (a) Baramundi fish scales and (b) HAp powder.](image)
The effect of variations in different concentrations of HAp 15, 20, 25, and 30% on the tensile strength of the PVA-HAp composite hydrogel resulting from the treatment of freeze-thaw and irradiation combinations of 0, 10 & 20 kGy is presented in Figure 2. It is seen that under conditions of increased HAp concentration (15 -30%) at a constant irradiation dose of 0 kGy, showed that PVA-HAp composites which were only treated in the freeze-thaw process increased linear tensile strength with increasing HAp concentrations (0.6 to 0.8 MPa). In addition, under conditions of increased irradiation dose from 0 to 20 kGy (constant HAp concentration) it is seen that there is an increase in tensile strength at each HAp concentration up to 1.003 MPa. This shows that at 30% HAp content and 20 kGy irradiation dose, there is no saturation of HAp in the PVA matrix which causes the composite to become brittle. An increase in the tensile strength of the PVA-HAp composite by increasing the HAp concentration during the freeze-thaw process for up to 3 cycles, this is due to an increase in the crystallinity of PVA in the composite. Whereas increasing irradiation dose causes cross-linking in composites to increase. Therefore, the combination of the freeze-thaw and irradiation treatment has a synergistic effect on the physical properties of the PVA-HAp composite. According to Gonzales et al. [27] the PVA-HAp composite with a tensile strength of 1.003 MPa should be considered as a candidate for application as a joint replacement with the required tensile strength of 275 ± 23 kPa (0.275 ± 0.023 MPa). An example of a freeze-thaw PVA-HAp blend and its irradiation can be cast on various desired shapes with strong physical characteristics not fragile (Figure 3).

**Figure 2.** Effect of HAp concentration vs the tensile strength (TS) of the composite PVA-HA prepared by gamma irradiation in the dose range of 0 kGy - 20 kGy.

**Figure 3.** Example of a type of bone implant that can be made from PVA-HAp composites prepared from freeze-thaw and gamma irradiation.
3.2. Elongation at break (EB) of PVA-HAp composites

Elongation at break and tensile strength are important mechanical properties of hydrogel composites to be used as biomaterials. The relationship between hydroxyapatite concentration with the elongation at breaks of the composite is presented in Figure 4. It is seen that with increasing HAp concentrations from 15 to 30% followed by an increase in irradiation doses from 0 to 20 kGy, the elongation at breaks composite has increased to a maximum at 30% concentration and dose irradiation of 20 kGy with elongation at break of 372.33%. This shows that the composite can be extended to 300% (3 times the original length). Thus it can be concluded that the PVA composite is relatively flexible.

![Figure 4. Effect of HAp concentration on elongation at break (EB) PVA-HAp composites with various irradiation dose 0 kGy, 10 kGy, and 20 kGy.](image)

3.3. Biodegradation

The degradation properties of the composite are one of the important factors should be tested, because this concerns the stability of the composites in a particular medium if used for biomedical purposes. The effect of HAp concentration from 15% to 30% on the percentage degradation of PVA-HAp composite hydrogel measured at intervals of 24 days is presented in Figures 5 A, B, C, D. It can be seen that with increasing HAp concentration from 15% to 30% followed by increasing the irradiation dose from 0 to 20 kGy, under constant immersion conditions the percentage degradation of composite hydrogels generally decreases by around 2%. It showed the increase in irradiation dose and HAp concentration resulted in the composite's biodegradation did not increase significantly as well as increasing immersion time in the PBS solution. This may be due to the erosion of some of the HAp that is not strongly bound in or on the surface of the hydrogel composite matrix which is a component of inorganic compounds. The occurrence of dissolving HAp from the composite, this certainly does not cause significant side effects because HAp can have a positive effect on the body that participates in the bloodstream containing Ca²⁺ ions.

![Figure 5. Effect of immersion time in PBS solution vs biodegradation of PVA-HAp hydrogel composites resulting from 0-20 kGy irradiation on variations in HAp content (a) 15%, (b) 20%, (c) 25%, (d) 30%.](image)
3.4. SEM of PVA-HAp composite

The Scanning Electron Microscopy (SEM) test is intended to test the porosity or microscopic structure arising from the PVA-HAp composite. By using SEM analysis it can be observed the pore size and surface morphology of the composite. The results of SEM observations on PVA-HAp composites with magnifications of 100-1000 times are presented in Figures 6 a, b, and c. It can be seen that the composite surface consists of white solid fibers with irregular pores (Figure 5a). Judging from a cross-section in a composite with a magnification of 500x (Figure 5b), visible layers / layers of layers in the composite cavity are not solid. The white solid coated sheet is HAp which is mixed with PVA resulting from a strong mixer and cavity due to evaporation of water from the solid mixture in the freeze dryer process. Based on the porosity of the PVA-HAp SEM composite photo with a magnification of 100 times, poricomposite size varies with heterogeneous size, the smallest pore size of the composite hydrogel around 20 µm.

![SEM images of PVA-HAp composite](image)

**Figure 6.** The photomicrograph composite of PVA-HAp hydrogel irradiated with (a) magnification of 100X, (b) 500X, and (c) 1000X.

4. Conclusion

From the results of this study it can be concluded that the biocomposite hydrogel PVA-HAp based on bio-nano HAp of fish scales was successfully prepared by gamma irradiation technique. Increased HAp concentrations from 15% to 30% followed by an increase in irradiation doses up to 20 kGy in the matrix causes the breaking stress and elongation of composite breaks to increase. Biocomposites of HAp-based hydrogel prepared by gamma irradiation are relatively difficult to degrade in PBS solutions. PVA-HAp biocomposite hydrogels can be considered as biomaterials.
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