

EFFECT OF GLUTARALDEHYDE AS A CROSSLINKER ON MECHANICAL CHARACTERISTICS OF CATGUT REINFORCED POLYVINYL ALCOHOL-HYDROXYAPATITE COMPOSITE AS BONE- FRACTURE FIXATION MATERIAL

Submission date: 07-Mar-2023 08:15AM (UTC+0700)
by Mochammad Taha Maruf

Submission ID: 2030745310

File name: Glutaraldehyd_jeas_0815_2414.pdf (559.4K)

Word count: 4208

Character count: 23051



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ABSTRACT

Metal (titanium) as a bone-fracture fixation material were characterized by very high strength and stiffness compared to mechanical bone, thus could lead to bone atrophy. This condition requires a secondary surgical procedure to remove bone-fracture fixation devices, leads to increase of treatment time, recovery time and nursing costs. In this study, we use absorbable material (biodegradable) to avoid the secondary surgical procedure. Previous study shows catgut reinforced PVA-HA (60:40) composite, did not reach sufficient mechanical strength as a bone-fracture fixation material. In this study, we used glutaraldehyde as a crosslinker to improve the mechanical characteristics of the composite. The specimens were prepared for tensile and bending test and immersed in phosphate-buffered saline (PBS) solution with pH 7.4 and incubated at 37 °C. The specimens were tested for mechanical strength during the period of 0 and 60 days. The results showed that the addition of glutaraldehyde (1%) as a crosslinker could significantly improve the mechanical strength of catgut reinforced Polyvinyl Alcohol (PVA)-hydroxyapatite (HA) composite as a bone-fracture fixation material.

Keywords: glutaraldehyde, crosslinker, polyvinyl alcohol, hydroxyapatite.

INTRODUCTION

The application of bone-fixation devices to manage facial and jaw fractures had become a routine procedure using metal plates and screws, such as titanium, titanium-alloy or cobalt chrome (Mazzonetto *et al.* 2004). Metal fracture-fixation devices have several advantages, and some disadvantages. Metal have very strength and stiffness characteristic compared to mechanical bone, thus could lead to bone atrophy (Kennady *et al.* 1989; Brodke *et al.* 2001). The other disadvantage, including the requirement of secondary surgical procedure to remove bone-fracture fixation devices, leads to increase of treatment time, recovery time and nursing costs. It is a great challenge to use a biodegradable polymer material with good mechanical characteristics and biocompatibility as jaw bone-fracture fixation material such as Polyvinyl Alcohol (PVA).

Polyvinyl Alcohol (PVA) has the excellent physicochemical characteristic, with superior surface interaction resistant to friction and wear, and has a smooth surface to prevent erosion and corrosion (Zheng *et al.* 1998; Stammen *et al.* 2001; Suci *et al.* 2004). Unfortunately, this material has the weak mechanical strength, thus it was used widely in low mechanical strength applications, including drug delivery, contact lenses, bone grafts, wound closure and articular cartilage (Noguchi *et al.* 1991; Pan *et al.* 2007). Therefore, an effort has been made to improve the mechanical characteristics of this material. The material used as a reinforcement to increase the mechanical characteristics was catgut, absorbable wound suturing material. Catgut derived from

sheep intestines collagen and has been used widely in the medical field, including orthopedics to cover deep tissue, and will be absorbed by the skin mucous. The thickness and stiffness of the polymer will be increased by the addition of hydroxyapatite (HA) as a filler in the composite. Hydroxyapatite is a bioactive and osteoconductive material and has a good biocompatibility (Suchanek and Yoshimura, 1998).

Previous study by Ma'ruf *et al.* 2013, shows that catgut reinforced PVA-HA (60:40) composite did not have sufficient mechanical strength as a bone-fracture fixation material. In this study, we added a crosslinker agent to improve the mechanical characteristics of the composite. PVA has a hydroxyl or amino groups in the main chain, thus the material that used as the crosslinker was glutaraldehyde (Swieszkowski *et al.* 2008). The aim of the study was to assess effects of glutaraldehyde as a crosslinker on mechanical characteristics of catgut reinforced PVA-HA composite for bone-fracture fixation material.

LITERATURE REVIEW

The composite material is a macroscopic combination of two or more distinct materials, having a recognizable interface between them (Vasiliev and Morozov, 2001). Biocomposite is a material consisting of two or more different components used to achieve the physicochemical, mechanical and biological characteristic on biomedical applications, and cannot be reached from the initial material (Ramakrishna *et al.* 2004). Composite materials are usually optimized to achieve a certain



balance of properties for various applications. The improved structural properties generally achieved by a load-sharing mechanism (Miracle and Donaldson, 2001).

Polyvinyl Alcohol (PVA) is a biodegradable material (Goodship and Jacobs, 2005), and has excellent biocompatibility (Covert *et al.* 2003; Kobayashi *et al.* 2005). The use of these materials in biomedical applications was limited to low requirement of mechanical strength sites such as articular cartilage (Noguchi *et al.* 1991; Pan *et al.* 2007). Polyvinyl Alcohol (PVA) has a high tensile strength and flexibility, oxygen barrier and good solvent resistance (Peppas and Merrill, 1977). The solubility of PVA in water was influenced by the molecular weight and degree of hydrolysis. Fully hydrolyzed PVA has a high hydrogen bonding due to no dispersion before melting or dissolution. The degree of hydrolysis related to the strength of chemical structures. PVA is characterized by the presence of polar alcohol (OH group) lead to increasing water solubility. Hydroxyl group was shown by high hydrogen bonding in water and the hydroxyl groups hindered the solubility overall (Goodship and Jacobs, 2005; Pal *et al.* 2007).

Catgut is a bioresorbable wound suture and biocompatible materials (Kulkarni, *et al.* 1966; Cutright, *et al.* 1971; Racey, *et al.* 1978). According to Tolga *et al.* (2004), catgut has a good tensile strength, so it has been used as a reinforcement on PVA-HA composite.

In polymer matrix composite, it oftenly composite with some additional materials, including a filler material. According Brydson (1999), the addition of filler will lead to increasing of polymer density. Hydroxyapatite particles as inert filler was influenced by the size, shape and distribution of the particles. The smaller the particle size, the higher mechanical strength, such as tensile strength, modulus and hardness. The results of sharing mechanisms such composite materials can enhance the structural properties, especially the strength and stiffness of the composite (Vasiliev and Morozov, 2001).

Crosslinker is a compound that can bind to the polymer chain with another chain, covalent or ionic bonds form (Gebben *et al.* 1985). The material that used as a crosslinker for hydrogels PVA which has hydroxyl or amino groups in the main chain was glutaraldehyde or $\text{CH}_2(\text{CH}_2\text{CHO})_2$ (Swieszkowski *et al.* 2008). Patel and Vavia (2010), showed that the improvement in the mechanical characteristics caused by the strong bond between the aldehyde and the OH group of PVA chains. Furthermore, according to Patel and Vavia (2010), an increase in the mechanical characteristics will also lead to an increase in the degree of PVA crystallinity on C-O stretching band with a broader absorption band formation, which was associated with ether (C-O) and acetal ring (C-O-C).

Based on the polymerization reaction, the polymer can be classified into three groups, namely polyaddition, polycondensation and polymerization through genetic engineering (Temenoff and Mikos, 2008). Polyaddition is a polymer occurs due to an addition

reaction or a chain reaction. In this polymerization requires three phases, consist of initiation, termination, and propagation. In the initiation phase, it requires an activation of the monomer through the addition reaction of monomer molecules bind to each other copies that are triggered by free radicals, ions, or cations. In propagation stage, further monomers bind to the polymer chain, resulting in increased molecular weight. At the termination stage, chains stop the destruction of a way of active through free radical reactions. Addition reaction usually takes place quickly by opening the double bond. PVA is a hydrophilic polymer as many have -OH group and the polymerization occur by way of polyaddition. Polycondensation is a polymer that occurs due to condensation reaction or reaction stages, is slow and does not require free radicals as initiation. The mechanism is identical to a condensation reaction with a low molecular weight compound that is a reaction of two active groups of two different monomer molecules interact with the release of small molecules (Temenoff and Mikos, 2008).

Mechanism of polymer degradation can occur in two ways, namely the dissolution mechanism (swelling/dissolution) and the chain scission mechanism. Biodegradation by dissolution occurs through the absorption of water into the polymer which causes the gaps between macromolecules. In this mechanism, the water acts as a plastizer which causes the polymer material becomes ductile, a reduction of secondary bonds between the chains. This condition affects the crystallinity of the polymer (Temenoff and Mikos, 2008). The parameters that used to monitor biodegradation are decrease in molecular weight as well as a decrease in the mechanical strength (Laitinen *et al.* 1992; Gijpferich, 1994). In addition Temenoff and Mikos (2008), indicators that used to monitor biodegradation are discoloration, cracks (crazes) and a decrease in mechanical strength.

Characterization test of the catgut reinforced PVA-HA (60:40) composite with glutaraldehyde as a crosslinker was a FT-IR studies. FT-IR observations indicate the intensity of the peaks associated with hydroxyl, carbonyl and vinyl groups. The increase in the peak of the carbonyl group is due to the strong bond between the aldehyde group of glutaraldehyde and the hydroxyl groups of polyvinyl alcohol as a result of PVA dissolved (Thirmizir *et al.* 2011). According to Patel and Vavia (2010), the aldehyde group of glutaraldehyde used in the reaction is a complete crosslink with hydroxyl groups of PVA chains. Absorption peak associated with the vinyl group, indicating a covalent bond between two carbon atoms (C-C). Increased peak indicates the degree of PVA crystallinity, which occurred a covalent bond between the carbon and oxygen (C-O) (Thirmizir *et al.* 2011). Patel and Vavia (2010) indicates the degree of PVA crystallinity will be replaced by the formation of acetal ring (C-O-C).

MATERIALS AND METHODS

This study used PVA (Mowiol) 56-98 grade, a fully hydrolyzed with 98.0-98.8 (mol %) hydrolysis rate



and 52–60 (mPa·s) viscosity (Aldrich–Germany). Hydroxyapatite powder obtained by synthesizing calcite powder (CaCO_3) with diammonium hydrogen phosphate (DHP, $(\text{NH}_4)_2\text{HPO}_4$; Merck–Germany) solution. Catgut used in this study was chromic catgut with 2/0 diameter. Crosslinker is used glutaraldehyde 25% (Merck–Jerman). In each specimen, it contained five layers chromic catgut 2/0 plait with horizontal orientation.

PVA and HA powder was weighted with PVA–HA (60:40) proportion. PVA is dissolved in distilled water at comparison 7.5% (w/v) using a stirrer machine (95 °C, 600 rpm, 30 minutes). After PVA dissolved, HA was added and stirred (60 °C, 300 rpm, 60 minutes). After the materials mixed, it was added with appropriate glutaraldehyde concentrations according to the groups. In prepared mold with catgut fibers plait, lay-up was done layer by layer. Each layer was heated with oven (70 °C, 15 minutes) until thickness desired. The specimen is heated using an oven (70 °C, 120 minutes) and removed from mold after that.

There are two treatment groups (T) with the variation of concentration glutaraldehyde as crosslinker on catgut reinforced PVA–HA (60:40) composite, where the T1 (glutaraldehyde 0.5%), T2 (glutaraldehyde 1%) and Control group, was catgut reinforced PVA–HA (60:40) composite without glutaraldehyde as a crosslinker. Biodegradation test was performed by immersing specimens in phosphate-buffered saline (PBS) solution with pH 7.4 and incubated at 37 °C. In 30 and 60 days, specimens were performed for mechanical strength tests, including tensile (ASTM D 638–99) and bending test (ASTM D 790–9913). In this period also performed characterization tests that Fourier Transform Infrared (FT-IR) spectroscopy observation to determine the changes of the molecular bonds picture.

RESULTS AND DISCUSSIONS

The specimens dried at 70 °C for 2 hours after immersing in PBS solution for 30 and 60 days period, in addition to mechanical strength tests, include tensile and bending tests. Mechanical strength mean of catgut reinforced PVA–HA (60:40) composite after biodegradation test for 30 and 60 days on Treatment and Control groups is shown in Table-1 and Figure-1.

Table-1. Mechanical strength mean of catgut reinforced PVA–HA (60:40) composite after biodegradation test for 30 and 60 days in treatment and control groups.

Mechanical Strength	Group	0 DAY	30 DAYS	60 DAYS
		σ (MPa)	σ (MPa)	σ (MPa)
Tensile	C	30.98±1.11	28.82±1.04	26.84±0.98
	T1	34.35±2.08	34.35±0.78	32.22±1.94
	T2	43.32±0.35	39.96±0.55	39.63±0.63
Bending	C	16.85±0.55	14.75±0.68	12.31±0.87
	T1	21.35±0.50	19.90±0.83	18.54±0.43
	T2	43.54±1.86	37.06±0.74	36.40±0.43

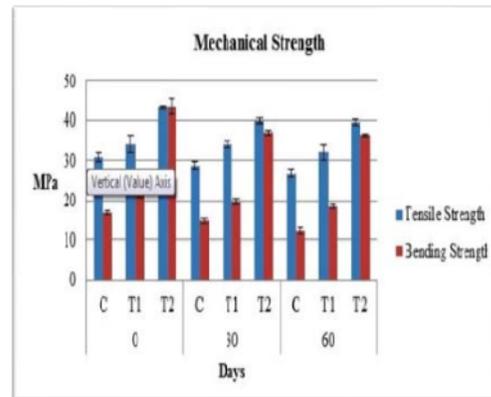


Figure-1. Mechanical strength mean of catgut reinforced PVA–HA (60:40) composite after biodegradation test for 30 and 60 days.

The mechanical strength test shows T2 group (catgut reinforced PVA–HA 60:40 with glutaraldehyde 1%) had the highest tensile (43.32 ± 0.35 MPa) and bending strength (43.54 ± 1.86 MPa) mean. One Way Anova test showed that the ratio of the concentration of PVA–HA (60:40) with glutaraldehyde 1% had significant effects ($p < 0.05$) on the tensile and bending strength tests before and after immersion for 30 and 60 days. After immersion for 30 and 60 days, the catgut reinforced PVA–HA (60:40) composite with glutaraldehyde 1% had still sufficient mechanical strength as bone fracture fixation material. These showed that the catgut reinforced PVA–HA (60:40) composite with glutaraldehyde 1% as a crosslinker having a stable mechanical characteristics after immersion for 30 and 60 days.

The high mechanical strength was due to the influence both of the hydroxyl groups of the PVA polymer and HA particle bonds. According to Pan and Xiong (2009), there were two binding modes between HA and PVA molecules existed in the composites: (1) The Ca^{2+} cation in the HA and inter-PVA molecules or intra-PVA molecule forms bond bridge, and this can improve the rigidity of the network in the composites. (2) The hydroxyl group of HA and the free hydroxyl group in PVA molecules linked by the forms of hydrogen bond, and this can improve the interfacial bonding strength between HA and PVA polymer (Figure-2). The formation of chemical bonding between HA and PVA, such as hydrogen bonding and/or hydroxyl–calcium–hydroxyl ($[\text{HO}-\text{Ca}^{2+}-\text{OH}]$), allows the uniform dispersion of HA in poly(vinyl alcohol) hydrogel matrix (Fenglan *et al.* 2004).

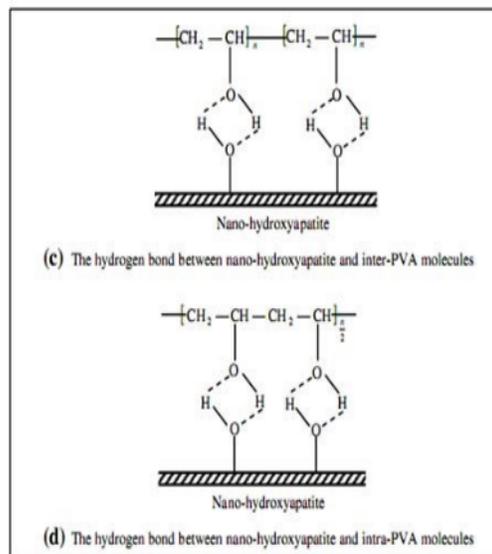
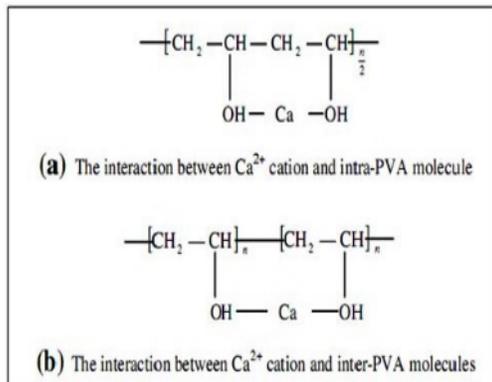


Figure-2. HA-PVA Interaction.

Catgut derived from the collagen gut (intestine) sheep and has been used extensively in the medical field, including orthopedic field to cover deep tissue, so it does not need to be taken back. Gelatin, which being a hydrolyzate of collagen, recently is also studied to substitute the expensive collagen. According to Wang *et al.* 2010, there was a complex network structure in HA/Gel/PVA composites, being existence of Ca^{2+} -COO- bond between HA and gelatine, and being OH---NH bond between gelatine (catgut) and PVA (Figure-3).

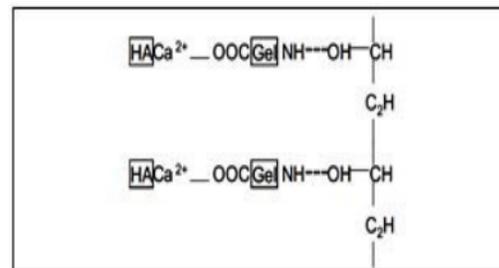


Figure-3. The complex network structure in HA/Gel/PVA composites.

The high mechanical characteristics are also influenced by the glutaraldehyde $[\text{CH}_2(\text{CH}_2\text{CHO})_2]$ concentration as a crosslinker to the PVA. The increase in the mechanical characteristics due to the strong bond between both the aldehyde group of glutaraldehyde with PVA chains. In addition to directly bind monomers in a polymer chain, a bond can also be formed between adjacent chains. Although not as strong or rigid as a bond in the chain, crosslinking has an important effect on the polymer. When the polymer is pulled, crosslink prevents single chain slide past each other. Can be a straight chain, but after the tension is removed the chain will return to the position and shape.

Crosslinker is a compound that can bind to the polymer chain with another chain, covalent or ionic bonds form. According to Gebben *et al.* 1985, two different modes of crosslinking can be expected when a crosslinking agent is added to a polymer solution. The first one is the crosslinking between different polymer molecules, called intermolecular crosslinking, which will lead to an increase in viscosity and finally to a gelation of the system. The second mode is the internal crosslinking of a single polymer molecule, called intramolecular crosslinking, leading to a decrease in viscosity due to volume contraction of the polymer coils. Intramolecular crosslinking occurs preferentially in dilute polymer solutions.

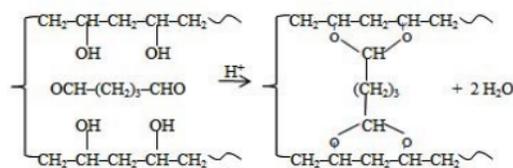


Figure-4. Chemical reaction of intermolecular crosslinking of the polyvinyl alcohol (PVA) and glutaraldehyde.

Furthermore, to determine changes in the molecular bonding picture was performed by Fourier Transform Infrared (FT-IR) Spectroscopy observation of PVA-HA composite (60:40) with glutaraldehyde (1%) as a crosslinker before and after immersion for 30 and 60 days (Figure-5).

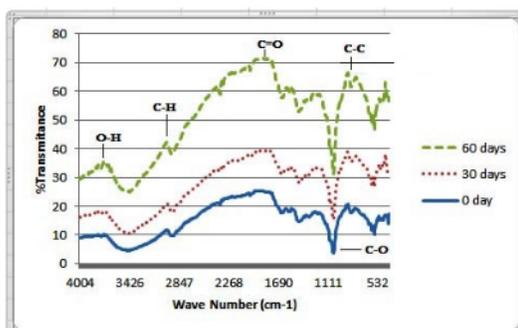


Figure-5. 13 FTIR spectra of PVA-HA (60:40) composite with glutaraldehyde (1%) as a crosslinker before and after immersion for 30 and 60 days.

Figure-5 shows the peak in the band near 3487 cm^{-1} wave number which shows the bond between the hydroxyl group ($-\text{OH}$) of polyvinyl alcohol and hydroxyapatite. After immersing for 30 and 60 days, there was a slight increase in the peak of the hydroxyl bonds caused the dissolution of PVA. Increased weak of this peak is due to the binding of the aldehyde of glutaraldehyde as a crosslinker with the hydroxyl groups of PVA. Absorption peaks associated with the vinyl group show at the band near the 847 cm^{-1} wave number, indicating a covalent bond between two carbon atoms ($\text{C}-\text{C}$). Increased this peak is due to chain scission of PVA after immersion for 30 and 60 days. Increased peak at band near 1065 cm^{-1} wavenumber indicates the degree PVA crystallinity, which occurs covalent bonds between carbon and oxygen atoms ($\text{C}-\text{O}$) after immersion for 30 and 60 days. Patel and Vavia (2010) indicates the degree of PVA crystallinity will be replaced by the formation of acetal ring ($\text{C}-\text{O}-\text{C}$) at wave number $980-1110\text{ cm}^{-1}$.

Figure-5 also shows the intensity of the peaks associated with a hydroxyl, vinyl and carbonyl groups. The increase in the bands near the 1715 cm^{-1} wave number shows the formation of a carbonyl group ($\text{C}=\text{O}$) after immersion for 30 and 60 days. The increase in the peak of the carbonyl group is due to the strong bond between the aldehyde group of glutaraldehyde and the hydroxyl group of polyvinyl alcohol as a result of the dissolution of PVA. According to Patel and Vavia (2010), that the aldehyde group of glutaraldehyde used in the reaction is complete crosslink with hydroxyl groups of PVA chains.

CONCLUSIONS

The results showed that the addition of glutaraldehyde (1%) as a crosslinker can improve the mechanical strength of catgut reinforced Polyvinyl Alcohol (PVA)-hydroxyapatite (HA) composite as bone-fracture fixation material significantly.

ACKNOWLEDGEMENT

The authors are thankful to Department of Industrial and Mechanical Engineering Gadjah Mada

University Yogyakarta, especially Bioceramics Laboratory for all support on the research.

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