ORIGINAL ARTICLE

Analysis of Characteristics and Immunogenic Response of Scafold Hydroxyapatite Gypsum Puger Combination of Cassava Starch as Bone Graft Material

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ABSTRACT

Introduction: A bone graft is a bone replacement material that is widely used in grafting damaged bone. The bone graft material being developed is a combination of hydroxyapatite (HAp) gypsum puger and cassava starch scaffold. Bone graft material must have the characteristics of the material according to standards, osteogenesis and not cause an immunogenic response. This study aimed to analyze characteristics and responses immunogenic from a combination HAp gypsum puger scaffold cassava starch as a bone graft material. **Materials and methods:** Scaffold HAp gypsum puger and Cassava starch were tested using XRD, compressive test, and immunogenic assay. Application of scaffolds to rats for 7 and 14 days. Blood samples were taken and tested for IgG levels using Elisa. **Results:** XRD test results, obtained high peaks in HAp gypsum puger scaffold and cassava starch. compressive strength test of HAp gypsum puger scaffold and cassava starch (50:50) of 2.07 MPa. The results of Ig G on day 14 on HAp scaffolding gypsum puger and cassava starch did not cause immunogenic reactions. **Conclusion:** In the XRD test, high peaks are produced indicating the purity of HAp. In the compressive strength test of the HAp gypsum scaffold group Cassava starch Puger 50:50 obtained 2.07 MPa.. immunogenic test analysis of scaffold HAp gypsum puger cassava starch in rats on day 14 did not cause immunogenic reactions.
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Keywords: XRD, Immunogenic, Gypsum HAp scaffold, Cassava starch, Bone graft

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INTRODUCTION

Bone graft is a bone replacement material that is widely used in grafting damaged bone. Bone graft is one of the alternative treatments for overcoming problem health bone. Several procedures related to medical health tooth clinical need graft bone are implant teeth, ridge augmentation, sinus tightening, preservation sockets, and periodontal surgery (1,2). When a tooth is extracted, the technique of preservation socket can used to minimize lost bone in the socket and maintain bone volume for placement implant teeth in the future. Bone graft is usually placed in the socket after the tooth is extracted for fill-in-room empty and guarding structure bones (3,4)

Some types of bone graft material include autograph uses network bone obtained from the body itself, allograft uses network bone donor, that is from others who have died or corpses that have been frozen, xenograft uses bone from animals besides species people, and materials synthetic from material ceramics, bioactive glass, polymer, hydroxyapatite synthetic (HAp) (5,6).

Bone graft material can used for the application manipulation of network bone. Tissue engineering technology is currently developing rapidly to facilitate tissue regeneration. In general, tissue engineering technology involves scaffold factors, cells, and growth factors. Scaffolds are a form of bone graph that is currently being developed (7,8,9). Synthetic bone graft is currently the choice because it has the advantages of good biocompatibility, is not toxic and does not damage cells in the body, does not cause immunogenicity, osteoconduction, ability injection, ability mold, manipulation easy, procedure minimally invasive its wide availability, because material the can with easily produced in scale large (10).

The synthetic bone graft that we have developed in previous research is characterized composition and microstructure of HAp gypsum puger freeze-dried scaffold with XRF test has the chemical formula (CaSO4.2H2O),

with XRD test shows rate purity 100% HAp and SEM test were obtained pores on the scaffold with average size 3 µm, results identical or contain the same pattern with HAp bovine scaffold as control (standard gold). The degradation process of the HAp gypsum puger freeze-dried scaffold occurred away slowly as you can influence proliferation cells and activities cells, so come in and grow become a scaffold for manipulation network bones (11). Scaffold HAp gypsum puger freeze-dried compared to freeze-dried HAp bovine scaffold no cause response inflammatory and immunogenic in mice through TNF-□ and IgG mediators (12). In other research regarding the mechanism regeneration of alveolar bone in mice, the expression of Stro-1, Runx-2, Osterix, and ALP is higher in the Hap gypsum puger group than in the group control on the day to 7th,14th, and 28th (13). However, this HAp puger gypsum scaffold still has weaknesses, namely low biomechanics, low porosity, and brittleness (14). Therefore, to improve the properties of this material, it needs to be further developed in combination with biopolymer materials. A natural material that includes biopolymer materials is cassava. Cassava with the Latin name Manihot utilissima is a tuber plant. Cassava is classified as a polysaccharide containing starch amylopectin and amylose. Cassava can be processed into cassava starch which is beneficial for health because it is a source of carbohydrates, protein, vitamins, and minerals to build bone mass (15).

In the development, study advanced k combination HAp gypsum puger and starch cassava generated a high amount of Ca with XRF test and had excellent interconnectivity between pores as well as No trigger response inflammation of the tissue through the mediator TNF-α (16).

The bone graft material should be own condition specifically so you can use and own optimal work among them that is characteristics material by standard, without sacrificing the donor area, stimulating osteogenesis, no There is response immune host, revascularization fast, stimulating osteoinductive, promotes osteoconductive and completely replaced with bone in similar quantity and quality with the host (4). In cases of bone damage, bone therapy is needed to accelerate the healing process and new bone formation with alternative tissue engineering materials in the form of bone grafts (17,18). The research aims to analyze characteristics and response immunogenic from a combination HAp gypsum puger scaffold cassava starch as an ingredient bone graft.

MATERIALS AND METHODS

This research was divided into several tests, namely XRD tests, mechanical tests, and immunogenic tests on mice.

Ethical test

Permission ethics study This has Approved Committee Ethics Faculty Jember University Dentistry with number letter no. 2015/UN25.8/KEPK/DL/2022.

Manufacturing HAp Gypsum Puger

Synthesis of HAp Gypsum Puger, Gypsum is prepared and then sieved to get a smooth texture. Next, make a Diammonium Hydrogen Phosphate (DHP) solution with a concentration of 0.5 M. Fine Gypsum powder is added to the DHP solution as much as 5 g. Next, homogenize the solution. After that, the solution is filtered while rinsing with distilled water to remove sediment. The precipitate was dried in an oven and weighed.

Creation Cassava Starch (Manihot esculenta Crantz)

1 kg of cassava is washed, peeled, and grated. Then, add 1 L of water to the grated cassava. Next, settle the solution for 12 hours. Drying the precipitate using an oven at a temperature of 50 ° Celsius for 6 hours. The powder was ground with a mortar and sieved with a 100 mesh size.

Sample Preparation Scaffolds combination HAp gypsum puger and Cassava Starch *(Manihot esculenta Crantz)*

Samples were made with HAp gypsum puger cassava starch scaffold ratios of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 . The gelatin solution was mixed with 4 mL of 2% acetic acid. HAp gypsum puger powder, cassava starch, and 0.1 M NaOH were added, then homogenized on a magnetic stirrer hot plate and sonic rupture. After the solution was homogeneous, the solution was put into the solution which was inserted using a micropipette into a cylindrical Eppendorf with a height of 30 mm and a diameter of 8 mm. Then it was frozen at -60 degrees Celsius for 24 hours and the freeze-drying process was carried out at -80 degrees Celsius for 24 hours. Got it Hap gypsum puger scaffold starch cassava in form The measuring cylinder has a diameter of 5 m and a height of 5 m, then Gamma radiation sterilization is carried out.

XRD Test

Analysis techniques are used to identify phase compounds as well as give information about the dimensions of cell crystals. The analyzed material was pounded smooth, and homogenized, The Sample was placed in the container sample, Next sample was pressed until flat surface. Then receptacle sample the placed in the sample holder and carried out radiation with an X-ray. Samples are analyzed using XRD.

Mechanical tests

The scaffold sample is then placed on the Universal Testing Machine test equipment arrange compression test time and speed namely 1 mm/min with pressure and insert scaffold height in the compression testing equipment software. This software tool automatically stops if already reaches 50% of tall scaffold samples. The results from this test compression test data are generated in N (Newton) form which is then converted

to the Megapascal formula.

Immunogenic test

Feasibility test ethics, application HAp gypsum puger scaffold starch cassava material on the socket mouse. Then wait until 7 and 14 days. Taking blood from the heart mouse then determine IgG levels using ELISA (Enzyme-Linked Immunosorbent Assay).

RESULTS

XRD characterization was used to identify the phase, lattice parameters, and degrees of crystallinity something a sample. Determination suitability structure crystals are formed with a match every peak that appears on the diffractogram to mark *d spacing*.

Test results using XRD show diffractogram *scaffold* HAp gypsum puger own point peak possible crystallinity seen in Figure 1 at angle 22.49 intensity 85, angle 23.41 intensity 85, angle 23.95 intensity 87. On *the Scaffold* HAp gypsum Puger starch cassava can seen in Figure 2 at angle 21.47 intensity 88, angle 22.37 intensity 88, and angle 24.07 intensity 89.

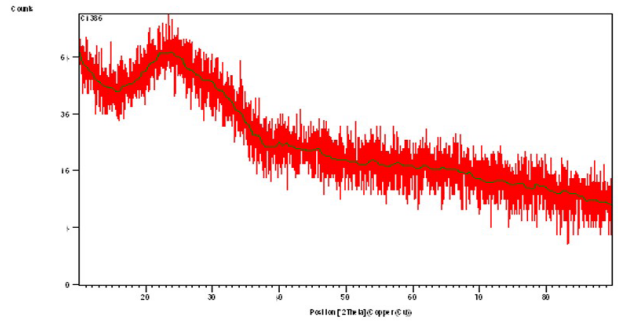

Figure 1: XRD difactogram pattern of the HAp gypsum puger scaffold

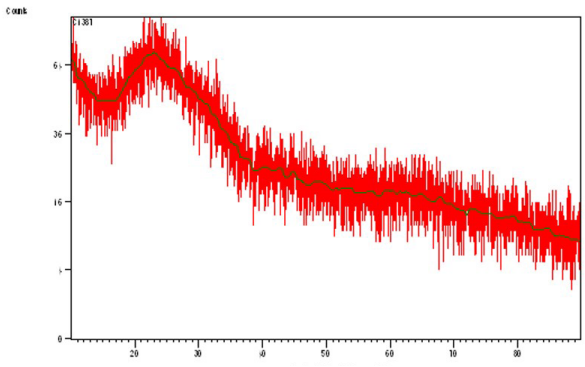

Figure 2: XRD difactogram pattern HAp gypsum puger cassava starch scaffold

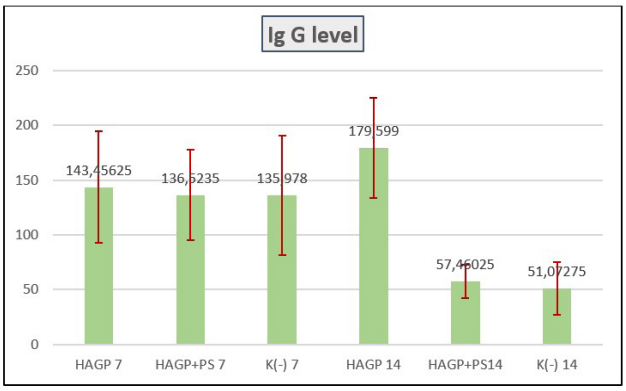

Figure 3: Bar diagram of Ig G levels in the HAp gypsum Puger, HAp gypsum Puger cassava starch, and control (-) on days 7 and 14.

On results mechanical test research or strong press HAp gypsum puger cassava starch scaffold (*Manihot Esculenta Starch*) with comparison ratio of HAp of puger gypsum and starch cassava (%w/w) , including HAp gypsum puger : Cassava starch (100:0), HAp gypsum puger : Cassava starch (50:50) , and HAp gypsum puger : Cassava starch (70:30). Average strength results press HAp gypsum Puger cassava starch scaffolds can seen in Table I.

Table I: Average Compressive Strength HAp gypsum puger scaffold: cassava starch

Group	Total sample	Mean ± standard deviation (MPa)
HAp gypsum puger scaffolds (100:0)	4	0.78 ± 0.08
HAp gypsum puger scaffolds : Cassava starch (7 0: 3 0)	4	1.78 ± 0.12
HAp gypsum puger scaffolds : Cassava starch (50:50)	4	2.07 ± 0.10

Table I shows that the group scaffolds that have average strength press highest in the group HAp gypsum puger scaffold: cassava starch (50:50) of 2.07 MPa followed by group HAp gypsum puger scaffold: cassava starch (70:30) of 1.78 MPa and group HAp gypsum puger scaffold: cassava starch (100:0) or HAp gypsum puger scaffold pure amounting to 0.78 Mpa, the data prove that addition starch cassava in HAp gypsum puger scaffold influential to strength press.

Data analysis was carried out on three group samples. Initial test namely the normality test and homogeneity test. The normality test used is the Shapiro-Wilk Test and results from the test show that the data is normally distributed because of its mark significance (p) >0.05. Homogeneity test was done using Levene's Test and the results are homogeneous because of own mark significance (p) >0.05. Because the data is normally

distributed and homogeneous so next with carry out a difference test for each sample using the one-way ANOVA statistical test and carrying out the Least Significance Difference (LSD) test. Based on one-way ANOVA test results obtained mark significance (p) <0.05 which means there is a significant difference between groups.

The results of the immunogenic response test with a mediator of Ig G levels in mice after administration of a HAp gypsum puger scaffold and HAp gypsum puger starch cassava scaffold can be shown in Table II.

Table II: Description of data on Ig G levels between treatment groups

Group	n	Ig G				ANOVA
		Means	SD	Minimum	Maximum	
HAGP 7	4	143.45625	50.924812	83,711	207,408	0.0 02
HAGP + PS 7	4	136.52350	41.065875	76,856	169,395	
K(-) 7	4	135.97800	54.809631	54,734	173,445	
HAGP 14	4	179.59900	45.659403	147,584	247,290	
HAGP + PS 14	4	57.46025	15.267137	45,075	78,414	
K(-) 14	4	51.07275	23.892520	16,410	70,936	

Description: significant at α=0.05 *superscript* shows no differences between groups using *multiple comparisons* LSD

The results of data analysis of Ig G levels using the Shapiro Wilk normality test obtained p > 0.05, this shows that the data was normally distributed followed by a homogeneity test with the Levene test obtained p > 0.05 This means that the data variance is homogeneous between groups, then continuing with the Anova test, it is found that p = 0.002, which means there are differences in Ig G levels between groups, followed by LSD multiple comparisons to find out which pairs of groups are different. Different test results were obtained in the groups' HAp gypsum puger 7 days, HAp gypsum puger cassava starch 7 days, Control (-) 7 days, HAp gypsum puger 14 days different significant with HAp gypsum puger cassava starch 14 days, Control (-) 14 days.

DISCUSSION

Study This uses HAp gypsum Puger starch cassava scaffold *(Manihot Esculenta Starch*). Analysis using XRD to learn the structure of crystals and non-crystal material. XRD methods can used to determine the size of crystals in condition certain (19). Analysis results in XRD pattern in the form of diffractogram can be analyzed with see the arrangement of lines or peaks with their intensity and position vary identified own sharp shape with high intensity, indicating that phase the compound formed form crystalline. Determination suitability structure crystals are formed done with matching every peak that appears on the diffractogram to mark certain d-spacings results analysis with data from the Joint Committee on Power Diffraction Standard (JCPDS) so can obtain information orientation field crystals are formed (20).

The phase to which the result belongs study sample scaffold is HAp which has the Name compound Calcium Phosphate Hydroxide Ca 10 (PO4) 6 (OH) 2. Phase the found on the HAp gypsum puger scaffold and HAp scaffolds gypsum puger cassava starch. This matters because of the content in both samples there is Originating HAp from gypsum Ca 10 (PO4) 6 (OH)2. Three intensity point peaks with varying crystallinity can be shown in Figure 1 – Figure 2. These results from each sample own difference This can be because of the difference in concentration used in each scaffold. This matter is supported by research by Rosalina (2017) that difference results in degrees of crystallinity producing hydroxyapatite with level different crystallinity. Differences can shown by happening subtraction wide peak and a rise in something intensity at each peak. The more tall level something crystallinity of the material shows that the more regular the internal atomic arrangement of material the. The size of something's intensity peak can seen in the number of atoms or ions present and their distribution within cell material units (21). Test results using XRD on the scaffold produce a diffractogram with high peaks which marked that the sample has succeed shaped crystal with different peaks. XRD peaks represent peak sharp with purity high and form round (19).

In the compressive strength test analysis the three sample groups had significant differences in compressive strength averages. seen from strength test results compressive strength obtained average strength press HAp gypsum puger scaffold without cassava starch with group added cassava starch show exists influence from the independent variable that is ratio addition starch cassava on puger gypsum HAp to the dependent variable that is strength press scaffolds. HAp gypsum puger scaffold pure own characteristic brothel or easy broke (9). Cassava starch has a content of amylose and amylopectin. Structure helix amylose and amylopectin which have structure group open hydroxyl OH will bond with Ca^{2+} group on HAp [$Ca10(PO_4)6(OH)2$] via bond hydrogen, Starch thus acts as a Reinforcer and forms porosity in the Scaffold because amylose has hydrophilic properties or soluble in water and amylopectin is hydrophobic or insoluble in water, so that in the process of making *scaffold* amylose will form porosity and amylopectin will bind to HAp gypsum puger through a branched cluster structure like an organized network with an interlocking mechanism, but amylose in the freeze drying process some form porosity

and some remain to form strong straight chain hydrogen bonds and the amylopectin branch groups cause it to expand and some form porosity cavities. So the higher the amylose content in starch, the greater the mechanical strength and the higher the porosity formed (22).

Strength test results press (Compressive strength) according to researcher previously that strength press cancellous bone physiological of 2 – 12 MPa. The ideal power for scaffold for network bone is vulnerable namely 0.01 MPa to 3 MPa. This result can made as a comparison with HAp gypsum puger scaffold as HAp with biopolymers. Hap content in the scaffold effect on strength press (19,23).

Based on the results strength test research press (*Compressive strength*) HAp gypsum puger: cassava starch scaffold composition (50:50) has more tall strength press because the percentage starch cassava as a binder and shaper porogen more bigger than in the (70:30) and (100:0) groups. The addition of gelatin also influences strength press Scaffolds because gelatin provides an effect layer elastic consequence bond group hydroxyl gelatin (COO-) with ions (CA 2+) on the scaffold. So in groups HAp gypsum Puger: cassava starch 50:50 response to pressure more capable muted without cracks, aside content the amino acids in gelatin make More scaffolding easy induce formation bone new because gelatin is a collagen protein (24). From the results show that the Hap gypsum puger scaffold cassava starch is suitable for development material substitution bone more carry on by characteristic strength desired mechanic.

An analysis Ig G levels was carried out on days 7 and 14 because the reaction causing immunity rejection reaction transplant usual graft material rejected in time 7-14 days (25). The average results of Ig G levels in the HAp gypsum puger 14 days group were very high compared to other groups, this is what caused significant differences between the HAp gypsum puger groups 14 days with HAp gypsum puger cassava starch 14 days, Control (-) 14 days. On Control (-)1 4 days own lowest Ig G levels because of the group this only tooth extraction is carried out without the application of graft material. So the reaction has very little resistance. On group HAp gypsum puger starch cassava 14 days generated Ig G levels are also low means the material can said safe the result was almost the same with group control (-) 14 days.

In the HAp gypsum Puger 7 days, HAp gypsum puger cassava starch 7 days, Control (-) 7 days found mark Ig G levels were high compared to other groups because on day 7 it was the initial immune response where rejection occurred after treatment. Exposure to the first (primary) antigen activates T cells and B cells, the B cells then differentiate and multiply to produce several IgG. IgG antibodies is the front line of adaptive immunity and also the body's specific humoral response to pathogens (25).

In the HAp group gypsum puger cassava starch day 14 and Control (-) 14 there was no significant difference, from the data obtained that the Ig G levels in the groups generated low levels and almost the same, which means that the HAp group of puger gypsum cassava starch 14 did not induce an immunogenic response compared with controls (-) 14. This is the HAp group gypsum puger cassava starch 14 contains the main ingredients HAp and cassava starch. HAp is biocompatible, low toxicity, biodegradable, non-immunogenic, and non-carcinogenic (26). HAp is a bioceramic-based composite material and has potential as a biodegradable bone implant. Meanwhile on starch cassava based on safety data information toxicology based on health that material cassava starch is not poisonous and cassava starch can also be beneficial for health because it is a source of carbohydrates, protein, vitamins, and minerals to build bone mass, so can say safe for body and not give rise to reaction immunogenic (27). This research is only limited to characteristic tests and immunogenic tests, so further research is needed on the Hap gypsum puger cassava starch scaffold material so that it can meet the ideal content of bone graft material.

CONCLUSION

In this research, the aim is to analyze the characteristics of material scaffold HAp gypsum puger and cassava starch with XRD test and compression test as well as immunogenic assay analysis in mice in a way in vivo. Analysis XRD is used for the determination of purity material And there is a sharp peak. In the XRD test it is produced exists sharp peak of group HAp gypsum puger cassava starch scaffold that shows purity tall from HAp. On strong test press group HAp gypsum Puger starch cassava scaffold with there is a 50:50 ratio strength press the corresponding 2.07 MPa with strength press bone in a way physiological. In immunogenic test analysis material, HAp gypsum puger starch cassava scaffold in mice obtained mark Ig G levels on the 14th no give rise to reaction immunogenic. The results show that the HAp gypsum puger cassava starch scaffold is suitable for the development of bone graph material more carry on by characteristics by XRD, properties strength mechanical and immunogenic tests for fulfill condition in accordance standard as candidate alternative bone graph material for manipulation network.

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