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The Effect of Additive Substitute of MgO Nanoparticle on the Characteristics of Exports as Bone Filler

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Abstract. Research has been conducted on the effect of adding MgO nanoparticle additives to the characteristics of porous hydroxyapatite as bone filler. The purpose of this study was to optimize previous research by replacing the ZnO nanoparticle additive with MgO nanoparticles accompanied by the use of the injection method. Porous hydroxyapatite synthesis is carried out by mixing nano-sized hydroxyapatite powder with MgO nanoparticles and making PVA solution. The composition of MgO nanoparticles varied at 8 wt%, 10 wt%, 12 wt% and 14 wt%. The hydroxyapatite-MgO mixture is dissolved in PVA solution so that the hydroxyapatite slurry is formed. Polyurethane foam is prepared with dimensions of 1 cm × 1 cm × 1 cm. Hydroxyapatite slurry is injected into polyurethane foam and followed by thermal treatments. The thermal treatments process was carried out in 3 stages, the drying stage at 80ºC for 2 hours, the phase of removal of PVA and polyurethane foam by heating in a temperature of 650ºC for 1 hour, and the sintering stage at 1200ºC for 3 hours. The effect of variations in the addition of MgO nanoparticles additives to the porous hydroxyapatite produced was characterized using porosity test, compressive strength test, morphological test with SEM, functional group test with FTIR, and cytotoxicity test using MTT assay. Based on the analyzes that have been done, it is known that the porous hydroxyapatite sample with the addition of 14 wt% of the MgO nanoparticle additive shows the best character with a porosity percentage of 68.60%, compressive strength value of 2.43 MPa, pore size of 100.3 - 314.3 µm, and not toxic.

Keywords: bioceramics, porous hydroxyapatite, magnesium oxide, additives, injection method.

1. Introduction

Bone cancer occurs because of cell dysfunction or uncontrolled bone cell growth. Bone cancer (osteosarcoma) is a systemic malignant disease that occurs in bone cells, hematopoietic components in bone, cartilage and fibrous or synoval material [7]. Technological developments in the medical industry encourage the development of biomaterials. Biomaterials aim to repair, restore, and replace damaged tissue or connect with the physiological environment of the body. Biomaterial as a bone implant application provides a better solution in handling cases of damage to bone tissue by tissue regeneration as an effort to repair and restore damaged tissue. The technique used is a network technique

that refers to the growth of new tissue using living cells that are controlled by the substrate structure of synthetic materials [8].

Bone filler is one of the biomaterials as an application in the process of repair, recovery, and bone reconstruction due to accidents, bone tumors, bone cancer, and other bone degradation diseases. Bone filler is used to fill damaged bone cavities so that it triggers bone tissue growth. Hydroxyapatite is the main constituent of bone filler. As one type of natural ceramic, pure hydroxyapatite has low mechanical characteristics. Addition of additives to hydroxyapatite is needed to make bone fillers with suitable mechanical characteristics and can be applied to bone. Porous hydroxyapatite synthesis using the addition of ZnO additives and the polymer foam immersion method was continued by sintering at 1200 ° C for 3 hours performed by Yunita [14] produced porous hydroxyapatite with pore size, porosity, and cytotoxicity levels that met medical application standards as bone filler with a compressive strength of 0.319 MPa which did not meet the medical application standard for spongious bone of 7.5 - 41 MPa .

Magnesium oxide nanoparticle or MgO nanoparticle is one of the candidates for additives because magnesium is one of the elements found in bones and good mechanical characteristics. MgO nanoparticle has been introduced as a sintering additive to improve the mechanical properties of hydroxyapatite. The addition of MgO nanoparticle additives will increase the value of compressive strength or hydroxyapatite compressive strength to 70% [3]. It is estimated that the addition of MgO nanoparticle additives to hydroxyapatite followed by the sintering process will produce bone filler products that have good properties and characteristics.

2. Experiment design

This research was carried out in two stages, namely the preparation stage and the stage of testing the porous hydroxyapatite sample. The preparation and sample preparation phase includes the manufacture of hydroxyapatite slurry, polyurethane foam injection process, drying, and sintering process. Hydroxyapatite powder mixed with MgO nanoparticles powder with variations in the number of additions is 8 wt%, 10 wt%, 12 wt% and 14 wt%. Hydroxyapatite and MgO nanoparticles powder mixture is added little by little to distilled water and PVA solution until hydroxyapatite slurry is obtained. The cut polyurethane foam is then injected with hydroxyapatite slurry. Next, the sample is dried in the furnace at temperature 80 ° C for 2 hours. The temperature in the furnace was increased to 650 ° C for 1 hour aimed at removing polyurethane foam and PVA. The sintering process in the sample was carried out at 1200 ° C for 3 hours. The phases of testing porous hydroxyapatite samples include FTIR test, SEM test, porosity test, compressive strength test, and MTT assay test.

3. Results and discussion

3.1 Synthesis Results

The process of making pure hydroxyapatite-based porous hydroxyapatite with addition of MgO nanoparticles additives to produce cubical porous hydroxyapatite with dimensions of 1 cm × 1 cm × 1 cm. Hydroxyapatite slurry injected on polyurethane foam as a pore frame has undergone reinforcement between particles of hydroxyapatite and MgO nanoparticles in the sintering process so that the porous hydroxyapatite solids are shown in Figure 1.

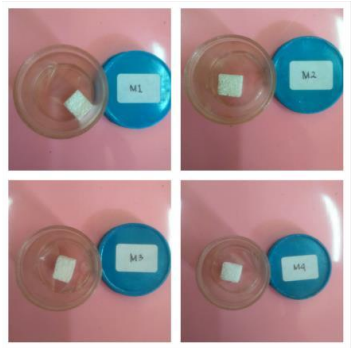
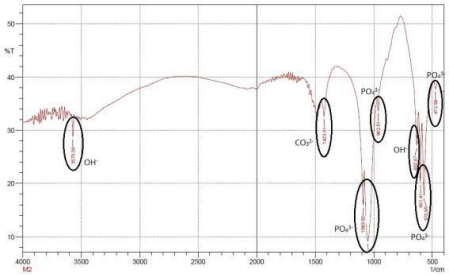


Figure 1. Porous Hydroxyapatite Samples with Addition of MgO Additives Nanoparticles

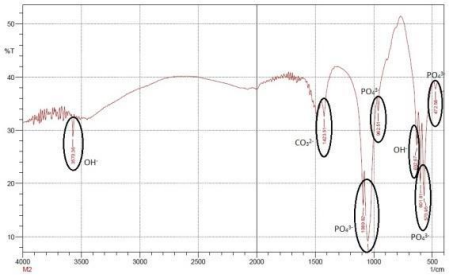
3.2 Characterization Results

3.2.1 Fourier Transform Infra-Red (FTIR) Test Results

FTIR testing was carried out as a qualitative analysis of identification of sample functional groups based on the absorbance of infrared rays. The main purpose of FTIR testing is to identify the presence or absence of PVA functional groups and polyurethane foams. PVA and polyurethane foam are expected to disappear due to the heat treatment process. The results of FTIR testing on hydroxyapatite samples are shown in Figure 2.



(a)



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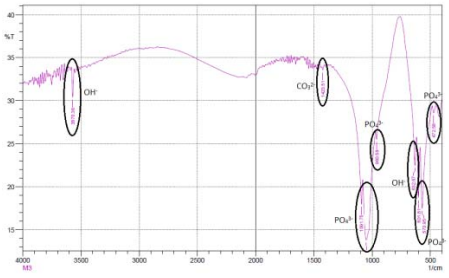

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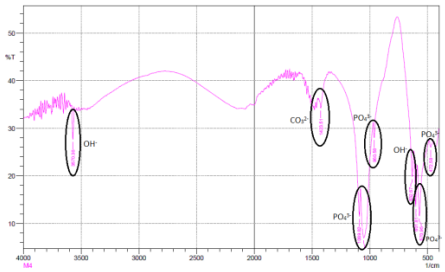


(d)

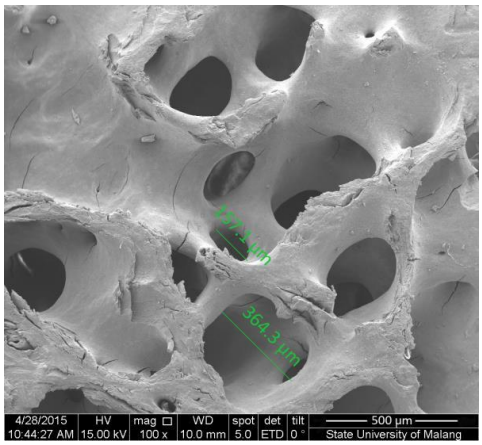
Figure 2. FTIR Test Results for Porous Hydroxyapatite Samples with Addition of Substances of MgO Nanoparticle Additives in total (a) 8 wt%, (b) 10 wt%, (c) 12 wt%, and (d) 14 wt%

The results of analysis of infrared absorption of porous hydroxyapatite samples showed the presence of hydroxyl (OH-) groups at wave numbers 3570 cm^{-1} and 633 cm^{-1}. The hydroxyl function group is characterized by the vibration of the H - O - H function group [9] [10]. Cluster found carbonate (CO_3^{2-}) on infrared absorption wave number 1423 cm^{-1}. The peak width at 1400-1550 cm^{-1} indicates a large quantity of carbonate ions [9]. The presence of peaks at 1455-1410 cm^{-1} shows the presence of CaCO3 on the surface [9]. The phosphate group (PO43-) is found in infrared absorption of wave numbers 1090 cm-1, 1050 cm^{-1}, 961 cm^{-1}, 602 cm^{-1}, 571 cm^{-1}, and 473 cm^{-1}. Phosphate group on Infrared uptake ranges from 470 cm^{-1}, 960 cm^{-1}, and 1090 cm^{-1} is characterized by symmetrical O-P-O compound bonds [9]. Infrared uptake in the range of 1050 cm^{-1} and 570 cm^{-1} showed the existence of asymmetric stretching mode and symmetric bending mode PO43- functional groups [2]. Infrared absorption in the range of 603 cm^{-1} shows a bending vibration and stretching from P-O to the $PO4^{3-}$ functional group [10].

The four porous hydroxyapatite samples with variations in the addition of MgO nanoparticles additives showed that infrared absorption results were not much different. This shows the identity of the compounds produced and shows the absence of new functional groups. There are three main functional groups of porous hydroxyapatite, namely hydroxyl groups, carbonate groups, and phosphate groups. The magnesium (Mg) function group does not appear in the results of the infrared absorption of porous hydroxyapatite samples. The use of small amounts of magnesium as an additive in porous hydroxyapatite samples causes absorption of magnesium functional groups not to be seen clearly or not. No infrared absorption from PVA functional groups and polyurethane foams were found in porous hydroxyapatite samples. This shows that PVA and polyurethane foam have melted and evaporated perfectly due to thermal treatments so that it does not cause impurities in porous hydroxyapatite samples.

3.2.2 Scanning Electron Microscopy (SEM) Testing Results

The results of SEM testing in the form of images or surface structure images that show the shape and pore diameter and the percentage of elemental composition in the porous hydroxyapatite sample with variations in the addition of MgO nanoparticles. The results of SEM testing are presented in Figure 3.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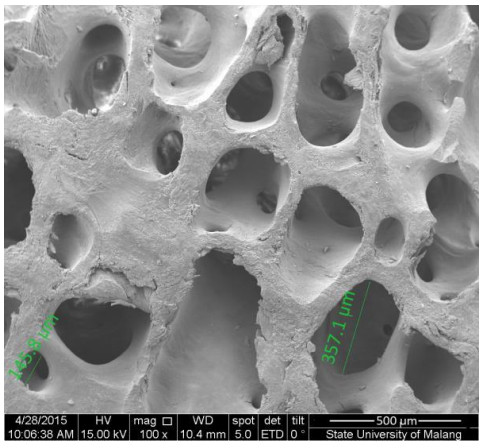
(a)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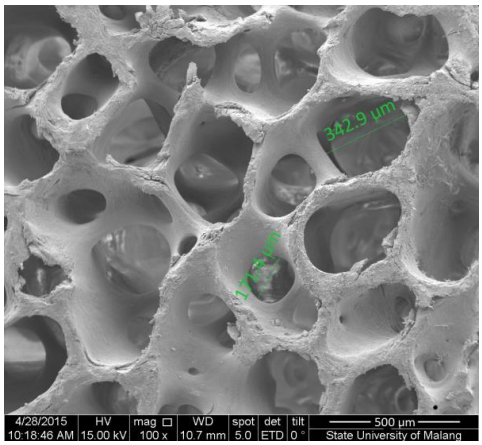
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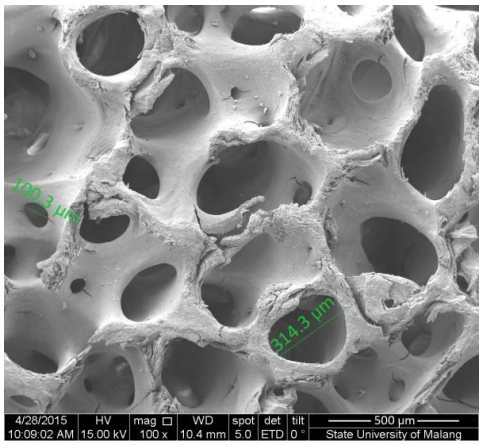

(d)

Figure 3. Morphology of Porous Hydroxyapatite Samples with Variations in Substance Addition of Additives MgO Nanoparticles (a) 8 wt%, (b) 10 wt%, (c) 12 wt%, and (d) 14 wt% Using SEM

Analysis of the results of the morphological testing of porous hydroxyapatite samples was focused on observing pore shape and size. The results of observations and pore size analysis are presented in Table 1.

Table 1. Results of Testing Porous Hydroxyapatite Sample Pore Size with Variations Composition of Substance Additions of MgO Nanoparticle Additives

Parameter	Additions of MgO Nanoparticle Additives			
	8 wt%	**10 wt%**	**12 wt%**	**14 wt%**
Pore size (µm)	157.1 – 364.3	145.8 – 357.1	171.4 – 342.9	100.3 – 314.3

The addition of the composition of the MgO nanoparticle additive has an effect on the pore size of the porous hydroxyapatite sample. The pore size of the hydroxyapatite sample decreases with the addition of the composition of the MgO nanoparticle additive. This matter caused by the administration of nano-sized MgO nanoparticle additives will fill the empty spaces between the hydroxyapatite particles so that they can reduce the pore size.

Pore size is an important parameter in bone filler. Bone filler is expected to be able to support the osteointegration process between cells in bone tissue with bone filler. Pore size characteristics that are suitable for triggering bone cell growth are 100 - 400 µm and no tissue grows in the pore below the size of 5 µm [7] [12]. The results of the pore size analysis of porous hydroxyapatite samples with variations in the composition of the addition of MgO nanoparticle additives showed that the fourth pore size of the porous hydroxyapatite sample fulfilled the pore size characteristics as a bone filler application.

3.2.3 Porosity Test Results

Porosity testing was carried out to determine the percentage porosity of the porous hydroxyapatite sample. Porosity test results for porous hydroxyapatite samples with variations in the addition of MgO nanoparticles additives are presented in Table 2.

Table 2. Test Results for Porous Hydroxyapatite Sample Porosity with Variations Composition of Substance Additions of MgO Nanoparticle Additives

Parameter	Addition of MgO Nanoparticle Additive			
	8 wt%	10 wt%	12 wt%	14 wt%
Porosity(%)	70,19	70,07	68,89	68,60

The percentage of porosity decreased with the addition of the composition of the MgO nanoparticle additive in the porous hydroxyapatite sample. This is due to the addition of nano-sized additives to hydroxyapatite to improve densification between particles [12]. The process of densification or compaction will reduce the amount of porosity in porous hydroxyapatite samples [14]. The pore existence of porous hydroxyapatite is controlled by polyurethane foam as a pore frame or frame. The deposition of polyurethane foam during heat treatment will leave the cavities in the porous hydroxyapatite sample followed by the sintering process to assist the process of diffusion and inter-particle densification of the MgO nanoparticle and hydroxyapatite additives.

Porous hydroxyapatite as a bone filler application for trabecular bone in the femur has a porosity characteristic of ± 70% [5]. From the results of porosity testing, it is known that the four porous hydroxyapatite samples meet medical application standards as bone fillers because they have a range of porosity values that are in accordance with the literature of bone filler porosity characteristics of ± 70%.

3.2.4 Compressive Strength Test Results

A compressive strength test is carried out to determine the resistance of the sample by giving a number of forces or loads. The results of testing the compressive strength of porous hydroxyapatite samples for each variation of the addition of MgO nanoparticles additives are presented in Table 3.

Table 3. Test Results for the Compressive Strength of Porous Hydroxyapatite Samples with Variation in Composition of Substance Additions of MgO Nanoparticles Additives

Parameter	Additions of MgO Nanoparticles Additives			
	8 wt%	10 wt%	12 wt%	14 wt%
Compressive strength (MPa)	2,3	2,39	2,40	2,43

The compressive strength of porous hydroxyapatite samples increases with increasing composition of MgO nanoparticle additives. This increase in compressive strength occurs because the nature of MgO nanoparticles as additives can improve the biochemical mechanical properties of brittle pure hydroxyapatite because MgO nanoparticles additives can improve the densification process along the grain boundary. Nano-sized magnesium oxide fills in the empty space between the hydroxyapatite particles thereby increasing the density of porous hydroxyapatite. The use of nano-sized additives contributes to better densification between particles [12]. In addition, the thermal treatment process carried out in temperature 1200ºC for 3 hours helps the process of combining and compacting particles optimally so that bond reinforcement occurs between items. Improvement of densification process has an effect on increasing mechanical properties of porous hydroxyapatite samples. Increased concentration of MgO nanoparticle additives on hydroxyapatite improves mechanical properties and increases the compressive strength of porous hydroxyapatite samples.

Compressive strength is an important parameter in bone filler. The bone filler strength required for spongious bone implant application is 7.5 - 41 MPa [13]. Samples with the composition of the addition of 14 wt% MgO nanoparticle additives have the best compressive strength value of 2.43 MPa. The four porous hydroxyapatite samples showed better compressive strength values compared to the results of previous studies conducted by Nurmanta [7] and Yunita [14]. In a study conducted by Nurmanta [7], the synthesis of pure hydroxyapatite without the addition of additives carried out by polyurethane foam

immersion method produced the best strength value of 0.5591 MPa. The use of MgO nanoparticles as additives on hydroxyapatite shows the results of compressive strength that are better than pure hydroxyapatite because additives are able to strengthen density and reduce porosity in the hydroxyapatite lattice thus increasing macroscopic properties such as hardness and compressive strength. Previously by Yunita [14], the synthesis of porous hydroxyapatite was carried out by the addition of ZnO nanoparticle additives by immersion method resulting in the best compressive strength value of 0.319 MPa. MgO nanoparticles as additives were able to increase the porous hydroxyapatite compressive strength compared to ZnO nanoparticles. This is caused by the mineral content of magnesium in bones which is more than zinc in bone. Magnesium as a bone mineral functions as a reinforcement of bone tissue located on the surface of hydroxyapatite bone crystals [1]. As much as 60% of magnesium minerals from total bone minerals other than calcium and phosphorus as the main form of hydroxyapatite, are stored in bone and 40% residual bone minerals such as Zn, Mn, Cu, etc. [4] [1]. Magnesium is also one of the main substitutes (predominant substitute) for calcium in biological apatites on hard tissues (enamel, dentine, and bone each containing 0.44 wt%, 1.23 wt%, and 0.73 wt%) [11]. Therefore, the addition of MgO nanoparticles additives in hydroxyapatite is considered more optimal in improving the mechanical properties of porous hydroxyapatite samples than ZnO additives.

The replacement of the immersion method using the injection method is expected to increase the compressive strength of the porous hydroxyapatite sample. Injection techniques using injections or syringes provide an external force so that hydroxyapatite slurry can enter into polyurethane foam optimally. From the analysis of the compressive strength of porous hydroxyapatite samples with the addition of MgO nanoparticles additives it was found that the best compressive strength value was 2.43 MPa. The value of the best compressive strength in porous hydroxyapatite samples from the results of this study still does not meet the standard bone filler application, but looks at the trend or tendency of increasing the value of compressive strength along with the addition of MgO additive composition nanoparticles, the value of compressive strength can be increased by increasing the composition of MgO nanoparticles. The presence of pores is a factor that weakens the porous hydroxyapatite mechanical strength. The presence of pores in medical application biomaterials is expected to help the osteointegration process with bone tissue. However, the presence of pores causes the formation of empty spaces which causes a decrease in the mechanical properties of hydroxyapatite. Pore existence in bone filler causes a decrease in compressive strength compared to bulk forms [14]. Material compressive strength is inversely proportional to the level of porosity. The value of compressive strength will increase as the porosity of the biomaterial decreases.

3.2.5 MTT Assay Test Results

MTT assay testing is a quantitative test to determine the level of cytotoxicity in vitro and shows the absence of a porous hydroxyapatite sample. Cytotoxicity testing using cellular-reduced MTT reagent based on the breakdown of yellow MTT tetrazolium salt into purplish formazan crystals. The color change method is used to detect cell proliferation. Cell mitochondria that experience proliferation will absorb MTT which causes the formation of purplish formazan crystals. Therefore, the intensity of purple that is formed is proportional to the number of living cells so that the intensity of purple that gets thicker will indicate an increasing number of living cells. Analysis of the results of cytotoxicity level testing using MTT assay on porous hydroxyapatite samples with variations in the composition of additives MgO nanoparticles additives of 8 wt%, 10 wt%, 12 wt%, and 14 wt% are presented in Table 4.

Table 4. Results of Testing of Porous Cytotoxicity Levels with Composite Variations of Substance Addition of MgO Nanoparticles Additives Based on Percentage of Live Cells

Parameter	Addition of MgO Nanoparticles Additives			
	8 wt%	10 wt%	12 wt%	14 wt%
Live Cells (%)	87.09	91.29	94.87	98.60

From Table 4 it is known that the percentage of living cells in the MTT assay for the four porous hydroxyapatite samples is more than 60%. This indicates that the four porous hydroxyapatite samples are not toxic to living cells. The inoxicity of porous hydroxyapatite samples is caused by the absence of polyurethane and PVA foams in the thermal treatments process in the form of polyurethane and PVA removal processes followed by the sintering process. The MTT assay test results reinforce the results of FTIR testing which shows that there are no groups forming polyurethane foam and PVA. From the analysis of the results of cytotoxicity testing using MTT assay it is known that the porous hydroxyapatite sample with the addition of 14 wt% of the MgO nanoparticle additive is a sample that shows the best percentage of living cells at 98.60%.

4.Conclusion

Based on the results obtained in this study, it can be concluded that:

1. Variation in the composition of MgO nanoparticle additives in the preparation of porous hydroxyapatite in this study affected porosity, compressive strength, and pore size of porous hydroxyapatite samples. The percentage of porosity and pore size decreased with increasing composition of the addition of MgO nanoparticle additives. The value of compressive strength of porous hydroxyapatite samples increases with increasing composition of the addition of MgO nanoparticle additives. Porous hydroxyapatite samples from this study are also non-toxic.
2. Addition of 14 wt% MgO nanoparticle additives in porous hydroxyapatite samples has the best character with a pore size of 100.3 - 314.3 µm, porosity percentage of 68.60%, compressive strength value of 2.43 MPa, and is non-toxic with a percentage of living cells of 98.60%. Porous hydroxyapatite samples with the addition of 14 wt% MgO nanoparticle additives have the potential to be applied as bone fillers because they meet medical application standards pore size parameters are 100 - 400 µm and porosity is ± 70%.

5.References

[1] Castiglioni, Sara., Cazzaniga, Alessandra., Albisetti, Alter., Maier, Jeanette A.M. 2013. *Magnesium and Osteoporosis: Current State of Knowledge and Future Research Direction*. Nutrients, Open Access Journal Vol. 5 pp. 3022 – 3

[2] Chandrasekar, Arunseshan., Sagadevan, Suresh., Dakshnamoorthy, Arivuoli. 2013.*Synthesis and Characterization of Nano-Hydroxyapatite (n-HAP) Using Wet Chemical Technique*. Academic Journal Vol 8 (32), pp. 1639 – 1645. International Journal of Physical Sciences. Chennai: Anna University, India.

[3] Demirkol, Nermin., Meydanoglu, Onur., Gokce, Hwan *et al*. 2012. *Comparison of Mechanical Properties of Sheep Hydroxyapatite (SHA) and Commercial Synthetic Hydroxyapatit (CHSA)-MgO Composite*. Switzerland: Trans-Tech Publication.

[4] Dewi, Setyautami. 2007. *Analisis Kuantitatif, Kekerasan, dan Pengaruh Termal Pada Mineral Tulang Manusia*. Skripsi Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Pertanian Bogor, Bogor.

[5] Keaveny, Tony M., Morgan, Elise F., Teh, Oscar C. 2004. *Mechanic of Human Body: Chapter 8 Bone Mechanic*. New York City: McGraw Hill Companies.

[6] Kulandaivelu, Ravichandran., R, Abinaya., B. Subha., A. Adhilakshmi., 2014. *Comparative Study on The Mg Doped Hydroxyapatite through Sol-Gel and Hydrothermal Techniques*. International Journal of Innovative Research in Science and Engineering. Department of Analytical Chemistry, University of Madras, Chennai, India.

[7] Nurmanta, Dica Aprilia. 2013. *Optimasi Parameter Waktu Sintering pada Pembuatan Hidroksiapatit Berpori untuk Aplikasi Bone Filler Pada Kasus Kanker Tulang (Osteosarcoma)*. Skripsi Departemen Fisika Fakultas Sains dan Teknologi Universitas Airlangga, Surabaya.

[8] Park, Joon B., Bronzino, Joseph D. 2007. *Biomaterials: Principle and Applications*.Washington DC: CRC Press.

[9] Rachmania, Aida. 2012. *Preparasi Hidroksiapatit dari Tulang Sapi dengan Metode Kombinasi Ultrasonik dan Spray Drying*. Tesis Fakultas Teknik Universitas Indonesia.

[10] Sedyono, Joko., Tontowi, Alva Edi. 2008. *Proses Sintesis dan KarakterisasiFTIR Hidroksiapatit dari Gipsum Alam Kulon Progo*. Media Mesin Vol. 9, No. 1. Jurnal Ilmiah Universitas Gadjah Mada, Yogyakarta.

[11] Stipniece, Liga., Salma-Ancene, Kristine., Jacovlevs, Dmitrijs., et al. 2013. *The Study of Magnesium Substitutian Effect on Physicochemical Properties of Hydroxyapatite*. Material Science and Applied Chemistry Journal Riga Technical University, Russia.

[12] Swain, Sanjaya Kumar. 2009. *Processing of Porous Hydroxyapatite Scaffold*. Thesis Department of Ceramic Engineering, National Institute of Technology Rourkela. Winarno, Eko. 2011. *Uji Sitotoksik Ekstrak Kapang Aspergilus sp. Terhadap Sel Kanker Payudara T47D*. Skripsi Program Studi Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Indonesia.

[13] Ylinen, Pekka. 2006. *Applications of Corraline with Bioabsorbable Containment and Reinforcement as Bone Graft Substitute.* Academic Dissertation of Department of Orthopaedics and Traumatology, Medical Faculty of The University of Helsinki, Helsinki.

[14] Yunita, Erma. 2014. *Pengaruh Penambahan ZnO Nanopartikel Terhadap Karakteristik Bone Filler Hidroksiapatit Berpori*. Skripsi Departemen Fisika Fakultas Sains dan Teknologi Universitas Airlangga, Surabaya.