Mixed Polymethylmethacrylate and Hydroxyapatite as a Candidate of Synthetic Graft Materials for Cleft Palate

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Abstract

This research aimed to investigate scaffolds of mixed polymethylmethacrylate (PMMA) and hydroxyapatite (HA) that can be considered as a candidate of synthetic graft materials for cleft palate.

Polymethylmethacrylate-hydroxyapatite (PMMA-HA) scaffold was done by weighing PMMA granules and HA powder for a 20:80, 30:70 and 40:60 ratio, that mixed and moulded in 5 mm of diameter and 10 mm of height, then freeze dried before being analyzed by X-Ray Diffraction (XRD), Fourier Transform Infra-Red (FTIR), Scanning electron microscope (SEM) and Energy dispersive X-ray (EDX) instruments.

XRD analysis in 20:80, 30:70 and 40:60 ratio has a peak that represents HA and an amorphous peak that represents PMMA, with the best ratio is 30:70.FTIR analysis showed the existence of HA functional groups namely phosphate (PO_4^{3-}), calcium carbonate (CO_3^{2-}), and hydroxyl (OH^-) in all three samples, with 20:80 ratio as the optimal composition. SEM observation showed visible interconnected pores and roughness, also various pore sizes that averaged by 278.71µm; 312.3µm; 332.8µm, and 1.267µm; 1.716µm; 1.951µm, that showed by 50x and 5000x magnification, with 20:80 ratio as the ideal environment. EDX analysis showed Calsium (Ca), Oxygen (O), Phosphate (P), Silicate (Si), and Aluminium (Al) contained in the PMMA-HA sample, with 20:80 ratio as the highest potention to osteoblast regeneration.

PMMA-HA scaffold with 20:80 ratio considered as the best ratio to be a candidate of synthetic graft materials for cleft palate.

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Introduction

Cleft lip and palate (CLP) is an anatomical abnormality orofacial suffered by a person since birth where a defect is formed in the palate and lips due to failure of the formation of the epithelial bridge, incomplete growth of the intermaxillary segment of the medial nasal prominence, or failure of the lateral palatine processes to fuse with each other or with the median palatine process.[1] The prevalence of cleft palate varies by ethnicity. The highest prevalence is Japanese at 2.1 per 1000 births, European ethnicity is 1:1000.[2] Impaired speech, hearing, swallowing, breathing, malpositioned teeth, and facial development as well as delay of growth[3] urge the need to permanently close the cleft. Tissue engineering technology allows children to get bone grafting in the area of their palate defect.[4] To date, autografts are the gold standard in ideal bone replacement materials due to their good osteoconductive and osteoinductive properties and are also a source of osteoprogenitor cells.[5] However, autografts require a secondary surgical procedure at the tissue extraction site and can cause several complications at the donor site, morbidity, and lead to scarring.[6]

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Alloplastic graft is an option that can be developed to form a scaffold that functions as a bone graft and has a material character that is close to the golden standard and allows it to have a porous composition and structure that resembles human bone. Alloplastic grafts are obtained from synthetic various biomaterials such as calcium carbonate (CO_3^{2-}), tricalcium phosphate, hydroxyapatite (HA) and polymers.[7] Polymethylmethacrylate (PMMA) is increasingly being applied in tissue engineering for scaffold materials,[8] because of its low toxicity, good biocompatibility when interacting with human tissues, as well as good mechanical stability, and cellular adhesion.[9] Various ways have been done to improve the mechanical and chemical functions of PMMA materials, one of which is by adding bioactive ceramic materials such as HA. The superior nature of HA is a consideration for many researchers to strengthen the function of PMMA with the addition of HA.[10]

In terms of scaffold sources, Indonesia has abundant HA mineral resources derived from local limestone mining materials.[11] HA has a composition very similar to natural apatite in bone and is known for its biocompatibility with body tissues and is a good structure for scaffolds.[12] We supposed that mixture of PMMA and HA can be useful as a candidate of synthetic graft material for cleft palate.

However, there has been limited research on the composition of the polymethylmethacrylate - hydroxyapatite (PMMA-HA) biomaterial as a synthetic graft on cleft palate (CP) with biodegradable properties and can also be used in smaller areas. We choose three ratio of PMMA-HA sample with consideration, 70% of the inorganic components of human bone are made up of HA.[13] As a basis for assessing the quality relative to natural bone and what properties and abilities it can have to support the closure of palatal defects, it is important to know the elements that make up a scaffold.[14] The goal of this study was to determine which of the three existing composition ratios was the best, so it is necessary to characterize the PMMA-HA biomaterial as a graft synthetic material. The characterization includes a test of the crystal structure and functional group of the PMMA-HA mixture to confirm the ingredients contained, as well as a test of morphology and composition of the constituent elements of various PMMA-HA compositions. The results of this study are expected to be the basis for the development and design of a new synthetic graft material using PMMA-HA biomaterial as a candidate for synthetic graft material in the cleft palate.

Materials and methods

Materials Preparation

HA powder preparation was purchased from the Center for Ceramics in Indonesia (Balai Besar Keramik Indonesia). HA powder is made and extracted from natural ingredients, in the form of limestone. PMMA granules preparation was purchased from “HiMedia Laboratories”, India.

Manufacture of PMMA-HA Scaffold

The procedure for making PMMA-HA scaffold is done by weighing 1 gram of PMMA, 2 ml of acetone and 4 grams of HA powder for a 20:80 ratio. Then 1.5 grams of PMMA, 3 ml of acetone and 3.5 grams of HA powder for a 30:70 ratio. Then 2 grams of PMMA, 4 ml of acetone and 3 grams of HA powder for a 40:60 ratio. Put PMMA granules into a bottle and mixed it with acetone, then stored in a 0˚C refrigerator for 1 x 24 hours. After that, continue by mixing the HA powder into the PMMA solution and then stir it on a magnetic stirrer until homogenic. Then pour it into a mold with 5 mm of diameter and 10 mm of height. After that, a freeze-drying process is carried out (supplementary data).

XRD analysis

X-Ray Diffraction (XRD) [X’Pert PRO PAN analytical; X’Pert³MRD; Malvern Panalytical Ltd., Malvern, United Kingdom] was used to determine phase identification. The results will be compared to the PMMA and HA standard.[15]

FTIR analysis

PMMA-HA was physically characterized in order to identify the functional groups with fourier transform infra-red (FTIR) [Thermo Scientific; Nicolet iS10 FTIR Spectrometer; Thermo Fisher Scientific Inc., Massachusetts, USA] using a 3D scaffold of PMMA-HA that was placed on the sample holder has been used to determine functional group identification.[15]

SEM analysis

The pore of the scaffold was observed using a scanning electron microscope (SEM) [Inspect S50 FEI Company, Hillsboro, OR, USA] set to 20 kV. The horizontal and vertical cross sections of the three PMMA-HA samples per ratio were used in the analyses.[16]

EDX analysis

To determine the composition of the constituent elements, 3D scaffold of PMMA-HA was placed on pin holder and attached to the sample using a carbon tip energy dispersive X-ray (EDX) [EDAX AMETEK; EDAX's Octane SDD Series for the TEM; EDAX Inc., New Jersey, USA] was used to determine the particle composition of the material.[15]

Results

XRD analysis

Showed that the PMMA-HA sample for 20:80; 30:70 and 40:60 ratio has a sharp peak that represents hydroxyapatite and an amorphous peak that represents PMMA in Figure 1.

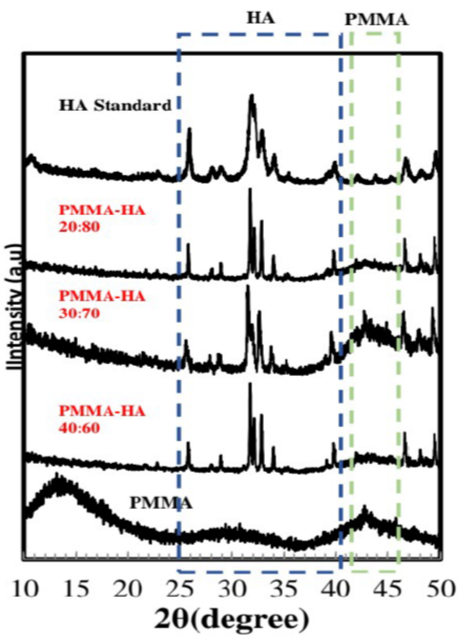

Figure 1. XRD pattern of pure PMMA, HA, and PMMA-HA sample for 20:80; 30:70 and 40:60 ratio.

FTIR analysis

With transmittance (%) as Y-axis and wavelength (cm^{-1}) as X-axis, the FTIR result revealed peaks of the functional group in the graph. The PMMA-HA sample for 20:80; 30:70 and 40:60 ratio shows the existence functional groups of PMMA and HA in all three samples namely, phosphate (PO_4^{3-}), carbonate (CO_3^{2-}) and hydroxyl (OH^-). From the results above, it is confirmed that these samples contain PMMA and HA in Figure 2.

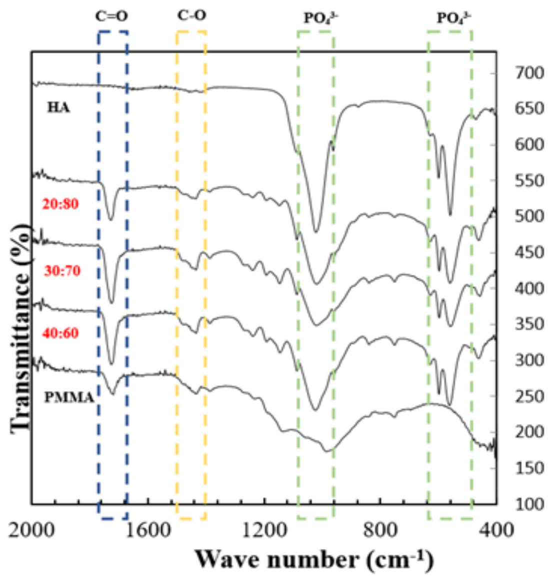

Figure 2. FTIR pattern of pure PMMA, HA, and PMMA-HA sample for 20:80; 30:70 and 40:60 ratio.

SEM Observation Results

The macropore size average increased by 278.71μm; 312.3μm; and 332.8μm respectively for PMMA-HA ratio of 20:80, 30:70 and 40:60 at 50 times magnification. SEM imaging is also able to capture the pore size at a magnification of 5000 times, which appears to be gradually increasing in micropores average, namely 1.267μm; 1.716μm and 1.951μm for a ratio of 20:80; 30;70 and 40;60 in Figure 3.

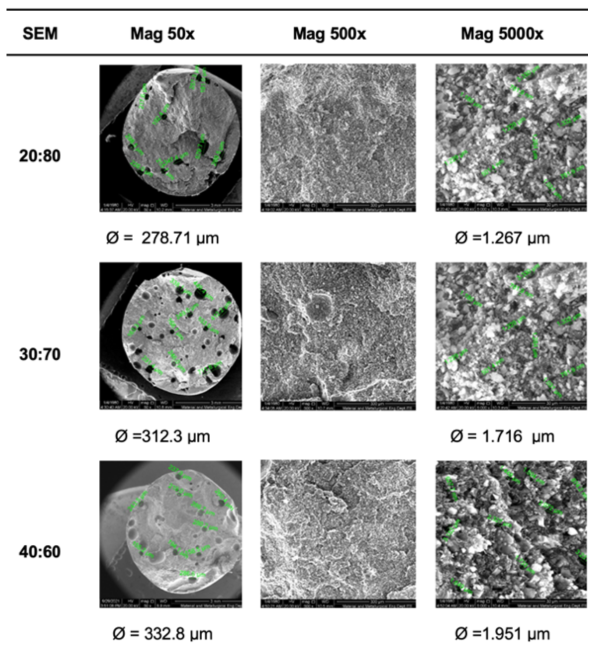

Figure 3. SEM cross sectional image results for 20:80 (A); 30:70 (B) and 40:60 (C) ratio, with its

pore's size average.

EDX Observation Results

Calsium (Ca), oxygen (O), phosphor (P), aluminium (Al), and silicate (Si) were detected in the PMMA-HA scaffold with weight percentation of each element as shown in the Figure 4. There is an increase in the amount of O level and decrease in Ca amount sequentially in the PMMA-HA ratio of 20:80, 30:70 to 40:60, unfortunately not significant. P, Al and Si doesn't show linearly percentage as shown in Figure 4.

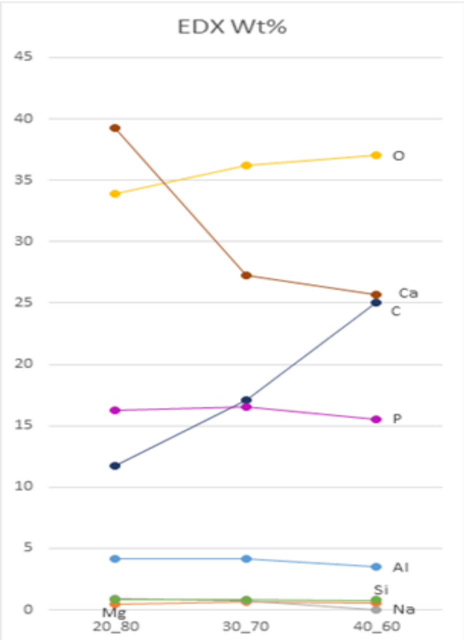

Figure 4. EDX line plot analysis of PMMA-HA sample for 20:80; 30:70 and 40:60 ratio, with n=3.

Discussion

This study has fabricated scaffolds from PMMA-HA mixture with three different compositions to be considered as a candidate of synthetic graft materials for cleft palate. To analyze the best ratio, characterizations have been carried out including XRD analysis to determine the crystal structure of the sample, FTIR to evaluate the functional groups of the sample, and SEM-EDX analysis to observe the surface morphology and elements present in the sample. Because of its mechanical, chemical and biological qualities, PMMA-HA composite biomaterials have been identified as a candidate of synthetic graft materials. PMMA-HA has the potential to promote osseointegration, being biocompatible, and reduce allergy and hypersensitivity symptoms.[17,18]

HA is able to manipulate with PMMA without losing its properties. This can be seen in Figure 1, that the XRD patterns of HA in the PMMA-HA sample do not change. Those three compositions show the sharp peak of crystalline phase that represents HA that was detected in the 2θ range 25° - 40°. In the same range, the amorphous peak of PMMA polymer was also detected. Those XRD patterns revealed that the HA insertion had no effect on the structural properties of PMMA and vice versa. Similar XRD patterns of HA and PMMA detected at 2θ range 25° - 49° also reported in other biomaterial mixtures as studied by Wijesinghe et al.[19] From XRD result, the 30:70 ratio is the best composition considering the peaks that similar to both PMMA and HA standards. There was no difficulty in manipulating the two materials, as evidenced by the absence of studies reporting changes in the properties of HA and PMMA after manipulation.

FTIR analysis was conducted to further determine the presence of functional groups that correspond to PMMA and HA. FTIR test with PMMA-HA sample reveals the presence of PO_4^{3-}, and OH^- functional groups of PMMA and HA in all three samples as shown in Figure 2. From the spectra it can be seen that the most dominant is the PO_4^{3-} group. The presence of OH^- and PO_4^{3-} functional groups indicates the formation of HA crystals in the sample. Other research that was studied by Moreno et al. (2014) also supports our study by showing a similar wave number of stretching and bending modes of PO_4^{3-}, CO_3^{2-}, and OH^- groups.[20]

The osteoconductive and osseoinductive properties of phosphate play a role in bone regeneration. Along with calcium ions, bone contains about 80% of phosphorus ions in the form of calcium phosphates. Phosphorus is most commonly found in the form of PO_4^{3-} which has a significant impact on tissue formation and growth, also osteoclast differentiation and bone resorption are inhibited.[21,22] The OH^- functional groups have an important role in ionic conduction, which can speed up the cytoskeleton remodeling in cells like osteoblasts.[23,24] Furthermore, CO_3^{2-} functional groups is a phosphate ion substitution indicating the production of type B apatite, which is the apatite present in biological bone hydroxyapatite. Carbonate groups also come from the carbonate phase of HA and CO_2 in the air, but they do not affect the human body, because the inorganic composition of human bones consists of carbonate about 4-6 Wt%. Carbonate has the ability to stimulate osteoblast responses and increase osteoblast development.[25-27] According to the FTIR results, it is confirmed that these samples contain PMMA and HA. It is also shows the optimal composition, which is a 20:80 ratio, showing the peaks of the functional group that are similar to both PMMA and HA standards.

Varied pore sizes are important in the success of tissue regeneration since each pore size has its own function.[28] As shown in Figure 3 that the three samples show visible interconnected pores and roughness that would be useful in osseointegration with the tissue. A pore size of 100 to 300 μm is the optimal environment for initiating bone formation and supporting osteochondral formation prior to the onset of osteogenesis; it is also the optimal size for cell attachment and osteoblast proliferation.[28-30] Nano-pores (<1 μm) are useful for increasing cell-surface interactions, while 1-3 μm micropores are the right environment for cell-to-cell communication.[29] Based on the optimal environment (100-300 μm) it is narrowing the best selection to 20:80 ratio, where the average pore size is within that range. Compositions of scaffold will support the process of osteogenesis, osteoconduction, osteoinduction and osseointegration.[31] In this experiment, EDX analysis showed that the PMMA-HA sample were composed of C, O, Al, Si, P, Na, Mg and Ca. However, from three ratio that were analyzed three times (n=3), only O, Ca, Al and Si that appears consistently. Therefore, C, Mg and Na omitted from Figure 4. This happens due to a misperception of C peak by EDX tool. On the other side, minimal amount of Mg and Na makes this tool cannot detect accurately, this also reported in other journal.[32] P, Si and Al were detected unlinearly also because of the minimal amount, as shown in Figure 4. The same information was reported on the company's data sheet of HA.

C and O bonded from PMMA material have high modulus of elasticity and tensile strength so that it will increase the structural integrity of a scaffold and support the mechanical stability of the bone graft in a large defect area.[9,33] Ca plays a role in increasing the signal of osteoblast proliferation and extracellular matrix, while P promotes osteoblast differentiation and growth, as well as inhibiting osteoclast differentiation and bone resorption.[21,34,35] These four characteristics are the abilities needed by a synthetic bone graft to support stem cells and growth factors interaction. The PMMA-HA scaffold is dominantly contained by O and Ca, but they are not significant between every ratio. However, the weight percentation of Ca in 20:80 ratio is higher than others, so we can conclude that the ideal composition based on the potential to support osteoblast regeneration is 20:80 ratio. Further in vivo research is needed to determine this ratio effect in bone regeneration related to palate defect.

Conclusions

PMMA-HA scaffold with 20:80 ratio considered as the best ratio to be a candidate of synthetic graft materials for cleft palate.

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Declaration of Interest

The authors report no conflict of interest.

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