The Characterization of nanoHA-Balik Wangi Rice Starch Tissue Engineering Scaffold

M. Riza Roslan[a], N.F. Mohd Nasir[b], E.M. Cheng[c], N. Mamat[d]
School of Mechatronic Engineering,Universiti Malaysia Perlis (UniMAP), Pauh Putra Campus,02600 Arau, Perlis, Malaysia.
[a]reza.roslan@yahoo.com,[b]nashrul@unimap.edu.my,[c]emcheng@unimap.edu.my,[d]normahira@unimap.edu.my

***Abstract*—Bone tissue scaffold had been ventured over the decades in compromising bone failure and trauma. Starch is the most common natural polymer that have been used to fabricate the scaffold and there is many resource of starch. The native starch contribute differently in their structure regarding the amylose content, interactions between granules, swelling power and solubility in which those differences mainly due to botanical and planted origin. In Malaysia itself, there is a various resources of rice starch. No research yet have been made in application Malaysian rice starch with bone tissue scaffold. Balik Wangi rice was reviewed due to high amylose content which correlate with the mechanical strength of scaffold. Via solvent casting and salt leaching technique, this experiment conducted to fabricate the scaffold based on Balik Wangi rice starch originally planted in Sarawak, Malaysia. From this experiment, porosity, density, and pore size achieved is relevant with the optimum value of natural bone. The interaction between Balik Wangi rice starch and hydroxyapatite shows a good combination.In a nutshell, Balik Wangi rice starch is a good resource of starch to be incorporated with hydroxyapatite to form tissue scaffolds.**

***Index Term*-- Balik Wangi Rice, Starch, Hydroxyapatite, Tissue Scaffold.**

I. INTRODUCTION

Extracellular matrices of hard bone tissue is usually comprises of the mineral phase which is made of Hydroxyapatite(HA). Thechemical structure of hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$ is similar to bone materials of human skeletal system. Thus, HA had been commonly used to develop biomimetic scaffolds which promotes new bone formation [1–5]. Unfortunately the brittle nature of HA has limits its used. Hence, consolidation of hydroxyapatite with other natural polymers may provide the solution for the brittleness issue. Starch has been ventured for the past few decades as a promising biopolymers in various biomedical applications due its criteria which are biocompatible, water soluble, abundant in nature, and inexpensive [6–8].

Sadjadi et al. claimed that the polar nature of starch promotes a good adhesion between starch and hydroxyapatite [6], [8]. From the research conducted by Sadjadi, the presence of starch has significant effect on the final morphology of hydroxyapitate due to the interaction of OH^- groups from starch and Ca^{2+} from hydroxyapatite. Ayza et.al suggested that the starch acted as the pore generator in membranes and other porous ceramics[9]. This porous criteria is crucial in bone tissue engineering as porous architecture directly related to the scaffolds' mechanical strength, cell migration, and this wouldensure bone oxygenation and angiogenesis [10-12]. However, not all starch share the same properties and the combination of starches from series of botanical sources is another method to improve the textural qualities of starch [13]. This differences influenced the starch characteristics including its concentration, mixing ratio, amylose content, interactions between granules, swelling power and solubility, and granule characteristics [14].

Upon the variety characteristics of starch, studies had been made in regards to the effect of starch type on certain properties. As for instance, Lawton et al. had studied the effect of different starch on the starch-PVA blend films. It is revealed that PVA blended with high amylose cornstarch hasgood elongation, tensile strength, tear resistance, and impact strength [15]. While, Ahmed et al. claimed that the addition of potato starch showed favourable effect on the hydroxyapatite suspension fluidity. Besides, increasing the amount of starch gradually would increase the density and compressive strength of scaffold [16]. Nasri-Nasrabadia et al. extracted the nanofibers from rice straw and starch to fabricate tissue scaffold by using salt leaching and freeze drying method. As the result, the Young's modulus of the newly fabricated scaffold was up to 325% and the tensile strength wasabout 60%, meanwhile the porosity was 66%. Thus, the addition of rice starch to cellulose nanofibers had improved the mechanical properties, porosity, hydrophilicity, and the degradation rate of the starch based scaffold [10].

Biojo et al. stated that the chemical modification of starch associated with the adaptation of its functional group mutually revamp its physico-chemical characteristics such as gelatinization, pasting, and retrogradation. Basically, the rate and productivity of chemical modification process is strongly correlated with the reagent type, botanical origin of starch, size and structure of granules and surface structure of starch granules [14], [17–22]. Hence, the objectives of this research is to develop nanoHA-starch based from Malaysian rice to produce the scaffold and to study its material and mechanical properties. Malaysian rice starch may consistof different amylose to amylopectin ratio where high amylose starch is claimed to have strong gelatinization behavior [23]. Here, Balik Wangi rice was chosen as a potential starch source for tissue scaffold application and this rice starch is expected to induce considerable porosity to the fabricated scaffold. The tissue scaffold was fabricated using solvent casting and salt leaching techniques. Interaction of the starch and hydroxyapatite blends were analyzed via Fourier Transform Infrared Spectroscopy (FTIR) and the porosity and density of

the scaffolds were examined using liquid displacement method while the morphologies and microstructures were evaluated by using Scanning Electron Microscopic (SEM). This research is mainly to expose the pontentially of native Balik Wangi rice starch to fabricate the scaffold since this rice is claimed to have high high amylose content hence attained its optimum strength. The good reaction in between rice starch and hyroxyapatite from the FTIR result convince that the rice starch is comparable with the lab grade starch. Using the rice starch instead of lab grade starch is cost effectively.

II. MATERIALS AND METHOD

A. Materials

Balik Wangi rice starch was obtained from Sarawak (Malaysia). Sodium Chloride (NaCl) was purchased from Sigma-Aldrich) which ranges 300µm used as porogens. Glutaraldehyde (50% in water) was supplied by Merck.

B. Scaffold Fabrication

Figure 1 shows the step to fabricate rice starch/hydroxyapatite scaffolds. Five different percentages of Balik Wangi rice starch were used to mix with respective amount of HA which are 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), and 90% (w/w). Table 1 shows the detail amount of the starch, HA, and NaCl for the respective percentages. At first, Balik Wangi rice starch powder was mixed with distilled water to make the starch solutions. After several minutes, the sodium chloride particle was added to the amount of starch with the ratio of 1 to 5. Later, the hydroxyapatite solution was added and the mixture was stirred for another few minutes. After stirring the composite homogeneously, the slurry composite was casted into a rectangular Teflon mold with the dimension of 25mm x 15mm x 15mm. The samples were oven dried for 48 hours at 80ºC. The dried scaffold samples was then immersed in 25% glutaraldehyde for crosslinking. This was performed approximately five hours and later was followed by immersing the samples in distilled water for another 72 hours to leach out NaCl and to remove the glutaraldehyde. The samples were dried at room temperature and placed in the desiccator to avoid moistures before further analysis.

C. Liquid Displacement

Liquid displacement method was used to obtained the expected porosity and density of the scaffolds[1-2], [24–27]. Liquid ethanol is used as the medium as ethanol is capable to penetrate the pores easily without shrinking or swelling the samples used [10], [28]. The samples were kept in desiccator for 5 minutes under vacuum. The density and porosity were calculated using equation 1 and 2 respectively ;

Density p,

$$\rho = \frac{W1}{V2 - V3} \qquad (1)$$

Where;
W1 = weight of scaffold
V2 = volume of scaffold immersed in ethanol
V3 = volume of residue ethanol

$$\varepsilon = \frac{V1 - V3}{V2 - V3} \qquad (2)$$

Expected Porosity ε ,
Where;
V1 = volume of ethanol
V2 = volume of scaffold immersed in ethanol
V3 = volume of residue ethanol

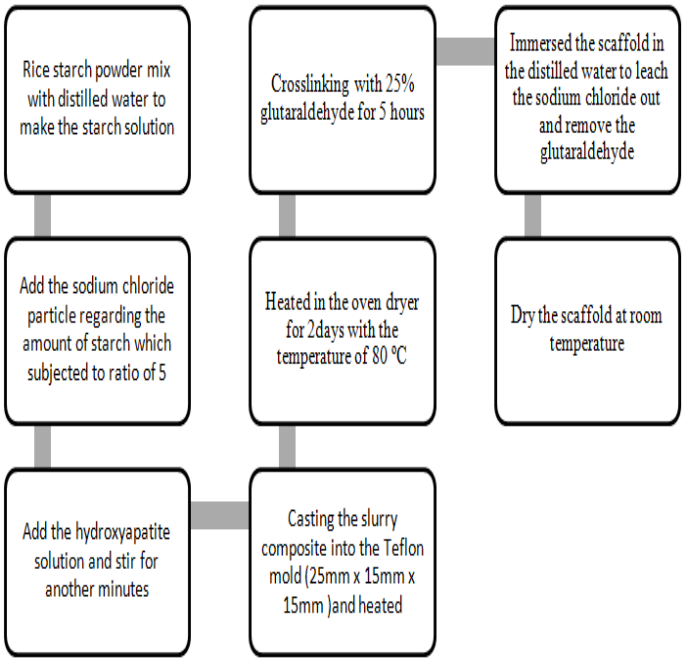

Fig.1.The fabrication of rice starch/hydroxyapatite scaffold

TABLE I
THE COMPOSITION OF BALIK WANGI RICE STARCH, HYDROXYAPATITE, AND SODIUM CHLORIDE

Starch percentage (wt%)	**Hydroxyapatite percentage (wt%)**	**Balik Wangi Rice Starch amount (g)**	**Hydroxyapatite amount (g)**	**Sodium chloride (NaCl) amount (g)**
50	50	5	5	25
60	40	6	4	30
70	30	7	3	35
80	20	8	2	40
90	10	9	1	45

D. Scanning Electron Microscopy

The samples for Scanning Electron Microscopy were cut symmetrically in the middle to observe the porosity atthe inner part. The SEM model used was TM3000 with the excitation

voltageof 15kV was bombarded upon the samples. The magnifications used were about100X to 300X.

E. Fourier Transform Infra Red Spectroscopy (FTIR)

The wavelength spectrum of Balik Wangi rice starch blended with HA for all the samples were obtained via the FTIR spectrometer Perkin Elmer Spectrum 65 with the frequency spectrum of 400 cm^{-1} to 4000 cm^{-1}.

III. Results and Discussion

A. Liquid Displacement

Porous Balik Wangi rice starch and HA scaffold was prepared based on the method aforementioned. The details regarding the density and the expected porosity of every each of the scaffolds made of Balik Wangi rice starch and HA with different percentage are listed in Table 2. Only samples with 50wt%, 60wt%, and 70wt% of rice starch were able to be fabricated but not for the 80wt% and 90wt% samples as they had cracked severely (as shown in Figure 2).

TABLE II
THE COMPOSITION OF RICE STARCH, HYDROXYAPATITE, AND SODIUM CHLORIDE USED TO FABRICATE SCAFFOLDS

Percentage of starch (wt%)	Percentageof hydroxyapatite (wt%)	W1 (g)	V1 (ml)	V2 (ml)	V3 (ml)	P (g/cm³)	ε (%)
50	50	2.130	10.0	12.5	8.0	0.4733	44.59
60	40	1.028	10.0	11.3	8.5	0.3671	54.33
70	30	0.261	10.0	10.3	9.0	0.2008	78.39

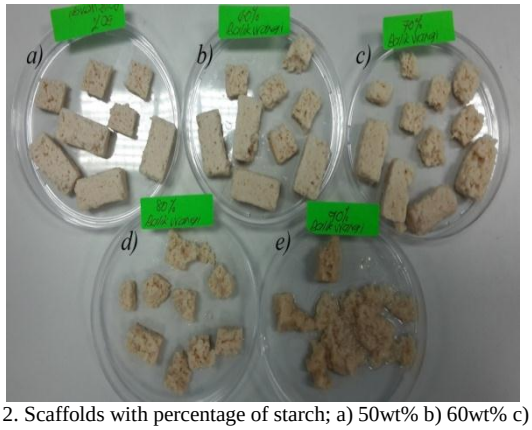

Fig. 2. Scaffolds with percentage of starch; a) 50wt% b) 60wt% c) 70wt% d) 80wt% e) 90wt%

Here, higher Balik Wangi starch percentages were responsible to the fragile structure of the scaffolds. Thus, the 80wt% and 90wt% Balik Wangi starch scaffolds was not capable to undergone tests such as liquid displacement experiments and Scanning Electron Microscopy due to their fragility. From the result, it is showed that by increasing the rice starch percentages, the expected porosity had increased from 44.59% to 78.39%. This trend is well understood as starch could act as pore former for the scaffold [9]. Ahmed et al. also agreed that the increasing porosity was mainly due to the increment of the starch content [16]. Hence increasing the Balik Wangi starch percentage is expected to increase the porosity of scaffold collectively.

In context of NaCl as the porogen agent, by increasing the starch percentage, this would also increase the amount of NaCl based on the 1 to 5 ratio. This is another justification on the increment trend of scaffold's porosity as the yield porosity and the pore size could be regulated by salt fraction and size [29–31]. Hence, it is strongly believe that the mechanical properties could be governed by manipulating the pores' structure [32].

Basically, the range of porosity percentage achieved in this research is about 44.50% to 78.39%. For scaffold with 50 wt% 60wt% and 70wt% of rice starch gave the corresponding porosity with spongy bone which is 30% to 90% [33]. From the experiment conducted, increasing the rice starch shows the decreasing trend of density which is from 0.4733 g/cm³, 0.3671 g/cm³, to 0.2008 g/cm³ for 50wt%, 60wt%, and 70wt% of rice starch respectively. Evans et al. claimed that the density of trabecular bone ranges from 0.14 g/cm³ to 1.10 g/cm³ [34]. Hence, the obtained densities were still in the range ofa typical trabecular bone density. Therefore,by increasing the starch content, this would increase the relative density of the scaffold[16] and higher density will increase the mechanical strength[35].

B. Scanning Electron Microscopy

From the liquid displacement results, it can be seen that thehigher amount of starch percentage would increase the porosity and the pore size of the scaffolds. Analyzing the pores' size from 50 wt% to 70 wt%, it is noticed that the pore size hadgetting bigger and at 70 wt% scaffolds, typical size of the pores found are 125 µm – 396µm. This range of pore size (10 µm – 400 µm) is capable to furnish the nutrient and osteoblast cellular infusion while preserve the structural term [36–40].

Higher amount of starch would lead to scaffold's cracking and rupture as Ahmed et al. claimed that a very high starch content would cause great dissemination throughout thescaffold, creating large cracks on the scaffold. The high quantity of starch granules also lead to shrinking during the drying process [16]. Hence, it makes sense that the scaffold with 80 wt% and 90 wt%Balik Wangi rice starch had cracked and ruptured severely due to the high starch content. Thus, using percentages of rice starch more than 70 wt% is not possible as this will cause scaffolds tocrack and rupture. While, the obtained pore size for samples with 50 wt%, 60 wt%, and 70 wt% of rice starch is 396 µm.

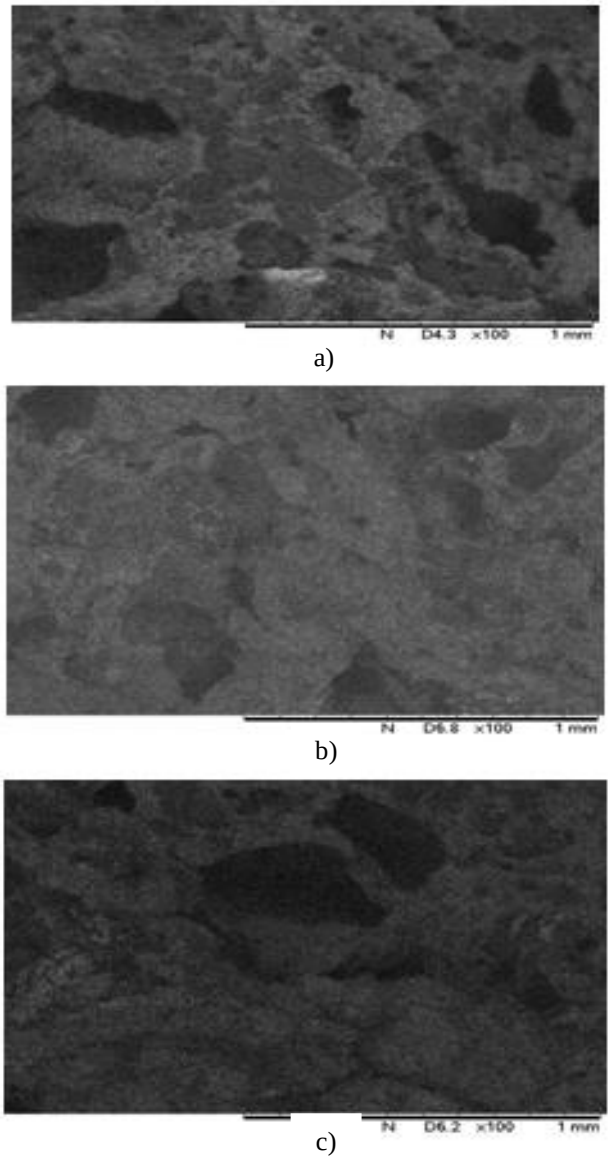

Fig.3.Scanning Electronic Microscopic (SEM) image ; a)50wt%, b)60wt%, and c)70wt%.

C. *Fourier Transfrom Infrared Spectroscopy (FTIR)*

The results of three samples are shown in Figure 4. The peak at 3435.74 $cm^{-1}$for 50 wt% balikwangi rice starch is characterized by the peak of O-H stretching of amylopectin where the width is attributed to the inter and intra-molecular hydrogen bonding [41]. At this range, for the 60 wt% and 70 wt% of Balik Wangi rice starch, the peak value is 3428.03 $cm^{-1}$and 3429.39 cm^{-1}respectively. The shifts is due to the difference of Balik Wangi rice starch concentration.

Bands at 2952 cm^{-1} and 2927 cm^{-1}arethe asymmetric stretching of C-H of starch bond [41, 42]. The peaks for 50 wt%, 60 wt%, and 70 wt% which are 2926.30 cm^{-1}, 2927.75 cm^{-1}, and 2927.48 cm^{-1} respectively arethe asymmetric stretching of C-H bond. Narrow peaks exist at 1155.42 cm^{-1}, 1160.41 cm^{-1}, and 1159.73 cm^{-1} for 50 wt%, 60 wt%, and 70 wt% rice starch respectively indicates C-O stretching [42]. Band at 1040 cm^{-1} indicates the asymmetric stretch modes of P-O bonds of phosphate group [43]. This band exists in all the blends with the values of 1030.86 cm^{-1}, 1026.69 cm^{-1}, and 1029.78 cm^{-1} respectively. Whereas the peak at 602 cm^{-1}indicates the phosphate vibration group for all the three percentages [7].

For all the three percentages, there are peaks in range of 1465 cm^{-1} – 1415 cm^{-1} which represent the characteristic peak of hydroxyapatite carbonate group. Those peaks imposed CaOandCa$(OH)_2$ used to get optimal stoichiometric relation between Ca/P materials. These elements are responsible for hindering the regeneration of bone tissue in the presence of implants. Carbonated hydroxyapatite has the capability to allow regeneration [43]. Last but not least, peak in the range of 1720$cm^{-1}$to 1740 cm^{-1} represents the free aldehyde groups of glutaraldehyde, however, since there is no such peak, it is suggested that glutaraldehydehad successfully cross-linked the samples [44].

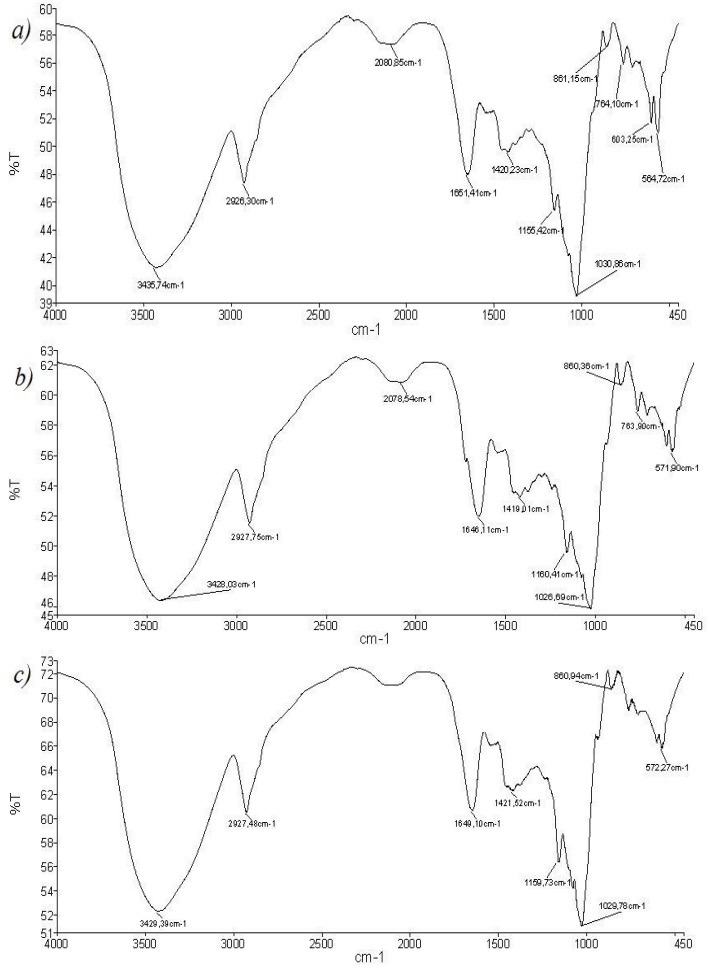

Fig.4. Fourier Transform Infrared Spectroscopy (FTIR) ; a)50wt%,b)60wt%, and c)70wt%

IV. CONCLUSION

As a conclusion, according to the analysis conducted, Balik Wangi rice starch could be a potential biomaterial in

fabricating bone tissue scaffold. Solvent casting and salt leaching technique had resulted a trend of the density, the porosity, and the pore size of the scaffold. The scaffold's porosities obtained through this study are in the range with the porosity of spongy bone which is approximately 30% to 90% and the density obtained is similar to trabecular bone. Increasing the starch percentage seems to increase the value of porosity and mutually decreasing the value of density. Increasing the starch content is applicable until 70 wt% percentage, as higher percentage would compromise the integrity of the scaffold's structure. Besides that, the higher salt ratio will increase the amount of porosity. Y. Ahmed fabricate the scaffold with potato starch and the apparent porosity obtained is about 53%, while in this research, porosity achieved is more which is 78.39%. Fourier Transform Infrared Spectroscopy (FTIR) analysis with different percentage of starch proposechanges in peak shift and the peak width amongst the starch percentage. The amylopectin compositon in Balik Wangi rice starch shows interaction through the O-H stretching, hence confirming starch and HA have a good interaction to produce a functional tissue scaffold.

ACKNOWLEDGMENT

This research is supported by fundamental research grant scheme (FRGS) 2014 (No. 9003-00487),School of Mechatronic Engineering, University of Malaysia Perlis.

REFERENCES

[1] F. Zhao, Y. Yin, W. W. Lu, J. C. Leong, W. Zhang, J. Zhang, M. Zhang, and K. Yao, "Preparation and histological evaluation of biomimetic three-dimensional hydroxyapatite/chitosan-gelatin network composite scaffolds," *Biomaterials*, vol. 23, no. 15, pp. 3227–3234, 2002.

[2] M. K. Narbat, F. Orang, M. S. Hashtjin, and A. Goudarzi, "Fabrication of Porous Hydroxyapatite-Gelatin Composite Scaffolds for Bone Tissue Engineering," vol. 10, no. October, pp. 215–223, 2006.

[3] J. Sundaram, T. D. Durance, and R. Wang, "Porous scaffold of gelatin-starch with nanohydroxyapatite composite processed via novel microwave vacuum drying," *Acta Biomater.*, vol. 4, no. 4, pp. 932–942, Jul. 2008.

[4] I. Sopyan and J. Kaur, "Preparation and characterization of porous hydroxyapatite through polymeric sponge method," vol. 35, pp. 3161–3168, 2009.

[5] C.-K. Chiu, J. Ferreira, T.-J. M. Luo, H. Geng, F.-C. Lin, and C.-C. Ko, "Direct scaffolding of biomimetic hydroxyapatite-gelatin nanocomposites using aminosilane cross-linker for bone regeneration.," *J. Mater. Sci. Mater. Med.*, vol. 23, no. 9, pp. 2115–26, Sep. 2012.

[6] H. J. M.S. Sadjadi, M.Meskinfam, "Hydroxyapatite-Starch Nano Biocomposites Synthesis and Characterization Hydroxyapatite - starch nano biocomposites synthesis and characterization," vol. 1, no. November, 2015.

[7] M. Shakir, R. Jolly, M. Shoeb, and H. M. Khan, "International Journal of Biological Macromolecules Nano-hydroxyapatite / chitosan – starch nanocomposite as a novel bone construct : Synthesis and in vitro studies," *Int. J. Biol. Macromol.*, vol. 80, pp. 282–292, 2015.

[8] M. S. Sadjadi, M. Meskinfam, B. Sadeghi, H. Jazdarreh, and K. Zare, "In situ biomimetic synthesis, characterization and in vitro investigation of bone-like nanohydroxyapatite in starch matrix," *Mater. Chem. Phys.*, vol. 124, no. 1, pp. 217–222, Nov. 2010.

[9] M.-M. Lorente-Ayza, M. J. Orts, V. Pérez-Herranz, and S. Mestre, "Role of starch characteristics in the properties of low-cost ceramic membranes," *J. Eur. Ceram. Soc.*, vol. 35, no. 8, pp. 2333–2341, Aug. 2015.

[10] B. Nasri-Nasrabadi, M. Mehrasa, M. Rafienia, S. Bonakdar, T. Behzad, and S. Gavanji, "Porous starch/cellulose nanofibers composite prepared by salt leaching technique for tissue engineering.," *Carbohydr. Polym.*, vol. 108, pp. 232–8, Aug. 2014.

[11] W. Ponds, "Biomimetic Porous Titanium Scaffolds for Orthopedic and Dental Applications," pp. 415–451.

[12] J. Gil-Albarova, M. Vila, J. Badiola-Vargas, S. Sánchez-Salcedo, a. Herrera, and M. Vallet-Regi, "In vivo osteointegration of three-dimensional crosslinked gelatin-coated hydroxyapatite foams," *Acta Biomater.*, vol. 8, no. 10, pp. 3777–3783, Oct. 2012.

[13] S. Charoenrein and N. Preechathammawong, "Effect of waxy rice flour and cassava starch on freeze-thaw stability of rice starch gels.," *Carbohydr. Polym.*, vol. 90, no. 2, pp. 1032–7, Oct. 2012.

[14] N. Seetapan, N. Limparyoon, C. Gamonpilas, P. Methacanon, and A. Fuongfuchat, "Effect of cryogenic freezing on textural properties and microstructure of rice flour / tapioca starch blend gel," *J. Food Eng.*, vol. 151, pp. 51–59, 2015.

[15] J. W. Lawton, "Effect of starch type on the properties of starch containing films," *Carbohydr. Polym.*, vol. 29, no. 3, pp. 203–208, Mar. 1996.

[16] Y. M. Z. Ahmed, E. M. M. Ewais, and S. M. El-sheikh, "Potato starch consolidation of aqueous HA suspension," *Integr. Med. Res.*, 2014.

[17] F. M. Fakhouri, D. Costa, F. Yamashita, S. M. Martelli, R. C. Jesus, K. Alganer, F. P. Collares-Queiroz, and L. H. Innocentini-Mei, "Comparative study of processing methods for starch/gelatin films.," *Carbohydr. Polym.*, vol. 95, no. 2, pp. 681–9, Jun. 2013.

[18] R. F. Tester, J. Karkalas, and X. Qi, "Starch—composition, fine structure and architecture," *J. Cereal Sci.*, vol. 39, no. 2, pp. 151–165, Mar. 2004.

[19] E. M. Biojo and A. Rodrigues, "StarOchuch : From Food to Medicine," 2005.

[20] F. Xie, E. Pollet, P. J. Halley, and L. Avérous, "Starch-based nano-biocomposites," *Prog. Polym. Sci.*, vol. 38, no. 10–11, pp. 1590–1628, Oct. 2013.

[21] E. C. Dario Puppi, Federica Chiellini, Mamoni Dash, "Biodegradable polymers for biomedical applications," no. August 2015, 2011.

[22] R. Colussi, S. Lisie, M. El, V. Zanella, J. Bartz, L. Carlos, R. Zavareze, A. Renato, and G. Dias, "LWT - Food Science and Technology Acetylation of rice starch in an aqueous medium for use in food," *LWT - Food Sci. Technol.*, pp. 1–7, 2015.

[23] A. A. Wani, P. Singh, M. A. Shah, U. Schweiggert-Weisz, K. Gul, and I. A. Wani, "Rice Starch Diversity: Effects on Structural, Morphological, Thermal, and Physicochemical Properties-A Review," *Compr. Rev. Food Sci. Food Saf.*, vol. 11, no. 5, pp. 417–436, Sep. 2012.

[24] J. Guan, K. L. Fujimoto, M. S. Sacks, and W. R. Wagner, "Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications.," *Biomaterials*, vol. 26, no. 18, pp. 3961–71, Jun. 2005.

[25] J. Venkatesan, R. Pallela, I. Bhatnagar, and S. Kim, "International Journal of Biological Macromolecules Chitosan – amylopectin / hydroxyapatite and chitosan – chondroitin sulphate / hydroxyapatite composite scaffolds for bone tissue engineering," *Int. J. Biol. Macromol.*, vol. 51, no. 5, pp. 1033–1042, 2012.

[26] S. Mallick, S. Tripathi, and P. Srivastava, "Advancement in Scaffolds for Bone Tissue Engineering : A Review," vol. 10, no. 1, pp. 37–54, 2015.

[27] S. Briefs and I. N. Materials, *Naznin Sultana Mohd Izzat Hassan Mim Mim Lim.* .

[28] J. Guan, K. L. Fujimoto, M. S. Sacks, and W. R. Wagner, "Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications.," *Biomaterials*, vol. 26, no. 18, pp. 3961–71, Jun. 2005.

[29] "Biomaterial scaffold fabrication techniques for potential tissue engineering applications," no. 3, pp. 141–159, 2010.

[30] H. Janik and M. Marzec, "A review: Fabrication of porous polyurethane scaffolds," *Mater. Sci. Eng. C*, vol. 48, pp. 586–591, Mar. 2015.

[31] C. S. Ki, S. Y. Park, H. J. Kim, H.-M. Jung, K. M. Woo, J. W. Lee, and Y. H. Park, "Development of 3-D nanofibrous fibroin scaffold with high porosity by electrospinning: implications for bone regeneration.," *Biotechnol. Lett.*, vol. 30, no. 3, pp. 405–10, Mar. 2008.

[32] S. Wu, X. Liu, K. W. K. Yeung, C. Liu, and X. Yang, "Biomimetic porous scaffolds for bone tissue engineering," *Mater. Sci. Eng. R Reports*, vol. 80, pp. 1–36, Jun. 2014.

[33] M. Mozafari, F. Moztarzadeh, M. Rabiee, M. Azami, S. Maleknia, M. Tahriri, Z. Moztarzadeh, and N. Nezafati, "Development of macroporous nanocomposite scaffolds of gelatin/bioactive glass prepared through layer solvent casting combined with lamination technique for bone tissue engineering," *Ceram. Int.*, vol. 36, no. 8, pp. 2431–2439, Dec. 2010.

[34] L. A. Evans, D. J. Macey, and J. Webb, "Calcium biomineralization in the radular teeth of the chiton, Acanthopleura hirtosa," *Calcif. Tissue Int.*, vol. 51, no. 1, pp. 78–82, Jul. 1992.

[35] M. K. Narbat, F. Orang, M. S. Hashtjin, and A. Goudarzi, "Fabrication of Porous Hydroxyapatite-Gelatin Composite Scaffolds for Bone Tissue Engineering," vol. 10, no. October, pp. 215–223, 2006.

[36] and S. M. S. K. Whang, K.E. Healy, D.R. Elenz, E.K. Nam, D.C. Tsai, C.H. Thomas, G.W. Nuber, F.H. Glorieux, R. Travers, "Engineering Bone Regeneration with Bioabsorbable Scaffolds with Novel Microarchitecture," *Tissue Eng.*, vol. Vol. 5, no. 1, pp. 35–51, 1999.

[37] A. Bignon, J. Chouteau, J. Chevalier, G. Fantozzi, J. Carret, Â. R. Laennec, and G. Paradin, "Effect of micro- and macroporosity of bone substitutes on their mechanical properties and cellular response," vol. 4, pp. 1089–1097, 2003.

[38] K. A. Hing, B. Annaz, S. Saeed, P. A. Revell, T. Buckland, B. Materials, and Q. Mary, "Microporosity enhances bioactivity of synthetic bone graft substitutes," vol. 6, pp. 467–475, 2005.

[39] B. D. Bovan, T. W. Hummert, D. D. Dean, and Z. Schwartz, "Role of material surfaces in regulating bone and cartilage cell response," vol. 17, no. 2, pp. 137–146, 1996.

[40] J. R. Woodard, A. J. Hilldore, S. K. Lan, C. J. Park, A. W. Morgan, J. A. C. Eurell, S. G. Clark, M. B. Wheeler, R. D. Jamison, and A. J. Wagoner Johnson, "The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity.," *Biomaterials*, vol. 28, no. 1, pp. 45–54, Jan. 2007.

[41] M. Shakir, R. Jolly, M. S. Khan, N. E. Iram, and H. M. Khan, "Nano-hydroxyapatite/chitosan-starch nanocomposite as a novel bone construct: Synthesis and in vitro studies.," *Int. J. Biol. Macromol.*, vol. 80, pp. 282–92, Sep. 2015.

[42] Z. Hadisi, J. Nourmohammadi, and J. Mohammadi, "Composite of porous starch-silk fibroin nanofiber-calcium phosphate for bone regeneration," *Ceram. Int.*, vol. 41, no. 9, pp. 10745–10754, Nov. 2015.

[43] L. Pighinelli and M. Kucharska, "Properties and Structure of Microcrystalline Chitosan and Hydroxyapatite Composites," no. April, pp. 128–138, 2014.

[44] Z. Hadisi, J. Nourmohammadi, and J. Mohammadi, "Composite of porous starch-silk fi broin nano fi ber-calcium phosphate for bone regeneration," 2015.