

การวิเคราะห์เปรียบเทียบคุณสมบัติทางกายภาพและเคมีของอาร์เอสยู-กราฟต์ จากกระดูกวัวกับกระดูกเทียมไบโอ-ออส Physicochemical characterization of RSU-Graft comparison with Bio-Oss®

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บทคัดย่อ

ไบโอ-ออสเป็นกระดูกเทียมจากวัวที่ใช้กันอย่างแพร่หลายในทางทันตกรรมเนื่องจากมีอัตราความสำเร็จที่สูงแต่ยังมีราคาแพง ทางกลุ่มผู้วิจัยจึงเห็นว่าเป็นประโยชน์อย่างมากที่จะพัฒนากระดูกเทียมที่มีประสิทธิภาพเทียบเท่ากับไบโอ-ออส วัตถุประสงค์ของงานวิจัยนี้คือการวิเคราะห์เปรียบเทียบคุณสมบัติทางกายภาพและเคมีของกระดูกเทียมอาร์เอสยู-กราฟต์กับกระดูกเทียมไบโอ-ออส โดยไบโอ-ออสเป็นกระดูกเทียมจากวัวที่ผ่านกระบวนการเผาที่อุณหภูมิ 300°C และมีขนาดแกรนูลเท่ากับ 0.25-1 มม. ส่วนอาร์เอสยู-กราฟต์เป็นกระดูกเทียมจากวัวที่ผ่านกระบวนการเผาที่อุณหภูมิ 900°C และมีขนาดแกรนูลเท่ากับ 0.25-1 มม. ใช้เครื่องมือทั้งหมด 4 ชนิดในการวิเคราะห์ ได้แก่ 1) scanning electron microscopy (SEM) 2) X-ray diffraction (XRD) 3) energy dispersive X-ray spectroscopy (EDX) และ 4) Fourier transform infrared (FTIR) ผลการศึกษานี้พบว่าอาร์เอสยู-กราฟต์มีสั-

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ส่วนแคลเซียมต่อฟอสฟอรัสมากกว่า 1.67 โดยไม่สามารถวัดขนาดรูพรุนที่ชัดเจนได้ และมีคุณสมบัติความเป็นผลึกไฮดรอกซีอะพาไทต์ที่มากกว่าไบโอ-ออสเล็กน้อย นอกจากนี้ ยังตรวจพบหมู่ OH- ในไบโอ-ออสซึ่งบ่งบอกถึงความชื้นในตัวอย่างชนิดนี้ ในขณะที่ไม่สามารถตรวจพบหมู่ OH- ซึ่งบ่งบอกถึงการระเหยที่สมบูรณ์ของน้ำในอาร์เอสยู-กราฟต์ ข้อสรุปคืออาร์เอสยู-กราฟต์มีคุณสมบัติบางประการที่คล้ายกับไบโอ-ออสแต่จะมีความเป็นผลึกที่มากกว่า และไม่สามารถวัดขนาดของรูพรุนได้ อย่างไรก็ตาม ต้องทำการทดสอบคุณสมบัติทางกายภาพและทางเคมีอื่นของอาร์เอสยู-กราฟต์ก่อนที่จะนำไปศึกษาต่อในสิ่งไม่มีชีวิตและมีชีวิต

คำสำคัญ: ไบโอ-ออส® กระดูกวัวเทียม คุณสมบัติทางกายภาพและเคมี เนื้อเยื่อปลูกถ่ายวิวิธพันธุ์ ไฮดรอกซีอะพาไทต์

Abstract Bio-Oss® is the deproteinized bovine bone graft material that has been widely used in dentistry because of its successful treatment result, but its cost is relatively high. Therefore, it is beneficial to develop bone substitute comparable to Bio-Oss®. Thus, the purpose of this research is to compare physicochemical properties of RSU-Graft and Bio-Oss®. Bio-Oss® are xenogenic granules (bovine bone heated to 300°C, sizes of granules are 0.25-1mm) and RSU-Graft is xenograft (bovine bones heated at 900°C, sizes of granules are 0.25-1mm). This study used 4 measurement methods, including 1) scanning electron microscopy (SEM), 2) X-ray diffraction (XRD), 3) energy dispersive X-ray spectroscopy (EDX) and 4) Fourier transform infrared (FTIR). For the results, RSU-Graft had the Ca/P ratio higher than 1.67 and did not have distinctive pore size to measure. The crystalline hydroxyapatite corresponded to HAp was slightly greater crystallinity than Bio-Oss®. Also, OH- groups were found in Bio-Oss® because there was small amount of water remaining in the sample, whereas RSU-Graft showed no OH- group which indicated complete dehydration. For conclusions, RSU-Graft had similar properties compared to Bio-Oss®. It had slightly higher crystallinity but it did not have distinctive pore size. Nevertheless, RSU-Graft needs further experiment on other physicochemical properties before *in vitro* and *in vivo* studies.

Keywords: Bio-Oss®, bovine bone graft, physicochemical, xenografts, hydroxyapatite

Introduction

The practice of reconstructive surgery in dentistry is widely used for patients with bone defects. There are several bone substitution materials that possess different physicochemical properties. Bone substitution materials can be classified into 4 groups according to source of bone substitute; 1) autograft

2) allograft 3) xenograft and 4) alloplast.⁽¹⁾

Inorganic bovine bones, the xenograft materials, are indicated as bone defect filling materials due to their osteoconductive properties.⁽²⁾ Xenografts were introduced to dentistry in 1936 by Buebe and Silvers. They used boiled cow bone powder to repair intra-bony defect caused by periodontal disease and the

results were excellent in humans. In 1956, Os purum, anorganic bovine bone graft, was developed by Forsberg and the results were mostly satisfying in humans.⁽³⁾ However, some case reports revealed unsatisfactory results of disease transmission and graft rejection from the organic residues of bovine bones. There is risk of prion disease transmission from bovine bone substitutes including the Creutzfeldt-Jakob disease, fetal prion disease in humans, and bovine spongiform encephalopathy (BSE). The transmission of the disease from bovine bones are caused by proteins including collagens which were detected in some anorganic bovine bone substitute.⁽⁴⁾ In the present, manufacturing processes can reduce the risk of disease transmission by deproteinization of the organic part from bovine bone substitutes.

Bio-Oss[®] is one of the commercial deproteinized bovine bone graft materials that is widely used in dentistry. It is manufactured by deproteinization at temperature of 300°C using sodium hydroxide⁽⁵⁾ and still able to maintain bony structure. Bio-Oss[®] structure is crystallized and its calcium phosphate ratio is classified as calcium deficient hydroxyapatite. The physicochemical properties of Bio-Oss[®] are similar to those of human bone. The components of Bio-Oss[®] are 7% of carbonate, $37.1 \pm 0.7\%$ of calcium and $17.8 \pm 0.5\%$ of phosphorus. The calcium phosphate ratio is 2.1 ± 0.1 . Bio-Oss[®] has 2 particle sizes which are 0.25-1 mm and 1-2 mm.⁽⁶⁾

According to Lee et al, in 2014, the InterOss[®] anorganic bovine bone was performed by chemical and heat treatment with low temperature process as equivalently as Bio-Oss[®]. From scanning electron microscope (SEM), surface area and porosity analyzer (BET), X-ray diffraction (XRD), Fourier transform infrared (FTIR), inductively coupled plasma (ICP) and energy dispersive X-ray spectrometer (EDS) anal-

ysis, comparative physicochemical characteristics of Bio-Oss[®] and InterOss[®] were very similar. The Ca/P ratio of both bones carried out by the ICP technique were close to the theoretical value of 1.67.⁽⁷⁾

Accorsi-Mendonca et al, in 2008, studied 2 commercial bovine bone Gen-Ox[®] and Bio-Oss[®] and reported that both of them were hydroxyapatite compounds. The sintering temperature of Gen-Ox[®] was at 950-1000°C, increasing of temperature led to more crystalline structure. Porosities through SEM were similar. Residual organic material of Bio-Oss[®] was higher than Gen-Ox[®]. The low crystallinity and presence of organic residual made Bio-Oss[®] had greater dissolution than Gen-Ox[®].⁽²⁾

Pripatnanont, in 2016, reviewed some commercial bovine bones processed under high temperature such as Osteograft N[®], Endobon[®] and Cerabone[®]. This high temperature process could eliminate organic substance and vital tissues, preventing antigenicity stimulation and transmission of bacteria, but retained similar properties such as pore size and volume of porosity. They also had high crystallinity and less degradation by osteoclasts. High crystallinity and containing of hydroxyapatite, resulted in lesser reabsorption of water into material. However, they had property of hydrophilicity which was measured by contact angle. Less contact angle showed better wettability which promoted bone formation in initial wound healing.⁽⁶⁾

Although Bio-Oss[®] is the leading bone substitute using worldwide, but its cost is not affordable by patient with low socioeconomic status. Nowadays, Thai researchers are still trying to develop bone substitute that are more affordable.

Chotika Bone was developed by Thai researchers, Punyanitya et al, in 2011 for regenerative purpose.⁽¹⁾ Chotika Bone is the production of the porous ceramic

from bovine bone powders strengthened by adding of glass ceramic for application of porous ceramic for the attachment of load bearing. Porosity in bone is key element to allow penetration and new bone formation around bone graft material. Pore size must be 100-500 microns with volume of 18-74%.⁽¹⁾ It was tested in rats and rabbits, there were no adverse reactions or inflammation inside animals testing.⁽⁸⁾ So if we can develop bone graft substitute that practically use in dental surgery, it bring more benefit for patients, environment and decrease import products in Thailand. Our studied aimed to develop RSU-Graft for bone substitute in dentistry.

Materials and methods

This study compared the physicochemical properties of 2 bone substitutes of Bio-Oss[®] and RSU-Graft

1) Bio-Oss[®] xenogenic granules (bovine bones heated to 300°C, sizes of granules were 0.25-1 mm)

2) RSU-Graft xenograft (bovine bones heated at 900°C, sizes of granules were 0.25-1 mm)

Bone preparation protocol

RSU-Graft, a femur bovine bone collected from a slaughterhouse, was manufactured from bovine bone (Punyanitya Medical Instrument). The bovine bone was prepared by cutting into bone blocks size 15 × 25 × 10 cm. The bone blocks were cleaned to remove all organic substances by boiling in NaOH solution (pH 14.0) 8 hours per day for 7 days, rinsed with distilled water 3 times for 1 hour and heat-dried with hot air oven at 200°C for 1 hour. Then, the bone blocks were sintered at 900°C for 3 hours, milled and grinded with mortar and sifted with sieve to gain the bone particle size of 250-1,000 microns.

The study methods

The 4 methods used in this study included

1. Scanning electron microscopy (SEM), using JSM-IT300 InTouchScope™ Scanning Electron Microscope, JEOL Ltd, Japan

The SEM was operated at 15kV of electron acceleration to obtain information about the samples surface structure, morphology of bone particles and measure the size of the pores. The magnification used were 70x, 1000x and 10,000x. The average size was observed in quantity within 5 numbers of particles and micropores.

2. X-ray diffraction (XRD), using Bruker D8

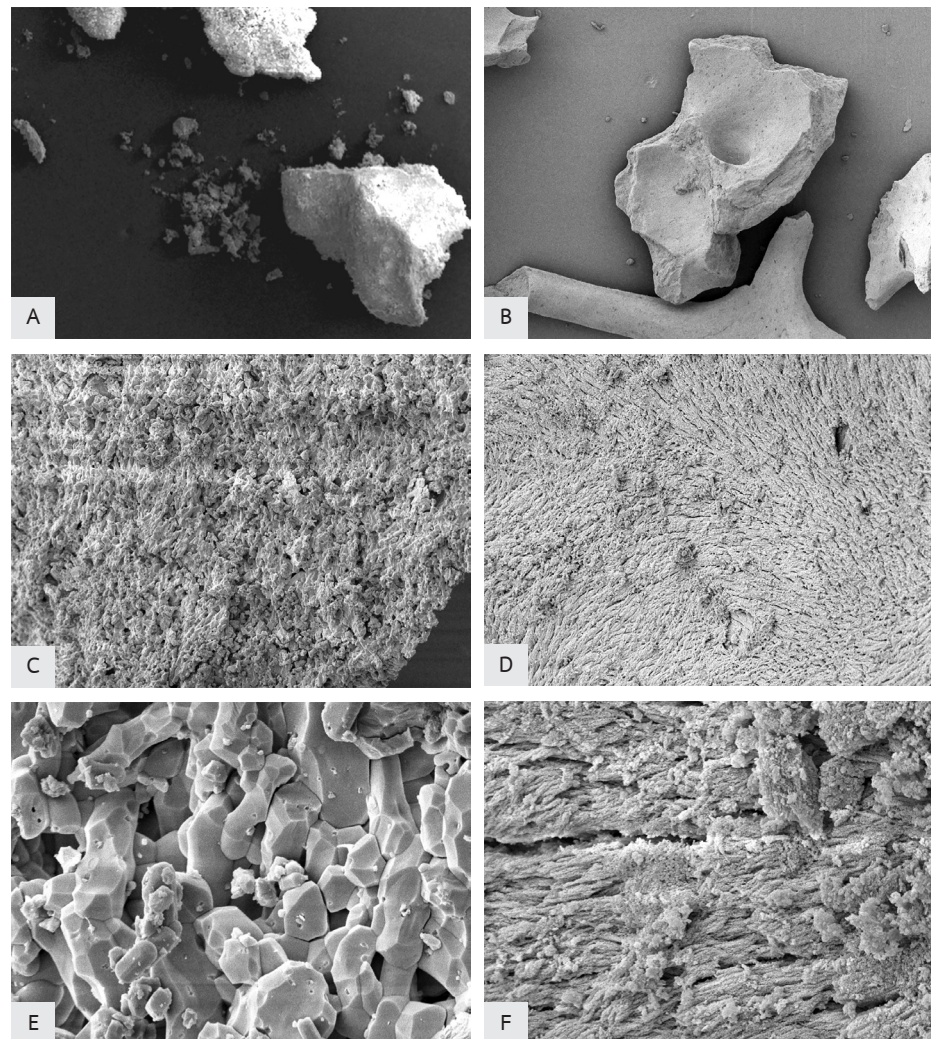
X-ray diffraction was used to identify crystalline phase of study materials. The crystallinity would affect the quality in bone tissue repair.⁽⁹⁾ An X-ray diffractometer was operated at 30kV, 15mA using Cu K α radiation and the data were collected from 0-80 degrees theta-2. The XRD pattern was compared with the PDF (Power Diffraction File pattern 09-0432).

3. Energy dispersive X-ray spectroscopy (EDX), using Oxford instruments X-MaxN 80

EDX was used to identify Ca/P ratio of RSU-Graft. The Ca/P ratio was associated with the dissolution rate or bioabsorption rate of the HAP materials. The EDX was operated at 20 kV, large-area 80 mm 2 sensor and resolution at 50,000 cps.⁽¹⁰⁾

4. Fourier transform infrared (FTIR), using Perkin Elmer, Spectrum 100

FTIR was used to identify the presence of certain functional groups in a molecule. It could also identify unknown substances and determine the amount of components in a given sample to get authenticated information about the vibrational origin of the phosphate and carbonate.⁽⁹⁾ This experiment were recorded in wavelength between 400-4,000 cm⁻¹, with 4 scans and a 16 cm⁻¹ resolution.



รูปที่ 1 ภาพกำลังขยาย 70 เท่าของกระดูกเทียมอาร์เอสยู-กราฟต์ (A) และไบโอ-ออส (B) ภาพกำลังขยาย 1,000 เท่าของกระดูกเทียมอาร์เอสยู-กราฟต์ (C) และไบโอ-ออส (D) ภาพกำลังขยาย 10,000 เท่าของกระดูกเทียมอาร์เอสยู-กราฟต์ (E) และไบโอ-ออส (F)

Fig. 1 RSU-Graft (A) and Bio-Oss® (B) images with magnification 70×, RSU-Graft (C) and Bio-Oss® (D) images with magnification 1,000× and RSU-Graft (E) and Bio-Oss® (F) images with magnification 10,000×.

Results

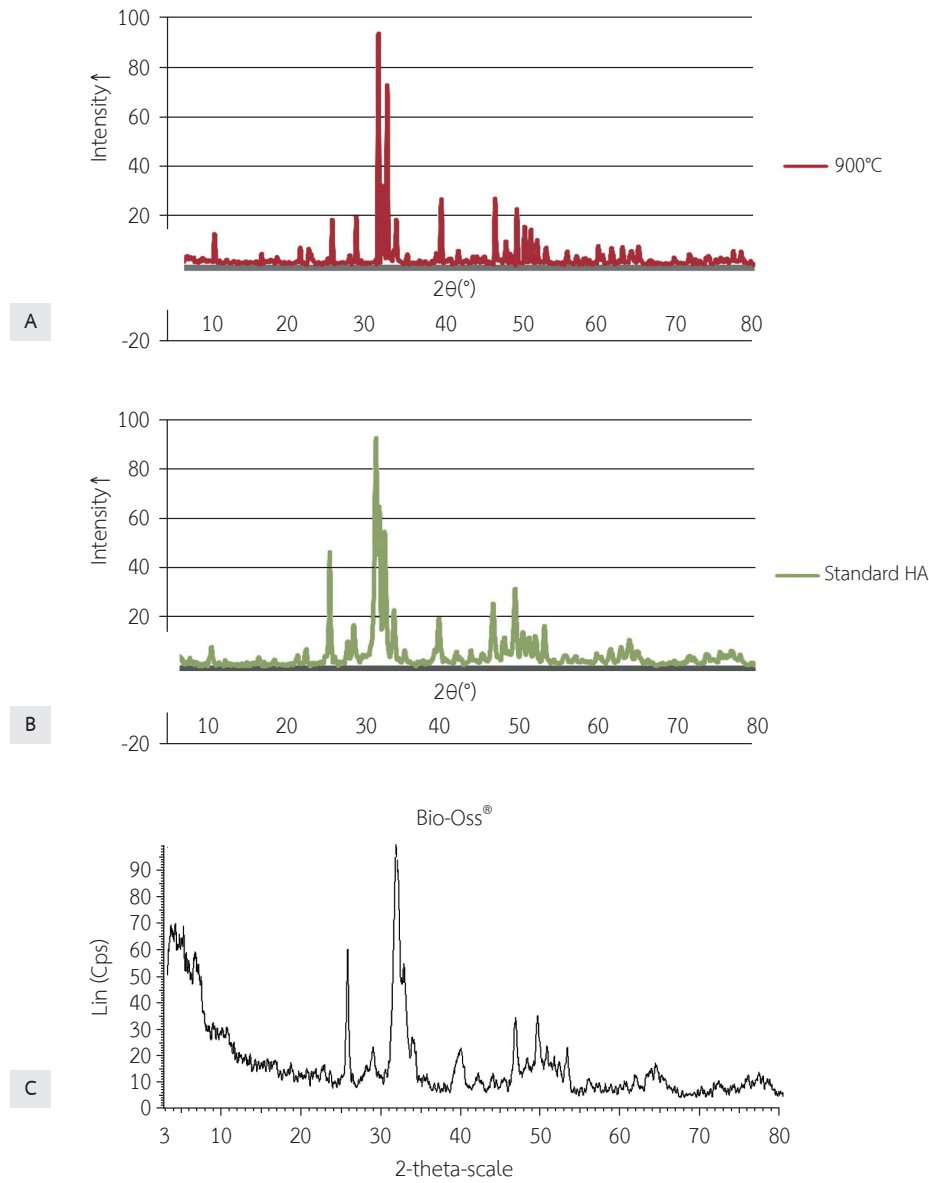
Scanning electron microscope (SEM)

The surface morphology and microstructure of RSU-Graft in Fig. 1A and 1B showed the SEM images of RSU-Graft sintered at 900°C and Bio-Oss® taken with magnification of 70×, both samples had irregular particles but RSU-Graft had more surface roughness. Fig. 1C and 1D images, taken with magnification of 1,000×, showed that RSU-Graft had coarser surface with more microporous than Bio-Oss®. Fig. 1E and 1F

images, taken with magnification of 10,000×, showed that RSU-Graft had polygonal particles with infiltrated inter-microporous, but Bio-Oss® had linear in shape with infiltrated inter-microporous. The average pore sizes of RSU-Graft sintered at 900°C and Bio-Oss® were 537 μm and 525 μm, respectively.

X-ray diffraction (XRD)

Individual X-ray diffraction patterns of RSU-Graft sintered at 900°C, standard HA and Bio-Oss® were showed in Fig. 2.⁽¹¹⁾ They represented intensity of



รูปที่ 2 A: ผลการวิเคราะห์กระดูกเทียมอาร์เอสยู-กราฟต์ที่ผ่านกระบวนการเผาที่อุณหภูมิ 900 องศาเซลเซียส เปรียบเทียบกับ B: รูปแบบเอกซอาร์ดีของไฮดร็อกซีอะพาไทต์มาตรฐาน และ C: กระดูกเทียมไบโอ-ออส⁽¹¹⁾

Fig. 2 A: RSU-Graft sintered at 900°C, B: individual XRD pattern of standard HA and C: Bio-Oss^{®(11)} (courtesy data from Berberi, A, et al. Physicochemical characteristics of bone substitutes used in oral surgery in comparison to autogenous bone. *BioMed Research International*. 2014.)⁽¹¹⁾

X-ray as a function of the diffraction angles (2 theta, θ).

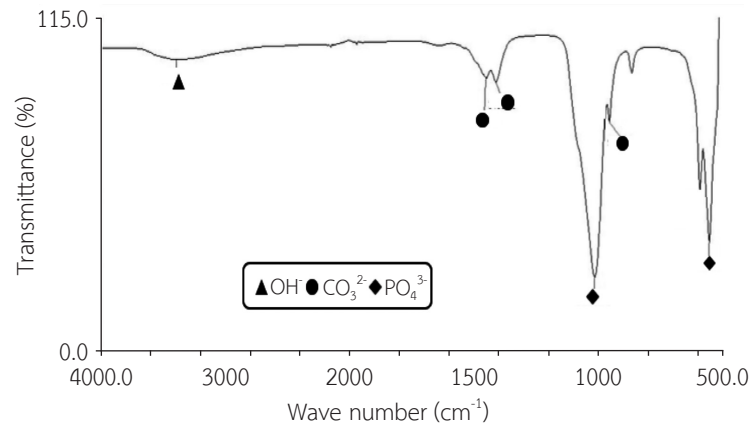
The results of X-ray diffraction experiments were indicated for the presence of crystalline phase. As expected, the XRD pattern of 900°C bone graft material corresponded to the pattern of standard HA with coincident peak positions as shown in Table 1.

In the patterns obtained for X-ray diffraction, ac-

ตารางที่ 1 แสดงตำแหน่งจุดสูงสุดของไฮดร็อกซีอะพาไทต์ เปรียบเทียบระหว่างไฮดร็อกซีอะพาไทต์มาตรฐานกับกระดูกเทียมอาร์เอสยู-กราฟต์ที่ผ่านกระบวนการเผาที่อุณหภูมิ 900 องศาเซลเซียส

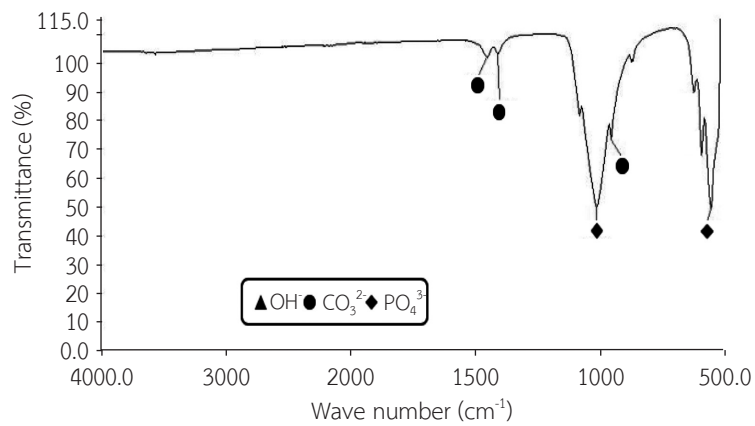
Table 1 Showed position of peaks of hydroxyapatite in standard HA and RSU-Graft sintered at 900°C.

	2θ(°)
Standard HA	31.780
900°C	31.979
Difference	0.199



รูปที่ 3 แสดงแถบการดูดกลืนแสงของกระดูกเทียมไบโอ-ออส

Fig. 3 Showed the absorption bands of Bio-Oss®.



รูปที่ 4 แสดงแถบการดูดกลืนแสงของกระดูกเทียมอาร์เอสยู-กราฟต์ที่ผ่านกระบวนการเผาที่อุณหภูมิ 900 องศาเซลเซียส

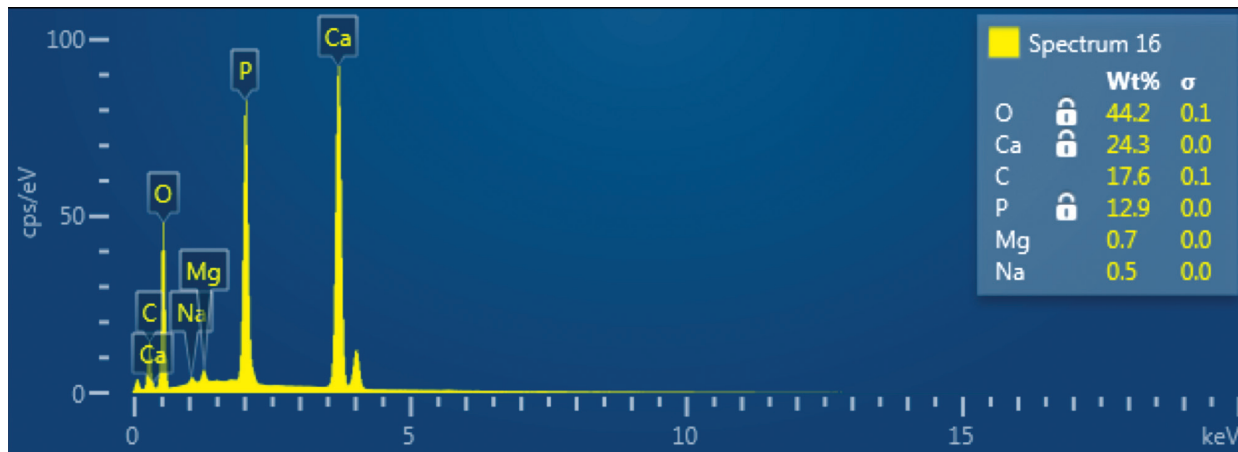
Fig. 4 Showed the absorption bands of RSU-Graft sintered at 900°C.

cording to card data JCPDS 9-432 (JCPDS-ICDD1, 1992) the major peaks were placed at $2\theta = 32^\circ, 33^\circ$ and 26° , with relative intensity of 100, 60 and 40%, respectively.⁽²⁾ From X-ray diffraction patterns of RSU-Graft sintered at 900°C , the major peaks at $2\theta = 32^\circ, 33^\circ$ and 26° had relative intensity of 100%, 78.2% and 19.9%, respectively. Therefore, X-ray diffraction of RSU-Graft sintered at 900°C at $2\theta = 32^\circ$ corresponded with card data JCPDS 9-432, but at $2\theta = 33^\circ$ and 26° were not consistent with card data JCPDS 9-432.

Fourier Transform Infrared (FTIR)

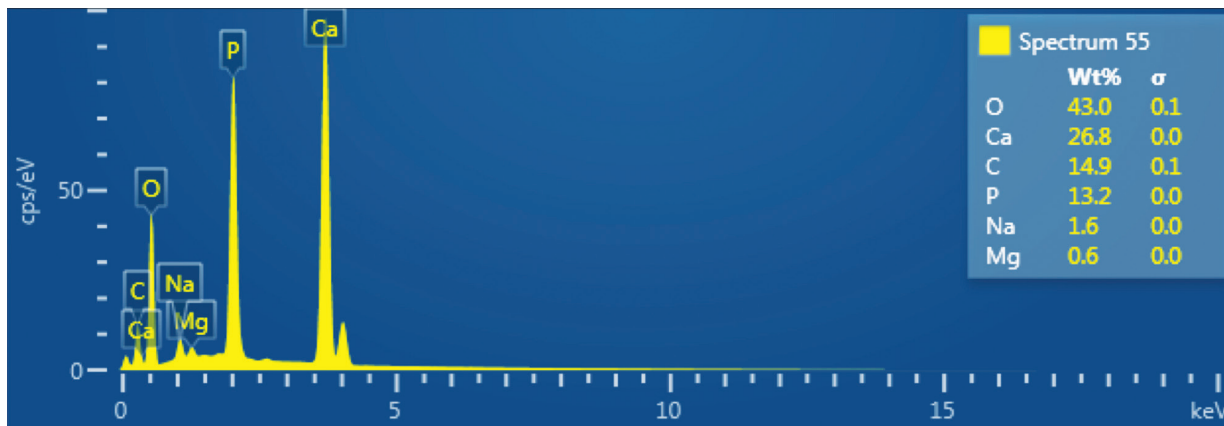
Fig. 3 showed the FT-IR spectra of Bio-Oss®. As indicated, bands could be identified in the wavelength

ranges of $1,416\text{-}1,456\text{ cm}^{-1}$, and 962 cm^{-1} corresponding to the functional group CO_3^{2-} of the HAp for Bio-Oss®. The orthophosphates PO_3^{4-} were observed at $1021\text{ cm}^{-1}, 563\text{ cm}^{-1}$ for Bio-Oss®. The wavelength ranges $961.5\text{-}961.9\text{ cm}^{-1}, 601.3\text{-}601.9\text{ cm}^{-1}, 570.9\text{-}571.7\text{ cm}^{-1}$ also matched bands in the hydroxyapatite reference spectrum.⁽⁹⁾ Furthermore, small amounts of adherent water might give rise to the stretching vibration of the hydroxyl groups (OH^-) that was observed at approximate $3,394\text{ cm}^{-1(9)}$ in Bio-Oss®. Fig. 4 showed the FT-IR spectra of RSU-Graft sintered at 900°C . As indicated, bands could be identified in the wavelength ranges of $1,412\text{-}1,456\text{ cm}^{-1}$, and 960 cm^{-1} corresponding to the functional group CO_3^{2-} of the HAp for RSU-Graft. The



รูปที่ 5 ผลการวิเคราะห์ที่อิตีเอ็กซ์ของไบโอ-ออส

Fig. 5 EDX analysis of Bio-Oss®



รูปที่ 6 ผลการวิเคราะห์ที่อิตีเอ็กซ์ของอาร์เอสยู-กราฟต์ที่ผ่าน

Fig. 6 EDX analysis of RSU-Graft sintered at 900°C.

orthophosphates PO_3^{4-} were also observed at $1,017\text{ cm}^{-1}$, 560 cm^{-1} for RSU-Graft. However, the stretching vibration of the hydroxyl groups (OH^-) was not observed in RSU-Graft indicating complete dehydration of this bovine bone. The wavelength ranges $1,090\text{ cm}^{-1}$, $961.5\text{--}961.9\text{ cm}^{-1}$ also matched bands in the hydroxyapatite reference spectrum.⁽⁹⁾

Energy dispersive X-ray spectroscopy (EDX)

The elemental composition of the bovine bone samples were investigated by EDX analysis. A typical EDX spectrum of the various elements presented in the bone matrix was shown in figure 5 and 6. In

ตารางที่ 2 แสดงสัดส่วนระหว่างแคลเซียมและฟอสฟอรัสของอาร์เอสยู-กราฟต์ และไบโอ-ออส

Table 2 Showed Ca/P ratio of RSU-Graft and Bio-Oss®

Type of bone	Ca/P ratio
RSU-Graft sintered at 900°C	2.04
Bio-Oss®	1.92

general, the analysis indicated that inorganic phases of both bovine bones composed mainly of Ca and P the major constituents with minor components of Na, Mg, O and C. Ca/P ratio of RSU-Graft was 2.04 and Bio-Oss® was 1.92 (Table 2), which were somewhat higher than stoichiometric value of 1.67.

Discussion

Xenografts, especially derived from bovine bone, are widely used as bone defect filling materials due to its osteoconductive property. Nowadays, researchers are still trying to develop bone substitutes that mimic the chemical composition, structural and functional properties of a human bone.

RSU-Graft is a bovine bone-derived xenograft sintered at 900°C, 0.25-1mm in size. The purpose of RSU-Graft is to develop bone substitute with the properties similar to human bones and Bio-Oss[®], anorganic bovine bone which has been successfully used for more than 10 years. Also, patients who had problem with severe bone loss can afford the treatment. In this research, RSU-Graft was tested for its physicochemical properties.

SEM images of RSU-Graft and Bio-Oss[®] had irregular particles but RSU-Graft had more surface roughness. In higher magnification, RSU-Graft had polygonal granules while Bio-Oss[®] had fiber-like structure. Morphology of RSU-Graft and Bio-Oss[®] were different because of different sinter temperatures. Pramanik et al, in 2013, reported a result that corresponded with our SEM results; the equiaxed and pure polycrystalline HAp with uniform porous microstructure could be produced from heat treated bovine bone at 900 °C while for bovine bone sintering at 350 °C, most of the collagen polymer molecules were melted and very few fibrils still remained in the material.⁽¹²⁾ Moreover Pripatnanont, in 2016, reported that xenografts which sintered below 500°C had collagen fibrils and prions that could cause disease transmission. Sintering the bovine bone above 500°C could completely remove organic part.⁽⁶⁾ Surface roughness of RSU-Graft possibly promoted tissues attachment and encouraged proliferation of bone cells. Although, RSU-Graft had no clearly visible pore size to measure at magnification 70x. SEM

image of RSU-Graft with magnification 10,000x had the character of microporous which tended to be interconnecting networks. The proper pore size that enhanced angiogenesis bone cells was 100 microns or larger.⁽⁶⁾ According to review of Yamada and Egusa in 2017, the appropriate particle size was 250 microns to 2 mm to maintain the volume of initial bone formation better than smaller particles. Particle sizes of both RSU-Graft and Bio-Oss[®] were within this range.⁽¹³⁾

The EDX analysis verified that the main component of both bone minerals were Ca and P which are main element of the human bone. The values of the Ca/P ratio of both bone minerals carried out were closely approached to the theoretical value of 1.67 for synthetic HAp structure.⁽⁷⁾ RSU-Graft exhibited Ca/P ratio at 2.04 while Bio-Oss[®] exhibited at 1.92. Ca/P ratio at 2 produces calcium tetraphosphate compounds.⁽¹⁴⁾ Several key factors identified by researchers could have contributed to the deviation of Ca/P ratio from the theoretical value of 1.67. One of the most significant factors was sintering temperature that had effect on type and amount of calcium phosphate compounds. This Ca/P ratio as well as the crystalline degree were associated with dissolution rate or bioabsorption rate of HAp materials. Another contributing factor was ions such as Ca²⁺, PO₄³⁻ and OH- which are known to exist in natural tissues to facilitate interchange of ions and their intensity may vary depend on the diet and nutrition of animal.⁽¹⁵⁾ In spite of deviation of Ca/P ratio from stoichiometric HA, calcium phosphate compound still exhibited the characteristic peaks in XRD trace that corresponds to stoichiometric HA.⁽¹⁶⁾ The RSU-Graft in the presented work strongly indicated key characteristics of HA phase from both FTIR and XRD results although exhibiting Ca/P ratios deviated from stoichiometric HA.

The XRD patterns of RSU-Graft sintered at 900°C,

standard HA and Bio-Oss[®] suggested that all three materials had similar crystallinity as seen from similar peak intensity. Pramanik et al, 2013 compared XRD patterns of heat-treated bone at 120, 350, 500, 750 and 900°C. The result revealed more crystalline pure HAp phase formations at 900°C as the peaks were more intense at 900°C⁽¹²⁾, which was similar to XRD pattern of RSU-Graft sintered at 900°C. If the bovine bone was sintered at temperature lower than 900°C, the peaks of XRD were less intense, indicating lesser crystallinity. Pripatnanont suggested that the bovine bone should not be sintered at temperature more than 1300°C to maintain the hydroxyapatite phase.⁽⁶⁾

The FTIR results, bands in both anorganic bones were similar with each other, and no band associated with other compounds excluding chemical groups corresponded to the HAp was observed.⁽⁷⁾ This finding indicated that all of organic compounds in RSU-Graft was completely removed through high-heated treatment process, which correspond to finding of Singh et al, in 2018⁽¹⁷⁾, while Bio-Oss[®] showed OH- group representing water that contained in it. The study of Berzina-Cimdina and Borodajenk, in 2012, proposed that OH- group would disappear with thermally treated CaP at temperature of 900°C.⁽¹⁸⁾ Moreover, the results showed typical absorption bands originated by specific mineral. Both anorganic bones presented intense

phosphate absorption and a low-intensity double band that corresponded to stretching vibrations of carbonate group, substituted for phosphate in the apatite lattice.

Conclusion

This study compared physicochemical characterization of bovine bone derived grafting materials; RSU-Graft sintered at 900°C and Bio-Oss[®]. The purpose is to develop properties of bone grafting materials for dental application. The properties included surface morphology, granule size, pore size distribution, crystallinity, element and functional groups. Differences found in surface morphology, pore size and some functional groups (-OH group) may result from different manufacturing process. The RSU-Graft was mainly composed of HA and some functional groups. Ca/P ratio of RSU-Graft was similar to Bio-Oss[®] and also with human bone. These results showed that RSU-Graft can be developed to use as bone substitute materials but still requires further experiments of other physicochemical properties such as biodegradation test, thermogravimetry for crystalline properties, Vickers microhardness test and Archimedes' principle for density analysis. These physicochemical properties should be tested before *in vitro* and *in vivo* studies.

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