# Comparative Study of PCL and PCL -TCP Scaffold as the Sinus Lift Grafting Material in Rabbit

*Thongchai Nuntanaranont * Srisurang Suttapreyasri * Jakchai Jantaramano **

***Abstract***

***Purpose:*** *To assess new bone formation using Polycaprolactone (PCL) and Polycaprolactone with Tricalcium Phosphate (PCL-TCP) scaffolds as the grafting material in rabbit sinus cavity.*
***Materials and Methods:*** *Nineteen New Zealand White rabbits male, weighing 3 - 3.5 kg were served as the experiment model. The mid-sagittal incision was performed to expose the nasal bone and nasoincisal suture line. A bony window was created into the sinus on both sides leaving the mucosa intact. The elevated sinus space was randomized augmented into 3 groups according to the type of grafting material. PCL, PCL-TCP and autogenous iliac bone were served as grafting material in Group A (n=12), Group B (n=12) and Group C (n=12) respectively. In group D (n=2), the sinus mucoa were raised without any augmentation material. The animals were sacrificed upon each interval at 2, 4 and 6 weeks. The section of bone including the grafting sinus cavities and surrounding bone was cut for gross morphological evaluation and radiographic study. The specimen was then fixed and process for H & E stain for histologic analysis.*
***Results:*** *New bone formation was found to gradual increase in both Group A and Group B without significant difference although the quantity had not achieved the similar level when compared with autogenous bone graft in Group C. Nevertheless the graft volume was better maintained by these two groups when compared to Group C which showed marked decrease in graft size. No newly formed bone was detected in Group D.*
***Conclusion:*** *PCL and PCL-TCP could be used as an alternative sinus lift grafting material. However the addition of osteoinduction agent should be considered to increase new bone formation to achieve the optimal level in clinical use.*

***Keywords:*** *Scaffold, Polycaprolactone, Tricalcium Phosphate, Sinus lift, Grafting*

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## Introduction

Bone grafting of the maxillary sinus in cases of trauma and osseous reconstruction after oncologic partial resection of the maxilla or after traumatic avulsion have been well established. Recently bone grafting in the sinus floor to increase the vertical height and volume of bone is the essential process to improve the bone quality and quantity for dental implant placement in the posterior maxillary region. This procedure particularly indicated in posterior edentulous maxilla patients who has the increased pneumatization of maxillary sinus resulted in closed approximation of maxillary sinus floor to the alveolar crestal bone or inadequate ridge width.[1] Sinus peumatization, which typically occurred with aging, often minimizes or completely eliminates the vertical bone available for implant placement. These factors will affect the stability and survival rate of the implants in the posterior maxillary region.

The use of bone grafting of maxillary sinus to increase osseous tissue was first proposed in the 1960s by Boyne (US Navy Dental School lectures to the postgraduates, 1965-1968). In that time it was used to increase the bulk of bone for subsequent maxillary posterior ridge reduction for optimal prosthodontic interarch distance. Then grafting of the antral floor for implant was originally developed and described by Tatum in the early of 1970s.[2-4] The modified Caldwell Luc procedure and infracturing of the sinus wall was used. An autogenous bone graft was then placed demonstrated bone formation in the maxillary antrum following placement of autogenous marrow and cancellous bone in the maxillary sinus.[5] The risk and morbidity of sinus lift grafting are low and bone response is excellent, and different graft materials produce bone that is demonstrable on histologic examination.[6] The graft and new bone appear to remodel in response to functional loading. The various prostheses in the area previously were occupied by the inferior third of the sinus. This technique provided adequate bone in the posterior maxilla for implant placement. In 1980, Boyne and James alternatives can be placed over implants within the sinus graft uneventfully.[1]

Many materials have been used for sinus lift procedures, including autogenous bone,[7-12] allograft,[13-19] and alloplasts such as tricalcium phosphate (TCP) hydroxyapatite,[20-22] bioactive glasses and polymer. Alloplastic material offers many potential advantages over the other bone grafting method. Since sources of autograft are limited and may leave local morbidity at the donor site and allografts may transmit unknown antigens, bacteria, or even viruses. Therefore the present study aims to assess two types of alloplastic scaffold materials, polycaprolactone (PCL) and polycaprolactone with tricalcium phosphate(PCL-TCP), in the ability of new bone formation enhancement when using as grafting material after sinus lift procedure comparing to the gold standard autogenous bone graft.

## Materials and Methods

Nineteen male New Zealand White rabbits, weighing 3 - 3.5 kg were served as the experiment model. Two types of scaffold, PCL and PCL-TCP produced by rapid prototyping technology fused deposition modeling process (FDM) size 3x4x6 mm were used as sinus lift grafting material. (Figure 1)

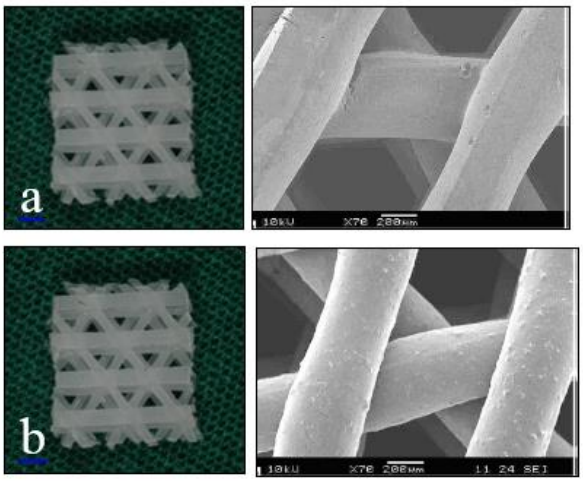


**Figure 1** Demonstrated the scaffold cut into **a** dimension of 3x4x6 mm block and SEM study. a: showed the uniform honeycomb filament structure of PCL. The similar structure was found in b: PCL-TCP combined with multiple small particles of TCP attached on PCL filaments.

PCL scaffold is fabricated from medical grade PCL using rapid prototyping technology fused deposition modeling process (FDM). The FDM method forms three-dimensional objects from computer generated solid or surface models like in a typical RP process. Models can also be derived from computer tomography scans, magnetic resonance imaging scans or model data created from 3D object digitizing systems. FDM uses a small temperature controlled extruder to force out a thermoplastic filament material and deposit the semi-molten polymer onto a platform in a layer by layer process.

The monofilament is moved by two rollers and acts as a piston to drive the semi-molten extrudate. At the end of each finished layer, the base platform is lowered and the next layer is deposited. The designed object is fabricated as a three dimensional part based solely on the precise deposition of thin layers of the landmark for specimen cutting. The wound was closed by layer with 4-0 resorbable extrudate. The deposition path and parameters for every layer are designated depending on the fabricated from medical grade PCL blended with 20% microparticles (100– 300 μm) of β-TCP ($Ca_3$ $[PO_4]_2$) using rapid prototyping technology fused deposition modeling process as previous mentioned to form composite scaffolds with both biodegradable and bioactive properties. Combining biodegradable polymers with an inorganic bioactive phase were of particular interests as tailored physical, biological and mechanical properties can be obtained. Both PCL and CPL-TCP manifested a lay-down pattern of 0/60/120˚ and porosity of 70%. Each scaffold is cut into a dimension of 3x4x6 mm.

The animal was sedated with Ketamine HCL 25 mg/kg and diazepam 5 mg/kg intramuscularly 15 minutes prior the operation. Then Thiopental 5mg/kg was given intravenously and titrated 2 mg/kg repeated every 15 minutes (maximum not exceed 30 mg/kg) until the rabbit is unconscious. Antibiotic premedication with Penicillin G sodium was injected before the surgical procedure. The mid-sagittal incision was performed to expose the nasal bone and nasoincisal suture line. A bony window 4x6 mm in dimension located approximately 20 mm anterior to the nasofrontal suture line and 10 mm lateral to the midline was created into the sinus on both sides leaving the mucosa intact. (Figure 2) The elevated sinus space was randomized augmented into 3 groups according to the type of grafting material. PCL, PCL-TCP and autogenous iliac bone were served as grafting material in Group A (n=12), Group B (n=12) and Group C (n=12) respectively. In group D (n=2), the sinus mucosa were raised without any augmentation material. The tiny bone holes were made adjacent to the created bony windows in the antero-posterior direction. These holes were filled up with gutta-percha as suture. Pethidine 10 mg/kg and Penicillin G sodium 100,000 unit/kg were given immediate postoperatively. The antibiotic was given daily for consecutive 5 days. Housing and feeding of the animal were performed according to standard animal care protocols. The animal experimental design was approved by the ethics committee of the Prince of Songkla University.

The animals were sacrificed upon each interval at 2, 4 and 6 weeks (Table 1). The skull of each rabbit was dissected and soft tissue removed carefully. The section of bone including the grafting sinus cavities and surrounding bone was cut in the coronal direction at the marks of gutta percha antero-posteriorly by the diamond saw. After gross morphological evaluation, the radiographic examination of the bone specimen was taken (10 mA, 50 KVP, 0.32 second 12FFD) with occlusal film. An aluminum step wedge was added in every film to calibrate the density of the radiographs. The custom made film holder was also used in order that same position and distance could be reproduced. All examination was taken by same radiographic machine (Gendex, Illinois, USA). Automatic film processor (Dent X 9000, DentX/Logetronics, GmbH, Kornberg, Germany) was used for all film processing procedure. The descriptive study of the radiographic examination was carried out by one investigator. Then the specimen was cut through the middle of the grafting region and fixed with 10% formalin for 2 weeks. After decalcified with 10% formic acid, the specimen was dehydrated in increasing concentration of alcohol until 100% concentration had been reached, finally embedded in paraffin. The section of 5 µm thickness was stained with hematoxylin and eosin for histologic analysis by conventional light microscope

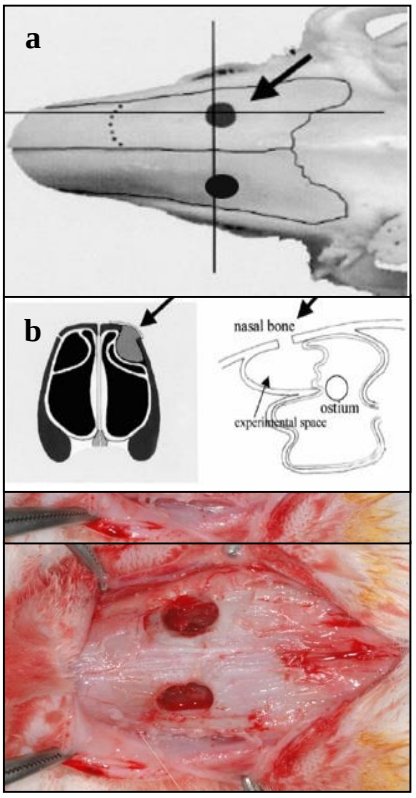


**Figure 2** a: Graphic and b: Surgical approach showing the creation of bony windows on both sides of the sinus via mid-sagittal incision. The elevated sinus mucosa spaces were grafted with various types of material depending on group.

## Results

The animals tolerated well with the surgical operation and the anesthesia. There was no any infection encountered in the surgical area and in the maxillary sinus. The animals were sacrificed upon each interval at 2, 4 and 6 weeks. After scarification the section of bone including the grafted sinus cavities and surrounding bone was cut in the coronal direction for gross morphological evaluation, radiographic and histological study. According to all of the examination, new bone formation was found gradual increase in both Group A: PCL and Group B: PCL-TCP. The maturation of new bone was increase according to time of of grafting. The remaining of PCL and PCL- TCP particles were observed in both groups.

**Table 1** Showed the number of sinus grafted with various types of materials in each time interval.

| Group | 2 Week | 4 Week | 6 Week | Total (n) |
|---|---|---|---|---|
| **A** | 4 | 4 | 4 | 12 |
| **B** | 4 | 4 | 4 | 12 |
| **C** | 4 | 4 | 4 | 12 |
| **D** | 0 | 0 | 2 | 2 |

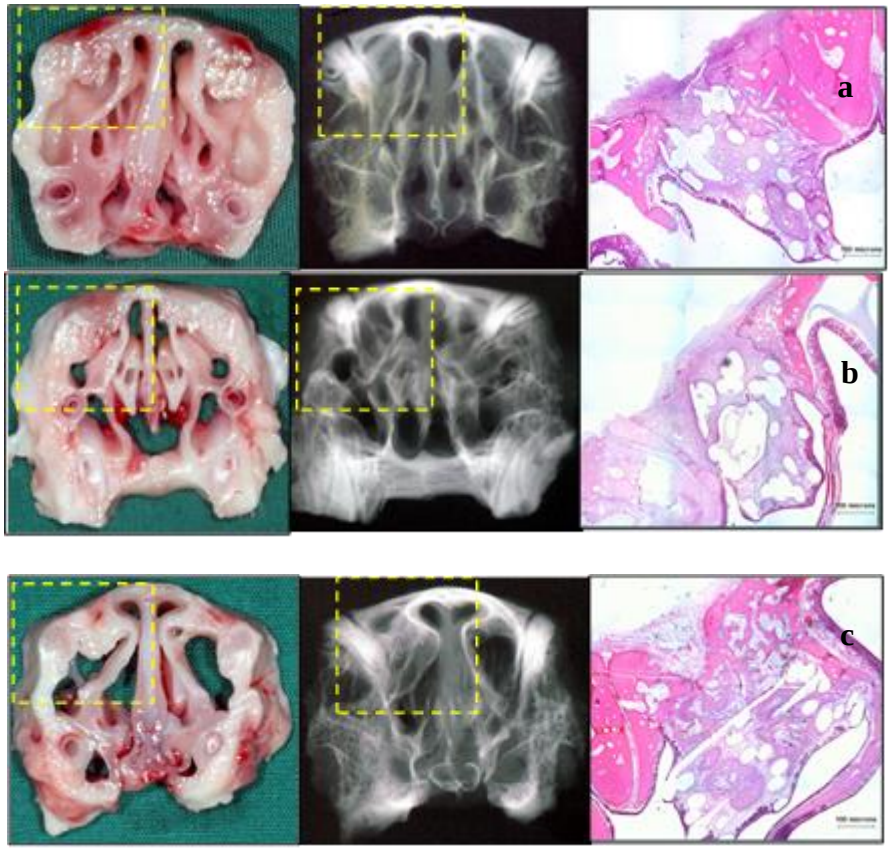


**Figure 3** Demonstrated the cross section gross morphological appearance, radiographic feature and histological finding (H&E stained) of grafting material in the sinus of group A: PCL for sinus augmentation material (rectangular dot line). a: 2 weeks group, b: 4 weeks group and c: 6 weeks group respectively. The newly formed bone was gradual increase according to the time of grafting. The remaining of PCL grafting material was noted in all interval

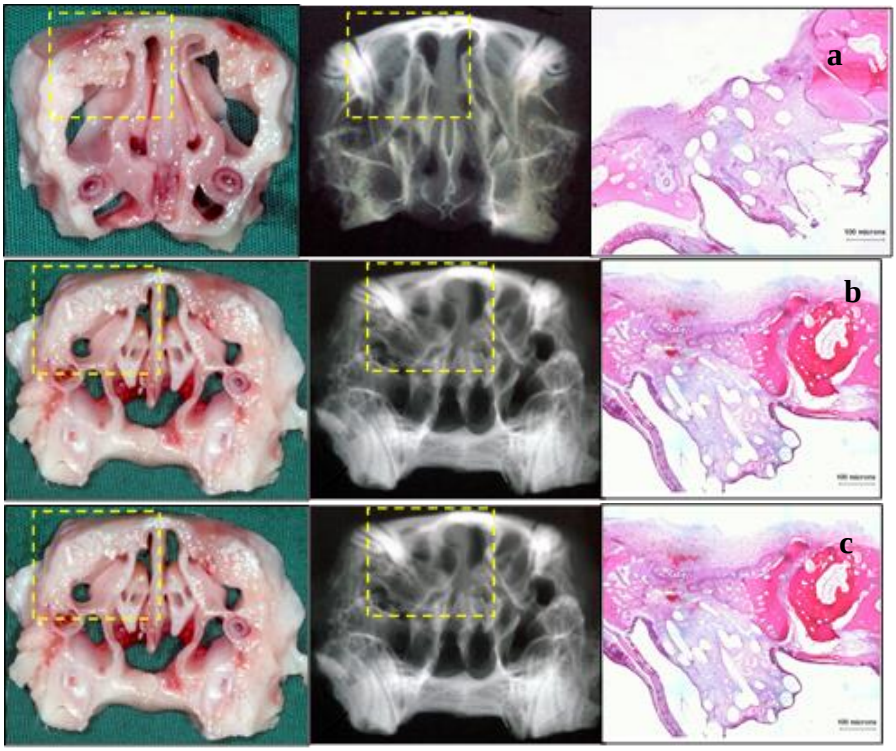


**Figure 4** Demonstrated the cross section gross morphological appearance, radiographic feature and histological finding (H&E stained) of grafting material in the sinus of group B: PCL-TCP as the sinus augmentation material (rectangular dot line) .a: 2 weeks group, b: 4 weeks group and c: 6 weeks group respectively. The newly formed bone was gradual increase according to the time of grafting. The remaining of PCL grafting material was noted in all intervals.

Minimum of inflammatory reaction cells were detected. (Figure 3, 4) The grafted sinus with autogenous iliac bone in Group C demonstrated rapid new bone formation and remodeling when compared to Group A and B. However marked grafted volume resorption was observed in late interval of the group. (Figure 5) The graft volume was better maintained by previous two alloplastic groups when compared to Group C, which showed marked decrease in graft size. In addition no newly formed bone was detected in Group D.

## Discussion

The use of bone or grafting material of maxillary sinus to increase osseous tissue is an essential part for further prosthodontic or dental implant purpose especially in the severe atrophic posterior maxillary region. Several materials have been used as sinus grafting material. To date, there is no official consensus as to which graft material or combination of materials is best for augmenting the sinus antral space created by the sinus lift operation.[1,23,24] Autogenous bone has long been considered the gold standard among grafting materials because

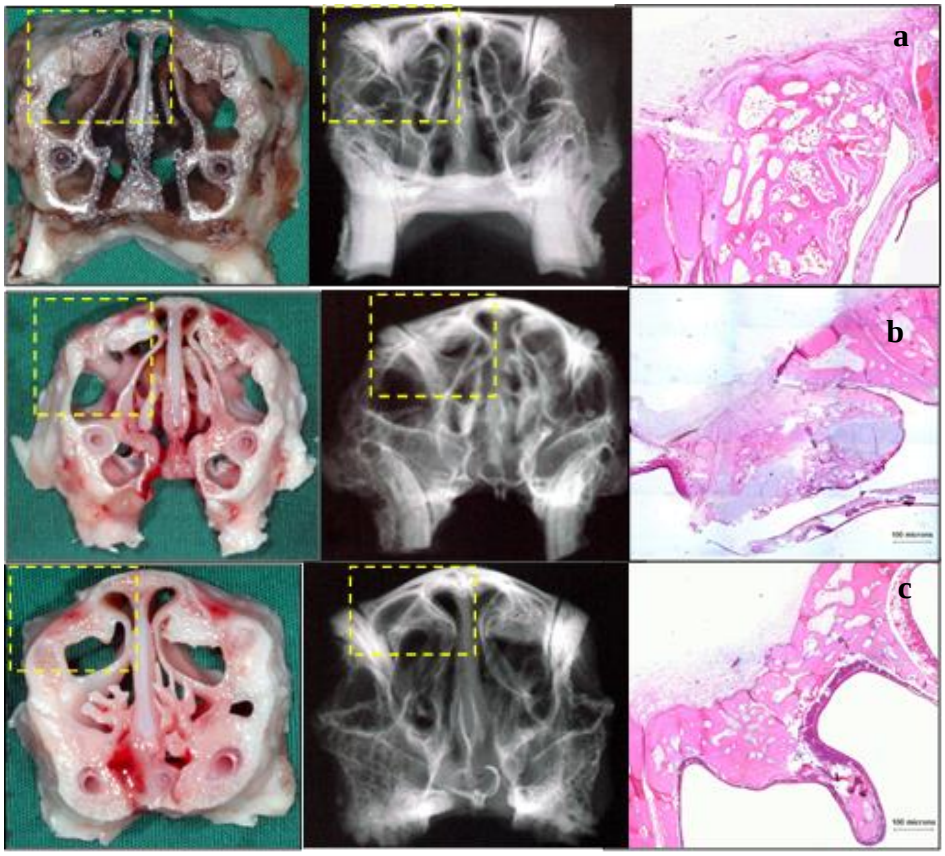


**Figure 5** Demonstrated the cross section gross morphological appearance, radiographic feature and histological finding (H & E stained) of grafting material in the sinus of group C: autogenous iliac bone as the sinus augmentation material (rectangular dot line). a: 2 weeks group, b: 4 weeks group and c: 6 weeks group respectively. The newly formed bone was rapidly increase and remodel to form the normal lamellar bone according to the time of grafting. Nevertheless the reduced initial graft size was demonstrated in the last experimental group.

of its highly osteogenic, osteoinductive, and osteoconductive properties, a combination not found in the alternatives.[25] These properties allow bone to form more rapidly as seen in Group C. However bone graft resorption were reported particularly from the iliac crest bone[9,26] and also demonstrated in the present study in 6 weeks interval of the autogenous iliac bone grafting group. The alloplastic material, PCL, used in the present study is a synthetic polymer. The advantages of polymer are biocompatibility, biodegradable and can be constructed into 3-dimensional scaffolds. This properties provides the different features(forms, porosities and pore size, rate of degradation, mechanical properties) to match tissue specific application.[27] There are three types of polymer-base materials, which are natural polymers, synthetic polymers and composites. Many natural polymers found in living organisms of known biocompatibility. Such polymers can be used to replace of regenerate native tissue structures and allows positive cell interactions with surrounding tissues. Conversely, synthetic polymers are formed through controllable chemical processes to achieve desirable material and chemical properties for a wide range of biomedical applications. The synthetic polymers as used in the present study have a promising advantage over the natural polymers for scaffold developments because their mechanical and proliferation properties are comparatively more predictive and reproducible. The PCL-TCP in the present study is the composite polymer-base scaffold

by mixing Tricalcium Phosphate particle on Polycaprolactone filament. The composite scaffolds are designed to achieve desirable properties and characteristics by taking advantages from each of the materials.[28] Beta-Tricalcium Phosphate is similar to hydroxyapatite, but is not a natural component of bone material. In the body, TCP is converted in part to crystalline hydroxyapatite. The rate of TCP resorption varies and appears to depend greatly on the material's chemical structure, porosity and particle size. TCP has osteoconductive and it is intended to provide a physical matrix that suitable for deposition of new bone. The material is resorbed completely and is generally replaced by natural bone in 3-24 month period, depending on the type of bone. TCP as grafting materials in sinus lift procedure was found in difference results. Suba et al reported that new bone density was not significantly different on the TCP and autogenous bone graft from iliac crest sides.[29] But the graft biodegradation was significant slower on the experimental side than the control side. However, in 2004 Zerbo et al. showed the lower rate of bone formation in beta-TCP than the autogenous bone graft.[30] In the present study new bone formation were demonstrated in both Group A: PCL and Group B: PCL-TCP, although the amount and rate of maturation are less than the autogenous bone grafting group. PCL and PCL- TCP scaffold in the present study are produced by advanced fused deposition modeling techniques.[31] These bioresorbable scaffolds possess a completely interconnected architecture with a regular porous morphology that is favorable for cellular conduction [32] as well as protein loading and releasing.[33,34] PCL scaffolds have been shown to support ectopic bone formation in rat model[35], repair critical-sized calvarial defects in a rabbit model[36], orbital defects in pig model[35], and bur hole defects in patients with chronic subduaral hematoma.[37] While the combination of PCL and TCP was reported to enhance new bone formation by supported vascularized connective tissue and bone infiltration in femur of rat model. The deposition of acellular osteoid and mineralization of newly developing bone and direct contact between the scaffold and the newly formed tissue was observed.[38] This implies good integration and nonrestricted infiltration of scaffold with surround bone. The present study showed new bone formation in air sinus cavity after using PCL and PCL-TCP as the sinus grafting material. The amount and maturation rate of the newly formed bone in these two groups are not noticeable different and seem less than in autogenous group C. This can be concluded that PCL or PCL- TCP have the promising ability to enhance new bone formation in air sinus cavity and will be an alternative choice especially when combine with the appropriated bioreactor material to increase rate and amount of new bone formation. Quantitative and long term study should be considered to detect the effect of mixing of TCP on PCL filament as the composite polymer-base scaffold. PCL-TCP scaffold were degraded slowly by a hydrolytic mechanism when immersed in simulated body fluids. Upon degradation, PCL-TCP scaffolds exhibited a bioactive nature where the formation of a calcium-rich surface layer was nucleated, beginning with an initial phase of tricalcium phosphate that served as a template for hydroxyapatite growth.[39] In vivo environment, PCL-TCP scaffolds degraded at a more rapid rate. At 24 weeks, PCL-TCP scaffolds demonstrated a significant extent of degradation, while maintaining mechanical properties that closely matched those of the cancellous bone.[40] In the present study remaining particles of PCL and PCL-TCP were still observed in the last group and needed more period of time for degradation. The minimum grafted sized reduction was noticed, since PCL scaffold is able to fabricated with the high porosity, large inner surface and rigid enough to withstand the pressure in the sinus cavity when using as the sinus augmentation materials. Minimum inflammatory cell reaction to this alloplastic materials confirmed that PCL scaffold material possess the excellent biocompatibility. Nevertheless long term study for the final situation of these alloplastic graft fate, including combining of osteoinductive

material such as BMP should be considered in the further studies.

**Conclusion**

Both PCL and PCL-TCP scaffolds demonstrated the capability in new bone formation enhancement in the rabbit sinus. It implies that these materials could be used as an alternative sinus lift grafting material. Nevertheless the provided amount of newly formed bone was still less than gold standard autogenous bone grafting. The addition of osteoinductive agents should be considered to increase new bone formation to achieve the optimal level in clinical use**.**

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# การศึกษาเปรียบเทียบประสิทธิภาพการใช้โครงร่างทดแทนกระดูก PCL และ PCL – TCP ในการปลูกกระดูกโพรงอากาศแม็กซิลลาในกระต่าย

*ธงชัย นันทนรานนท์* ศรีสุรางค์ สุทธปรียาศรี * จักรชัย จันทรมโน **

***บทคัดย่อ***

***วัตถุประสงค์:*** *เพื่อประเมินการสร้างกระดูกใหม่ด้วยการใช้โครงร่างทดแทนกระดูกชนิดพอลิคาร์โปแลคโตน(PCL) และพอลิคาร์โปแลคโตนผสมไตรแคลเซียมฟอตเฟส(PCL-TCP)ในโพรงอากาศแม็กซิลลาในกระต่าย*

***วัสดุอุปกรณ์และวิธีการ:*** *การศึกษาครั้งนี้ใช้กระต่ายพันธุ์นิวซีแลนด์เพศผู้น้ำหนักระหว่า 3 -3.5 กิโลกรัมจำนวน 19 ตัว ทำการผ่าตัดเพื่อเปิดกระดูกเนซัลโดยทำการลงรอยผ่าในแนวระนาบข้างหลังจากนั้นทำการเจาะกระดูกเพื่อสร้างเป็นช่องหน้าต่างโดยไม่ทำลายเนื้อเยื่อบุโพรงอากาศ หลังจากนั้นทำการยกพื้นโพรงอากาศแม็กซิลลาและทำการปลูกกระดูกโดยการใช้วัสดุ 3 กลุ่มได้แก่ กลุ่ม A ใช้ PCL, กลุ่ม B ใช้ PCL-TCP และกลุ่ม C ใช้กระดูกของตัวกระต่ายที่ทำการเก็บจากกระดูกสะโพก และมีกลุ่มทดลองกลุ่ม D ซึ่งเป็นกลุ่มที่ไม่มีการใช้วัสดุปลูกกระดูก โดยทั้ง 4 กลุ่มจะใช้กลุ่มตัวอย่างกลุ่มละ 12 ตัว หลังจากนั้นทำการเก็บตัวอย่างจากกระต่ายในช่วง 2, 4, และ 6 สัปดาห์ ชิ้นตัวอย่างที่ได้จะถูกนำไปศึกษาเนื้อเยื่อด้วยการย้อมสีแบบธรรมดา และทำการประเมินด้วยภาพรังสี*

***ผลการทดลอง:*** *พบว่ามีการสร้างกระดูกใหม่อย่างค่อยเป็นค่อยไปในกลุ่ม A และ B แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญกับกลุ่ม C แต่อย่างไรก็ตามพบว่าการรักษาปริมาณกระดูกที่สร้างใหม่ในกลุ่ม A และ B ดีกว่ากลุ่ม C โดยพบว่ากลุ่ม C มีการละลายตัวของกระดูกที่สร้างขึ้น และไม่พบการสร้างกระดูกในกลุ่ม D*

***สรุปผลการทดลอง:*** *การใช้โครงร่างทดแทนกระดูก PCL and PCL-TCPสามารถนำมาเป็นวัสดุทดแทนกระดูกสำหรับการปลูกกระดูกในโพรงอากาศแม็กซิลลาในกระต่ายได้ แต่อย่างไรก็ตามการนำมาใช้ในมนุษย์ต้องอาศัยการศึกษาเพิ่มเติมต่อไป*

***คำสำคัญ*** *โครงร่างทดแทนกระดูก พอลิคาร์โปแลคโตน ไตรแคลเซียมฟอตเฟส โพรงอากาศแม็กซิลลา*

**ภาควิชาศัลยศาสตร์ช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์*