PRE-CLINICAL *IN VIVO* EVALUATION OF MALAYSIAN-MADE "OSTEOPASTE" SELF HARDENED SYNTHETIC BONE CEMENT IN CRITICAL SIZE BONE DEFECTS

BY

CHE NOR ZARIDA BINTI CHE SEMAN

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ABSTRACT

Calcium phosphate is an ideal bone substitute material that is widely used for bone repair due to its excellent biological properties including biocompatibility and osteoconductivity. In order to improve the properties of calcium phosphate materials for clinical use, a new injectable self-hardened synthetic bone cement (Osteopaste) was developed. Osteopaste consists of tetra-calcium phosphate (TTCP) and tri-calcium phosphate (TCP) powder. It was intended for the treatment of bone fracture or reconstruction of bone defects. The objective of this study was to compare bone formation between Osteopaste and commercialized synthetic bone grafts; JectOS (calcium phosphate) and MIIG–X3 (calcium sulphate) at three different assessment periods. The first phase of the study was to establish the critical size defect in New Zealand White rabbit model. The second phase involved the implantation of Osteopaste, JectOs and MIIG-X3 in critical size defects. Thirty-nine New Zealand White rabbits were divided into four groups (Osteopaste, JectOs, MIIG-X3 and sham). Each group was further divided into three subgroups according to the assessment period either at 6, 12 or 24 weeks. Each subgroup consisted of four rabbits except the sham group which consisted of only one rabbit. A critical size defect of approximately 4.5 mm (width) X 9.0 mm (length) was created at the proximal tibial metaphysis of rabbit's right leg and then implanted with either Osteopaste, JectOs or MIIG-X3. At each assessment period, plain radiograph and computed tomography (CT) scan were performed before the animals were sacrificed for undecalcified histology, histomorphometry and scanning electron microscopy assessments. Using the histomorphometric data, the mean percentage of new bone areas and the length of unbridged defects were compared between groups. In this study, a simple and safe method for performing critical size defect at proximal tibial metaphysis was established. The Osteopaste group exhibited radiographic density in between JectOS and MIIG-X3. The critical size defect in Osteopaste group was bridged by new bone at 12 weeks. In MIIG-X3 group, the defect was bridged at 24 weeks whereas in JectOS group, the defect was not bridged at all assessment periods. New bone area was the largest in MIIG-X3 group followed by Osteopaste and JectOS groups. Osteopaste had formed direct bonding with host bone without intervening soft tissue compared to JectOS and MIIG-X3. There were significant differences in new bone area percentages between Osteopaste, JectOs and MIIG-X3 at 6, 12 and 24 weeks post-surgery ($P<0.0001$). In conclusion, the performance of Osteopaste to promote new bone formation is in between JectOS and MIIG-X3.

خلاصة البحث

فوسفات الكالسيوم هو مركب مثالي للاستعمال كبديل للعظام وقد تم ضمنه على نطاق واسع لإصلاح العظام نظرا لخصائصه البيولوجية الممتازة بما في ذلك التوافق الحيوي والربط العظمي. من أجل تحسين خصائص المواد المصنعة من فوسفات الكالسيوم للاستخدام السريري، تم تطوير أسمنت عظمي اصطناعي جديد متصلب ذاتيا وقابل للحقن وتم تسميته أوستيوبيست (Osteopaste). يتكون Osteopaste من فوسفات رباعي الكالسيوم ومسحوق ثلاثي فوسفات الكالسيوم، وكان مخصصا لعلاج كسور العظام أو إعادة بناء عيوب العظام. كان الهدف من هذه الدراسة هو مقارنة تكوين العظام بين تلك المستعملة لـ Osteopaste وبين الطعوم العظمية التجارية أخرى وهي: JectOS (فوسفات الكالسيوم) و MIIG-X3 (كبريتات الكالسيوم) على ثلاث فترات تقييم مختلفة. تم في المرحلة الأولى من الدراسة تحديد عيب الحجم الحرج في نموذج الأرنب الأبيض النيوزيلندي، وتضمنت المرحلة الثانية زراعة كل من Osteopaste و JectOs و MIIG-X3 في عيوب الحجم الحرجة. تم تقسيم تسعة وثلاثين من الأرانب البيضاء النيوزيلندية إلى أربع مجموعات (Osteopaste، JectOs، MIIG-X3، والمجموعة الضابطة). تم تقسيم كل مجموعة إلى ثلاث مجموعات فرعية وفقًا لفترة التقييم وهي 6 أو 12 أو 24 أسبوعًا. تكونت كل مجموعة فرعية من أربعة أرانب باستثناء المجموعة الضابطة والتي تكونت من أرنب واحد فقط. تم إنشاء عيب الحجم الحرج بمساحة حوالي 4.5 مم (عرض) × 9.0 مم (طول) عند الكردوس الظنبوبي القريب للساق اليمنى للأرانب ثم تم زرعها إما باستخدام Osteopaste أو JectOs أو MIIG-X3. تم في كل فترة تقييم إجراء تصوير شعاعي عادي وتصوير مقطعي محوسب (CT) قبل التضحية بالأرانب من أجل تقييم الأنسجة والقياس النسجي والمسح المجهري الإلكتروني. باستخدام البيانات النسيجية تمت مقارنة متوسط النسبة المئوية للمناطق العظمية الجديدة وأطوال العيوب غير المرتبطة بين المجموعات. تم في هذه الدراسة إنشاء طريقة بسيطة وآمنة لإجراء عيوب الحجم الحرج في الكردوس الظنبوبي القريب. أظهرت مجموعة Osteopaste كثافة إشعاعية كميتها تقدر بين تلك التي في JectOS و MIIG-X3. تم سد عيوب الحجم الحرج في مجموعة Osteopaste بعظام جديدة في 12 أسبوعًا. في مجموعة MIIG-X3، تم سد العيوب في 24 أسبوعًا بينما في مجموعة JectOS لم يتم سد العيوب في جميع فترات التقييم. كانت منطقة العظام الجديدة هي الأكبر في مجموعة MIIG-X3 تليها مجموعة Osteopaste و JectOS. شكل Osteopaste رابطة مباشرة مع العظم المضيف دون تدخل الأنسجة الرخوة مقارنة بـ JectOS و MIIG-X3. كانت هناك اختلافات كبيرة في نسب مساحات العظام الجديدة بين Osteopaste و JectOs و MIIG-X3 في الأسابيع 6 و 12 و 24 ما بعد الجراحة (P<0.0001). في الختام فقد اتضح أن أداء Osteopaste لتعزيز تكوين العظام الجديدة كان بين JectOS و MIIG-X3.

APPROVAL PAGE

The thesis of Che Nor Zarida binti Che Seman has been approved by the following:

Zunairah binti Buyong
Supervisor

Zamzuri bin Zakaria @ Mohamad
Co-Supervisor

Ahmad Razali bin Md Ralib @ Md Raghib
Co-Supervisor

Aminudin bin Che Ahmad
Internal Examiner

Mohamad bin Abd Razak
External Examiner

Fauziah binti Othman
External Examiner

Deny Susanti binti Darnis
Chairman

DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Che Nor Zarida binti Che Seman

Signature..... Date 29 JULY 2021

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Date

This thesis is dedicated to my loving family;

To my respectful and loving parents; Maimunah Abd Rahman, Allahyarham Che Seman Che Jusoh and Mamat bin Ismail (my step father), thank you very much for allowing me to pursue my dreams and supporting me all the way. Without your blessing I will never be where I am today. Thank you for believing in me.

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LIST OF SYMBOLS

%	Percentage
<	Less than
=	Equal to
±	Standard Deviation
°C	Degree Celsius
µm	Micrometre
cc	Cubic Centimeter
cm	Centimeter
g	Gram
HU	Hounsfield
kg	Kilogram
kV	Kilovolt
M	Molar
mA	Milliampere
minute	Min
mg	Miligram
mL	Mililiter
mm	Millimetre
mmHg	Millimetre of Mercury

MPa	Megapascal Pressure Unit
ms	Milisecond
nm	Nanometer
Wt/vol	Weight/volume

LIST OF ABBREVIATIONS

3D	3 Dimension
ABM	Anorganic bovine-derived hydroxyapatite
ACB	Autologous iliac crest bone
AP	Anteroposterior
AMREC	Advance Research Material Centre
ASTM	American Society for Testing and Materials
ANOVA	Analysis of Variance
BMD	Bone mineral density
BP	Bovine pericardium
BMP	Bone morphogenic protein
BMSC	Bone marrow stromal cell
CDHA	Calcium-deficient hydroxyapatite
CHAP	Carbonated hydroxyapatite
CSH	Calcium sulfate hemihydrate
CNS	Central nervous system
CPC	Calcium phosphate cement
CSC	Calcium sulphate cement
CSH/nHAC	Calcium sulfate hemihydrate collagen and nano-hydroxyapatite
CT	Computed tomography
DBM	Demineralized bone matrices
DCPD	Dicalcium phosphate dehydrated

e-PTFE	Polytetrafluoroethylene
ECG	Electrocardiogram
EGF	Endothelial growth factor
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FTIR	Fourier Transform Infrared
Gelatin	Gel
HA	Hydroxyapatite
HFL	Human fascia lata membrane
HFT	Human fascia temporalis
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
HP	Human pericardium
IACUC	Institutional Animal Care and Use Committee
IIUM	International Islamic University Malaysia
IGF	Insulin-like growth factor
IM	Intramuscular
IN	Intranasal
ISO/IEC	International Organization for Standardization / International Electrotechnical Commission
IV	Intravenous
KTX	Ketamine, Xylazine and Tilatamine/Zolazepam mixture
L	Lateral
L-PRF	Leukocyte platelet-rich fibrin
MHC	Major histocompatibility complex

Micro-CT	Micro-computed tomography
MIDA	Malaysian Industrial Development Authority
MOSTI	Ministry of Science, Technology and Innovation
MIIX-X3	Minimally Invasive Injectable Graft
ML	Mediolateral
MSC	Mesenchymal stem cell
nano-HA	Nanoscale hydroxyapatite
NBF	Neutral buffered formalin
NDA	New Development Application
nHA/PA	Nano-hydroxyapatite/polyamide composite
NPRA	National Pharmaceutical Regulatory Agency
NTPT	Non-treated porous titanium
OCP	Octacalcium phosphate
OPG	Osteoprotegerin
P-15	Binding peptid
PBCP	Porous biphasic calcium phosphate
PBS	Phosphate-buffered saline
PCL	Poly-ε-caprolactone
PLGA	Poly(lactic-co-glycolic acid)
PDGF	Platelet derived growth factor
PMMA	Polymethylmethacrylate
PO	Oral
PTFE	Polytetrafluoroethylene
PTG	Porous titanium granules
PTMC	Poly(trimethylene carbonate)

PRF	Platelet-rich fibrin
PRP	Platelet-rich plasma
RANKL	Receptors for activation of nuclear factor kappa B
rBMMSC	Rabbit bone marrow mesenchymal stem cell
rhbmp-2	Recombinant human bone morphogenetic protein-2
SC	Subcutaneous
SD	Standard deviation
SF	Silk fibroin
SEM	Scanning electron microscopy
Si-αTCP	Si-αtricalcium phosphate
SrCPC	Strontium modified calcium phosphate cement
STPT	Microstructured porous titanium
TCP	Tricalcium phosphate
TGF	Transforming growth factor
TTCP	Tetracalcium phosphate
VEGF	Vascular endothelial growth factor
X-ray	Plain radiograph
XRD	X-ray Diffraction

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Bone healing continues to pose challenges for researchers and clinicians working in the field of orthopaedics. Autogenous and allogenous bone graft is widely used as bone graft material in orthopaedics but each has serious limitations by material availability and by the possible risks of infection or donor site morbidity. As an alternative, calcium phosphate cements is used as a bone graft material because their calcium/phosphorus ratios are close to that of natural bone and they are relatively stable in physiological environment (Al-Sanabani, Madfa & Al-Sanabani, 2013). In fact, the mineral component of calcium phosphate containing calcium ions (Ca^{2+}), orthophosphates (PO_4^{3}), metaphosphates or pyrophosphates ($P_2O_7^{4}$) and occasionally hydrogen or hydroxide ions is similar to the mineral component of bone (Dorozhkin, 2016).

Calcium phosphate cements have been evaluated as one of the potential materials for bone tissue engineering (Poulus et al., 2011; Silva et al., 2011; Sun & Yang, 2015). An advantage of calcium phosphate cement is that they can be directly injected into the bone defect and allowed to set *in situ* (Al-Sanabani, Madfa & Al-Sanabani, 2013). Calcium phosphate is widely used in numerous dental and craniofacial procedures, including the reconstruction of frontal sinus, augmentation of craniofacial skeletal defects, endodontics and the repair of periodontal bone defects and tooth defects due to its osteoconductivity and bone replacement capability (Xu et al., 2017; Xu et al., 2002).