FUNCTIONAL AND BIOCOMPATIBILITY OF ORTHOPAEDIC METAL IMPLANT COATED WITH SILVER

BY

NURUL HAFIZA BINTI MOHD JAN

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Kulliyyah of Allied Health Sciences
International Islamic University Malaysia

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ABSTRACT

The prevalence of orthopaedic implant-related infections remains high. Bacterial colonisation and biofilm adhesion on the implant can cause infection at the underlying bone and surrounding tissues. The management of this incidence poses major challenges in orthopaedic. Although several conventional strategies were taken to control the incidence, none of them is effective in all cases. The application of orthopaedic metal implant coated with silver composite (OMICS) has been suggested as an alternative to reduce or prevent implant-related infections. This study aimed to (1) determine the antibacterial properties of OMICS against Staphylococcus aureus (S. aureus); (2) evaluate the toxicology effects of OMICS on in vitro cellular and in vivo animal models; (3) perform and validate surgical approach using an open fracture model that expose bone to the environment for six hours to induce osteomyelitis in the New Zealand White (NZW) rabbits; and (4) evaluate the efficacy of OMICS as antibacterial agent and its biocompatibility in animal models. The silver composite was extracted from OMICS using two different immersion techniques, namely water bath and magnetic stirrer. The potential of OMICS as an antibacterial agent against S. aureus was investigated. The toxicity and biocompatibility studies were conducted in an accredited testing laboratory operating in accordance with the ISO 10993 to validate the biosafety aspect and ISO 17025 to validate the biocompatibility of OMICS. The OMICS were implanted in NZW rabbits after six hours of tibial bone exposure following validation of the open wound surgical approach. The rabbits were euthanised at week three and week six post-operatively. The OMICS-implanted tibia was excised en bloc and evaluated through post-mortem of microbial assessment for signs of infection as well as the post-mortem of radiographic evaluations, gross inspection and histological analysis for quality implantation assessment. The results showed that the variance for OMICS silver release extraction was significant with value $F(1,10) = 4.996$, $p = 0.034$, $\eta^2 p = 0.285$. As for the analysis variance of anti-microbial, it showed that the effect of silver release was significant with value $F(1,10) = 11.071$, $p = 0.003$, $\eta^2 p = 0.356$. The OMICS group halted the S. aureus growth “significantly” better than the control group indicative of antibacterial properties of the OMICS against the bacteria. The OMICS does not produce any mutagenic and toxicity effects after exposure in both cellular and tissue level. Besides, OMICS did not induce skin sensitisation after exposed to animal models. The post-mortem of the microbial assessment showed no signs of infection isolated at week six. The post-mortem of radiographic evaluations, gross inspection and histological analysis showed there is good integration between bone and OMICS implant at surrounding tissue. The OMICS is thus shown to be effective to reduce infection during implantation. In conclusion, the above results showed that OMICS is biocompatible and holds potential to reduce infection during implantation.

خلاصة البحث

لا يزال معدل انتشار العدوى المرتبطة بالتطعيم العظمي مرتفعًا، حيث يمكن أن يسبب الاستكثار البكتيري والتصاق الأغشية الحيوية على مادة التطعيم التهابًا في العظام والأنسجة المحيطة. يشكل التعامل مع هذه الحالات تحديات كبيرة في طب العظام، وعلى الرغم من اتخاذ العديد من الاستراتيجيات التقليدية للسيطرة عليها، لم تكن أيا منها فعالة في جميع الحالات. تم اقتراح تطبيق زرع العظام المعدنية المغلفة بمركب الفضة (OMICS) كبديل للحد من أو منع الالتهابات المرتبطة بالتطعيم. هدفت هذه الدراسة إلى (1) تحديد خواص الـ OMICS المضادة لبكتيريا المكورات العنقودية الذهبية؛ (2) تقييم الآثار السمية لـ OMICS في النماذج المختبرية الخلوية والحيوانية؛ (3) إجراء الطريقة الجراحية والتحقق من صحتها باستخدام نموذج الكسر المفتوح الذي يتم فيه تعريض العظم للبيئة المفتوحة لمدة ست ساعات لحث التهاب العظم في الأرانب النيوزيلندية البيضاء (NZW)؛ و (4) تقييم فعالية الـ OMICS كعامل مضاد للميكروبات وتقييم التوافق الحيوي في النماذج الحيوانية. تم استخراج المركب الفضي من الـ OMICS باستخدام تقنيتي غمر مختلفتين، وهما حمام الماء والهزاز المغناطيسي. تم التحقيق في إمكانية الـ OMICS كعامل مضاد للميكروبات ضد المكورات العنقودية الذهبية. أجريت دراسات السمية والتوافق الحيوي في مختبر معتمد يعمل وفقًا لمعيار ISO 10993 للتحقق من السلامة الأحيائية ومعيار ISO 17025 للتحقق من التوافق الحيوي لـ OMICS. تم زرع الـ OMICS في الأرانب النيوزيلندية البيضاء بعد ست ساعات من تعريض عظام الظنبوب بعد التحقق من صحة الطريقة الجراحية للجرح المفتوح. تمت تضحية الأرانب بالقتل الرحيم في الأسبوع الثالث والأسبوع السادس بعد الجراحة. تم استئصال الظنبوب المحتوي على الـ OMICS بالكامل وتقييمه من خلال تحليل الجثة الميكروبي لعلامات العدوى وكذلك تحليل الجثة الشعاعي، والتقييم الإجمالي، والتحليل النسيجي لتقييم جودة التطعيم. تم استكشاف الجانب الأخلاقي باستخدام دراسة مكتبية مصممة ذاتيا. أظهرت النتائج أن التباين في استخلاص الفضة من الـ OMICS كان كبيرا بقيمة F قدرها (1،10)=4.996، p=0.034، η^2 p=0.285. أما بالنسبة إلى تحليل تباين الخواص المضادة للميكروبات فقد كان تأثير إطلاق الفضة ذا أهمية بقيمة قدرها F قدرها (1،10)=11.071، p=0.003، η^2 p=0.3356. ثبطت مجموعة الـ OMICS نمو المكورات العنقودية الذهبية "بشكل ملحوظ" وبنحو أفضل من المجموعة الضابطة مشيرة إلى خصائص الـ OMICS المضادة للميكروبات. لم تنتج الـ OMICS أي تأثيرات مطفرة وسمية بعد التعرض لها على كل من المستوى الخلوي والنسيجي. لم تحفز الـ OMICS أيضا حساسية في الجلد بعد تعريضها للنماذج الحيوانية. لم يظهر تحليل الجثة الميكروبي أي علامات للعدوى عندما عزلت في الأسبوع السادس. وأظهرت عمليات التشريح اللاحقة للتقييمات الإشعاعية، والتقييم الإجمالي، والتحليل النسيجي وجود توافق جيد بين العظم والـ OMICS في الأنسجة المحيطة. وبالتالي فقد كان الـ OMICS فعالا في تقليل العدوى أثناء التطعيم. ختاما أظهرت النتائج المذكورة أعلاه أن الـ OMICS متوافق حيوياً ولديه القدرة على تقليل العدوى أثناء التطعيم.

APPROVAL PAGE

The thesis of Nurul Hafiza Mohd Jan has been approved by the following:

_____
Prof. Dr. Ahmad Hafiz Zulkifly
Chairman of Supervisory Committee

_____
Asst. Prof. Dr. Munirah Sha'ban
Supervisor

_____
Prof. Dr. Nor Fadilah Rajab
Co-Supervisor

_____
Prof. Dr. Jamaluddin Abdul Rahman
Co-Supervisor

_____
Prof. Dr. Muhammad Nor Omar
Internal Examiner

_____
Prof. Dr. Md Zuki Abu Bakar @ Zakaria
External Examiner

_____
Prof. Dr. Ismail Zainol
External Examiner

_____
Prof. Dr. Md Zaidul Islam Sarker
Chairperson

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.

NURUL HAFIZA MOHD JAN

Signature……. Date ….....

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Affirmed by Nurul Hafiza Mohd Jan

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Signature Date

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LIST OF ABBREVIATIONS

2D	2-Dimension
3D	3-Dimension
AG	German Aktiengesetz
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BR	Basic Research
Co.	Company
CT	Computed Tomography
DC	Direct Current
DNA	deoxyribonucleic acid
E	Erythema
EDTA	Ethylenediaminetetraacetic acid
EMEM	Eagle's Minimum essential Medium
FOV	field of view
HCA	hexylcinnamaldehyde
HDPE	high-density polyethylene
IACUC	Institutional Animal Care and Use Committee
IBM	IBM Corporation
ICP-MS	Inductive Coupled Plasma Mass Spectrometry
ICR	Institute of Cancer Research
IIUM	International Islamic University Malaysia
ISO/IEC	International Organization for Standardization / International Electrotechnical Commission
KTX	A combination of drugs Ketamine, Tilatamine / Zolazepam and Xylazine for anaesthasia

Micro-CT	Micro-Computed Tomography
MOSTI	Minister of Science, Technology and Innovation
NBF	Natural Buffered Formalin
NBRC	NITE Biological Research Center
NZWR	New Zealand White Rabbit
O	Oedema
OM	Osteomyelitis
OMICS	Orthopaedics Metal Implant Coated with Silver
ORS	Orthopaedic Society Research
PBS	phosphate buffered saline
PMMA	polymethylmethacrylate
POP	Plaster of Paris
PPB	part per billion
PSD	particle size distribution
Pty Ltd.	Propriety / Private Limited
PVD	physical vapor deposition
qPCR	Quantitative Polymerase Chain Reaction
ROI	Region of interest
S.A.W	Sallalahu Alaihu Wassallam
S.W.T	Subhanahu wa ta'ala
SPSS	Statistical Package for the Social Science
USA	United States of America

LIST OF SYMBOLS

%	Percentage
<	Less than
=	Equal to
≠	Not equal to
>	More than
±	Standard deviation
≤	Less than or equal to
≥	More than or equal to
®	Registered patent
°C	Degree Celsius
µA	Microampere
µg/ml	Microgram per millilitre
µm	Micrometre
1^{st}	First
Ag	Argentum/silver
CFU/ml	Colony-forming units per milliliter
Cm	Centimetre
cm^2	Square Centimetre
CO_2	Carbon dioxide
d*f*	Degree of freedom
F	Fisher–Snedecor distribution
g	Gram
g/ml	gram per millilitre
Ha	Alternative Hypothesis

Ho	Null Hypothesis
kg	Kilogram
kV	Kilovolt
M	Mean
MD	Mean different
mg	Milligram
mg/ml	Milligram per millilitre
ml	Millilitre
ml/kg	Millilitre per Kilogram
mm	Millimetre
mM	Millimolar
mm^3	Cubic Millimetre
mmHg	Millimetre Mercury
ms	Millisecond
NaCl	Natrium Chloride
rpm	Rotation per minute
SD	Standard deviation
SE	Standard error
™	Trademark
v/v	Volume per volume
w/v	Weight/volume
w/w	Weight per weight
Z	Normal distribution score
$\eta 2p$	Effect size
$\chi 2$	Chi-squared test

LIST OF EQUATIONS

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Orthopaedic implants are widely used either for bone fixation or joint replacement (Campoccia et al., 2018; Li & Webster, 2018). Besides, the application of orthopaedic implant in modern orthopaedic and trauma surgery has successfully improved the quality of life for patients, either by supporting the rapid and effective bone healing after bone fractures or restoring mobility after joint replacement (Elniel & Giannoudis, 2018; Gimeno et al., 2015; Li & Webster, 2018; Sansone, Pagani, & Melato, 2013; Vilardell et al., 2015).

To date, implant-related with infection poses a significant challenge in the orthopaedic field (Campoccia et al., 2018; Harasser, de Wild, et al., 2016; Jorge-Mora et al., 2018; Li & Webster, 2018; Tschudin-Sutter et al., 2016). The pathogenicity of this incidence begins when the race of the surface started between bacterial adhesion and tissue cell integration on the surface of the implant after implantation (Gallo et al., 2014; Gallo et al., 2016; Odekerken et al., 2013; Ribeiro et al., 2012; Romanò et al., 2015). When the host protein deposited rapidly at the foreign body, it promotes the adherence of thick bacteria community or known as a biofilm to attach at the surface of the implant. In the biofilm, bacteria are protected against environmental stresses, antimicrobial treatment, and the host immune system (Birt et al., 2017; Gallo et al., 2014; Hobley et al., 2015; Ribeiro et al., 2012). Osteomyelitis is one of the human infections that implicated by the biofilm. It can be described as infection and inflammation of the bone. This inflammatory bone disorders mainly caused either by microbial infections or auto-inflammatory processes. It can occur at preferred

localisations in the human skeleton in all different ages (Gomes et al., 2013; Groll et al., 2018; Prieto-Pérez et al., 2014).

Bacteria response for osteomyelitis usually invades bone-forming osteoblasts, leading to inflammation, necrosis and bone destruction at the sites of infection. As often rebellious to treatment and recurrent, osteomyelitis is considered as one of the most challenging medical conditions for orthopaedic surgeons (Liu et al., 2017). *S. aureus* is the most prevalent species in implant-related with infection cases isolated in bone infection (osteomyelitis) with accounts between 20% to 30% cases of infection after fracture fixation, followed by coagulase-negative staphylococci with range between 20% to 40% of cases (Gaudin et al., 2011; Gomes et al., 2013; Kaur et al., 2014; Lu et al., 2016; Moriarty et al., 2016; Pande, 2015). Other species involved were gram-negative bacteria (6%-17%) and followed by anaerobes (including Propionibacteria and Peptostreptococci) with range 4%-5% (El Din et al., 2016; Hotchen et al., 2017; Li & Webster, 2018; Moriarty et al., 2016; Percival et al., 2015).

The incidence that approximately happened in the United States were between 1%-2% and was more widespread in developing countries, with 2% of the high rate of mortality (Lu et al., 2016). Despite the best practices in medical and surgical management to reduce this incidence in all cases, however, it gave a negative impact to clinical outcome and significantly increased the healthcare expenditure (Li & Webster, 2018; Moriarty et al., 2016; Sharma, 2010). Common sophisticated practice and prevention have been developed for the past two decades to reduce the risk of infectious complications in implant surgery. Examples were through a pre-operative procedure such as sterilisation surgical instruments and implants, application of laminar with ultraclean air and short of operation duration (Moriarty et al., 2016; Walley et al., 2016). Besides, the use of routine antimicrobial prophylaxis (Kuehl et