IN-VIVO ANTIMICROBIAL AND BIOCOMPATIBILITY ANALYSIS OF ORTHOPAEDIC METAL IMPLANT COATED WITH SILVER (OMICS)

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Abstract. Implant-related infections and their management pose a major challenge in the orthopaedic field. The colonisation of bacteria and biofilm adhesion on implant surfaces may lead to infection at the implantation site. The infection risk may be overcome by applying OMICS as an alternative strategy in managing cases of implant-related infection. This study aims to evaluate OMICS's efficacy as an antibacterial implant and its biocompatibility properties in an animal model. All rabbits were implanted with OMICS as well as conventional plates and screws. The implanted tibia was excised en bloc and evaluated using microbiological swab and histological analysis for any effects of infection and its biocompatibility respectively. After three and six weeks of post-implantation, microbial analysis showed that no colonies were noted in OMICS groups compared to control. The histological analysis showed no bone reaction with no indication of the presence of microbial in both groups. No periosteal reaction was observed at the surrounding of the implanted area in both groups. This data showed that OMICS implant had antibacterial properties, biocompatible and provided good osteoconductivity comparable to conventional plates and screws. It can be concluded that the OMICS have potential to be served as antibacterial implant to prevent bacterial infection during implantation.

Keywords: Implant-related infection, OMICS, antibacterial, biocompatibility

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Introduction

The management of open fractures remains a challenge in the orthopaedics field. Bone and tissue infections still represent one of the major complications when patients sustain open fractures. The main goal for orthopaedic surgeons is preventing infection by giving immediate treatment to remove any tissues that may lead to infection [1]. The principle of treatment for open fracture consists of antimicrobial therapy, debridement, and follow-up care that includes stabilisation of the bone and coverage of soft tissue.

To stabilise the fracture fragments, the application of implants is needed to provide mechanical stability so that optimal alignment and function of bone can be maintained during the physiologic loading of bones and joints. An effort is made, such as treating patients quickly by giving immediate treatment, which begins from surgery or during an operative surgical procedure by removing any tissues that may be affected by the healing event [2]. Furthermore, the conventional method of administrating antibiotics, tetanus cover, and inducing soft tissue coverage is implemented to avoid infectious complications [1-2].

The administration of antibiotic therapy is a common treatment given to a patient with an open fracture to reduce the risk of post-traumatic infection. A high parenteral dose of antibiotic is needed to achieve optimum therapeutic effect [3]. However, prolonged use of high antibiotics dosage and prolonged treatment can lead to systemic toxicity and increase antibiotic resistance [4]. Hence, to overcome this, an alternative treatment strategy using silver coating technology as an antibacterial agent has been introduced [4-5]. The silver coating on the orthopaedic implant is one of the technologies employed to reduce infection in orthopaedic cases involving implants [6-8]. Silver has shown inhibition of biofilm formation, particularly in its adhesion stage.

It is widely known that before a material can be declared clinically usable, it must undergo biological tests to prove its biocompatibility. One must think that a foreign material placed in the human body will always cause a reaction, but if the material is "inert", the response solely consists of the formation of the fibrous [4,6-7]. Although OMICS containing 3 % of silver has been successfully produced, the full data of its ability as an antibacterial agent and its biocompatibility (in vivo) through the pulsed DC magnetron sputtering coating technique remains scarce. Animal studies are needed before OMICS implants can be translated into a clinical trial. To evaluate the full potential of OMICS implant that coated with silver through pulsed DC magnetron sputtering technique, the implantation of OMICS was performed on rabbit models. In this research, the potential effect of an orthopaedic metal implant coated with silver (OMICS) against infection and its biocompatibility was investigated through in vivo experimental setting from the histological analysis. The performance of OMICS was compared to conventional non-coated implants.

Materials and Methods

The animal study and surgical procedures were conducted at the Advanced Orthopaedic Research Laboratory (ORL), Department of Orthopaedic, Traumatology, and Rehabilitation, Kulliyyah of Medicine, International Islamic University Malaysia (IIUM), Kuantan, Pahang. The management system of ORL in accredited with the ISO/IEC 17025:2005 standards. This study was approved by the Institutional Animal Care and Use

Committee, International Islamic University Malaysia (IACUC-IIUM), with approval letter reference number: IIUM/IACUC Approval/2016/(9)(52).

Experimental Animal

A total of eight New Zealand White Rabbits (NZWR) weighing between 2.5 and 3.0 kg were used in this study. Animals were acclimatised for fourteen days. The animal behaviour, posture, extremities, food, and water intake, urine, and faeces were monitored and recorded during acclimatisation. All rabbits underwent open tibial fracture via the anterolateral approach by exposing the underlying tibial bone for six hours to induce open fracture-related infection. The rabbits were divided randomly into 2 groups; Group 1 (treated with OMICS plate and screws as the study group), and conventional plate (Synthes® Titanium DCP® Plate, 42 mm) and screws (Synthes® Titanium Cortex Screw Ø 2.0 mm) as the control group. One orthopaedic surgeon performed the surgical procedures. All groups were observed for microbiology and histology result interpretation after three and six weeks.

Anaesthesia Procedure

Anaesthesia was administrated by well-trained staff. Before the pre-operative procedure, the animals were weighed to determine the proper amount of medication and anaesthetic drugs. The animals were sedated with a composition of 2.5 ml Ketamine (Ketapex, Apex Laboratories Pty Ltd., Australia), 250 mg of Tiletamine/Zolazepam (Zoletil-50, Virbac Laboratories, Carros, France) freeze-dried and 2.5 ml Xylazine (Ilium Xylazil-100, Australia) with dosage 0.2 ml/kg intramuscularly for the induction and maintained at 0.1 ml/kg intravenously via marginal veins of the ear. Surgery was performed using standard aseptic techniques.

Pre-Operative Procedure

The animals were positioned in the supine site. Under intramuscular anaesthesia, the rabbit fur (surgical site from) ankle to femur was shaved. The animals were then transferred to the Animal Operating Room for surgery. The surgical site was then disinfected with povidone-iodine and draped in the standard sterile procedure. The entire surgical procedure was performed under the aseptic technique to avoid infection. A record of animal surgery was maintained in the institutional animal surgery form.

Surgical Technique

The tibial tuberosity was palpated. The surface marking incision site was along the medial shaft tibia of the right and left tibia, about 6 cm below the tibia tubercle and 1.2 cm above the ankle joint. Then, periosteal stripping was made at the shaft, exposing the underlying tibial bone. The haemostasis was secured with gauze and diathermy. The same approach was made at the other leg of the rabbit. The animals were awakened and placed in the cage. The surgical site was exposed to the environment for six hours [1] to induce infection. After six hours of exposure to induce open fracture creation, the animal was reanaesthetised as described above. Under sterile conditions, the wounds were exposed. A plate (OMICS or non-coated plate) was then inserted and fixed with screws using a drill and screwdriver. Before suturing, the tibia with plate was washed with 50 ml of 0.9 % sodium chloride. Then deep fascia and skin tissue layers were closed in layers using Safil 3/0. The rabbit was then transferred to a cage.

Post-Operative Management

The animals were housed individually in a cage with access to food and water. Ambulatory status, local wound inspection, and localised or systemic infection (fever and wound swelling or colour changes) were observed. There was no post-operative restriction on activity, and no supportive orthotic devices were used. The subjects were monitored twice daily. Tramadol 1 mg/kg will be given intramuscularly daily for up to seven days post-operatively. No antibiotics were given to all subjects in this study.

Sample Harvesting

Animals were sacrificed at three and six weeks post-operatively by overdose (3 ml/rabbit) of 100 mg/ml ketamine hydrochloride drugs by intravenous injection. Under aseptic conditions, the tibia was harvested by making an incision of the skin. Then, the bone sample was taken and fixed in a 10 % natural buffered formalin (NBF) solution. All rabbits were euthanised for histological analysis according to the interval selected for bone infection and biocompatibility evaluation. All assessments were conducted after the specimen had been harvested.

Post-Mortem Microbial Analysis

As for the microbial analysis, the post-mortem swabs analysis was taken between bone and implants (during harvesting sample). The swabbed then from both groups were retrieved on nutrient agar for inoculation. All samples then were inoculated and incubated (CO_2 incubator, Innova CO-170, New Brunswick Scientific) at 37 ˚C for 24 hours. This procedure was conducted to evaluate the capability of OMICS to reduce infection.

Histological Interpretation

The harvested tibia was sliced in the coronal plane into a few pieces at least 0.5 cm thick for each section with the plate in situ using the EXAKT cutter machine. The samples were then dehydrated in a graded series of alcohol and infiltrated with a series of methyl methacrylate (Technovit® 7200, Heraeus Kulzer Co., Germany). The samples were embedded in methyl methacrylate (Technovit® 7200, Heraeus Kulzer Co., Germany), sectioned at a thickness of 80 μm by EXAKT 310 Cutter Machine and EXAKT 400 CS Micro grinder (EXAKT Apparatebau systems, Norderstedt, Germany), and stained with Masson Goldner Trichrome staining. All sections were visualised and captured using a motorised transmitted light research microscope (Nikon Eclipse Ni, Japan) for histological interpretation.

Results and Discussion

Post-Mortem Microbial Analysis

The results of the Microbial Analysis show that OMICS implants have antibacterial activity from three and six weeks after implantation. Figure 1 shows that after 24 hours of incubation, there was no evidence of microbial development in the OMICS group samples compared to the conventional implants. Six weeks after implantation revealed no microorganism colonies found in OMICS group compared to control (Figure 2). These findings demonstrated that silver coating technology can act as an antimicrobial agent by

reducing infection during implantation [4-5,7,11]. Silver, an inorganic compound ion, has been established used as an antimicrobial agent [6-7,11]. The ions have been selected as a promising candidate for coating materials for devices because they have a broad antimicrobial spectrum as an antibacterial agent against polymicrobial species such as Gram-positive, Gram-negative, multi-drug resistant and more [7-9]. Theoretically, it acts as an antibacterial agent by attacking bacterial thiols, protein amino groups, cell membranes, and nucleic acids. This mechanism causes iron sulphur to be disrupted and inhibits the respiratory chain [7-9]. As a result, it will initiate a bacteriostatic or bactericidal process. This is the main reason silver was chosen as the substrate for coating orthopaedic implants in order to reduce the risk of infection during implant placement [7,9]. The findings suggest that using DC magnetron sputtering technology to coat orthopaedic implants could help reduce implant-related infection cases during implantation for open fracture-related infections in orthopaedic cases. The approach of applying coating technology to implant surfaces may thus be a promising option for reducing infection cases during implantation in orthopaedic cases.

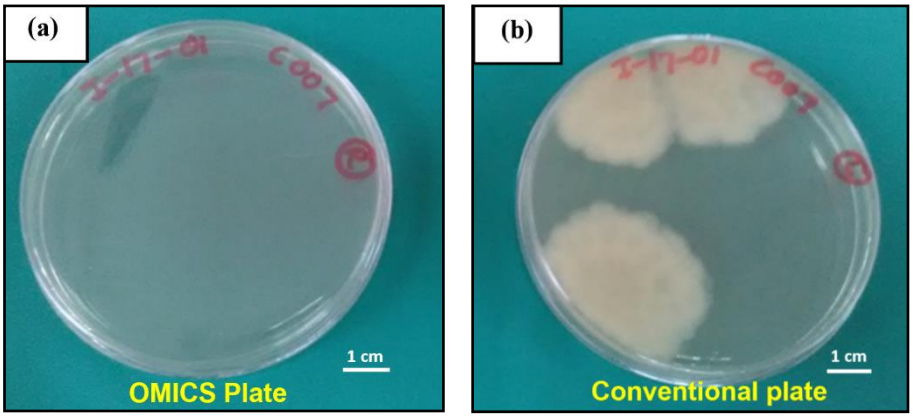

Figure 1: The post-mortem microbial analysis of (a) the OMICS plate and (b) the conventional implants

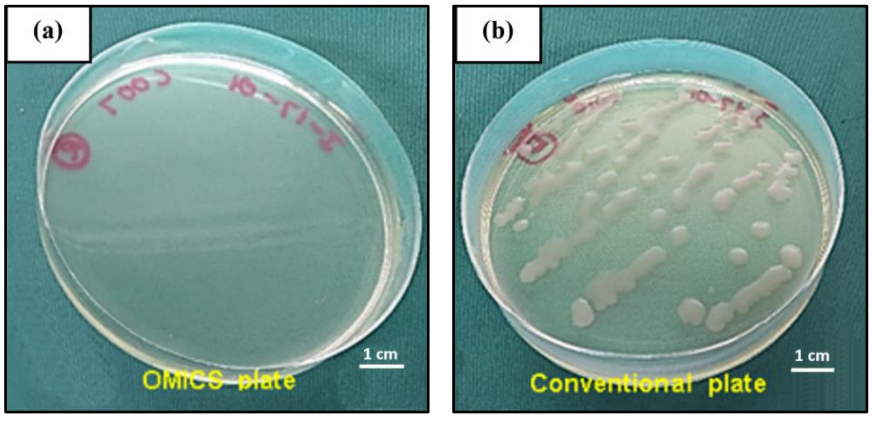

Figure 2: The post-mortem microbial analysis of (a) the OMICS implants and (b) the conventional implants

Histological Analysis

The interaction between the OMICS implants and bone samples was confirmed by histologic analysis. Masson Goldner Trichrome stained sections of the OMICS group revealed normal tibial cortex morphology three weeks after implantation. In comparison to the control group, there was good contact between the bone and the OMICS implants with no obvious changes and no adverse reaction (Figure 3). There was no incompatibility found in either group. Histologic analysis at six weeks post-implantation revealed no morphological changes in either group, as shown in Figure 4. There were no adverse reactions or abnormality changes in the bone apposition on the implant surface in either group. Both groups showed bone remodelling near the screw at this interval. In both groups, no incompatibility and no significant histological findings were observed. These in vivo findings demonstrated direct contact healing between the OMICS implant and bone. As shown in Figure 4, this result indicated that OMICS was biocompatible and comparable to conventional non-coated implants. The ability of the metal and the body to tolerate each other, particularly in host-specific situations, is referred to as biocompatible interaction [4-5,10-11].

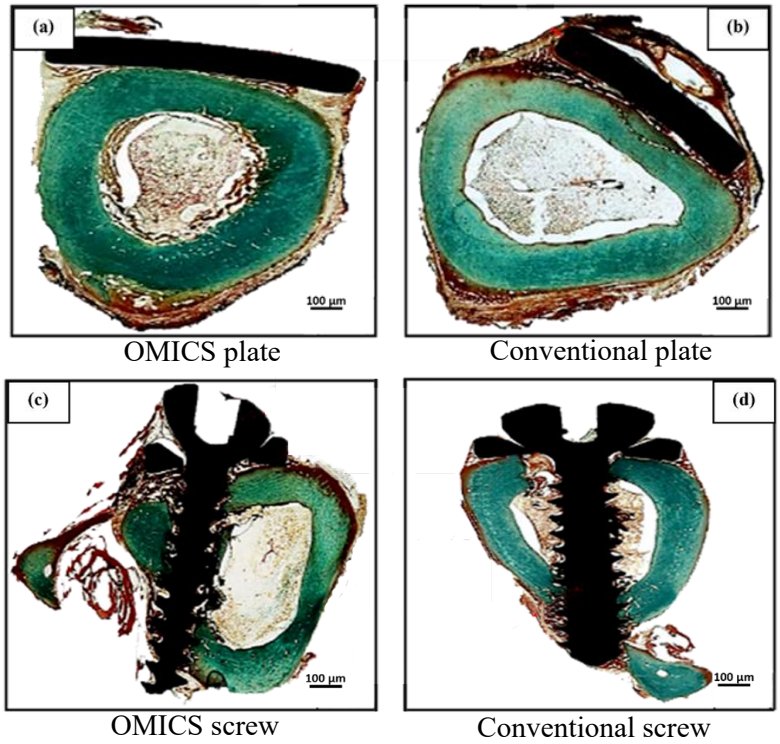

Figure 3: Histological evaluation after three weeks of implantation with the OMICS plate and screw (a and c) and conventional implants (b and d) at the transverse view (Masson Goldner Trichrome stained)

In this study, the biocompatibility of OMICS via DC sputtering was investigated in vivo using the rabbit tibia model. This portion of the study was carried out to assess the interaction between bone and OMICS implants. Masson Goldner Trichrome staining was used to demonstrate the distinct interaction between the bone and the OMICS implant. At both time intervals, histology evaluations revealed good interaction between bone and OMICS at the implantation site (three and six weeks). Normal bone architecture was also observed in OMICS groups at both time intervals. It was clear that OMICS and bone were compatible. Based on the data collected, it was determined that the OMICS implant was

bioinert. These findings also support the notion that OMICS was biocompatible with conventional non-coated materials.

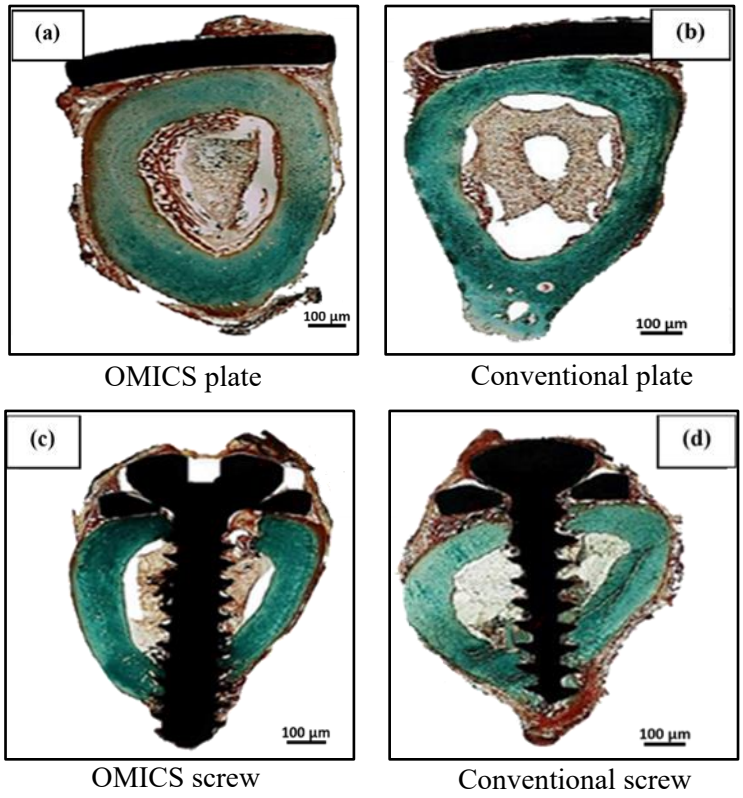

Figure 4: Histological evaluation after six weeks of implantation with the OMICS plate and screw (a and c) and conventional non-coated implants (b and d) at the transverse view (Masson Goldner Trichrome stained)

Overall, this study demonstrated that the OMICS implant developed using DC magnetron sputtering technology remained in situ, straight, stable, made good contact with bone, and possessed antibacterial properties. Furthermore, this technology would be an excellent candidate as an orthopaedic implant coating technique to reduce implant-related infection cases during implant placement in orthopaedic cases. If this finding is applicable for future clinical use, it is practically certain that it will be implemented as an alternative strategy to reduce the risk of implant infection cases in the future. Although this intervention had a promising outcome, it is recommended that it be evaluated further using the polymerase chain reaction (PCR) diagnostic method. This analysis is recommended in future because it can evaluate and identify the overall community of bacteria attached to the OMICS implant surface and provides a sensitive and accurate method for detecting specific genes.

Conclusions

The study has shown a good outcome of using silver as substrate for orthopaedic implant coating. In conclusion, results show the potential of OMICS through DC magnetron sputtering coating technology to reduce infection during implantation. This is supported by the result obtained from the microbial analysis. Histological findings demonstrate that

OMICS is biocompatible and might have the potential to prevent implant-related infections. Overall, these findings may pave the opportunity for clinical trials and applications.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards. The ethical approval was obtained from the IIUM Animal Care and Use Committee (I-ACUC), with approval letter reference number: IIUM/IACUC Approval/2016/(9)(52).

References

[1] Kyle, P. K., Qian, K. K., Xuejun, W. & Yueheui, H. A. (2013). Novel Rabbit Model for the Evaluation of Open Tibia Fractures; Effect of Delayed Surgery on Infection Rate. *The Duke Orthopedic Journal*. 3(1), 48-53.

[2] Diwan, A., Eberlin, K. R. & Malcolm, R. (2018). The Principles and Practice of Open Fracture Care. *Chinese Journal of Traumatolog*y. 21(4), 187–192.

[3] Leah, H. C., Emily, M. M. & Lauren, B. P. (2020). Therapeutics and Delivery Vehicles for Local Treatment of Osteomyelitis. *Journal of Orthopaedic Research*. 28(10), 2091-2103.

[4] Martin, F., Amir, A. M., Christine, F., Henriettet, J., Markus, Z., Thomas, L. & Jacobus, J. A. (2020). Antimicrobial Silver Multilayer Coating for Prevention of Bacterial Colonization of Orthopaedic Implants. *Materials*. 13(6), 1415.

[5] Guy, V. M., Jakub, K., Jonathan, G. & Lee, J. (2017). The Use of Silver-Coated Orthopaedic Implants: Are All Silvers the Same?.*Current Orthopaedic Practice*. 28(6), 532-536.

[6] Chenhao, P., Zubin, Z. & Xiaowei, Y. (2018). Coatings as the Useful Drug Delivery System for the Prevention on Implant-Related Infections. *Journal of Orthopaedic Surgery and Research*. 13(1), 220.

[7] Yi, H. H., Kuen, S. L., Wan, J. K., Chien, T. H., Chao, L. C., Dong, Y. T. & Shih, T. L. (2015). The Antimicrobial Properties of Silver Nanoparticles in *Bacillus subtilis* Are Mediated by Released Ag+ Ions. *PLoS ONE*. 10(12), 1-17.

[8] Masaya, U., Hiroshi, M., Masatsugu, T., Shuichi, E., Iwao, N., Takeo, S., Tomoki, K., Motoki, S. & Masaaki, M. (2016). Silver-Containing Hydroxyapatite Coating Reduces Biofilm Formation by Methicillin-Resistant *Staphylococcus aureus* In Vitro and In Vivo. *BioMed Research International*. 2016, 8070597.

[9] Myriam, K. S., Ballo, S. R., César, P., Nancy, H., Aurélie, B., John, K., Philippe, M., José, M. E, & Alain, B. (2016). In Vitro and In Vivo Effectiveness of an Innovative Silver-Copper Nanoparticle Coating of Catheters to Prevent Methicillin-Resistant *Staphylococcus aureus* Infection. *Antimicrobial Agents and Chemotherapy*. 60(9), 5349-5356.

[10] Tadatsugu, M., Hirohito, H., Shuichi, E., Akira, H., Sakumo, K., Takaomi, K., Masatsugu, T., Tomohito, Y., Yu, T. & Masaaki, M. (2022). Development of Silver-Containing Hydroxyapatite-Coated Antimicrobial Implants for Orthopaedic and Spinal Surgery. *Medicina (Kaunas).* 58(4), 519-539.

[11] Richard, K., Priscilla, S. B., Anne, K. W., Massiom, V., Zarko, R., Juerg, V., Luigi, T., Katharina, M. F. & Nina, K. (2016). Preventing Implant-Associated Infectious by Silver Coating. A*ntimicrobial Agents and Chemotheraphy*. 60(4), 2467-2475.

[12] Penn, B. J. G., Murray, C. K. & Wenke, J. C. (2012). Early Antibiotics and Debridement Independently Reduce Infection in an Open Fracture Model. *Journal of Bone and Joint Surgery British Volume*. 94(1), 107-112.

[13] Nurul, H. M. J., Zulkifly, A. H., Mohd, Z. I., Munirah, S. & Mohd, T. M. R. (2019). The In-Vitro and In Vivo Evaluations of Orthopedic Metal Implant Coated with Silver Composite: Potential Treatment Modality for Implant Related Infection. *Materials Today: Proceedings*. 16 (4), 2003-2009.