

## Effects of a noble metal alloy coating on fibroblast-mediated tissue repair

Aisha S. Ahmed 1, Amina Hamzatova 1, Stefan Grass 2\*, Katrin Ljunggren 2, Vaso Basinou 2, Paul W. Ackermann 1

1. Department of Molecular Medicine and Surgery, Karolinska Institutet, 171 76 Stockholm, Sweden

2. Bactiguard AB, Tullinge, Sweden

\* corresponding author: [stefan.grass@bactiguard.com](mailto:stefan.grass@bactiguard.com)

Disclosures: SG, KL and VB are full time employees of Bactiguard. The study has been funded by Bactiguard.

### INTRODUCTION

Implant-associated infections are serious complications in orthopaedic surgery, with potential severe consequences for peri-implant tissue healing and osseointegration. A noble metal alloy coating (Bactiguard®, Sweden) has been shown to reduce the risk of implant-associated infections when applied on titanium orthopaedic nails (1). This coating technology prevents bacterial adhesion, does not interfere with bony union, and shows superiority in soft tissue peri-implant reactions compared to other noble metal coatings (2). The effects of coated implants on tissue repair are of high clinical importance. This study aimed to establish an *in vitro* model to investigate the impact of Bactiguard® (BG)-coated titanium on fibroblast-mediated tissue repair. Fibroblasts proliferate and migrate to the injury site, where they produce collagens like Collagen type I (COL I) and type III (COL III), which both are essential proteins to regulate tissue repair (3). The objectives of this study were to assess the *in vitro* effects of BG-coating on fibroblast proliferation, migration, and collagen production.

### METHODS

Human fibroblasts (fHDF/TERT166) were cultured with 1.) BG-coated titanium coins, 2.) non-coated (NC)-titanium coins and 3.) without any coins as controls. Cell proliferation was studied by counting the number of cells at different timepoints after seeding in the presence of BG-coated and NC titanium coins. A cell scratch assay was applied to mimic tissue injury. Cell migration was determined by the rate of scratch recovery in BG-coated, NC titanium coins and control cultures. Western blot analysis was used to study Col I and III expression in protein lysates extracted from cultures with BG-coated, NC-titanium coins and normal controls. All statistical analyses were performed using SPSS software (IBM SPSS, v26.0). Between group comparisons were made by the Student's t-test or one-way analysis of variance followed by LSD.  $p < 0.05$  was considered significant.

### RESULTS SECTION

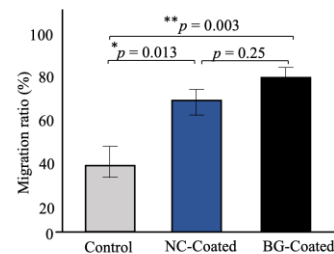
Cell proliferation experiments at 24 or 36 hours detected no significant differences between the number of cells in cultures with BG-coated and NC titanium coins. In subsequent experiments a defined area close to the coin was selected, and cell numbers were again non significantly different between the two different coins. Interestingly, a significantly higher migration rate was observed in both the BG-coated (54%,  $p = 0.003$ ) and NC-coated coin (46%,  $p = 0.013$ ) cultures compared to the normal control (Fig 1). However, no statistically significant difference in migration rate was observed between BG-coated and NC-coated cultures ( $p = 0.25$ ). Western blot analysis revealed no significant differences among BG-coated, NC coins or control cultures in the amount of Col I and Col III expression (Fig 2A, B). However, in comparison to normal control there was a trend for increased (9% and 19%) COL III and decreased (13% and 12%) COL I expression in the BG-coated and NC coin cultures, respectively, compared to normal control (Fig 2A and 2B). Subsequently, the COL III/COL I ratio was non-significantly higher in the coated (26%,  $p = 0.075$ ) and NC (14%,  $p = 0.419$ ) coin cultures, compared to the normal control (Fig 2C). During tissue regeneration, COL III is being successively replaced by COL I. Thus, the COL III/ COL I ratio is an indicator of tissue repair regulation.

### DISCUSSION

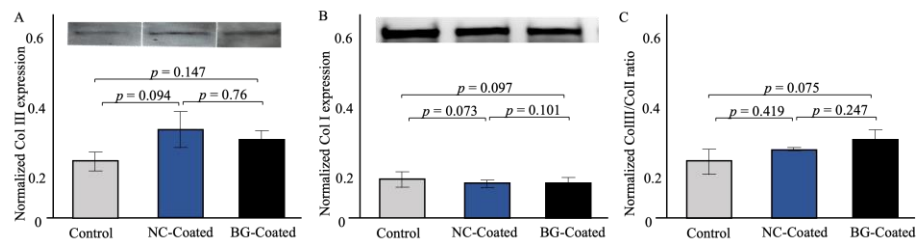
The study results of this *in vitro* model on fibroblast-mediated tissue repair suggest that the BG-coating does not exert any adverse effects on tissue repair. The impact of BG-coating on cell proliferation, cell migration and collagen production was comparable to control. In contrast to silver-releasing coatings which have been shown to significantly reduce the fibroblast proliferation, viability and COL I synthesis, the BG coating, which is non-releasing, did not show any cytotoxic effects. The higher migration ratio and slightly higher COL III/COL I ratio on the coin cultures (i.e., BG coated and NC) compared to normal control, indicates inflammatory healing response from placing a coin on a fibroblast cell culture. Our proposed tissue repair model has some potential limitations, like the manually defined area used for cell migration calculations type. Future studies may benefit from cell cultures of bone cells, osteoblasts and primary cells, to investigate long-term effects on cell growth and cell death.

### SIGNIFICANCE/CLINICAL RELEVANCE:

Implant-associated infections have severe clinical consequences for patients. Coated implants can help reduce infections, but may increase the risk of toxicological effects. Thus, non-toxic, infection-prevention coatings that do not disrupt the normal cell repair mechanisms are preferable.



**Fig 1.** Semi-quantitative analysis of cell migration ratio in control, Non-coated (NC) and Bactiguard (BG)-coated cultures at 24 hrs. Data reported as mean  $\pm$  SEM with  $n = 3$  replicates. \* $p < 0.05$ , \*\* $p < 0.005$ .



**Fig 2.** The representative western blot images and semi-quantitative analysis of A) Col III, B) Col I, and C) Col III/I ratio from protein lysates generated by control, Non-coated (NC) and Bactiguard (BG)-coated cultures at 36 hrs. Signal intensity was used for analysis and the intensity of the house-keeping gene (beta-actin) used for normalization. Data reported as mean  $\pm$  SEM with  $n = 3$  replicates.

### REFERENCES

1. Karupiah, Thevarajan, Aik Peng Yong, Ze Wee Ong, Heng Keat Tan, Wei Chern Tang, and Hishamuddin Bin Salam. 2022. Use of a Novel Anti-Infective Noble Metal Alloy-Coated Titanium Orthopedic Nail in Patients with Open Fractures: A Case Series from Malaysia. *Antibiotics* 11, no. 12: 1763.
2. Suska, F.; Svensson, S.; Johansson, A.; Emanuelsson, L.; Karlholm, H.; Ohlander, M.; Thomsen, P. 2010. *In vivo* evaluation of noble metal coatings. *J. Biomed. Mater. Res. B Appl. Biomater.* 92, 86–94.
3. Chen, J., Wang, J., Hart, D. A., Ahmed, A. S., and Ackermann, P. W. 2022 Complement factor D as a predictor of Achilles tendon healing and long-term patient outcomes. *FASEB J* 36, e22365.
4. Zou SB, Yoon WY, Han SK, Jeong SH, Cui ZJ, Kim WK. Cytotoxicity of silver dressings on diabetic fibroblasts. *Int Wound J.* 2013;10(3):306-312.