

# Improving Osseointegration by local co - delivery of a novel Prostaglandin EP4 Agonist and Zoledronic Acid in a Rat distal femur defect model

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**INTRODUCTION:** A stable fracture fixation by orthopaedic implants is essential, to achieve optimal conditions for bone regeneration. Indeed, robust osseointegration is crucial for implant stability and fracture healing.[1] Advanced age, female sex, and metabolic comorbidities can compromise stable fixation due to reductions in bone volume (BV) and bone mineral density (BMD), ultimately resulting in impaired fracture healing.[2] Systemic administration of bisphosphonates like Zoledronic acid (ZA) effectively suppresses bone resorption, but is associated with potential severe adverse effects. Consequently, local delivery strategies are desirable. In addition, previous experiments revealed local application of the novel Prostaglandin EP4 receptor agonist KMN-159 as a promising small molecule in enhancing local bone formation. Therefore, the aim of this study is the evaluation of combined therapeutic effects on bone formation and osseointegration by the local co-delivery of KMN-159 and ZA within a mineralized collagen matrix (MCM) scaffold in a rat distal femur defect model. Additionally elucidating sex-specific differences and comparing local with systemic administration of ZA is of major importance.

**METHODS:** In total 84 male and female, 12-weeks-old Wistar rats were randomized into 7 different groups with 6 animals of each sex per group. In the surgical procedure, a 1.8 mm drill hole was created in the distal, lateral femur. Following, the group - specific drug - loaded MCM - scaffold and a 2.3 mm titanium screw was implanted. The groups received either treatment with local or systemic ZA administration ± local application of KMN - 159, within the MCM - scaffold. The groups with only screw ± MCM scaffold implantation served as control groups. After six weeks of observation, animals were sacrificed, and femora were explanted. µCT - imaging was used to assess bone formation in the peri-implant region. To evaluate bone formation homogenously, a cylindrical region of interest (ROI) was defined and a threshold was set to exclude the MCM - scaffold as the minimal - and the titanium screw as the maximal intensity value. The included interval was defined as newly formed bone tissue and analyzed for Bone Volume (BV) and Bone Mineral Density (BMD) using Dragonfly - Software (ORS Inc., Montreal, Canada). Furthermore, high-resolution Synchrotron recordings were captured to evaluate the bone-implant interface. All present data were analyzed using Graph Pad Prism 10.5.0 (Inc., San Diego, USA). All quantitative results are expressed as mean ± standard deviation (SD). Differences between multiple groups were tested using two-way ANOVA with Tukey's post hoc test. Differences were considered significant when p < 0.05 (\*). All experiments were performed in adherence to the National Institutes of Health Guidelines for the Use of Experimental Animals and were approved by the Local Animal Care Committee [TVV33/24].

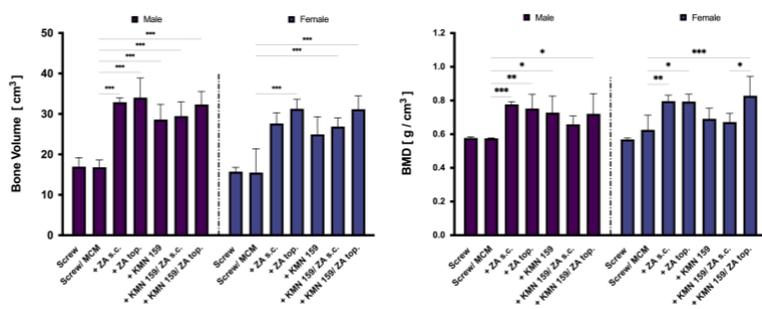
**RESULTS:** All 84 animals survived the surgery and observation period. After sacrifice, BV [cm<sup>3</sup>] and BMD [g/cm<sup>3</sup>] in the peri - implant region was quantified. All groups receiving drug - administration showed a highly significant increase in BV compared to the control groups in both sexes, respectively (p < 0.01). In female animals, the highest BV was measured in the groups receiving local application of either ZA or ZA with co - delivery with KMN - 159. The same observation applied for male animals. Furthermore, in female specimens the local co - delivery of KMN - 159 and ZA showed significantly higher BV than mono - delivery of KMN - 159. (p = 0.03). Whereas in male animals, the group treated with systemically ZA - administration showed similar newly formed bone volume compared to local co - delivery of KMN - 159 and ZA. Surprisingly, the only significant sex - specific difference was observed in the groups receiving systemically ZA administration (p = 0.008). In female samples, BMD - analysis revealed that the groups with either systemically or local ZA administration and local co - delivery of KMN - 159 and ZA presented a significantly higher BMD compared to the control group (p < 0.05). In male specimens, a significant increase in BMD in all groups receiving systemically or local delivery of drugs, except for co - delivery of KMN - 159 and systemically administration was observed. (p < 0.05; p = 0.72). Notably, local co - delivery of KMN - 159 and ZA in the MCM scaffold in female animals showed the highest total BMD value in all groups. (0.83 g/cm<sup>3</sup>, p < 0.001). The high-resolution Synchrotron recordings qualitatively support the bone formation process in drug treatment groups similar to the observations with µCT. Especially in the groups with co - delivery of KMN - 159 and ZA an increased bone - implant contact area was observed.

**DISCUSSION:** The study revealed the local co - delivery of the Prostaglandin - EP4 receptor agonist KMN - 159 in combination with ZA in an MCM scaffold as a valuable option to increase BV and BMD in the peri - implant region. Therefore, possible adverse effects of systemically treatment may be mitigated. In the presence of multiple risk factors, the local combination of the two pharmaceuticals on bone formation could improve implant stability and decrease the risk of impaired fracture healing. Future measurements, including biomechanical pull-out testing will be carried out to determine implant stability. Subsequently histomorphometrically analysis will be performed to assess osteoblastogenesis, osteoclastogenesis and angiogenesis to corroborate recent results. Future experiments are essential to validate the observed effects in conditions of delayed fracture healing in old or osteoporotic animals.

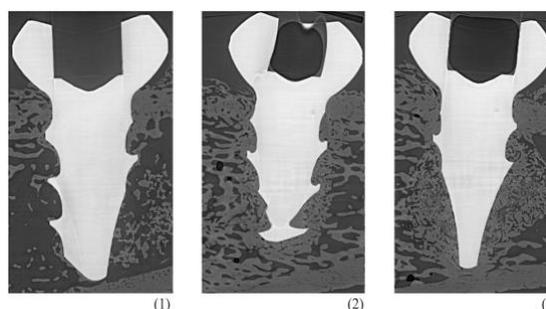
**SIGNIFICANCE/CLINICAL RELEVANCE:** Local co - delivery of the Prostaglandin EP4 - Agonist KMN - 159 and ZA in a mineralized collagen matrix is a promising therapeutic option for improving osseointegration and fracture healing.

**REFERENCES:** [1] Einhorn, T., Gerstenfeld, L. *Nat Rev Rheumatol* 11, (2015). [2] Claes, L., Recknagel, S. & Ignatius, A. *Nat Rev Rheumatol* 8, (2012).

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**Figure 1** Comparison between different groups receiving implantation of the MCM scaffold and titanium screw different combinations of the selective EP4 - Receptor Agonist KMN 159 and Zoledronic Acid within the scaffold or systemic injection. Especially groups receiving local co - delivery of KMN - 159 and Zoledronic Acid showed significant increase in bone volume and bone mineral density



**Figure 2** High resolution Synchrotron recordings indicating superior bone formation and increased bone - implant area in groups with co - delivery of KMN - 159 and Zoledronic Acid within the MCM scaffold (3) or with systemical injection(2) compared to control group (1)