EXTRACELLULAR VESICLES FUNCTIONALIZED POROUS PHOSPHATE SCAFFOLD ENHANCES BONE REGENERATION IN SHEEP METHAPHYSEAL TIBIAL DEFECT

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SUMMARY

SIGIT DARU CAHAYADI. Extracellular Vesicles Functionalized Porous Phosphate Scaffold Enhances Bone Regeneration In Sheep Methaphyseal Tibial Defect. Supervised by **BERRY JULIANDI, YESSIE WIDYA SARI, ARIEF BOEDIONO,** and **IFTY AHMED**

A critical bone defect is a loss of bone structure that exceeds the critical size of the bone's ability to regenerate. They are commonly caused by trauma, chronic inflammation, metabolic disorders, or post-tumor resection. Bone defects not only interfere with the bone healing process but also aggravate morbidity for the patient, thus becoming one of the main problems in the field of orthopedics. In principle, bone healing requires mechanical stability, osteoinductive, osteoconductive, and osteogenic cells which are lost in critical bone defect conditions. To overcome this problem, a substitute material is needed as an intermediary between the bone and the regeneration process. Not only as a mechanical bridge, the replacement material must have good bioactivity, biocompatibility and biodegradability. Phosphate-Based Porous Bioactive Glass (P30) has been investigated to have the required characteristics. In addition, the addition of biologically active agents can enhance osteoinductive and osteogenesis. Currently, the focus of biological active agent research is shifting from cell-based to non-cell-based, one of which is Extracellular Vesicle (EV). EVs have stem cell-like characteristics in terms of bone healing efficacy but don't served as cell itself in terms of structure and differentiation. with low immunogenicity and can be mass produced. In this study, the effectiveness of EVs and various concentration P30 were tested under in vitro and in vivo conditions.

Phosphate Based Porous Bioactive Glass (P30) as EV-loading biomaterials were fabricated via the speroidization method. Meanwhile, EVs were obtained from purified human umbilical cord secretome as extraction source. In the invitro test, extracellular vesicles (EV) will be loaded on porous bioactive glass microspheres with various concentrations, then the efficacy of EV on microspheres based on osteogenesis, EV uptake, and cell migration will be evaluated. The *in vivo* test used local female sheep (*Ovis Aries*) as the experimental animal. A cylindrical defect measuring 15 mm x 8 mm was made by drilling using a drill, then three treatments will be applied to the bone defect including hydroxy apatite, Phosphate- Based Porous Bioactive Glass (P30) and P30+EV. Radiology, osteogenesis, and angiogenesis measurements were taken on days 28 and 56.

Based on *in vitro* studies, observation of osteogenesis on days 7 and 14 showed that P30 concentrations of 100 µg were significantly higher than the control group. Similarly, in the EV uptake test results, concentrations of 100 µg were significantly higher than the control group. While in the cell migration test, P30 500 µg concentration had a significantly higher value. The findings in the *in vitro* test revealed that the highest effectiveness for osteogenesis and EV uptake was obtained at P30 levels of 100 µg. Optimal osteogenesis was obtained from balanced P30 levels for

biodegradability and mineralization. Correspondingly, effective EV uptake was obtained with the speed of EV release in accordance with the cell's capability for EV uptake. While at a concentration of 500 µg, the bioactive signal was strong enough to induce pre-osteoblastic migration.

Based on the *in vivo* study, the defect size in the radiological test group was significantly smaller than the negative control at day 28 and 56. Correspondingly, the highest number of osteoblasts was also found in the P30+EV group. Furthermore, osteogenesis analysis based on RUNX2 expression showed the area with the highest RUNX2 positivity in P30+EV. Similarly, in angiogenesis analysis, the highest CD31 positive area was found in P30+EV. IRS Score analysis also showed similar results. The findings in the*in vivo* test prove that the combination of P30+EV can be applied in sheep animal models with good effectiveness.

Keyword: *Sheep bone defect, Extracellular Vesicle, Phosphate Scaffold, Bone Regeneration*

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PREFACE

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May this scientific work be beneficial to those in need and contribute to the advancement of science and knowledge.

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