Use of a Biomimetic Polymer Adhesive Improves Tibia Bone Fracture Healing in Rats

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INTRODUCTION: Bone healing occurs through primary or secondary ossification to restore the functional integrity of the affected bone [1] and involves numerous parameters. Even slight imbalances in any of these factors can critically or completely inhibit the regenerative potential of a fractured bone [2]. While fractured bones are capable of repair and regeneration, approximately 10% of fractures result in delayed union, malunion, or nonunion [3]. Approaches like tissue engineering, gene therapy, and bone repair enhancement are used to enhance bone fracture healing. However, there is a need to develop novel treatments as alternatives or adjuncts to the standard methods used for bone regeneration [4]. In this study, we used a biomimetic polymer adhesive developed in our lab to improve bone fracture healing.

METHODS: Amino acids - glycerol - based (Lysine, Phenylalanine, Cysteine, Glycerol) biomimetic adhesive polymer was synthesized in Mechanobiology Laboratory. It exhibits non-toxic, biodegradable, biomimetic, and bio-adhesive properties. After polymer ingredients are mixed in syringe mixer polymerization starts forming a foam. A foam filling the gap between fracture fragments serves as a scaffold for cells migration – osteoconductive factor, and Lysine, Phenylalanine and Cysteine amino acids serve as osteoinductive factor attracting cell migration. Partial tibia fracture model was made by sawing of rat tibia shaft 0.5 mm thick * 3 mm deep, n=3 rats/group (**Fig. 1**), followed by wound suturing in control group, whereas in treatment group 10 µl of biomimetic polymer injected in the gap (**Fig. 1B**) before wound sutures application. The fracture healing process was assessed in term 8 weeks post – surgery using Micro-CT analysis of new bone formation and histological slides assessment.

RESULTS: Analysis of micro-CT images shows intensive formation of sponge bone in fracture healing site in control group, with formation of deformed cortical layer, what is typical for early bone callus remodeling phase (**Fig. 2A**), whereas in polymer group we observed intensive thick and smooth cortical layer formation, what is typical for late remodeling phase (**Fig. 2B**). Also, we show increase of newly formed bone tissue volume in polymer group – 36.27 mm^3 compared to 26.10 mm^3 in control group (**Fig. 2C**). Histological slides of fracture healing site stained in Safranin O / Fast Green indicated formation of disorganized sponge – like bone tissue with excessive inclusions of fibro – vascular and connective tissue, confirming early bone callus remodeling phase (**Fig. 3B, red arrows**). At the same time in polymer group, we observed remodeled and organized cortical bone layer formation, which indicates late remodeling phase (**Fig. 3D, green arrows**), some inclusions of fibro-vascular and connective tissue (**Fig. 3D, red arrows**), and proteoglycans expression in periosteum area, indicating that remodeling phase is still ongoing (**Fig. 3D, blue arrows**).

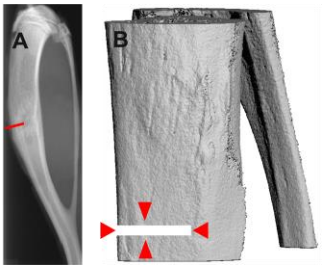

Fig. 1 Tibia fracture model. X-ray image shows schematic partial bone fracture position, red line (**A**). Micro-CT image shows the scheme of bone fracture modeling by sawing a defect 0.5*3 mm in tibia shaft, red arrows (**B**).

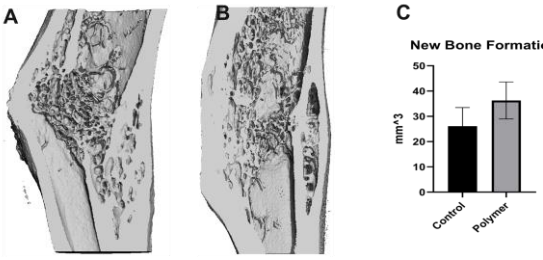

Fig. 2 New bone tissue formation measurements. Micro-CT of control group (**A**) and Polymer group (**B**). Increase of new bone formation volume in polymer group compared to control group presented at (**C**).

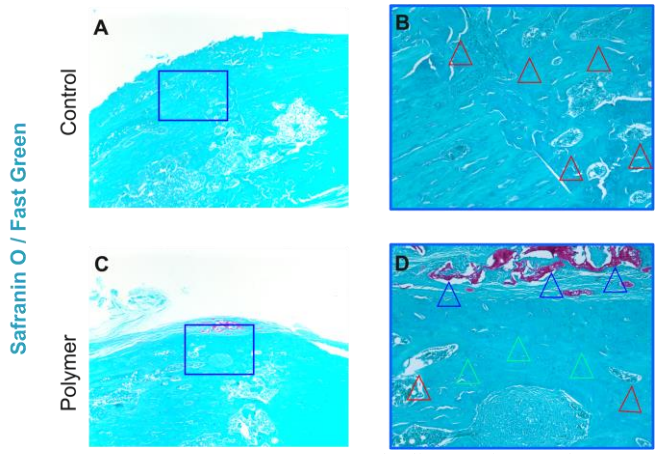

Fig. 3. Bone fracture healing site. Fracture healing area in control group presented by disorganized bone tissue, mostly sponge – like (**A**) with massive inclusions of fibro-vascular and connective tissue, red arrows (**B**), stage of early callus remodeling. In polymer group fracture healing site (**C**) we observed cortical bone formation, green arrows, some inclusions of fibro-vascular and connective tissue, red arrows, and superficial expression of proteoglycans in periosteum area, blue arrows (**D**), late remodeling stage.

DISCUSSION: In this study, we used our novel biomimetic amino acid-glycerol-based polymer bone adhesive and demonstrated that its biomimetic properties stimulate bone tissue formation at the fracture healing site compared to the control group. The foam formed as a result of polymerization serves as an osteoconductive and osteoinductive scaffold, promoting bone tissue formation while inhibiting excessive fibrovascular tissue formation. The use of this polymer adhesive increased newly formed bone volume and enhanced fracture healing by accelerating the bone callus remodeling phase.

SIGNIFICANCE: Our amino - acids - glycerol - based biomimetic polymer adhesive may be used as a new tissue engineering approach to enhance bone fracture healing.

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