KERATIN-ASSOCIATED PROTEIN MICROMATERIALS AS A NEW TYPE OF WOUND DRESSING IN DIABETIC MOUSE MODEL

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Introduction

Regenerative medicine pursues the reconstruction of damaged tissues by the controlled growth of cells, either *in vitro* followed by implantation or directly *in vivo*. As a pivotal concept supporting this approach, cells must be cultured on biocompatible substrates that should ideally provide the right combination of mechanical and biological stimuli for colonization, proliferation and, eventually, for differentiation.

In this context protein biomaterial a specially keratin associated protein (*KAP*) obtained from wool, fur, hair have became a promising candidate due to their above-mentioned properties (biodegradability, biocompatibility and ability to support skin cells growth). We apply KAP's derived from mouse fur as a positional bandage for wound healing in diabetic mouse model.

Material and Methods

Chemical studies:

Procedure of preparation of keratin associated protein micromaterials from mice fur was carried out according method proposed by Lipkowski *et al.*[1] (using different enzyme: pepsin, papain, pankreatyn). Next, the obtained mice-KAP's (m-KAP'S) was impregnate by silver nanoparticles (antimicrobial properties), which was prepared by chemical reduction according to the description of A. Sileikaite *et al.*[2] At the some time in mice pharmacologically induced diabetes according method K.K.Wu *et al.*[3]

In vivo studies

STZ diabetic model and surgical procedure:

Protocols involve i.p. administration of multiple, low dose of STZ (80 mg/kg) to C57BL6 male mice on by 5 consecutive days, to cause diabetes. Mice from control group administrated equal volume of citrate buffor (pH 4.5). Mice were considered diabetic when 3 consecutive measurements were above 250 mg/dL (Fig.4). When diabetes were stable two incisions wounds were made, and KAP's bandage was tested.

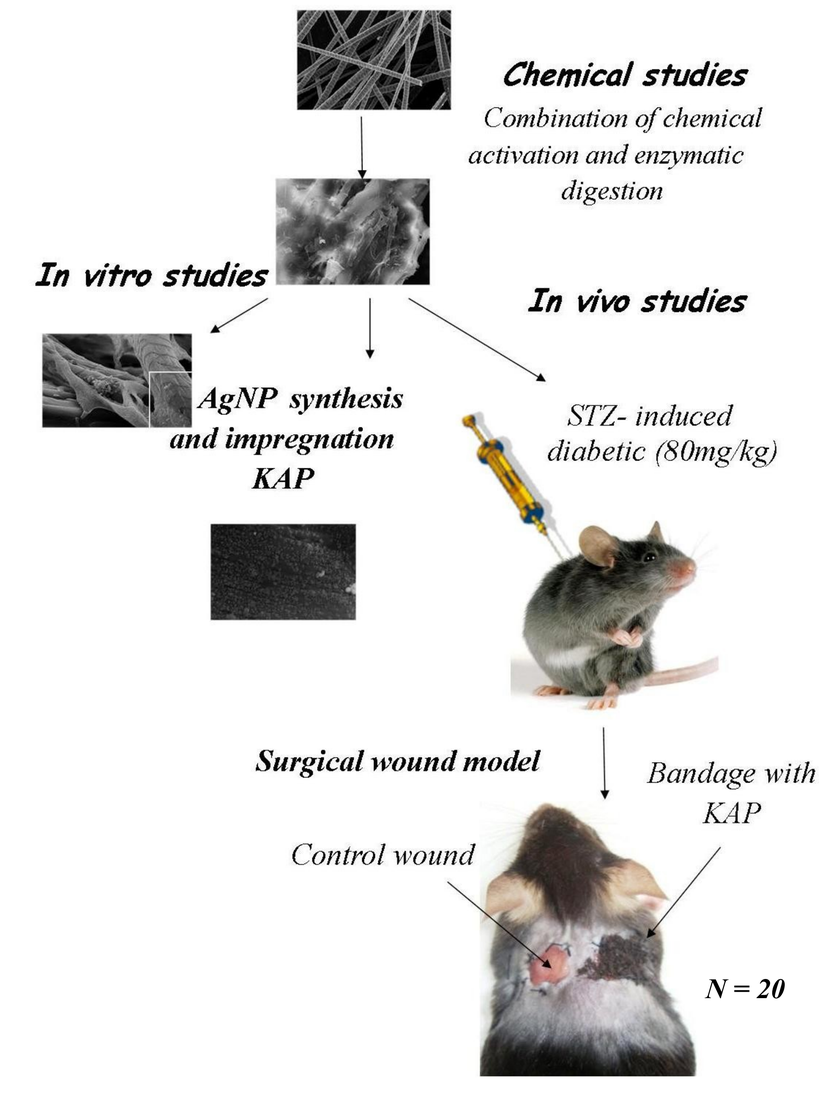

Fig. 1 *Schematic representation of the research.*

Scanning electron microscope

two-way ANOVA, followed by Bonferroni post tests (*** p<0.001)

JSM-6390LV (SEM) has been used to examine the shape and

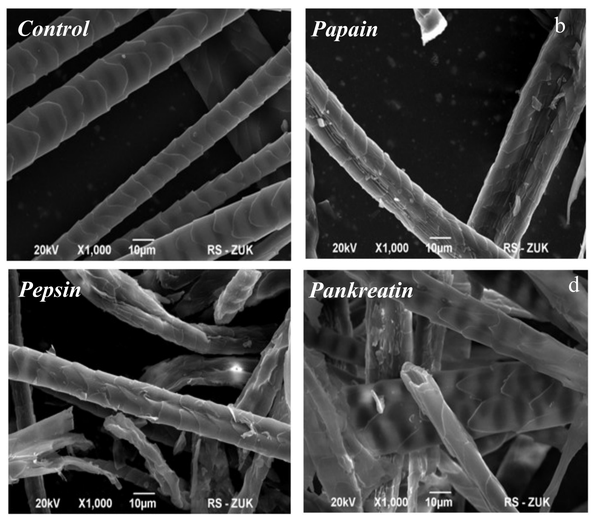

Fig. 2 *SEM images of mice-KAP's (control (a) and digested by: papain (b), pepsin (c), pankreatin (d) .*

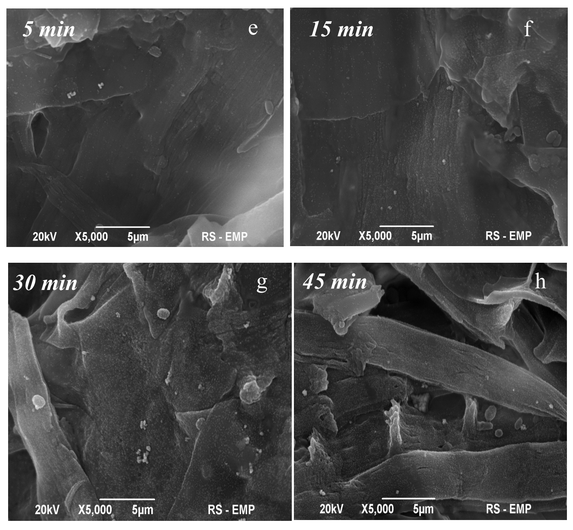

Fig. 3 *SEM images (e-h) of m-KAP's impregnate by AgNP .*

Results

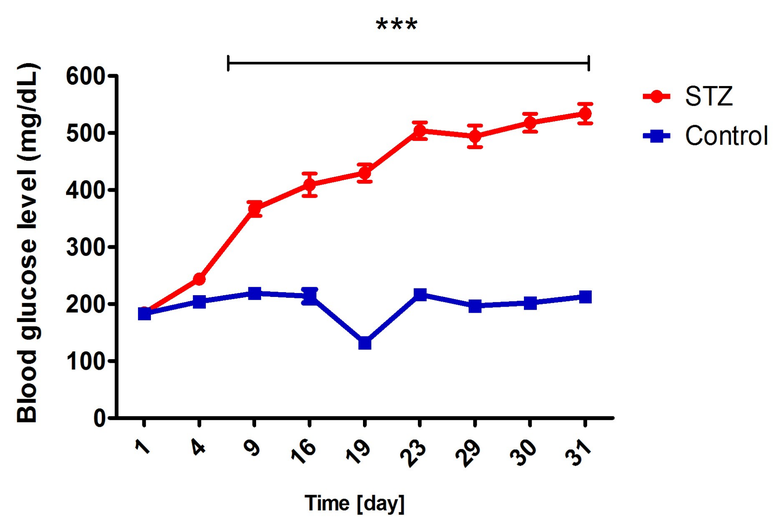

*Fig. 4 Schematic representation of the time ocures of multiple, low-dose STZ-induced diabets in mice (*two-way ANOVA, followed by Bonferroni post tests (*** p<0.001).

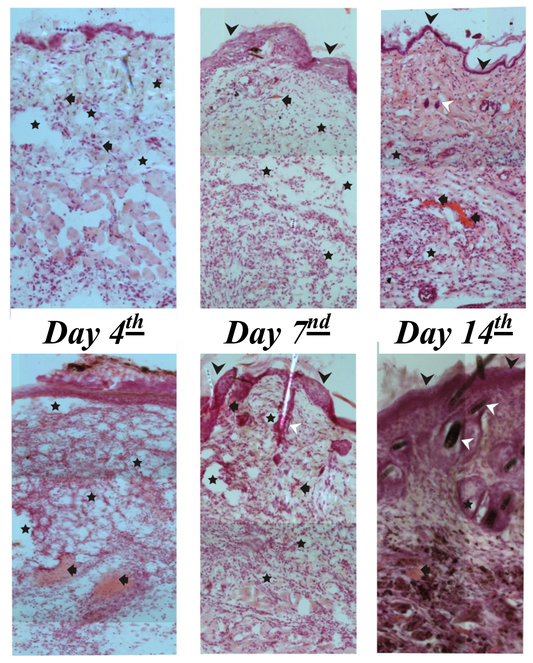

Fig. 5 Histological tissue sample derived from control (upper panel) and m-KAP-treated (down panel) wound stained by H&E.

Conclusions

- SEM results show that KAP's with highest porosity was obtained after digestion by pepsin, future studies this type of scaffold were taken.
- SEM measurements show that silver nanoparticles stick to the KAP's material, and form aggregate, bandages impregnate by it exhibit antimicrobial properties.

Preliminary in vivo studies:

- STZ in dose 80mg/kg is optimal for induce diabetic state during whole experiment.
- During healing process bio-dressing was incorporated into restored tissue.
- Applied KAP's bandage exhibit good biocompatibility with "patient" tissue, and changing them is a painless, relative to conventional dressings. KAP's dressing well absorbs exoduses, while maintaining a moist environment inside the wound area.
- During application KAP's dressing wasn't observed an exacerbation or inflammation of the wound tissue.
- KAP's bandage can be a scaffold for cells migrating from the edge and deeper layers of the wound minimizing formation of scar tissue.

References:

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