28. Summary

Occurrence of hard-to-heal wound is very frequent especially in association with diseases that is why the wound care specialists are interested in rapid tissue reparation. Generally, in the wound treatment moist interactive wound healing is preferred. It is represented by various dressing products with appropriate properties. This kind of dressing helps to create moist wound environment and optimal pH, supports protection against infection, facilitates elimination of inflammatory cytokines and an increase of the local growth factors concentration. Hydrophilic materials such as carboxycellulose or other saccharides have been launched into the market as individual material or in combination e.g. with collagen. Modern textile manufacturing provides various layered textile forms exhibiting antimicrobial properties. Moreover, these materials are able to remove excess exudate from wound and support cell migration and proliferation, and moreover extracellular matrix formation together with angiogenesis which result in optimal healing.

A new substance, a patent of Alltracel laboratories (Ireland, Dublin) microdispersed oxidised cellulose (MDOC™), was employed in our study. MDOC™ is a random copolymer of polyanhydroglucose and polyanhydroglucuronic acid (PAGA) which was identified to be effectivelly used as a biocompatible and completely absorbable haemostatic agent. This material exhibits low toxicity and is usually used in the powder, spray, gel or textile form. No acid character is manifested due to COO^- neutralization by Ca^{2+}/Na^+ ions. MDOC™ forms in water the colloidal disperse system or gel. Further it can be also used as a polymeric ion or drug carrier (e. g. antibiotics), which was also explored in our project.

An experimental Apo-E ($^{-/-}$) deficient mouse model of atherosclerosis revealed that short term administration of MDOC™ in the diet possesses mild an antiinflammatory and hypolipidemic effect. From the point of atherogenesis especially adhesion molecule were studied because these molecules are important for transendothelial leukocyte migration and for the maintenance of normal endothelial permeability. So the aim of our next project was detailed exploratory study of lipid spectrum and adhesive molecules expression (VCAM – 1 and ICAM – 1) after the MDOC™ administration in the diet in the apo-E deficient ($^{-/-}$) mouse model of atherosclerosis. Moreover, we investigated the possible hypolipidemic mechanism of MDOC™ action

For the better understanding this dissertation thesis was divided into the part dealing with locally applied MDOC™ effects on the wound healing process employing the rat and pig model and into another part concerning orally applied MDOC™ effects on the lipid spectrum and atherogenesis in the mouse model of atherosclerosis.

The particular aims of this dissertation thesis were:

1. Investigate an effect of locally applied MDOC™ on acute cutaneous wound healing by light microscopy in the experimental rat model.
2. Compare the efficiency of various MDOC™ forms (powder, spray, gel, and textile variant).
3. Explore the expression of proinflammatory markers in the wound by immunostaining technique.
4. Create a pig model of infected wound healing (*Staph. aureus, Ps. aeruginosa, E. coli*) and investigate changes in the healing process after the locally PAGA application in combination with ATB gentamicine by light microscopy.
5. Investigate the potential hypolipidemic effects of MDOC™ on atherogenesis in the apo-E deficient mouse model employing lipid spectrum analysis in blood and additionally carry out an expression analysis of cell adhesion molecule VCAM-1 in atherosclerotic plaques by immunostaining and stereology techniques.
6. Find out the MDOC™ mechanism of possible hypolipidemic effect and determine whether MDOC™ possesses action as a soluble dietary fiber.

In the experimental rat model we studied the locally applied MDOC™ effect on wound healing process. For skin changes detection and identification was used gross pathology, specifically for wound contraction observation. Moreover, light microscopy with basic staining was used for detection of cell localization and, abundance, granulation tissue properties or collagen mass, and finally we used immunohistochemistry technique for detection of specific markers (TNFRI, CCR2, TGF-β RII) expression changes in all phases of wound healing. However our results (obtained the third, seventh, and fourteenth day after injury) did not show any significant differences after the application of any form of MDOC™. Mild difference was observed when applying the powder and gel into the wound. In this case prolonged wound contraction but without any influence on the final scar formation was revealed. Studied substance showed haemostatic action at the beginning of experiment which might positively affect the subsequent healing phases. Localization of wound healing infiltrate (remarkable reaction) involving accumulation of neutrophils, lymphocytes and macrophages

after the MDOC™ application on the border between hemorrhagic crust and the dermis, indicated their important role in the acute wound healing phase. Mass of macrophages in the dermis were detected in the seventh day after the injury treated with the textile MDOC™ form application, which can be explained by the chemoattractive properties of any oxidised cellulose. The maximum of epidermis recreation with abundant presence of epidermal cells was seen when the MDOC™ powder form was used. Moreover, we revealed a mass of fibroblasts with organized collagen fibers in the extracellular matrix in the control group during the seventh day after the injury, while in the MDOC™ group was this observation apparent till the fourteenth day.

Immunohistochemical analysis of specific proinflammatory markers did not also confirmed any significant influence of MDOC™ substance on the wound healing process. In accordance with the similar analysis published it was proven that TNFRI expression is visible in macrophages, lymphocytes and endothelial cell during the acute healing phase in the third, seventh day in both MDOC™ treated group and the control group. Expression of TNFRI was also detected in the migrating basal keratinocytes in the ECM during the fourteenth day, in the proliferating fibroblasts without any distinct intensity in immunohistochemical staining comparing MDOC™ and control groups. Moreover, we confirmed presence of TGF-β RII in the third day post injury in the MDOC™ treated group, whereas the most intensity of inflammatory cells reaction on the border between hemorrhagic coagulum and granulation tissue, further, TGF-β RII was more expressed in the fibroblasts and endothelial cells of dilated vessels. During the wound healing process TGF-β RII was also expressed by the inflammatory cells in the MDOC™ group and more intensively by the fibroblasts in the control group. In the fourteenth day this factor was also confirmed in the control group expressed by fibroblasts and additionally by epidermal cells in both groups, and in the hair follicles of intact tissue. CCR2 expression was captured in the inflammatory cells of the control group on the border of crust. Seventh and fourteenth day CCR2 expression was more pronounced in the epidermal and endothelial cells more of MDOC™ group.

PAGA with attached antibiotic gentamicine (kindly provided by Alltracel lab, Ireland) or already established Garamycin Schwamm® used as a sort of positive control, were tested in the pilot wound healing study on porcine infectious wound healing model. Wounds were created on the backs of pigs, infected with experimental inoculum of *P. aeruginosa, S. aureus and E. coli* and covered with studied products. Gross analysis confirmed main positive benefit of PAGA substance, which meant biocompatibility and completely resorption in the seventh day after the operation. In the control group with Garamycin Schwamm® prolonged

resorption was visible mainly in the infected wounds with signs of infection mainly the seventh day after the injury. However, light microscopy showed the granulation tissue formation, accumulation of proinflammatory cells infiltrate (mainly macrophages) in the centre and in the wound surroundings, as well as the high rate of epithelialization, angiogenesis and fibroplasia, but these results did not confirmed any wound healing progression in comparison with the control group. Statistical analysis of microbiology results acquired by CFU counting from primo cultures from seventh day after the operation did not show any significant differences among the applied microbes. Only in the *E. coli* group, both PAGA and Garamycin Schwamm® showed mild effect on infection elimination. Microbiological findings indicate the secondary wound contamination probably caused by insufficient gaps between the wounds. However other investigations must be completed to clarify precisely the effects of PAGA with attached gentamicine, e.g. studies focused on antibiotic dose precise identification, technological textile PAGA form modification or experimental conditions adjustment (bigger wound distances or changing material in the wound with respect to maintenance moist microenvironment).

After the short term administration of MDOC™ in the diet (50mg/kg/day) in the C57BL/6J apo-E deficient mouse model of atherosclerosis, was revealed only mild hypolipidemic effect. Total cholesterol level was slightly influenced and VLDL and LDL levels were not significantly changed when compared to control group with administration of standard diet. Stereological analysis of immunohistochemistry staining revealed significant decreasing of endothelial VCAM-1 and ICAM-1 expression. We concluded that this effect could be probably marked after the long term MDOC™ administration, because the lipid metabolism in mice has slower turn-over, so fourth weeks administration was insufficient.

The results from apo-E/LDLRKO deficient mice showed that administration of 5% MDOC™ for eight weeks in atherogenic diet led to a significant decrease of total cholesterol and VLDL level, and increase of HDL level compared to control group. Analysis of acquired results from apo-E deficient C57BL/6J mice showed that administration of 5% MDOC™ for eight weeks with an atherogenic diet resulted in a significant decrease of total cholesterol and TAG level when compared to control group. However, total cholesterol and TAG level decrease did not influence the size of atherosclerotic lesions. Stereology also showed insignificant VCAM-1 expression after the MDOC™ administration. The aim of our next studies was to find out the possible mechanism of hypolipidemic action of MDOC™ and whether MDOC™ possesses effect as a soluble dietary fiber. Determination of MDOC™ influence on cholesterol absorption in the small intestine in C57BL/6J mice showed that this

substance did not affect this process compared with positive control (Ezetimibe group). Results also showed that neither MDOC™ nor pectin (control group as a soluble fiber) affected bile acid elimination in cholesterol-fed C57BL/6J mice. Contrary, we demonstrated that MDOC™ and pectin significantly decrease cholesterol content in the liver when compared with control animals. *In vivo* analysis revealed increased fermentation in the large intestine after the MDOC™ administration. Moreover, *in vitro* experiment showed that MDOC™ is a fermentable substance under the conditions mimicking the conditions in the large intestine. MDOC™ probably acts increase the SCFAs production, which in turns inhibits the synthesis of cholesterol in the liver. This mechanism of action is of MDOC™ may be species-/strain-specific to mice, and therefore other studies with MDOC™ in different species, e.g., rats or guinea pigs, have to confirmed to reveal other possible mechanism of hypolipidemic effect of MDOC™.