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BIOCOMPATIBILITY AND EFFECTS OF A COMPOSITE NANOMATERIAL CONTAINING SILVER

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Abstract. Our previous studies revealed the ability of composite materials based on Ag nanoparticles to induce hair follicle regeneration. As a step forward in this research, the present study investigates the biocompatibility of composite materials consisting of polyurethane-urea nanofibers functionalized with silver nanoparticles by subcutaneous implantation in rabbits. In the same time, we recorded the influence of these materials on hair follicles. Macroscopically we noticed acceleration of surgical wound cicatrisation and of hair growth in the area located above the composite material implant. Microscopic analysis revealed that the inflammatory response produced by implantation was minimal, the nanofibrilar matrix serving as support for the multiplying of young fibroblasts, and above it the hair follicles proliferate in the hypodermis and dermis. The biological effects suggest that these composite materials could be used as dressings for teguments covered with hair.
Keywords: polyurethane-urea nanofibers, silver nanoparticles, hair follicles

Introduction

The antimicrobial, anti-inflammatory and antiallergic activity of silver, in the form of ions and metal nanoparticles is well-known [1, 2]. The present study is a continuation of our previous investigations, which suggested that subcutaneous implantation of bulk membranes made of polyurethane-urea functionalized with silver nanoparticles are able to induce stimulation of hair follicle stem cells [3, 4, 5, 6]. In this work we investigate the biocompatibility and hair follicles generation effects of nanofibers functionalized with silver nanoparticles.

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Materials and methods

The non woven nanofiber membranes were prepared by electrospinning using a solution of polyurethane-urea in dimethylformamide with a concentration of 12% polymer. The silver nanoparticles were obtained during the polymer synthesis stage. The polyurethane macromolecules acted as dimensional stabilizers. The nanofibers' morphology analysis performed through atomic force microscopy (AFM) (figure 1) showed that the resulting nanofibers have diameters in the range 200–270 nanometers, and are arranged in a three-dimensional structure with pores of 2-4 micrometers, with three-dimensional connectivity which is ideal for supporting and developing oriented cells growth. The silver nanoparticles' (figure 2) dimensional distribution was centered around 10 nanometers, which is known as limit for virucide [7] and probably regenerative [3, 4, 5, 6] effects.

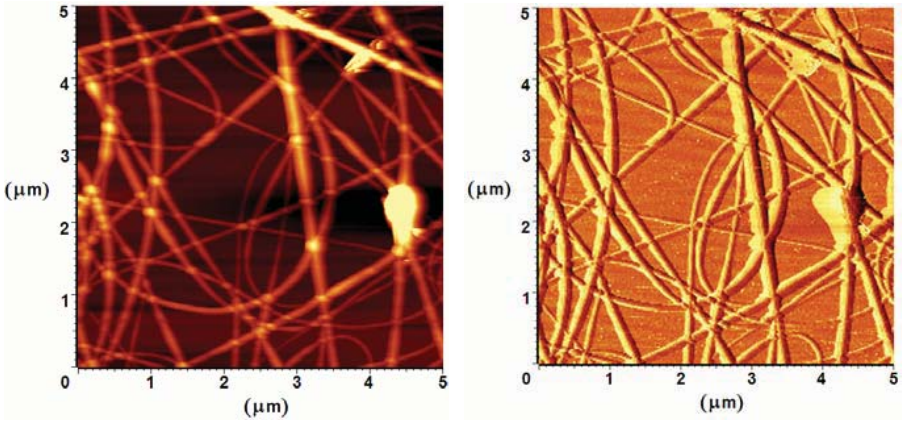

Figure 1. Atomic force microscopy (AFM) image of polyurethane-urea nanofibers: topography (left), phase (right)

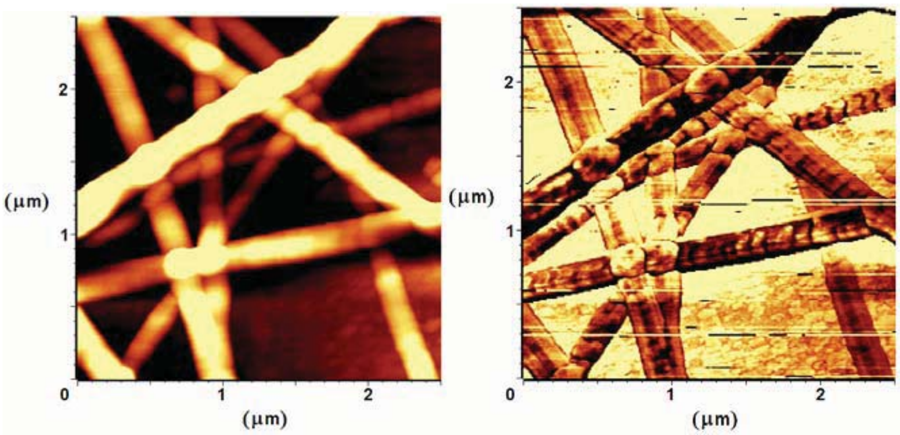

Figure 2. AFM image of polyurethane-urea nanofibers functionalized with silver nanoparticles: topography (left), phase (right)

Investigations were performed on 12 male New-Zealand rabbits, age 2, on which skin implants in the lumbar area were performed. Six of these rabbits were implanted with polyurethane-urea nanofiber non-woven samples of 1x1 cm^2 and 0.30 mm thick, and the rest with samples of composite material made of polyurethane-urea functionalized with silver nanoparticles. Above the implant region the hair was removed by cutting on a surface of 5x5 cm^2. The implantation operation was performed under anesthesia with procaine according to animal protection regulations. The samples were placed subcutaneously, in lateral positions with respect to the skin incision point. After 2 weeks of clinical observation of the animals, the samples were extracted together with the surrounding tissues under the action of the same anesthetic. The composite material samples with tissue fragments were fixed in neutral formalin (15% concentration), then processed through inclusion in paraffin, followed by microtome cutting in sections of 4 μm and stained using Hematoxylin-Eosin (HE) for microscopic analysis.

Results

Macroscopically, the healing of surgical wounds occurred more rapidly in the animals implanted with composite material samples made of polyurethane-urea nanofibers functionalized with silver nanoparticles compared to those implanted with polyurethane-urea nanofibrilar material only (11 to 14 days). Moreover, at the exact place of skin tumescence produced by the subcutaneous implant of the silver nanoparticles doped material the hair grew at a speed much higher than the rest of the depilated region (figure 3).

Microscopic examination of the histological sections revealed that the inflammatory reaction produced by the nanofiber material doped with silver nanoparticles was minimal (figure 4), while the nanofibers made out of the original polyurethane-urea caused intense rejection reaction (figure 6). The implant of polyurethane-urea functionalized with silver nanoparticles was infiltrated predominantly with young fibroblasts (round shape), presenting large dislocations produced by wide regions of

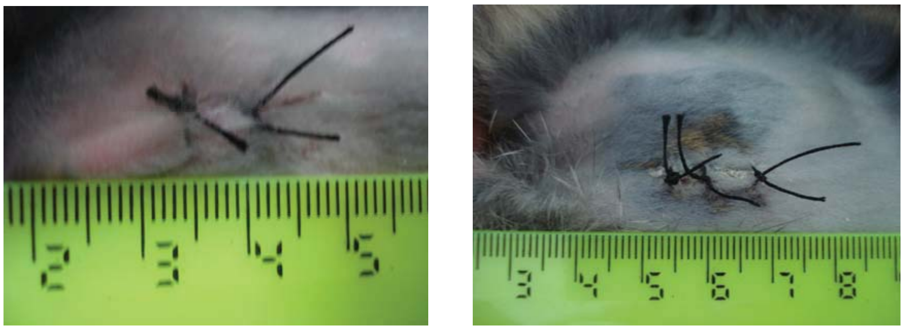

Figure 3. The macroscopic aspect of the area where a silver doped polyurethane-urea nanofiber sample was implanted, immediately after the operation (left) and after two weeks (right)

neoformed connective vascular tissue with lymphocytes, predominantly adult oblong fibroblasts, and foreign-body giant cells (figure 5).

Microscopic analysis showed numerous hair follicles above the implant area, located in the dermis and hypodermis, unlike in the neighboring regions where the hair follicles of predominantly lower density were found in the dermis (figure 4).

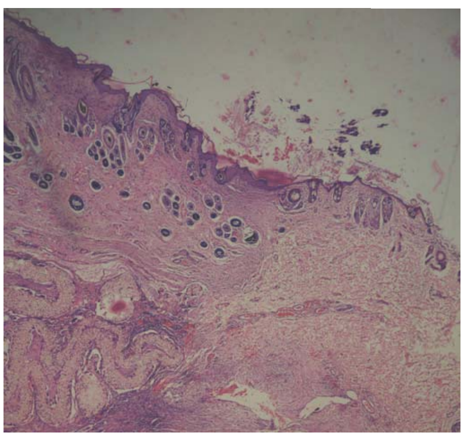

Figure 4. Hair follicles proliferation in derma and hypoderma located above the an implant made of polyurethane-urea/silver nanoparticles composite material. Histological section (HEx4)

Discussion

Given that at the contact areas between the nanofibrilar material doped with silver nanoparticles with the skin layers the histological expression of the inflammatory reaction is low (figure 4), the silver playing an anti-inflammatory role [1], it can be considered that the young fibroblasts proliferate inside the nanofibrous matrix (figure 5). Moreover, this effect has been found by our research team also in the case of intramuscular implants with this material [8]. After a hypothesis launched by Robert Becker in 1985, the regenerative action of silver catalyzed iontophoresis is due to fibroblasts' dedifferentiation, the non-differentiated cells thus created being turned into various cell types depending on the tissue [9]. According to this hypothesis, the proliferation of young fibroblasts inside the silver doped polyurethane-urea nanofibers matrix and their dedifferentiation could explain the abundance of hair follicles that seem to emerge from the material (figure 4). These hair follicles contain stem cells that can turn into keratinocytes that help skin regeneration, or into other hair follicles [7, 10]. On the other hand, in the cicatrisation process,

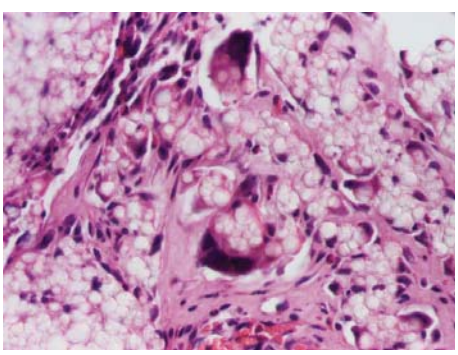

Figure 5. Young fibroblasts proliferation between polyurethane-urea nanofibres functionalized with silver nanoparticles. Histological section (Hex200)

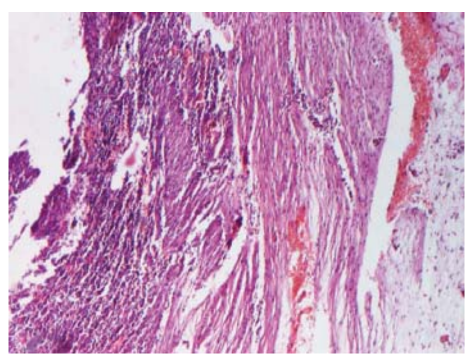

Figure 6. Inflammatory reaction produced by subcutaneous implantation of a polyurethane-urea nanofiber membrane sample. Histological section (Hex40)

the dermal cells can develop not only from mesenchymal stem cells but also from fibroblasts [11]. In comparison with the silver doped composites which we previously used for experimental purposes for their regenerative effect [6, 7, 8], this material presents the advantage of a nanofibrilar matrix which serves as support for fibroblasts proliferation, which are cells with essential role in healing. In addition, the silver nanoparticles included in composite materials confer bactericidal properties [12]. Given the anti-inflammatory, bactericidal and regenerative properties, and the property of stimulating hair follicles' proliferation, we appreciate that this composite material made of polyurethane-urea nanofibers functionalized with silver nanoparticles is indicated for the production of dressings designed for teguments covered with hair.

Conclusions

1. Subcutaneous implantation in rabbits consisting of a composite material made of polyurethane-urea nanofibers functionalized with silver nanoparticles determines an acceleration of surgical wounds' cicatrisation and of hair growth in the area located above the implant, which is macroscopically evident.
2. Histological analysis revealed that this composite material produces a minimal inflammatory reaction and that the nanofiber matrix serves as substrate for young fibroblasts' proliferation, which are essential cells in the healing process.
3. Microscopic examination showed a wealth of hair follicles - reservoirs of stem cells that can differentiate into keratinocytes and other hair follicles - in the dermis and hypodermis above the implant, compared to neighboring areas, where hair follicles were found in a lower density, predominantly in the dermis.
4. The biological effects of this composite material recommend it as a dressing for teguments covered with hair.

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