In vivo study of the nanofiber-based composite wound dressing intended for treatment of deep skin wounds

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Summary

Electrospinning technique was used for preparation of two-layer wound dressing consisting of a layer of aliphatic copolyamide nanofibers and a layer including chitosan nanofibers and chitin nanofibrils. Experimental studies (*in vivo* treatment of deep skin wounds with this dressing) demonstrated that complete epithelialization of wound surface was achieved after 28 days. Histological analysis of scar tissue indicated the presence of insignificant amount of capillaries and low amount of infiltrate cells. The survival of animals was 100%. At the same time, in the control group of animals, the lethality was observed in 10% of cases, and suppurative complications were observed in 100% of cases.

Riassunto

Attraverso l'elettrofilatura è stata realizzata una nuova medicazione avanzata costituita da due strati; uno esterno formato da nanofibre di copoliammide alifatica ed uno interno basato su nanofibre di chitosano e nanofibrille di chitina. I nostri studi sperimentali in vivo (trattamento di ferite profonde con tale medicazione) hanno dimostrato la completa riepitelizzazione della ferita in 28 giorni.

Le analisi istologiche condotte sul tessuto cicatriziale hanno posto in evidenza una presenza non significativa di capillari e un basso quantitativo di infiltrati cellulari.

La sopravvivenza degli animali è stata del 100%. Al contrario, nel gruppo di controllo è stata verificata una mortalità del 10% accompagnata da complicanze suppurative pari al 100%.

INTRODUCTION

Nowadays, healing of skin and soft tissue wounds is a topical problem in cosmetology. The main factors which inhibit epithelialization and granulation processes are tissue dystrophy, oxidative damage, moisture imbalance in wound, infections and other complications in the area of surgical resection, trauma or burn. Formation of scar tissue or other structural defects at the wound site reduces a patient's quality of life. Currently, there are a number of techniques which allow intensification of wound healing process. In the majority of these methods, wound dressings of various compositions and structures are used (1-3).

Wound dressing should facilitate the optimal gas and moisture exchange which is necessary for cell vital activity, should reproduce wound surface relief, i.e. possess elasticity and provide ease of surgical manipulations. Another important property of modern wound dressings is atraumaticity; that is, the formed epithelium layer should not be traumatized or destroyed during removal of a dressing from wound. Finally, a dressing should prevent wound infection (invasion of pathogenic microflora from external medium).

These properties can be achieved in porous type films based which are obtained by electrospinning of polymeric solvents. This method allows preparing fibers with diameters ranging from tens to hundreds of nanometers from various polymers. Nanofiber-based films possess low density, high porosity, water permeability and gas permeability (4-7). Recently, these porous films have found practical use as matrices for cell technologies. Chemical structure and porosity of these materials facilitate adhesion of stem or somatic cells onto fiber surface, and promote metabolic processes which are necessary for efficient cell proliferation and differentiation. Preparation of nanofibers from alcohol-soluble aliphatic copolyamide (CoPA) (copolymer of poly(ε-caprolactam) [-NH-(CH2)5-CO-]n and poly(hexamethylene diamine adipinate) [-NH(CH2)6NHCO(CH2)4CO-]n) is described in (5). Good adhesion of stem cells and the absence of cytotoxicity were demonstrated.

Chitosan is another polymer which is widely used in developing materials for medicine. This biocompatible and biodegradable polysaccharide consists of β-(1-4)-D-glucosamine and N-acetyl-D-glucosamine units (8). Products of chitosan biodegradation are non-toxic; as decomposition proceeds, these products are involved in usual metabolic reactions of organism. However, it is known (8-10) that processing fibers from chitosan solutions by electrospinning is difficult, since this polymer is a polyelectrolyte. For preparation of chitosan-based fibers by electrospinning, water-soluble polymers, e.g. poly(ethylene oxide), poly(vinyl alcohol), methyl cellulose, poly(vinyl pyrrolidone), are introduced into aqueous solution of acetic acid. Addition of these polymers (up to 50 wt.% with respect to mass of chitosan) has a detrimental effect on properties of the end product, e.g. leads to increase in hygroscopicity and deterioration in mechanical properties.

Some authors (11-13) described a patented method of preparing chitin nanofibrils intended for use in cosmetology. Chitin nanofibrils were used as a filler in chitosan-based composite fibers obtained by wet spinning (14) also. It was demonstrated that the chitosan-based composite fibers containing chitin nanofibrils with a diameter of 20 nm and a length ranging from 600 to 800 nm possess enhanced mechanical strength and elasticity. Besides, introducing chitin nanofibrils into chitosan spinning solution stabilizes the spinning process.

Properties and structure of the composite nanofibers obtained by electrospinning and containing chitin nanofibrils as a filler are described in (15). It was established that introducing chitin nanofi-

brils into chitosan solution leads to significant increase in the rate of nanofiber formation, and results in the formation of materials with lesser amount of defects. In addition, introducing chitin nanofibrils into chitosan-based composite nanofiber allowed lowering the necessary amount of poly(ethylene oxide) (PEO), which is water-soluble polymer added into solution in order to facilitate formation of fibrous structures during electrospinning.

In the present work, we suggest the method for preparation of the composite wound dressing consisting of a layer of CoPA-based nanofibers and a layer of composite nanofibers including chitosan and chitin nanofibrils. Nanofibers from non-resorptive CoPA impart the necessary mechanical properties to wound dressing, facilitate metabolism between a wound and external environment and create a barrier for pathogenic microflora. The layer of chitosan nanofibers and chitin nanofibrils which is in direct contact with wound surface simultaneously affords hemostatic and bactericidal action and atraumaticity of dressing. In the process of integration with active biological medium, gradual resorption of chitosan and chitin nanofibrils occurs. After removing dressing, non-resorptive CoPA layer is separated from wound surface, while the chitin-chitosan layer remains and facilitates epithelialization of a wound.

The aim of the present work was development of the wound dressing based on CoPA nanofibers and composite nanofibers containing chitosan and chitin nanofibrils as well as *in vivo* studies of these materials as wound dressings for treatment of deep skin wounds.

MATERIALS & METHODS

The materials based on CoPA and chitosan nanofibers were obtained from polymeric solutions by electrospinning (5). The method of processing composite nanofibers from chitosan and fillers (chitin nanofibrils), and the properties of these materials are described in (16). Films were prepared by electrospinning of composite chitosan-based nanofibers onto the surface of porous film made from CoPA nanofibers. The thickness of CoPA nanofibers was 150 μm, and the thickness of the layer of composite nanofibers was 50 μm. Electrospinning was performed using a NANON 01 instrument (Japan) at a voltage of 18 kV; the distance between electrodes was 0.15m.

The structure of nanofibers and nanofiber-based materials was studied by scanning electron microscopy. The measurements were carried out using a Supra 55VP electronic microscope (Carl Zeiss, Germany).

Composite wound dressings based on nanofibers were tested on scalped wounds of skin. For this purpose, the model of a full-thickness skin wound in small laboratory animals (rats) was developed. The incision in the back of an animal was made with a scalpel; then separation of skin from surface fascia was performed with the use of scissors and surgical tweezers (Fig. 1a). Skin edges of a wound were fixed with interrupted musculocutaneous inverting sutures to its own fascia (Fig. 1b). Diethyl ether was used for inhalation anesthesia in animals. This method allows to avoid early wound closing by primary intention and to reliably assess efficiency of wound treatment preparations.

Eighteen animals were used in the experiments (Wistar-Kyoto male rats, mass 200-250 g). The animals were divided into two groups (the control group and the experimental group, each including 9 animals). In the experimental group, after modeling full-thickness mechanical skin wound (10% from the animal surface area), wound surface was covered with chitosan/copolyamide dressings. The animals were observed for four weeks.

In 28 days, the experiments were completed; tissue samples were taken from wound area, and

morphological studies of this tissue were carried out. Tissue samples were fixed in 10% solution of neutral formalin in phosphate buffer (pH = 7.4) for 24 hrs, then treated with ethanol solutions of increasing concentrations and embedded in paraffin. Paraffin sections (5 μm thick) were dyed with hematoxylin and eosin (Bio-Optica, Italy). Microscopy analysis and registration of images were performed with the use of a Leica DM750 light microscope (Germany).

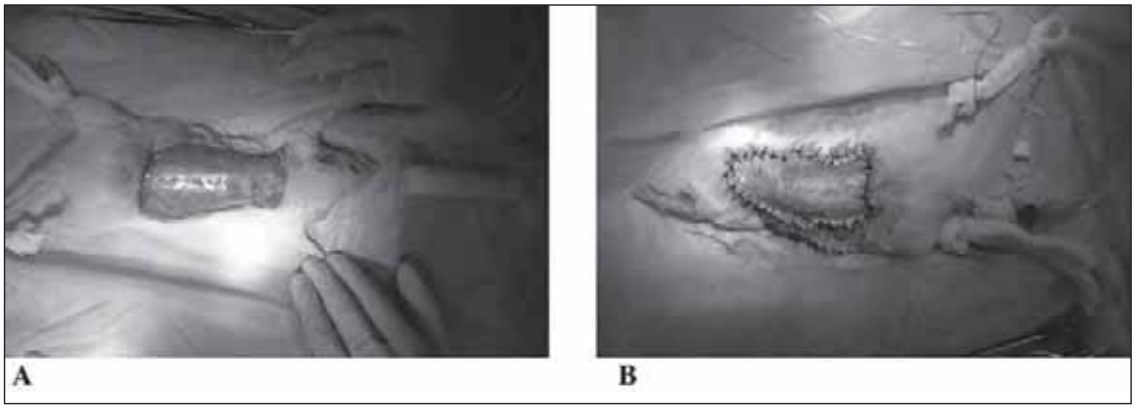

Fig. 1 *Formation of a scalped wound in rat: before suturing (a); after suturing (b).*

RESULTS AND DISCUSSION

Figure 2 presents microphotographs of CoPA nanofibers and chitosan-based composite nanofibers containing 20 wt.% of chitin nanofibrils. It can be seen that the average diameter of CoPA fibers is about 600 nm, and the average diameter of chitosan-based composite fibers is 150 nm.

Two-layer composite material was applied to wounds in such a way that the layer containing nanofibers of chitosan and chitin nanofibrils was in direct contact with a wound.

All animals from the experimental group, which were treated with the two-layer wound dressing, were alive in 28 days after beginning of the experiment; complete epithelialization of wound surface was observed in all cases (Fig. 3a). Scar areas were significantly smaller than those in the animals from the control group, which were not treated with the developed wound dressing (Fig. 3b). Histological studies of scar tissue demonstrated the presence of a small amount of capillaries and insignificant amount of infiltrate cells (Fig. 4).

Observation of the animals from the control group demonstrated that lethality occurred in 11% of cases; suppurative complications were registered in 100% of cases. In 11% of cases, large amounts of pus were accumulated under scar tissue (Fig. 3b). Histological studies of scar tissue (Fig. 4) revealed the presence of a large amount of capillaries and dense infiltrate.

Efficiency of using chitosan nanofibers is confirmed by the experiments in which we employed wound dressings prepared from CoPA nanofibers without the layer of composite nanofibers. In all animals with deep skin wounds which were treated with these dressings, large amounts of pus were accumulated under scars.

Morphological studies showed that in the animals of the control group, in the scar area, neutrophilous leukocytes prevailed over other infil-

trate cells; maturation of granulation tissue was less pronounced. This granulation tissue covers rather large area compared to that of the experimental group.

At the same time, only in some cases from the experimental group, complete epidermization of wound surface (Fig. 5) and formation of new connective tissue (Fig. 6) were observed; the connective tissue occupied rather small area in comparison to that of the control group. These morphological features may be indicative of more intense regeneration of wound surface in experimental animals as a result of using the developed wound dressing.

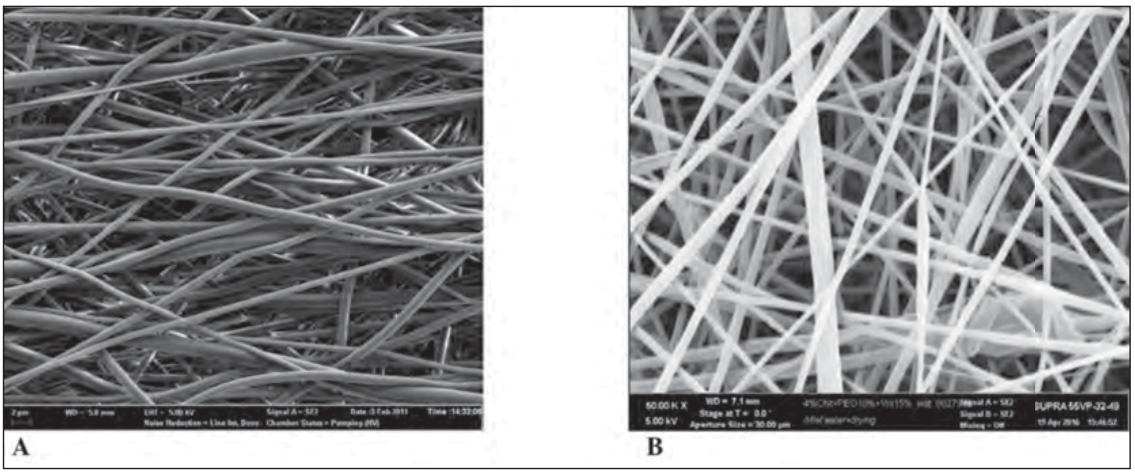

Fig. 2 *Microphotographs of CoPA fibers (a) and chitosan-based composite nanofibers containing chitin nanofibrils (b).*

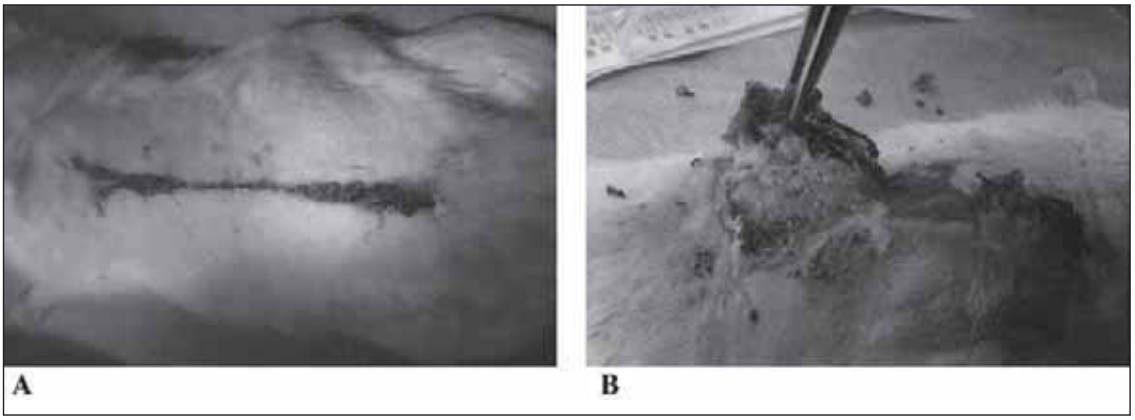

Fig. 3 *Visual appearance of the wound surface in an animal after applying the composite wound dressing (a) and in an animal from the control group (b).*

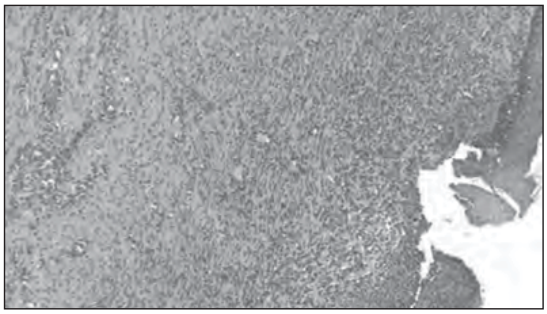

Fig. 4 *Histological view of the scar area of a rat from the control group (magnification X10).*

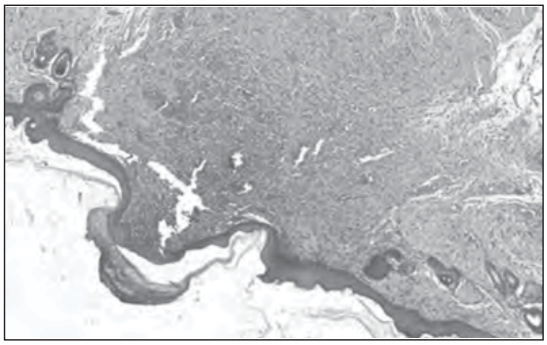

Fig. 5 *Histological view of the scar area of a rat from the experimental group (magnification X5).*

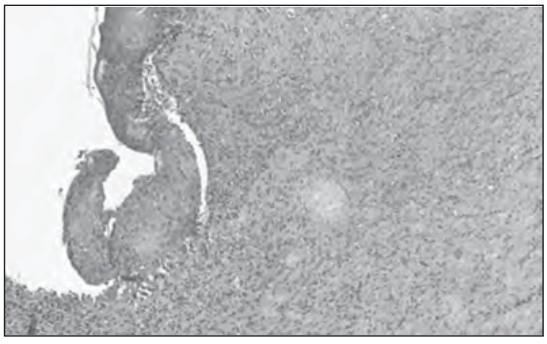

Fig. 6 *Histological view of the scar area of a rat from the experimental group (magnification X10).*

CONCLUSIONS

The two-layer wound dressing including a layer of fibers from non-resorptive polymer (aliphatic copolyamide) and a layer of chitosan nanofibers combined with chitin nanofibrils was developed. The *in vivo* experiments demonstrated high efficiency of this dressing in treatment of deep skin injuries.

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