Use of Carbonized Rise Shell for the Local Treatment of Wounds

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Abstract

On the model of musculocutaneous wound in rats, the effect of applicative sorption by carbonized rise shell (CRS) on the healing of festering wound was studied. It has been shown, that cytological changes end with rapid scar formation. The use of CRS at the period of severe purulent wound contributes to its favorable course, prevents the development of complications of the animals from sepsis.

Introduction

With the increasing interest to carbonized materials received on the basis of vegetative raw materials and their applications in biological and medical research, it has become a focus to investigate the influences of these materials on human cells and environment. Some number of *in vitro* studies has demonstrated that carbonized materials can induce cytotoxicity in relation to different cells and, still other studies reported that they are non-toxicity. The influences of carbonized materials on some other important cellular physiological events such as cell adhesion and migration are not well-defined.

Over the last decades, in connection with introduction of new highly effective sorption materials for medical purpose, sorbents began to be applied to treatment of purulent wounds.

High effectiveness of novel ways of wound clarification from microorganisms and their metabolites, necrotic tissues are well accepted. Methods such as elimination of devitalized tissues by drainage and sorption, proteolytic enzymes and new chemical antiseptics are actively studied. Necessity of such research is caused not only by the growth of number of pyoinflammatory diseases and postoperative purulent complications, but also deterioration of outcome of purulent surgical changes in etiological structure of purulent surgical infection, re-organization of biological characteristics of wound microflora, increase in its pathogenicity and resistance to effect of many antibacterial preparations. Therefore, questions of wounds and wound infections treatment remain in the main focus of practical doctors and scientists.

The main advantage of this method consists that the sorbent at direct contact with wound surface is capable to absorb toxic metabolites, microbic cells and bacterial toxins from wounds and wound cavities, showing antitoxic and antibacterial properties. This process promotes normalization of biological reactions of organism in response to damage.

It has been observed that application of sorbents allows to decrease the quantity of microorganisms in a wound 100-1000 fold in average, in comparison with traditional dressings.

The treatment by sorption of purulent wounds has a number of advantages, such as efficiency, profitability, availability, simplicity, possibility to influence a current wound process. Due to these advantages, researches in the field of new sorption materials development for medicine are rapidly increasing.

In this article the results of study of wound-healing effect of a new sorbent based on carbonized rice shell are presented. The sorbent was derived by

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high-temperature carbonization of rise shell at Institute of Combustion Problems of al-Farabi Kazakh National University under the guidance of professor Z.A. Mansurov.

In this study, human dermal fibroblasts from primary cultures are used as *in vitro* models to investigate their interactions with carbonized materials in terms of cell viability, adhesion and wound healing ability. The fibroblasts are used here because carbonized materials more close the skin and open wounds. The fibroblasts also play an important role in the cell renewing and would repair process.

Materials and methods

Due to properties of surface, CRS characterized as active sorbent and by electronic microscopy it was established that the nanoparticle surface has numerous pores and folds. The formation of nanostructural particles appeared when the temperature of carbonization was about 500-800ºC. Carbon materials are used in oil processing, petroleum chemistry, wine making, butter and fats production, etc. They are increasingly used in medicine, for example, to remove some toxic components from physiological liquids and to purify blood.

In vivo experiments were carried out on fifteen male rats with weight of 200-230g. On right and left-hand side of the top part of a back musculocutaneous wound in length - 2 cm and depth - 4 mm was made by medical scalpel. During the experiment the rats were kept in separate cages on a standard diet until their wound were fully healed. Two forms of preparations on the base of carbonized rice shell – powdered and suspension (in lanolin oil) were used.

Experiments were carried out in three variants (5 rats in each): 1 variant - A right-hand wound treated with powdered form of CRS and left side untreated (control); 2 variant- A right-hand wound treated with suspension form of CRS in lanolin oil, whereas left side treated only lanolin oil; 3 variant - Wounds for the purpose of formation of the artificial purulent environment infected with bacterial suspension (*Staphylococcus aureus 817)* and after 24 hours a right-hand wound treated with suspension CRS, left side - served as the control.

Wounds were daily photographed on a digital camera and with the soft ImageJ 1.35r (NIH, the USA) defined its area.

Primary human dermal fibroblasts (NHDF) were obtained from spare skin tissues of 22 years old patients with his consensus and were used *in vitro* between passages 5 and 13. The cells were maintained in regular growth medium consisting of high-glucose DMEM supplemented with 10 % fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a 5 % CO_2 humidified environment. To make the results more close to the physiological conditions, NHDF were suspended in growth medium supplemented with 10 % serum in all the cell relevant experiments.

The influence of the CRS on the cell viability was assessed using assay of microscopic count of viable cells. Briefly, cells in 100 μl medium were seeded into a well of a culture plate with grids and incubated overnight. Then, the cells were treated with CRS of variable concentrations (weight/volume of solution): 5, 10 and 50 μg/ml for 24 h, and maintained in fresh medium containing 0.5 mg/ml of MTT for another 4 h at 37ºC. The fibroblasts were washed with PBS, and then used Diff Quick method. After staining the cells were photographed under an optical microscope. The cell number was counted at 12 different areas. Data were averaged from three parallel experiments, which were normalized to that of the control.

For study the cell migration 70-80% confluent cells were treated with CRS of variable concentrations for 24 h, and then scraped with a pipette tip to create a linear wound. The culture wells were washed twice with PBS and incubated with fresh regular medium. The wound closure of cells was observed and photographed using an optical microscope at t=0h and after 6h, 12h, respectively.

Results and discussions

It was been observed that at animals of the experimental groups treated with active preparations (powdered form of CRS and CRS in lanolin oil) the general condition was significantly improved. During the experiment it was also observed that necrotizing tissues turned in porridge-like mass and easily removed at healing of wounds. Efficiency of treatment estimated on rate of disappearance of the basic symptoms (edema reduction, necrosis, pus, intoxication symptoms).

After 10 daily experiments it was observed that at rats of the first group the right-hand wound treated with powdered CRS was healed in 8 days (its length from 2 cm for 4 days was shorten to 1 cm, and for 6 days - to 0,5 cm) whereas left side (control) healed 10 days (Fig. 1).

Initial state 2 days 4 days 6 days 8 days (a)

Initial state 2 days 4 days 6 days 8 days 10 days (b)

Fig. 1. Dynamics of wound healing; (a) treated with powder of CRS and (b) control

Thus, application of powder of CRS on a wound surface lead to reduction of quantity of wound exudate and the reduction of tissue edema around wounds.

In following experiments wound healing activity of a suspension of CRS in lanolin oil was studied. Pure lanolin oil was used in a control variant.

According to the results, a right-hand wound treated with the suspension of CRS in lanolin oil was healed in 6 days (fig. 2, a) while healing of the wound treated with lanolin oil is observed only in 9 days (fig. 2, b). In Fig. 2 it is visible that the effect of suspension form of CRS reduces wound healing for three days in comparison to control (lanolin oil). Speed of wound defect healing at use of CRS in lanolin oil was registered both on volume, and on the area of wound surfaces.

Initial state 2 days 4 days 6 days 8 days (a)

Initial state 2 days 4 days 6 days 8 days 10 days (b)

Fig. 2. Dynamics of wound healing treated with (a) suspension form of CRS in lanolin oil and (b) with lanolin oil alone

Thus, efficiency of a preparation on the basis of CRS in lanolin oil is registered in the form of decrease in frequency of development of a purulent inflammation in wounds and accelerations of their clarification from wound detritus.

The wound of the third variant were preliminary infected with suspension of *Staphylococcus aureus 817* to make a purulent detritus. After 24 hours, the right-hand wound was treated with the preparation of CRS suspended in lanolin oil, left side wound was untreated and served as a control. Purulent wound surface of other group of rats was treated with powdered CRS.

Fig. 3 - Dynamics of wound healing infected with *Staphylococcus aureus 817* (a – treated with suspension form of CRS, b – control (untreated), c – treated with powdered form of CRS)

As it is shown in Fig. 3, wound healing of the infected *Staphylococcus aureus 817* suspension form of CRS has reduced the duration of healing for 2-3 days, whereas powdered form of CRS has reduced this period for 4-5 days. After 7-8 days, the processes of high-grade granulation was developed in wounds.

During experiments it was revealed that in studying medical effect of new kinds of sorbents on the basis of vegetative raw materials it is necessary to consider that various forms of preparations show efficiency in various situations; the powdered form is effective at treatment of purulent wounds, whereas the suspension form is effective at treatment of primary (pure) wounds.

There are wide variety of options in extensive wounds treatment and the choice depends on a phase of wound process. In a phase of regeneration, a leading role played by endothelium capillaries and fibroblasts. Fibroblasts, the main component of

granulation tissues forming collagenic fibres, provides healing (scarring) of a wound.

Microscopic count of viable cells by Diff Quick assay was used to investigate the cytotoxic effect of the CRS on fibroblasts (HNDF). As shown in Fig.4, after culturing with different concentrations of CRS for 24h, there was a dose-dependent decrease in viability with increasing dose of the CRS.

It was shown that the NHDF is less sensitive to the CRS stimulation.

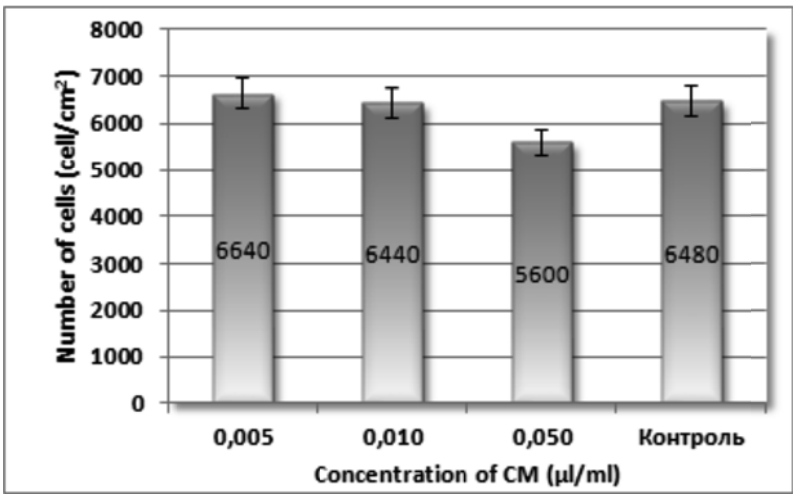

Fig 4. Relative viability of NHDF in the presence and absence of CRS

The results show that the morphologies, attachment and spreading behavior of the CRS-treated cells were similar to the untreated control cells.

To study the CRS influence the wound healing process, the fibroblasts were exposed to different concentrations of CRS for 24 h, and then scratched to form a linear wound. As shown in Fig.5, the untreated cells migrated into and recovered the exposed surface within a shorter time period.

The cells treated with 5 µm/ml CRS movement was the same like control. However, the cells treated with 10 µm/ml CRS moved slowly and needed longer closure time.

The current studies demonstrated that the CRS exposure caused no cytotoxicity on human skin fibroblasts. Dose-dependent decrease in cell viability along with the increase of CRS for NHDF was observed.

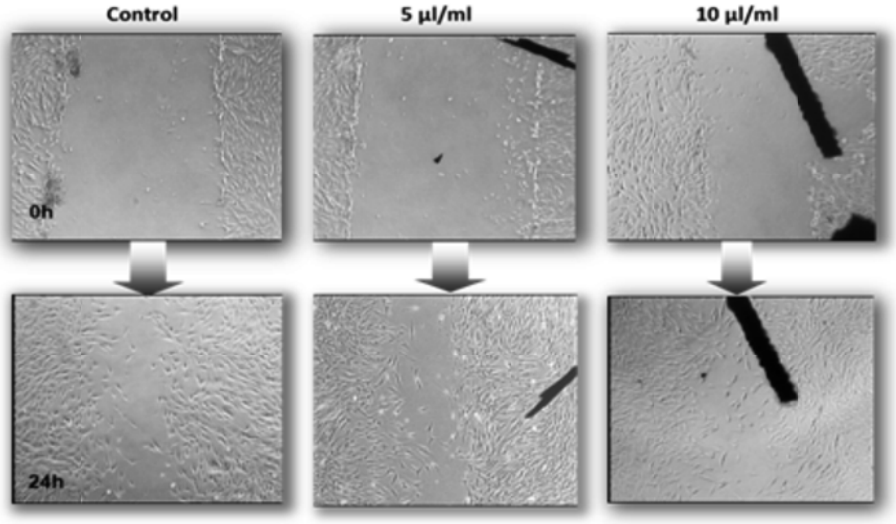

Fig 5. The wound healing process of NHDF observed by using an optical microscope

Cell migration is a central process in the development and maintenance. Tissue formation during wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations. In the initial period after injury, the fibroblasts from neighboring tissue proliferate and migrate into the wound area. According to the results of wound healing assay in this study, the migration ability of fibroblasts was not impaired after CRS treatment.

Conclusion

In the experiments on rats with musculo-cutaneous wound, it has been shown that the use of CRS provides timely haemostasis in the damaged tissues, prevents the development of traumatic edema, decreases the intensity of inflammatory process and facilitates rapid healing of wounds. The treatment-and-prophylactic effectiveness of CRS is associated with its ability for rapid and irreversible sorption of biologically active substances forming in the wound tissues, and as well with protection of a wound against secondary infection.

This study is very important for obtaining new medical preparations, which have a high wound-healing ability.

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