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# Wound Healing Activity of Carbonized Rice Husk

N.Sh. Akimbekov[1,a], Z.A. Mansurov[2,b], J. Jandosov[2,c], I.E. Digel[3,d], M.Gossmann[3,e], G.M. Artmann[3,f], A. Temiz Artmann[3,g], A.A. Zhubanova[1,h]

[1]al-Farabi Kazakh National University, 71 al-Farabi av., 050039, Almaty, Kazakhstan

[2]Institute of Combustion Problems, 172 Bogenbai Batyr St., 050012, Almaty, Kazakhstan

[3]Aachen University of Applied Sciences, 1 Heinrich-Mussmann St., 52428, Julich, Germany

[a]akimbeknur@gmail.com, [b] ZMansurov@kaznu.kz, [c] jandosovj@gmail.com, [d] digel@fh-aachen.de, [e]gossmann@fh-aachen.de, [f]artmann@fh-aachen.de, [g]a.artmann@fh-aachen.de, [h]Azhar_1941@mail.ru

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**Abstract.** The carbonized rice husk (CRH) was evaluated for its wound healing activity in rats using excision models. In this study, the influences of CRH on wound healing in rat skin *in vivo* and cellular behavior of human dermal fibroblasts *in vitro* were investigated. The obtained results showed that the CRH treatment promoted wound epithelization in rats and exhibited moderate inhibition of cell proliferation *in vitro*. CRH with lanolin oil treated wounds were found to epithelize faster as compared to controls.

## Introduction

The use of ashes for the treatment of skin diseases is an ancient practice described in many cultures [1]. The traditional practice of using ashes is being questioned as this material has not been scientifically proven. The method is commonly referred to as "vulnerosorption". There are very few scientific reports available on the effect of ashes-based preparations on skin diseases [2, 3].
In the present study preparations based on carbonized rice husk and lanolin oil were applied to assess the effectiveness of treatment. The choice of CRH to be applied topically was made as it worked quite effectively [4] since it has both active carbon and high amount of ash, mostly silica[5]. The use of CRH in lanolin oil showed antiseptic activity during the wound healing process.

## Materials and methods

*Carbonized rice husk*

CRH was received from the Institute of Combustion Problems (Almaty, Kazakhstan) and used as the basis for making preparations for further trials. To obtain CRH, washed and dried rice husk was loaded into rotary steel reactor and heated in Ar atmosphere (flow rate- 5L/h) at a ramp rate of temperature: 10-12 °C/min until it reached 700°C, at which carbonization was sustained for 20 min.

*Physicochemical methods of investigation of CRH*

Moisture capacity was determined by immersion of samples in water until complete saturation following by calculation: $V_{H2O}=(m_1-m_o)/m_o$; where $m_1$ and $m_0$ refer to mass of wet and dry samples. Mercury-injection porosimetry (MIP) method was applied using "AutoPore IV 9500" porosimeter. On Autopycnometer-1320 **(**Micrometrics, USA), true density (ρ) was measured by He-pycnometry. Porosity was calculated as follows: $\varepsilon=V_\Sigma\times\rho\times100\%/ (V_\Sigma\times\rho+1)$, where $V_\Sigma$ is MIP-total pore volume. "VARIO ELEMENTAR III" elemental analyzer was used to determine C,H,N-elemental content. X-ray fluorescence spectroscopy, using a VRA-30 analyzer equipped with a Cr anode of X-ray tube and SPRUT-001 energy dispersive analyzer, was employed to measure silicon content.
Scanning electron microscope JSM 6460LV (JEOL, Japan) was used to obtain SEM-images.

*Experimental animals*

Young albino rats of either sex weighing between 200 to 225 g were used. The animals had been closely checked and those which showed signs of infection were separated and excluded from the study. Rats were maintained under hygienic conditions and provided with commercial food pellets

and tap water. Cleaning and sanitation work were done on every three days. Sawdust was provided as bedding material, which was changed every two days. The cages were maintained clean and all experiments were conducted between 10 am to 5 pm.

*Excision wound model*

Excision wounds were made on the dorsal region 1 cm away from vertebral column and 5 cm away from ear on the rat. The corresponding skin area was shaved one day prior to the experiment. The skin was excised to the full thickness to obtain a wound area of about 20 mm. Animals were divided into four groups 6 animals each. Group I: treated with powdered CRH (1g/kg). Group II: CRH with lanolin oil (1g/kg). Group III: lanolin oil (1g/kg). Group IV: control without treatment. Wound area was measured on days 0, 2, 4, 6, 8, 10 and 12 days for all the groups using a millimeter scale graph paper.

*Dose and treatment period*

First CRH alone, then CRH with lanolin oil and only pure lanolin oil were used for topical application in excision model. They were applied to cover the entire wounded area. The treatment period was 10 days.

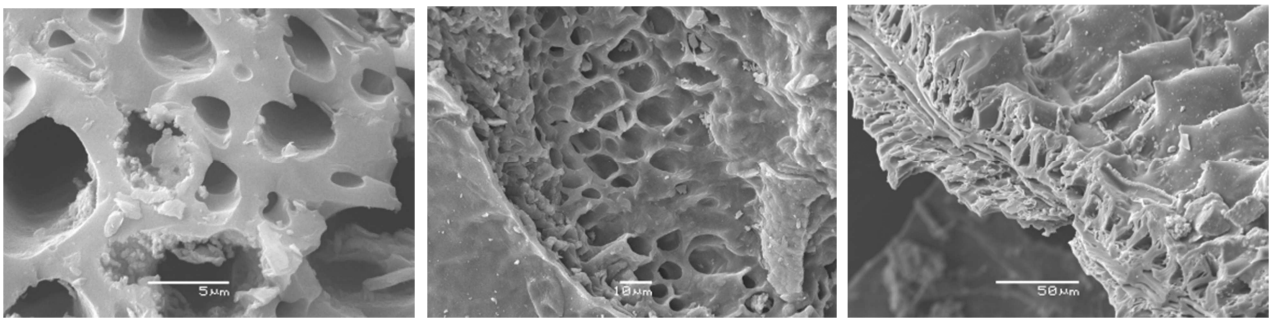
*Cell culture*

Primary normal human dermal fibroblasts (NHDF) were used 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.

The influence of the CRH on the cell viability was assessed using 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 CRH of variable concentrations (weight/volume of solution): 5, 10 and 50 μg/ml for 24 h, and maintained in fresh medium for another 24 h at 37ºC. The fibroblasts were washed with PBS, and then Diff Quick staining method was used. 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 and normalized respectively to the control.

**Results and Discussion**

According to the results of both surface texture/morphology and chemical analyses, the material possesses a set of unparalleled adsorption properties. Moisture capacity of the CRH is up to 3.8 $cm^3/g$. According to MIP-results, its pore volume is up to 1 $cm^3/g$ with pore size ranging from 180 to 360 μm. According to combined results of MIP and He-pycnometry, the porosity of CRH reaches 68%. Furthermore, CRH has a well-balanced network of macro-, meso- and micropores according to low-temperature $N_2$ adsorption studies [5]. Its chemical composition is unique of its own, since it exhibits presence of both active carbon and silica at ratio of ca. 50/50, while only traces of the other "ash" elements are presented. Nitrogen and hydrogen have partial content of about 0.5 % each. SEM-images of CRH are shown in Fig.1. The figure reveals sophisticated morphology of the CRH samples, e.g.: button-like structures on outer walls as well as virtually round channel structure of supermacropores [5].




(a) (b) (c)

Fig.1. SEM-images of CRH, magnification: (a) –x 10 000; (b) –x 5 000; (c) –x 1 000

Hence CRH could serve as both an adsorbent and a desiccant to drain suppuration (pus) from wounds. That is why this composite nanostructured adsorbent was chosen as the subject of this study to check its eventual activity in accelerating wound healing while employing both *in vitro* cell and *in vivo* animal trials [6].

A significant decrease in duration of wound healing was observed in CRH treatment groups of rats when compared to control groups. After 10 days of the experiment it was observed that in the first group treated with powdered CRH the healing process completed upon 8 days (Fig.2.1). Comparative analysis revealed that CRH with lanolin oil had highest wound healing activity. In excision wound models during 10 days the wounds healed significantly faster in all groups when compared to control (Fig.3a).

After culturing with different concentrations of CRH for 24h, there was a dose-dependent decrease in viability of NHDF with increasing dose of the CRH (Fig.3b).

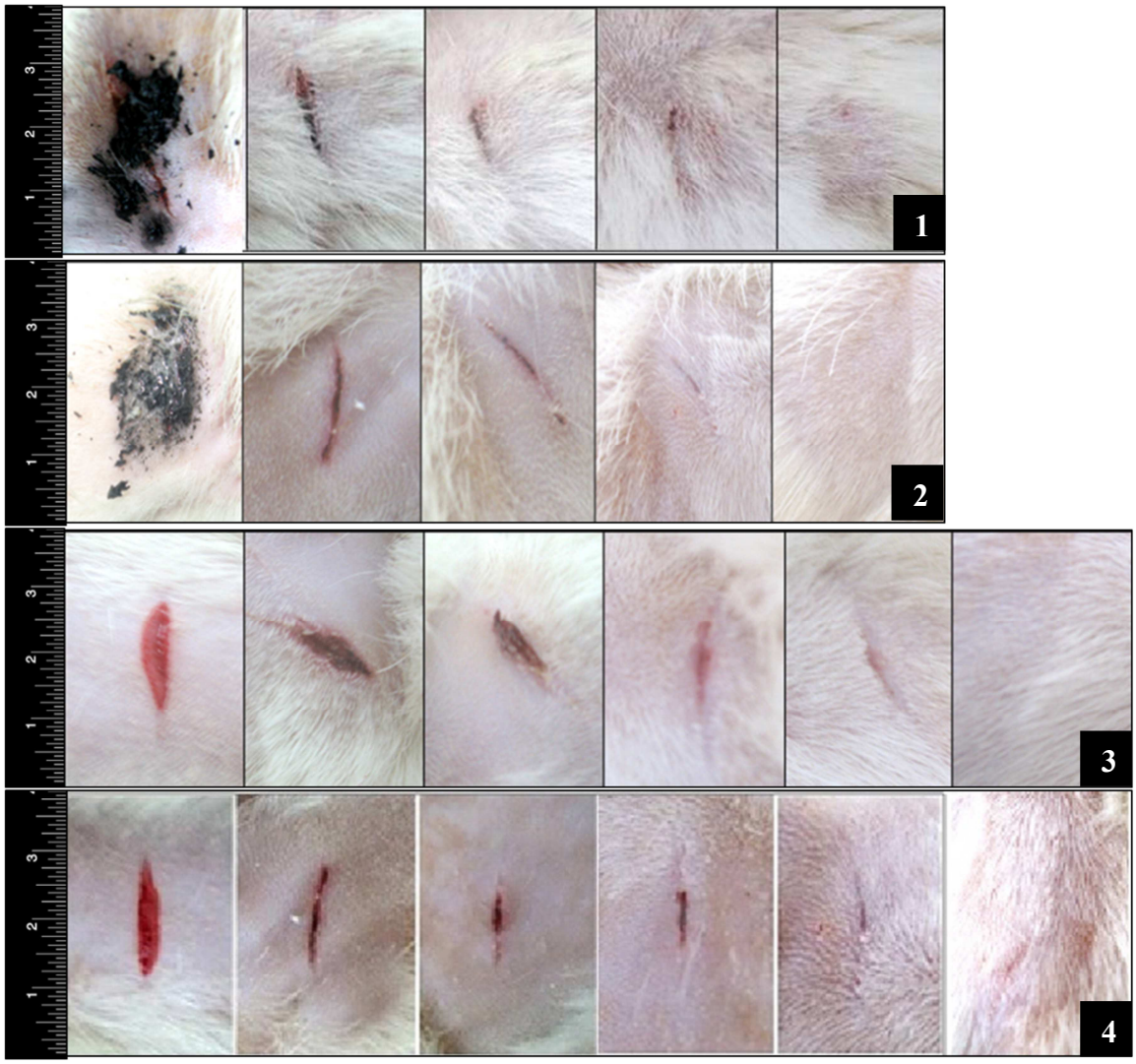
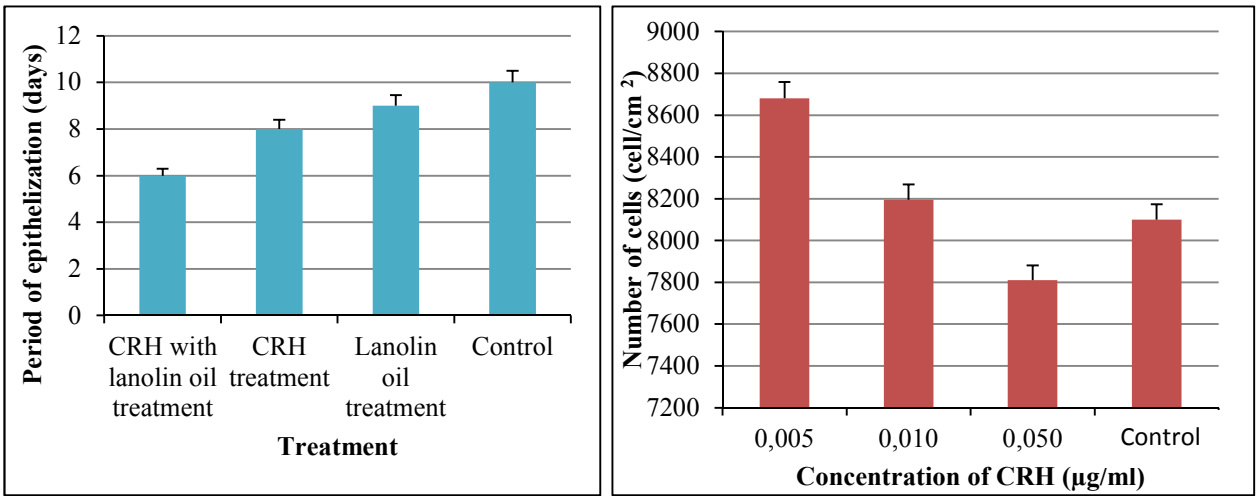



Fig.2. Dynamics of wound healing treated with CRH (1); suspension form of CRH in lanolin oil (2); lanolin oil (3) and control (4)




(a) (b)

Fig.3. a- Effect on period of epithelization; b- Viability of NHDF in presence of CRH

During the experiment the group of rats treated with a combination of CRH and lanolin oil, the necrotizing tissues turned in porridge-like mass and easily removed during the healing of wounds. Efficiency of treatment estimated on rate of disappearance of the basic symptoms as edema reduction, necrosis, pus, intoxication symptoms.

The combination of CRH with lanolin oil showed good wound healing properties after its topical application in rats. The wound size reduced as early as Day 7 in animals with wounds in the control animals (Fig.2.2.).

The adhesive ability of the CRH-treated NHDF cells was quantified by comparing the CRH-treated adhesive NHDF cells to the control adhesive NHDF cells. The results showed that the morphologies, attachment and spreading behavior of the CRH-treated NHDF cells were similar to those of the untreated control cells.

This work also demonstrated that the CRH exposure has no significant cytotoxicity in respect of NHDF. Rather a dose-dependent decrease in NHDF proliferation was observed (Fig.3).

## Conclusions

The present study indicated that the group of experimental animals treated with CRH had a facilitated healing process. The group treated with a combination of CRH and lanolin oil presented the highest epithelization rates among the experimental groups. We concluded that suspension form of CRH in lanolin oil is effective for treatment of primary (pure) wounds.

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