Creation of Polymer Hydrogel Dressings with Herbal Medicinal Substance "Alkhydin" and their Properties

G.E. Temirkhanova[1*], G.Sh. Burasheva[1], Zh.A. Abilov[1], G.S. Irmukhametova[1], A.G. Mun[1], Zh.I. Beksultanov[2], Zh.K. Myktybayeva[1], V.S. Shnaukshta[1]

[1] Al-Farabi Kazakh National University, 050000, al-Farabi, 71. Almaty, Kazakhstan
[2]Institute of Nuclear Physics, Almaty, Kazakhstan

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Abstract

New hydrogel dressings containing the herbal medicinal substance "Alkhydin", obtained from the Kirghiz (Alhagi kirghisorum Schrenk), grown in Kazakhstan, were obtained by radiation irradiation of the initial reaction mixture (IRM). Poly-N-vinylpyrrolidone (PVP) with a molecular weight (MM) of 1 ppm was used to prepare hydrogel dressings as the main gelling polymer for IRM, agar-agar and low-molecular polyethylene glycol MM = 600 were used as auxiliary substances. The main regularities of the formation of the three-dimensional structure of PVP under conditions of irradiation of IRM are investigated. It is shown that with increasing PVP in IRM, as well as irradiation dose, the yield of gel fraction increases and the degree of swelling decreases. Obviously, these effects are due to an increase in the degree of cross-linking of the polymer network formed. For the obtained hydrogel dressings containing the herbal medicinal substance Alkhydin, cytotoxic effects on the culture of mouse embryonic fibroblasts obtained by primary trypsinization were studied. The results obtained in this case testify to the absence of a cytotoxic effect on the cell culture. In conditions "in vivo" in rats using the model of thermal skin burn, the wound healing effect of hydrogel dressings with "Alkhydin" was studied. It is shown that such bandages show a pronounced wound-healing effect. This is evidenced by a higher rate of reduction in the area of the burn wound treated with a hydrogel dressing compared to the control.

1. Introduction

The use of hydrogels to replace damaged tissues is the main incentive for their synthesis and detailed investigation. The polymer gels have a very low modulus of elasticity, and therefore cause minimal mechanical irritation. They usually show good biocompatibility in contact with blood, body fluids and tissues [1–3]. Copolymer hydrogels or multipolymer hydrogels make them attractive because they impart a variety of the chemical and physical properties for medical applications by combining the different monomeric units [4–6]. Natural polymers such as chitin, chitosan, and alginate are used for wound because they offer an important role for the healing process. However, natural polymers are considered to be a limitation in their applications for wound dressing material because of their shortage of processing and mechanical properties. The combination of natural and synthetic polymers can endow optimal properties for wound repair. PVP hydrogel has excellent transparency and biocompatibility. PVP is used as a main component of temporary skin covers or wound dressings [7]. The object of our research is the aerial part of the grass camel thorn of the Kyrgyz variety Alhagi Adans. From this plant is derived a substance "Alkhydin", where the active principle is a polymer proanthocyanidins. Substance "Alkhydin" (RC-LS-3-No.004762) it is registered in the Republic of Kazakhstan as a new domestic drug. It is now generally believed that the moist healing environment is beneficial for wound healing, and moist wounds heal 40% faster than wounds which

*Corresponding author. E-mail: guldenka_t@mail.ru

are exposed to the air. Irradiation is recognized as a very suitable tool for the formation of hydrogels. The radiation process has various advantages, such as easy process control, the possibility of joining hydrogel formation and the sterilization in one technological step, no necessity to add any initiators and crosslinkers which are possibly harmful and difficult to remove. They make irradiation the method of choice in the synthesis of hydrogels [8].

2. Experimental

2.1. Materials

Poly(vinylpyrrolidone)(PVP)(molecular weight M_w = 1 000 000–1 500 000, Kollidon 90F, «BASF group», Germany), poly(ethylene glycol) molecular weight M_w = 2 000 «Fluka Chemika», Switzerland) were of analytical grade of purity and were used as received. Microbiological agar (Sigma Aldrich, USA) was used without further purification.

2.2. Synthesis of PVP-alkhydin polymers by radiation method

Poly-N-vinylpyrrolidone (PVP) with a molecular weight (MM) of 1 ppm was used as the main polymer for the preparation of hydrogel premixes. Agar and low molecular weight polyethylene glycol MM = 600 were used as auxiliary substances. The powder of alkhydin was dissolved in glycerin/etanol/water (6/2/2 composition), and then mixed by mechanical stirrer at room temperature to give solution of alkhydin. All components were dissolved in distilled water by heating and vigorous stirring. Solutions of definite concentration of PVP, agar, PEG and alkhydin were prepared with constant stirring in the beakers. All the reactants were mixed in the reactor for half an hour to get a homogeneous mixture. The reaction mixture was irradiated with gamma rays from a Co-60 source at the Institute of Nuclear Physics. These polymers then were then dried in an oven at 40 °C for the swelling test. The optimum reaction parameters were evaluated for synthesis of hydrogels by varying the concentration of alkhydin, PVP, agar and PEG. In order to determine the optimum reaction parameters, the content of alkhydin was varied from 3.0 g to 5.0 g, PVP was varied from 8.0 g to 11.0 g, agar was varied from 1.5 g to 3.0 g, total radiation dose was varied from 25 kGy to 50 kGy and water content was varied from 50 mL to 75mL during the synthesis of hydrogels.

2.3. The degree of swelling

The degree of swelling was studied by a gravimetric method. The samples were immersed in water with the proportionality of mass of gels, to the mass of water about 1:500 at room temperature. Swelling continued until a constant mass weight of gel was reached. Before weighing the sample, any surface water was removed with filter paper. The swelled gel was then slowly dried to the constant weight. The weight of dry substances in the gel was determined on an analytical balance Sartorius (Germany), after drying the sample in a vacuum oven to constant weight to an accuracy of 0.0001 g.

The degree of swelling was calculated as follows:

$$\alpha = \frac{W_s - W_d}{W_d} \times 100\%,$$

where W_d and W_s represent the weight of the hydrogel in dry and swollen state.

2.4. Determination of the cytotoxic effect on mouse embryonic fibroblasts

A study of the cytotoxic effect of hydrogel dressings was carried out on a mouse embryonic fibroblast culture obtained by primary trypsinization. A monolayer fibroblast culture was prepared by growing trypsinized cells on glass mattresses with a capacity of 50 ml and vials using the nutritional medium IGLA-MEM with 10% of normal blood serum of cattle. The inoculated concentration of cells was regulated at 200 thousand cells/cm^3. In all mattresses, fibroblasts formed a uniform monolayer of cells. The test samples were at the highest concentration in the form of extracts in a monolayer culture of fibroblast cells and cultured at a temperature of 37 °C. Extracts from the test samples were prepared in accordance with GOST R ISO 10993-5-2009 in a ratio of 1:100 and injected into a volume of 1 ml per 1 ml of the culture medium of fibroblasts of mouse embryos.

2.5. Study of wound healing and burns properties of polymeric materials structured by alkhydin

The wound healing effect of hydrogel dressings PVP-Agar-PEG-alkhydin was studied in RSE "National Center for Examination of Medicines, Medical Devices and Medical Equipment" (Almaty, Kazakhstan) on the model of thermal skin burn. In the experiment, we used non-native rats weighing 180–200 g, contained in standard vivarium conditions. The model of thermal burn in pre-depilated rats was reproduced under ether anesthesia by applying a metal plate with a total area of 113 mm^2, heated to 67.0 ± 0.5 °C. Exposure time is 5 sec. Defects of the skin remained open throughout the observation period.

3.Results and discussion

3.1. The degree of swelling

In this work, as the initial reaction mixture to receive hydrogel dressings aqueous solution was used, containing poly-N-vinylpirrolidon (PVP), agar, polyethylene glycol (PEG) and phytopreparation "Alkhydin". They were investigated basic patterns forming PVP crosslinked structure under conditions of radiation exposure. The results are shown in Tables 1 and 2.

Table 1
Dependence of the equilibrium degree of swelling (α) and PVP hydrogels release gel fraction of PVP content of the feed (D = 30 kGy)

The content of PVP in IRM, wt.%	α, g	Gel fraction, %
5.0	17.2	33.0
7.0	15.9	45.7
9.0	13.3	56.5
11.0	11.2	63.5
13.0	9.8	71.1

Table 2
Dependence of the equilibrium degree of swelling (α) of PVP hydrogels and the yield of the gel fraction on the dose of irradiation D (content of PVP in IRM = 7 wt.%)

The dose of irradiation D, kGy	α, g	Gel fraction, %
25.0	18.2	30.0
37.0	14.9	51.7
53.0	10.3	59.9
62.0	8.2	73.7
75.0	6.8	87.1

As can be seen from the data in the Tables 1 and 2 with the increase in the content of PVP in the IRS or the irradiation dose of the gel fraction yield increases, and the degree of swelling decreases. The observed effects are obviously due to an increase in the degree of cross-linking of the polymer network.

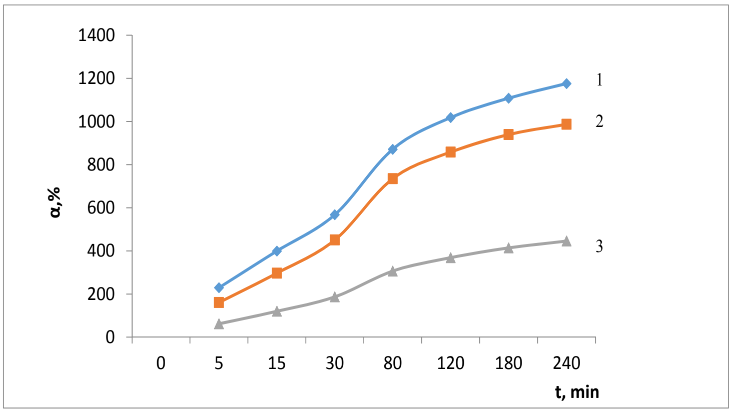

Fig. 1. Kinetics of swelling in water hydrogel dressings: the content of PVP in IRM = 5 (1), 7 (2), 11 (3) wt.%, D = 30 kGy.

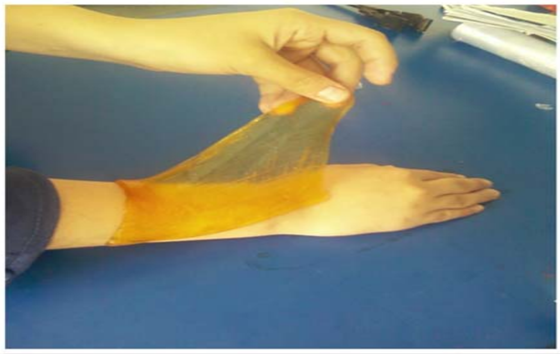

Fig. 2. Photo of hydrogel dressing containing Alkhydin.

Figure 1 presents data on the kinetics of swelling of hydrogel dressings obtained with different contents of alkhydin in the initial reaction mixture. It can be seen that with an increase in the content rate of alkhydin and the equilibrium swelling value increases, apparently due to a decrease of crosslinking density of the polymer network formed.

Dressings have a high elasticity, transparency, have a yellowish color, due to the presence in their Alkhydin screen (Fig. 2).

3.2. Determination of the cytotoxic effect on mouse embryonic fibroblasts

As a positive control, a monolayer of fibroblasts was used in the culture medium without the addition of pharmacological compounds (Fig. 3). Primary trypsinized cells are murine embryonic fibroblasts regardless of the inoculation concentration of a uniform monolayer formed on an inner surface of the mattress. The photographic image of a monolayer of cells obtained after 24 h after seeding, is shown in Fig. 3. As can be seen from the figure, a uniform monolayer of cells without degenerative changes.

A negative control was obtained by introducing a standard cytotoxic substance, phenol, onto a monolayer fibroblast cell culture obtained 72 h after cultivation of the seeded trypsinized cells at a temperature of 37 °C (Fig. 4). Then incubated at 37 °C and cultured for 24 h (until complete evaluation results).

As seen in Fig. 4, 24 h after addition of phenol (negative control) cytopathogenic effects subjected to the maximum number of monolayers of cells, and the majority of these cells were torn away from the glass surface, destroying the monolayer structure. Upon subsequent culturing this structure collapsed completely and remained on the glass surface only single cells.

When administered to all hoods 24 h later the cell monolayer remained the same complete and uniform. After adding the test preparations disorders marked by the absence of morphological structures fibroblasts, and their proliferation, what indicate the absence of the toxic effect of introduction of the sample (Figs. 5 and 6).

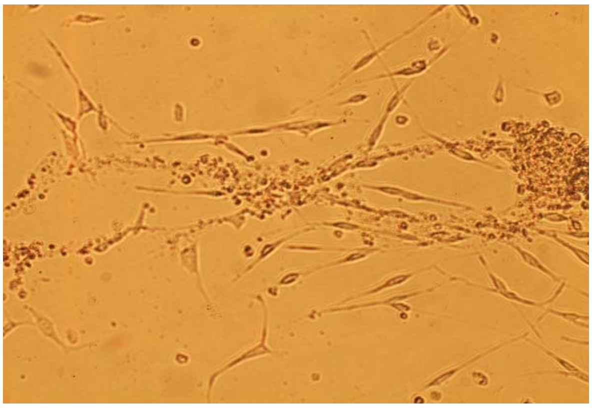

Fig. 3. A monolayer of fibroblast primary trypsinized murine embryos at 24 h of culture (Positive control).

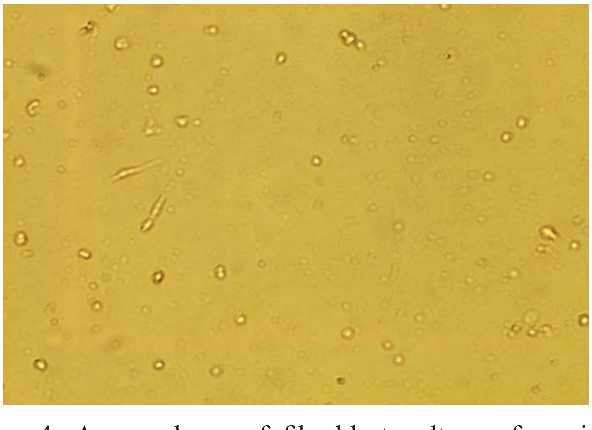

Fig. 4. A monolayer of fibroblast culture of murine embryos 24 h after the addition of phenol (negative control).

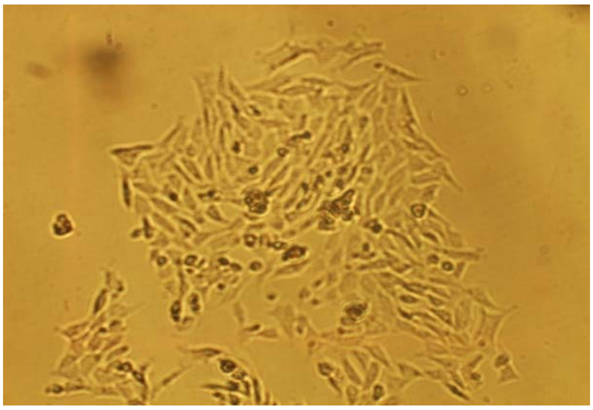

Fig. 5. Culture of fibroblasts 24 h after the addition of Alkhydin gel 10%.

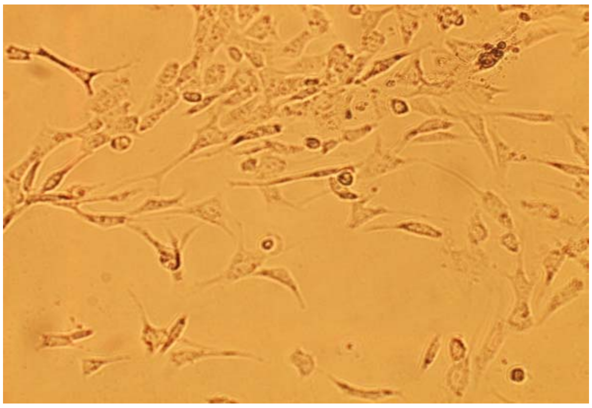

Fig. 6. Culture of fibroblasts 24 h after the addition of the gel Alkhydin gel 5%.

Thus, the cytological studies carried out testify to the absence of a cytotoxic effect on the cell culture of the gels under study and hydrogel dressings (in the ratio 1:100 and above).

3.3. Study of wound healing and burns properties of polymeric materials structured by alkhydin

The dynamics of the burn injury was judged by the area of the wound. The rate of healing of burns in rats of different groups was assessed by weight periodically traced on a tracing wound configuration. Every day the appearance of wounds, the presence and nature of the discharge, the timing of healing were assessed. Statistical processing of the obtained results was carried out using the method of least squares. Defects of the skin remained open throughout the observation period Fig. 7.

Assessment of the general condition of animals was carried out on the basis of behavioral reactions, appetite, body weight, survival. Observation of the healing process of burn wounds was carried out daily, and the magnitude of burn defects was

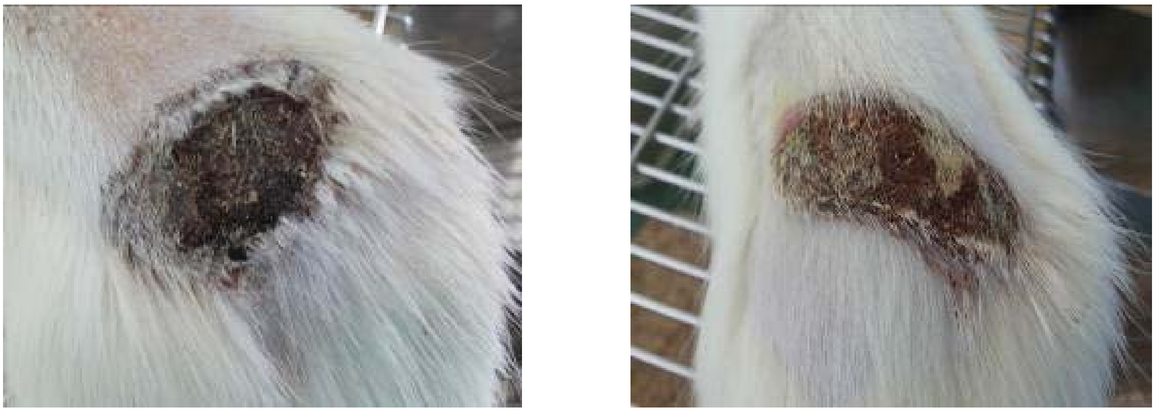

Fig. 7. Model of "thermal burn". The inflammatory reaction of soft tissue of rat back. 2nd day of the experiment

(a)

(b)

Fig. 8. The state of the wound surface of the skin of rats during the study of the wound-healing activity of wound dressing with Alkhydin. 22 day of the experiment; (a) – untreated animals; (b) – the wound was daily treated with a polymer hydrogel wound dressing with Alkhydin (5 mass.%)

measured periodically throughout the study. Evaluation of the wound healing effect was carried out according to the nature of the clinical course (presence of suppuration, time of complete rejection of the scab, time and dynamics of complete fusion of the edges of the wound) at 2, 5, 10, 15, 22, 25, 28 days of observation.

The investigated polymer hydrogels were applied daily to the wound surface of the animals in the remaining experimental groups. The drugs were applied starting from the second day after the burn was applied until the wound was completely healed. The process of regeneration was studied at discrete intervals by the measuring the change in the area of the wound surface, the state of the wound. Control animals underwent spontaneous wound healing.

A day after the burn, the condition and the area of the wound surface of the control and experimental animals were practically the same: pronounced hyperemia, edema, and necrosis elements.

From the 2nd day of the experiment the application of polymer hydrogel samples was started. As can be seen, from the data in Fig. 8, hydrogel dressings exhibit a pronounced wound healing effect. This is evidenced by a higher rate of reduction in the area of the burn wound, treated with wound dressing with Alkhydin, compared to the control.

4. Conclusions

New hydrogel dressings containing the herbal medicinal substance "Alkhydin", grown in Kazakhstan, were obtained by the method of radiation crosslinking on the basis of poly-N-vinylpyrrolidone. It is shown that with increasing PVP in IRM, as well as irradiation dose, the yield of gel fraction increases and the degree of swelling decreases, which is obviously due to the increase in the cross-linking density of the PVP polymer network formed by irradiation of the reaction mixture with fast electrons. As a result of the medical and biological tests, it was shown that dressings containing the herbal medicinal substance "Alkhydin" show a pronounced wound-healing effect. This is evidenced by a higher rate of reduction in the area of the burn wound treated with a hydrogel dressing compared to the control.

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