EXPERIMENTAL BIOLOGY

Effect of Xenogenic Immobilized Bone Matrix on the Course of Wound Process

P. E. Anfimov, Yu. V. Zimin, V. M. Denisov, Yu. I. Bushuev, S. I. Pylaeva, and N. S. Krasnova

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The effect of xenogenic demineralized bone matrix as a component of film coating on healing of a full-thickness skin wound was studied. Regenerative processes were more intensive in wounds dressed with a complex film containing demineralized bone matrix than in uncovered wounds and wounds covered with films without bone matrix. Film coating with antibiotics suppressed the development of wound microflora. *In vitro* experiments demonstrated different effects of demineralized bone matrix on some enzymes in rat skin and liver homogenates. Biological activity of xenogenic demineralized bone matrix as a component of wound coating is shown.

Key Words: *skin wound; xenogenic demineralized bone matrix; complex film coating*

A variety of dressing materials are used for local treatment of skin wounds by means of covering the wound surface: biological tissues of allogenic and xenogenic origin, cell cultures, sprays, films, and sponges from synthetic and natural compounds [1, 3,7]. Collagen-based wound dressing is widely used [6]. Allogenic demineralized bone matrix (DBM), consisting mainly from type I collagen dispersed on the wound surface activates angiogenesis, maturation of granulation tissue, and stimulates healing of soft tissue and skin wounds [2].

A complex film coating (CFC), consisting of the outer polysaccharide layer with an antiseptic and inner layer made from dermal collagen impregnated with finely dispersed allogenic DBM with antibiotic was developed at the Nizhny Novgorod Institute of Traumatology and Orthopedics.

We evaluated the effects of xenogenic DBM immobilized in the film coating on the course of wound process.

Nizhny Novgorod Institute of Traumatology and Orthopedics, Ministry of Health of Russian Federation

MATERIALS AND METHODS

The study was carried out on 80 outbred male albino rats (200-250 g). Full-thickness scalped wound (3 cm^2) on the back was inflicted under ether narcosis. The wound process was observed in open wounds (control) and in wounds dressed with polysaccharide-collagen-based materials: two-layer film (group 1), CFC impregnated with DBM xenogenic for rats (group 2), and CFC containing, in addition to DBM, gentamicin or fortum (0.01 mg/cm^2 coating) (groups 3 and 4).

Planimetric, histomorphological, and cytological studies were carried out every 3 days from the moment of wound infliction until its complete epithelialization, bacteriological studies were carried out directly after surgery and on days 3, 6, 9, and 12. Parameters of cell immunity were evaluated in the peripheral blood of controls and experimental animals from groups 1 and 2 using monoclonal antibodies ICO-101, ICO-109, and ICO-10, the concentration of transforming growth factor-β (TGF-β) was measured by enzyme immunoassay. The effect

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of finely dispersed DBM on activities of lactate dehydrogenase (direct and reverse reactions), aldehyde and alcohol dehydrogenases in rat skin and liver homogenates was studied *in vitro*.

RESULTS

The area of the wound increased in all animals on day 1 from the moment of its infliction because of centrifugal traction of the skin. This was followed by gradual shrinking of the wound surface; complete epithelialization was observed on days 28-31 in controls, on days 18-21 in experimental group 1, and on days 12-16 in other groups.

On day 3 of the experiment the cytograms of the wounds in controls and experimental group 1 were typical of the inflammatory phase of the wound process. In rats of other groups a trend to transition from the inflammatory to regeneratory phase was observed during this period. On day 9 the cytograms of animals of all experimental groups corresponded to regeneratory phase of the wound process, while in control animals this picture was observed on day 14.

The formation of capillaries and the appearance of few histiocytes and young fibroblasts were observed in experimental rats 3 days after the moment of injury. After 2 weeks the wounds in all animals were replaced by fibrous tissue (more mature in the experimental group) containing new capillaries. Epidermal regenerates had a trend to the formation of full-thickness structure with the basal layer. At the final stage of healing the wound defect consisted of tissue of the maturing cicatrix type. Degenerative changes in the epidermal regenerates were more pronounced in the control. In experimental animals hair shafts and bulbs were forming in the subepidermal zone of regenerating tissue, sometimes with new sebaceous glands.

Peripheral blood counts of $CD4^+$, RT-Ia^+ and Thy^+ lymphocytes decreased on day 3 after the injury in comparison with intact animals. Shrinkage of the wound area was paralleled by an increase in the counts of these immunocompetent cells (Table 1).

Normalization of the counts of these cells coincided with the period, when the proliferative regeneratory processes started to predominate over inflammatory processes, which was confirmed by cytological and histomorphological findings.

TGF-β is a cytokine essential for wound healing [4]. Its serum concentration decreased on day 6 of the experiment in control animals. By day 14

TABLE 1. Peripheral Blood Levels of $CD4^+$, RT-Ia^+ and Thy^+ Lymphocytes (% of Total Lymphocyte Count; *M*±*m*)

Lymphocytes		Day			
		0	3	6	9
$CD4^+$	control	16.6±1.2	8.7±1.0*	10.0±1.2**	16.6±1.2
	group 1	19.3±1.5	8.9±1.2*	13.6±0.9**	19.7±0.9
	group 2	19.7±1.3	8.2±0.9	19.9±1.1	21.3±0.8
RT-Ia^+	control	29.5±1.3	24.5±1.8**	21.0±0.9**	29.5±1.3
	group 1	31.1±1.1	24.3±1.3**	29.1±0.6**	30.9±1.0
	group 2	30.0±1.2	23.7±1.4*	31.3±1.8	33.5±0.7
Thy^+	control	6.3±1.1	5.6±0.9	4.0±0.6**	6.3±1.1
	group 1	6.1±0.5	4.2±1.4**	5.0±0.7	6.2±1.5
	group 2	6.6±1.3	4.8±1.0	4.6±0.8**	6.6±1.0

Note. Group 1: wounds covered by polysaccharide film without DBM; group 2: wound covered by CFC. *$p<0.005$, **$p<0.05$ compared to control.

TABLE 2. Levels of Wound Microflora (CFU/cm^2)

Lymphocytes	Day			
	0	3	6	9
Control	63 000±21 700	12 880±6082*	5507±2313*	863.3±176.0
1	0	410.0±286.4	4.00±2.45	
2	9.00±2.19	16.00±6.65	3.60±0.67	3.70±3.08

Note. *$p<0.05$ compared to day 3.

TABLE 3. Effects of Xenogenic DBM on the Activities of Lactate Dehydrogenase (LDH), Aldehyde Dehydrogenase (ALDH), and Alcohol Dehydrogenase (ADH) in Rat Skin and Liver Homogenates *In Vitro* (nmol/min×mg Protein)

Enzyme	LDH, direct reaction	LDH, reverse reaction	AIDH	ADH
Skin homogenate	133.59±2.71	344.11±8.19	167.72±3.07	
Homogenate+DBM	152.07±3.51*	465.80±11.33*	465.80±11.33*	
Liver homogenate	570.46±7.06	1492.58±22.44	328.87±9.81	75.70±1.55
Homogenate+DBM	475.16±5.68*	1812.37±26.26*	643.78±6.32*	25.25±0.58*

Note. *$p<0.005$ compared to homogenate.

the content of TGF-β reached the minimal value (264.0±20.4 pg/ml), and then increased. In group 2 the concentration of TGF-β tended to decrease on day 14, while on day 21 its level surpassed the initial values. Presumably, this was due to more active secretion of TGF-β by cells under the effect of DBM during the period of intense regeneration.

Initially all wounds were sterile. On days 3-6 abundant bacterial contamination of the wounds was observed in the control group; the bacterial flora was presented mainly by staphylococci and streptococci, often in association with gram-negative enteric flora. A significant decrease in the content of wound microflora in comparison with its maximum vales was observed on day 9 in all experimental animals (Table 2). In groups 1 and 2 the wound microflora was scanty during this period, without enteric flora representatives. In groups 3 and 4 the wounds remained sterile throughout the experiment.

Pulverized DBM added to skin homogenate activated direct and reverse lactate dehydrogenase and partially inhibited aldehyde dehydrogenase. Activities of direct lactate dehydrogenase and alcohol dehydrogenase decreased in liver homogenate, while activities of reverse lactate dehydrogenase and aldehyde dehydrogenase increased (Table 3). It can be hypothesized that bioactive compounds released into the wound medium from DBM collagenic structure activated energy metabolism and stimulated the detoxifying function of the liver, specifically, inhibits production of acetaldehyde and accelerates its degradation.

DBM biodegradation products modify cells migration and interactions [5]. Presumably, these substances activate metabolism and production of cytokines (proliferative processes regulators), this resulting in acceleration of epithelialization by on average 5 days in comparison with wounds covered with films containing no DBM and by 10 days in comparison with wounds healing under natural crust. Rapid healing of wounds covered with CFC with antibiotics can be attributed to the effect of DBM inducing regeneration and to absence of complications caused by secondary infection.

Hence, xenogenic DBM can be used as a bioactive component of dressing for the treatment of skin wounds.

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