A Study of Collagen-Based Bioactive Dressings

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Laboratory studies of collagen-based compositions demonstrated digispon to be the most effective agent for clinical use. Digispon is permeable to vapor and air, plastic, ensures prolonged dosed release of dioxydine into the wound, stimulates the growth of granulation tissue, accelerates marginal and islet epithelialization, and reduces the likelihood of the formation of coarse scar tissue. Radioautographic study of wound granulation tissue in experimental animals with modeled nonsuppurative inflammation showed that the principal processes ensuring wound healing during the application of collagen-based agents occur in the early stages, with an increase in the number of fibroblasts, including the proliferating variety, and blood vessels.

Key Words: *collagen-based compositions; regenerative processes; electron-microscopic radioautography*

The development of methods for the local treatment of wounds has led to the creation of bioactive compositions initially characterized by the capacity to influence the course of healing. Collagen-based biopolymers deserve special attention among this group of dressings. They exert a favorable impact on angiogenesis and on the mobility of epithelial cells and possess a unique ability to stimulate the repair processes in the wound by creating conditions for the active proliferation of fibroblasts, an increase of the content of collagen and glycosaminoglycans in tissues, and the accumulation of amino acids.

MATERIALS AND METHODS

Laboratory studies of the collagen-based agents combutec-2 and digispon were carried out. The properties of these agents determining their therapeutic effect, which results from prolonged and effective action on the wound process, were examined. Their efficacy is due to the creation of a moist state and the maintenance of a constant temperature on the wound surface, the weakly acid reaction of the medium, satisfactory gas exchange, controlled discharge of the wound exudate, elimination of bacteria, and reliable insulation of the wound from the outside environment.

The agents were evaluated at the State Center of Surgical Dressing, Suturing, and Polymeric Materials, A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, by the following laboratory methods determining:

- the absorption capacity for water and biological fluids;
- the rate of wetting;
- shape stability upon wetting;
- the absorption kinetics;
- the kinetics of release of antibacterial drugs from the dressing;
- antibacterial activity.

Experiments were carried out for preliminary assessment of the agents with a view toward their clinical use: in the first series purulent wounds were modeled after a previously described method [1], in the second series wounds with nonsuppurative in-

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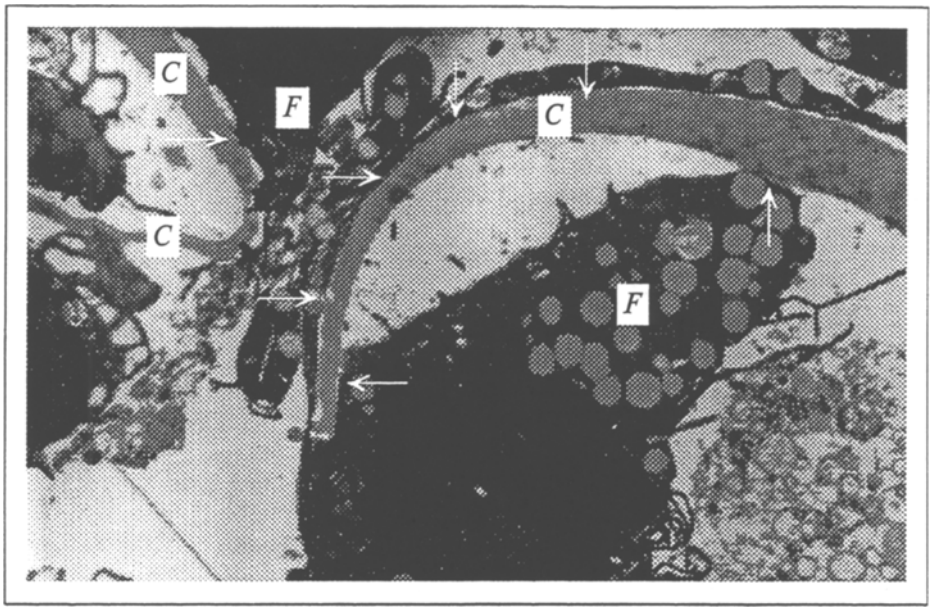

Fig. 1. Rat granulation tissue on day 5 of digispon treatment. Fibroblasts (*F*) are situated near exogenous collagen (*C*) fibers. Arrows show sites where fibroblasts are closely adhering to collagen fibers. ×9000.

flammation were modeled in animals using a method we developed.

The experimental studies were carried out under aseptic conditions in specially equipped operating and dressing rooms. Male white rats weighing 150 to 200 g were used.

The course of the wound process was monitored on the basis of local clinical data, the rate of wound healing, dynamic bacteriological control, and cytological and histological findings.

Morphological studies were carried out using electron microscopy and radioautography.

RESULTS

Evaluation of the physicochemical properties of the tested materials vis-a-vis water and blood showed digispon to have an excellent absorbing capacity, in contrast to combutec-2, while in gentacicol (which was taken as a control agent, Table 1) these properties were less well expressed.

The addition of antibacterial agents to the compositions led to an appreciable increase of their activity toward pathogenic flora, an important factor in boosting their therapeutic effect. After a culture suspension was covered with digispon, no microflora was found in the pores of the dressing, in contrast to experiments with the collagen sponge combutec-2 (Table 1).

Study of the absorption properties for model fluids showed that digispon possesses a better retaining capacity, becomes more evenly wetted, and is characterized by a relatively better draining activity and structural stability in the wet state.

Ultrastructural study of the biosynthetic material digispon showed that, due to its synthetic basis, its initial structure is characterized by a stable carcass and a more homogeneous composition in comparison with combutec-2, whose structure is heterogeneous, whose swelling ability varies, and under which biologically limited spaces are liable to form.

TABLE 1. Comparison of the Biosynthetic Dressing Digispon with Known Collagen Dressings

Parameter	Digispon	Combutec-2	Gentacicol
Absorption capacity, g/g:			
for water	45±5	40±5	36±3
for blood	65±5	45±7	41±5
Antibacterial activity (arrest of growth zone, mm):			
Staphylococcus aureus	No growth	8.5	6
Pseudomonas aeruginosa	No growth	16.0	13
Shape stability	Preserves initial size and shape	Vertical bulk is reduced	
Rate of wetting, sec:			
for water	15	15	15
for blood	30	20	25

A study of the time course of dioxydine release from digispon into model media showed that the antiseptic is desorbed gradually over 48 hours. This may be conducive to prolonged action on the wound flora (not less than 2 days).

The study demonstrated digispon to be the most effective of the studied agents and a promising candidate for clinical use.

Digispon is permeable to vapor and air, plastic, ensures prolonged dosed release of dioxydine into the wound, stimulates repair processes in infected wounds and the growth of granulation tissue, accelerates marginal and islet epithelialization, and reduces the likelihood of formation of coarse scar tissue.

A modest therapeutic effect was observed during treatment of modeled suppurative wounds with collagen-based preparations. Inflammatory phenomena persisted in the wounds: soft tissue edema, hyperemia, and continued purulent discharge from the wound. Microbiological study showed that the level of microflora on day 5 did not differ from that before the beginning of treatment and was 10^4 to 10^5. This bespoke an inadequate therapeutic effect and attested to the undesirability of using such preparations during the first phase of the wound process.

In the second series of experiments with the treatment of modeled nonsuppurative wounds the animals were divided in two groups, experimental and control. In the experimental group the treatment was carried out using the tested materials. No treatment was administered to controls.

Starting from day 3 the wounds were covered with granulation tissue and active marginal epithelialization was observed in the experimental group, in contrast to the control, in which these processes were better expressed on day 5.

On day 7 the dynamics of repair processes was well expressed in experimental group, the wounds being half as large. In the control the wounds had shrunk 35-36% at this time.

Complete healing was observed on days 9-10 in the experimental group and on days 12-13 in the control.

Radioautographic study of granulation tissue revealed that in the experimental animals the fibroblasts, including the proliferating variety, and blood vessels were localized around exogenous collagen fibers representing the carcass of the collagen-based dressings (Fig. 1). Endotheliocytes exhibited a high functional and proliferative activity: incorporation of ^{3}H-uridine (RNA synthesis) and ^{3}H-thymidine (DNA synthesis) in their nuclei was indicative of the growth of blood vessels.

The principal processes ensuring wound healing during the application of collagen-based preparations occur in the early stages, when the number of fibroblasts, including proliferating ones, and blood vessels increases. In our experiment fluctuations in the counts of proliferating fibroblasts were synchronous with fluctuations in the number of vessels. As early as on day 3 the proliferative and functional activity of fibroblasts had increased and vessels were invading the granulation tissue. This was confirmed by the results of quantitative analysis: the total number of fibroblasts was 2.5 times higher than in the control, the number of proliferating fibroblasts was 3 times higher, and the number of vessels twice as high as in the control ($p<0.001$). In the control group of animals the number of proliferating fibroblasts was maximal on day 5, the total number of fibroblasts on day 7, and of vessels on day 9.

Our studies showed that the effects of the collagen-based preparations are achieved through the following mechanisms:

1. Collagen degradation products cause a marked proliferation of cellular elements (the phenomenon of chemotaxis underlying this) and become the raw material for the synthesis of their own collagen.

2. The retained collagen fibers represent the "stroma" for the growing granulation tissue. Blood vessels thread into the collagen pores, and young cells (histocytes, polyblasts, and fibroblasts) accumulate in them, closely adhering to the surface of the fibers.

Hence, we may conclude that local treatment of wounds should be aimed at suppressing the microflora, arresting the inflammatory process, and eliminating the pyonecrotic mass; at the same time, the regenerative processes should be stimulated as early as possible during the phase of inflammation, thus promoting the growth of granulation tissue and accelerating epithelialization. This confirms the notion that stimulation of the early phases of inflammation leads to more intensive repair whereas, conversely, sluggish leukocytic and macrophagal reactions inhibit healing and collagen formation [2].

REFERENCES

1. S. P. Glyantsev, *Development of Modern Enzyme-Containing Dressing Materials and Improvement of Methods of Their Application in Combined Therapy of Suppurative Wounds* [in Russian], Author's synopsis of Doct. Med. Sci. Dissertation, Moscow (1992).
2. M. I. Kuzin and L. L. Shimkevich, in: *Wounds and Wound Infection* [in Russian], eds. M. I. Kuzin and B. M. Kostyuchenok, Moscow (1990), pp. 108-113.