https://doi.org/10.35630/2199-885X/2019/9/3.24

POTENTIAL USE OF CHITOZAN-BASED MULTILAYER WOUND COVERING IN DENTAL PRACTICE

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ABSTRACT — A promising area in dentistry is the development of modern biotechnological wound dressings based on chitosan, which, depending on the molecular weight and three-dimensional structure, is able to change its physico-mechanical properties from the state of a hydrogel to a dense frame structure with a different degree of swelling, and at the same time perform a depot function the drug introduced into it. Two new types of wound dressings based on chitosan with an immobilized 10% aqueous solution of iodopyrone were developed and their effectiveness was studied in an experiment on animals (rabbits) for dental practice. A comparative analysis was carried out with the well-known wound dressings *Alvostaz*, *Gelatamp* by introducing the studied samples into the well of the tooth after its extraction. The high efficiency of the proposed wound dressings based on chitosan was established.

KEYWORDS — wound coverings, porous polymer materials, biopolymer, biotechnology, surgery, stomatology, chitosan, medicine

INTRODUCTION

Despite a significant range of therapeutic measures employed to treat inflammatory diseases in the maxillofacial area, much of the effort falls short of effective suppression of the infection and proper regeneration as the outcome of the pathological process [1–6]. The development of optimal wound covering that would contribute to fast and complete recovery of the damaged structures appears an urgent issue in modern medicine. Scientific items, monographs and patents offer a wide range of materials used to create wound covering, with over 400 various types of sponges, films, combined collagen/gelatin/oxidized cellulose/starch-based implants, etc., described nowadays [7–10].

Of the well-known new-generation biodegradable synthetic and natural materials, a special place belongs to chitosan — a derivative of chitin — a natural polysaccharide obtained through its partial deacetylation, consisting of residues of D-glucosamine and N-acetyl-D-glucosamine units connected by β-1,4-glycosidic bonds. Extremely promising here is the development of biotechnological wound covering based on chitosan, which, depending on the molecular weight and a three-dimensional structure, can change its physicochemical and strength properties from the state of a hydrogel to a dense frame structure with a different degree of swelling and water absorption, as well as perform a depot function for the drug introduced into it. The potential of its use is due to a number of features which are inherent in this polymer and materials based on it, i.e. biocompatibility, a minimum number of side effects, high wound-healing activity, moisture- and air permeability, high porosity, mechanical stability along with simultaneous plasticity, controlled bioresorption time in the body, and the ability to act as biodegradable depot carriers of the drugs [11]. Gradual biodegradation of chitosan in the alteration zone, along with diffusion through the swollen walls of the hydrogel, will promote gradual release of the immobilized pharmacological agent within controlled time intervals, and replacement with native cells and tissues, which will result in a step-by-step prolonged medication therapy depending on the pathophysiological processes underway in the wound. A number of authors, too, claim high antimicrobial and hemostatic activities observed in chitosan and its derivatives [12, 13]. This means that a wound covering synthesized on its basis will feature action polyvalence.

Aim of study

to evaluate the effectiveness of chitosan-based multilayer wound covering and that of the popular wound coverings *Alvostaz* and *Gelatamp* developed for dental practice.

MATERIALS AND METHODS

The study included 12 ten-month-old male rabbits with a body weight of 2500 (±50) g. The animals were kept in a vivarium with free access to water and food, which corresponds to the standards of GOST 33044-2014 PRINCIPLES OF GOOD LABORATORY PRACTICE (approved by the Federal Agency for Technical Regulation and Metrology, order # 1700-st of November 20, 2014), entered into force on August 1, 2015.

2 popular wound coverings were selected for the control group of drugs (*Alvostaz* and *Gelatamp*).

The experimental samples taken for comparison were multilayer wound coverings based on chitosan, core-shell type (molecular mass — 600 kDa; porosity — 98%, and the following pore size: a denser core, 20–45 microns; more loose area close to the shell, 70–200 microns, different in the structure with immobilized iodopyron in the loose part of the samples; specially designed at the Kurchatov Research Institute) (Fig. 1). Porous materials were obtained through the technology of chitosan solutions freeze drying. A specific feature of the core and shell materials was the pore shape: in Sample 1c, the shell had isotropic pores, while the core was composed of longitudinal pores along the cylinder axis. In Sample 2c, the pores were initially oriented longitudinally, along the cylinder axis.

Fig. 1. *Experimental multilayer samples of chitosan-based wound coverings — Sample 1c (a), Sample 2c (b).*

Through the experiment all the studied chitosan samples revealed high biocompatibility, biodegradability, bioadhesive capacity and permeability. However, the degree of adhesion and biodegradation depended significantly on the molecular weight and the specifics of the internal orientation that pores had in the sample. The chitosan samples, while featuring moderate strength, density, elasticity, and the ability to maintain the desired shape and initial size, showed varying biodegradation rates. Sample 1c, externally, had a higher porosity chitosan, while inside it contained a denser material; the external loose shell was completely biodegradable on Day 7; the denser core — on Day 14. Due to its large porosity and hygroscopic properties, the outer shell ensured high adhesion of the sample to the surrounding tissues when introduced into the surgical area. The core of Samples 1c and 2c was modified via introducing into the central part a 10% iodopyron aqueous solution. The capillary effect helped the drug penetrate deep into the sample, which led to swelling and increased chitosan elasticity in this area; subsequent drying of the sample through the surface tension of the solution resulted in larger pores in the central (axial) part. All this ensured a draining and a prolonged antiseptic effects. The external, unmodified and more rigid shell served the frame function for the biomaterial.

Sample 2c, on the contrary, was a structure, whose outer layer contained a more dense porous material, while the inner one featured a lower specific density.

Under aseptic conditions and general anesthesia (premedication: Atropine Sulfate 0.1 mg/kg; Prednisone 0.1 mg/kg; Sedamidine 0.05 mg/kg and anesthesia: Telazole 0.05 mg/kg intramuscular) the animal (according to the developed method) was fixed on the operating table in a position on its side. After the surgical field was prepared (triple treatment of the perioral area with a 5% aqueous solution of iodopyron, once — the oral mucosa with a 3% aqueous solution of chlorhexidine bigluconate), the upper central incisor (right or left) was extracted with the experimental and control samples introduced into the hole to a predetermined depth (Fig. 2).

The effectiveness of wound coverings was evaluated both in an aseptic wound and under purulent inflammatory conditions. In the latter case, after the tooth extraction, the bacterial culture Ps. aeruginosa was introduced into the hole (concentration – 109 CFU/ml) 3 days before the introduction of the wound covering sample. The gum mucous membrane above the hole was sutured with apposition interrupted sutures (atraumatic Prolene 3/0). In order to reduce the sedative effect of the injected drugs, after the procedure, the animals were injected with Antisendan (0.05 mg/kg). To relieve the pain in the early postoperative period, the animals were administered Flexoprofen (2.5%, 0.08 mg/kg). Through the 14 postoperative days, the animals were monitored with free access to water and food.

The experimental and control samples were studied in the operating room on Day 14.

MORPHOLOGICAL ANALYSIS

The preparations fixed for 7 days in a 10% solution of neutral formalin were washed in running water for 60 minutes, after which they were placed in a SoftiDec decalcifying solution (ErgoProduction, Russia), with the degree of demineralization in the peripheral areas checked with a metal needle every 20 minutes. After decalcification, experimental specimens were cut out. Then, an incision was made in the sagittal

Oral cavity treatment prior to extracting the incisor

The hole after the incisor extraction

Introduction of the samples into the dental hole

Gum soft tissue suture

Fig. 2. *Experiment stages*

plane through the middle of the tooth hole filled with the sample. The materials were processed and paraffin-embedded employing an automatic method using a Leica TP1020 histoprocessor (Germany) subject to a standard procedure. Then, the samples were poured into paraffin to obtain paraffin blocks on a Leica EG1150H modular unit (Germany). 5 μm thick paraffin sections were obtained using a Leica RM2235 rotational microtome (Germany). For general histological evaluation, the preparations were dewaxed and hydrated. The obtained sections were stained with hematoxylin and eosin following the standard methods. The micropreparations were studied on an Olympus CX41 microscope (Japan).

RESULTS AND DISCUSSION

The wound covering Gelatamp proved to be satisfactory for an aseptic wound, serving a good frame function to stabilize the blood clot. In an animal experiment with a purulent wound, this covering did not provide good antimicrobial effect, despite the presence of silver as an antiseptic, which was proven histologically (multiple microabscesses in soft tissues (Fig. 3a).

Compared to the chitosan Sample 1c, the Alvostaz wound covering provides a skeleton function due to a homogeneous, denser fibrous structure, as well as chitosan Sample 2c. Almost complete biodegradation of the material was noted on Day 14 into the experiment. The antimicrobial effect was unsatisfactory: histologically verified microabscesses were observed in the drug injection area. The underlying muscle tissue was ischemic; phenomena of venous stasis and transvascular lympho-macrophage infiltration were to be observed (Fig. 3b).

A comparative evaluation of chitosan samples revealed higher efficiency regarding conventional wound coverings (*Alvostaz*, *Gelatamp*) in animal experiments (rabbits). The developed wound coverings provided a skeleton function, revealed permeability, biodegradability and the ability to be a carrier matrix for the drug introduced into their structure. At the same time, Sample 2c, if matched against Sample 1c, proved the most effective performing a fusing function due to a looser internal structure. During the first 3 days, complete rehabilitation of the wound hole surface was observed, with further effective drainage maintained. The dense outer surface of the structure avoided premature adhesion of soft tissues through further observation time, too. On Day 14 into the experiment, almost complete biodegradation of the material was to be observed in the tissues. No inflammatory changes, be that in the bone or soft tissues, were registered (Fig. 3c.).

The above means that the proposed samples of chitosan-based wound coverings revealed sufficient efficiency on the model of a wound process in the hole of an extracted tooth. Sample 2c was recognized as most reliable because, due to the dense external material, the structure would retain frame functions for a long time, offering proper drainage from the pathological focus. At the same time, the loose chitosan material filling the inner diameter of the endoprosthesis undergoes earlier biodegradation, providing, on the one hand, moderate compliance and elasticity of the external shape, while on the other hand (due to the transformation of chitosan into a gel with a highly ordered micellar-type internal nanostructure) its prolonged function was ensured as a matrix carrier of the drugs introduced into the wound covering during implantation. This type of wound covering proved the most reliable to be used in case of a purulent inflammatory process.

Sample 1c was recognized as more promising in cases where no inflammatory process was observed in the extracted tooth hole, where initially high adhesion with surrounding tissues was required, which ensures tightness and keeps the blood clot in the wound. Note to be made that due to the rapid biodegradation of the outer shell, the sample lost its shape, which created conditions for its coming out of the tooth hole on Day 3—5.

CONCLUSION

An analysis of the obtained data suggests the following conclusion: the studied experimental chitosan-based materials revealed high wound healing activity, biocompatibility, biodegradability, bioadhesive capacity and permeability. In addition, a comparison of chitosan-based samples with conventional wound coverings (*Alvostaz*, *Gelatamp*) done through experiments involving laboratory animals (rabbits), revealed that a wound covering Sample 2c showed higher efficiency in both clean and purulent wounds (with preliminary introduction of a 10% aqueous solution of iodopyrone into the wound covering). Due to the combination in the wound coverings of two different types of chitosan, which differ in biodegradation rate and other physical & chemical properties, the tested sample was found as promising for dental practice, in particular, in the surgical treatment of purulent inflammatory maxillofacial issues. The external frame of the sample, due to higher rigidity and later biodegradation (up to 14 days) provides adhesion to the wound walls as well as drainage of the surgical area; the internal loose chitosan offers a sustained local pharmacological effect, while serving a depot for medications. Further on, the sample proved completely biodegradable without impeding healing in the surgery area. Sample 1c was recognized as promising in cases featuring no inflammation in the extracted tooth hole, where initially high adhesion of the biomaterial to the surrounding tissues is required.

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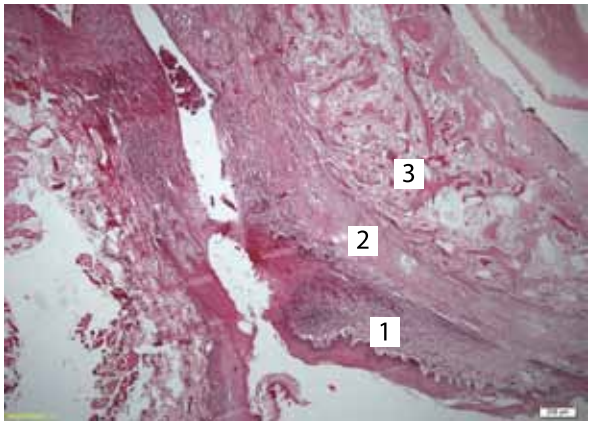

Fig. 3a. *Morphological status after administration of the Gelatamp preparation (mucous membrane of the cavity with a partially biodegraded implant (1); microabscesses in soft tissues (2); spongy bone, capillaries hyperemic, with stasis (3).*

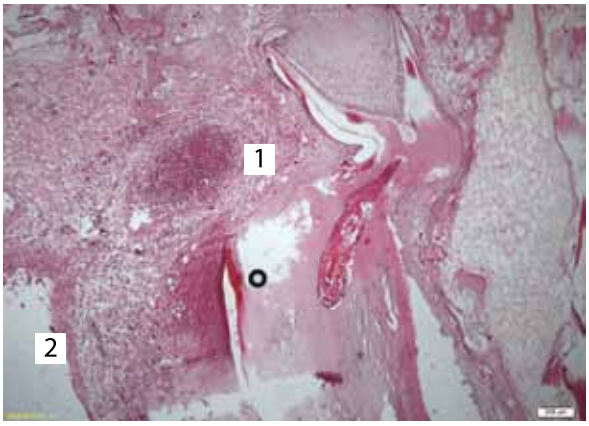

Fig. 3b. *Morphological status after administration of the Alvastaz (abscess wall (1), consisting of granulation tissue with an increased number of cellular elements — a macrophage row of thin-walled capillaries with stasis effects; implant (2) is biodegradable; surrounding adipose tissue featuring a state of fat necrosis).*

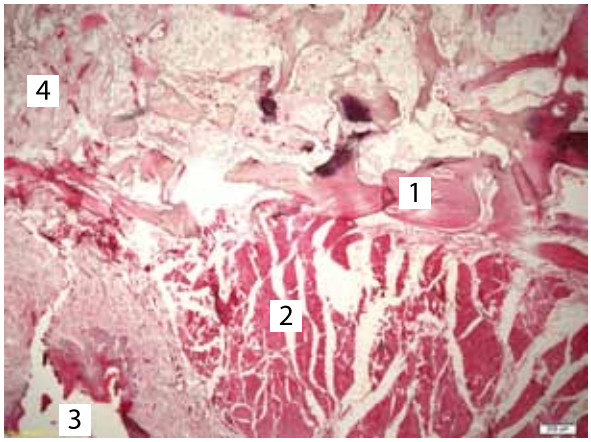

Fig. 3c. *Morphological status after introduction of chitosan Sample 2c (the mucous membrane of the oral cavity reveals no sign of inflammatory change (1). In the submucosal layer and the adjacent loose fibrous connective tissue, through its entire length, infiltration of lymphocytes and macrophages can be observed; muscle tissue shows signs of edema (2); lamellar bone (3); spongy bone, capillaries are hyperemic, with stasis (diameter 16–80 microns). Total biodegradation of chitosan can be observed).*

Fig. 3. *Morphological analysis of tissues on Day 14 into the implantation of the compared samples of wound coverings (stained with hematoxylin and eosin. Magnification, x 200).*

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