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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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EFFECTIVENESS OF THE APPLICATION OF THE DEVELOPED BIOPOLYMER FIBROUS MATRIX WITH CENOBONE® BIOGEL FOR THE RECONSTRUCTION OF BONE TISSUE DEFECTS OF THE JAWS

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Abstract.

Aim: The aim of the research was to study the framework ability of the fibrous non-woven PCL matrices we've created during the restoration of bone tissue.

Materials and methods of the research: There were performed some spectroscopic, histological and immunohistochemical, radiological and clinical analyses of the effectiveness of microfibrous non-woven PCL polycaprolactone matrices developed by us, in the work.

Results: The obtained results of morphological studies of bone tissue in the experiment of the implantation of a fibrous matrix indicated an increase in reparative osteogenesis in the form of an increase in osteoid areas up to 34.38% ($p<0.05$) at an early period.

The analysis of clinical data showed the effectiveness of the frame developed by us, which was confirmed by the absence of pronounced compaction of bone tissue in group III, in contrast to group II, where, on the contrary, the use of granulate based on hydroxyapatite and tricalcium phosphate has led to a significant increase in density indices of 974.53+19.74 HU $p<0.05$, which did not exceed 36.8% of indices of the group III 615.17+24.53 HU $p<0.05$.

Conclusions: The matrix material developed by us is not only a means of delivering some other substances and materials into the damaged area, but also serves as a kind of framework for the restoration of bone tissue.

Key words. Matrix materials, polycaprolactone, histological analysis, bone tissue, radicular cyst.

Introduction.

One of the relevant aspects of modern medicine is the timely detection, prevention, and rehabilitation of patients with chronic inflammatory processes of the facial skeletal bones. Among chronic inflammatory processes, chronic forms of osteomyelitis and radicular cysts are the most frequent and problematic in the rehabilitation program since these pathologies most often lead to the destruction of bone tissue and require the use of reconstructive plastic surgery [1-3].

Surgical dental interventions in chronic inflammatory processes leading to destructive changes of the facial skeleton, often involve the use of implant materials based on the tricalcium phosphate and hydroxyapatite for the additional structural support during the restoration of bone tissue [4-6]. However, when using these materials, there is a problem of restoring the full-fledged structure of bone tissue, which is associated with untimely degradation of the granulate and the development of bone tissue hyperostosis or the formation of connective tissue around the implanted granules. In addition, the granular matrix cannot effectively serve as a local delivery system for medicines or growth factors. A number of studies assign an important role in the reconstruction of tissues, the structure of a functional carrier for these tissues or cells, namely, a matrix based on the biocompatible biodegradable materials [7,8].

Today, in addition to hydroxyapatite and tricalcium phosphate, natural polymers (hyaluronic acid, collagen, gelatin, fibrinogen, chitosan, pectins, agarose, alginates, cellulose) and synthetic materials (polycaprolactone and polylactide) are considered to be the promising tools for the controlled reconstructive tissue repair [9-12]. Microfibrous implants made of such bioinert materials can eliminate the disadvantages of granular material. Currently, a new direction is being pursued in medicine, which includes the combination of fibrous materials with therapeutic agents, as delivery system for medicines and living cells [13-15]. This approach allows for purposeful management of the structural-functional condition of cells involved in regenerative processes.

When creating a tissue-engineered implant, it is important to provide the matrix material with a complex three-dimensional fibrous frame structure (non-woven scaffold) with a high ratio of surface area to the total volume, which imitates the intercellular tissue matrix. A unique method of forming a porous non-woven matrix is the technology of electrospinning [16-19]. The three-dimensional frame of the implant due to its architectonics and the presence of active functional groups (which is determined by the type of polymer material) promotes the adhesion and migration of cells to the area of tissue defect, provides complex cascades of intercellular signaling interactions that underlie the angiogenesis, trophicity and repair.

In general surgery, porous matrices are used as frameworks for growing vessels, valves, etc., as well as the local delivery systems of antimicrobial medicines to the damaged area [20-22]. However, electrospinning technology as a method is a quite energy-intensive one, and due to the very small pore sizes between fibers, through which vascularized tissue cannot grow within large volumes, it cannot be used for bone tissue regeneration. Instead, the use of biogels based on the demineralized bone matrix eliminates the problem of hyperostosis of bone tissue, but due to its fluidity, it significantly affects the stability of the framework in the wound. Only the combination of the gel component and the fibrous structure of the matrix can provide the stability of such a hybrid frame and provide it with the necessary osteoinductive and osteoconductive properties.

The aim of the research was to study the framework ability of the fibrous non-woven PCL matrices we've created, during the restoration of bone tissue.

Materials and Methods.

Microfibrous non-woven matrices made according to the method developed by us, out of polycaprolactone PCL (invention patent of Ukraine № 119958), were used in the work.

Fibers were obtained by heating to a temperature of 180°C and centrifuging a melt of PCL polycaprolactone granules and sucrose powder. The fibers of sucrose and polymer obtained as a result, were immersed into sterile physiological solution with a temperature of no more than 40°C until the complete dissolution of sucrose. The synthesis method developed by us, made it possible to obtain a microfiber matrix with a fiber size from 1 to 10 μm (Figure 1).

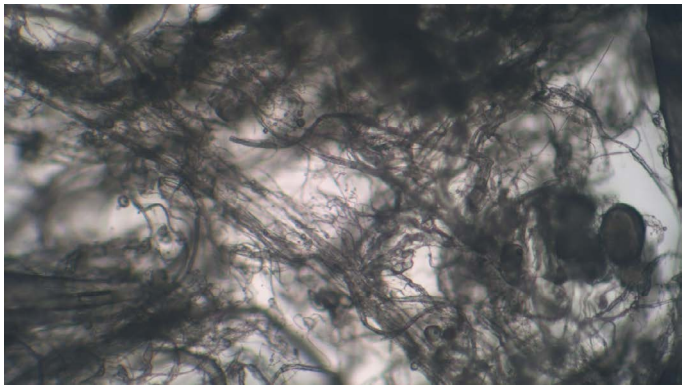


Figure 1. *Photo of microfibers: Magnification: ocular lens 10, field lens 40.*

Microfibers produced by us, were divided into fragments, dried in a thermostat at a temperature of 35°C for 10-20 minutes; after this they were hermetically packed into double "Medicom" bags with a thickness of 0.6 mm (in accordance with the standards of EN 868-5, ISO 11140-1, ISO 11607-1). Sterilization of microfibrous matrices using γ-radiation was performed using the "Elektronika ELU-4" linear accelerator.

The identity and purity of the material obtained out of the original raw material, were determined using infrared spectroscopy of the samples. For this purpose, the spectroscopy of the control samples (granules of thermoplastic biopolymer PCL and crystals or sucrose powder) and 10 samples of the created fibrous matrix from PCL was first performed. Fourier transform infrared spectroscopy was performed using IR Affinity-1 spectrometer, Shimadzu ATR attachment.

To study the osteoconductive properties of the matrix, we've performed experimental research using animals. The experimental part of the research using laboratory animals was performed with the use of adult, sexually mature male rabbits weighing 1100-1400 g, having been kept in a vivarium on a regular diet. Animals were kept and manipulated in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals (Strasbourg, 1985), "Ethical Guidelines for the Use of Animals in Research" adopted by the First National Congress of Bioethics (Kyiv, 2001), and the Law of Ukraine "On the Protection of Animals from Cruelty" (2006).

Experimental animals were divided into 2 groups. The main group I included 30 animals having been implanted with a polymeric fibrous non-woven matrix made of polycaprolactone PCL, into the bone tissue. The comparison group II included 30 animals having been surgically formed a defect in the bone tissue followed by sutures. For this, there was performed an intravenous premedication with atropine sulfate solution0.1% – at a dose of 0.22-0.27 mg/kg; Diphenhydramine 1% – at a dose of 4.6-5.2 mg/kg; droperidol 0.25% – at a dose of 1.25 mg; ketorolac trimethamine 1% – at a dose of 0.1 ml. Propofol 1% – at a dose of 15 mg/kg intravenously was used as induction of anesthesia. Propofol 1% – at a dose of 25-30 mg/kg/h intravenously was also used to maintain anesthesia. On the left in the middle and slightly below the knee joint of the rabbit hindlimb, there was made a skin incision, soft tissues were separated up to the periosteum, and the proximal condyle of the tibia was skeletonized on the anteromedial side. Using a drilling machine, a bone cutter was used to form a defect in the bone tissue with a diameter of up to 5 mm; and a polymeric fibrous non-woven matrix was placed into it. The wound was sutured in layers. Material was collected out of bone tissue in both groups during the 1st, 2nd, 3rd, 4th and 5th months of the experiment. While taking the material the experiment was completed by an overdose of 2% sodium thiopental solution at a dose of 1.5 ml IV.

To perform a general histological examination, special histological examinations, fragments of bone tissue were fixed in a 10% solution of neutral formalin (pH-7.0). Histological sections of bone tissue were stained with hematoxylin and eosin, and according to Masson. To assess osteoinductive properties and bone remodeling, immunohistochemical determination of osteocalcin and osteopontin expression was performed. The prepared sections were deparaffinized in xylene. Endogenous peroxidase was blocked in 80% ethanol with 1% hydrogen peroxide for 30 min at a room temperature. Unmasking of antigens was carried out using the Triton X100 detergent with the addition of EDTA for 45 min at ambient temperature. After blocking nonspecific immunoreactivity with normal goat serum for 20 min, the sections were incubated with the primary antibodies for 12 h at a temperature of 4°C with a titer of 1:800 for osteocalcin and 1:400 for osteopontin. Anti-mouse biotinylated sera were used as secondary antibodies. The ABC-method (Vectastain Elite Kit, Vector) was used to visualize the reaction followed by the reaction of a chromogenic substrate (DAB Kit, Vector). The reaction product was visualized as a brown colour in the areas of expression of the studied markers. Histological studies were performed using a Leica DME light-optical microscope. In order to objectify quantitative studies, computer morphometry and densitometry of objects in histological preparations were performed. Digital copies of images were analyzed using the computer program Image Tool 3.0 for Windows (free license).

There were selected 90 patients aged 20 to 50 with radicular cysts of the jaws for clinical studies. For a reliable analysis of the dynamics of defect recovery, the size of the defect, which did not exceed 17 mm, was also taken into account when selecting patients. All the patients examined by us, were practically healthy without accompanying somatic pathology, without the presence of orthopedic structures in the areas of surgical correction, pathological condition of the oral cavity vestibule, tobacco abuse, unsatisfactory oral hygiene, that is, factors that could directly or indirectly affect the results of the studies performed.

In order to compare the efficiency of the fibrous matrices developed by us, all patients were divided into three groups,

taking into account the use of one or the other material. Thus, the group I included (n=30) patients who were planned to perform surgical correction, which included cystectomy using only the isolating collagenous Collprotect® membrane (manufactured by Botiss Biomaterials). Group II included (n=30) patients who were performed cystectomy in combination with bone grafting with osteoplastic material in the form of CeraBone® granulate (manufactured by Botiss Biomaterials) and the isolating collagenous Collprotect® membrane. Group III included (n=30) patients who were performed cystectomy in combination with bone grafting with a microfibrous matrix made of a biopolymer based on PCL polycaprolactone (developed by us), which was mixed with CenoBone® biogel (manufactured by Cenobiologics Ltd) and covered with an insulating collagenous Collprotect® membrane.

The control group (comparison group) included the analysis of archival data of computer tomography of intact areas of the jaws of 30 practically healthy individuals without pathology of the maxillofacial area.

The effectiveness of the fibrous matrices developed by us, was evaluated by X-ray examination. X-ray analysis was performed before surgery and 6 and 12 months after surgical treatment. Computed tomography (TOSHIBA Aquilion PRIME 160-slices MODEL TSX-302A/1C equipment) was used to assess bone tissue density and structure. Analysis of bone tissue density according to Hounsfield GN (1919), HU and its structure were performed in the SimPlant Pro 11.04 software.

In all patients, during the cystectomy surgery, operative access was performed through an angular or trapezoidal incision. With the help of a fissure drill, resection of the apex of the root followed by cystectomy, was performed (Figure 2).

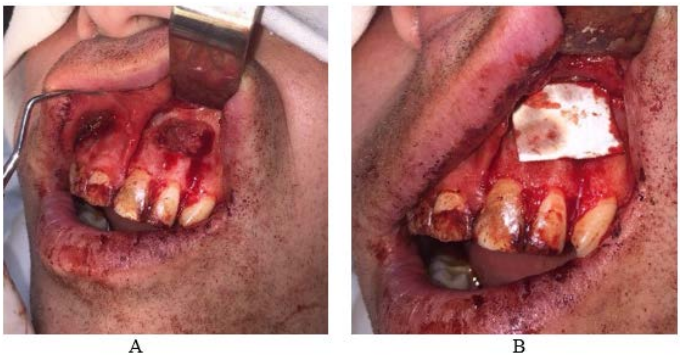


Figure 2. *Patient M., aged 36, cystectomy combined with the resection of the tops of the roots of the teeth 21and 22, angular surgical access: a – without a collagenous membrane, b – with a collagenous membrane.*

The bone defect was either left under a blood clot and covered with a collagenous membrane or filled with osteoplastic material and covered with a collagenous membrane. The mucosal-occipital flap was located in place and fixed with sutures.

Statistical analysis of numerical data was performed using Microsoft Excel 2019 software (Microsoft Office 2019 (Microsoft). All the quantitative data obtained in the study corresponded to the normal type of distribution according to the Shapiro-Wilk's W-test, and therefore the interval (M±m) was used to represent their central tendency: arithmetic mean (Mean) ± Standard error. To assess the reliability of the differences in the results obtained in comparison with the control group, the parametric t-criterion (Student's test) was used. The reliability of the difference in qualitative data between the comparison groups was determined according to the results of calculating the Chi-squared test with Yates's correction for continuity. A value of $p<0.05$ was considered probable.

Results.

Direct analysis of microfibrous polymer matrix samples showed compliance with absorption spectra of PCL polycaprolactone without sucrose impurities. This result was confirmed by the fact that there are valent vibrations of adsorbed water in the range of 3600-3200 cm^{-1}. As in the control samples, in the matrix, the valent vibrations of C-H bonds are located in the range of 3200-2800 cm^{-1}. The frequency of C=O oscillations in the spectrum corresponded to 1720-1730 cm^{-1}. The bands at 1458, 1390, and 1163 cm^{-1} were attributed to the deformational torsional vibrations of the methylene groups of the cis-isomers. The bands at 1470, 1395, and 1193 cm^{-1} belonged to the methylene groups of the trans-isomers. The bands at 1235 and 1275 cm^{-1} were attributed to the deformation ester group. Comparative analysis of the spectral characteristics of control samples of PCL granules and PCL matrix showed the identity of the location of the bands in the 1000-900 cm^{-1} range, which are caused by C-O-C valent vibrations. In the range of 1600-700 cm^{-1} there are valent vibrations of the polymer chain (C-C-C-C) (Figure 3).

Therefore, the results of infrared spectroscopy showed the absence of impurities and changes in the polymer, which indicates the high purity of the synthesis of the fibrous matrix obtained by our technology.

The results of the performed histological and immunohistochemical analysis of bone tissue at different times of implantation of the polymeric fibrous non-woven matrix indicated a certain nature of bone tissue formation (Tables 1&2).

Table 1. *Part of osteoid per 1 μm^2 of tissue area.*

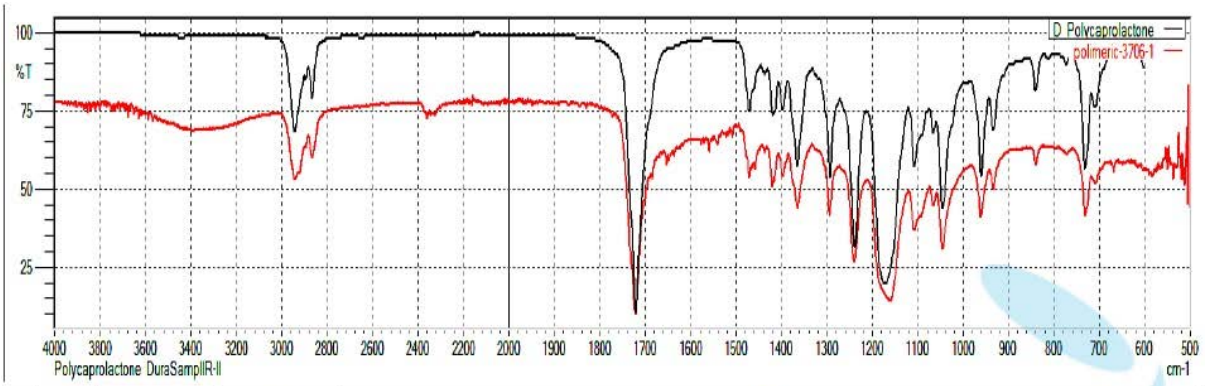
	Research group	**Control group**
1 month	18.96%	13.24%
2 month	34.38%	20.33%
3 month	8.91%	13.51%
4 month	3.30%	5.87%
5 month	0.13%	0.94%

Table 2. *Optical density of immunohistochemical expression of osteoinductive markers.*

	Osteocalcin		**Osteopontin**	
	Experimental group, CU	Control group, CU	Experimental group, CU	Control group, CU
1 month	132.73±1.79*	151.32±2.31	157.79±2.09*	148.0±2.72
2 month	117.09±2.64*	138.83±2.90	135.27±2.84	140.11±1.85
3 month	150.56±2.49*	162.9±2.16	158.46±2.32	166.0±3.07
4 month	163.56±1.95	165.62±1.84	165.23±1.53*	172.0±2.24
5 month	172.0±2.54	174.22±2.43	175.13±2.12	175.64±2.14

*Note: * – $p<0.05$ when comparing indices with the control.*

Histological examination of the bone tissue in 1 month after the implantation of the polymer matrix showed the growth of



	Score	Library	Name	Comment
1	918	143 - ATR-Polymer2	D_Polycaprolactone	Polycaprolactone DuraSamplIR-I
2	874	137 - T-Polymer2	T_Polycaprolactone	Polycaprolactone Transmission(Microscope)
3	785	22 - A_FoodAdditives2	A_Isoamyl Formate-4	Isoamyl Formate(Sales origin;TOKYO CHEMICAL INDUSTRY CO.,LTD.) @DuraSamplR2(diamond)
4	744	138 - T-Polymer2	T_Polydiallyl_Isophthalate	Poly(Diallyl Isophthalate) Transmission(Microscope)
5	743	142 - ATR-Polymer2	D_Polybutyl_Methacrylate	Poly(Butyl Methacrylate) DuraSamplR-I
6	734	136 - T-Polymer2	T_Polybutyl_Methacrylate	Poly(Butyl Methacrylate) Transmission(Microscope)
7	734	89 - ATR-Polymer2	D_Acrylic	Acrylic Adhesive DuraSamplR-I

Figure 3. *Infrared spectra of the polymer fibrous matrix sample with the identity spectrum of polycaprolactone.*

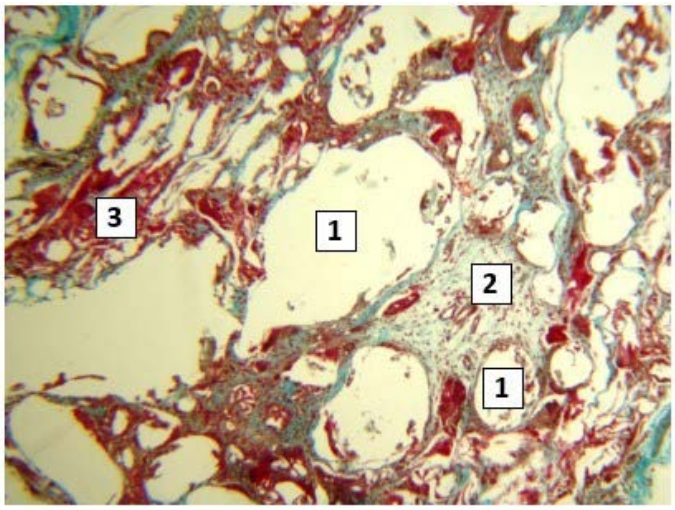


Figure 4. *Bone defect within 1 month after implantation of the polymer matrix. Staining: according to Masson. Magnification: ocular lens 10, field lens 20. 1 – location of polymer implant fibers, 2 – connective tissue fibers, stained blue, 3 – osteoid, stained red.*

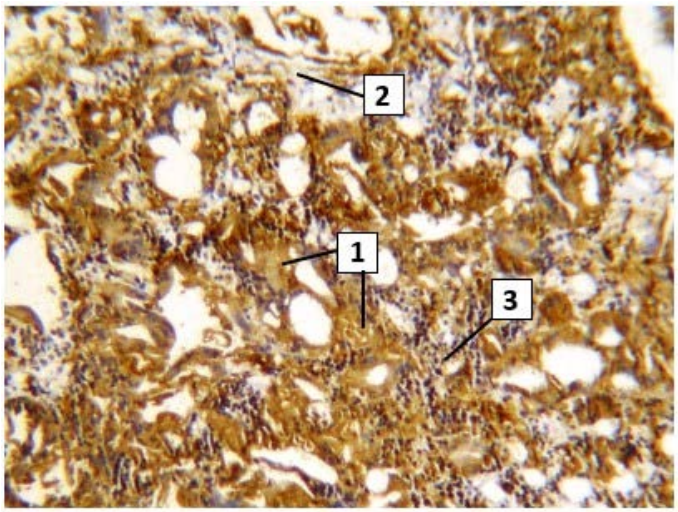


Figure 5. *Expression of osteocalcin, 1 month after implantation of the polymer matrix. Immunohistochemical study: antibodies to osteocalcin. Magnification: ocular lens 10, field lens 20.1 – positive marked expression of osteocalcin in the form of brown areas in osteoid areas, 2 – connective tissue elements, 3 – connective tissue cells.*

connective tissue with a loose arrangement of connective tissue fibers mainly in the central and peripheral parts of the defect. Multiple osteoid cells were also noted – 18.96% per 1 μm^2 in a close contact with the fibrous non-woven polymer matrix, which indicated the beginning of bone mineralization and regeneration processes in the area of the defect. Immunohistochemically, the optical density of osteocalcin in osteoid zones was at the average level (132.73±1.79) CU (control group – (151.32±2.31) CU) (Figures 4&5).

Expression of osteopontin was observed in osteoid, osteoblasts, as well as diffusely in fibroblasts of connective tissue and bone marrow cells, the optical density of which was on average (157.79±2.09) CU (control group – (148.0±2.72) CU).

In 2 months after implantation, the process of osseointegration of the matrix and bone tissue increased, which is confirmed by the increased growth of circularly located tightly fitting collagenous fibers to the polymer matrix and the increase of osteoid up to 34.38% ($p<0.05$), which was significantly different from the previous term. The presence of osteocalcin in the osteoid areas, which according to the results of the study was (117.09±2.64) CU, which at 11.8% exceeded the corresponding index at the previous stage of the study and at 15. 6% was greater compared to the control group ((138.83±2.90) CU, – this presence was the evidence of actively ongoing reparative regeneration of bone tissue. A proportional increase in the expression of osteopontin was noted, the optical density of which was on average (135.27±2.84) CU, which at 14.3% and at 3.5% exceeded the indices of the previous group and the control group, respectively ((140.11±1.85) CU).

At the end of the 3rd-4th months, mineralized lamellar bone tissue was noted in the area of bone defect. The presence of a large number of osteocytes, in our opinion, indicated the completion of the osteogenesis process and the presence of already formed bone. The share of unmineralized bone plates was only 8.91% ($p<0.05$), which is significantly lower than the corresponding index at the end of the 2nd month of the experiment and reflected the process of active mineralization and compaction of bone tissue (Figure 6).

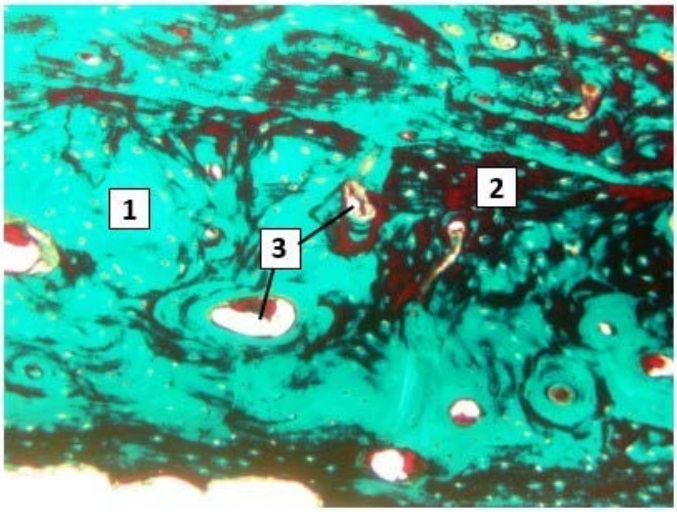


Figure 6. *Bone defect within 3 months after implantation of the polymer matrix. Staining: according to Masson. Magnification: ocular lens 10, field lens 20. 1 – mineralized bone matrix, 2 – osteoid, 3 – central canals of osteons.*

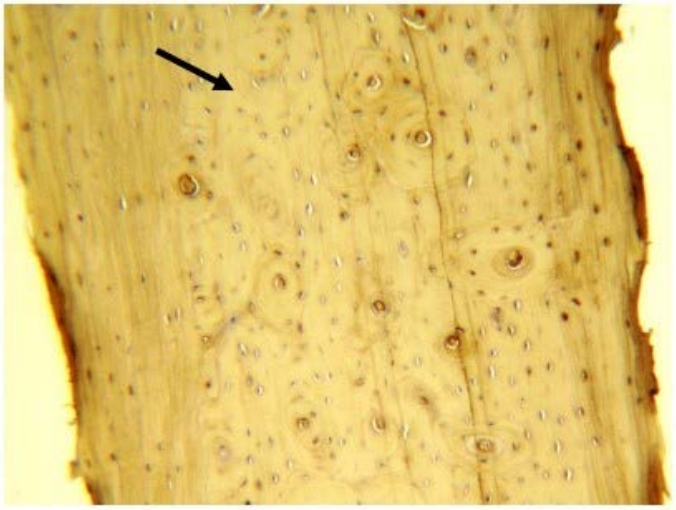


Figure 7. *Positive expression of osteocalcin in the bone matrix, 4th month after implantation of the polymer material. Immunohistochemical study: antibodies to osteocalcin. Magnification: ocular lens 10, field lens 20.*

The completion of the mineralization process in the 4th month was also reflected in the decrease in the expression of osteocalcin, which was (163.56±1.95) CU, which is lower when compared with the 3rd month of the experiment at 8.6%, and at the same time insignificantly higher at 1.2%, as in the control group ((165.62±1.84) CU) (Figure 7).

Osteopontin was verified in the bone matrix of both calcified mature lamellar bone tissue and in osteoid islands and averaged (165.23±1.53) CU of optical density, which is less than during the 3rd month of the experiment, and also it slightly exceeded the index of the control group ((172.0±2.24) CU).

During the 5th month of the experiment, the presence of fully mineralized bone tissue with lysis of the fibers of the non-woven polymer matrix and the presence of microosteoid cells was noted in the defect area, the share of which was 0.13% ($p<0.05$), which is almost 25-fold lower than in the previous experimental group and at 86% less than the index of the control group (the share of osteoid of the control group is 0.94%). Densitometrically, insignificant expression of both osteocalcin ((172.0±2.54) CU) and osteopontin ((175.13±2.12) CU) was found in the bone matrix. The optical density was lower when compared with the 4th month of the experiment (5.1% and 5.9%, respectively, for osteocalcin and osteopontin) and practically did not differ from the indices of the control group ((174.22±2.43) and (175, 64±2.14) CU, respectively).

Therefore, the results of our experimental studies confirmed the possibility of the fibrous matrix application in the clinic.

The analysis of the data of cone beam computed tomography of patients in the group I showed that after 6 months in 10 of 26 patients (38.4%) the bone cavity was completely filled with trabeculae of bones. In the remaining 16 (61.6%) patients, the defect was filled at only 2/3 of its size. In other 4 (13.3%) patients, there were no signs of the bone tissue defect restoration. After 1 year, in 18 (60%) patients, the bone tissue completely filled the bone defect, in the remaining 8 (26.7%) patients, the defect was filled only at 2/3 of the primary size of the cavity.

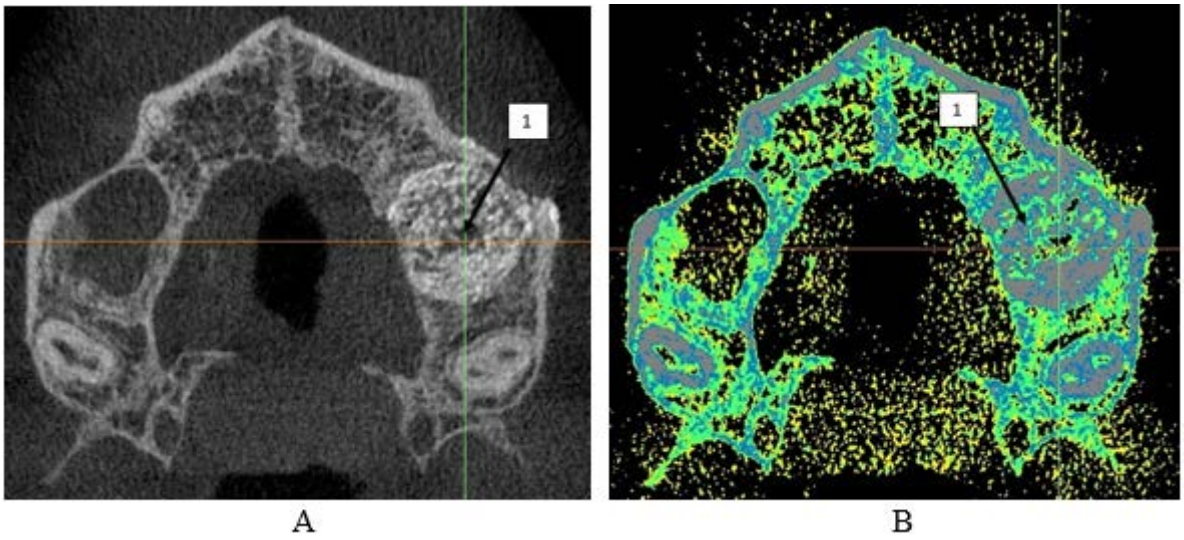


Figure 8. *Patient M. aged 28, condition in 6 months after cystectomy and bone grafting (1) in the area of upper molars: a - without visualization of density fields; b - with visualization of density fields.*

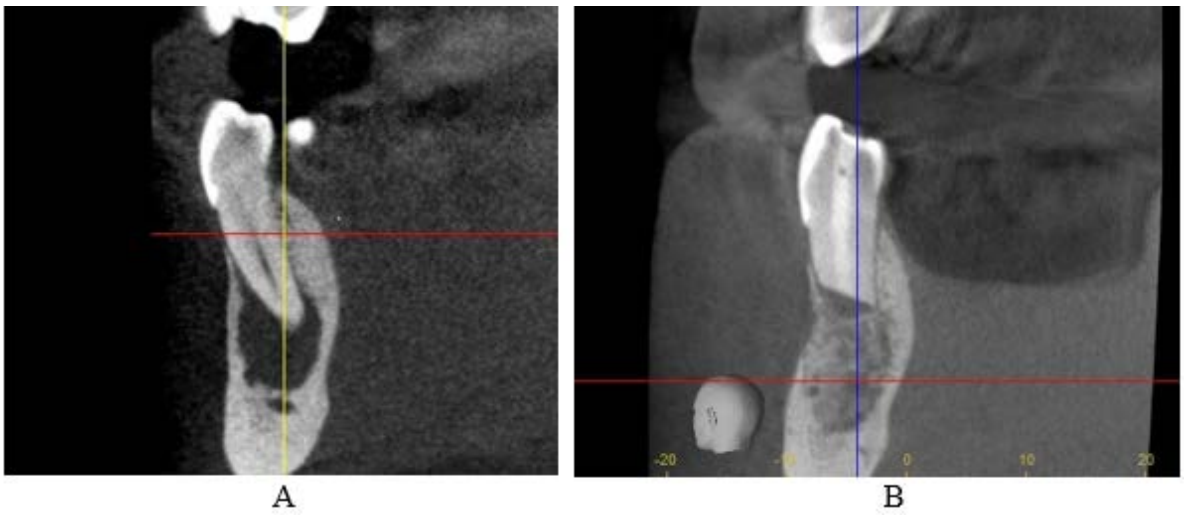


Figure 9. *Patient L. aged 21, the condition in 6 months after cystectomy and bone grafting in the area of lower premolars: a - before surgery; b - after surgery.*

Table 3. *Indices of bone tissue density in Hounsfield units (HU) in patients of groups I, II and III in 6 and 12 months after surgery.*

Time of operation	Group I	Group II	Group III	Control group
6 months	543.42±19.43 p>0.05	974.53±19.74 p<0.05	615.17±24.53 p<0.05	654.76±17.52
12 months	613.46±17.56 p>0.05	811.45±16.43 p<0.05	678.17±21.36 p<0.05	

The performed analysis of computed tomography data in the group II during the 6th month after the surgical intervention, showed that in 3 (10%) patients, there were no signs of recovery of the bone defect, confirmed by the progression of inflammatory changes in the form of periodically occurring swellings in the operated area and secretions of exudate. In 4 (13.3%) operated patients, there was a violation of the dense parietal contact of the granulate, which was noted both on axial sections and on reconstructions, in the form of a small gap of up to 0.5 mm in size between the material and the defect walls. In our opinion, in this case, the invasion of connective tissue with the subsequent encapsulation of the material took place. Only 23 (76.6%) patients showed signs of replacement by bone tissue, which was evidenced by the close parietal contact of the material with the present area of osteosclerosis (Figure 8).

In 6 months after cystectomy and bone grafting, out of 28 patients in the group III, in 22 (78.6%) patients the defect was completely restored by bone tissue, in the remaining 6 (21.4%) patients bone tissue regeneration took place only at 2/3 of the bone cavity volume. One year after the surgical intervention, bone tissue recovery occurred in all 28 patients (Figure 9).

Analysis of bone tissue density was performed during the 6th and 12th months after surgery (Table 3).

Analyzing the dynamics and nature of growth of bone tissue density indices in the postoperative period in patients with radicular cysts, a clear tendency can be noted only in the area of the former bone tissue defect. During the 6th month of the postoperative period, in comparison with the clinical group I, the density of bone tissue in the group III did not exceed 11.7% of the indices of the group I, which, in our opinion, is due to the use of polymeric degradable fibrous material, which in terms of its structure and X-ray contrast corresponded to connective tissue matrix. In the group II, on the contrary, the use of granulate based on hydroxyapatite and tricalcium phosphate has led to a significant increase in density indices, which did not exceed 36.8% of the indices in group III.

This tendency is caused on the one hand by the untimely resorption of the granulate, on the other hand – by the compact arrangement of the granules, which, in our opinion, has led to the compaction of the spongy structure of bone and the formation of a kind of hyperostosis in the area of bone grafting. During the comparison of indices, a tendency to the reduction of the difference almost two-fold between the indices of groups II and III in the 12-months postoperative period, compared to the same indices, in 6 months after the surgical intervention, was also noted. The indicated difference between the groups II and III in the one-year postoperative period did not exceed 16.3%, which is associated with a decrease in the bone density of the group II. In our opinion, this fact is caused by the final degradation of the material and some decrease in bone compaction due to its reconstruction.

Discussion.

The results of the indicated above experimental and clinical studies confirm the effectiveness of the microfibrous polymer matrix developed by us. In our opinion, the effectiveness is due, first of all, to the structural peculiarities of the frame itself, namely the thickness, structure and chaotic arrangement of its microfibers, which form a kind of mesh with micropores; this creates conditions for the regeneration of cells along it. This matrix effect was confirmed by the compact and circular arrangement of the collagenous fibers around groups of polymer microfibers at the early stages of the experiment with the percentage of osteoid (34.38% ($p<0.05$)) and the subsequent creation of a formed and organized bone structure in three mutually perpendicular directions. That is, a group of polymer fibers created a kind of substrate for building bone tissue on it. The active synthesis of bone tissue at the early stages of the experiment was also indicated by the growth of the osteoinductive proteins of osteocalcin and osteopontin.

A decrease in the activity of osteoinductive markers of osteocalcin and osteopontin after the 3rd month of the experiment indicated the inhibition of the activity of bone tissue synthesis in the experimental and control groups, however, an increase in the densitometric optical density of osteoinductive markers compared to the control group, indicated an increase in the strength (probable strength) of the reparative tissue in the defect area and accelerated the processes of its subsequent compaction. This, in turn, in our opinion, was a sign of the pronounced framework function of the polymeric microfiber matrix synthesized by us.

The fibrous structure of the polymer frame synthesized by us, also made it possible to attach a bone substitute biogel based on demineralized bone of allogeneic origin to it, which made it possible to stabilize its fluid consistency and improve the osteogenic functions of the entire hybrid frame. This effect was also confirmed by clinical data, namely, the quality of the structure of the newly formed bone tissue with a more pronounced trabecular pattern in the group III, in contrast to the group II, where the compact structure of the bone prevailed. In our opinion, this fact is due to the presence of non-resorbed granules of material that form a compact framework for bone growth. This tendency was confirmed by the works of other authors [4-6].

Conclusion.

1. The results obtained of the histological examination of the bone tissue in the experiment with the implantation of the fibrous matrix indicated an increase in reparative osteogenesis in the form of an increase in osteoid zones up to 34.38% and the concentration of osteocalcin and osteopontin.

2. The slight difference in bone structure and density between the groups I and III indicated an unchanged bone regeneration process, however, the frequency and speed of complete repair of the defect in the group III testified to the effectiveness of the applied matrix.

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РЕЗЮМЕ

ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ РАЗРАБОТАННОГО БИОПОЛИМЕРНОГО ВОЛОКНИСТОГО МАТРИКСА С БИОГЕЛЕМ CENOBONE® ДЛЯ РЕКОНСТРУКЦИИ ДЕФЕКТОВ КОСТНОЙ ТКАНИ ЧЕЛЮСТЕЙ.

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Цель исследования: изучение каркасной способности созданых нами волокнистых нетканых PCL матриксов при восстановлении костной ткани.

Материалы и методы исследования: В работе проведен спектроскопический, гистологический, иммуногистохимический, рентгенологический и клинический анализ эффективности разработанных микроволокнистых нетканых матриксов из поликапролактона PCL. Эксперимент проведен на двух группах лабораторных животных (кролях), из которых 30 основная и 30 контрольная. Гистологический анализ в обеих группах проводили на 1-м, 2-м, 3-м, 4-м и 5-м месяцах эксперимента. Клиническое исследование проводилось на 90 больных в возрасте от 20 до 50 лет с радикулярными кистами челюстей.

Результаты: Полученные результаты морфологических исследований костной ткани в эксперименте при имплантации волокнистого матрикса свидетельствовали об усилении репаративного остеогенеза посредством увеличения зон остеоида до 34,38% ($p<0.05$) в раннем периоде. Значительное усиление минерализации и компактизации костной ткани в зоне дефекта отражалось уменьшением доли остеоида на 0,13% ($p<0.05$) в позднем периоде.

Анализ клинических данных показал эффективность разработанного нами каркаса, которая подтверждалась отсутствием выраженной компактизации костной ткани в III группе, в отличие от II, где наоборот применение гранулята на основе гидроксиапатита и трикальцийфосфата приводило к существенному росту показателей плотности 974,53+19,74 HU $p<0,05$, которые не превышали 36,8% показателей III группы 615,17+24,53 HU $p<0,05$.

Выводы: Разработанный нами матриксный материал является не только средством доставки веществ и материалов в зону повреждения, но и выступает своеобразным каркасом для восстановления костной ткани.

Ключевые слова: матриксные материалы, поликапролактон, гистологический анализ, костная ткань, радикулярная киста.