УДК 616.71-089.844-092
https://doi.org/10.17816/PTORS16480

EXPERIMENTAL EVALUATION OF THE EFFICIENCY OF CHITOSAN MATRIXES UNDER CONDITIONS OF MODELING OF BONE DEFECT *IN VIVO* (preliminary message)

© ***S.V. Vissarionov[1], M.S. Asadulaev[1], A.S. Shabunin[1, 2], V.E. Yudin[2], M.B. Paneiakh[3], P.V. Popryadukhin[2], Yu.A. Novosad[2], V.A. Gordienko[3], A.G. Aganesov[4]***

[1] H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery, Saint Petersburg, Russia;
[2] Peter the Great Saint Petersburg Polytechnic University, Saint Petersburg, Russia;
[3] Saint Petersburg State Pediatric Medical University, Saint Petersburg, Russia;
[4] Russian Scientific Center of Surgery named after academician B.V. Petrovsky, Moscow, Russia

■ For citation: Vissarionov SV, Asadulaev MS, Shabunin AS, et al. Experimental evaluation of the efficiency of chitosan matrixes under conditions of modeling of bone defect *in vivo* (preliminary message). *Pediatric Traumatology, Orthopaedics and Reconstructive Surgery*. 2020;8(1):53-62. https://doi.org/10.17816/PTORS16480

Received: 17.10.2019 Revised: 04.02.2020 Accepted: 10.03.2020

Background. Despite the wide range of studies, the development of osteoplastic material, which has not only osteoconductive but also osteoinductive properties, remains an extremely topical issue in modern medical materials science. This work is devoted to experimental evaluation of the effectiveness of synthetic osteoplastic composite material based on chitosan and hydroxyapatite.

Aim. This study aimed to determine the effects of spongy implants based on chitosan and its composite with hydroxyapatite nanoparticles in an amount of 50 wt. % on early osteogenesis in the area of the through defect of the ileum.

Materials and methods. The studied materials were sponge implants based on chitosan and its composite with hydroxyapatite nanoparticles in an amount of 50 wt. %. Comparison groups include those without implant placement and those with replacement with commercial Reprobone osteoplastic material. Materials were implanted into the zone of the through defect of the ileum of rabbits for a period of 28 days.

Results. A high rate of resorption of materials based on chitosan in bone tissue and active growth of reticulofibrotic bone tissue along the edges of the defect was established, and the formation of cartilaginous islands and bone marrow was recorded in the group of chitosan implants with hydroxyapatite. The aseptic effect was observed with the use of implants made of chitosan and hydroxyapatite.

Conclusions. The data obtained allow us to argue about the osteoconductivity of the studied materials and the prospects for further development in this direction.

Keywords: bone defect; bioresorbable material; experiment; defect modeling; traumatology; orthopedics.

ЭКСПЕРИМЕНТАЛЬНАЯ ОЦЕНКА ЭФФЕКТИВНОСТИ ХИТОЗАНОВЫХ МАТРИЦ В УСЛОВИЯХ МОДЕЛИРОВАНИЯ КОСТНОГО ДЕФЕКТА *IN VIVO* (предварительное сообщение)

© ***С.В. Виссарионов[1], М.С. Асадулаев[1], А.С. Шабунин[1, 2], В.Е. Юдин[2], М.Б. Панеях[3], П.В. Попрядухин[2], Ю.А. Новосад[2], В.А. Гордиенко[3], А.Г. Аганесов[4]***

[1] Федеральное государственное бюджетное учреждение «Национальный медицинский исследовательский центр детской травматологии и ортопедии имени Г.И. Турнера» Министерства здравоохранения Российской Федерации, Санкт-Петербург;
[2] Федеральное государственное автономное образовательное учреждение высшего образования «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург;

[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Санкт-Петербургский государственный педиатрический медицинский университет» Министерства здравоохранения Российской Федерации, Санкт-Петербург;
[4] Федеральное государственное бюджетное научное учреждение «Российский научный центр хирургии имени академика Б.В. Петровского», Москва

■ Для цитирования: Виссарионов С.В., Асадулаев М.С., Шабунин А.С., и др. Экспериментальная оценка эффективности хитозановых матриц в условиях моделирования костного дефекта *in vivo* (предварительное сообщение) // Ортопедия, травматология и восстановительная хирургия детского возраста. – 2020. – Т. 8. – Вып. 1. – С. 53–62. https://doi.org/10.17816/PTORS16480

Поступила: 17.10.2019 Одобрена: 04.02.2020 Принята: 10.03.2020

Обоснование. Несмотря на широкий спектр проводимых исследований, разработка костнопластического материала, обладающего не только остеокондуктивными, но и остеоиндуктивными свойствами, остается крайне актуальным вопросом современного медицинского материаловедения. Данная работа посвящена экспериментальной оценке эффективности синтетического костнопластического композиционного материала на основе хитозана и гидроксиапатита.

Цель — исследование воздействия губчатых имплантатов на основе хитозана, а также его композита с наночастицами гидроксиапатита в количестве 50 масс. % на ранний остеогенез в зоне сквозного дефекта подвздошной кости.

Материалы и методы. В основной группе применяли губчатые имплантаты на основе хитозана и его композита с наночастицами гидроксиапатита в количестве 50 масс. %. В группе сравнения использовали имплантаты и выполняли замещение коммерческим костнопластическим материалом Reprobone. Материалы имплантировали в зону сквозного дефекта подвздошной кости кроликов на 28-е сутки.

Результаты. Установлены высокая скорость резорбции материалов на основе хитозана в костной ткани и активное разрастание ретикулофиброзной костной ткани по краям дефекта, а в группе имплантатов из хитозана с гидроксиапатитом происходило образование островков хрящевой ткани и костной мозоли. Имплантаты из хитозана и гидроксиапатита оказывали асептическое действие.

Заключение. Полученные данные свидетельствуют об остеокондуктивности исследованных материалов и перспективности дальнейших разработок в данном направлении.

Ключевые слова: костный дефект; биорезорбируемый материал; эксперимент; моделирование дефекта; травматология; ортопедия.

Bone defect filling of various etiologies is one of the most important and significant tasks in the practice of orthopedic traumatologists [1, 2]. Today, autologous bone is traditionally used for bone grafting of bone defects, as it currently remains the gold standard for replacing bone defects of different localizations [3, 4]. However, specialists always face many problems associated with the occurrence of complications in the area of graft collection of a donor wound, especially in the postoperative period. And attention should be paid to such negative phenomena, including pain in the postoperative wound area, infectious complications, hematoma in the graft collection area, fracture of the donor bone, a cosmetic defect in bone structures and skin in this area, and a limited amount of the donor resource [5]. In addition, it should be borne in mind that in patients with an orthopedic disorder, osteoporosis is often a concomitant diagnosis, which significantly reduces bone density and is a reason for searching for osteoplastic material alternatives.

The second most common material for replacing bone defects is an allograft [1, 3, 6–8]. However, despite contemporary methods of processing biological materials, this option carries a risk of transmitting human immunodeficiency virus, as well as hepatitis B and C viruses. In addition, the problems associated with resorption and remodeling of allograft in the recipient's body remain relevant [5].

A detailed study of the physiological mechanisms of bone transformation and remodeling and tissue engineering led to the development of new synthetic implants as an osteoplastic material [3]. In addition to the earlier requirements for new synthetic materials being developed, several general necessary conditions can be considered, including ease of use, accessibility, flexibility, and the presence of osteoinductive and osteoconductive properties [3, 9].

Tissue engineering aims to regenerate tissues, including bone tissue, using various matrices (scaffolds) based on synthetic materials, mostly

polymer, stem cells, and biomodulators [1, 10]. An ideal scaffold should contain intertrabecular spaces sufficient for cell adhesion and growth to ensure the transit of nutrients and metabolites. The rate of graft degradation should not exceed the rate of normal bone tissue regeneration. The mechanical properties of the implant must correspond to possible loads and not lead to additional trauma to the marginal zones of the defect [11].

Despite the variety of bone-substituting materials, today, it is impossible to single out an "ideal" one that is suitable for use in various fields of surgery [10]. When developing new biomaterials for repairing defects, the mechanisms of reparative regeneration of bone tissue should be taken into account. Bone replacement materials should not only serve as a framework for the forming bone (osteoconduction) and stimulate bone cell maturation (osteoinduction) but also initiate these processes at the appropriate stage of reparative regeneration.

Chitosan (CS) is a promising polymer for biomedical applications [12–15], as it is a heteropolysaccharide obtained by deacetylation of a natural polymer, chitin. CS has many useful physicochemical and biological properties, in particular, bioresorbability, biocompatibility, and hemostatic properties, and it lacks cytotoxicity [16]. The product of CS resorption is D-glucosamine, which is a normal component of synovial fluid that can facilitate the normal deposition of Ca^{2+} in bone tissue. The combination of all these positive properties enables to consider the prospectivity of using CS in surgery for bone tissue regeneration.

Hydroxyapatite (HA) is a normal component of bone tissue that provides its strength. HA is located between collagen fiber bundles in the form of parallel plates. Composite biomaterials containing nanosized HA have enormous potential for the repair and synthesis of bone tissues [17–19]. The idea of using HA as part of composite materials for bone grafting was implemented in a wide range of works on modern materials science in the field of biomedicine [18, 20–23]; therefore, modification of spongy CS matrices using HA can be a promising solution to the problem to not only increase the strength characteristics of the implant but also form a structure more analogous to the native one.

Although a wide range of *in vitro* studies was conducted to evaluate various aspects of the new materials, a full-fledged study of the process of reparative osteogenesis *in vitro*, *in situ*, and *in silico* is currently not possible because of complexity. Thus, *in vivo* testing is the only option for an adequate assessment of the reparative regeneration of bone defects.

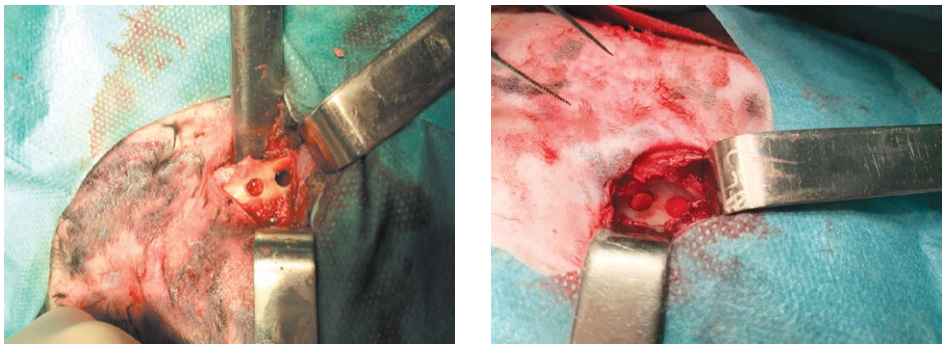
The **study aimed** to analyze the effect of CS-based spongy implants, as well as its composite with HA nanoparticles in the amount of 50 mass percentage on early osteogenesis in the site through an iliac bone defect.

Materials and methods

The studied materials were obtained by lyophilization of a 3% solution of CS (Heppe, Germany, an average molecular weight of 168 kDa and deacetylation degree of 90%) in 2% acetic acid, followed by conversion to the basic form by leaching in a 10% NaOH solution. Similarly, sponges based on HA CS were obtained; and 50 mass percentage HA nanoparticles (Institute of Silicate Chemistry RAS, Russia) were also added to the initial CS solution. The resulting sponges were shaped into a cylinder with a diameter of 4–5 mm and a height of 1 cm, after which they were sterilized by autoclaving. Reprobone (Ceramisys Ltd, UK), used in clinical practice, consisting of 60% HA and 40% β-tricalcium phosphate, was used as a commercial comparison material.

An experimental study was conducted on 18 female chinchilla rabbits aged 8 to 12 months, with an average weight of 2000–2500 g, and bred at the Laboratory Animal Nursery Rappolovo (series and number of veterinary certificate: 247 No. 0150967 of 06/19/2018). This work was performed in the experimental laboratory of the Turner Scientific and Research Institute for Children's Orthopedics in compliance with international requirements for this type of research.

Iliac bone injuries were modeled by applying two through holes with a diameter of 5 mm located at a distance of 1 cm from each other (Fig. 1). The total duration of the experimental study was 28 days because after 2–4 weeks, bone resorption ceases, and osteoclasts undergo apoptosis and englobing. After which, there is a short rest period, when the formed cavity is covered with osteoblasts and filled with osteoid tissue during the next 3 months.



a *b*

Fig. 1. The appearance of the modeled defect site: (*a*) site of the defect before implantation of the materials under study; (*b*) defect site with implanted chitosan-based spongy materials

In total, 16 defects were filled with a sponge material based on CS, 16 perforation holes with CS with HA (CHA), and 16 perforation holes of positive control with Reprobone material. Also, 16 defects of the negative control group were modeled, where after modeling, the perforations remained hollow in the animals (Table). Reprobone material by Ceramisys consists of HA (60%) and tricalcium phosphate (40%).

All manipulations with animals were performed under adequate anesthesia. Animals were anesthetized with a 1:1 mixture of tiletamine/zolazepam (Zoletil 100, Vibrac, France) and xylazine (Rometar, Bioveta, Czech Republic). The dosage for anesthetic induction was 0.15 mL Zoletil 100 per 1 kg of animal weight and 0.1 mL of Rometar per 1 kg of animal weight. The maintenance dose was half the induction dose. To calculate the dosage of the preparations, the experimental animals were pre-weighed.

Distribution of animals in groups

Group of animals	Materials and localization	Number of animals
CS/CM	Left: CS, 2 pcs. Right: CM, 2 pcs.	3
CS/CHA	Left: CS, 2 pcs. Right: CM, 2 pcs.	3
CHA/CM	Left: CHA, 2 pcs. Right: CM, 2 pcs.	3
CS/CNTR	Left: CS, 2 pcs. Right: control, without treatment, 2 pcs.	3
CHA/CNTR	Left: CHA, 2 pcs. Right: control, without treatment, 2 pcs.	3
CM/CNTR	Left: CM, 2 pcs. Right: control, without treatment, 2 pcs.	3
	Total	18

Note. CS, chitosan sponge; CHA, chitosan sponge with hydroxyapatite; CM, commercial material Reprobone; CNTR, control group, without treatment.

The surgical field was prepared after the animal was introduced into the surgical anesthesia stage. The hair was cut and shaved, and then, the skin was treated three times with a skin antiseptic solution with an exposure of 5 min, and the intervention site was bordered with sterile drapes. Additionally, infiltration anesthesia was performed with 0.1% lidocaine hydrochloride solution.

The pelvic bone spine was determined by palpating the dorsal surface. Then, under sterile conditions, a 2.5-cm-long skin incision was made in the projection of the pelvic bone spine with a bellied scalpel. The pelvic bone was separated in a layer-by-layer dull and sharp manner. After an incision of the periosteum, the iliac wing was skeletonized using a straight raspatory. The bone was perforated under the protection of soft tissues with Buyalsky wound scoops.

During the manipulation, a sterile cutter with a diameter of 5 mm, fixed in a drill, was rigidly positioned at an angle of 90° to the iliac bone plane. The angle of a perforation hole was measured using an angular gauge. After stabilizing the drill position at a 90° angle to the plane of the bone, a through perforation of the iliac bone wing was made. The manipulation was repeated twice on each side, resulting in two bone defects with a diameter of 5 mm at a distance of 10 mm from each other, which were filled with the test material in accordance with the experimental group.

Four experimental groups were formed depending on the materials implanted, and six different pair combinations of materials were obtained to take into

account the individual differences of the animals, which were placed in different zones of defects to avoid cross-exposure (Table). A total of 16 iliac bone defects were modeled in groups of synthetic materials and positive and negative controls.

It was convenient to fill the iliac bone defects with CS and CHA. The materials were easily compressed and placed in a bone defect using surgical forceps. Within 5 s, the initial shape was restored, and the materials filled densely and fully the bone defect. All this was accompanied by additional mechanical hemostasis of the bone defect.

When implanting the Reprobone material, which is represented by granules, the implant (5–6 granules) was placed into the bone defect with forceps and pressed tightly. At that, the material was located by thrust apart the bone defect. It should be noted that at the time of suturing, bleeding continued from the edges of the iliac bone in the perforation area of the wound.

In the negative control group, the perforations remained hollow after modeling.

Then, hemostasis was performed, and the wound was sutured in layers. In the postoperative period, the wound was treated with an antiseptic solution daily for 3 days. Before and after the surgery, cefuroxime solution was injected intramuscularly at a dose of 30 mg/kg.

During the study, animals were examined daily. On day 28, digital radiography was performed with frontal and lateral views for visualization and radiological assessment of the zone of the bone defect modeling in the iliac wing area. At the end of the indicated period, the animals were withdrawn from the experiment following the international standards of humane treatment of animals. An autopsy was performed, and autopsy samples were taken from the defect zone.

The main indicator of the effectiveness of experimental samples was the results of the morphological evaluation of autopsy samples of the defect zone, including determining the degree of the graft resorption, assessing the severity and activity of inflammatory reactions in the area of bone tissue damage, and determining the stage of regeneration in the defect zone by the presence (or absence) of cartilaginous islets and their rearrangement in the reticulofibrous bone tissue.

To compare adequately the experimental data, taking into account the individual characteristics of the animals, these manipulations were performed on both sides, and two bone defects were modeled on each side. At the same time, only materials of the same type were implanted into neighboring defects to avoid a cross-exposure.

The autopsy samples obtained were decalcified in an ethylene diamine tetraacetate-based solution (Solution B, Bio Vitrum, Russia) for 45 days, followed by treatment with alcohols of increasing concentrations in a Thermo Scientific Excelsior AS tissue processor (Thermo Fisher Scientific, UK) for 12 h. Paraffin embedding was performed at Thermoscientific Histo Star station (Thermo Fisher Scientific, GB), and paraffin blocks were cut using Microm HM 430 microtome (Thermo Fisher Scientific).

The sections obtained were stained in an automatic histological station Microm HMS 740 with eosin, hemalum, and picro-fuchsin, according to van Gieson (Bio Vitrum, Russia). After staining the histological specimen, a morphological examination was performed by light microscopy using a Carl Zeiss microscope (Germany), model Axio Lab.A1.

Results

During the experiment, there were no significant deviations in animal behavior, as well as fatal outcomes and any complications after the implantation procedure of the materials under study.

As a result of a visual assessment of the surgical intervention site on a gross specimen after the animals were withdrawn from the experiment and autopsy was taken 28 days after the surgery, no gaping bone defect was revealed in the zones of the CS materials implantation.

A bone defect of up to 0.2 mm in size and implanted samples of synthetic material were found in the positive control group with implanted Reprobone material.

In the negative control group, a macroscopic assessment of the operative area showed the presence of unfilled bone defects of up to 0.3 mm in size.

CS materials in their initial form have not been revealed. In the positive control group, the implanted samples practically retained their original appearance.

During the histological study of autopsy samples, the following data were obtained.

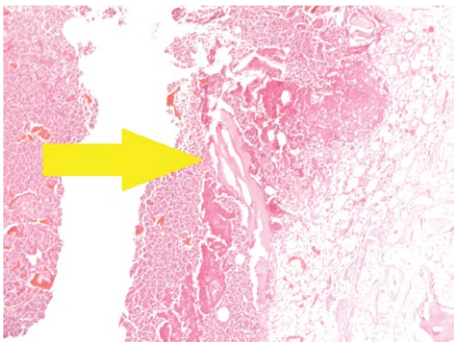


Fig. 2. Control group: necrotic detritus with dense neutrophilic leukocyte infiltration and adjacent bone trabeculae and periosteum. Hematoxylin and eosin staining, magnification ×40

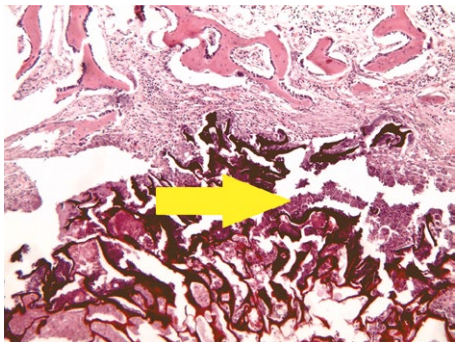


Fig. 3. Positive control group: spongy structure of Reprobone filling the bone defect area. In the cells of a foreign body, there is a pronounced infiltration with neutrophilic leukocytes. Hematoxylin and eosin staining, magnification ×40

- *Negative control group with perforations* (Fig. 2).

In the bone defect area, there was a large amount of necrotic detritus with multiple crowded neutrophilic leukocytes. The periosteum and small fragments of striated muscle tissue with focal lymphohistiocytic infiltration and giant multinucleated cells, of foreign body type, which are single in the field of vision, were adjacent to the defect zone and surrounding bone tissue.

- *Positive control group with the use of Reprobone material.*

In the studied material, on day 28 of the experiment, there was a large defect of the tabular bone tissue, and the iliac bone wing was filled with a spongy, sharply eosinophilic material. Among the sponge cells, a pronounced mixed-cell exudative reaction was noted; exudate of neutrophilic leukocytes with foci of finely lumpy decays predominated at the periphery of the material. In the central and paracentral cells of the sponge, the exudate mainly consisted of lymphocytes and macrophages. Between the bone tissue and the foreign body, there was a rim of coarse-fibrous connective tissue with foci of lymphomacrophage infiltration with an admixture of giant multinucleated foreign body type cells (Fig. 3). There are signs of the granulomatous inflammation in the periosteum.

- *A group of CS-based spongy material* (Fig. 4).

On day 28 in the defect zone, an area filled with fibrous cartilage was registered, and no elements of the synthetic material test sample were found. At the edges of the bone defect, the transition of fibrous cartilage into reticulofibrous bone tissue was visible.

- *A group of spongy material based on CS and HA* (Fig. 5).

On day 28 in the defect zone, there was a site filled with coarse-fibrous connective tissue with numerous giant cells of foreign bodies formed around small foci from the residues of the test sample. Bone callus with small and few larger cartilaginous islets was adjacent to the coarse-fibrous connective tissue.

Discussion

In the course of the work, it was possible to create an experimental model of bone defect of the iliac wing in laboratory animals and to conduct

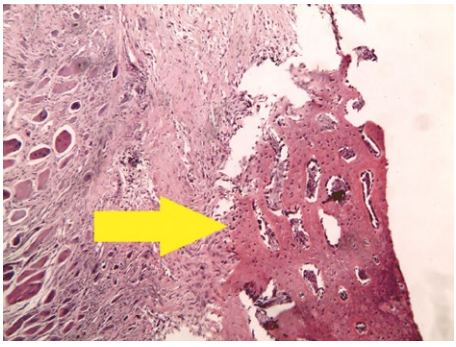


Fig. 4. The group with chitosan: proliferation along the edges of the defect of reticulofibrous bone tissue. Hematoxylin and eosin staining, magnification ×40

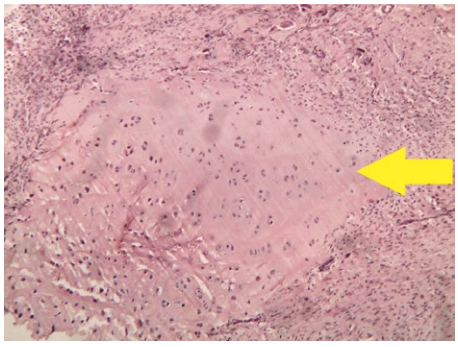


Fig. 5. A group with a chitosan prosthesis with hydroxyapatite: cartilaginous islets among the coarse fiber tissue in the area of the bone defect. Hematoxylin and eosin staining, magnification ×40

a comparative analysis of the use of various implant types as osteoplastic materials.

In the negative control group, where bone defects remained unfilled, the presence of necrotic detritus with dense infiltration by neutrophilic leukocytes was noted in the defect area during the morphological examination of the samples.

The positive control group was characterized by an extremely low rate of sample resorption. Since in the experiment, a defect was modeled, which was not critical, by definition, the low resorption rate restricted the repair process. Moreover, the histological examination revealed an exudative reaction in the defect zone and granulomatous inflammation with giant multinucleated foreign body type cells in the adjacent periosteum, which suggests a negative tissue reaction to this material in the early stages of healing. Nevertheless, this type of reaction may also be due to intraoperative wound contamination with bacterial agents.

CHA sponge materials showed a rather high resorption rate. In the initial form, the samples were not detected on the gross specimen; the remnants of the implanted materials were determined only by using a morphological study of biopsy samples of the defect zone.

CS materials at the stage of introduction into the experiment were characterized by their ease of use due to the spongy structure. The use of cylindrical samples with a diameter slightly larger than the initial hole of the defect enabled to achieve tight fixation of the defect zone sample with minimal impact on its edges due to the high elastic properties of the initial materials.

In the CHA group, there was a fairly high degree of resorption with mild manifestations of chronic inflammation in response to the foreign body introduction. Along with this, the cartilaginous tissue islets were found in the defect area, which supported the osteoinduction stimulation, as well as bone callus formation signs.

CS grafts proved to be the best in terms of morphological studies. In addition to the maximum resorption rate, the formation of reticulofibrotic bone tissue and the complete absence of traces of a negative response from adjacent tissues were also noted in the experiment. At that, the fast material resorption rate can also be a significant drawback. Since this study aimed to assess qualitatively the tissue response and osteoinductive properties of the materials, modeling a defect that is not critical was acceptable and was evaluated in a significantly shorter time. However, in the case of critical bone defects involving a significantly longer recovery period, excessively fast resorption of samples becomes a severely negative factor.

The results correlate with published data on the bioresorption of other CS and CHA porous matrices for the repair of bone defects [13, 17, 18]. One research [18] had a general similarity of the material studied, and the size and concentration of its HA exceeded significantly the corresponding parameters in our study. As a result, a more pronounced negative response of adjacent tissues was recorded. The use of HA nanoparticles in a concentration of 50 mass percentage enabled to obtain less brittle material, which ensured both ease of use and fixation compared with a denser and more brittle commercial analog, as well as a more positive reaction of the adjacent tissue and increased osteoconductivity of the materials under study.

Study limitation

The study limitations include the following factors.

- The influence of the developed materials in the early stages of reparative osteogenesis was studied. Short experimental terms do not allow an unambiguous conclusion about the long-term effect and complete comparison of the positive and negative aspects of the use of materials with a longer period of resorption (Reprobone material group).
- The purpose of the radiography of the surgical intervention area was control over the implantation site. Also, it was of a survey nature; as a result, it was impossible to quantify reliably the change in the defect zone.
- The size and localization of the initial defect did not enable us to make a supposition on the positive or negative effect of the viscoelastic properties of spongy materials in the case of mechanical loads in the defect area.

Conclusion

The study evaluated the biological properties, including bioresorption, of experimental materials based on CHA in both the positive and negative

controls. A positive effect of these materials on the process of bone regeneration and a mild negative reaction of adjacent tissues to the implant were confirmed. Also, it was revealed that the CS materials in bone tissue are resorbed extremely quickly, which can have both positive and negative values, depending on the defect size and application method of the above materials. At that, CS, in comparison with the other materials studied, has the strongest stimulating effect on bone tissue regeneration, whereas CHA manifested to be the weakest stimulator of osteoinduction in the ratio used. The aseptic effect when using CHA should also be emphasized.

The study results indicate that further research and material refinement are necessary. The simplicity of the process of obtaining the studied materials by freeze-drying in combination with their efficiency enabled us to consider the prospects of further developments in the field of bone implantation of tissue-engineering structures in the form of CHA-based composites.

Additional information

Source of funding. Grant of the Russian Science Foundation 19-7330003.

Conflict of interests. The authors declare no conflict of interest.

Ethical statement. The pilot study was approved by the local ethics committee. Extract from the protocol No. 19-1 of the meeting of the local ethics committee of the Turner Scientific and Research Institute for Children's Orthopedics dated 07/01/2019.

Author contributions

S.V. Vissarionov developed the methodology and study design and wrote the text of the article.

M.S. Asadulaev took part in the development of the study design, prepared and conducted surgical interventions, processed experimental data, and wrote the text of the article.

A.S. Shabunin took part in the development of the study design, processed the experimental data, maintained related documentation, and wrote the text of the article.

V.E. Yudin developed the research materials.

M.B. Paneiakh was involved in the production of histological preparations, performed morphological examination of materials, and wrote the text of the article.

P.V. Popryadukhin performed development and production of the materials under study.

Yu.A. Novosad was involved in the development and production of the materials under study and took part in surgical interventions.

V.A. Gordienko took part in surgical interventions and wrote the text of the article.

A.G. Aganesov took part in the development of research design and processed the experimental data.

All authors made a significant contribution to the research and preparation of the article, read and approved the final version before publication.

Acknowledgements. We express our gratitude for the help and assistance in conducting the experiment to the scientific researcher Natalya Borisovna Fomina, as well as to the employees of the laboratory of experimental surgery, the surgery suite head Olga Konstantinovna Kartseva, Tatyana Petrovna Maslennikova (Institute of Silicate Chemistry RAS), X-ray laboratory assistants, as well as the radiologist Tatyana Valerevna Murashko, students of Saint Petersburg State Pediatric Medical University Lola Aydarovna Davletova, Andrey Mikhailovich Fedyuk, Petr Leonidovich Suschinsky, and a resident physician of Saint Petersburg State Pediatric Medical University Biika Narimanovna Atabieva.

References

1. Анастасиева Е.А., Садовой М.А., Воропаева А.А., Кирилова И.А. Использование ауто- и аллотрансплантатов для замещения костных дефектов при резекциях опухолей костей // Травматология и ортопедия России. – 2017. – Т. 23. – № 3. – С. 148–155. [Anastasieva EA, Sadovoy MA, Voropaeva AA, Kirilova IA. Reconstruction of bone defects after tumor resection by autoand allografts (review of literature). *Travmatologiia i ortopediia Rossii*. 2017;(23):148-155. (In Russ.)]
2. Котельников Г.П., Колсанов А.В., Щербовских А.Е. Реконструкция посттравматических и постоперационных дефектов нижней челюсти // Хирургия. Журнал им. Н.И. Пирогова. – 2017. – № 7. – С. 69–72. [Kotel'nikov GP, Kolsanov AV, Shcherbovskikh AE. Reconstruction of posttraumatic and postoperative defects of lower jaw. *Khirurgiia (Mosk)*. 2017;(7):69-72. (In Russ.)]. https://doi.org/10.17116/hirurgia2017769-72.
3. Garcia-Gareta E, Coathup MJ, Blunn GW. Osteoinduction of bone grafting materials for bone repair and regeneration. *Bone*. 2015;81:112-121. https://doi.org/10.1016/j.bone.2015.07.007.
4. Гайворонский И.В., Губочкин Н.Г., Микитюк С.И., и др. Анатомические обоснования формирования

костных трансплантатов на мышечно-сосудистой ножке в нижней трети предплечья и возможностей их перемещения // Вестник Российской военно-медицинской академии. – 2016. – Т. 3. – № 55. – С. 129–134. [Gayvoronskiy IV, Gubochkin NG, Mikityuk SI. Anatomic substantiation of formation of bone grafts on muscle-pedicle in lower third of the forearm and the possibility of their transplantation. *Vestnik Rossiiskoi voenno-meditsinskoi akademii*. 2016;3(55):129-134. (In Russ.)]

5. Предеин Ю.А., Рерих В.В. Костные и клеточные имплантаты для замещения дефектов кости // Современные проблемы науки и образования. – 2016. – № 6. – С. 132–146. [Predein YA, Rerikh VV. Bone and cellular implants for replacement bone defects. *Sovremennye problemy nauki i obrazovaniya*. 2016;(6):132-146. (In Russ.)]
6. Лекишвили М.В., Склянчук Е.Д., Акатов В.С., и др. Костнопластические остеоиндуктивные материалы в травматологии и ортопедии // Гений ортопедии. – 2015. – № 4. – С. 61–67. [Lekishvili MV, Sklyanchuk ED, Akatov VS, et al. Osteoplastic osteoinductive materials in traumatology and orthopaedics. *Genij ortopedii*. 2015;(4):61-67. (In Russ.)]
7. Хватов В.Б., Свищев А.В., Ваза А.Ю., и др. Способ изготовления лиофилизированного аллотрансплантата кости // Трансплантология. – 2016. – № 1. – С. 13–18. [Khvatov VB, Svishchev AV, Vaza AY. Sposob Method of manufacturing a lyophilized allograft bone. *Transplantologiia*. 2016;(1):13-18. (In Russ.)]
8. Кирилова И.А., Подорожная В.Т., Шаркеев Ю.П., и др. Свойства деминерализованного костного матрикса для биоинженерии тканей // Комплексные проблемы сердечно-сосудистых заболеваний. – 2017. – Т. 6. – № 3. – С. 25–36. [Kirilova IA, Podorozhnaya VT, Sharkeev YP, et al. Properties of the demineralized bone matrix for bioenginery of tissue. *Copmplex issues of cardiovascular diseases*. 2017;6(3):25-36. (In Russ.)]
9. Кирилова И.А., Садовой М.А., Подорожная В.Т. Сравнительная характеристика материалов для костной пластики: состав и свойства // Хирургия позвоночника. – 2012. – № 3. – С. 72–83. [Kirilova IA, Sadovoy MA, Podorozhnaya VT. Comparative characteristics of materials for bone grafting: composition and properties. *Spine surgery*. 2012;(3):72-83. (In Russ.)]
10. Кирилова И.А. Деминерализованный костный трансплантат как стимулятор остеогенеза: современные концепции // Хирургия позвоночника. – 2004. – № 3 – С. 105–110. [Kirilova IA. Demineralized bone graft as an osteogenesis stimulator: current literature review. *Spine surgery*. 2004;(3):105-110. (In Russ.)]
11. Roseti L, Parisi V, Petretta M, et al. Scaffolds for bone tissue engineering: state of the art and new perspectives. *Mater Sci Eng C Mater Biol Appl*. 2017;78:1246-1262. https://doi.org/10.1016/j.msec.2017.05.017.
12. Deepthi S, Venkatesan J, Kim SK, et al. An overview of chitin or chitosan/nano ceramic composite scaffolds for bone tissue engineering. *Int J Biol Macromol*. 2016;93(Pt B):1338-1353. https://doi.org/10.1016/j.ijbiomac.2016.03.041.
13. Balagangadharan K, Dhivya S, Selvamurugan N. Chitosan based nanofibers in bone tissue engineering. *Int J Biol Macromol*. 2017;104(Pt B):1372-1382. https://doi.org/10.1016/j.ijbiomac.2016.12.046.
14. Logith Kumar R, Keshav Narayan A, Dhivya S, et al. A review of chitosan and its derivatives in bone tissue engineering. *Carbohydr Polym*. 2016;151:172-188. https://doi.org/10.1016/j.carbpol.2016.05.049.
15. Dobrovolskaya IP, Yudin VE, Popryadukhin PV, et al. *In vivo* studies of chitosan fiber resorption. *J Appl Cosmetol*. 2015;33:81-87.
16. Rinaudo M. Chitin and chitosan: properties and applications. *Prog Polym Sci*. 2006;31(7):603-632. https://doi.org/10.1016/j.progpolymsci.2006.06.001.
17. Sharma C, Dinda AK, Potdar PD, et al. Fabrication and characterization of novel nano-biocomposite scaffold of chitosan-gelatin-alginate-hydroxyapatite for bone tissue engineering. *Mater Sci Eng C Mater Biol Appl*. 2016;64:416-427. https://doi.org/10.1016/j.msec.2016.03.060.
18. Zhang J, Liu G, Wu Q, et al. Novel mesoporous hydroxyapatite/chitosan composite for bone repair. *J Bionic Eng*. 2012;9(2):243-251. https://doi.org/10.1016/s1672-6529(11)60117-0.
19. Danoux CB, Barbieri D, Yuan H, et al. *In vitro* and *in vivo* bioactivity assessment of a polylactic acid/hydroxyapatite composite for bone regeneration. *Biomatter*. 2014;4:e27664. https://doi.org/10.4161/biom.27664.
20. Cox SC, Thornby JA, Gibbons GJ, et al. 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. *Mater Sci Eng C Mater Biol Appl*. 2015;47:237-247. https://doi.org/10.1016/j.msec.2014.11.024.
21. Dutta SR, Passi D, Singh P, Bhuibhar A. Ceramic and non-ceramic hydroxyapatite as a bone graft material: a brief review. *Ir J Med Sci*. 2015;184(1):101-106. https://doi.org/10.1007/s11845-014-1199-8.
22. Ratnayake JTB, Mucalo M, Dias GJ. Substituted hydroxyapatites for bone regeneration: A review of current trends. *J Biomed Mater Res B Appl Biomater*. 2017;105(5):1285-1299. https://doi.org/10.1002/jbm.b.33651.
23. Oliveira HL, Da Rosa WLO, Cuevas-Suárez CE, et al. Histological evaluation of bone repair with hydroxyapatite: a systematic review. *Calcif Tissue Int*. 2017;101(4):341-354. https://doi.org/10.1007/s00223-017-0294-z.

Information about the authors

Sergei V. Vissarionov — MD, PhD, D.Sc., Professor, Corresponding Member of RAS, Deputy Director for Research and Academic Affairs, Head of the Department of Spinal Pathology and Neurosurgery, H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery, Saint Petersburg, Russia. https://orcid.org/0000-0003-4235-5048. E-mail: vissarionovs@gmail.com.

Сергей Валентинович Виссарионов — д-р мед. наук, профессор, член-корр. РАН, заместитель директора по научной и учебной работе, руководитель отделения патологии позвоночника и нейрохирургии, ФГБУ «НМИЦ детской травматологии и ортопедии имени Г.И. Турнера» Минздрава России, Санкт-Петербург. https://orcid.org/0000-0003-4235-5048. E-mail: vissarionovs@gmail.com.

Marat S. Asadulaev — MD, clinical resident, laboratory assistant in the Laboratory of Experimental Surgery, H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery, Saint Petersburg, Russia. https://orcid.org/0000-0002-1768-2402. E-mail: marat.asadulaev@yandex.ru.

Anton S. Shabunin — PhD student, Peter the Great Saint Petersburg Polytechnic University; laboratory assistant in the Laboratory of Experimental Surgery, H. Turner National Medical Research Center for Children's Orthopedics and Trauma Surgery, Saint Petersburg, Russia. https://orcid.org/0000-0002-8883-0580. E-mail: anton-shab@yandex.ru.

Vladimir E. Yudin — Dr. Phys.-Math. Sci., Professor, Director of Laboratory of Polymeric Materials for Tissue Engeneering and Transplantology, Peter the Great Saint Petersburg Polytechnic University, Saint Petersburg, Russia. https://orcid.org/0000-0002-5517-4767. E-mail: yudin@hq.macro.ru.

Moisei B. Paneiakh — assistant of the Department of Pathological Anatomy with a course of forensic medicine, Saint Petersburg State Pediatric Medical University, Saint Petersburg, Russia. https://orcid.org/0000-0002-2527-9058.

Pavel V. Popryadukhin — PhD, Senior Researcher of Laboratory of Polymeric Materials for Tissue Engeneering and Transplantology, Peter the Great Saint Petersburg Polytechnic University, Saint Petersburg, Russia. https://orcid.org/0000-0001-5478-5630. E-mail: pavelpnru@gmail.com.

Yury A. Novosad — student, Peter the Great Saint Petersburg Polytechnic University, Saint Petersburg, Russia. https://orcid.org/0000-0002-6150-374X. E-mail: yurynovosad@gmail.com.

Vasili A. Gordienko — Research Assistant of the Laboratory of Experimental Surgery, Saint Petersburg State Pediatric Medical University, Saint Petersburg, Russia. https://orcid.org/0000-0003-0590-2137. E-mail: chet1337@gmail.com.

Aleksandr G. Aganesov — MD, PhD, D.Sc., Professor, Head of the Department of spine surgery of the Russian Scientific Center of Surgery named after academician B.V. Petrovsky, Moscow, Russia. https://orcid.org/0000-0001-8823-5004.

Марат Сергеевич Асадулаев — клинический ординатор, лаборант лаборатории экспериментальной хирургии, ФГБУ «НМИЦ детской травматологии и ортопедии имени Г.И. Турнера» Минздрава России, Санкт-Петербург. https://orcid.org/0000-0002-1768-2402. E-mail: marat.asadulaev@yandex.ru.

Антон Сергеевич Шабунин — аспирант, ФГАОУ ВО СПбПУ; лаборант лаборатории экспериментальной хирургии, ФГБУ «НМИЦ детской травматологии и ортопедии имени Г.И. Турнера» Минздрава России, Санкт-Петербург. https://orcid.org/0000-0002-8883-0580. E-mail: anton-shab@yandex.ru.

Владимир Евгеньевич Юдин — д-р физ.-мат. наук, профессор, заведующий лабораторией полимерных материалов для тканевой инженерии и трансплантологии, ФГАОУ ВО СПбПУ, Санкт-Петербург. https://orcid.org/0000-0002-5517-4767. E-mail: yudin@hq.macro.ru.

Моисей Бениаминович Панеях — ассистент кафедры патологической анатомии с курсом судебной медицины, ФГБОУ ВО СПбГПМУ Минздрава России, Санкт-Петербург. https://orcid.org/0000-0002-2527-9058.

Павел Васильевич Попрядухин — канд. техн. наук, старший научный сотрудник лаборатории полимерных материалов для тканевой инженерии и трансплантологии, ФГАОУ ВО СПбПУ, Санкт-Петербург. https://orcid.org/0000-0001-5478-5630. E-mail: pavelpnru@gmail.com.

Юрий Алексеевич Новосад — студент, ФГАОУ ВО СПбПУ, Санкт-Петербург. https://orcid.org/0000-0002-6150-374X. E-mail: yurynovosad@gmail.com.

Василий Аркадьевич Гордиенко — лаборант-исследователь лаборатории экспериментальной хирургии, ФГБОУ ВО СПбГПМУ Минздрава России, Санкт-Петербург. https://orcid.org/0000-0003-0590-2137. E-mail: chet1337@gmail.com.

Александр Георгиевич Аганесов — д-р мед. наук, профессор, руководитель отделения хирургии позвоночника, ФГБНУ «РНЦХ им. акад. Б.В. Петровского», Москва, Россия. https://orcid.org/0000-0001-8823-5004.