Известия Саратовского университета. Новая серия. Серия: Физика. 2023. Т. 23, вып. 3. С. 245–253
Izvestiya of Saratov University. Physics, 2023, vol. 23, iss. 3, pp. 245–253
https://fizika.sgu.ru https://doi.org/10.18500/1817-3020-2023-23-3-245-253, EDN: NGCWHC

Article

Composite hydrogel gellan gum-based materials with $CaCO_3$ vaterite particles

M. S. Saveleva✉, P. A. Demina

Saratov State University, 83 Astrakhanskaya St., Saratov 410012, Russia

Mariia S. Saveleva, mssaveleva@yandex.ru, https://orcid.org/0000-0003-2021-0462
Polina A. Demina, polina.a.demina@list.ru, https://orcid.org/0000-0002-9203-582X

Abstract. ***Background and Objectives***: Hydrogels are cross-linked three-dimensional polymeric structures containing a large amount of water. Hydrogel materials based on natural and/or synthetic biocompatible polymers are capable of imitating the structure and properties of the extracellular matrix of living tissues. Therefore, hydrogel-based materials are widely studied and developed as functional materials in various fields of biology and medicine, including the creation of biomaterials for transplantation and tissue engineering. However, hydrogels have a number of disadvantages, such as a low biomineralization capacity, low biomechanical properties, and weak ability to form biointerface with hard tissues. These properties make hydrogel-based materials unsuitable for hard tissue engineering, particularly, bone regeneration. Currently, approaches to overcome these limitations, in particular, to improve the biological activity and biomineralization of hydrogels are currently being widely developed. ***Materials and Methods***: This study reports an efficient approach of hydrogels mineralization based on the ultrasound-assisted synthesis of calcium carbonate $CaCO_3$ in the gellan gum hydrogel material. ***Results***: The composite hydrogel materials based on the gellan gum with $CaCO_3$ micron-sized particles in the vaterite polymorph, uniformly distributed within the hydrogel matrix, have been obtained. The fraction of $CaCO_3$ in the hydrogel can easily be controlled by the number of ultrasound treatment procedures. The morphology and structure of the obtained hydrogel materials, especially the structure and distribution of the inorganic phase $CaCO_3$, have been studied by scanning electron microscopy and X-ray diffraction. ***Conclusion***: The proposed strategy for the hydrogel mineralization allows for to create functional composite materials with the potential for application for the tissue engineering, especially bone regeneration.

Keywords: composite hydrogel, vaterite, gellan gum, mineralization

Acknowledgements: This work was supported by the scholarship of the President of the Russian Federation (No. SP-727.2022.4). Authors thank Bogdan V. Parakhonskiy (Ghent University, Ghent, Belgium) and Timothy E. L. Douglas (Lancaster University, Lancaster, UK) for the assistance and support.

For citation: Saveleva M. S., Demina P. A. Composite hydrogel gellan gum-based materials with $CaCO_3$ vaterite particles. *Izvestiya of Saratov University. Physics*, 2023, vol. 23, iss. 3, pp. 245–253. https://doi.org/10.18500/1817-3020-2023-23-3-245-253, EDN: NGCWHC

Научная статья
УДК 54

Композитные гидрогелевые материалы на основе геллановой камеди и частиц ватерита $CaCO_3$

М. С. Савельева✉, П. А. Демина

Саратовский национальный исследовательский государственный университет имени Н. Г. Чернышевского, Россия, 410012, г. Саратов, ул. Астраханская, д. 83

Савельева Мария Сергеевна, младший научный сотрудник лаборатории «Дистанционно управляемые системы для тераностики» Научного медицинского центра, mssaveleva@yandex.ru, https://orcid.org/0000-0003-2021-0462
Демина Полина Анатольевна, кандидат химических наук, старший научный сотрудник лаборатории «Дистанционно управляемые системы для тераностики» Научного медицинского центра, polina.a.demina@list.ru, https://orcid.org/0000-0002-9203-582X

Аннотация. Гидрогели представляют собой трехмерные полимерные связанные структуры, содержащие большое количество воды. Материалы на основе гидрогелей широко используются для тканевой инженерии. Однако низкая степень минерализации, слабые биомеханические свойства и слабая способность образовывать связь с костной тканью делают гидрогели непригодными для применения в качестве имплантов для регенерации костей. В настоящее время активно разрабатываются подходы к повышению биологической активности гидрогелей и их способности к минерализации. В данном исследовании описывается эффективный метод минерализации гидрогелей, основанном на синтезе карбоната кальция в гидрогелевой матрице при обработке ультразвуком. Были сформированы гидрогели на основе геллановой камеди с микрочастицами $CaCO_3$ в полиморфной модификации ватерита, равномерно распределенными в матрице гидрогеля. Содержание $CaCO_3$ в гидрогеле возможно контролировать количеством процедур при обработке ультразвуком. Таким образом, предложенная стратегия минерализации гидрогеля позволяет создавать функциональные композиционные материалы, перспективные для применения в инженерии костной ткани.

Ключевые слова: композитный гидрогель, ватерит, геллановая камедь, минерализация

Благодарности: Работа выполнена при финансовой поддержке стипендии Президента Российской Федерации (№ СП-727.2022.4). Авторы выражают благодарность Богдану Владиславовичу Парахонскому (Гентский университет, Гент, Бельгия) и Тимоти Дугласу (Ланкастерский университет, Ланкастер, Великобритания) за помощь в проведении исследования.

Для цитирования: *Савельева М. С., Демина П. А.* Композитные гидрогелевые материалы на основе геллановой камеди и частиц ватерита $CaCO_3$ // Известия Саратовского университета. Новая серия. Серия: Физика. 2023. Т. 23, вып. 3. С. 245–253. https://doi.org/10.18500/1817-3020-2023-23-3-245-253, EDN: NGCWHC

Introduction

Biocompatible hydrogels are among the most intensely research topics in the biomaterials development and tissue engineering due to their unique properties including structural resemblance to natural extracellular matrices [1], adjustable physical-chemical properties [2], and the fact that hydrogels can be easily incorporated by functional additives (such as inorganic materials, biomolecules and drugs) due to the high amount of water [3,4]. However, hydrogels have the low ability to mineralize, and, therefore, do not support the formation of interfacial bonds with hard tissues such as bone. Mineralization of hydrogels with appropriate inorganic minerals will enhance their bioactivity, osteoconductivity and mechanical properties [5]. Many examples of hydrogel mineralization have been reported so far, including mineralization with calcium phosphate [6], calcium carbonate and magnesium carbonate [7–9].

Among various inorganic materials, vaterite, the metastable $CaCO_3$ polymorph, is of particular interest due to its ability to be an efficient source of calcium ions Ca^{2+} [10], rapid degradability [11], and high drug loading capacity for the various range of drugs and biologically active molecules [12, 13]. Moreover, vaterite promotes hydroxyapatite formation upon incubation in simulated body fluid (SBF) [14]. Also it is osteoconductive and supports osteogenesis, along with widely used hydroxyapatite [15].

The gellan gum (GG) is a green naturally-derived biopolymer which is able to form water-swollen hydrogels with multivalent cations [16]. Ability to absorb and retain significant volume of aqueous phase allows for efficient encapsulation of drugs. Therefore, GG hydrogels are extensively used in pharmaceutics and cosmetics as carrier and suspending agent [16], as well as biomaterial in the soft tissue engineering [17].

As a result, the composites based on the combination of biopolymer GG hydrogels with the $CaCO_3$ vaterite is potentially interesting materials for bone regeneration. At the moment, there are several protocols for such systems formation [9, 18, 19] which can be generally summarized in the following approaches: (i) mixing pre-synthesized particles into a GG solution, and (ii) particles *in situ* synthesis during GG hydrogel incubation in solution containing Ca^{2+} and CO_3^{2-} ions, including physical stimuli such as the ultrasonic (US)-assisted technique [20]. This work presents the first comparative study of these various approaches to GG hydrogel mineralization and revealing of the most effective one. In the current study, the US-assisted treatment is demonstrated as a suitable and efficient technique for obtaining the composite hydrogel materials aimed at the biomedical applications.

1. Materials and methods

1.1. Materials

Gellan gum (GG, Gelzan™ CM, molecular weight 200–300 kDa), $CaCl_2$ and Na_2CO_3 were purchased from Sigma-Aldrich and used as received. Milli-Q water (specific resistivity higher than 18.2 MΩ cm^{-1}) was obtained from a Millipore filtration system and used in all experiments.

1.2. Composite hydrogels preparation

For preparation the blank GG hydrogel, the GG aqueous solution (0.8 % wt.) was prepared as described in Ref. [21]. The GG gelation was carried out by mixing of the GG and $CaCl_2$ (0.02 M) aqueous solutions for 30 sec at vigorous agitation and heating at +70°C. After that, the obtained mixture was cooled down to the room temperature (+22°C) and, thus, the gelation procedure was completed.

For preparation a composite GG/$CaCO_3$ hydrogel, the three approaches were used.

(i) Addition of preliminarily synthesized $CaCO_3$ vaterite microparticles to the GG solution in course of the gelation.

The $CaCO_3$ vaterite microparticles prepared as described previously in Ref. [22] were added to the

GG aqueous solution under the vigorous agitation and heating at +70°C. The agitation of obtained mixture was continued to 1 min until the homogenous distribution of $CaCO_3$ microparticles in the GG solution. After that, the obtained mixture was cooled down to the room temperature (+22°C).

(ii) Synthesis of $CaCO_3$ microparticles *in situ* in the GG solution in course of the gelation.

At the first step, an aqueous Na_2CO_3 solution (0.33 M) was added to the GG solution under the vigorous agitation and heating at +70°C. After that, an aqueous $CaCl_2$ solution (0.33 M) was added to this mixture. The agitation of mixture was continued to 1 min until the completion of $CaCO_3$ crystallization. Then, the obtained mixture was cooled down to the room temperature (+22°C).

(iii) Ultrasonic-assisted mineralization of the GG hydrogel in solutions saturated with Ca^{2+} and CO_3^{2-} ions.

The GG hydrogel mineralization was performed using the technique of US-assisted $CaCO_3$ synthesis as described in the previous study [20]. Hydrogel samples were immersed in an aqueous $CaCl_2$ solution (0.33 M) in a tube which was then placed in the ultrasonic bath. The hydrogel sample was pretreated with an ultrasound (US) (working frequency 35 kHz and radiation intensity 0.64 W/cm^2) in a $CaCl_2$ solution for 1 min. Then, an aqueous Na_2CO_3 solution (0.33 M) was added to the $CaCl_2$ solution under continuous US treatment. The synthesis of $CaCO_3$ particles in the GG hydrogel was carried out for 1 min in the presence of US treatment.

All the obtained GG hydrogel materials were stored in a refrigerator at the temperature of +5°C.

1.3. Characterization methods

The surface morphology of hydrogel materials was studied by the scanning electron microscopy (SEM) with using a Tescan MIRA II LMU setup (Tescan, Czech Republic). The hydrogel mass was monitored with a Sartorius Quintix 35–18 (Germany) microbalance prior to US-assisted mineralization treatment, after each mineralization procedure, and after following drying in a drying oven for 4 hours, at +60°C in air. Powder X-ray diffraction analysis of the preliminary dried and grinded samples was performed with a Rigaku Minilex-600 diffractometer (Rigaku Corporation, Tokyo, Japan) using Cu-K_α radiation (40 kV, 15 mA, Ni-K_β filter) in the 2θ range 5–60° at a scan speed 1°/min. The XRD data obtained were compared with the literature crystallographic data for vaterite [23] and calcite [24].

The average diameter of $CaCO_3$ particles and the filling factor of $CaCO_3$ in composite GG hydrogels were measured by analyzing SEM images with the use of the Image J software (https://imagej.nih.gov/ij/index.html). The areas occupied with $CaCO_3$ were extracted from the total area of the hydrogel cross-section by the area selection function. Then, the filling factor was calculated as the ratio of the $CaCO_3$-filled area to the total area [25].

2. Results and discussion

Hydrogel matrices based on anionic polysaccharide gellan gum were prepared by GG ionotropic gelation with the divalent calcium ions Ca^{2+} as a cross-linking agent, which is promote the site binding of carboxylate groups of GG polysaccharide helical chains [26]. Moreover, the addition of calcium ions lowers the negative charge of the GG helical chains and, thus, facilitate their electrostatic internal interaction and leading to gelation as well [26, 27]. In this way, a three-dimensional network of a water-insoluble GG polysaccharide hydrogel is formed. Photo and SEM images of the resulting GG hydrogel are shown in Fig. 1, *a*, *b* and *c*, respectively.

The modification of GG hydrogels by $CaCO_3$ microparticles was performed using three different approaches. A comparison study of these approaches allows to optimize the method of hydrogels mineralization, and, thus, to obtain composite hydrogel materials with the most optimal structural and functional characteristics, particularly, with $CaCO_3$ in the vaterite polymorph. Vaterite is able to provide the required functionality of a composite material, including Ca^{2+} release capacity [10] and high drug loading efficiency [13], which is significantly important for biomedical applications, especially for bone tissue engineering [28].

In the first approach, $CaCO_3$ microparticles were added to GG aqueous solution under rapid homogenization of the mixture (Fig. 1, *d*). In this case, the presence of water environment initiates the process of partial dissolution of $CaCO_3$. Therefore, an amount of free Ca^{2+} ions are occurred in the solution near the surface of the $CaCO_3$ particles, which promotes the ionotropic gelation of GG. Thus, the addition of $CaCl_2$ for gelation is not required in this case. It can be observed that the $CaCO_3$ microparticles in the hydrogel have the form of cubic crystals, which indicates that the vaterite has recrystallized

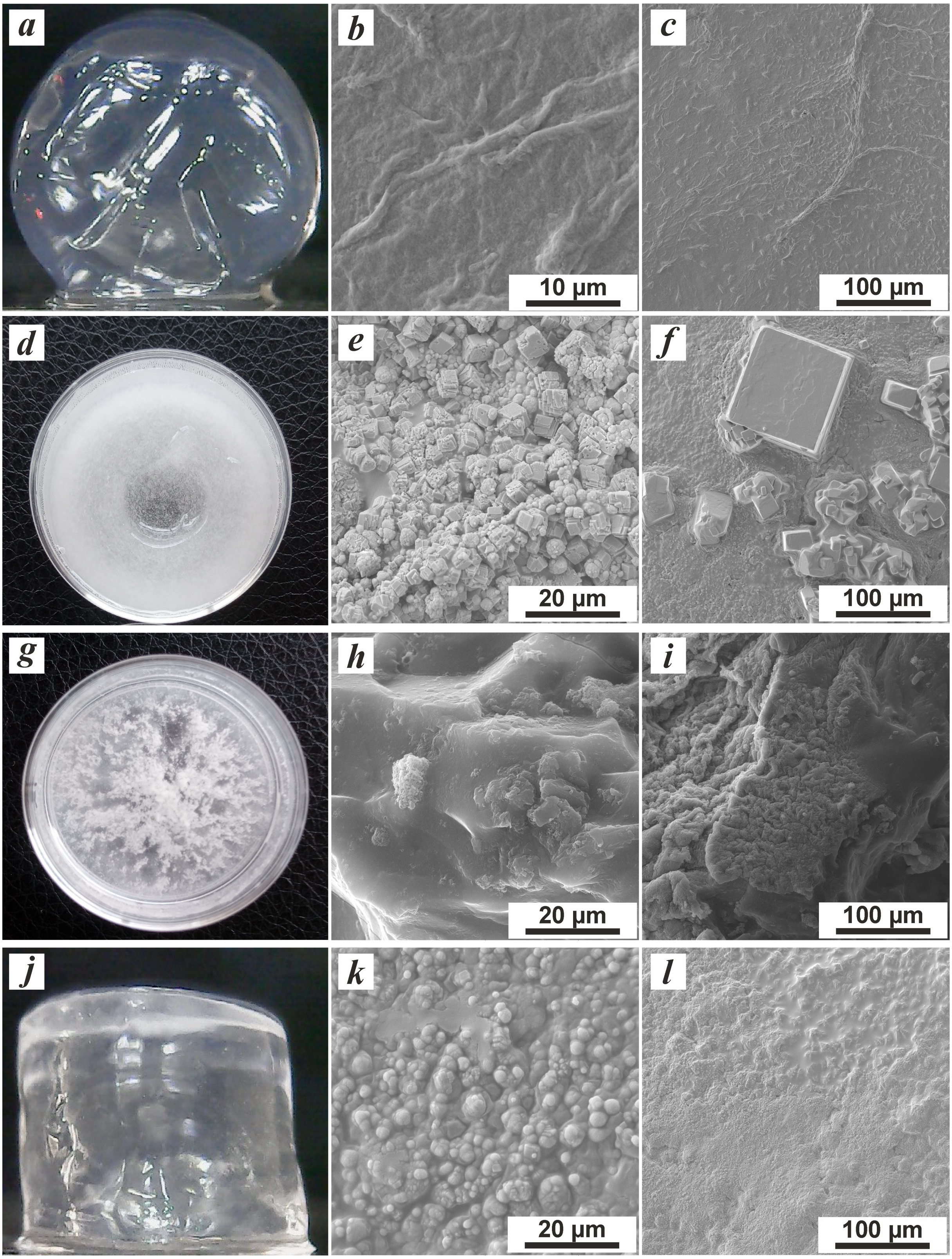

Fig. 1. *a* – photo of the blank GG hydrogel, *b* and *c* – SEM images of the blank GG hydrogel; *d* – photo of the composite GG hydrogel in a petri dish, prepared by the addition of preliminarily synthesized $CaCO_3$ microparticles to the GG solution, *e* and *f* – SEM images of this composite GG hydrogel; *g* – photo of the composite GG hydrogel in a petri dish, prepared by the *in situ* synthesis of $CaCO_3$ microparticles in the GG solution in course of the gelification, *h* and *i* – SEM images of this composite GG hydrogel; *j* – photo of the composite GG hydrogel, prepared by the ultrasonic-assisted mineralization of the GG hydrogel in solution saturated with Ca^{2+} and CO_3^{2-} ions, *k* and *l* – SEM images of this composite GG hydrogel (color online)

into calcite [29] (Fig. 1, *e*, *f*) because of the dissolution process. The $CaCO_3$ particles are distributed in the hydrogel in the form of agglomerates.

In the second approach, a Na_2CO_3 aqueous solution was added to GG solution for providing carbonate CO_3^{2-} ions which are necessary for the growth of $CaCO_3$ microparticles. Further, the following addition of Ca^{2+} ions to the mixture initiates two parallel processes. First, the electrostatic and site binding of GG molecular chains by calcium ions is resulting in the gelation of GG. Second, the interaction with carbonate ions is resulting in the formation of $CaCO_3$ particles in GG hydrogel. In this way, the "embedding" of $CaCO_3$ inorganic particles into the GG hydrogel matrix occurs during its gelation and results in the formation of a composite material. One can observe the inhomogeneous distribution of the $CaCO_3$ inorganic phase in the hydrogel (Fig. 1, *g*). The $CaCO_3$ exhibits cubic crystals characteristic for calcite polymorph [29] as well as an amorphous [30] phase (Fig. 1, *h*, *i*).

In the third approach, the pre-formed GG hydrogel was pretreated in a $CaCl_2$ solution under ultrasound irradiation, which initiates the diffusion of Ca^{2+} ions from solution into the hydrogel network. The following addition of carbonate CO_3^{2-} ions initiates the US-assisted growth of $CaCO_3$ in the GG hydrogel. The scheme of US-assisted process of hydrogel modification is shown in the Fig. 2. The spherical particles with a porous surface morphology, which is typical for vaterite polymorph, as well as a uniform distribution of particles in the hydrogel can be observed (Fig. 1, *j*, *k*, *l*).

Thus, a comparison of the structure of composite GG hydrogel materials formed by different methods allows us to conclude that the US-assisted mineralization provides the most optimal structure of a composite material with respect to the polymorphic modification of $CaCO_3$ microparticles and their distribution in the material. In terms of functionality, $CaCO_3$ in the polymorphic modification of vaterite is preferable to calcite, since it has a higher bioactivity, osteogenic potential [28], and a higher capacity for immobilization of biologically active moieties [13].

Further, to study the effect of the US-assisted $CaCO_3$ synthesis on the composite material structure, three procedures of the such US treatment of GG hydrogels were carried out. On SEM images of hydrogel samples after 1st, 2nd, and 3rd US treatment procedures (Fig. 3, *a*), it can be observed that as a result of an increase in the number of procedures, the size and amount of $CaCO_3$ particles in the material increase as well (Fig. 4, *a*, *b*). The statistically significant increase in average diameter of $CaCO_3$ particles occurs after 3rd US treatment procedure, while filling factor drastically increases already after 2nd procedure. After the third procedure, the cubic calcite microparticles are observed in the hydrogel. The weight of samples (both wet and dried) showed that the water content in the hydrogels increased (Fig. 4, *c*) along the statistically significant decrease of dry mass (Fig. 4, *d*) after the 1st US treatment procedure. It is worth noting that there is no statistically significant difference in changing of dry mass amount for samples after 1st, 2nd and 3rd US treatment procedures. Also, the following

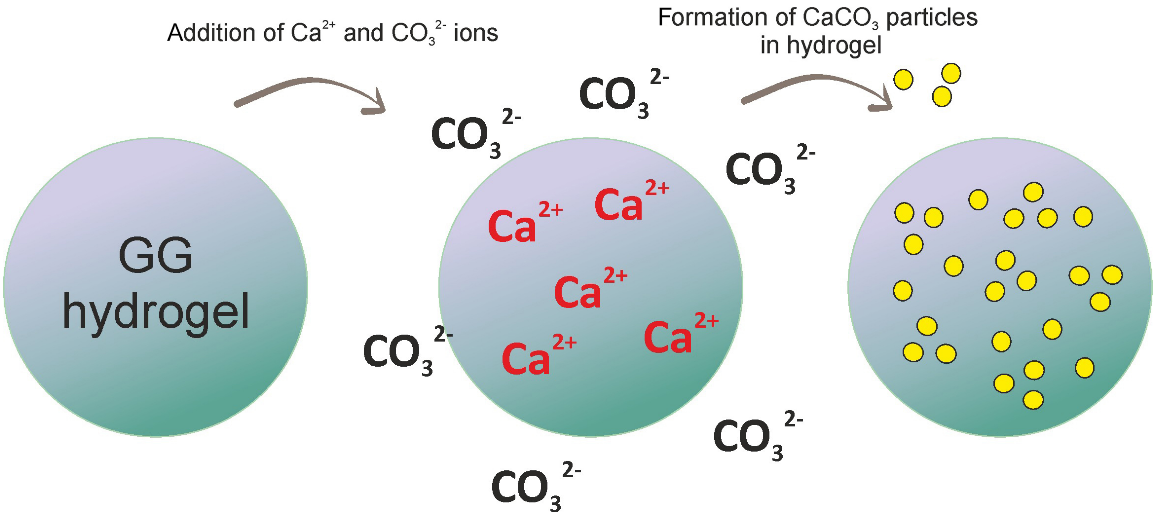

Fig. 2. Scheme of the process of ultrasonic mineralization of GG hydrogel by $CaCO_3$ vaterite particles (color online)

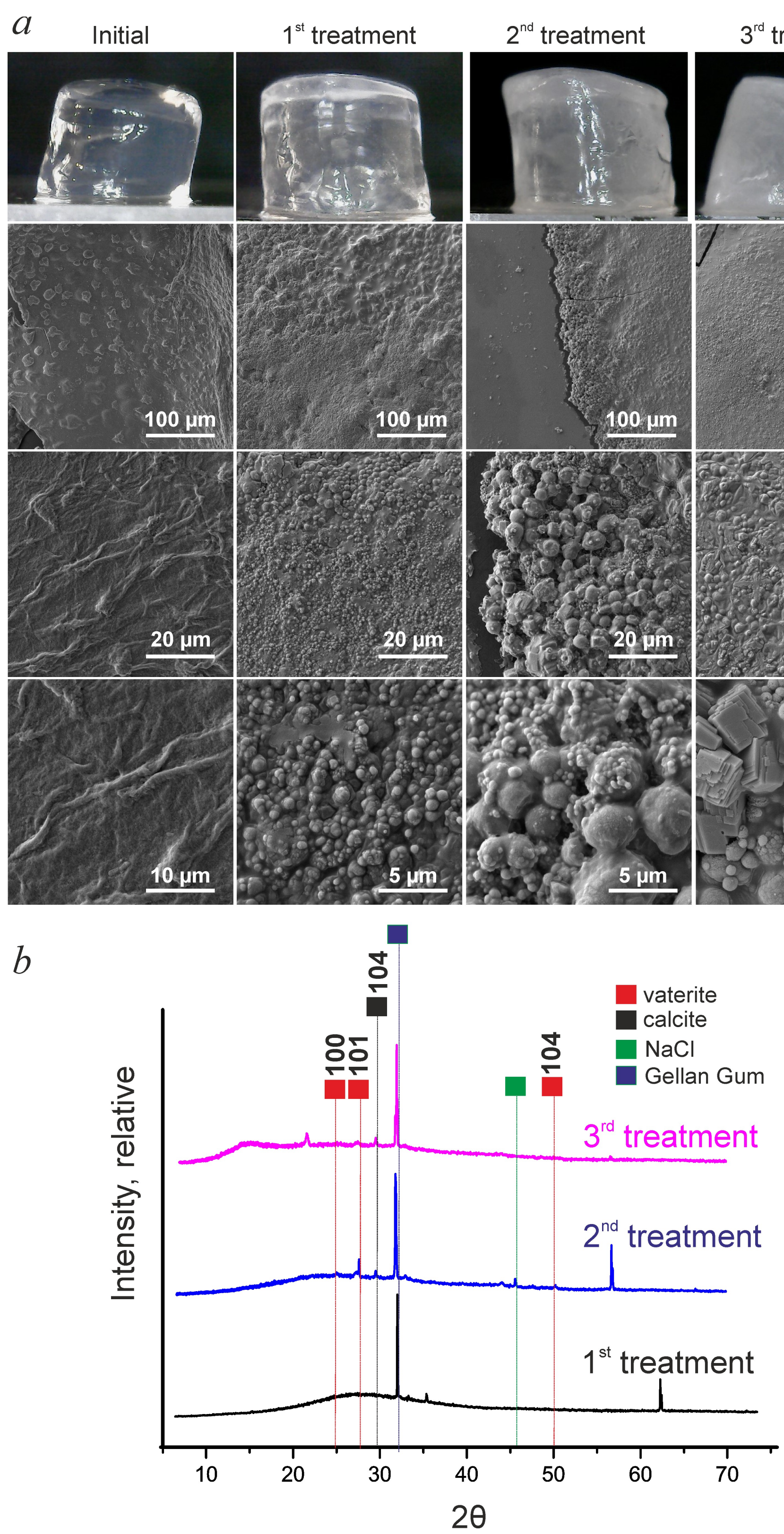

Fig. 3. *a* – photo and corresponding SEM images of initial GG hydrogels, and composite GG hydrogels after one, two and three US-assisted $CaCO_3$ mineralization procedures, *b* – diffraction patterns of composite GG hydrogels after one, two and three US-assisted $CaCO_3$ mineralization procedures (color online)

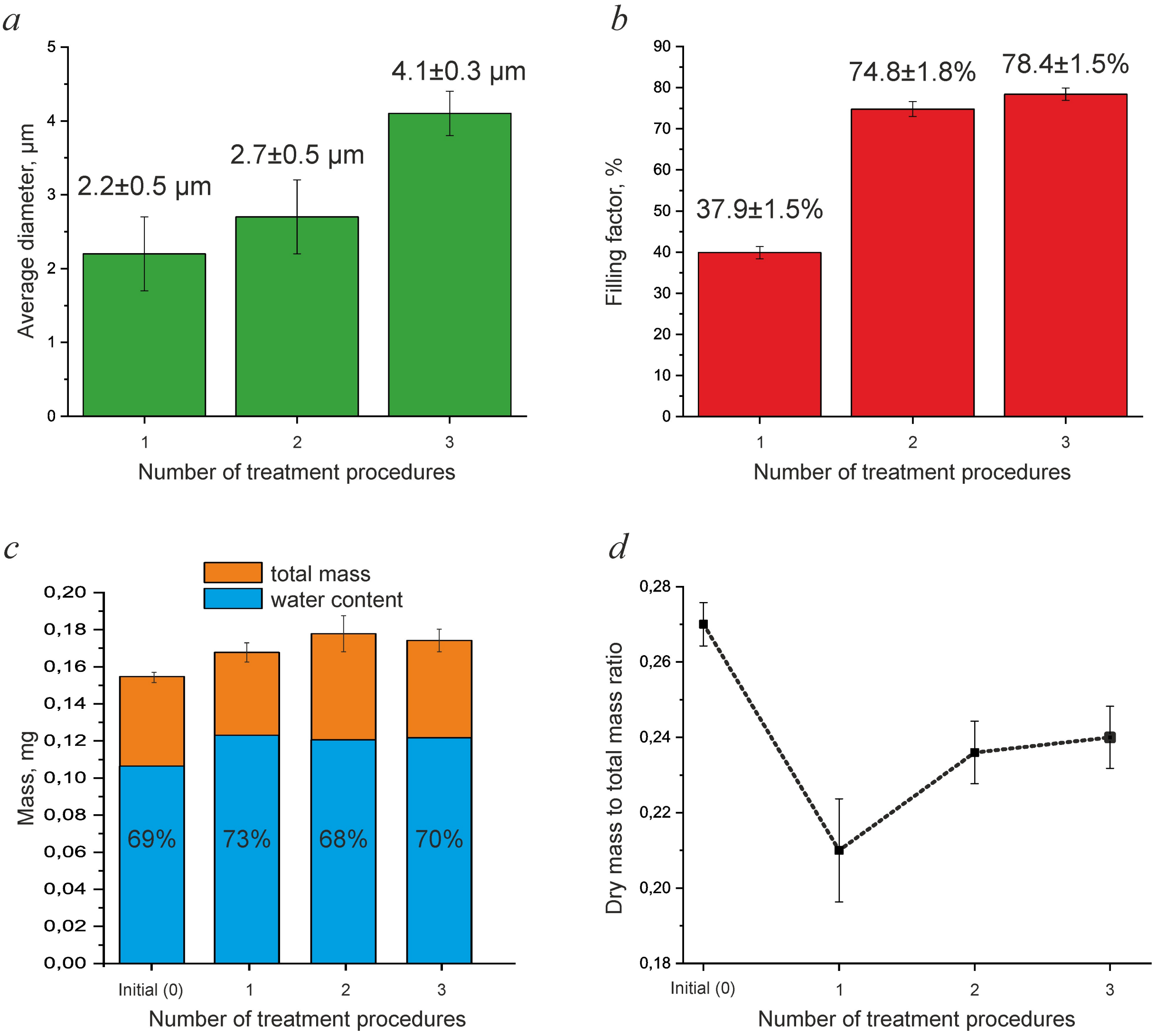

Fig. 4. *a* – average diameters of $CaCO_3$ particles and *b* – filling factor of $CaCO_3$ particles in composite GG hydrogel samples after one, two and three US-assisted $CaCO_3$ mineralization procedures; *c* – mass measurements of wet GG hydrogel samples (initial and after different number of US-assisted $CaCO_3$ mineralization procedures) and corresponding water content in hydrogels; *d* – the ratio of dry mass to total mass of initial GG hydrogel and composite GG hydrogels after one, two and three US-assisted $CaCO_3$ mineralization procedures (color online)

(2nd and 3rd) treatment procedures did not cause any statistically significant change in the water content in the hydrogels. The diffraction patterns of these samples (Fig. 3, *c*) show peaks characteristic of vaterite, which confirm the presence of $CaCO_3$ in the vaterite polymorph in the composite material. The diffraction patterns for samples after second and third procedures show the presence of calcite peaks as well.

Thus, one can conclude that a consistent increase in the number of US treatment procedures of GG hydrogel initiate the process of recrystallization of vaterite particles into calcite. Therefore, the two US treatment procedures were considered as optimal for the formation of a stable $CaCO_3$ phase in composite GG hydrogel, from the point of view of the vaterite polymorph of $CaCO_3$ and particles distribution in the hydrogel matrix.

Conclusions

In the presented study, gellan gum hydrogels have been functionalized with $CaCO_3$ microparticles in the porous vaterite polymorphic form. The comparative study of three approaches to the formation of a composite material based on gellan gum hydrogel and $CaCO_3$ microparticles has been performed. The structure, polymorphic modification of $CaCO_3$ particles, as well as their distribution in the hydrogel matrix depend on the conditions of their synthesis. It has been shown that the most efficient approach of hydrogel mineralization is the ultrasonic (US)-assisted treatment of GG hydrogel in Ca^{2+} and CO_3^{2-} containing solutions. The US treatment stimulates penetration of these ions into the polymeric hydrogel network and facilitates porous vaterite microparticles formation inside the hydrogel. It has been found that the presence of ultrasonic treatment during the $CaCO_3$ particles synthesis allows to obtain particles with the necessary polymorph (vaterite) and a uniform distribution in the GG hydrogel. The effect of the US treatment procedures number on the structure of the composite GG hydrogel has also been studied, and it has been shown that the two procedures are optimal in terms of the structure and distribution of $CaCO_3$ particles in the hydrogel matrix. Composite GG hydrogels functionalized with vaterite can be promising for application as the implantable materials for bone tissue regeneration.

References

1. Radulescu D.-M., Neacsu I. A., Grumezescu A.-M., Andronescu E. New Insights of Scaffolds Based on Hydrogels in Tissue Engineering. *Polymers (Basel)*, 2022, vol. 14, iss. 4, article no. 799. https://doi.org/10.3390/polym14040799
2. Chauhan N., Saxena K., Jain U. Hydrogel based materials: A progressive approach towards advancement in biomedical applications. *Mater. Today Commun.*, 2022, vol. 33, article no. 104369. https://doi.org/10.1016/j.mtcomm.2022.104369
3. Hoffman A. S. Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.*, 2012, vol. 64, pp. 18–23. https://doi.org/10.1016/j.addr.2012.09.010
4. Kailasa S. K., Joshi D. J., Kateshiya M. R., Koduru J. R., Malek N. I. Review on the biomedical and sensing applications of nanomaterial-incorporated hydrogels. *Mater. Today Chem.*, 2022, vol. 23, article no. 100746. https://doi.org/10.1016/j.mtchem.2021.100746
5. Gkioni K., Leeuwenburgh S. C. G. G., Douglas T. E. L. L., Mikos A. G., Jansen J. A. Mineralization of Hydrogels for Bone Regeneration. *Tissue Eng. Part B. Rev.*, 2010, vol. 16, pp. 577–585. https://doi.org/10.1089/ten.teb.2010.0462
6. Douglas T., Wlodarczyk M., Pamula E., Declercq H., Mulder E. de, Bucko M., Balcaen L., Vanhaecke F., Cornelissen R., Dubruel P., Jansen J., Leeuwenburgh S. Enzymatic mineralization of gellan gum hydrogel for bone tissue-engineering applications and its enhancement by polydopamine. *J. Tissue Eng. Regen. Med.*, 2014, vol. 8, pp. 906–918. https://doi.org/10.1002/term.1616
7. Lopez-Heredia M. A., Lapa A., Mendes A. C., Balcaen L., Samal S. K., Chai F., Voort P. Van der, Stevens C. V., Parakhonskiy B. V., Chronakis I. S., Vanhaecke F., Blanchemain N., Pamuia E., Skirtach A. G., Douglas T. E. L. Bioinspired, biomimetic, double-enzymatic mineralization of hydrogels for bone regeneration with calcium carbonate. *Mater. Lett.*, 2017, vol. 190, iss. 1, pp. 13–16. https://doi.org/10.1016/j.matlet.2016.12.122
8. Schroder R., Pohlit H., Schuler T., Panthofer M., Unger R. E., Frey H., Tremel W. Transformation of vaterite nanoparticles to hydroxycarbonate apatite in a hydrogel scaffold: Relevance to bone formation. *J. Mater. Chem. B*, 2015, vol. 3, pp. 7079–7089. https://doi.org/10.1039/C5TB01032B
9. Abalymov A., Lengert E., Meeren L. Van der, Savelеva M., Ivanova A., Douglas T. E. L., Skirtach A. G., Volodkin D., Parakhonskiy B. The influence of Ca/Mg ratio on autogelation of hydrogel biomaterials with bioceramic compounds. *Mater. Sci. Eng. C*, 2022, vol. 133, article no. 112632. https://doi.org/10.1016/j.msec.2021.112632
10. Tolba E., Müller W. E. G. Abd El-Hady B. M., Neufurth M., Wurm F., Wang S., Schröder H. C., Wang X. High biocompatibility and improved osteogenic potential of amorphous calcium carbonate/vaterite. *J. Mater. Chem. B*, 2016, vol. 4, pp. 376–386. https://doi.org/10.1039/C5TB02228B
11. Campbell J., Ferreira A. M., Bowker L., Hunt J., Volodkin D., Vikulina A. Dextran and Its Derivatives: Biopolymer Additives for the Modulation of Vaterite $CaCO_3$ Crystal Morphology and Adhesion to Cells. *Adv. Mater. Interfaces*, 2022, vol. 9, article no. 2201196. https://doi.org/10.1002/admi.202201196
12. Trushina D. B., Bukreeva T. V., Kovalchuk M. V., Antipina M. N. $CaCO_3$ vaterite microparticles for biomedical and personal care applications. *Mater. Sci. Eng. C*, 2014, vol. 45, pp. 644–658. https://doi.org/10.1016/j.msec.2014.04.050
13. Svenskaya Y. I., Fattah H., Zakharevich A. M., Gorin D. A., Sukhorukov G. B., Parakhonskiy B. V. Ultrasonically assisted fabrication of vaterite submicron-sized carriers. *Adv. Powder Technol.*, 2016, vol. 27, iss. 2, pp. 618–624. https://doi.org/10.1016/j.apt.2016.02.014
14. Maeda H., Maquet V., Kasuga T., Chen Q. Z., Roether J. A., Boccaccini A. R. Vaterite deposition on biodegradable polymer foam scaffolds for inducing bone-like hydroxycarbonate apatite coatings. *J. Mater. Sci. Mater. Med.*, 2007, vol. 18, pp. 2269–2273. https://doi.org/10.1007/s10856-007-3108-4
15. Vuola J., Göransson H., Böhling T., Asko-Seljavaara S. Bone marrow induced osteogenesis in hydroxyapatite and calcium carbonate implants. *Biomaterials*, 1996, vol. 17, iss. 18, pp. 1761–1766. https://doi.org/10.1016/0142-9612(95)00351-7
16. Das M., Giri T. K. Hydrogels based on gellan gum in cell delivery and drug delivery. *J. Drug Deliv. Sci. Technol.*, 2020, vol. 56, article no. 101586. https://doi.org/10.1016/j.jddst.2020.101586
17. Stevens L. R., Gilmore K. J., Wallace G. G., in het Panhuis M. Tissue engineering with gellan gum. *Biomater.*

Sci., 2016, vol. 4, pp. 1276–1290. https://doi.org/10.1039/C6BM00322B

18. Lopez-Heredia M. A., Łapa A., Reczyńska K., Pietryga K., Balcaen L., Mendes A. C., Schaubroeck D., Voort P. Van Der, Dokupil A., Plis A., Stevens C. V., Parakhonskiy B. V., Samal S. K., Vanhaecke F., Chai F., Chronakis I. S., Blanchemain N., Pamuia E., Skirtach A. G., Douglas T. E. L. Mineralization of gellan gum hydrogels with calcium and magnesium carbonates by alternate soaking in solutions of calcium/magnesium and carbonate ion solutions. *J. Tissue Eng. Regen. Med.*, 2018, vol. 12, pp. 1825–1834. https://doi.org/10.1002/term.2675
19. Abalymov A., Meeren L. Van der, Skirtach A. G., Parakhonskiy B. V. Identification and Analysis of Key Parameters for the Ossification on Particle Functionalized Composites Hydrogel Materials. *ACS Appl. Mater. Interfaces*, 2020, vol. 12, iss. 35, pp. 38862–38872. https://doi.org/10.1021/acsami.0c06641
20. Savelyeva M. S., Abalymov A. A., Lyubun G. P., Vidyasheva I. V., Yashchenok A. M., Douglas T. E. L., Gorin D. A., Parakhonskiy B. V. Vaterite coatings on electrospun polymeric fibers for biomedical applications. *J. Biomed. Mater. Res. Part A*, 2017, vol. 105, pp. 94–103. https://doi.org/10.1002/jbm.a.35870
21. Douglas T. E. L., Piwowarczyk W., Pamula E., Liskova J., Schaubroeck D., Leeuwenburgh S. C. G., Brackman G., Balcaen L., Detsch R., Declercq H., Cholewa-Kowalska K., Dokupil A., Cuijpers V. M. J. I., Vanhaecke F., Cornelissen R., Coenye T., Boccaccini A. R., Dubruel P. Injectable self-gelling composites for bone tissue engineering based on gellan gum hydrogel enriched with different bioglasses. *Biomed. Mater.*, 2014, vol. 9, article no. 045014. https://doi.org/10.1088/1748-6041/9/4/045014
22. Parakhonskiy B. V., Haase A., Antolini R. Sub-Micrometer Vaterite Containers: Synthesis, Substance Loading, and Release. *Angew. Chemie*, 2012, vol. 124, pp. 1221–1223. https://doi.org/10.1002/ange.201104316
23. Bail A. Le, Ouhenia S., Chateigner D. Microtwinning hypothesis for a more ordered vaterite model. *Powder Diffr.*, 2012, vol. 26, iss. 1, pp. 16–21. https://doi.org/10.1154/1.3552994
24. Sitepu H. Texture and structural refinement using neutron diffraction data from molybdite (MoO_3) and calcite ($CaCO_3$) powders and a Ni-rich $Ni_{50.7}Ti_{49.30}$ alloy. *Powder Diffr.*, 2009, vol. 24, iss. 4, pp. 315–326. https://doi.org/10.1154/1.3257906
25. Saveleva M., Prikhozhdenko E., Gorin D., Skirtach A. G., Yashchenok A., Parakhonskiy B. Polycaprolactone-Based, Porous $CaCO_3$ and Ag Nanoparticle Modified Scaffolds as a SERS Platform With Molecule-Specific Adsorption. *Front. Chem.*, 2020, vol. 7, pp. 1-11. https://doi.org/10.3389/fchem.2019.00888
26. Bellini D., Cencetti C., Meraner J., Stoppoloni D., D'Abusco A. S., Matricardi P. An *in situ* gelling system for bone regeneration of osteochondral defects. *Eur. Polym. J.*, 2015, vol. 72, pp. 642–650. https://doi.org/10.1016/j.eurpolymj.2015.02.043
27. Robinson G., Manning C. E., Morris E. R. Conformation and Physical Properties of the Bacterial Polysaccharides Gellan, Welan, and Rhamsan. In: Dickinson E., ed. *Food Polymers, Gels and Colloids*. Woodhead Publ., 1991, pp. 22–33. https://doi.org/10.1533/9781845698331.22
28. Saveleva M. S., Ivanov A. N., Chibrikova J. A., Abalymov A. A., Surmeneva M. A., Surmenev R. A., Parakhonskiy B. V., Lomova M. V., Skirtach A. G., Norkin I. A. Osteogenic Capability of Vaterite-Coated Nonwoven Polycaprolactone Scaffolds for *In Vivo* Bone Tissue Regeneration. *Macromol. Biosci.*, 2021, vol. 21, article no. 2100266. https://doi.org/10.1002/mabi.202100266
29. Svenskaya Y. I., Navolokin N. A., Bucharskaya A. B., Terentyuk G. S., Kuz'mina A. O., Burashnikova M. M., Maslyakova G. N., Lukyanets E. A., Gorin D. A. Calcium carbonate microparticles containing a photosensitizer photosens: Preparation, ultrasound stimulated dye release, and *in vivo* application. *Nanotechnologies Russ.*, 2014, vol. 9, pp. 398–409. https://doi.org/10.1134/S1995078014040181
30. Saveleva M. S., Lengert E. V., Verkhovskii R. A., Abalymov A. A., Pavlov A. M., Ermakov A. V., Prikhozhdenko E. S., Shtykov S. N., Svenskaya Y. I. $CaCO_3$-based carriers with prolonged release properties for antifungal drug delivery to hair follicles. *Biomater. Sci.*, 2022, vol. 10, pp. 3323–3345. https://doi.org/10.1039/D2BM00539E

Поступила в редакцию 20.03.2023; одобрена после рецензирования 02.05.2023; принята к публикации 15.06.2023
The article was submitted 20.03.2023; approved after reviewing 02.05.2023; accepted for publication 15.06.2023