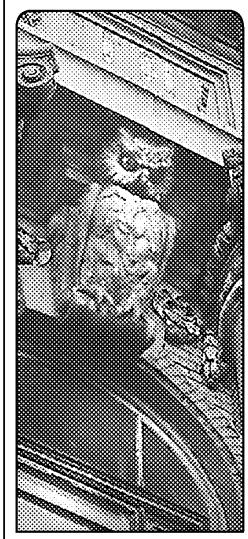

НАУЧНЫЙ ОТДЕЛ

# НАНОТЕХНОЛОГИИ, НАНОМАТЕРИАЛЫ И МЕТАМАТЕРИАЛЫ

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Article

# Composite mesoporous vaterite-magnetite coatings on polycaprolactone fibrous matrix

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**Abstract.** ***Background and Objectives***: Based on polymers and inorganic components, hybrid nanostructured materials are used in biomedicine, including tissue engineering and drug delivery with the controlled release. This research aims to develop the method for forming composite coating of vaterite and MNPs on electrospun PCL fibers, which will maintain sensitivity to magnetic fields for a period for the use of magnetotherapy and to control the rate of drug release. ***Materials and Methods:*** Three methods of $CaCO_3$ + magnetic nanoparticles coating formation on the surface of policaprolacton fibers were tested. The coating recrystallization time of $CaCO_3$ (transformation from the vaterite polymorph to calcite) on policaprolacton fibers was determined. ***Results:*** For samples obtained by $CaCO_3$ and magnetite coprecipitation and US-assisted methods, the time of complete recrystallization is 5 hours, which is less by 2 times than the recrystallization rate of the control sample. ***Conclusion:*** The crystallization-induced method is most effective, proved by the recrystallization time of magnetic $CaCO_3$ microparticles on the surface of PCL fibers, which is comparable to the control sample. Obtained by the method of salt co-precipitation with magnetite and US-assisted method, inorganic coatings on PCL fibers have a shorter recrystallization period.

**Keywords:** scaffold, polycaprolactone, magnetic nanoparticles, calcium carbonate, bone regeneration

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**Композитные мезопористые ватерит-магнетитовые покрытия, выращенные на матрице из волокон поликапролактона**

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**Аннотация.** Представлены методы модификации кальций карбонатного покрытия, сформированного на волокнах поликапролактона, наночастицами магнетита. Разрабатываемая структура может быть использована в качестве тканеинженерного каркаса и одновременно средства доставки лекарственных веществ с возможностью контроля процесса высвобождения, что позволит использовать её в регенерационной медицине. Определено время перекристаллизации покрытий на волокнах поликапролактона, состоящих из микрочастиц карбоната кальция, из полиморфного состояния ватерит в кальцит. Использование метода адсорбции, индуцированной кристаллизацией, является наиболее эффективным, что доказывается временем перекристаллизации микрочастиц карбоната кальция, модифицированных наночастицами магнетита, выращенных на поверхности волокон поликапролактона, которое сравнимо с контрольным образцом. Композитные покрытия на волокнах поликапролактона, полученные методом копреципитации солей и магнетита и методом ультразвуковой обработки, имеют более короткий период перекристаллизации.

**Ключевые слова**: тканеинженерные конструкции, поликапролактон, магнитные наночастицы, карбонат кальция, регенерация костной ткани

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## Introduction

Based on polymers and organic components, hybrid nanostructured materials are used in biomedicine, including tissue engineering [1] and drug delivery [2] with the controlled release [3]. Their ease of formation and improved mechanical parameters together with features of inorganic components, such as biocompatibility, and biodegradability are advantageous properties of mineralizing fibers with inorganic particles [4]. Due to these features and their specific morphology, hybrid nanostructured materials are very promising for regenerative medicine [5]. The structure of the polymeric shell in nanocomposite materials has decisive importance for the growth and morphology of inorganic components [6, 7]. Polymeric materials obtained by electrospinning (ES) have great potential in the biomedical field. Due to nanoscale structuring, fibers are similar to extracellular matrixes of living tissues and can be obtained from a wide range of polymers [8], both natural (chitosan [9], collagen [10]) and synthetic (polycaprolactone (PCL) [11], polyurethane [12]). Electrospun fibers have been exploited as tissue engineering materials, including skin regeneration medicine [13], bone tissue [14], and cartilage reconstruction [15].

Modification of electrospun fibers surface allows acquiring of new properties (namely, the ability to load drugs, biological activity), which may be useful in the development of new tissue engineering scaffolds [8]. The incorporation of metallic nanoparticles (including gold, silver, etc.) into the spinning solution for fiber production is a well-known and proven approach for obtaining composite ES materials, but it has one significant disadvantage, that is the inhomogeneous distribution of the embedded nanoparticles in the electrospun fibers. Another approach of modification is based on surface functionalization, i.e. formation of functional coatings, by special physicochemical treatment of the surface [16, 17]. Modifications of fibers can be performed by inorganic materials such as hydroxyapatite, CaP, $CaCO_3$, $MgCO_3$, being components of biomineralized tissues of living organisms [18, 19]. $CaCO_3$ microparticles can be deposited on fibers by their incubation in saline solutions [20–22].

Porous inorganic $CaCO_3$ microparticles in polymorphic form of vaterite are well known as containers used for drug storage and delivery [23, 24]. Due to its high porosity and large surface area, vaterite has a higher loading capacity in comparison with adsorption on smooth surfaces of polymers [25]. The modification of polymeric fibers surface with the porous $CaCO_3$ microparticles allows obtaining nanostructured composite materials not only with improved osteoconductivity but also with the capability of drug delivery. The technique of homogeneous $CaCO_3$-mineralized coating formation on the surface of PCL fibers is described in [7, 26].

Magnetite nanoparticles (MNPs), in particular, iron oxide nanoparticles, have been used for various biomedical purposes *in vivo*, e.g. as a colloidal solution to increase contrast and enhance diagnostic sensitivity in magnetic resonance imaging [27], and as nanoparticles for hyperthermia in alternating magnetic fields [28] and tissue engineering [29]. The inclusion of MNPs into the structure of porous vaterite coatings on PCL fibers will provide various magnetic effects that can be exploited for biomedical purposes, such as MRI visualization [30, 31], magnetothermal release/therapy [32, 33], etc. MNPs may affect the regeneration process [34], as well as stimulate the immune system response [35]. Nanostructured materials modified with MNPs have been studied to determine the recrystallization rate from vaterite to calcite, which also influences the release rate of a substance, immobilized in vaterite, for the developing of vaterite-based drug delivery systems.

This research aims to develop the method for forming composite coating of vaterite and MNPs on electrospun PCL fibers, which will maintain sensitivity to magnetic fields for a period for the use of magnetotherapy and to control the rate of drug release.

## 1. Materials and methods

### *1.1. Materials*

For the synthesis of $CaCO_3$ microparticles, aqueous solutions of calcium chloride dihydrate ($CaCl_2$, Sigma-Aldrich) and sodium carbonate ($Na_2CO_3$, Sigma-Aldrich) were used. To obtain polymeric fibers we used: polymer – polycaprolactone($[–(CH_2)_5–CO_2–O–]_n$), with molecular weight – 80 a.u.m. Formic acid (HCOOH) and acetic acid ($CH_3COOH$). Sodium oxide hydrate (NaOH, Sigma-Aldrich), iron chloride (II) ($FeCl_2$, Sigma-Aldrich), iron chloride (III) ($FeCl_3$, Sigma-Aldrich), citric acid ($C_6H_8O_7$, Sigma-Aldrich) were used to produce magnetite nanoparticles.

### *1.2. Formation of organic polycaprolactone fibers*

The electroforming method was used to produce organic fibers. PCL solution with a concentration of 10% wt. was prepared by dissolving PCL pellets in a mixture of methane and acetic acid (weight ratio 1 : 1). The PCL pellets and solvent mixtures were continuously mixed for 2 hours at room temperature to produce a homogeneous spinning solution. This mixture was transferred to a syringe that was placed in an experimental electroforming unit. The formation of organic fibers was carried out within 3 hours at the applied voltage of 75 kV and a feed rate of 7.8 ml/hour. The distance between the needle and the assembly screen was 25 cm.

### *1.3. Production of colloidal magnetite solution*

To obtain a colloidal solution of $FeCl_3$–$6H_2O$ (1.3 g) and $FeCl_2$–$4H_2O$ (0.48 g) magnetite salt solution, it was dissolved in 25 ml of deionized water at room temperature. Five minutes before synthesis gaseous nitrogen was passed through all solutions in a closed container. First, 150 ml of 0.1 M NaOH solution was injected into the reaction chamber, which was then heated to 40°C in a closed nitrogen atmosphere. To ensure oxygen displacement, the medium pressure has been increased. The iron salt solution was then injected into the reaction chamber for a few seconds and left for 30 seconds to be mixed. At the contact of solutions of sodium hydroxide and salts of iron the origin of magnetite began, reflected in the formation of a dark brown deposit of iron oxide (III) nanoparticles. 25 ml of citric acid was injected to stabilize the sludge with a constant mixing process. To remove excess citric acid and prevent further dissolution, the magnetite hydrosol was dialyzed with a slow, constant mixing in deionized water for 3 days. The concentration of the obtained solution was 0.4 mg/ml, the diameters of nanoparticles were $16 \pm 4$ nm, determined by dynamic laser light scattering.

### *1.4. Formation of $CaCO_3$ coating on organic PCL fibers*

One of the methods described in [26] with changes in some technological parameters was used to form $CaCO_3$ coating on PCL fibers. $CaCO_3$ particles were formed from a mixture of saturated $CaCl_2$ and $Na_2CO_3$ solutions of equal concentrations (0.5 M).

Before the mineralization of the PCL nanofibers, they were pre-treated with $CaCl_2$ solution in an ultrasonic bath for 1 minute. A sample of $2 \times 2$ cm PCL fibers was placed in a tube with

1 ml of $CaC_2$ solution, then 1 ml of $Na_2CO_3$ solution was added. The system was ultrasonically treated for 1 minute and set aside for completion of the crystallization process. The experiment was carried out in an ultrasonic bath ("Sapphire", Russia) at an operating frequency of 35 kHz and an intensity of 0.64 W/cm$^2$. Then the sample was taken out of the tube, washed with ethanol, and dried in a drying oven at 45°C for 5–10 minutes. The homogeneous $CaCO_3$ coating formation on PCL fibers was carried out through triple repetition of mineralization stages, carried out similarly, starting from the sample treatment with calcium chloride solution.

### *1.5. Methods of modification PCL fibers with nanoparticles of magnetite*

Three methods of $CaCO_3$ + MNPs coating formation on the surface of PCL fibers were tested. The first method of modification PCL fibers with nanoparticles of magnetite is the method of co-precipitation with MNPs. The method of co-precipitation with MNPs involved the mixture of $CaCl_2$ and $Na_2CO_3$ salt solutions with the MNPs suspension in the ratio of 2 : 1. Further, synthesis was carried out using well-known technology. In the second method – the US-assisted method – the PCL fibers were subjected to ultrasonic treatment in an MNPs suspension for 5 minutes, after which the $CaCO_3$ synthesis was performed, as shown in previous experiments. The third method – the crystallization-induced method was based on the process described in [36]. This approach was based on MNPs incorporation into the pores of calcium carbonate particles, formed around the fibers. The $Fe_3O_4$ nanoparticles were pushed out by the water crystallization front and concentrated around the vaterite microparticles surfaces. Finally, MNPs were pressed into the surface of $CaCO_3$ microparticles by the increasing pressure of the forming ice. To modify the nanoparticle coating, the PCL/$CaCO_3$ scaffold was placed in a tube containing a colloidal magnetite solution, then was frozen in a freezer for 1.5 hours, after which it was extracted from the tube, washed with ethanol, and dried in a drying oven for 10 minutes at 45°C.

### *1.6. Investigation of morphology and the recrystallization process of PCL fibers*

The recrystallization process of $CaCO_3$ coatings on PCL fibers from vaterite polymorph to calcite is described in [37]. The scaffold was divided into 12 equal fragments. The fragments were placed in the deionized (DI) water. One fragment was removed from DI water after 1 hour of the incubation, washed with ethanol, and dried in a drying oven for 10 minutes at 45°C.

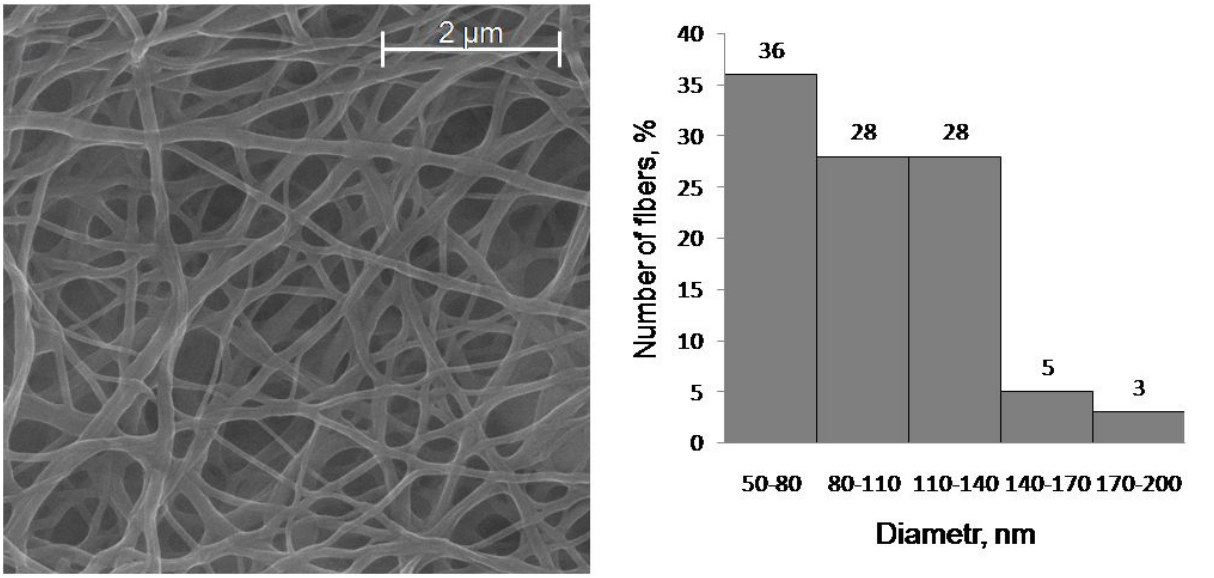
To characterize PCL/$CaCO_3$+MNPs scaffolds morphology, scanning electron microscopy (SEM) was used (analytical complex based on high-resolution scanning electron microscope Mira II LMU of "TESCAN" company, Czech Republic). SEM studies were carried out in the mode of secondary electrons. The accelerating voltage was 30 kV, the beam diameter was 3.2 nm. The samples were sprayed with gold. 100 diameters of fiber samples and mineralization shells were measured for the obtained SEM images of the samples using Image J software [7]. The percentage ratio of calcite microparticles on the total number of microparticles and time dependence was determined.

## 2. Results and discussion

The mineralization process by porous $CaCO_3$ microparticles was described in [7]. The biocompatibility of these PCL/$CaCO_3$ scaffolds was studied *in vivo*; the concept of drug release capability of PCL/$CaCO_3$ scaffolds *in vivo* was confirmed [36]. The transition from metastable vaterite polymorph to stable calcite form determines the release of molecules immobilized in vaterite pores due to the restructuration of $CaCO_3$. Additional modification of $CaCO_3$-mineralized fibers by MNPs allows controlling of the scaffold properties by the external electromagnetic field.

The morphology of electrospun PCL fibers is shown in Figure 1, *a*. The percentage distribution of fiber diameters is illustrated in Figure 1, *b*. The average diameters of most PCL fibers are 50–80 nm, 36%, and the smallest number of PCL fibers with diameters is 170–200 nm, 3% similar to the size of extracellular matrix fibers in living tissues (50–500 nm) [38].

Mesoporous morphology of vaterite allows it to encapsulate various substances: ibuprofen [39], lysozyme [40], fluorescent dyes [41], nanoparticles of magnetite [42]. Micron- and submicron-sized $CaCO_3$ particles are biocompatible and biodegradable materials [43], which can be successfully used in targeted drug delivery systems [44]. We assume that the deposition of porous $CaCO_3$ coating on the polymeric fiber surface enables the scaffold to deliver immobilized drugs and nanoparticles. MNPs are very promising in medicine [45], as a contrast substance for MRI [46]. Nanoparticles embedded in the structure of microcapsules are used for magnetothermal release [47] and magnetotherapeutic



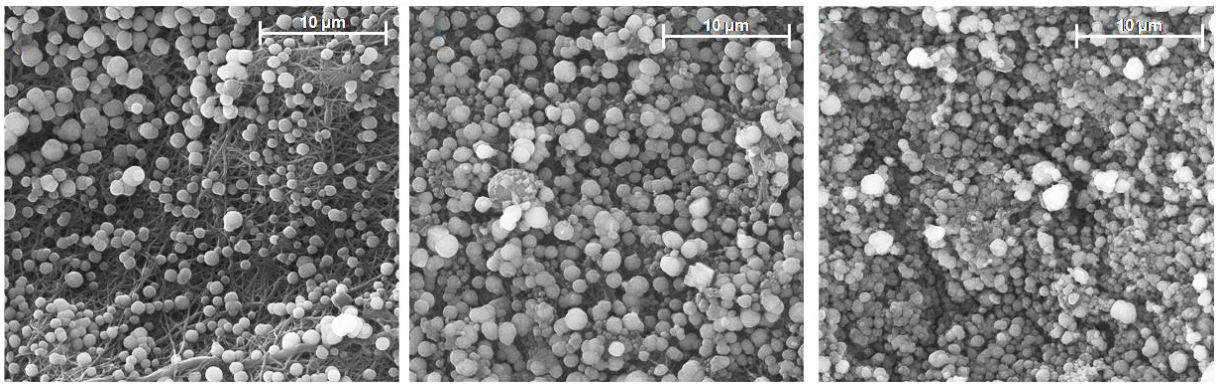

*a* *b*

Fig. 1. SEM image of the obtained polycaprolactone fibers (*a*) and the size distribution of PCL scaffold fibers (*b*)

purposes [48]. Mineralized fibers with encapsulated nanoparticles have some useful regenerative medical properties: biocompatibility, biodegradability, the usage in drug delivery systems, as well as sensitivity to magnetic fields.

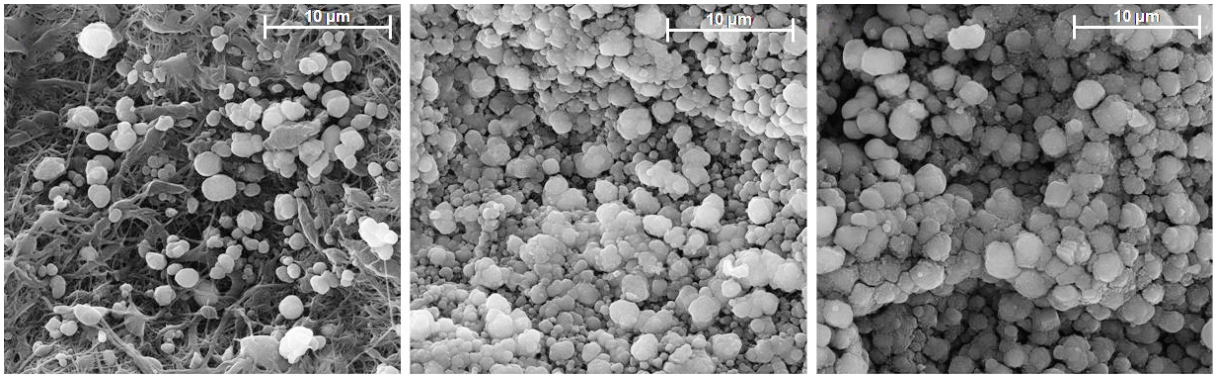
Using the proposed synthesis method, the vaterite-mineralized tissue-engineered scaffolds were obtained (Figure 2). The number of $CaCO_3$ microparticles and the total density of the $CaCO_3$ coating is increasing with the increase in the number of mineralization stages. $CaCO_3$ microparticles occur in vaterite polymorph, which corresponds to the results obtained in [7], with their diameter being $0.96 \pm 0.23$ μm. As the number of treatments increases, the polymorph type of $CaCO_3$ microparticles is harder to distinguish. The described phenomena confirm the increase of $CaCO_3$ amount and, consequently, the surface area of the formed $CaCO_3$ microparticles, which should increase the loading capacity of the functional molecules.

The morphology of PCL/$CaCO_3$ + MNPs scaffold, obtained by the method of co-precipitation with magnetite, is shown in Figure 3. The morphology of PCL/$CaCO_3$ + MNPs scaffold, obtained by the US-assisted mineralization method, is illustrated in Figure 4. The coating density and uniformity, as well as the average diameter of $CaCO_3$ microparticles ($1.44 \pm 0.39$ μm for the method of co-precipitation with magnetite and $1.21 \pm 0.38$ μm for US-assisted mineralization method), are increased with each subsequent treatment. It should be noted that



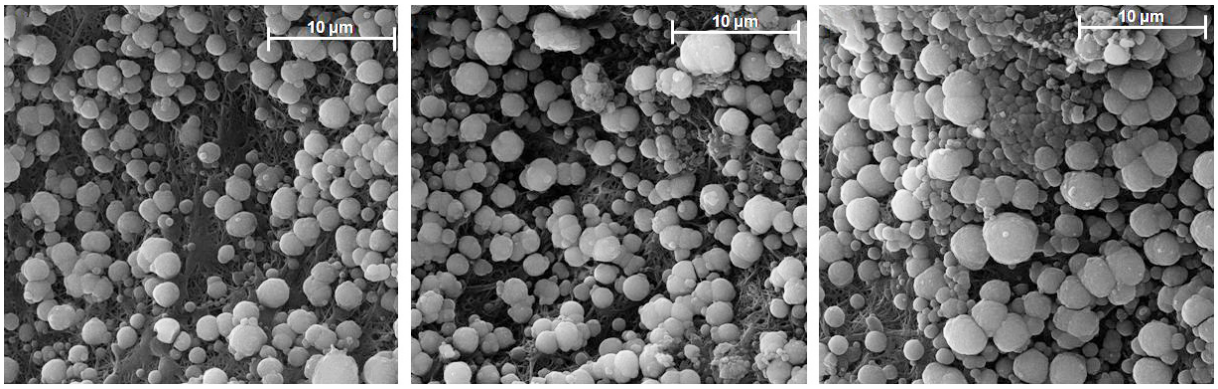

*a* *b* *c*

Fig. 2. SEM image of the porous vaterite $CaCO_3$ coating on the PCL fibrous scaffold after the first (*a*), the second (*b*), and the third mineralization stage (*c*)




*a* *b* *c*

Fig. 3. SEM image of the PCL/$CaCO_3$ + MNPs scaffolds, produced by coprecipitation with magnetite after the first (*a*), the second (*b*), and the third mineralization stage (*c*)




*a* *b* *c*

Fig. 4. SEM image of the PCL/$CaCO_3$ + MNPs scaffolds, produced by the US-assisted method, after the first (*a*), the second (*b*), and the third mineralization stage (*c*)

the difference of average microparticle diameter in these methods complicates the control of the loading capacity and drug release rate from vaterite.

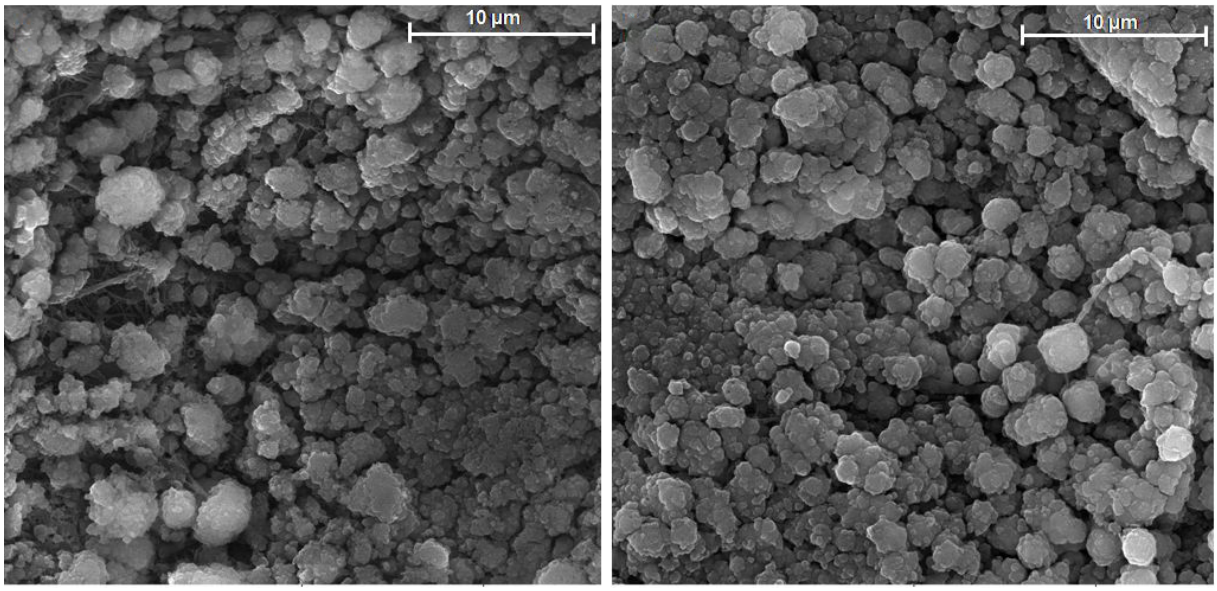
SEM images of the PCL/$CaCO_3$ + MNPs scaffold before and after the adsorption induced by crystallization from $Fe_3O_4$ colloidal solution are shown in Figure 5. Our findings suggest that after crystallization-induced adsorption $CaCO_3$ microparticles retain their geometric dimensions. This modification method is the most promising because it induces no change in the structure and geometric dimensions of the resulting $CaCO_3$ microparticles.

Using all the above mentioned methods, it is possible to get a uniform coating of vaterite $CaCO_3$ microparticles on the fibrous PCL scaffold surface. Due to their porosity, these structures might be used as containers for targeted drug delivery, and after conversion to calcite – as a nutritional component for the osteoblasts formation. MNPs integration allows to control the rate of drug release from the formed $CaCO_3$ microparticles through magneto therapy, as well as to apply this structure for magnetotherapy purposes.

Figure 6 compares the average diameters of composite vaterite $CaCO_3$ + MNPs microparticles, obtained by different methods, on PCL nanofibers surface. The similarity of these average diameters can be observed.

The presence of magnetite nanoparticles in the samples formed by the presented methods was confirmed using a permanent magnet. All synthesized samples, with the exception of the control sample, were attracted to a permanent magnet.

Formed on PCL nanofibers, $CaCO_3$ microparticles in the polymorphic form of vaterite are unstable, turning into calcite. Therefore, scaffolds might lose their drug adsorption and transportation properties, as well as sensitivity to magnetic fields, when vaterite turns to calcite due to the release of MNPs and immobilized functional molecules. In this regard, the next stage of the study is the determination of the vaterite recrystallization rate, because this process plays a key role in immobilized substances release




*a* *b*

Fig. 5. SEM image of the PCL/$CaCO_3$ + MNPs scaffold before (*a*) and after (*b*) the crystallization-induced adsorption method

and the magnetic sensitivity of the structure. The technique for determine the percentage of calcite modification microparticles to the total amount of calcium carbonate microparticles described in article [37] was applied. This method allows us to study the recrystallization process with sufficient reliability. The ratio of the number of calcite microparticles to the total number of microparticles using the obtained SEM images for each sample at control time points was determined. The dependence of calcite amount in the total number of microparticles by the time of scaffold incubation in water is shown in Figure 7. The control sample is a PCL/$CaCO_3$ with no MNPs.

The recrystallization time of the control sample is 8 hours; the recrystallization process starts in the time interval between $3^{th}$ and $4^{th}$ hours of the experiment. This process is more intensive in the last 2 hours, and by 7 hours vaterite recrystallization to calcite is almost complete. For samples obtained by $CaCO_3$ and magnetite coprecipitation and US-assisted methods, the time of complete recrystallization is 5 hours, which is less by 2 times than the recrystallization rate of the control sample. Also, it is worth noting that the increase in the average diameter of $CaCO_3$ + MNPs microparticles negatively affects the porosity of the vaterite structure. The sample, obtained by the crystallization-induced method, is recrystallized into calcite within 8 hours, but, the results suggest, that calcite occurs only at $5^{th}$ hour, and the process intensity is the highest during the last hour of the experiment.

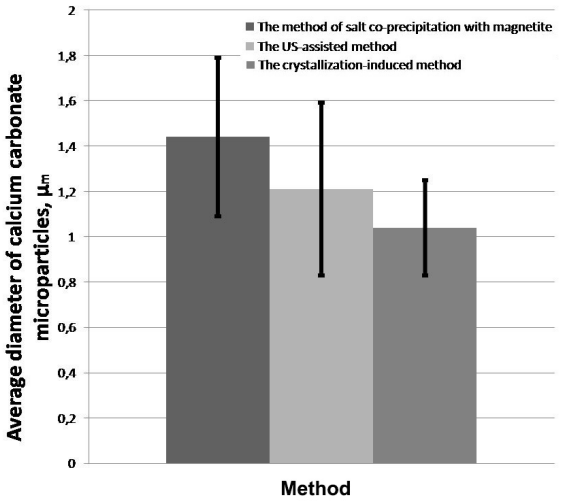



Fig. 6. Comparison of average diameters of the calcium carbonate micro-particle formed on PCL fibers after its modification with magnetite nanoparticles according to the above described methods: black – the method of salt co-precipitation with magnetite, light gray – the US-assistend method, dark gray – the crystallization-induced method

Thus, the structures, obtained by the US-assisted method, have the highest rate of recrystallization, as by 4 hours more than 90% of initial vaterite microparticles are transformed to calcite. The tissue-engineered scaffolds obtained by the crystallization-induced method show the lowest recrystallization rate of calcium carbonate microparticles, comparable with the recrystallization rate of the control sample. The decrease of the calcite formation dynamics may lead to the conclusion about

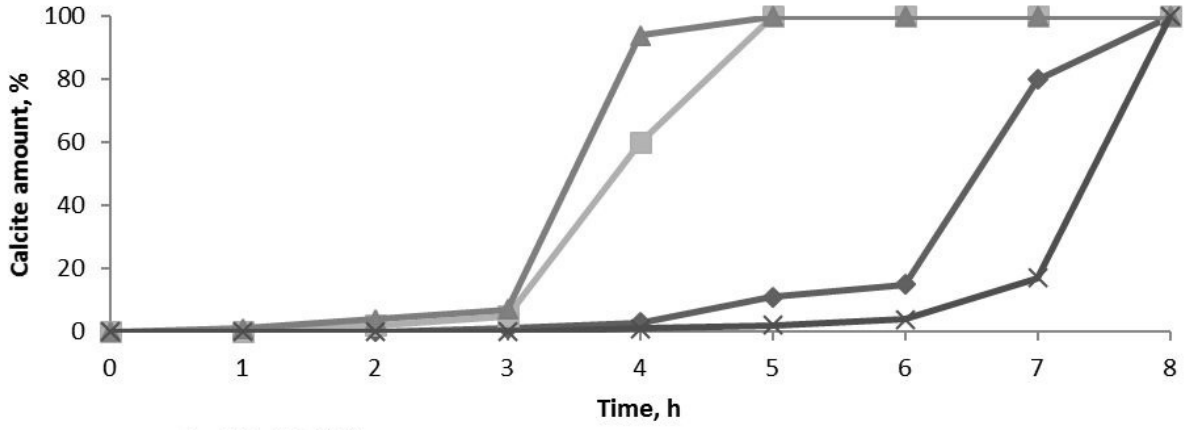



Fig. 7. Dependence of the rate recrystallization vaterite to calcite on time in PCL/$CaCO_3$ + MNPs scaffolds, developed by different methods: black – PCL/$CaCO_3$; light gray – PCL/$CaCO_3$ + MNPs (the method of salt co-precipitation with magnetite); gray – PCL/$CaCO_3$ + MNPs (the US-assisted method); dark gray – PCL/$CaCO_3$ + MNPs (the crystallization-induced method)

the improvement of some physical properties of the resulting structure.

## Conclusion

Three methods of modifying electrospun PCL scaffolds surface by composite nanostructured vaterite $CaCO_3$ + MNPs coatings were tested, namely, the method of salt co-precipitation with MNPs, the US-assisted method, and the crystallization-induced method. Vaterite $CaCO_3$ coatings with MNPs, embedded in their structure, on the PCL fiber surface, retain their porous structure, corresponding to the polymorphic form of vaterite. The coatings, obtained by the method of co-precipitation with MNPs and the US-assisted method, are transformed into calcite in a humid medium within 5 hours. Coatings formed by the crystallization-induced method are transformed to calcite within 8 hours, which corresponds to this process for the coating of calcium carbonate microparticles with no MNPs. Thus, the application of the crystallization-induced method is the most advantageous, because the resulting structure retains its properties much longer than the other samples. This material can be used for magnetotherapy, as well as for antibiotics and antiseptics delivery and controlled release, thus reducing inflammation in the postoperative period.

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