Architecture of biohybrid organosilicon materials using various structure-controlling agents

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Research Article

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Abstract

The article describes the immobilization of yeast cells *Ogataea polymorpha* VKM Y-2559 into organosilicon matrices based on tetraethoxysilane and dimethyldiethoxysilane using polyethylene glycol, polyvinyl alcohol and chitosan as structure-controlling agents. The influence of the structure-control agent on the time of formation of the sol-gel capsule around the cells and on the morphology of the hybrid material was determined. The formation of organosilicon material was confirmed using IR spectroscopy. Using the method of energy-dispersive X-ray spectroscopy, it was proven for the first time that the structure-controlling agent is not incorporated into the structure of the organosilicon shell.

1. Introduction

Currently, the field of creating hybrid materials is intensively developing [1–3]. The sol-gel method is widely used to create porous materials that have applications in drug delivery systems [4], for tissue repair [5], for corrosion protection [6], for the immobilization of biomaterials, for 3D printing [7], for use in biosensors [8] and biofilters [9], for the adsorption of heavy metals [10]. For a number of applications, it is necessary that the formed architecture be characterized by uniform, repeatable porosity for loading the active substance.

Multifunctional materials with controlled structural parameters under optimal reaction conditions and suitable reagents can be created using sol-gel technology [11, 12]. The structure of the resulting matrices strongly depends on the structure of silane precursors (alkoxysilanes and alkylalkoxysilanes with non-hydrolyzable Si-C bonds) [13], their concentration and ratio, pH of the environment [14], type of catalyst [15], as well as the presence of organic solvents [16], amphiphilic compounds, water-soluble polymers and cells [17] in the system. Also, the use of different ratios of silane precursors with hydrolyzable and non-hydrolyzable bonds makes it possible to obtain materials with different porosities [18, 19].

One of the methods for obtaining homogeneous porous matrices for loading pharmaceuticals and antiseptic agents is the use of template molecules with their further removal [20, 21]. Surfactants are used as templates, which determine the morphology and porosity of mesoporous silica nanoparticles. Surfactants belong to the so-called soft templates. The basis for the formation of most porous silicon-containing materials is the self-assembly of surfactants (or templates) into micelles. Free pores can be obtained by removing the template substance [22].

Despite its convenience, the method has a disadvantage, since particle agglomeration occurs during sintering [23]. In addition, particle size may increase due to high temperature, which leads to a decrease in mesopore size and particle surface area due to particle growth. This has a negative impact on the overall payload, material uniformity and control over the release of active substances [24]. Template molecules can be replaced with microbial cells [25–27]. They have a number of advantages, such as a rich variety of forms, low cost, availability, the possibility of rapid growth of biomass, high repeatability of
morphology, and environmental friendliness [28]. This allows microorganisms to be used as an alternative to molecules used as templates.

Also, in addition to the nature of the precursors and their ratio, it is necessary to study the possibilities of using various polymers as structure-controlling agents. Degradable synthetic polymers or natural biopolymers, including polyvinyl alcohol (PVA) [29], chitosan [30], gelatin [31], collagen [32], polyethylene glycol (PEG) [33], poly(ε-caprolactone) (PLC) [34] are often used to widely improve the properties of the formed material, and are also used for medical purposes and biotechnological processes.

In this work, the immobilization of yeast cells *Ogataea polymorpha* VKM Y-2559 into organosilicon sol-gel matrices based on tetraethoxysilane and dimethyldiethoxysilane was carried out. Polyethylene glycol, polyvinyl alcohol, and chitosan were used as structure-controlling agents. They have different structural structures and different functional groups, which can affect the architecture of the formed matrices, increasing the porosity of the material or its homogeneity. The influence of structure-controlling agents is important because encapsulated microbial cells can be used as templates and create a material with controlled porosity for certain tasks.

### 2. Experimental part

**Formation of biohybrid material**

Cultivation of yeast cells *Ogataea polymorpha* VKM Y-2559 was carried out similarly to the article [35]. 50 µl of a suspension of yeast cells (1.3 ± 0.1 10⁹ CFU/ml) in a phosphate buffer solution (20 mmol/l, pH 6.8) was added to 20 µl of a structure-controlling agent (1% chitosan, 5% polyvinyl alcohol or 5% polyethylene glycol) and stirred for 5 minutes (Elmi CM-70M07, Poland) to form biohybrid materials. 100 µl of a mixture of dimethyldiethoxysilane (DMDES) and tetraethoxysilane (TEOS) in a volume ratio of 85%/15% was added, stirred for 5 minutes, then 10 µl of a 0.02 mol/l NaF catalyst solution was added, and stirred for 15 minutes.

**Optical microscopy**

An optical microscope Nikon Eclipse Ci-L (Nikon, Japan) equipped with a Color Camera Nikon DS-Fi3 (Nikon, Japan) was used to record the process of encapsulation of yeast cells in an sol-gel matrix.

**IR spectroscopy**

IR spectra of samples of biohybrid materials were obtained with an FMS 1201 Fourier IR spectrometer (Monitoring, Russia) using a multiple attenuated total internal reflection (MATIR) attachment of horizontal type with a zinc selenide prism (resolution 4 cm⁻¹).

**Scanning electron microscopy and energy dispersive X-ray analysis**
Before microscopy, samples of biohybrid materials were placed on an aluminum stage, secured with conductive carbon tape, and coated with a thin layer (15 nm) of carbon in a sputtering unit. Observations were made using a Hitachi SU8000 field emission scanning electron microscope (FE-SEM). EDX-SEM studies were carried out using an Oxford Instruments X-max 80 EDX at an accelerating voltage of 10 kV.

3. Result and discussion

Study of obtained biocomposites by optical microscopy and IR spectroscopy

Organosilicon precursors tetraethoxysilane (TEOS) and dimethyldiethoxysilane (DMDES), and yeast cells Ogataea polymorpha VKM Y-2559 were used to form biohybrid materials. Polyethylene glycol (PEG), polyvinyl alcohol (PVA) and chitosan were added as structure-controlling agents (SAs).

The organosilicon matrix must be formed in such a way that the pore size ensures high reliability of attachment of microorganism cells. Therefore, it is important to estimate the time of matrix formation in each selected precursor system, which was carried out using the method of optical microscopy (Fig. 1).

Sol particles begin to form on the surface of the cells and the number of particles gradually increases over time, regardless of the nature of the structure-controlling agent in the first 30 minutes (Fig. 1). However, sol-gel formation ends at different times when different structure control substances are used. When using chitosan as an organic component during the formation of the matrix, after 2 hours the particles completely cover the surface of the cells, but this is not observed when using PEG and PVA. The formation of the sol-gel ends after 3 hours in the presence of PVA, and in 5 hours in the presence of PEG. This is probably connected with the number of functional groups in the composition of the structure control agent (the more groups are contained, the faster chemical processes and aging of the material occur). However, this requires additional research. In further work, to study the structure of the resulting material, we used the material obtained by using TEOS: DMDES: PEG 5 hours after the start of the formation of the material, TEOS: DMDES: PVA after 3 hours, and TEOS: DMDES: chitosan after 2 hours.

The process of polymer materials formation by the sol-gel method was confirmed by IR spectroscopy. Figure 2 shows the IR spectra obtained for matrices based on TEOS and DMDES using SAs polyvinyl alcohol, polyethylene glycol and chitosan.

The absorption band at 1262 cm\(^{-1}\) shows deformation vibrations of Si-(CH\(_3\))\(_2\); it is identical for all three matrices, since the same ratio of silane precursors DMDES and TEOS (85 and 15 vol.%) is used. The absorption band around 2965 cm\(^{-1}\) in the polymer is attributed to asymmetric stretching vibrations of CH\(_3\) groups. Si-O-Si bonds are located in the region of 1100 cm\(^{-1}\) [36], the presence of this band confirms the formation of organosilicon polymers, since the formation of Si-O-Si bonds occurs during the polycondensation of TEOS and DMDES.

Surface study of obtained biocomposites by SEM and EDX methods
The biocomposites was studied by scanning electron microscopy to determine the influence of structure-controlling agents on the resulting materials. Figure 3 shows SEM photographs of the surface of organosilicon matrices with various structure-controlling agents.

Figure 3 (a,b,c) shows yeast cells immobilized into spheres ranging in size from 1 to 5 µm (a,c) and into the thickness of the material (b). When using PEG, spherical homogeneous spheres with a size of about 0.1 µm are observed, closely located to each other (Fig. 3a). When chitosan is added as an organic component to the sol-gel matrix, yeast cells are coated with a smooth silicon organic polymer structure. The structure is not fractal, but closer to monolithic (Fig. 2b). However, individual cells of microorganisms are observed encapsulated in a sol-gel matrix, which is important when using microorganisms as templates. The use of PVA (Fig. 3c) as a structure-controlling agent leads to the formation of spheres, apparently with immobilized yeast cells, which are located on the rough surface of the sol-gel matrix. Therefore, various structure-controlling agents make it possible to obtain different morphologies in the forming sol-gel matrices, which should be taken into account when choosing a material for creating a porous support.

Elemental analysis (Fig. 4) carried out using energy-dispersive X-ray spectroscopy (EDX) demonstrates the distribution of elements on the surface of a heterogeneous biocomposite with yeast cells immobilized in it using various SAs.

Figure 4 shows a typical view of the energy-dispersive spectrum of the formed biohybrid material. As shown by EDX, Si, C and O are the main constituent elements of the silicon matrix. The ratio of silane precursors DMDES:TEOS during encapsulation of yeast cells is 85:15 vol.%, therefore the theoretical distribution of elements on the surface of the test sample is Si:O:C = 24:35:41 (%). The actual distribution is shown in Fig. 5.

Other elements include P, Na, K. Phosphorus is present as the phosphate ion from phosphate buffer solution, similarly for sodium and potassium ions. It should be noted that the surface of the matrix does not contain nitrogen, which indicates that the yeast cells are in an immobilized state and immersed in the thickness of the matrix.

The distribution of elements on the surface of the organosilicon matrix correlates with the theoretically calculated one and does not change with the addition of PEG, PVA or chitosan. This proves that the structure-controlling agent does not form covalent bonds with the silicone matrix, but rather envelops the biomaterial to prevent excessive compaction of the material. This is important when forming matrices with clearly defined pores, which are formed when microorganisms are removed.

4. Conclusion

The work involved the immobilization of yeast cells into organosilicon sol-gel matrices based on tetraethoxysilane and dimethyldiethoxysilane; polyethylene glycol, polyvinyl alcohol or chitosan were used as structure-controlling agents. These structure-controlling agents have different structures and
different functional groups, which affects the architecture of the formed matrices, increasing the porosity of the material or pore sizes.

The method of optical microscopy showed that the organosilicon matrix is formed around yeast cells at different times in the presence of different structure-controlling agents. Therefore, 5 hours are required for matrix formation after sol-gel synthesis reactions using PEG, 3 hours when adding PVA, 2 hours when adding chitosan. The formation of an organosilicon polymer has been proven using IR spectroscopy.

The EDX method showed that the structure-controlling agent does not form covalent bonds with the organosilicate matrix, but envelops the biomaterial to prevent excessive compaction of the material.

Thus, various structure-controlling agents make it possible to obtain different morphologies in the forming sol-gel matrices, which should be taken into account when choosing a material for creating a porous support. This is important when forming matrices with clearly defined pores that appear when microorganisms are removed.

Electron microscopy characterization and determination of the local composition were performed in the Department of Structural Studies of Zelinsky Institute of Organic Chemistry, Moscow.

**Declarations**

**Author Contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Elizaveta A. Lantsova, Pavel V. Rybochkin, and Evgeniya A. Saverina. The first draft of the manuscript was written by Olga A. Kamanina and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**References**


**Figures**
Figure 1

Figure 2

IR spectroscopy of organosilicon matrices obtained using precursors TEOS and DMDES and SAs: pink – PEG, blue – PVA and green – chitosan.
Figure 3

Figure 4

Energy dispersive spectrum of the formed biohybrid material using PVA.

Figure 5

<table>
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<tr>
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Other
Map of the distribution of elements in matrices when using SAs: A – theoretically calculated value; B – PEG, C – PVA, D – chitosan