Hybrid functional materials for tissue engineering: synthesis, in vivo drug release and SERS effect

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Abstract. The research presents the designing new hybrid biocompatible materials aimed to bone tissue engineering with enhanced osteoconductivity and functionality. The scaffolds consisted of electrospun polymeric matrix, modified with porous calcium carbonate (vaterite) coatings, were developed and studied. The subcutaneous implantation tests in vivo with white rats demonstrated the high degree of biocompatibility of vaterite-mineralized scaffolds. Moreover, the performed in vivo release of bioactive molecules, immobilized in mineral coating of scaffold, allowed to control the regeneration process in tissues in the implantation area. Also, the decoration of mineralized scaffold with silver nanoparticles exhibited the capability of exploiting these materials as effective substrates with providing surface enhanced Raman scattering (SERS) for precise detection of low concentrations of analyte. In this way, developed scaffolds can be promising materials with enhanced functionality of tissue regeneration, in vivo drug release and detection for designing novel smart devices for biomedicine.

1. Introduction

At the current time, searching and designing of novel materials and methods for healing of disorders of human musculoskeletal system are the issue of heighten importance, as well as enhancing, controlling and monitoring of regeneration processes [1]. Recently, the novel class of implant materials based on biocompatible polymers (polycaprolactone (PCL) [2]) are being actively developed and investigated due to their advantages including biocompatibility, bioresorbability and ability to restructuration in a body. However, most of these polymers are bioinert materials, which lack functional groups supporting adhesion of specific proteins and cells, which would have facilitated the formation of a biological interface supporting bone cell and tissue growth. The bioinertness of bone implants is one of critical factors that possible to cause implant failure and rejection. In order to solve this problem, deposition of bioactive coatings is investigated with the goal of improving the osseointegration of the implants and reducing the possibility of their rejection or failure. Regarding to bone tissue engineering, inorganic bioceramics (hydroxyapatite, calcium phosphate, calcium
carbonate) are promising candidates for coating, because of their similarity of physical-chemical properties to the natural mineral of bone – apatite. Thus, hybrid composites consisted of inorganic and organic components possess the property set that could structurally and functionally mimic the natural bone tissues composed of organic (collagen) and inorganic (apatite) phases. Among various inorganic materials, the porous calcium carbonate polymorph called vaterite due to its beneficial properties is one most functional and advanced candidate material for using as the component of hybrid biomaterials for bone recovering. Together with osteoconductivity, vaterite due to its interconnected porous structure is perfect substrate for a functional payload storage and release. Along with the using as a carrier for therapeutic molecules and biological macromolecules delivery, the vaterite also could be exploited as effective template for metallic nanoparticles synthesis, which can be used as plasmon resonance substrates for supporting SERS effect. In this way, along with tissue engineering capability, the vaterite-mineralized scaffolds with Ag nanoparticles could provide the function of precise detection of molecules, performing the monitoring function.

2. Polymeric electrospun scaffold mineralized with vaterite

2.1. Mineralization technique

The aim of this study is the preparation of a functional tissue engineering scaffold based on vaterite-mineralized PCL fibrous matrix. The electrospun polymeric matrix consisting of fibers with average diameter 0.5±0.1 µm (Fig. 1 A) is similar to natural extracellular matrices. Vaterite coatings on fibers can provide an enhanced functionality of the scaffold due to beneficial properties of its material. The high degree of porosity and mild conditions of synthesis and decomposition allow to incorporate various bioactive substances (therapeutics, proteins, metallic nanoparticles) into vaterite structure. Porous CaCO₃ is biocompatible and able to biodegrade rapidly, so it can be successfully used as a drug delivery system. Moreover, the vaterite is able to transform to hydroxyapatite under physiological conditions, which allow the vaterite-coated scaffold to support bone regeneration. In this way, the vaterite coating on scaffolds can provide drug delivery functionality and osteoconductivity.

![Figure 1. Scanning electron microscopy images of blank PCL scaffold (A), vaterite-mineralized PCL scaffold (B). Optical microscopy images of histological sections of scaffolds after 21-day subcutaneous implantation: PCL/CaCO₃ scaffold (C) and PCL/CaCO₃+TA scaffold (D)](image)

The CaCO₃ can be precipitated on PCL fibers in vaterite form by using the classical route of CaCO₃ synthesis by reaction between Ca²⁺ and CO₃²⁻ ions in water solution. The process are assisted with ultrasound (US) treatment of the working solution and supplemented with presence of polymeric...
fibers immersed in this solution and serving as CaCO$_3$ growth centers. The vaterite shell-like coatings on fibers can be obtained by this way (Fig. 1 B). The shell-like formation can be accounted for by the ultrasonic influence on the crystalization process and CaCO$_3$ particle nucleation. Also, this method [3] allows to perform the homogeneous coating over all fibrous scaffold surface, as well as scaffold interior due to US stimulation of ions to penetrate into scaffold pores.

2.2. In vivo subcutaneous implantation with white rats

The vaterite-mineralized PCL/CaCO$_3$ scaffolds were studied in vivo in course of 21-day subcutaneous implantation tests with white rats [4]. The results of scaffolds examination after explantation (Fig. 1 C) revealed, that scaffolds were colonized with connective tissue cells and elements, and intensively vascularized. No signs of inflammation response were observed. Thus, the PCL/CaCO$_3$ scaffolds showed full biocompatibility and transplantability.

The excessive vascularization of the scaffold pronouncing in increased number of new-formed fragile blood vessels and capillaries along with enhanced blood filling, resulted in an amount of hemorrhages in scaffold and surrounding tissues. For the purpose of angiogenesis moderation of the scaffold, the natural antioxidant tannic acid (TA) was immobilized into vaterite coatings of the scaffold. This TA-loaded scaffold PCL/CaCO$_3$+TA was studied in subcutaneous implantation in vivo (Fig. 1 D) similarly as the blank PCL/CaCO$_3$ scaffold. The results of explanted scaffold examination showed the decrease of the blood vessels number and absence of hemorrhages, while the cell population of scaffold remained unchanged in comparison with the unloaded PCL/CaCO$_3$. The incorporation of TA molecules in vaterite allowed to control the angiogenesis process by TA release from vaterite in vivo due to recrystallization of vaterite to calcite. In such manner, the concept of exploiting of vaterite-mineralized polymeric matrix as tissue engineering scaffold with in vivo drug release functionality was demonstrated.

3. Mineralized scaffolds as SERS platform

The PCL/CaCO$_3$ scaffolds were decorated with silver Ag nanoparticles through Ag reducing from Tollen’s reagent. The performing of Ag reduction on the blank PCL matrix and vaterite-mineralized PCL/CaCO$_3$ scaffold showed, that vaterite coatings promoted the more uniform spreading of Ag nanoparticles on the scaffold surface and narrow nanoparticles size distribution, while in case of blank PCL matrix, reduced Ag was situated in agglomerated form and spreaded on matrix in unregular manner. Such behavior of Ag reducing can be accounted for the fact, that pores of vaterite surface serve as growth centers for nucleation of Ag nanoparticles, providing the homogeneous nanoparticle distribution and preventing nanoparticles agglomeration. The small and separate Ag nanoparticles provide the higher average enhancement factor for mineralized scaffold PCL/CaCO$_3$ (2*10$^4$) in comparison with unmodified PCL (5.3*10$^3$).

Figure 2. Scanning electron microscopy images of blank PCL scaffold modified with Ag (A), vaterite-mineralized PCL scaffold modified with Ag (B).
4. Conclusion
The novel functional hybrid materials based on porous CaCO$_3$-mineralized electrospun scaffolds were designed, studied and tested. These materials developed have the following advantageous properties:

- The high degree of biocompatibility and supporting cell colonization in vivo, which provides the transplantability of materials;
- The capability of in vivo drug release and controlling the regeneration process by this way;
- The mineralized scaffold with Ag nanoparticles provides the significant enhancement of the SERS effect.

So, the developed hybrid material PCL/CaCO$_3$ has the promising perspectives for exploiting in biomedicine.

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