dynamic bioreactor (which enables higher colonization of the inside of the scaffolds), on the depth of penetration of cells inside the scaffolds and on osteogenic cell differentiation. engineering. However, these first conclusions need further deeper investigation, e.g. focused on cell cultivation in a dynamic bioreactor (which enables higher colonization of the inside of the scaffolds), on the depth of penetration of cells inside the scaffolds and on osteogenic cell differentiation.

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SILK-COLLAGEN-INSPIRED COPOLYMER: PROMISING BIOMATERIAL PRODUCED BY YEASTS

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Introduction

Biomimetic recombinant protein polymers are a new group of materials introduced to regenerative medicine. Due to the high level of precision in the production using recombinant DNA technology, accurate control over material structure and properties is ensured. Importantly, biofunctional domains can be incorporated in a recombinant way. Therefore, the approach may lead to fully functional scaffolds with properties adjusted to a particular biomedical use. In the last three decades several recombinant protein modules for biomedical application were designed, produced and characterized [1,2]. A broad range of them is inspired by nature, such as elastin-like [3,4], collagen-like [3] and silk-like protein sequence [5]. Depending on the DNA sequence, most commonly used hosts organisms to achieve optimal expression are Escherichia coli or yeasts such as Saccharomyces cerevisiae and Pichia Pastoris.

The recombinant protein used in this study is a silk-collagen-inspired copolymer, denoted further as CSC. CSC is expressed by Pichia pastoris in a methanol fed-batch fermentation process and consists of two types of blocks. The silk-inspired block (S) is rich in histidine and is responsible for pH-responsive protein gelation. The S block is flanked by collagen-inspired blocks (C), which stabilize the hydrogel network, due to their hydrophilicity and random coil formation. The resulting CSC protein is soluble in water at low pH, whereas after increasing the pH to physiological values it self-assembles into fibers and at higher concentrations forms a physical hydrogel.

The aim of this study was to synthetize, characterize and evaluate the biological performance of CSC protein polymers. In addition, biofunctionalization of the block copolymer by active sites, such as integrin and proteoglycans binding domains, was performed and the modification effects investigated. It was shown that the obtained scaffolds are self-supporting at low protein concentrations. Our biomaterial appeared to be non-cytotoxic and able to support attachment and proliferation of bone cells. Moreover, the ability to induce a desired cell response by incorporating biofunctionalization was confirmed.

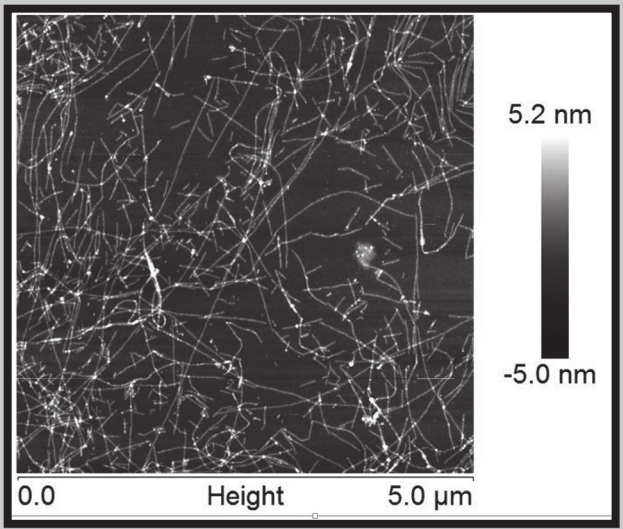

FIG. 1. AFM picture of 1% CSC solution at pH 7.4.

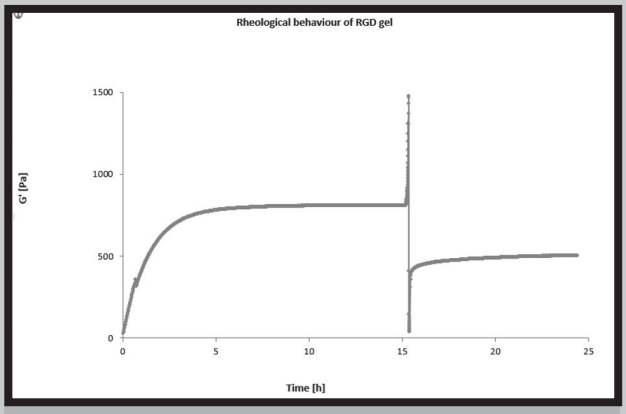

FIG. 2. Rheological data on assembly and healing of 2% CSC-RGD gel at pH 7.4

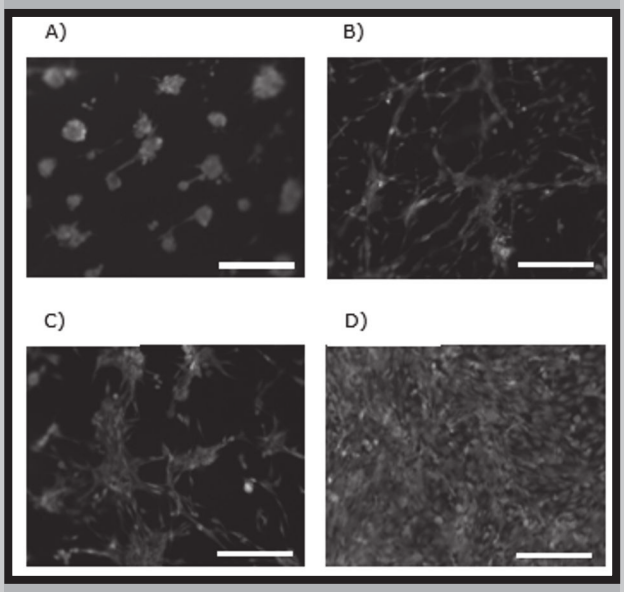

FIG. 3. Cell shape after 5 days of cell culture, visible after staining with Live/Dead assay: A) 100% CSC gel; B) mix of 50% CSC and 50% CSC-RGD gel; C) mix of 50% CSC and 50% CSC-HB gel; D) 100% CSC-HB gel. Scale bar = 200 µm.

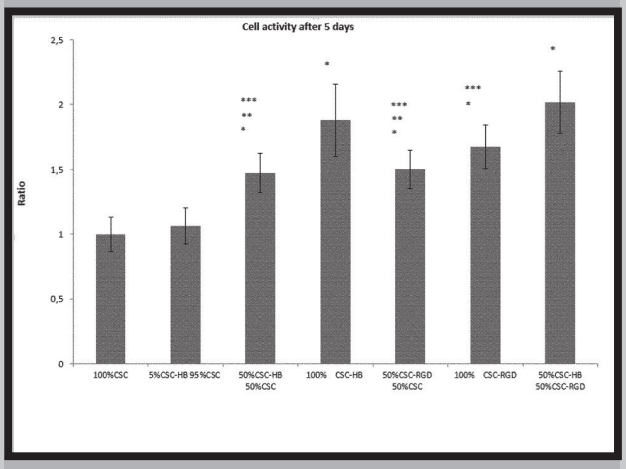

FIG. 4. Cell activity relative to cells on CSC gels after 5 days of cell culture.
*** significant difference in comparison to cells on CSC gels;**
**** significant difference in comparison to cells on 100% CSC-HB gels;**
***** significant difference in comparison to cells on 50% CSC-HB and 50% RGD mixed gels**

Results

Production of CSC protein, and its variants, with recombinantly incorporated integrin binding domains (denoted as CSC-RGD) or heparin binding domains (denoted as CSC-HB), consisted of fixed, consecutive steps, typical for recombinant DNA technology. The process started with the design of peptide sequence, and was followed by construction of encoding genes, transfection to a host organism Pichia pastoris and expression of proteins in methanol fed-batch fermentation of yeasts. To obtain the final product, selective ammonium sulphate precipitation was used for purification, after which the protein was lyophilized. The materials were characterized with a variety of techniques: SDS-PAGE gel analysis, amino acid composition and sugar content analysis, N-terminal sequencing and MALDI-TOF MAS. The analysis confirmed that CSC, CSC-RGD and CSC-HB proteins were successfully obtained in a recombinant way.

To induce fibril formation, an amount of protein was dissolved at low pH, followed by an increase in pH to physiological value of 7.4. The AFM picture (FIG.1) of 1% protein solution (protein concentration 10 g/l) at physiological pH shows fiber formation by the protein after 24 hours incubation time. The driving force in the assembly is stacking of S blocks with uncharged histidine.

The cell culture study showed that cells could survive for 24 days at all variants of CSC gels (FIG.3.). The differences in cell behaviour and activity, depending on the presence and amount of added specific bioactive domains, were clearly visible after 5 days of culture. Although cells were fully viable on the all gel types, plain CSC did not enhance cells spreading. Addition of 50% material with integrin binding domains increased cell spreading; the cell shape was more elongated. Addition of heparin binding domains improved cells spreading and proliferation. The cells seemed to spread a little less but the number of cells was higher on the gels with heparin binding domains. The cell activity test (FIG.4.) confirmed the effectiveness of biofunctional domain incorporation into our biomaterial. Cell activity was significantly higher on the gels containing functional domains than the cell activity of pure CSC gel. However, addition of 5% material with heparin binding domains appeared to be insufficient to improve material performance. According to the obtained results, the addition of heparin binding domains in high concentrations or a combination of heparin and integrin binding domains was most profitable for cells.

Conclusions

Biocompatible scaffolds composed of silk-collagen inspired copolymer were produced in a fermentation process of genetically engineered yeasts. Incorporation of bioactive domains in recombinant way was successful. The obtained fibrillar hydrogel scaffolds were strong enough at 2% protein concentration to perform a cell culture study. An in vitro study conducted with MG 63 cells cultured on top of the hydrogels showed that cells survived on the scaffolds and attached to the surface. The study proved that biological properties of artificial proteins can be nicely tuned by incorporation of specific bioactive domain. Well- designed recombinant protein polymers are promising materials for use in regeneration medicine to induce desired cellular response.

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EFFECT OF INTERNAL ARCHITECTURE ON MECHANICAL PROPERTIES OF POLYCAPROLACTONE SCAFFOLDS FOR TISSUE REGENERATION

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Abstract

The aim of the study was to investigate the influence of internal architecture of 3D printed scaffolds on their mechanical properties. The polycaprolactone scaffolds with six different internal architectures fabricated by rapid prototyping method were tested in this study. The scaffolds were plotted using a 330 μm dispensing needle, layer by layer with lay-down pattern of the fibers: 00/150/300; 00/300/600; 00/450/900, 00/600/1200, 00/750/1500 and 00/900/1800. Morphological analyses and mechanical properties examinations were performed. The obtained scaffolds had structures with high open porosity (50-60%) and interconnected pores ranging from 380 to 400 μm. The different lay-down pattern and the angle deposition of successive fiber layers resulted in different internal architecture and pore shape of the constructs, what was confirmed by scanning electron microscopy and microtomography analyzes. The geometries 00/900/1800 and 00/600/1200 were characterized with the most regular shape of pores between all analyzed architectures. The pores for 00/150/300 and 00/300/600 were not regular and arranged as a ladder-like helicoid structures. The lay-down pattern of the fibers affected significantly the mechanical properties of the scaffolds. The Young's modulus (E) of the scaffolds was increasing with increase of the angle deposition between successive layers. The scaffolds were also subjected to cyclic loading and again geometry and mechanical properties were under investigation. For all type of scaffolds the differences of mechanical properties after dynamic compression have been noticed. The geometries 00/900/1800 and 00/600/1200 exhibited the highest Young's Modulus after dynamic compression according to the rest of analyzed samples. According to the conducted research there is a clear correlation between internal architecture of polymeric scaffolds and their mechanical properties.

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Introduction

The number of critical bone defects caused by injury, cancer or aging of the world population is increasing. Techniques currently used to repair these defects suffer from several disadvantages, such as a lack of mechanical and biological matching of bone characteristics, the requirement of second surgery and the risk of pathogen transmission. Scaffolds made of bioresorbable polymers are a promising alternative as they temporarily support regeneration of the damaged site and undergo complete degradation after new tissue is formed. Fabrication of bioresorbable polymers scaffolds for tissue engineering becomes a popular research topic in present days. Biodegradable and biocompatible scaffolds are required for 3D implants as a temporary support for cell growth and cell adhesion. There are several fabrication methods currently used for creating 3D porous structures with high porosity and interconnected pores. A rapid prototyping (RP) is one of the most interesting one. It allows for fabrication scaffolds with predesigned external geometry and internal architecture as well as required mechanical properties. For the cell culture survival on the scaffolds 3D constructs needs characterize with interconnecting porous to allow the culture media 3D flow in order to ensure continuous supply of nutrients and metabolites. Tissue formation is generated on porous scaffolds. Mechanical strength of the human body implant is directly connected with internal architecture of the scaffold and has to be tailored according to the different implant application. The goal of the present study was to determine the changes of the mechanical properties of fibrous PCL scaffolds with differnet internal architecture. Scaffolds with different lay-down pattern were investigated to select the optimal fiber lay-down orientation for bone tissue engineering.

Materials & methods

Cylindrical porous scaffolds (height: 4mm, diameter: 6mm) with three-dimensional orthogonal periodic porous architectures, were manufactured by Bioscaffolder® machine (SYSENG, Germany) from ε- polycaprolactone granulate (Sigma Aldrich PCL, average Mn ca. 70-90kDa), (FIG.1). The melted polymer was plotted with a 330μm dispensing needle layer by layer, with lay-down pattern of the fibers: 00/150/300; 00/300/600; 00/450/900, 00/600/1200, 00/750/1500 and 00/900/1800. The temperature of the fabrication process was between 900 and 1000C. After samples fabrication the internal architecture were investigated by