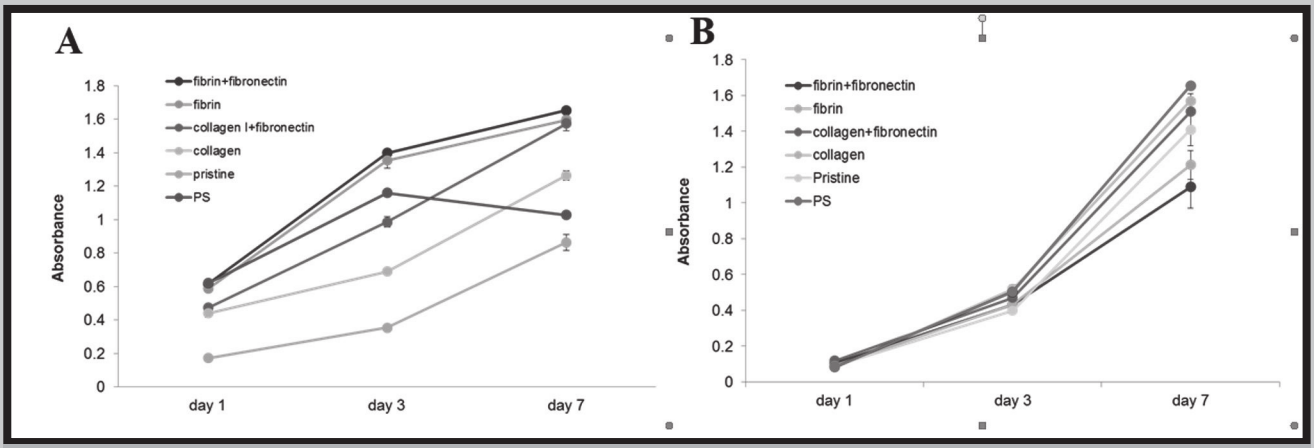

FIG. 3. Mitochondrial activity of human dermal fibroblasts (A) and human HaCaT keratinocytes (B) determined by MTS assay on day 1, 3 and 7 after cell seeding on pristine PLA membranes, PLA membranes with fibrin and fibronectin, fibrin, collagen and fibronectin or collagen. Standard cell culture polystyrene dish (PS) served as a reference material. Arithmetic means ± S.E.M from 9 measurements made on three independent samples for each experimental group and time interval.

Results

Results indicate that PLA nanofibrous membrane promoted adhesion and growth of the skin cells. Fibrin (FIG.1) and collagen structures on PLA membranes further improved adhesion, proliferation and metabolic mitochondrial activity of the skin cells. The human dermal fibroblasts preferentially adhered and were more spread on the membranes coated-with fibrin, fibrin with attached fibronectin on its surface or collagen I with fibronectin than on the membranes coated only with collagen or on the membranes in pristine form (FIG.2A). Moreover, the metabolic activity of human dermal fibroblasts was the highest on the membranes coated with fibrin or fibrin with fibronectin (FIG.3A). In addition, fibrin structures on PLA membranes stimulate fibroblasts to produce collagen I. The membranes coated with collagen I or collagen I with fibronectin promoted spreading of the HaCaT keratinocytes and increased the cell metabolic activity in comparison with pristine membranes or membranes coated with fibrin or fibrin with fibronectin (FIG.2B, 3B). Viability (determined by a Live/Dead assay) of the fibroblasts and the keratinocytes on the membranes was almost 100% on all samples.

Acknowledgements

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BLACK ORLON AS PROMISING MATERIAL FOR BONE TISSUE ENEGINEERING

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Introduction

Black Orlon is a promising material for applications in tissue engineering and regenerative medicine. Chemically, it is a carbonized polyacrylonitrile (PAN) containing fibrous ladder structure with chemical functional groups containing oxygen (e.g., hydroxyl, carboxyl), created by heating of Orlon above 300° C in the air atmosphere [1]. Although the biomedical applications of this materials started relatively early (in the seventieth), its potential for these applications has not yet been fully explored. In 1976, black Orlon was tested for construction of blood-contacting anticoagulant surfaces in the form of atrial patches implanted into hearts of experimental dogs [2].

Black Orlon can be relatively easily processed into three-dimensional (3D) scaffolds with microporous structure [3], and also nanofibrous structure created by electrospinning [4]. Microporous 3D scaffolds of carbonized PAN showed excellent osteoinductivity, i.e., they promoted osteogenic differentiation of human bone marrow mesenchymal stem cells without addition of other osteogenic factors [3]. The nanofibrous structure is also of a great importance for bone tissue engineering. It has been reported that nanos-

tructured materials adsorb preferentially vitronectin, which is then recognized by osteoblasts through the KRSR amino acid sequence in the vitronectin molecule (for a review, see [5]). Other properties, which make the black Orlon attractive for bone tissue engineering, are its mechanical resistance and electrical conductivity [4].

Thus, in the present study we investigated the osteoconductivity of microporous 3D black Orlon scaffolds in terms of the adhesion and subsequent growth of human osteoblast-like MG 63 cells in cultures on these materials.

Material and methods

Two types of black Orlon scaffolds were prepared for cytocompatibility tests in vitro, i.e. containing 5 wt.% or 10 wt.% of PAN in succinonitrile, which acted as a porogen. Both mixtures were heated to 35°C, and after stiffening of the material, the succinonitrile was washed out in methanol. The resulting PAN scaffolds with different porosities were further heated to 300 °C, which resulted in the material carbonization, oxidation and establishment of its electrical conductivity.

The materials were cut into samples of 10·10·2 mm in size, sterilized with 70% ethanol for 1 hour, inserted into 24-well plates (TPP, Switzerland; well diameter 1.5 cm) and seeded with human osteoblast-like MG 63 cells (30 000 cells/well, i.e. 17 000 cells/cm^2). Each well contained 1.5 ml of a medium DMEM with 10% of fetal bovine serum and 40 µg/ml of gentamicin. On days 1, 3 and 7 after seeding, the cell number and morphology were evaluated. For each experimental group and time interval, four samples were used. For evaluating the cell number, the cells were trypsinized and counted in Bürker hemocytometer. For evaluating the cell morphology, i.e. the cell shape and the size of cell spreading area, the cells were fixed with 70% ethanol (-20°C, 10 min) and stained with a combination of fluorescence dyes Texas Red C2-maleimide, which stains the cell membrane and cytoplasm, and Hoechst #33342, which stains the cell nuclei. The number and morphology of cells on the sample surface were then evaluated on pictures taken under a Leica TCS SPE DH 2500 confocal microscope. As reference materials, standard tissue culture polystyrene dishes were used.

Results and discussion

Generally, the numbers of cells on the scaffolds prepared from the mixture with 10 wt.% of PAN were significantly higher than on samples with 5 wt.% of PAN. Also the cell spreading areas on 1 day after seeding were significantly larger on the samples with 10 wt.% PAN, and the shape of these cells was mostly polygonal, while the shape of the cells on samples with 5 wt.% of PAN was often rounded (FIG.1). These findings could be explained by a more homogeneous and relatively large size of the pores in the scaffolds based on 10 wt.% of PAN (FIG.2). Our earlier studies revealed that for successful ingrowth of MG 63 cells inside the scaffolds, the pore diameter should be more than 100 µm, optimally 400-600 µm. Smaller pores were usually spanned by cells on the scaffold surface, which led to a lower cell population densities in the scaffolds [6,7].

Nevertheless, in the following days, the cell spreading on samples with 5% of PAN improved, and the cells on both types of scaffolds materials grew continuously without visible cell damage (FIG.2). The cell growth was probably supported by the presence of oxygen-containing groups on the material surface, which promote the adsorption of cell adhesion-mediating proteins in favorable geometrical con-

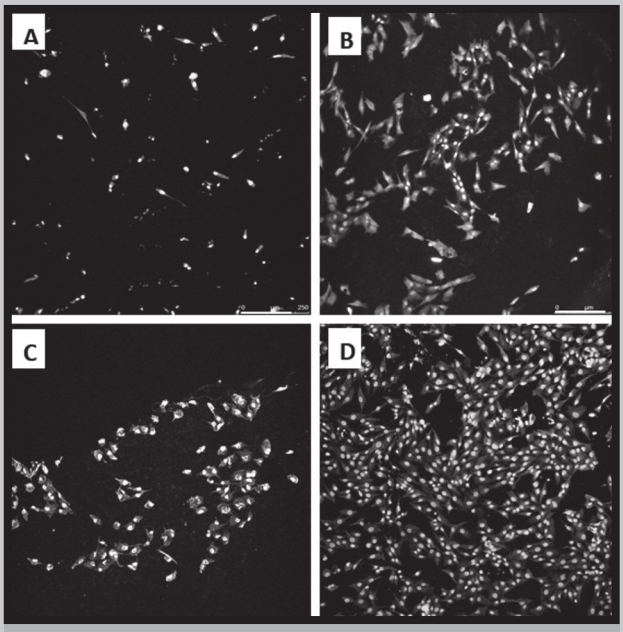

FIG. 1. Morphology of human osteoblast-like MG 63 cells on day 1 (A, B) and on day 3 (C, D) after seeding on black Orlon scaffolds prepared from a mixture containing 5 wt.% of PAN (A, C) or 10 wt.% of PAN (B, D) in succinonitrile. Cells stained with Texas Red C2-maleimide and Hoechst #33342. Leica TCS SPE DH 2500 confocal microscope, obj. 10.0x0.30, bar = 250 µm.

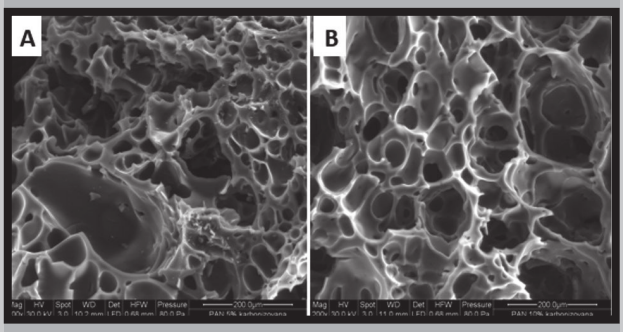

FIG. 2. Morphology of black orlon scaffolds prepared from a mixture containing 5 wt.% of PAN (a) or 10 wt.% of PAN (b) in succinonitrile. Sem microscope quanta 200 feg (fei), bar = 250 µm.

formation. The specific sites in these molecules, e.g. amino acid sequences such as RGD, are better accessible for cell adhesion receptors (for a review, see [5]). Also the material carbonization, i.e. a relative increase of carbon content in the material, and its electrical conductivity can contribute to the enhanced cell adhesion and growth. Similar results were obtained in vascular smooth muscle and endothelial cells grown on polymers modified with ion implantation, also resulting in the polymer oxidation and carbonization [8, 9], or on polymers doped with carbon black [10

Conclusion

It can be concluded that porous scaffolds made of black Orlon provided good support for the adhesion and growth of human bone-derived cells, particularly if they are prepared from matrix with a higher content of PAN, and thus this material is promising for construction of scaffolds for bone tissue 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. 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.

Acknowledgements

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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.

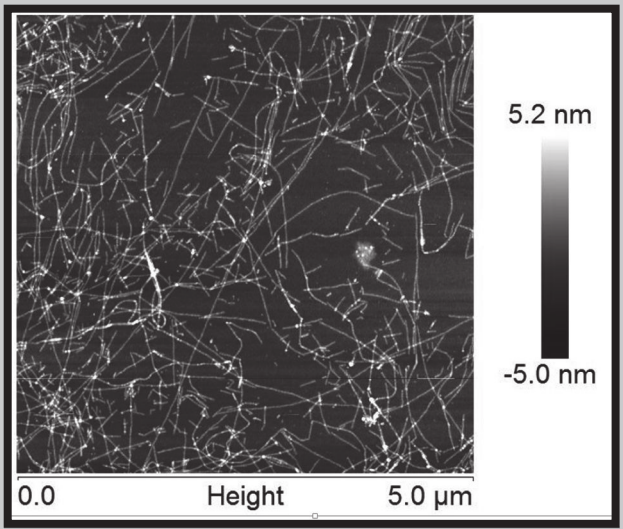

FIG. 1. AFM picture of 1% CSC solution at pH 7.4.