The adhesion and growth of cells on the scaffolds can be improved by further modifications, e.g. plasma treatment or coating the scaffold fibers with biomolecules that are normally present in the natural skin (collagen, hyaluronic acid), or that occur during wound healing (fibrin). Modification by plasma leads to changes in the physical and chemical properties of the material surface (i.e., surface wettability, morphology, electric conductivity, roughness, morphology, mechanical properties) [3].

In our experiment we evaluated the interaction of human HaCaT keratinocytes with PLA nanofibrous meshes that were modified by plasma irradiation or by coating with collagen, fibrin and hyaluronan of low (70-120 KDa) or high (1000-1250 KDa) molecular weight. For plasma irradiation, PLA nanofibers were exposed to O_2, CH_4 or Ar plasma for different times, with various ranges of power. For more detailed studies, O_2 plasma was chosen, because this type of plasma best supported the adhesion and growth of cells. PLA nanofibrous meshes were prepared with different densities of the fibres (5 g/m^2, 9 g/m^2, 16 g/m^2, 30 g/m^2). The potential damage to the fibres after plasma modification was observed using scanning electron microscopy (SEM). The cell adhesion, growth and metabolic activity were evaluated by the number of cells, their morphology, the amount of cellular DNA (PicoGreen ds DNA assay kit, Invitrogen®) and the XTT test (Roche) on days 1, 3 and 7 after seeding.

The results indicated that polylactide nanofibrous scaffolds promote adhesion and growth of HaCaT keratinocytes. Modification in plasma further improved the proliferation of cells on PLA nanofibers. The cells proliferated better on PLA meshes with lower densities of the fibers (5 g/m^2, 9g/m^2). SEM showed that damage to the fibers increased with the length of the period of plasma treatment. The collagen deposited on the fibers changed the morphology of the cells. The cells on the control unmodified fibers adhered in clusters, but on the collagen-coated fibers they were spread homogeneously. We can conclude that polylactide nanofibrous membranes are a promising material for the construction of temporary carriers for skin cells, particularly after they have been physically or biologically modified.

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APPLICATION OF CELLULOSE-BASED BIOMATERIALS IN VASCULAR TISSUE ENGINEERING – A REVIEW AND OUR EXPERIENCE

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Abstract

Artificial vascular replacements used in current clinical practice are fabricated from polyethylene terephthalate (PET, e.g. Dacron) or polyterafluoroethylene (PTFE, e.g. Teflon). Older materials used earlier for constructing vascular prostheses are polyamide (Nylon), polyvinyl alcohol (Ivalon) and polyacrylonitrile (Orlon). New promising materials include polyurethane and a wide range of biodegradable synthetic or nature-derived polymers, which are usually designed as temporary scaffolds for vascular cells forming a new regenerated blood vessel wall (for a review, see [1]). One of the nature-derived polymers is cellulose and its derivatives and composites with other materials.

Cellulose is the most abundant biopolymer on Earth. It is a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units [2,3]. Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. In plant cells, cellulose microfibrils are synthesized at the plasma membrane by hexameric protein complexes, also known as cellulose synthase complexes [4]. Some species of bacteria secrete cellulose to form biofilms.

For industrial use, cellulose is mainly obtained from wood pulp and cotton. For tissue engineering applications, bacterial cellulose has been predominantly used, mainly that synthesized by Acetobacter xylinum. Bacterial cellulose is identical to plant cellulose in chemical structure, but it can be produced without contaminant molecules, such as lignin and hemicelluloses, and does not require intensive purification processes. In addition, it is remarkable for its mechanical strength, its ability to be engineered structurally and chemically at nano-, micro-, and macroscales, its biocompatibility and chemical and morphologic controllability [5]. Bacterial cellulose has been used for experimental engineering of bone tissue [6], cartilage [7], skin [8], heart valve [9], and also for urinary reconstruction and diversion [10].

One of the first attempts at vascular tissue engineering was made with cellulose fibers, which were used for constructing three-dimensional vascularized tissue in vitro. These fibers were immobilized with fibronectin in order to improve cell adhesion, and were seeded with bovine coronary artery smooth muscle cells.

These cells proliferated on the scaffolds and, after they formed multilayers on the fibers, the fibers were removed by enzymatic digestion using cellulase. The remaining smooth muscle cell aggregates maintained lumens after this procedure, and thus mimicked newly-formed blood vessels [11]. Similarly, three-dimensional nanofibrous scaffolds with micropores made of bacterial cellulose allowed attachment and proliferation of human saphenous vein smooth muscle cells on the surface and also in the inside of the scaffolds [12]. In addition, the mechanical properties of nanofibrous bacterial cellulose scaffolds, evaluated by the shape of the stress-strain response, were reminiscent of the properties of the carotid artery, most probably due to the similarity in architecture of the nanofibril network [13].

The adhesion and growth of vascular endothelial cells was also supported by cellulose-based scaffolds, namely by nanofibrous bacterial cellulose or cellulose acetate scaffolds, especially if these scaffolds were functionalized with RGD-containing oligopeptides, i.e. ligands for integrin adhesion receptors on cells [14, 15], or if they were combined with chitosan [16]. The angiogenic response to bacterial cellulose was also observed under in vivo conditions, i.e. after implantation of these scaffolds in the form of dorsal skinfold chambers into Syrian golden hamsters [17].

Cellulose has also been used for creating tubular structures designed for replacing small-caliber vessels. Hollow-shaped segments of bacterial cellulose were created with a length of 10 mm, an inner diameter of 3.0-3.7 mm and a wall thickness of 0.6 - 1.0 mm. These grafts were used to replace the carotid arteries of eight pigs. After a follow-up period of 3 months, seven grafts (87.5%) remained patent, whereas one graft was found to be occluded. All patent grafts developed a single inner layer of endothelium with a basement membrane and a thin layer of collagen, followed by a concentric medial layer containing smooth muscle cells and cellulose, and an outer layer of fibrous cells [18].

Similarly, bacterial cellulose grafts 4 cm in length and 4 mm in internal diameter were implanted bilaterally in the carotid arteries of eight sheep. Although 50% of the grafts occluded within 2 weeks, all patent grafts developed a confluent inner layer of endothelial-like cells [19]. In addition, the mechanical properties of tubular structures created from bacterial cellulose seemed to be advantageous for vascular tissue engineering. For example, these structures exhibited a compliance response similar to that of human saphenous vein [20].

In our experiments, we have concentrated on cellulose-based materials modified with oxidation and/or functionalization with biomolecules. We have prepared fibrous scaffolds made of non-oxidized viscose, dialdehyde cellulose and 6-carboxycellulose with 2.1 wt.% or 6.6 wt.% of –COOH groups. In addition, all these material types were functionalized with arginine, i.e. an amino acid with a basic side chain, or with chitosan, in order to balance (compensate) the relatively acid character of oxidized cellulose molecules. Two groups of samples with and without functionalization were then seeded with vascular smooth muscle cells (VSMC) derived from the rat thoracic aorta by an explantation method [21].

We found that the oxidized cellulose with 2.1 wt.% of –COOH groups was the most appropriate of all the tested materials for colonization with VSMC. The cells on this material achieved an elongated shape, while they were spherical in shape on the other materials. In addition, the numbers of cells obtained in one week after seeding and the concentration of alpha-actin and SM1 and SM2 myosins, measured per mg of protein, were significantly higher on oxidized cellulose with 2.1 wt.% of –COOH groups. Functionalization with arginine and chitosan improved the cell adhesion, but usually only slightly. The most apparent increase in cell number after functionalization was observed on oxidized cellulose with 2.1 wt.% of –COOH groups functionalized with chitosan, and on viscose functionalized with chitosan or arginin. However, the cells on all samples proliferated slowly and with a non-significant increase in cell population densities from day 1 to 7 after seeding. This suggests that cellulose-based materials can be used in applications where high proliferation activity of vascular smooth muscle cells is not desirable. They can therefore be used on vascular prostheses, where excessive VSMC proliferation can lead to the restenosis of the graft. Alternatively, cell proliferation might be enhanced by some other more efficient modification. This would require further research.

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POTENTIAL ACTIVATION OF THE IMMUNE SYSTEM ON METALLIC MATERIALS FOR BONE IMPLANTS

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Titanium and stainless steel are metallic materials that have been in use for a long time in orthopedics, traumatology and stomatology. These metals are strong, corrosion-resistant and biocompatible. However, metallic materials have some disadvantages in comparison with the natural bone, particularly their relatively high specific weight and toughness. For example, the Young's modulus of AISI 316L stainless steel, Co-Cr alloys and Ti-6Al-4V alloy, i.e. materials frequently used for implantation into bone, ranges between 110-220 GPa, while the Young's modulus of bone tissue is 10-40 GPa [1]. In addition, these metals can release cytotoxic, allergenic and immunogenic ions, which can affect their biocompatibility [2, 3]. Implantation is a special type of transplantation process, in which the implant is inserted into the body, usually in order to replace an irreversibly damaged tissue. However, the immune system recognizes the implant as a foreign substance and attacks it with its effector mechanisms. Just as it can reject other types of transplants, the immune system can reject an artificial implant. To prevent rejection of an implant, it is important to study the potential activation of the immune system.

This study has investigated the biocompatibility of samples made of pure titanium (according to quality standard ISO 5832-2) and corrosion-resistant steel (quality standards ISO 5832-1 and AISI 316L), obtained from Beznoska Ltd. (Kladno, Czech Republic), and the potential activation of the immune system by these materials. In addition to Fe, the steel samples contained C (max. 0.025 wt.%), Si (0.6 wt.%), Mn (1.7 wt.%), P (max. 0.025 wt.%), S (max. 0.003 wt.%), Cr (17.5 wt.%), Ni (13.5 wt.%), Mo (2.8 wt.%), and Cu (max. 0.1 wt.%). The materials were used in the form of square samples (9x9 mm or 30x30 mm, thickness 1 mm). Both the Ti samples and the steel samples were ground with SiO2. The surface of the steel samples was then treated by polishing with Al_2O_3 paste (grain size up to 1 um), while the surface of the Ti samples, i.e. a material not suitable for polishing, was finished by brushing using another type of Al_2O_3 paste with slightly larger grains. Thus, the surface of the steel samples was finally smoother and glossy, while the Ti surface was rougher and matte.

For the in vitro biocompatibility tests, human osteoblast-like MG 63 cells (European Collection of Cell Cultures, Salisbury, UK) were used. The smaller samples (9x9 mm) were inserted into polystyrene 24-well cell culture plates (TPP, Trasadingen, Switzerland; well diameter 1.5 cm). Each well contained 25 000 cells (approx. 14 150 cells/cm^2) and 1.5 ml of Dulbecco's Modified Eagle Minimum Essential Medium (DMEM; Sigma, USA, Cat. No. 10270-106) supplemented with 10% foetal bovine serum (FBS; Gibco, Cat. No. 10270-106) and gentamicin (40 µg/ml, LEK, Slovenia). These samples were used for evaluating the size of the cell spreading area (day 1), and for evaluating cell shape and cell viability (days 1, 4 and 7 after seeding). The size of the cell spreading area was measured using Atlas Software (Tescan Ltd., Brno, Czech Republic). The viability of the cells was determined by the LIVE/DEAD viability/cytotoxicity kit for mammalian cells (Invitrogen, Molecular Probes, USA).

The larger samples (30x30 mm) were inserted into GAMA polystyrene dishes (diameter 5 cm; GAMA Group Joint-Stock Company, Ceske Budejovice, Czech Republic) and seeded with 300 000 cells/dish (approx. 15 300 cells/cm^2) suspended in 9 ml of the above mentioned culture medium. These samples were used for evaluating the cell number on days 1, 4 and 7 after seeding, using a Beckman Vi-CELL XR Cell Analyser automatic cell counter.

For the in vitro analysis of markers of osteogenic differentiation and cell immune activation, human osteoblast-like MG 63 cells (European Collection of Cell Cultures, Salisbury, UK) were used. The samples (9x9 mm) were inserted into polystyrene 24-well cell culture plates (TPP, Trasadingen, Switzerland; well diameter 1.5 cm). Each well contained 25 000 cells (approx. 14 150 cells/cm^2) and 1.5 ml of Dulbecco's Modified Eagle Minimum Essential Medium (DMEM; Sigma, USA, Cat. No. 10270-106) supplemented with 10% foetal bovine serum (FBS; Gibco, Cat. No. 10270-106) and gentamicin (40 µg/ml, LEK, Slovenia). The cells were cultured for 1, 4, or 7 days at 37°C in a humidified atmosphere of 5% of CO_2 in the air. On day 4 after seeding, the medium was changed; one half of the samples contained standard medium DMEM with 10% foetal bovine serum and gentamicin (40 µg/ml) mentioned above, and the second half contained osteogenic medium, i.e. the standard medium further supplemented with β-glycerophosphate, L-glutamin, ascorbic acid, dihydroxyvitamin D3, dexamethason, 10% foetal bovine serum and gentamicin (40 µg/ml).

Using an Enzyme-Linked ImmunoSorbent Assay (ELISA), we measured the concentration of the Intercellular Adhesion Molecule-1 (ICAM-1, a marker of cell immune activation) and osteocalcin (a marker of osteogenic cell differentiation). These measurements were performed in homogenates of cells on days 4 and 7 after seeding, and the concentration of both