THE INFLUENCE OF TEMPERATURE AND PORE SIZE ON CELL GROWTH AND PROLIFERATION ON HYDROXYAPATITE SCAFFOLDS

Dalibor Soukup[1], Marketa Bacakova[1], Willi Pabst[2], Eva Gregorova[2], Lucie Bacakova[1]

[1]Department of Biomaterials and Tissue Engineering,
Institute of Physiology, Academy of Sciences of the Czech Republic,
Videnska 1083, 142 20 Prague 4-Krc, Czech Republic
[2]Department of Glass and Ceramics,
Institute of Chemical Technology in Prague,
Technicka 5, 166 28 Prague 6-Dejvice, Czech Republic

Abstract

Porous biomaterials, especially synthetic porous ceramics, are of significant importance in bone tissue engineering, and there has been rapid growth in the medical use of these biomaterials over the last 50 years. The reason is that they are relatively easy to prepare and are available in unlimited supply, unlike the allografts and autografts that are used in clinical practice. Various hydroxyapatite (HAp) scaffolds can be prepared, using various pore-forming techniques and firing temperatures. The firing temperature significantly affects microstructural parameters such as total porosity, pore size, the interconnected pore network, and also the chemical and phase composition. Last but not least, it also affects the mechanical properties of the samples. Knowledge about these factors is therefore essential for designing a sample with the desired controlled microstructure and properties.

In this work, uniaxial pressing has been used for preparing HAp disks from nanocrystalline HAp powder, using saccharose as a pore-forming agent. The highest porosity achieved (after partial sintering at 800°C) was in the range of 64.7-70.6%. The firing temperature significantly affects porosity, pore size, grain size and mechanical strength, whereas the dwell time has only a minor effect on these parameters. After firing, XRD confirmed more than 98.4% HAp in all cases. Mercury porosimetry confirmed the presence of nanosized interstitial voids for partially sintered materials and pore throat sizes of approximately 100μm (much smaller than the pore cavities), which is adequate for bone cell penetration and further ingrowth. After firing at 1200°C, the matrix is more or less fully sintered, and nanosized pores are absent or closed.

The biological part of the paper summarizes the results from cell-seeding and cultivation experiments to determine the cell adhesion, proliferation, viability, mitochondrial activity and osteogenic cell differentiation on the scaffolds, and thus the biocompatibility and bioactivity of the scaffolds. The highest values for all these parameters, particularly the number of cells, were on HAp fired at 1200°C. The samples fired at 1200°C were prepared with various pore sizes (in the range of 100 – 800μm). We found that pore size has a non-significant effect on cell colonization, whereas the firing temperature has a major influence. All tested HAp samples showed a remarkable ability to adsorb proteins on their surfaces, namely albumin and fibronectin, and to promote cell adhesion. Some cytotoxic activity was observed on the samples fired at 800 and 1000°C. Possible reasons for this cytotoxicity have been discussed. However, it can be concluded that the HAp samples created in this study and fired at 1200°C hold great promise for bone tissue engineering.

[Engineering of Biomaterials, 116-117, (2012), 127

Acknowledgements

Supported by the Grant Agency of the Czech Republic (grant No. 106/09/1000) and the Ministry of Industry and Trade of the Czech Republic (grant No. FR-TI3/088).

POLYLACTIDE NANOFIBERS IN SKIN TISSUE ENGINEERING

Marketa Bacakova[1], Marian Varga[2], Tomas Riedel[3], Denisa Stranska[4], Lucie Bacakova[5]

[1]Institute of Physiology,
Academy of Sciences of the Czech Republic,
Videnska 1083, 142 20 Prague 4 – Krc,
[2]Institute of Physics,
Academy of Sciences of the Czech Republic,
Cukrovarnicka 10, CZ-16253 Prague 6, Czech Republic
[c]Institute of Macromolecular Chemistry,
Academy of Sciences of the Czech Republic,
Heyrovsky Sq. 2, 16206 Prague 6, Czech Republic
[4]Elmarco Ltd., V Horkach 76/18, 460 07 Liberec, Czech Republic
MAILTO: marketa.bacakova@biomed.cas.cz

Abstract

Various artificial or natural biomaterials can be used for constructing a scaffold suitable for treating skin injuries. Artificial skin replacements are made from polyhema, polybutylene terephthalate, nylon, polylactic acid and polyglycolic acid or their copolymers. The most widely applied natural biomaterials are collagen, chitin, hyaluronic acid and chondroitin sulfate [1]. In recent tissue engineering, nanofibrous scaffolds have been very attractive because they better simulate the architecture of natural extracellular matrix. In skin tissue engineering, nanofibrous membranes can be used for constructing a bilayer of fibroblasts and keratinocytes [2]. These membranes will separate the two cell types, ensuring their physical and humoral communication; thus the layer of fibroblasts will serve as a feeder for keratinocytes. For our study, we chose nanofibers made of polylactide (PLA), prepared in external collaboration with Elmarco Ltd. (Liberec, Czech Republic). The main advantage of PLA is its biodegradability; it is slowly resorbed in the organism, and is finally replaced by regenerate tissue.

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.

[Engineering of Biomaterials, 116-117, (2012), 127-128]

Acknowledgements

Supported by the Grant Agency of the Czech Republic (grants No. P108/10/1106, P108/12/1168 and P108/12/G108). Mr. Robin Healey (Czech Technical University, Prague, Czech Republic) is gratefully acknowledged for his language revision of the abstract.

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

Lucie Bacakova[1*], Katarina Novotna[1], Martin Parizek[1], Pavel Havelka[2], Tomas Sopuch[3*]

[1]Dept. of Biomaterials and Tissue Engineering, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague 4 – Krc, Czech Republic;
[2]VUOS a.s., Rybitvi 296, 533 54 Rybitvi, Czech Republic
[3]SBU Nitrocelluloza, Synthesia a.s., Pardubice-Semtin, Czech Republic
*MAILTO: lucy@biomed.cas.cz

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.