Role of Electrospun Nanofibers in Stem Cell Technologies and Tissue Engineering

Ulrich Boudriot,[1] *Bernhard Goetz*,[1] *Roland Dersch*,[2] *Andreas Greiner*,[2] *Joachim-H. Wendorff**[2]

[1]Department of Orthopaedic Surgery, Philipps-University, D-35033 Marburg, Germany
E-mail: boudriot@post.med.uni-marburg.de
[2]Institute of Nuclear Physical and Polymer Chemistry, Philipps-University, D-35032 Marburg, Germany

Summary: Tissue engineering is a promising tool to manage structural and functional defects in bone and cartilage. To provide optimal conditions for three-dimensional cell growth the use of a scaffold is necessary. The aim of the study was to prove osteogenic differentiation of human mesenchymal stem cells (hMSC) onto a three-dimensional, nanostructured scaffold of electrospun poly (l-lactide)-nanofibers. HMSC were seeded onto this matrix and differentiated for 21 days. Cells clearly preferred a guided growth along the nanofibers and revealed no signs of cell-death. Osteogenic differentiation of hMSC onto the matrix has been demonstrated. Due to the fine structure, electrospun nanofibers promises to be an ideal scaffold for tissue engineering. They can be enriched with additives and are equally biocompatible and biodegradable.

Keywords: biomaterials; mesenchymal stem cells; nanofibers; nanotechnology; tissue engineering

Introduction

The aim to replace damaged parts of the body with new, fully functional tissues is becoming increasingly realistic. The emerging scientific field of tissue engineering has to be divided into two major categories: on the one hand in-vitro construction of bioartificial tissues, which are composed of allogenic or homogenic cells, growing on a synthetic or biologic scaffold, and, on the other hand, tissue engineering in vivo.

One fundamental component of any bioengineered tissue is the cell. For various types of tissues different cell-lineages are indispensable. The existence of undifferentiated, so-called precursor cells in bone marrow is well known [15]. Their inherent potential to differentiate into various lineages qualifies them for tissue engineering purposes. It has been demonstrated that the fate of stem cells is rather determined by extrinsic factors, such as growth factors [5], matrix types [1] and mechanical loading [3]. Various growth factors have been described but their complex interactions are not yet fully understood.

 DOI: 10.1002/masy.200550702

The majority of mammalian cells are anchorage dependent. Most of them are derived from mesenchymal stem cells (MSC). In order to proliferate and differentiate MSC depend on a matrix. The adhesion of cells is mediated by extracellular matrix proteins, such as fibronectin, vitronectin [17], collagen and various glucosaminoglycans. The adsorption of these proteins seems to be one of the key factors influencing cell attachment and growth on sythetic materials.

A fundamental question is whether to use synthetic or natural materials is furthermore, they can be easily processed into various structures. The advantage of synthetic materials is that they are cheap and reproducible manufacturing. It is possible to control various properties such as mechanical strength, hydrophobicity and degradation rate of the material whereas natural materials sometimes demonstrate a limited range of physical properties. Another problem is unfavourable host tissue response.

Scaffolds have to provide sufficient mechanical support to create space for cell growth in order to allow free transport of growth factors and nutritional components. The matrix can be designed to provide these functions for a defined period of time before biodegradation occurs. However, for certain purposes, non-degradable scaffolds are needed. In order to reconstruct complex bio artificial tissues, it is necessary to have a matrix allowing simultaneous proliferation and differentiation of MSC into different cell lineages. Other matrices used so far only supported the differentiation into one cell-lineage.

Electrospinning allows the formation of polymeric fibers in the micro- and nanometer range [7]. A polymer solution is pumped through a capillary tip under the influence of a electric field. A thin liquid jet emerges from the tip, is accelerated towards a counter electrode and arrives there as a solid polymer fiber due to solvent evaporation during this process[19]. During the spinning process of nanofibers it is possible to incorporate various additives, such as growth and differentiating factors, which can enable the parallel differentiation into multiple cell-lineages. Several biomedical applications of electrospun nanofibers have been described [8,10,11,20].

Materials and Methods

Human mesenchymal stem cells (hMSC) were obtained by density gradient centrifugation of human bone marrow derived from the femoral neck. Cells were propagated in

Dulbeccos´s Modified Eagle Medium (DMEM), low glucose, containing 10% mesenchymal stem cell supplement (MCGS, Cambrex®) and 50,000 I.E. penicillin/streptomycin.

Electrospun polylactide fibers were obtained from poly-l-lactide L 210 from Boehringer. A 3,5% solution in dichloromethane was spun at a flow rate of 0,016 mL/min, with an applied voltage of 20-30 kV and a distance of 15 cm. Samples of nonwoven poly-l-lactide nanofibers were attached to 19 mm glass slides and placed into 12-well cell culture plates. Sterilisation was achieved via UV-irradiation. This was performed over eight hours, under the clean bench. A quantity of 30,000 hMSC/cm^2 were seeded onto the scaffolds. At day four, the proliferation medium was changed into a differentiation medium. The latter consisted of DMEM, 10% MCGS, 50,000 I.E. penicillin/streptomycin, 10 mM β-glycerolphosphate, 0,05 mM ascorbic acid and 0,1 µM dexamethasone according to the protocol of Pittenger et al.[15]. Medium was replaced every second or third day. Samples were differentiated over three weeks and then fixated for van Kossas´s staining, immunofluorescence staining (osteocalcin) and scanning electron microscopy.

Results

Scanning electron micrographs which were obtained prior to cell seeding show a three-dimensional scaffold of non-woven, non-orientated nanofibers. Fiber diameters ranged from 400 to 4,000 nm (Fig. 1). Detection of cells by light microscopy during the culturing process is difficult as the dense mesh of nanofibers hides unstained cells (Fig. 2).

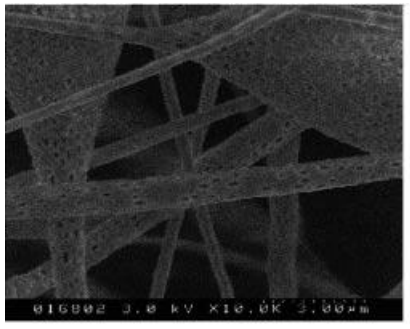

Fig. 1. Morphology of poly-l-lactide nanofibers prior to cell culture.

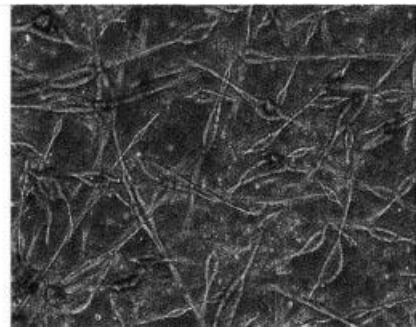

Fig. 2. hMSC, unstained, 200x.

Previous tests with human osteoblast-like cells (MG-63) demonstrated a clearly orientated cell growth along the fibers. Fluoresceindiacetate staining showed viable cells within the matrix. Van Kossa staining revealed calcified matrices as a sign of osteogenic differentiation (Fig. 3). Immunofluorescence staining proved the expression of osteocalcin, a further marker for osteogenic differentiation.

Scanning electron micrographs showed a dense network of cells penetrating the scaffolds (Fig. 4 and 5). Parts of the scaffolds were completely enclosed by spread cells and their extracellular matrix (Fig. 6). Cells appeared to attach to bundles of nanofibers using the preformed structures for guided growth.

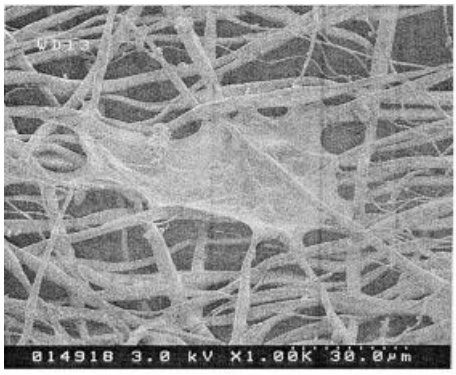

Fig. 3. hMSC morphology on nanofiber scaffold.

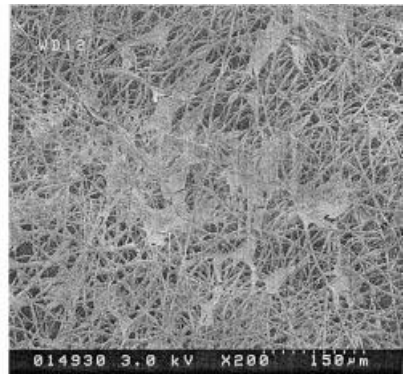

Fig. 4. Seeded hMSC.

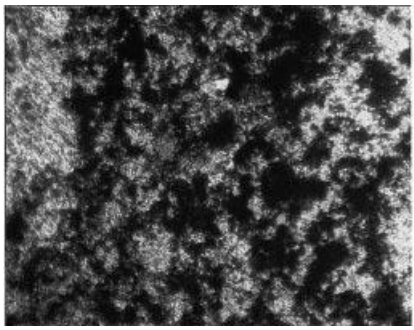

Fig. 5. Van Cossa-staining for calcified extracellular matrix, x160.

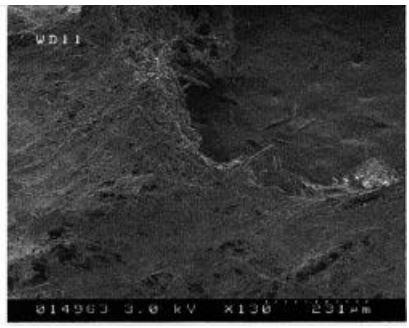

Fig. 6. Dense network of hMSC penetrating the poly-l-lactide scaffold after 21 days of osteogenic differentiation.

Discussion

The replacement of damaged tissues and organs is becoming an increasing influence in human medicine. The shortage of donor tissues as well immunological problems lead to the necessity of artificial biological replacement. In the past decade enormous progress has been made in the understanding of cell proliferation and differentiation processes. Emerging stem cell technologies provide the basis for ex-vivo reconstruction of artificial tissues. The prerequisite for three-dimensional cell growth is a matrix on which cells can attach and proliferate. Cell growth in conventional, two-dimensional cell culture systems is limited by contact inhibition.

The matrix influences cell growth and differentiation. Mechanical as well as physico-chemical properties of the matrix have crucial importance for cell binding processes, proliferation and differentiation. Thus, matrix modification is an indispensable tool for any engineered tissue. Each single characteristic of the material can modify the behaviour of growing cells. Kieswetter et al.[9] demonstrated that surface roughness of titanium implant materials affects proliferation, differentiation and extracellular matrix production of human osteoblast-like cells (MG-63). Surface roughness of scaffolds smaller than 10 µm positively influences the attachment of cells [1,2]. Different collagen matrices affect the morphology and cell activity of seeded chondrocytes [12]. Papadaki et al.[14] found differences in cell attachment, proliferation and differentiation of chondrocytes and

skeletal muscle cells on scaffolds of segmented block copolymers depending on the weight percentage and molecular weight of the components [poly(ethylene glycol) and poly (butylene terephthalate)].

The efficacy of supplementary substances for cell proliferation and differentiation has been demonstrated by many authors. Dalby et al.[4] varied the amount of hydroxyapatite in poly(methylmethacrylate) (PMMA) bone cement and demonstrated improved cell adhesion and increased expression of osteoblastic phenotype and extracellular bone matrix. The effects of various different additives, such as bone morphogenetic protein-2 [13], human growth hormone [5], transforming growth factor beta-1 [6], hepatocyte growth factor [21] and others has been analysed.

The fundamental mechanism influencing cell adhesion is mediated by integrins. Integrin function depends on divalent cations such as magnesium and calcium ions [18,22]. Another possibility by which to influence cell adhesion and differentiation is the adsorption of biomimetic proteins onto the matrix surface [16].

Manufacturing of electrospun biomaterials with defined characteristics is a major advantage. The modification of nanoscaled scaffolds in terms of the diameter as well as the pore density and the pore diameter presumably leads to a different "behaviour" of MSCs. The unique possibility to obtain electrospun nanofibers with incorporated growth factors or other biomimetic substances is a major advantage in the field of biomaterials.

Conclusions

Electrospun nanofibers represent a new class of biomaterials. The possibility to modify mechanical as well as physico-chemical properties of the matrix allows the design of optimized scaffolds for various tissue engineering applications. We demonstrated osteogenic differentiation of human mesenchymal stem cells onto a three-dimensional matrix of electrospun poly-l-lactid nanofibers.

Acknowledgements

We would like to thank ***Deutsche-Arthrose-Hilfe*** for financial support.

[1] K. Anselme, M. Bigerelle, B. Noel, A. Iost and P. Hardouin, Effect of grooved titanium substratum on human osteoblastic cell growth. *J.Biomed.Mater.Res.*, **2002**, 60, 529-540.

[2] M. Bigerelle, K. Anselme, B. Noel, I. Ruderman, P. Hardouin and A. Iost, Improvement in the morphology of Ti-based surfaces: a new process to increase in vitro human osteoblast response. *Biomaterials*, **2002,** 23, 1563-1577.

[3] L. E. Claes and C. A. Heigele, Magnitudes of local stress and strain along bony surfaces predict the course and type of fracture healing. *J.Biomech.*, **1999**, 32, 255-266.

[4] M. J. Dalby, L. Di Silvio, E. J. Harper and W. Bonfield, Increasing hydroxyapatite incorporation into poly(methylmethacrylate) cement increases osteoblast adhesion and response. *Biomaterials*, **2002**, 23, 569-576.

[5] J. Guicheux, O. Gauthier, E. Aguado, D. Heymann, P. Pilet, S. Couillaud, A. Faivre and G. Daculsi, Growth hormone-loaded macroporous calcium phosphate ceramic: in vitro biopharmaceutical characterization and preliminary in vivo study. *J.Biomed.Mater.Res.*, **1998**, 40, 560-566.

[6] Q. Huang, J. C. Goh, D. W. Hutmacher and E. H. Lee, In Vivo Mesenchymal Cell Recruitment by a Scaffold Loaded with Transforming Growth Factor beta1 and the Potential for in Situ Chondrogenesis. *Tissue Eng*, **2002**, 8, 469-482.

[7] Z. M. Huang, Y. Z. Zhang, M. Kotaki and S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites Science and Technology*, 63, **2003**, 2223-2253.

[8] M. S. Khil, D. I. Cha, H. Y. Kim, I. S. Kim and N. Bhattarai, Electrospun nanofibrous polyurethane membrane as wound dressing. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, **2003**, 67B, 675-679.

[9] K. Kieswetter, Z. Schwartz, T. W. Hummert, D. L. Cochran, J. Simpson, D. D. Dean and B. D. Boyan, Surface roughness modulates the local production of growth factors and cytokines by osteoblast-like MG-63 cells. *J.Biomed.Mater.Res.*, **1996**, 32, 55-63.

[10] W. J. Li, K. G. Danielson, P. G. Alexander and R. S. Tuan, Biological response of chondrocytes cultured in three-dimensional nanofibrous poly(epsilon-caprolactone) scaffolds. *J.Biomed.Mater.Res.*, **2003**, 67A, 1105-1114.

[11] W. J. Li, C. T. Laurencin, E. J. Caterson, R. S. Tuan and F. K. Ko, Electrospun nanofibrous structure: A novel scaffold for tissue engineering. *J.Biomed.Mater.Res.*, **2002,** 60, 613-621.

[12] S. Nehrer, H. A. Breinan, A. Ramappa, G. Young, S. Shortkroff, L. K. Louie, C. B. Sledge, I. V. Yannas and M. Spector, Matrix collagen type and pore size influence behaviour of seeded canine chondrocytes. *Biomaterials*, **1997**, 18, 769-776.

[13] T. Noshi, T. Yoshikawa, Y. Dohi, M. Ikeuchi, K. Horiuchi, K. Ichijima, M. Sugimura, K. Yonemasu and H. Ohgushi, Recombinant human bone morphogenetic protein-2 potentiates the in vivo osteogenic ability of marrow/hydroxyapatite composites. *Artif.Organs*, **2001**, 25, 201-208.

[14] M. Papadaki, T. Mahmood, P. Gupta, M. B. Claase, D. W. Grijpma, J. Riesle, C. A. van Blitterswijk and R. Langer, The different behaviors of skeletal muscle cells and chondrocytes on PEGT/PBT block copolymers are related to the surface properties of the substrate. *J.Biomed.Mater.Res.*, **2001**, 54, 47-58.

[15] M. F. Pittenger, A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig and D. R. Marshak, Multilineage potential of adult human mesenchymal stem cells. *Science*, **1999**, 284, 143-147.

[16] D. A. Puleo, R. A. Kissling and M. S. Sheu, A technique to immobilize bioactive proteins, including bone morphogenetic protein-4 (BMP-4), on titanium alloy. *Biomaterials*, **2002**, 23, 2079-2087.

[17] C. H. Thomas, C. D. McFarland, M. L. Jenkins, A. Rezania, J. G. Steele and K. E. Healy, The role of vitronectin in the attachment and spatial distribution of bone-derived cells on materials with patterned surface chemistry. *J.Biomed.Mater.Res.*, **1997**, 37, 81-93.

[18] T. J. Webster, L. S. Schadler, R. W. Siegel and R. Bizios, Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. *Tissue Eng*, **2001**, 7, 291-301.

[19] C. Y. Xu, R. Inai, M. Kotaki and S. Ramakrishna, Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. *Biomaterials*, **2004**, 25, 877-886.

[20] H. Yoshimoto, Y. M. Shin, H. Terai and J. P. Vacanti, A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. *Biomaterials*, **2003**, 24, 2077-2082.

[21] G. Zambonin, C. Camerino, G. Greco, V. Patella, B. Moretti and M. Grano, Hydroxyapatite coated with heaptocyte growth factor (HGF) stimulates human osteoblasts in vitro. *J.Bone Joint Surg.Br.*, **2000**, 82, 457-460.

[22] H. Zreiqat, C. R. Howlett, A. Zannettino, P. Evans, G. Schulze-Tanzil, C. Knabe and M. Shakibaei, Mechanisms of magnesium-stimulated adhesion of osteoblastic cells to commonly used orthopaedic implants. *J.Biomed.Mater.Res.*, **2002**, 62, 175-184.