J Mater Sci: Mater Med (2010) 21:1363–1369
DOI 10.1007/s10856-009-3947-2

# RGD-functionalisation of PLLA nanofibers by surface coupling using plasma treatment: influence on stem cell differentiation

**Jürgen Rudolf Josef Paletta · Sarah Bockelmann · Andreas Walz · Christina Theisen · Joachim Heinz Wendorff · Andreas Greiner · Susanne Fuchs-Winkelmann · Markus Dietmar Schofer**

Received: 4 August 2009 / Accepted: 16 November 2009 / Published online: 27 November 2009


**Abstract** The aim of this study was to functionalize the surface of synthetic poly-(l-lactic) (PLLA) nanofibers with RGD peptide, in order to promote growth and osteogenic differentiation of human mesenchymal stem cells (hMSC) in vitro. The cRGD was coupled onto PLLA nanofibers using oxygen plasma combined with EDC/sulfo-NHS activation. Matrices were seeded with hMSC and cultivated over a period of 22 days under growth conditions and analyzed during the course of cultivation. The plasma activation of PLLA nanofibers resulted in a reduction of hydrophobicity as well as a formation of carboxyl groups on the surface of the fibers. Furthermore, maximum load, but not young's modulus was influenced by the treatment with oxygen plasma. When hMSC were cultured onto the cRGD functionalized scaffolds, cells showed no increased proliferation or cell density but an induction of genes associated with the osteoblast lineage. In brief, this study indicates that functional peptides of the extracellular matrix can be coupled onto PLLA nanofibers using plasma treatment in combination with EDC/sulfo-NHS treatment. These groups are accessible for the growing cell and mediate probably some osteoinductive properties of collagen nanofibers.

J. R. J. Paletta (✉) · S. Bockelmann · C. Theisen · S. Fuchs-Winkelmann · M. D. Schofer
Department of Orthopedics, University of Marburg, 35043 Marburg, Baldingerstraße, Germany
e-mail: paletta@med.uni-marburg.de

A. Walz · J. H. Wendorff · A. Greiner
Department of Chemistry, University of Marburg, 35032 Marburg, Hans-Meerwein-Straße, Germany

## 1 Introduction

In many native tissues, collagen represents a principal structural element of the extra cellular matrix [1]. It can be isolated from a variety of sources, is highly conserved and relatively non-immunogenic. Therefore, it has been used in a variety of tissue engineering applications.

Collagen can be easily electrospun to a 3-D, non-woven network that supports the growth of keratinocytes [2], coronary artery smooth muscle cells [3], dermal fibroblast [4], endothelial cells [5] myoblasts [6] and as well the differentiation of mesenchymal stem cells [7, 8].

Collagen I interacts with the integrin receptor and its interaction is sufficient to induce osteogenic differentiation of human mesenchymal stem cells (hMSC), even in the absence of exogenous soluble stimuli [9, 10]. Since the biologic action of the collagen can be reduced to an RGD motive (R: arginine; G: glycine; D: aspartic acid) numerous materials have been RGD functionalized for medical applications [11–16]. With respect to bone healing, in vitro studies showed evidence that RGD covered surfaces resulted in an increase of both osteoblast cell number and differentiation [17–20]. Furthermore, the presence of signalling peptide allows a favourable micro environment for hMSC to differentiate into osteoblasts [21–23]. Here the direct incorporation of RGD peptides into PLLA nanofibers during electrospinning [24] mediated the osteogenic potential to some extent, although the incorporated peptides were distributed randomly within the nanofiber.

Hence, we designed this study in order to functionalize PLLA nanofibers with cyclic RGD (cRGD) peptides using

plasma activation and surface coupling with EDC/Sulfo-NHS.

## 2 Materials and methods

### 2.1 Construction of nanofibers and characterization

The preparation of PLLA nanofibers by electrospinning has already been reported in detail earlier [24].

In order to incorporate cyclic Arg-Gly-Asp (Cyclo (-RGDfK) AnaSpec, Inc. San Jose, USA) onto the surface, PLLA nanofibers were activated using plasma treatment. Nanofibers were electrospun onto cover slips and subjected to plasma treatment in a power generator (Edwards MPG 137 RF-power generator). The samples were clamped between both electrodes and the chamber was evacuated to 0.0133 mbar and filled with oxygen to a pressure of 0.133 mbar prior to the treatment. Plasma treatment was performed using a grid current of 6 mA, a plate current of 2 A, a forward power of 100 W and reflected power of 30 W over a period of 2 min. The pressure of the plasma chamber was kept at 0.133 mbar by controlling the working gas flow.

The plasma activated scaffolds (carboxyl groups) were incubated in an 0.1 M aqueous 2-(N-morpho)-ethanesulfonic acid solution (pH 5.3) for 30 mins at room temperature by shaking. After this washing step the 0.1 M aqueous 2-(N-morpho)-ethanesulfonic acid solution was removed and the activated nanofiber mashes were incubated with 8.69 mM EDC and 4.35 mM Sulfo-NHS in an 0.1 M aqueous 2-(N-morpho)-ethanesulfonic acid solution (pH 5.3) for 90 mins at room temperature by shaking to activate the carboxylic acid groups into creating a semi-stable amine-reactive sulfo-NHS-ester.

After the reaction the solution was removed and the chemically activated nanofiber meshes were incubated with 0.1 mg/ml cRGD solution (165.8 μM) in 0.1 M PBS for 6 h at room temperature to create cRGD-functionalized fibers by amide bond formation. After 6 h, the cRGD solution was removed and the weakly bound, physically absorbed cRGD was removed by washing with PBS. In some experiments cRGD was replaced by a fluorescein isothiocyanate (FITC)-labeled RGD, in order to analyze the distribution within the nanofibers.

### 2.2 Contact angles

Static contact angles of water were measured using the sessile drop method with a G10 drops shape analysis system (Krüss, Hamburg, Germany) and calculated using Data Physics SCA20 contact angle analyzer software.

### 2.3 Scanning electron microscopy

For scanning electron microscopy (SEM), samples were sputter-coated with platinum and examined in a SEM (JSM-7500F, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 2–5 kV in the SEI mode.

### 2.4 Specimen preparation and mechanical testing

Mechanical testing was performed using a uni-axial testing machine (zwicki-Line, Zwick GmbH & Co. KG, Ulm, Germany). Test specimens of 10 mm breadth and 50 mm gauge length were prepared from orientated fibres and stretched to failure at a cross head speed of 10 mm/min without any pre-tension or preconditioning. Load (N) and extension (mm) were recorded and tensile strength, strain, young's modulus were calculated using test expert software (Zwick GmbH & Co. KG, Ulm, Germany).

### 2.5 Human mesenchymal stem cell isolation, characterization and culture

Human mesenchymal stem cells were obtained from consenting patients with the approval of the institutional review board. The indication for surgery was primary osteoarthritis of the hip with increasing pain, decreased range of motion, and signs of progressive osteoarthritis in radiographs. The patients had no evidence of other bone or auto-immune diseases. The routinely removed bone was obtained from the proximal femur, while preparing the implant bed. Mesenchymal stem cells were isolated and cultured according to the preparation of Pittenger et al. [25], with minor modifications as described by Brendel et al. [26]. Further treatment was done as described by Schofer et al. [8].

### 2.6 Gene expression analysis

RNA extraction, cDNA synthesis and quantitative polymerase chain reaction (PCR) analysis were performed as described earlier [8]. Cycle temperatures and incubation times for human alkaline phosphatase (ALP), osteopontin (OP), collagen I (COLI), osteocalcin (OC), and 18 s rRNA were previously described [8, 27, 28]. Purity of the single PCR products was verified by melting point analysis and expression levels were calculated using ΔΔCT method [29].

### 2.7 Immunofluorescence microscopy

Immunefluorescence analysis was carried out as described earlier [8]. Slices were analyzed using a Leica DM5000.

Microphotographs of at least three different areas were made. The intensity and area of fluorescence was determined using Quips analysis software. The total cell count of DAPI stained nuclei was obtained. The proliferation index was calculated as a ratio of Ki-67 positive versus total cells.

### 2.8 Statistics

All values were expressed as mean ± standard error of at least 3 different patients and compared using students' *t*-test or ANOVA with Bonferroni as a post hoc test. Values of $P < 0.05$ were considered to be significant.

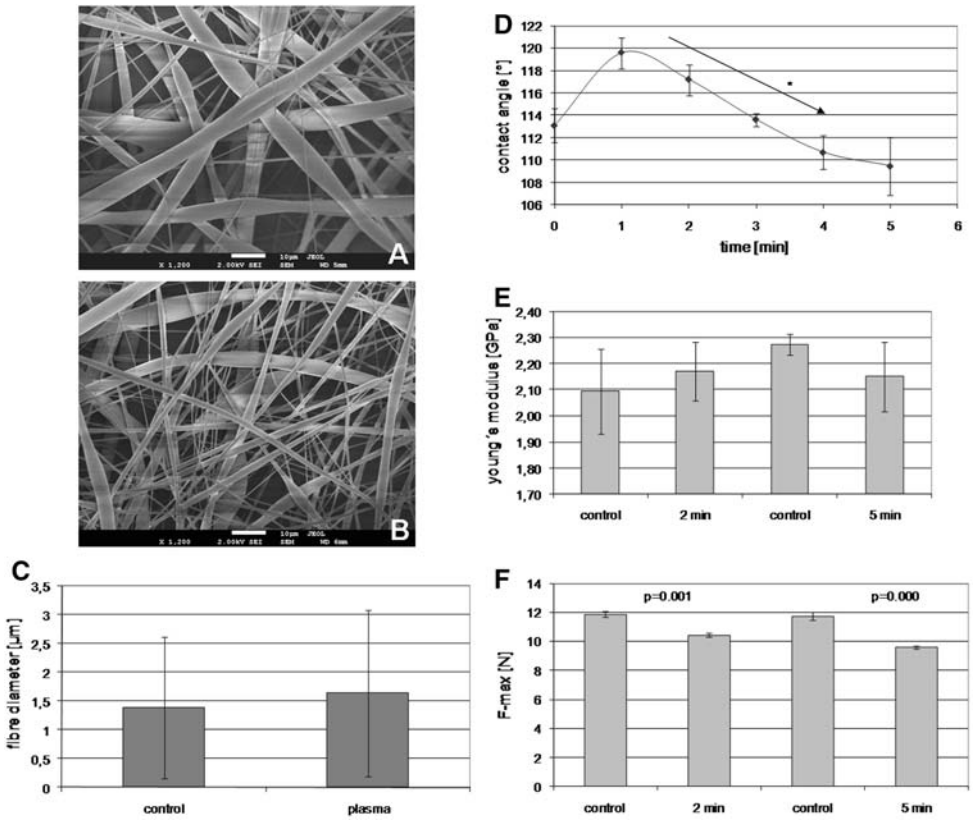


**Fig. 1** Influence of plasma treatment on nanofiber scaffolds. SEM analysis of PLLA (**a**) and PLLA nanofibers after 2 mins. of oxygen plasma treatment (**b**). Time dependent influence of plasma treatment on the contact angle (**d**), young's modulus (**e**), maximum load (**f**) and fiber diameter (**c**)

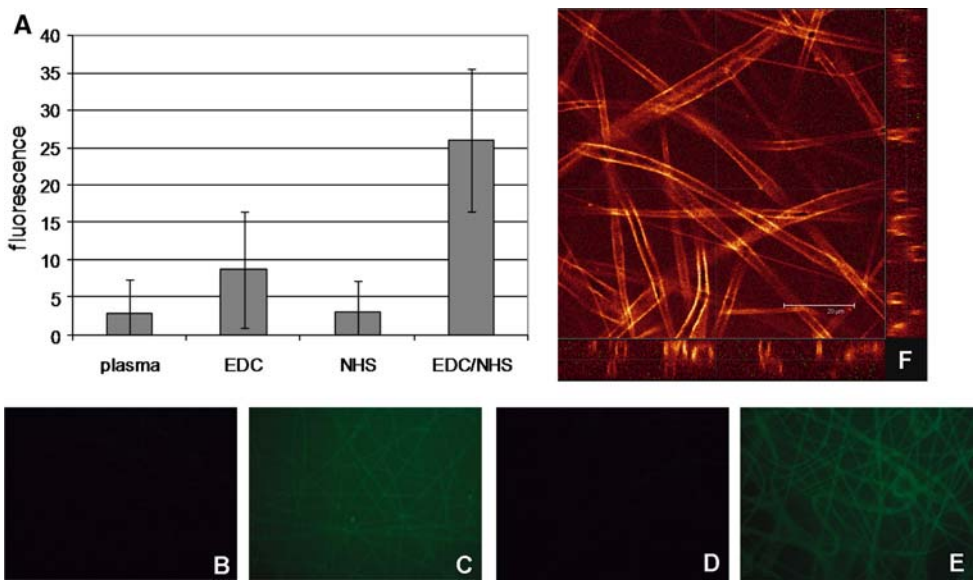


**Fig. 2** Coupling of fluorescent RGD on the surface of PLLA nanofibers using EDC/sulfo-NHS. Fluorescence intensity of nanofibers after different coupling protocols (**a**) and microphotographs of plasma treatment (**b**), EDC treatment (**c**), sulfo-NHS treatment (**d**) and a combination of EDC/sulfo-NHS (**e**). Fibres without any treatment were not detectable at all. Confocal microscopy of the EDC/sulfo-NHS RGD coupled fibres indicating a surface coupling of the RGD with an equal distribution (**f**)

## 3 Results

### 3.1 Characterization of cRGD incorporated PLLA nanofibers

In order to achieve functional groups on the PLLA nanofiber surfaces, scaffolds were subjected to oxygen plasma for different periods of time. As shown in Fig. 1 this treatment resulted in a time dependent decrease of water contact angle, indicating the formation of functional groups on the surface of the fibers. When plasma treated PLLA nanofibers specimens were subjected to mechanic forces they lost part of the stability, demonstrated by a significant loss in maximum load. However, stiffness was achieved (Fig. 1). No significant differences in fiber diameter could be observed (Fig. 1) using scanning electron microscopy. In order to demonstrate the efficiency of the coupling process and the influence of EDC and sulfo-NHS, the plasma activated PLLA nanofiber scaffolds were conjugated with fluorescein derivate (FITC) labeled RGD in presence of EDC, sulfo-NHS and a combination of both. Nanofibers exhibited higher level of fluorescence (Fig. 2) when treated with sulfo-NHS/EDC combinations. This fluorescence accumulated on the surface of the fibers as demonstrated by confocal microscopy. The individual chemicals had only a minor influence on the coupling reaction. Furthermore, no appreciable absorption either on PLLA or plasma treated PLLA nanofibers were found.

### 3.2 Growth and proliferation of hMSC cultured on nanofibers

In order to describe the biological effects of the incorporation of cRGD into PLLA nanofibers, we first analyzed the growth and proliferation of hMSC on the desired scaffolds when cultured under both growth and osteoinductive conditions. Cells were seeded at a density of approximately 12500 cells/cover slip on the scaffolds and allowed to grow over a period of 4 and 22 days. As shown in Fig. 3, during this time no significant differences in either cell densities or proliferation rate between cells cultured on PLLA, plasma treated PLLA as well as PLLA-cRGD nanofiber scaffolds were observed.

### 3.3 Expression of genes associated with the osteoblast lineage

In order to examine the influence of cRGD coupling on the surface of PLLA nanofibers on osteoblastic differentiation of hMSC, we compared the expression levels of cells grown on PLLA nanofibers with cells grown on PLLA nanofibers after plasma treatment, as well as cRGD coupling using EDC/sulfo-NHS treatment.

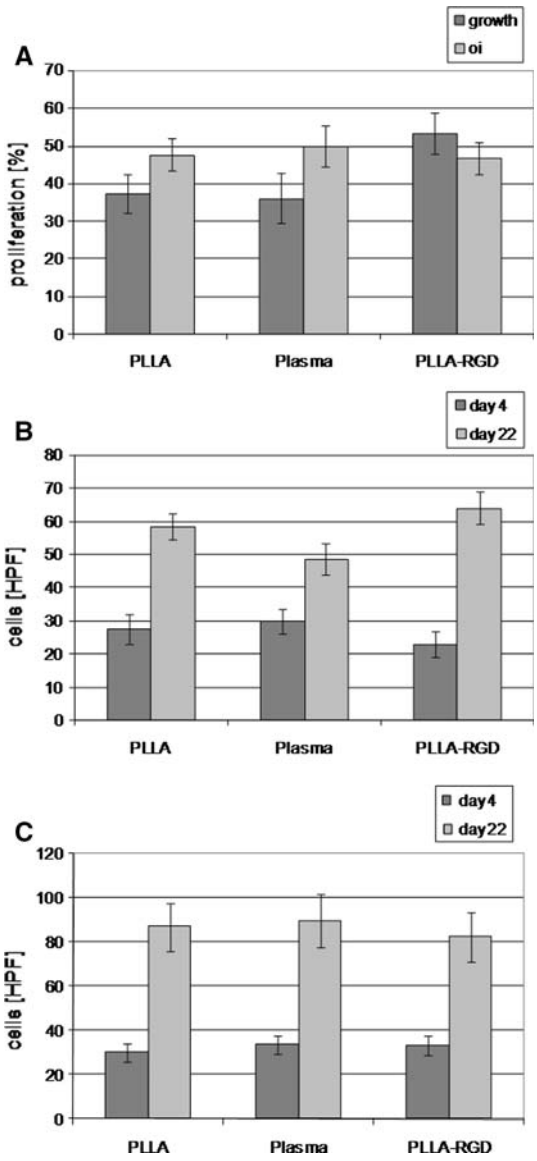


**Fig. 3** Influence of plasma treatment and cRGD coupling on proliferation and cell densities of hMSC cultured under growth and osteoinductive conditions. Proliferation obtained under osteoinductive (oi) and growth conditions (growth) after 4 days of cultivation (**a**). Cell densities after culture under growth (**b**) or osteoinductive conditions (**c**) over a period of 4 and 22 days

Regardless of whether the cells were cultured under growth or osteoinductive conditions, the plasma treatment of PLLA nanofibers resulted in an increased expression of genes associated with osteoblast linkage.

On the other hand, the chemical attachment of cRGD to the nanofiber surface resulted in an initial decrease in gene expression. However, after 10 days of cultivation, gene expression levels were equal or, in case of osteocalcin, significantly higher compared to plasma treated fibers alone (Fig. 4).

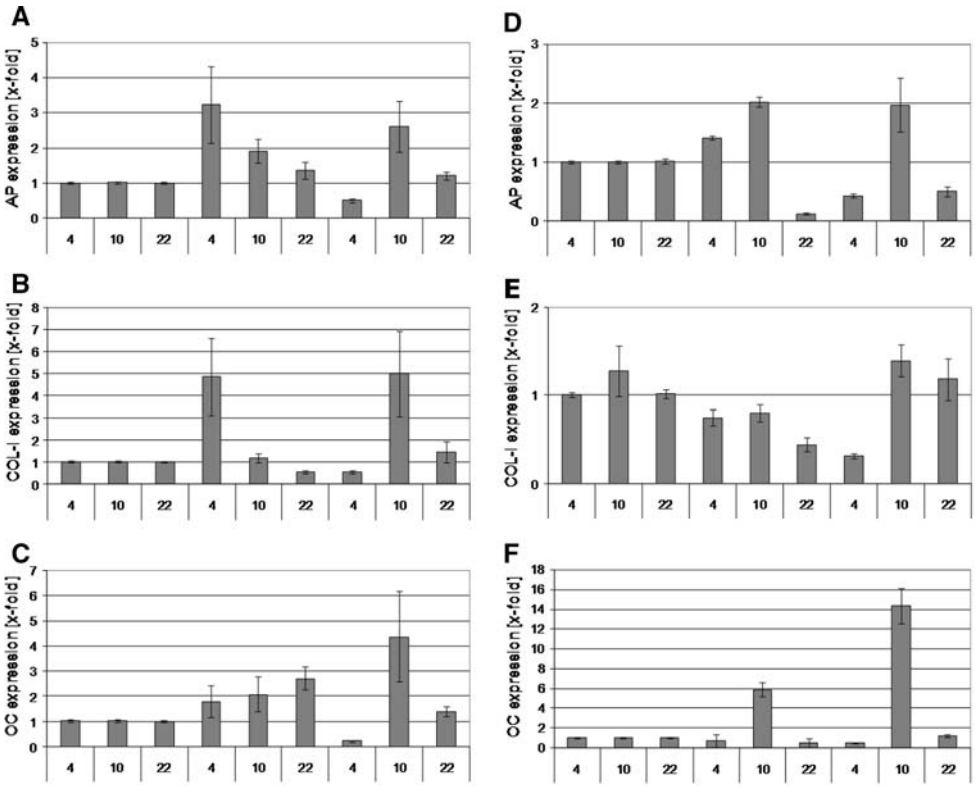


**Fig. 4** Influence of plasma treatment and cRGD coupling on expression of genes associated with the osteoblast lineage of hMSC cultured under growth and osteoinductive conditions. Time course of gene expression of hMSC, cultured under growth (**a**–**c**) and osteoinductive conditions (**d**–**f**) on PLLA nanofibers, compared to plasma treated and RGD coupled nanofibers. Alkaline phosphatase (**a**, **d**) collagen I (**b**, **e**) and osteocalcin (**c**, **f**)

## 4 Discussion

Scaffolds made of PLLA nanofibers represent an appropriate matrix for osteoblast growth and osteogenic differentiation of hMSC in principle [30–32]. However, we reported earlier that during the differentiation of hMSC the initial gene expression of osteoblast marker genes decreased. In order to overcome this disadvantage, a functionalization of PLLA nanofibers using bioactive motives of collagen, the main component of bone, might be a suitable tool. Indeed it has been shown that collagen nanofibers support growth and differentiation of hMSC [7, 8] even in the absence of osteogenic stimuli. Therefore, the fibers mediate, at least in part, the osteoinductive potential of collagen [9, 10]. In addition, PLLA nanofibers can be improved by either coating [33] or blending either with gelatin [34] or collagen [35]. These properties of collagen were mediated by the RGD sequences within the polymer chain. Furthermore, a direct incorporation of RGD peptides into PLLA nanofibers mediated, at least in part, the osteogenic potential of collagen (determined as the expression and deposition of collagen and osteocalcin) to some extent [24].

The direct coupling using the plasma EDC/sulfo-NHS system represents a method of coupling RGD sequences on the surface of nanofibers.

In contrast to other physical or chemical surface modifications the plasma action is limited to 10 nm below the surface and does not affect the bulk properties of the material [36, 37]. Nevertheless, these changes seem to be sufficient to weaken the PLLA nanofiber scaffolds (as demonstrated by maximum load measurements) and to decrease the hydrophobicity of the scaffold, presumably due to the induction of polar chemical groups [38, 39]. It is not clear, however, which of these groups is most effective in the cell-material interaction, although there is some evidence that hydroxyl groups are involved in cell adhesion [40]. However, in this study we found no effect on the proliferation and cell densities of hMSC. With respect to this, it should be realized that the effects of plasma treatment on polylactide scaffolds can be transient and/or permanent. The transient effects, such as surface activation and the formation of free radicals, disappear during storage, and will not be perceived by the cells in culture or in vivo [41]. On the other hand, we found some impact of plasma treatment on the differentiation of hMSC as demonstrated by an increased gene expression of genes associated with the osteoblast lineage, which might lead to the speculation that different groups on the surface may elicit different cellular response.

However, the formation of polar chemical groups was sufficient to couple RGD peptides on the surface of the nanofiber mash using a combination of EDC and sulfo-NHS comparable to Noga et al. [42].

Such RGD functionalized nanofibers were known to enhance cell attachment [24, 43, 44], but we and others

[45] demonstrate that this had no benefit during the course of cultivation.

Nevertheless, the presence of cRGD on PLLA nanofiber surfaces promoted the differentiation of hMSC to some extent. Therefore, the functionalisation of nanofibers by RGD has similar effects to the functionalisation of other surfaces by RGD peptides [46–48]. We interpret this finding to mean that the RGD is coupled on the fiber surface in a bioactive form in principal. However, as compared to the gene expression elicited by collagen nanofibers [8] or collagen blended PLLA nanofibers [35] the observed effect was small and limited to the expression of osteocalcin. One explanation here might be that the osteoinductive effect of RGD functionalized scaffolds depends on the concentration of RGD on the surface [49], and other bioactive sequences in the collagen. Within this context, further studies are needed in order to optimize the density of RGD on the surface of the nanofibers.

## 5 Conclusion

Taken together, this study clearly indicates that functional peptides of the extra cellular matrix can be coupled onto PLLA nanofibers using plasma treatment. These groups are accessible for the growing cell and mediate the expression of osteocalcin, indicating that the osteoblast linkage of hMSC is promoted to some extent.

**Acknowledgments** This work was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation, Grant No. BO 3065/1-1). We would like to thank M. Hellwig for SEM and Dr. F. Grolig for confocal fluorescence microscopy measurements.

## References

1. Parry DA. The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue. Biophys Chem. 1988;29:195–209.
2. Rho KS, Jeong L, Lee G, Seo B-M, Park YJ, Hong S-D, et al. Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing. Biomaterials. 2006;27:1452–61.
3. Venugopal J, Ma LL, Yong T, Ramakrishna S. In vitro study of smooth muscle cells on polycaprolactone and collagen nanofibrous matrices. Cell Biol Int. 2005;29:861–7.
4. Venugopal JR, Zhang Y, Ramakrishna S. In vitro culture of human dermal fibroblasts on electrospun polycaprolactone collagen nanofibrous membrane. Artif Organs. 2006;30:440–6.
5. Telemeco TA, Ayres C, Bowlin GL, Wnek GE, Boland ED, Cohen N, et al. Regulation of cellular infiltration into tissue engineering scaffolds composed of submicron diameter fibrils produced by electrospinning. Acta Biomater. 2005;1:377–85.
6. Gersbach Charles A, Byers Benjamin A, Pavlath Grace K, Guldberg Robert E, García AJ. Runx2/Cbfa1-genetically engineered skeletal myoblasts mineralize collagen scaffolds in vitro. Biotechnol Bioeng. 2004;88:369–78.
7. Shih Y-RV, Chen C-N, Tsai S-W, Wang YJ, Lee OK. Growth of mesenchymal stem cells on electrospun type I collagen nanofibers. Stem Cells. 2006;24:2391–7.
8. Schofer MD, Boudriot U, Wack C, Leifeld I, Gräbedünkel C, Dersch R, et al. Influence of nanofibers on the growth and osteogenic differentiation of stem cells—a comparison of biological collagen nanofibers and synthetic PLLA fibers. J Mater Sci Mater Med. 2008;20(3):767–74.
9. Salasznyk RM, Williams WA, Boskey A, Batorsky A, Plopper GE. Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. J Biomed Biotechnol. 2004;2004: 24–34.
10. Mizuno M, Kuboki Y. Osteoblast-related gene expression of bone marrow cells during the osteoblastic differentiation induced by type I collagen. J Biochem. 2001;129:133–8.
11. Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials. 2003;24:4385–415.
12. Bacakova L, Filova E, Kubies D, Machova L, Proks V, Malinova V, et al. Adhesion and growth of vascular smooth muscle cells in cultures on bioactive RGD peptide-carrying polylactides. J Mater Sci Mater Med. 2007;18:1317–23.
13. Alvarez-Barreto JF, Sikavitsas VI. Improved mesenchymal stem cell seeding on RGD-modified poly(L-lactic acid) scaffolds using flow perfusion. Macromol Biosci. 2007;7:579–88.
14. Ho MH, Lee JJ, Fan SC, Wang DM, Hou LT, Hsieh HJ, et al. Efficient modification on PLLA by ozone treatment for biomedical applications. Macromol Biosci. 2007;7:467–74.
15. Ho MH, Hou LT, Tu CY, Hsieh HJ, Lai JY, Chen WJ, et al. Promotion of cell affinity of porous PLLA scaffolds by immobilization of RGD peptides via plasma treatment. Macromol Biosci. 2006;6:90–8.
16. Hsu SH, Chu WP, Lin YS, Chiang YL, Chen DC, Tsai CL. The effect of an RGD-containing fusion protein CBD-RGD in promoting cellular adhesion. J Biotechnol. 2004;111:143–54.
17. Dee KC, Rueger DC, Andersen TT, Bizios R. Conditions which promote mineralization at the bone-implant interface: a model in vitro study. Biomaterials. 1996;17:209–15.
18. Healy KE, Rezania A, Stile RA. Designing biomaterials to direct biological responses. Ann NY Acad Sci. 1999;875:24–35.
19. Kantlehner M, Schaffner P, Finsinger D, Meyer J, Jonczyk A, Diefenbach B, et al. Surface coating with cyclic RGD peptides stimulates osteoblast adhesion and proliferation as well as bone formation. Chembiochem. 2000;1:107–14.
20. He X, Ma J, Jabbari E. Effect of grafting RGD and BMP-2 protein-derived peptides to a hydrogel substrate on osteogenic differentiation of marrow stromal cells. Langmuir. 2008;24: 12508–16.
21. Shin H, Temenoff JS, Bowden GC, Zygourakis K, Farach-Carson MC, Yaszemski MJ, et al. Osteogenic differentiation of rat bone marrow stromal cells cultured on Arg-Gly-Asp modified hydrogels without dexamethasone and beta-glycerol phosphate. Biomaterials. 2005;26:3645–54.
22. Yang F, Williams CG, Wang DA, Lee H, Manson PN, Elisseeff J. The effect of incorporating RGD adhesive peptide in polyethylene glycol diacrylate hydrogel on osteogenesis of bone marrow stromal cells. Biomaterials. 2005;26:5991–8.
23. Meinel L, Karageorgiou V, Hofmann S, Fajardo R, Snyder B, Li C, et al. Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. J Biomed Mater Res. 2004; Part A 71:25–34.
24. Schofer M, Boudriot U, Bockelmann S, Walz A, Wendorff J, Greiner A, et al. Effect of direct RGD incorporation in PLLA nanofibers on growth and osteogenic differentiation of human mesenchymal stem cells. J Mater Sci Mater Med. 2009;20:1535–40.

25. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7.
26. Brendel C, Kuklick L, Hartmann O, Kim TD, Boudriot U, Schwell D, et al. Distinct gene expression profile of human mesenchymal stem cells in comparison to skin fibroblasts employing cDNA microarray analysis of 9600 genes. Gene Expr. 2005;12: 245–57.
27. Frank O, Heim M, Jakob M, Barbero A, Schafer D, Bendik I, et al. Real-time quantitative RT-PCR analysis of human bone marrow stromal cells during osteogenic differentiation in vitro. J Cell Biochem. 2002;85:737–46.
28. Martin I, Jakob M, Schafer D, Dick W, Spagnoli G, Heberer M. Quantitative analysis of gene expression in human articular cartilage from normal and osteoarthritic joints. Osteoarthr Cartil. 2001;9:112–8.
29. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-[Delta][Delta]CT method. Methods. 2001;25:402–8.
30. Boudriot U, Dersch R, Goetz B, Griss P, Greiner A, Wendorff JH. Elektrogesponnene Poly-L-Laktid-Nanofasern als resorbierbare Matrix fur Tissue-Engineering. Biomedizinische Technik. 2004; 49:242–7.
31. Boudriot U, Dersch R, Greiner A, Wendorff JH. Electrospinning approaches toward scaffold engineering–a brief overview. Artif Organs. 2006;30:785–92.
32. Boudriot U, Goetz B, Dersch R, Greiner A, Wendorff HJ. Role of Electrospun Nanofibers in Stem Cell Technologies and Tissue Engineering. Macromol Symp. 2005;225:9–16.
33. Hu J, Liu X, Ma PX. Induction of osteoblast differentiation phenotype on poly(L-lactic acid) nanofibrous matrix. Biomaterials. 2008;29:3815–21.
34. Kim Hae-Won, Yu Hye-Sun, Lee H-H. Nanofibrous matrices of poly(lactic acid) and gelatin polymeric blends for the improvement of cellular responses. J Biomed Mater Res A. 2008;87A:25–32.
35. Schofer MD, Boudriot U, Leifeld I, Sutterlin RI, Rudisile M, Wendorff JH, et al. Characterization of a PLLA-collagen I blend nanofiber scaffold with respect to growth and osteogenic differentiation of human mesenchymal stem cells. ScientificWorldJournal. 2009;9:118–29.
36. Ibnabddjalil M, Loh IH, Chu CC, Blumenthal N, Alexander H, Turner D. Effect of surface plasma treatment on the chemical, physical, morphological, and mechanical properties of totally absorbable bone internal fixation devices. J Biomed Mater Res. 1994;28:289–301.
37. Baker SC, Atkin N, Gunning PA, Granville N, Wilson K, Wilson D, et al. Characterisation of electrospun polystyrene scaffolds for three-dimensional in vitro biological studies. Biomaterials. 2006; 27:3136–46.
38. Albino M, Elisabete DP, Susana F, Iva P, Alexandra PM, Rui LR, et al. Surface modification of electrospun polycaprolactone nanofiber meshes by plasma treatment to enhance biological performance. Small. 2009;5:1195–206.
39. Cui N-Y, Brown NMD. Modification of the surface properties of a polypropylene (PP) film using an air dielectric barrier discharge plasma. Appl Surf Sci. 2002;189:31–8.
40. Curtis AS, Forrester JV, McInnes C, Lawrie F. Adhesion of cells to polystyrene surfaces. J Cell Biol. 1983;97:1500–6.
41. Zbigniew G, Sylwester G. Attachment, growth, and activity of rat osteoblasts on polylactide membranes treated with various low-temperature radiofrequency plasmas. J Biomed Mater Res A. 2006;76A:288–99.
42. Noga DE, Petrie TA, Kumar A, Weck M, Garcia AJ, Collard DM. Synthesis and modification of functional poly(lactide) copolymers: toward biofunctional materials. Biomacromolecules. 2008;9:2056–62.
43. Grafahrend D, Lleixa Calvet J, Salber J, Dalton PD, Moeller M, Klee D. Biofunctionalized poly(ethylene glycol)-block-poly(epsilon-caprolactone) nanofibers for tissue engineering. J Mater Sci Mater Med. 2008;19:1479–84.
44. Choi WS, Bae JW, Lim HR, Joung YK, Park JC, Kwon IK, et al. RGD peptide-immobilized electrospun matrix of polyurethane for enhanced endothelial cell affinity. Biomed Mater. 2008;3:44104.
45. Kim TG, Park TG. Biomimicking extracellular matrix: cell adhesive RGD peptide modified electrospun poly(D, L-lactic-co-glycolic acid) nanofiber mesh. Tissue Eng. 2006;12:221–33.
46. Sofia S, McCarthy MB, Gronowicz G, Kaplan DL. Functionalized silk-based biomaterials for bone formation. J Biomed Mater Res. 2001;54:139–48.
47. Dettin M, Conconi MT, Gambaretto R, Pasquato A, Folin M, Di Bello C, et al. Novel osteoblast-adhesive peptides for dental/orthopedic biomaterials. J Biomed Mater Res. 2002;60:466–71.
48. Yang XB, Roach HI, Clarke NM, Howdle SM, Quirk R, Shakesheff KM, et al. Human osteoprogenitor growth and differentiation on synthetic biodegradable structures after surface modification. Bone. 2001;29:523–31.
49. Esmaiel J, Xuezhong H, Mani TV, Alireza SS, Weijie X. Material properties and bone marrow stromal cells response to < I > In situ </I > crosslinkable RGD-functionlized lactide- < I > co </I > -glycolide scaffolds. J Biomed Mater Res A. 2009;89A:124–37.