ADHESION AND GROWTH OF HUMAN OSTEOBLAST-LIKE CELLS ON ALIPHATIC POLYESTERS WITH DIFFERENT CHEMICAL COMPOSITION, SURFACE ROUGHNESS AND MODIFICATION WITH HYDROXYAPATITE

Barbora Vagaska*, Lucie Bacakova*, Elzbieta Pamula, Vera Lisa*, Piotr Dobrzyński*****

*Institute of Physiology, Acad. Sci. Cr, Videnska 1083, 142 20 Prague 4-krc, Czech Republic
**AGH-UST, Faculty of Materials Science and Ceramics, Department of Biomaterials,
Al Mickiewicza 30, 30-059 Kraków, Poland
***Centre for Polymer Chemistry,
Polish Academy of Sciences,
ul. Curie-skłodowskiej 34/20, 41-819 Zabrze, Poland
E-mail: Lucy@biomed.cas.cz

Abstract

In this study we have investigated the effect of three groups of polymeric foils on the behavior of MG 63 osteoblast-like cells. These included (1) poly(L-lactide) (PLLA) compared with newly synthesized copolymer of L-lactide and trimethylene carbonate (PLTMC 50:50), (2) three samples made of glycolide and ε-caprolactone copolymer (PGCap) with different surface roughness and topography, and finally (3) co-polymer of glycolide with L-lactide (PGLA) compared with its modification with hydroxyapatite deposits. On the 1st and 4th day of cultivation the cell number on all of the samples was lower than on control polystyrene culture dish. However, on day 8 after seeding, the values on the tested samples caught up with the control polystyrene. In the first group the cell number on PLTMC was higher than on polystyrene or PLLA. In the second group, the number of cells on PGCap samples of the lower surface roughness (RRMS 130 and 180 nm) was significantly higher than that on the control polystyrene, whereas on the PGCap samples with the roughness in micrometers, it was comparable to the value on the polystyrene. Moreover, the surface roughness influenced the cell adhesion area. The cells on the sample with the highest roughness index were roundly shaped and their adhesion area was significantly lower, because the cells were restricted in their spreading by the surface structure of the material. In the last group, the number of cells on day 8 on the polymer with hydroxyapatite deposits was significantly higher than on standard tissue culture polystyrene dish, as well as on unmodified PGLA foil, which suggests that hydroxyapatite supports cell proliferation.

Key Words*: Glycolide, L-lactide, ε-caprolactone, trimethylene carbonate, polyethylene glycol, hydroxyapatite, wettability, roughness, bone cells, cell adhesion, cell proliferation*

[Engineering of Biomaterials, 58-60, (2006), 4-7]

Introduction

Aliphatic polyesters such as polylactides, polyglycolides, poly-epsilon-caprolactone, and their copolymers are perspective and widely used materials for various advanced biomedical applications because of their advantageous properties such as biocompatibility, non-toxicity and biodegradability. The perspective fields for application of these materials are controlled drug release (Lysaght and Hazlehurst 2004), cell transfection with DNA constructs (Haas et al. 2005) or tissue engineering of bone (Yoon et al. 2004), cartilage (Savaiano and Webster 2004), vascular wall (Bacakova et al. 2003, Venugopal et al. 2005), skin (Chung et al. 2006), liver (Yoon et al. 2002) and even peripheric and central nervous tissue (Bryan et al. 2004, Han et al. 2005). Extensive studies of aliphatic polyesters over the past two decades have shown that surface chemistry, topography and roughness, and surface modifications influence biological properties such as protein adsorption, and consequently cell adhesion, spreading and proliferation, ultimately affecting new tissue formation (Bacakova et al. 2003, Savaiano and Webster 2004, Venugopal et al. 2005, Chung et al. 2006). Therefore, in this study we have investigated the effect of three groups of aliphatic polyester foils, differring in chemical composition and physicochemical surface properties, on the behavior of MG 63 osteoblast-like cells in cultures on these materials.

Materials and methods

Polymeric foils

The first group of samples contained foils with different chemical composition. We compared poly(L-lactide) (PLLA; Purac, Biochem, The Nederlands, Mv=410 kDa; Pamula et al. 2001) with a newly synthesized copolymer of L-lactide and trimethylene carbonate (PLTMC 50:50) (Dobrzyński and Kasperczyk 2006).
In the second group all the samples were made of glycolide and ε-caprolactone (PGCap 10:90), but had different surface roughness and topography (referred as PGCap up, PGCap down, PGCap membrane).
Finally we compared copolymer of glycolide and L-lactide (PGLA 18:84) and its modification with hydroxyapatite deposits (PGLA-HAp), produced by biomimetic method from simulated body fluid (Pamula and Buczynska 2005).
As a control material well supporting cell adhesion and growth, tissue culture polystyrene (TCPS) (TPP, Switzerland; diameter 22 mm) was used. As a negative control, we applied a blend of PGCap and 30 wt% of polyethylene glycol (PEG, Mn ca. 400 Da, Aldrich, Cat. No 20,239-8), referred as PGCap+PEG blend. It is known that PEG is a protein repulsive material and therefore non-appropriate for cell adhesion.

Measurements of physicochemical surface properties

The water contact angle was measured by sessile drop method by an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany). UHQ - water (produced by Purelab UHQ, Elga) of resistivity 18 MΩ/cm, was used for experiments. The surface topography and roughness were evaluated by atomic force microscopy in contact mode (AFM Explorer, ThermoMicroscopes, Vecco, USA) in the scan areas of 100 μm x 100 μm. The samples which were too rough to be studied in AFM were evaluated by scanning electron microscopy (SEM, JSM 5400, JEOL, Japan).

Cells and culture conditions

Human osteoblast-like cells from the cell line MG 63

(European Collection of Cell Cultures, Salisbury, UK) were seeded on the polymer foils at the initial density of 13 000 cells/cm^2 into 2 ml of Dulbecco-modified Eagle Minimum Essential Medium with 10% of fetal bovine serum. The cells were cultured at 37°C in humidified atmosphere containing 5% of CO_2 for 1, 4 and 8 days.

Cell adhesion and growth

In order to evaluate the cell morphology and distribution over the material surface, the samples were rinsed with phosphate-buffered saline (PBS), fixed in 70% ethanol, stained with propidium iodide and then observed using inverted microscope Olympus IX 50, equipped with a digital camera DP 70, obj. 10x.

To measure the number of cells, the cells were detached from the polymer by trypsin- EDTA solution (Sigma, U.S.A, cat. N° T4174) and counted in Bürker haemocytometer (3 measurements for each sample). The doubling time of cells was calculated according to the formula: $DT=(t-t_o)\log 2/\log Nt-\log Nt_o$, where to and t represent earlier and later time intervals after seeding respectively Nto and Nt the number of cells at these intervals (i.e., day 1, 4 or 8 after seeding). For cell adhesion area measurements on day 1, the cells were fixed in 70% ethanol and stained with hematoxylin-eosin. Ten pictures were taken from each sample using inverted microscope Olympus IX 50, with digital camera DP 70, obj. 20x. The images were analyzed using software Atlas (Tescan, Brno, CR).

Statistics

Data were presented as averages ±S.E.M. (Standard Error of Mean). The statistical significance of the differences was evaluated by the Student's t test for unpaired data.

Results and discussion

Properties of the polymer foils

Water contact angle and roughness indexes of the studied materials are provided in TABLE 1. The highest contact angle was found on PLTMC and on the samples containing poly-epsilon-caprolactone, which is generally known by its hydrophobicity. All caprolactone-containing samples also were relatively rough. As measured by AFM, their roughness indexes ranged from more than 100 nm to several μm (the latter in the case of PGcap membrane), whereas the surface roughness of standard tissue culture polystyrene, PGLA and PLTMC was in the range of nanometers or tens of nanometers. Also the HAp-enriched PGLA was relatively rough, thus its surface roughness was not measurable using AFM. The roughness of PLLA was in the range of more than 100 nm. These results are further documented on morphological pictures of the material surface topography obtained by AFM or SEM (FIG.1). The caprolactone-containing copolymers and polyL-lactide based polymers showed well apparent irregularities on their surface, while PGLA samples and polystyrene were almost completely flat.

Cell culture on the polymer foils

On day 1 after seeding, the cell number on all of the samples was significantly lower than on TCPS and comparable with the negative control (PGCap+PEG blend, FIG.2). In the first group of samples PLTMC had significantly higher number of cells than PLLA. In the second group cell number did not seem to be affected by the surface roughness. However, the surface topography influenced the cell adhesion area. The cells on the sample with the highest roughness index were roundly shaped and their adhesion area was significantly smaller (FIG.2). That could be explained by adhesion of the cells in the "valleys" on the material, which limited the cell spreading. In the third group the cell number was comparable on both materials, i.e. on PGLA and PGLA with hydroxyapatite deposits.

From day 1 to 4 after seeding, the cell population doubling time (TAB.2) on all tested polymeric samples was similar or even longer than on the control tissue polystyrene. As a results, on day 4 the cell number on all of the samples was still significantly lower than on TCPS, but already noticeably higher than on negative control (PGCap+PEG blend, Fig. 2). The latter sample did not allow cell colonization because of its extreme hydrophilicity, which was indicated by non-measurable water drop contact angle due to extreme spreading of the water drop on the material surface (TAB.1).

After eight days of cultivation, the cell number on most of the samples equaled or even exceeded the value found on the control TCPS (FIG.2). This result is in good correlation with a markedly shorter doubling time between the days 4 and 8 on all polymeric samples in comparison with TPCS (TAB.2). In the first group of samples, i.e. in samples of different chemical composition, the cell number on PLTMC was higher than on polystyrene or PLLA. In the second group, i.e. PGCap samples with different roughness, the number of cells on samples with a lower surface roughness (RRMS

Materialgroup	Controls		Group 1		Group 2			Group 3	
Parameter measured	TCPS	PGCap +PEG	PLLA	PLTMC	PGCap down	PGCap up	PGCap membr	PGLA	PGLA +HAp
Water contact angle [degree]	60.0±3.7	n.m.*	77.3±1.6	79.5±1.3	80.6±2.3	79.9±2.6	80.7±3.4	72.9±2.6	57.8±2.2
R_{RMS} [nm]	13.5	460	170	42	130	180	n.m.**	11.3	n.m.**

n.m.* = non-measurable (because of extreme spreading of the water drop); n.m.** = non-measurable (because of too high roughness to be analysed by AFM); Mean ± S.D. from 10-12 measurements of individual drops.

TABLE 1. Water contact angle and R_{RMS} roughness on polymeric materials (explanation of the abbreviations see Materials and methods).

Material group/ parameter	Controls		Group 1		Group 2			Group 3	
DT (h)	TCPS	PGCap +PEG	PLLA	PLTMC	PGCap down	PGCap up	PGCap membr	PGLA	PGLA +HAp
1.-4.	23.9 ±0.6	140.2±174.0	24.3±1.6	23.6±2.1	25.6±2.9	26.2±1.7	30.7±4.2	34.4±5.3	38.3±3.0
4.-8.	105.8 ±9.6	62.9±8.7	43.9 ±2.6	50.7±1.9	37.4±0.9	39.1±1.8	46.9±5.0	42.1±5.7	29.9±1.0

TABLE 2. Doubling time (DT) of MG 63 populations on polymeric materials (see Materials and methods) between days 1 to 4 and 4 to 8 after seeding. Mean ± S.E.M. from 6-9 measurements.

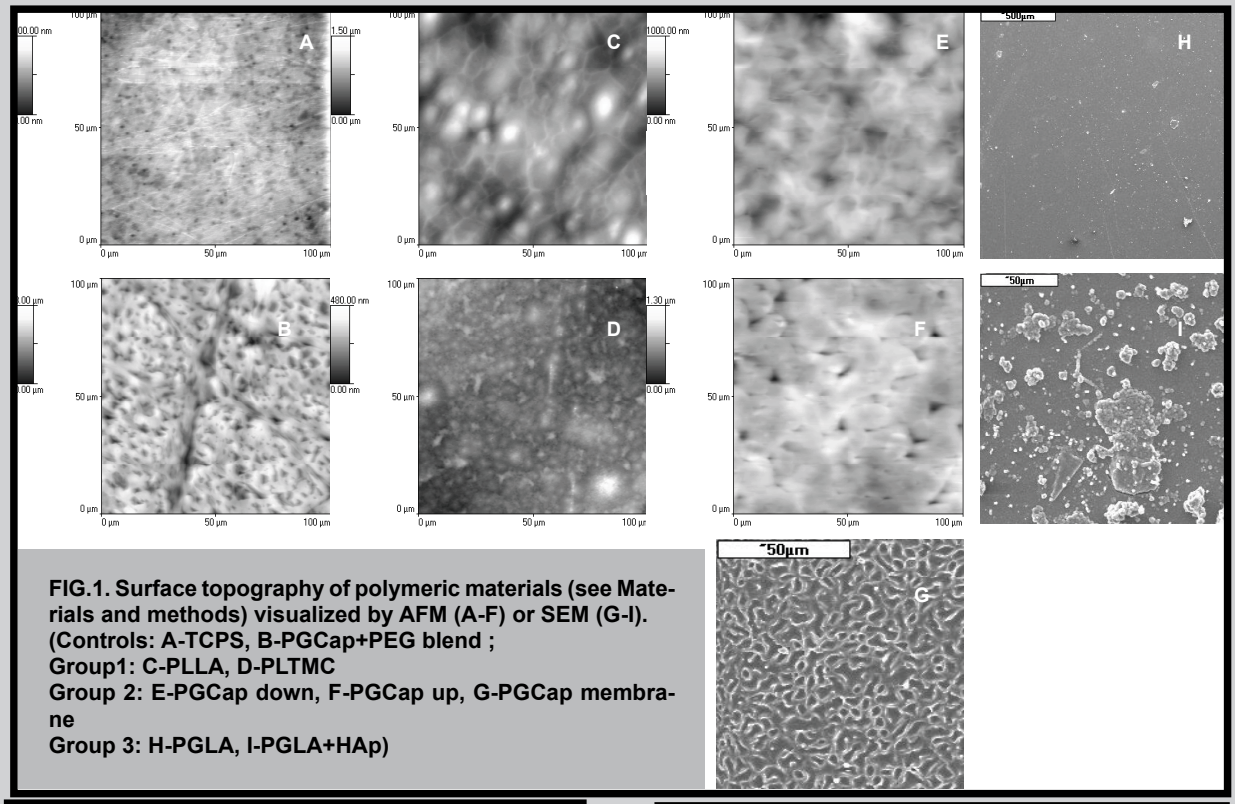

FIG.1. Surface topography of polymeric materials (see Materials and methods) visualized by AFM (A-F) or SEM (G-I).
(Controls: A-TCPS, B-PGCap+PEG blend ;
Group1: C-PLLA, D-PLTMC
Group 2: E-PGCap down, F-PGCap up, G-PGCap membrane
Group 3: H-PGLA, I-PGLA+HAp)

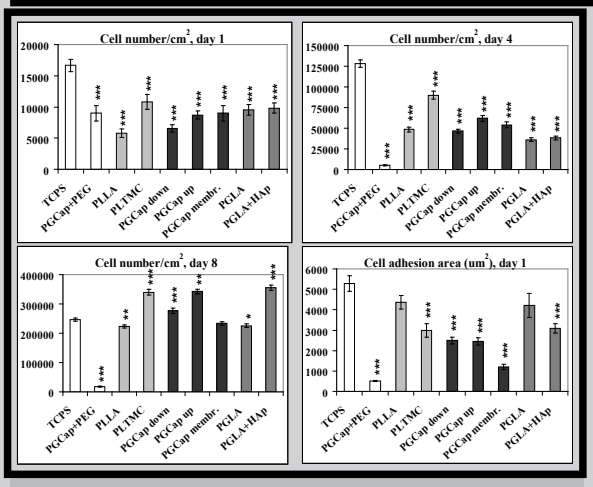

FIG.2. Cell number on day 1, 4 and 8 and cell adhesion area on day 1 on polymeric materials (see Materials and methods).
Means ± S.E.M. from 36 to 54 measurements obtained from 2-3 independent samples (cell number) or 13 to 20 cells for each experimental group (cell adhesion area). Student´s t-test for unpaired data. Statistical significance: *p≥0.05, **p≥0.01, ***p≥0.001 in comparison with the value on TCSP.

130 and 180 nm) was significantly higher than that on the control polystyrene, whereas on the PGCap samples with the roughness in micrometers, it was similar as the value found on the polystyrene. In the last group of samples, the number of cells on PGLA with hydroxyapatite deposits was significantly higher than on standard tissue culture dish, as well as on the unmodified PGLA foil, which suggests that hydroxyapatite supports cell proliferation (Bacakova et al. 2004).

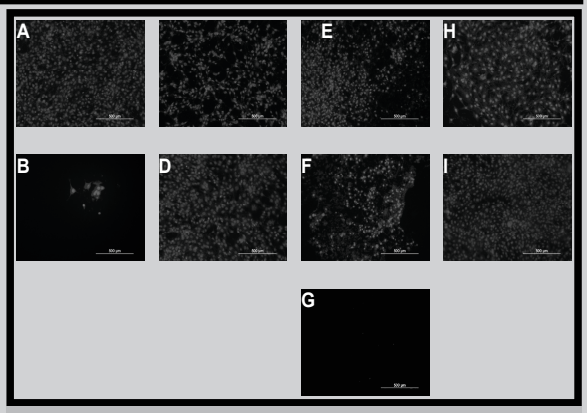

FIG.3. Morphology of MG 63 on polymeric materials (see Materials and methods) on the 8th day after seeding. Fixed with ethanol, stained with propidium iodide, fluorescence microscope Olympus IX 50, digital camera DP 70. Bar=500 µm.
(Control samples: A-TCPS, B-PGCap+PEG blend;
Group 1: C-PLLA, D-PLTMC;
Group 2: E-PGCap down, PGCap up, PGCap membrane;
Group 3: H-PGLA, I-PGLA+HAp

The morphology of MG 63 cells adhering to the studied polymers on day 8 after seeding is shown in FIG.3. It is apparent that on flat materials such as tissue culture polystyrene and PGLA, PLLA or PLTMC foils, the cells are distributed homogeneously while on rough materials (caprolactone-containing samples and hydroxyapatite-modified PGLA) the cells are concentrated in the depressions among the protuberances on the material surface. On the PGCap+PEG blend, only occasional cell aggregates were found.

Conclusion

Newly synthesized copolymer of L-lactide and trimethylene carbonate (PLTMC 50:50) promoted faster growth and proliferation of MG 63 osteoblast-like cells in comparison with both standard culture tissue dish and commercially available poly(L-lactide). Surface roughness and topography had no significant effect on the cell adhesion and growth on poly(glycolide-epsilon-caprolactone) from day 1 to 4 after seeding. However, on day 8, the cells reached a higher population density on samples of the surface roughness between 130-180 nm, whereas on the samples of the roughness in micrometers, this density remained similar as on the polystyrene. In addition, a higher surface roughness decreased the size of the cell adhesion area. Modification of the polymeric material by hydroxyapatite deposits improved the cell proliferation.

Acknowledgements

Supported by the Grant Agency of the Czech Republic (grant No.106/06/1576) and by Polish Ministry of Scientific Research and Higher Education (grant No.3 T08D 019 28). We also thank to Mrs. Ivana Zajanova for her excellent technical assistance.

References

[1] Bacakova L, Lapcikova M, Kubies D, Rypacek F: Adv Exp Med Biol 534: 179-189, 2003.
[2] Bačáková L, Jungova I, Slosarczyk A, Zima A, Paszkiewicz Z: Inz Biomater –Eng. Biomater, 7 [38-42]: 15-18, 2004.
[3] Bryan DJ, Tang JB, Doherty SA, Hile DD, Trantolo DJ, Wise DL, Summerhayes IC: J Neural Eng 1: 91-98, 2004.
[4] Chung TW, Wang YZ, Huang YY, Pan CI, Wang SS: Artif Organs 30: 35-41, 2006.
[5] Dobrzynski P, Kasperczyk J, J Pol Sci Part A, , 44: 3184-3201, 2006.
[6] Haas J, Ravi Kumar MN, Borchard G, Bakowsky U, Lehr CM: AAPS PharmSciTech 6: E22-E30, 2005.
[7] Han DW, Sub Lee M, Park BJ, Kim JK, Park JC: Biotechnol Lett 27: 53-58, 2005.
[8] Lysaght M, Hazlehurst A: Tissue Eng 10: 309-320, 2004
[9] Pamuła E, Błażewicz M, Paluszkiewicz C, Dobrzyński P: J Mol. Struct 596: 69 – 75, 2001.
[10] Pamuła E, Buczyńska J, Ceramika-Ceramics 91/1: 577-584, 2005
[11] Savaiano JK, Webster TJ: Biomaterials 25: 1205-1213, 2004.
[12] Venugopal J, Ma LL, Yong T, Ramakrishna S: Cell Biol Int 29: 861-867, 2005.
[13] Yoon JJ, Nam YS, Kim JH, Park TG: Biotechnol Bioeng 78 :1-10, 2002.
[14] Yoon JJ, Song SH, Lee DS, Park TG Biomaterials 25: 5613-5620, 2004.

VASCULAR SMOOTH MUSCLE CELLS IN CULTURES ON SYNTHETIC POLYMERS WITH ADHESIVE MICRODOMAINS

Martin Parizek*, Lucie Bacakova*, Vera Lisa*, Olga Kubova, Vaclav Svorcik**, Johannes Heitz*****

*Institute of Physiology, Acad. Sci. CR,
Videnska 1083, 142 20 Prague 4-Krc, Czech Republic
**Institute of Chemical Technology,
Technicka 5, 166 28 Prague 6 – Dejvice
***Angewandte Physik, Johannes Kepler Universität,
Altenbergerstr. 69, A-4040 Linz, Austria
E-mail: Parizek.M@seznam.cz, lucy@biomed.cas.cz

Abstract

Polyethylene terephtalate was modified by UV light irradiation produced by a Xe_2^*-excimer lamp for 10, 20 or 30 min in an acetylene atmosphere. For creation of microdomains for selective cell adhesion, a contact nickel mask (apertures of the diameter of 500 μm, centre-to-centre distance 2 mm) was used. The material was then seeded with rat aortic smooth muscle cells (passage 3, 17 000 cells/cm^2). After 1, 3 and 7 days of cultivation, the cells were homogeneously distributed on the samples without any preference of the irradiated microdomains. Moreover, on day 1, the number of initially adhered cells was similar on all tested samples. However, on day 3, the number of cells on the irradiated samples was significantly higher than that on control unmodified PET and increased proportionally to the time of exposure to UV light. On day 7 after seeding, however, the cell number on the unmodified PET exceeded the value on all irradiated samples. In the second set of experiments, polyethylene (PE) was irradiated by Ar^+ ions in order to create the adhesive microdomains (dose 10^{12}-10^{14} ions/cm^2, energy 150 and 15 keV, contact mask with holes of 100 μm diameter and distance 200 μm). The highest selectivity of the adhesion and growth of rat aortic smooth muscle cells (89% of all cells) was found on the microdomains created at the energy of 150 keV and the dose of $3 \cdot 10^{12}$ ions Ar^+. The lowest selectivity (30 %) occurred on samples irradiated with 150 keV Ar^+ ions of the dose of $3 \cdot 10^{14}$ ions/cm^2. Therefore, both methods seem to be suitable for modification of materials with highly hydrophobic surfaces in purpose to increase the cell colonization, for example when constructing bioartificial vascular replacements. The second method can be also used for the creation of domains for a regionally selective adhesion and growth of cells on biomaterials.

Key words: *UV light irradiation, ion implantation, patterned surfaces, adhesive microdomains, vascular smooth muscle cells*

[Engineering of Biomaterials, 58-60,(2006),7-10]

Introduction

Synthetic materials, such as polyethylene, polystyrene, polyurethane or polyethylene terephtalate, are commonly used in various industrial applications as well as in biology and medicine. They serve not only as growth supports for cell cultures *in vitro*, but also they can be used for a con-