THE INFLUENCE OF CHEMICAL STRUCTURE OF ALIPHATIC POLYESTERS ON ADHESION AND GROWTH OF OSTEOBLAST-LIKE MG63 CELLS

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Abstract

Degradable copolymers of glycolide and L-lactide (PGLA), glycolide and ε–caprolactone (PGCap) and terpolymer of glycolide, L-lactide and ε–caprolactone (PGLCap) were synthesized by ring opening polymerization using zirconium acetylacetonate ($Zr(acac)_4$) as a biocompatible initiator. The structure and physicochemical surface properties of the materials were studied by NMR spectroscopy, gel permeation chromatography, X-ray photoelectron spectroscopy and sessile drop. The interaction of polymeric films produced by slip-casting with osteoblast-like MG63 cells was tested in vitro. The number of adhering cells, their shape and the size of cell-material contact area were evaluated from day 1 to 7 after seeding. It was found that the cell behaviour on PGLA and PGLCap was very similar as on control tissue culture polystyrene (TCPS). On PGCap, however, the number of initially adhering cells was significantly lower (by 84% than on TCPS) and cell spreading area smaller (by 50% than on TCPS). On day 7 after seeding, these cells reached the lowest population density (by 30% smaller than on TCPS). We hypothesize that the lower cell adhesion and growth of MG63 cells on PGCap was caused by the highest hydrophobicity of this material among all studied samples.

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Introduction

Aliphatic polyesters such as polylactides, polyglycolide, poly-ε-caprolactone and their copolymers have been widely used in medicine as materials for sutures, prostheses or implants for internal fixation of bone fractures [1]. More recently these polymers have received considerable attention as carriers for controlled release of drugs and biodegradable scaffolds for tissue engineering [2].
It was recently shown that such materials can be synthesized by the use of initiators of lower toxicity, instead of commercially used, but highly toxic tin compounds. These initiators are represented by compounds of zinc, calcium, iron and zirconium [3-6].

Extensive studies of aliphatic polyesters over past two decades have shown that surface chemistry, wettability, topography and roughness markedly influence biological properties such as protein adsorption, cell attachment, spreading and proliferation, ultimately affecting new tissue formation [1, 2, 7, 8].
In the present study we characterize chemical structure and surface properties of three aliphatic polyesters synthesized with the use of zirconium acetylacetonate, and investigate the behaviour of osteoblast-like MG 63 cells contacting these materials in vitro.

Materials and methods

Synthesis

Preparation of substrata was described in detail earlier [6,9]. Briefly, copolymerization of glycolide and L-lactide (Purac, Netherlands) (PGLA), glycolide and ε- caprolactone (Fluka, Germany) (PGCap) and terpolymerization of glycolide, L-lactide and ε-caprolactone (PGLCap) were performed in bulk with a zirconium (IV) acetylacetonate $Zr(acac)_4$ (Aldrich Corp., Germany) molar ratio of 1.25×10^{-3} at 100°C by a conventional method using a vacuum line for degassing and sealing of the ampoules. The resulting materials were ground and shaken with methyl alcohol in order to remove non-reacted monomers and then dried in vacuum at 50°C.

The films were cast from 10% (w/v) polymer solution in methylene chloride on glass Petri dishes, followed by vacuum drying for 72h.

Measurements

The compositions of terpolymer and copolymers were determined by 1H NMR (Varian Unity Inowa spectrometer) at 300 MHz and a 5-mm sample tube. Dried dimethyl sulfoxide-d6 was used as a solvent. The molecular masses and polydispersity indices were determined by gel permeation chromatography (GPC) with the aid of Spectra Physics SP 8800 chromatograph (chloroform was used as the eluent, flow rate, 1 mL/min, Styragel colums 104, 103 and 500A and a Shoedex SE detector). Surface chemical composition was studied by X-ray photoelectron spectroscopy (XPS) (SSI X-Probe spectrometer, Surface Science Instruments, Mountain View, CA, USA) according to the method described previously [10]. The 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.

Cell culture conditions

The polymer samples were placed into Nunclon Multidishes (24 wells with diameter of 15 mm, Nunc, Denmark) and sterilised with ultraviolet irradiation for 1h from both sites. The MG63 osteoblast-like cell line (European Collection of Cell Cultures, Salisbury, UK; passage 156) were seeded on the foils at the initial density of 25,000 cells/cm^2 (i.e. 45,000 per well) in 1.5 mL of Dulbecco-modified Eagle Minimum Essential Medium supplemented with 10% foetal bovine serum and gentamicin (40μg/mL). Nunclon TCPS wells were used as control material. For each ex-

perimental group and time interval 2 samples were used. The cells were cultured for 1, 3 and 7 days at 37°C in humidified air atmosphere containing 5% of CO_2.

Cell adhesion and growth

The samples were rinsed with phosphate buffered saline (PBS), fixed in 4% formaldehyde in PBS for 5 min, stained with Gill's hematoxylin for 5 min, aqueous eosin Y for 2 min, and mounted in Glycerol Gelatin (all chemicals provided by Sigma Diagnostics, U.S.A.). The number of adhering cells on day 1 was counted under phase-contrast microscope (Opton, Germany) in 10 randomly selected microscopic fields of 1 mm^2 homogenously distributed on each sample. On day 7, because of high cell population density, the cells were detached by trypsin-EDTA (Sigma) and counted in Coulter Particle Counter (Coulter Electronics LTd, Florida, U.S.A.; 3 measurements for each sample). For evaluation of cell shape and spreading area, images from 5 to 6 regions on samples 1 and 3 days after seeding were captured by Olympus IX 51 inverted microscope equipped with digital camera DP 70 and DP Control Software (objective x20, captured area of 1.376 mm^2). Atlas Tescan Digital Microscopy Imaging (Tescan Co., Brno, CR) was used for the analysis of cell area (13-50 cells per sample). Cells forming cell-cell contact were excluded from the evaluation.

Data were presented as averages ± SEM (Standard Error of Mean) from 6 to 50 measurements obtained from 2 independent experiments. The statistical significance of the differences was evaluated by the Student's t test for unpaired data and by one-way analysis of variance (ANOVA) using SigmaStat software (Jandel Corp., U.S.A.).

Results and discussion

Properties of substrata

The physical and chemical properties of the newly constructed biomaterials are provided in TABLE 1. The obtained data indicate that synthesised materials have different chemical structure, but quite similar molecular masses (Mn). Their surface composition, as determined by XPS, reveals that the highest amount of oxygenated functions (about 40 mole%) was detected for PGLA and PGLCap. On the other hand, only 27 mole% of oxygen was measured on the surface of PGCap. These results go along with the values of contact angles: the lowest contact angles, slightly above 70°, were measured on PGLA and PGLCap, contrary to PGCap which had contact angle of about 80°.

Cell culture

The morphology of MG63 cells adhering to examined substrata on 1, 3 and 7 days after seeding is shown on FIGURES 1, 2 and 3. On day 1, the cells on all samples, except PGCap, were mainly spindle-shaped and partially

Sample	N	Mn	d	S[d] mole%		θ
	mole %	kDa		C	O	deg
PGLA	18:82[a]	34	2.5	58	42	72.9±2.6
PGCap	9:91[b]	53	1.8	73	27	80.6±2.3
PGLCap	10:70:20[c]	48	1.3	62	38	71.7±3.4

N – molar ratio of [a] glycolide to L-lactide, [b] glycolide to ε-caprolactone and [c] glycolide to L-lactide and ε-caprolactone; Mn – number average molecular mass; d – polydispersity coefficient (Mw/Mn); [d] surface composition excluding hydrogen - studied by XPS; θ – contact angle

TABLE 1. Properties of substrata

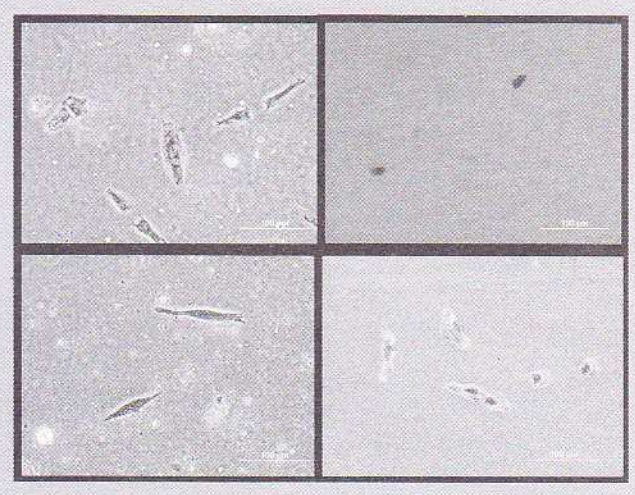

FIG. 1. Morphology of MG63 cells adhering to PGLA (A), PGCap (B), PGLCap (C), and Nunclon TCPS (D) on day 1 after seeding. Hematoxylin - eosin staining. Olympus IX51 inverted microscope with DP Controller software, objective 20x, bar 100 μm.

spread, while on PGCap, the cells were round and non-spread [FIG. 1]. The spreading area of cells on day 1 after seeding was also significantly smaller on PGCap in comparison to TCPS, PGLA and PGLCap [FIG. 4].

Similarly, in 3-day-old cultures on PGLA, PGLCap and TCPS, the cells were more flattened, mainly polygonal in shape. On the other hand, on the PGCap the cells were less spread [FIG. 2]. On day 7, the cells on all substrata formed monolayers [FIG. 3].

The number of cells adhered on PGLA and PGLCap on day 1 and 7 after seeding [FIG. 5 and 6 respectively], was comparable to that on TCPS and significantly higher than that on PGCap.

The lower cell adhesion and subsequent growth of MG63 cells on PGCap could be explained by a relatively high water contact angle found on this substrate, which is a sign of its hydrophobicity. Similar results were obtained on human dermal fibroblasts and myoblasts, Baby Hamster Kidney (BHK) cells or vascular endothelial cells cultured

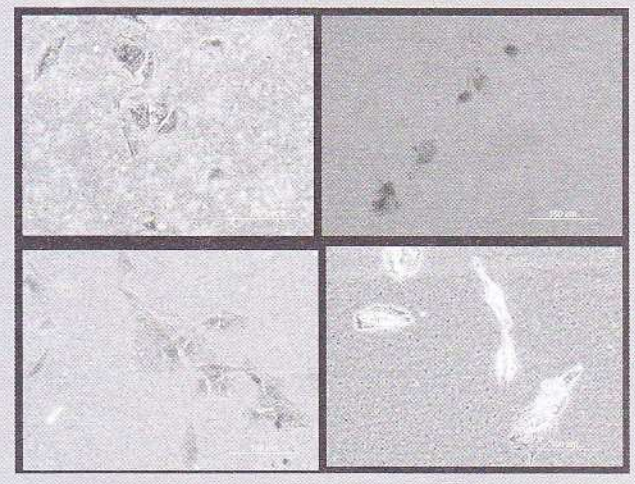

FIG. 2. Morphology of MG63 cells adhering to PGLA (A), PGCap (B), PGLCap (C), and Nunclon TCPS (D) on day 3 after seeding. Hematoxylin - eosin staining. Olympus IX51 inverted microscope with DP Controller software, objective 20x, bar 100 μm.

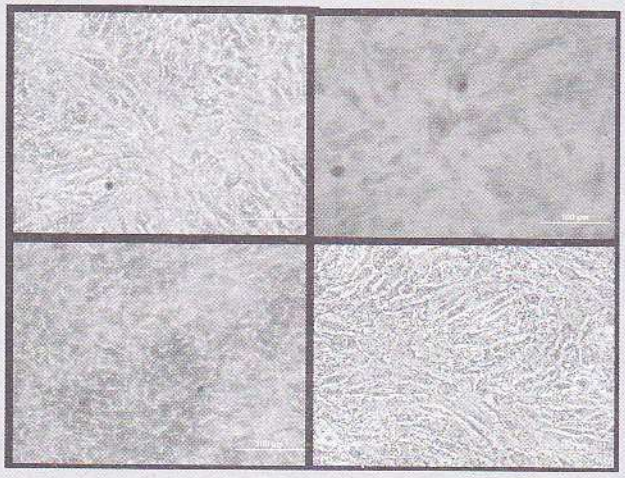

FIG. 3. Morphology of MG63 cells adhering to PGLA (A), PGCap (B), PGLCap (C), and Nunclon TCPS (D) on day 7 after seeding. Hematoxylin - eosin staining. Olympus IX51 inverted microscope with DP Controller software, objective 20x, bar 100 μm.

on pure poly-ε-caprolactone or its copolymers with poly(L-lactide) or poly(ethylene glycol) (PEG). When the hydrophilicity of these materials was enhanced by physical and chemical surface modification, such as treatment by plasma discharge, polymerization with acrylic acid, hydrolytic etching or increased content of PEG, the colonization of these substrates with cells markedly improved [11-13].

The surface wettability of polymer samples in this study was positively correlated with the concentration of oxygen-containing chemical functional groups on the material surface, which are well known to support adhesion, growth and differentiation of various cell types [14]. The increase of the material surface wettability is probably not the only mechanism by which these groups improve the cell adhesion on biomaterials. In our earlier study [14] the number of vascular smooth muscle cells initially adhered to polyethylene implanted with O+ or C+ ions cells appeared to be positively correlated with the amount of the oxygen group present at the polymer surface rather than with the surface hydrophilia arising from other reasons. Oxygen groups may have a direct influence on adsorption of cell adhesion-mediating extracellular matrix molecules (e.g., fibronectin, vitronectin, collagen provided by the serum in the culture media) to the materials [14].

Other important surface features, which might influence the cell adhesion on our polymers, were the surface roughness and topography. It has been reported that nanostructured surfaces, i.e. surfaces with nanometer features, e.g. grains, markedly promoted cell adhesion, which was explained by adsorption of cell adhesion-mediating extracellular matrix proteins in appropriate conformation allowing their good accessibility by adhesion receptors on cells [15].

Conclusions

The number and spreading of MG63 cells, comparable to that on control TCPS, was found on foils made of PGLA and PGLCap. On the contrary, significantly lower

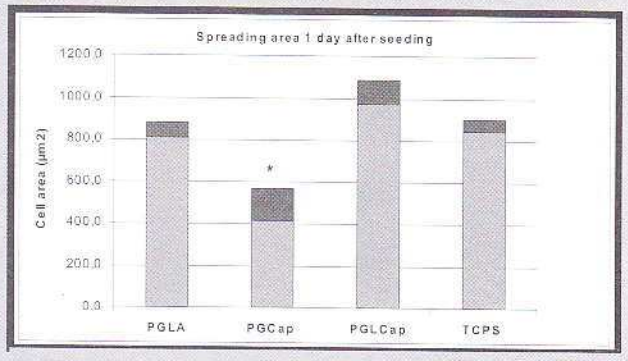

FIG. 4. Spreading area of MG63 cells adhering to PGLA, PGCap, PGLCap, and Nunclon TCPS on day 1 after seeding. Averages +/- SEM from 13 to 50 measurements on two independent samples; Student's t-test for unpaired data, *P<0.01 compared to TCPS.

number of cells, different cell morphology and smaller spreading area were observed for PGCap.

Such differences are likely due to much higher hydrophobicity of PGCap surface, resulting from lower content of surface oxygen. The correlation between physico-

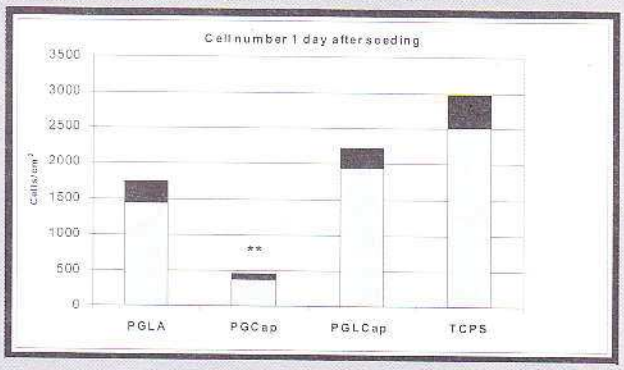

FIG. 5. Number of MG63 cells adhering to PGLA, PGCap, PGLCap, and Nunclon TCPS on day 1 after seeding. Averages +/- SEM from 20 measurements on two independent samples; Student's t-test for unpaired data, **P<0.001 compared to TCPS.

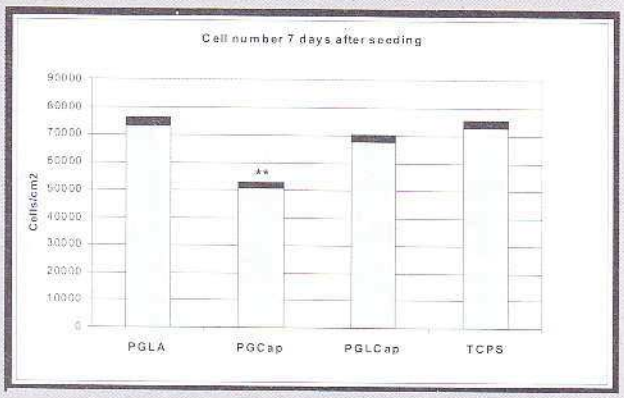

FIG. 6. Number of MG63 cells adhering to PGLA, PGCap, PGLCap and Nunclon TCPS on day 7 after seeding. Averages +/- SEM from 6 measurements on two independent samples; Student's t-test for unpaired data, **P<0.001 compared to TCPS.

chemical surface properties and cell behaviour, provided by the present study, may help in better understanding of the phenomena at the interface of a biomaterial and its biological environment. Moreover, it may provide some idea how to modify cell behaviour by simple changing the material chemical composition.

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INFLUENCE OF SURFACE PROPERTIES OF CARBON BASED MATERIALS ON CELL SPREADING

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Abstract

Carbon materials are generally well tolerated by animal cells. The possibility of applying carbon fiber reinforced carbon (CFRC) composite materials is given by their excellent biocompatibility and porosity, coupled with a modulus which can be tailored to be similar to that of bone. This makes them an attractive material for bone plates and implants in orthopaedic and dental surgery. It is known that the volume properties of a material usually have little or no influence on the surrounding living tissue cells. In general, biocompatibility is controlled mainly by the interface between biomaterial and living tissue cells.

The literature and our study indicate that the interaction at the interface is specifically controlled by the surface morphology, (i.e., especially by surface roughness), and by the chemical state of the surface - by hydrophobia (wettability), free chemical bonds and present chemical groups, etc. Never-theless, biocompatibility can be improved by a suitable change of these parameters. There are several possible methods for influencing the roughness and chemical state of the surface. One way to change the surface properties is by preparing a suitable coating. The properties of the surface are controlled by process technology, and the grinding and polishing of the substrate can be used for roughness control.

Till now we studied the influence of the surface on the cell adhesion and on the rate of the cell growth. There, we have studied the influence of a surface coating of CFRC using a several types of layers on the base of carbon. In our present contribution we continue in this work using the surfaces of CFRC in native and polished states, both covered by layers of amorphous carbon, or titanium with carbon or pyrolitic graphite. The vascular smooth muscle cells were grown on these surfaces. The purpose of this paper is to find the influence of the surface on the important parameter of tissue cell growth - the spreading of cells.

The main topic of this work is therefore the measurement and statistical evaluation of the cell area on the various types of surfaces. It will be shown, that the cell spreading is strongly influenced by various surface roughness and also its chemical state.

[Engineering of Biomaterials, 37, (2004), 17]