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Biological behaviour of an endothelial cell line (HPMEC) on vascular prostheses grafted with hydroxypropylgamma-cyclodextrine (HPγ-CD) and hydroxypropylbeta-cyclodextrine (HPβ-CD)

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Abstract The cytocompatibility of cyclodextrins (CDs) grafting on vascular polyester (PET) prostheses for further loading with biomolecules was investigated in this study. Viability tests demonstrated no toxicity of HP-CDs and PolyHP-CDs at 4,000 mg/l with survival rates of 80 to 96%. Proliferation tests using the human pulmonary microvascular endothelial cell line (HPMEC-ST1) revealed an excellent biocompatibility for Melinex® (Film form of PET). For Polythese® and Polymaille®, a good proliferation rate was observed at 3 days (60–80%) but decreased at 6 days (56–73%). For all CD-grafted samples, low proliferation rates were observed after 6 days (35–38%). Vitality tests revealed excellent functional capacities of HPMEC cells after 3 and 6 days for all samples. Adhesion kinetics tests showed a similar adhesion of HPMEC cells on control and Melinex®. A low adhesion was observed on Polythese® and especially on Polymaille® compared to control. After CD grafting, the cell adhesion was decreased. The woven or knitted architecture and CD grafting were the most likely causes of this weak adhesion. The adhesion kinetic test was confirmed by SEM observations and immunocytochemistry. The low proliferation of HPMEC on virgin prostheses and especially on grafted prostheses was not due to a cytotoxic effect, but to the physical surface characteristics of the prostheses.

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1 Introduction

Vascular polyester (PET, Dacron®) prostheses have been implanted by vascular surgeons since 40 years to replace damaged arteries (atherosclerotic or aneurysmal lesions) [1]. After implantation, several complications have been documented, in particular infections, which are associated with a high morbidity rate [2, 3]. To reduce this risk of infection, multiple techniques of impregnation and stabilizing antibiotics and other antibacterial agents have been applied on vascular grafts and evaluated in different experimental models [4–6].

As our primary aim is to reduce or to hinder the formation of a biofilm, we developed a new-concept drug delivery system, involving the grafting hydroxypropylgamma-cyclodextrine (HPγ-CD) and hydroxypropylbeta-cyclodextrine (HPβ-CD) on PET vascular prostheses [7–9]. All studies on CDs performed up to now did not exhibit any toxicity. Moreover, many investigations on animals revealed neither oral nor i.v. toxicity from CDs even at very high concentrations. For example, a β-CD uptake in dogs induced no oral toxicity up to 1,831 mg/kg/day

[10, 11]. These grafted CDs act as cage molecules and can release previously loaded bioactive molecules over a prolonged period on account of the external hydrophilic and internal hydrophobic interactions between HPγ-CD or HPβ-CD and different bioactive molecules [12–14]. Previous investigations demonstrated a long span of releasing the antibiotic vancomycin during 40 days in so-called physiological solutions, e.g. 0.9% NaCl [15]. Biocompatibility of vascular prostheses was also studied previously with the epithelial cell line L132. A low proliferation of these epithelial cells was reported on vascular prostheses grafted or ungrafted with CDs [16]. We ascribed this low proliferation to the woven structure of vascular prostheses, and to the collagen coating that is inadequate for the growth of the epithelial cell line. The aim of the present work was to evaluate the cytocompatibility of these grafted prostheses using an endothelial cell line immortalized by one of the cooperation partners in 2001 [17]. They have generated a permanent cell line from human pulmonary microvascular endothelial cells (HPMEC) isolated from adult donors. This cell line maintains important characteristics of the endothelial phenotype.

2 Materials and methods

Polyester (polyethyleneterephtalate, PET) prostheses were manufactured by Laboratoires Perouse (Ivry-Le-Temple, France). They are commercialized as Polythese® (woven fibres, 1 yarn of 84 dTex and 1 yarn of 167 dTex, surfacial weight = 133 g/m^2) and Polymaille® (knitted fibres, 2 yarns of 100 dTex, surfacial weight = 255 g/m^2).

Melinex® was supplied by Dupont Teijin Films™. This material is free of any additives and serves as film-covers-like overhead films with a thickness of 250 μm and a biaxial orientation. Before sterilization, the PET films were ultrasonically cleaned with ethanol during 20 min.

Hydroxypropyl-γ-CDs (HPγ-CDs) were provided by Wacker Specialties GmbH (Cavamax®). Hydroxypropyl-β-CDs (HPβ-CDs) were provided by Roquette (Kleptose®). Citric acid (CTR) and sodium dihydrogen hypophosphite were supplied by Aldrich chemicals.

For biological tests, 15 mm diameter disks were cut from Melinex® and from untreated and grafted PET prostheses. PolyHPβ-CD and PolyHPγ-CD were synthesized with CTR, catalyst and CDs at respective concentrations of 80, 10 and 100 g/l. Two soluble polymers were obtained. After neutralization of residual CTR with sodium carbonate at 4 g/l, a viability test was performed [9, 18]. PET prostheses were grafted at 6.7 wt.% and then coated with a thin collagen layer. Negative and positive controls were, respectively, Thermanox® (VWR International) and nickel (99.9999% pureness, Goodfellow).

2.1 In vitro experiments

In vitro cell tests were performed with the human pulmonary micro vascular endothelial cell line HPMEC-ST1, according to the International and European standards (ISO 10993-5/EN 30993-5) [19]. HPMEC cells were cultured in Endothelial Cell Growth Medium MV (Promocell), supplemented with 15% foetal calf serum (FCS, Gibco-Invitrogen) [18]. All media contained streptomycin (0.1 g/l) and penicillin (100 IU/ml). All in vitro cell incubations were performed at 37°C in 5% CO_2 atmosphere and 100% relative humidity in a CO_2 incubator (CB 150/APT line/Binder) with high stability of all technical parameters.

2.2 Viability test

The viability tests evaluated the relative plating efficiency (RPE) and subsequently the 50% lethal concentration LC50 (or RPE 50) by using the colony-forming method with L132 cells [18, 20]. The epithelial cell line (L132 cells) was selected for its good reproducibility and cloning efficiency of about 37% [21]. Cells were continuously exposed in MEM medium supplemented with 10% FCS to gradually increasing concentrations of HPβ-CDs, HPγ-CDs, polyHPβ-CDs and polyHPγ-CDs (0, 25, 50, 100, 200, 400, 1,000, 2,000, 4,000 mg/l) without renewal of the growth medium during the experiments. After a 9-day period, the medium was removed and the colonies were stained with crystal violet. The clones were then counted under a binocular microscope. At least eight repeated experiments were performed in triplicates for each concentration. Results are expressed as mean values ± SD with respect to the control (100%), and are compared with nickel as positive control.

2.3 Cell proliferation

The growth period for cell proliferation tests was 3 and 6 days without renewal of the medium [18]. PET disks were placed in the bottom of 24-well plates (Costar®, Starlab) after gamma sterilization. Viton® rings (Radiospares) were added to maintain the samples on the bottom of the wells and subsequently to avoid cells growing beneath the test samples. Then 10^4 cells were gently seeded in each well. Wells filled with cell suspension only served as control. After detachment of cells in a trypsin-EDTA solution (Gibco-Iinvitrogen), their number was determined using a cell counter Z1 (Coulter Electronics). Three samples of each group were used for one test. Results are expressed as the mean percentage of six separate triplicate assays for each PET test material.

2.4 Cell vitality

Cell vitality was assessed with the non-toxic Alamar Blue dye simultaneously with the proliferation test [22]. Three or 6 days after the cell seeding, and just before cell counting, the culture medium was removed from each well. About 500 µl diluted fluorescent dye (Interchim) was deposited in each well. After 3 h incubation, the solutions were transferred into 96-well plates (VWR International), and the absorbence was measured by fluorometry (Twinkle LB970™—Berthold) at 560 nm. The cell vitality rate was calculated as the absorbence of living cells on PET samples divided by the absorbence of control cells. Data were expressed as the mean percentage ± SD with respect to the control culture (100%).

2.5 Cell adhesion

For cell adhesion tests, the number of attached cells were quantified using a hexosaminidase assay. Briefly, 4×10^4 growing cells were gently seeded in each well. After 2 h incubation at 37°C in 5% CO_2, the media in the wells were aspirated, and 300 µl of a dye (*p*-NPP: *para*-Nitrophenyl phosphate, Sigma) were added [23]. After 3 h incubation at 37°C, 150 µl of 1 N NaOH were added in each well to develop the colour reaction and to stop the enzymatic reaction. Then 200 µl were removed from the wells and transferred into a 96-well plate (VWR International). The absorbence was measured by spectrophotometry at 405 nm (Apollo LB911™—Bertold). Six different triplicate assays were run for each test. The results of cell adhesion were compared to the control (wells without test samples).

2.6 Confocal laser scanning microscopy

Two different proteins have been visualised by confocal laser scanning microscopy (CLSM): cytoskeletal actin and vinculin contained in focal adhesion contacts. After 3-day culture on test samples, the cells were fixed for 20 min in 2% *para*-formaldehyde at room temperature, permeabilized in a PBS-Triton X-100 buffer (Sigma), and then blocked with 1% bovine serum albumin (Sigma) in PBS (Sigma).

Cytoskeletal organization: Actin filaments were directly labelled with 1.2 µg/ml FITC-phalloidin (Sigma).

Focal contact formation: cells were immunostained with 300 µl of a 1:50 diluted mouse anti-chicken monoclonal antibody against vinculin (Sigma) followed by FITC-rabbit-anti-mouse antibody (Sigma) [24].

After rinsing in buffer, the specimens were embedded in PBS-Glycerol-DABCO (1:1) (Sigma) mounting medium and examined by CLSM (LEICA TCS NT). For these observations, cell nuclei were previously labelled red using propidium iodide (Sigma).

2.7 Scanning electron microscopy

Cells were fixed with 2.5% glutaraldehyde (Sigma) in 0.1 M sodium phosphate buffered (Sigma), pH 7. After two washes in the rinsing buffer, they were post-fixed with 1% OsO_4 (VWR International) in saturated $HgCl_2$ (Sigma), then dehydrated in graded ethanol, critically point dried, and finally sputtered with gold/palladium. Surface morphology of untreated and grafted PET prostheses and cell morphology were observed with a SEM (JEOL J-SM-5300), using an accelerating voltage of 30 kV and a current of 100 µA [25].

2.8 Statistic analysis

One-way ANOVA (SPSS 11.0 software) was applied for all quantitive data from cell proliferation, vitality and adhesion test, in order to determine the statistical significance of the differences observed between test groups: $P < 0.05$ was considered as significant.

3 Results

3.1 Viability test

The first biological test was carried out to confirm the non-toxicity of HP-CDs and poly HP-CDs. Soluble powders of HPβ-CD and HPγ-CD demonstrated an excellent biological behaviour up to extremely high concentrations (4,000 mg/l) with respective survival rates of 96 and 92% (Figs. 1 and 2). In addition, the soluble PolyHP-CDs revealed no toxicity at the same extremely high concentration (4,000 mg/l) and produced 80 and 79% survival rates, respectively, for PolyHPβ-CD and PolyHPγ-CD.

3.2 Cell proliferation

Cell proliferation assays in direct contact with Melinex® showed an excellent proliferation of HPMEC-ST1 cells after 3 and 6 days. The proliferation was 71% at 3 days and increased to 86% after 6 days. On Polythese®, a good proliferation of endothelial cells was observed after 3 days (79%), but it decreased after 6 days (69%), which was probably due to the textile configuration of the PET. Finally, on HP-CDs grafted Polythese®, a lower

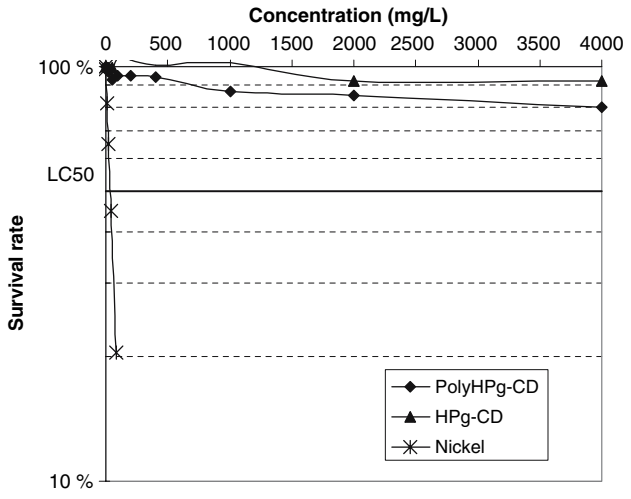

Fig. 1 Survival rate of L132 cells after exposure to HPγ-CD and PolyHPγ-CD

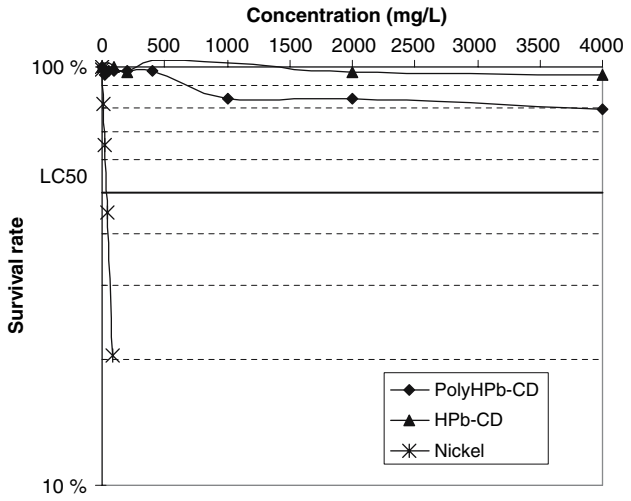

Fig. 2 Survival rate of L132 cells after exposure to HPβ-CD and PolyHPβ-CD

proliferation was observed on incubation compared to ungrafted Polythese®. In fact, respectively, 36 and 56% proliferation were obtained with cultures on Polythese® HPβ-CD and Polythese® HPγ-CD (Fig. 3). On Polymaille®, a similar pattern could be observed. Proliferation rates of 38 and 35% were obtained for cells cultured for 6 days on Polymaille® HPβ-CD and Polymaille® HPγ-CD, respectively, whereas 55% proliferation was obtained on ungrafted Polymaille®. The lower proliferation on grafted vascular prostheses compared to virgin prostheses was not a toxic effect of HP-CDs, since viability tests revealed no toxicity of the CD polymer even at very high concentration. This test demonstrated that the low proliferation of the HPMEC-ST1 cell line on Polythese® and Polymaille® compared to Melinex® was probably a result of the woven or knitted structure of the vascular prosthesis, and was similar to the reaction of L132 epithelial cells [16]. The low proliferation of grafted prostheses compared to virgin prostheses could be explained by the roughness of the surface coated with the CD polymer.

3.3 Cell vitality

Vitality tests were performed at the same time as the proliferation test after 3 and 6 days of incubation. An excellent vitality of HPMEC cells was observed on Melinex® after 3 and 6 days (respectively, 94 and 100%). The cell vitality confirmed the good proliferation on Melinex®. The same pattern of cell vitality on Polythese® (94%), Polythese® HPβ-CD (83%) and Polythese® HPγ-CD (102%) was observed, corresponding to the good cell proliferation after the 6-day incubation. Cells grown for 6 days on Polymaille® (94%), Polymaille® HPβ-CD (74%) and Polymaille® HPγ-CD (88%) demonstrated a similar cell vitality compared with Polythese® (Fig. 4). These vitality rates showed

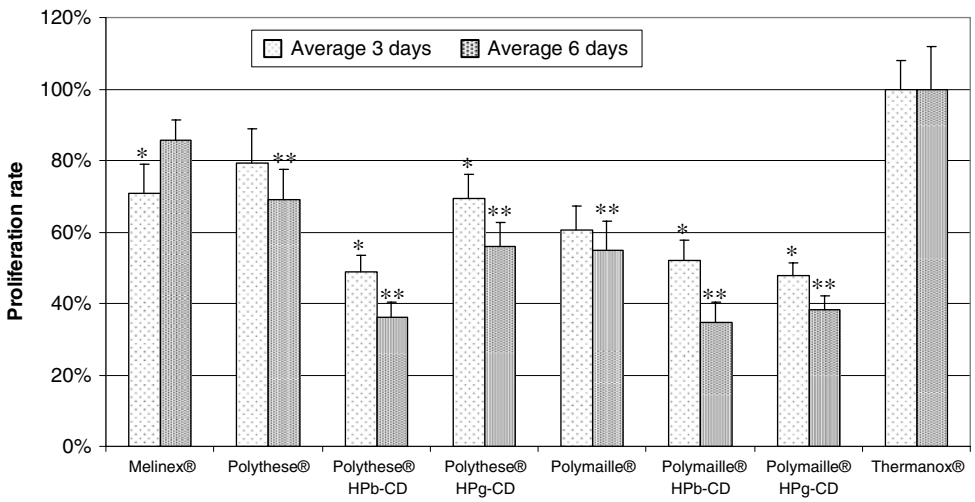

Fig. 3 Proliferation rate of HPMEC cells grown on Polythese® and Polymaille® grafted with HP-CDs after 3 and 6 days incubation. Statistical significance was assessed relative to Thermonox for all other groups with denoting * (for 3 day) and ** (for 6 day) ($P < 0.05$)

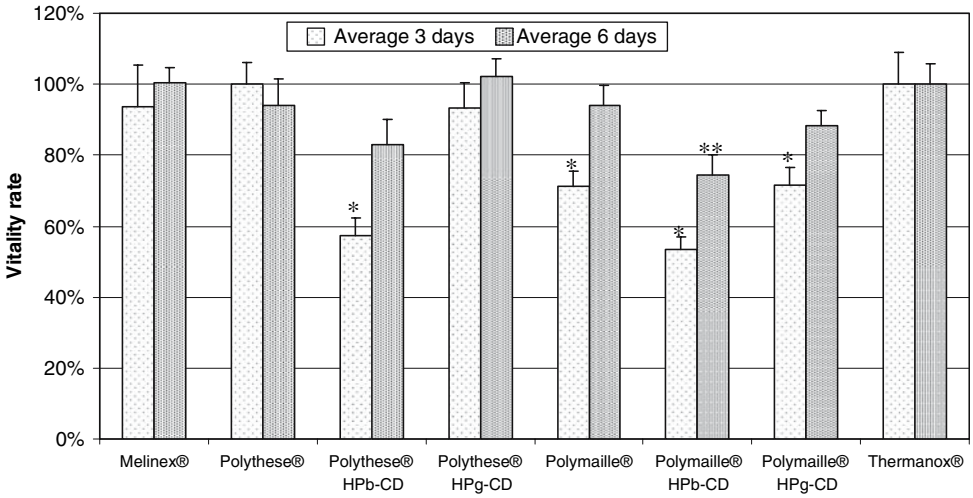

Fig. 4 Vitality rate of HPMEC cells grown on Polythese® and Polymaille® grafted with HP-CDs after 3 and 6 days incubation. Statistical significance was assessed relative to Thermonox for all other groups with denoting * (for 3 day) and ** (for 6 day) ($P < 0.05$)

a slight increase of cellular activity with respect to cell proliferation, in particular for grafted prostheses. CD grafting had a positive effect on cell vitality.

3.4 Cell adhesion

The kinetics of cell adhesion were similar for all samples (treated or untreated Melinex®, Polythese® and Polymaille®) and Thermanox®. The rate of cell adhesion was similar on Melinex® and on the TCPS control, and for each time point the number of attached cells was the same. On woven and knitted PET, the kinetics of adhesion were slower than that on Thermanox® and Melinex®. After 2 h adhesion, the numbers of attached cells were less on Polythese® compared to Melinex® and less on Polymaille® compared to Polythese® (Fig. 5). The woven and especially the knitted form of PET had an unfavourable effect on cell adhesion. Moreover, after CD grafting and on both structural prosthesis types, the cell attachment decreases for each time point examined. This "weak" adhesion on grafted prostheses was probably due to the roughness of the CD polymer on the surface of the fibres.

3.5 Morphology

Cell morphology was assessed by SEM after a 3- and a 6-day culture period without renewal of the culture medium (Fig. 6). Cells grown on TCPS control and on Melinex® had a rather flattened and wide spread form, and exhibited multiple cytoplasmic prolongations on the cell periphery and short microvilli on the cell surface (Fig. 6a, b). Cells grown on Polythese® and on Polythese® HP-CD were found mostly in the tracks of the fibrils, they had still the wide spread form covering sometimes two or three fibrils (Fig. 6c, e). They exhibited, however, only very few cytoplasmic prolongations at their periphery and the microvilli on the control cells have changed to spherical structures on the cell surface. Cells grown on Polymaille®

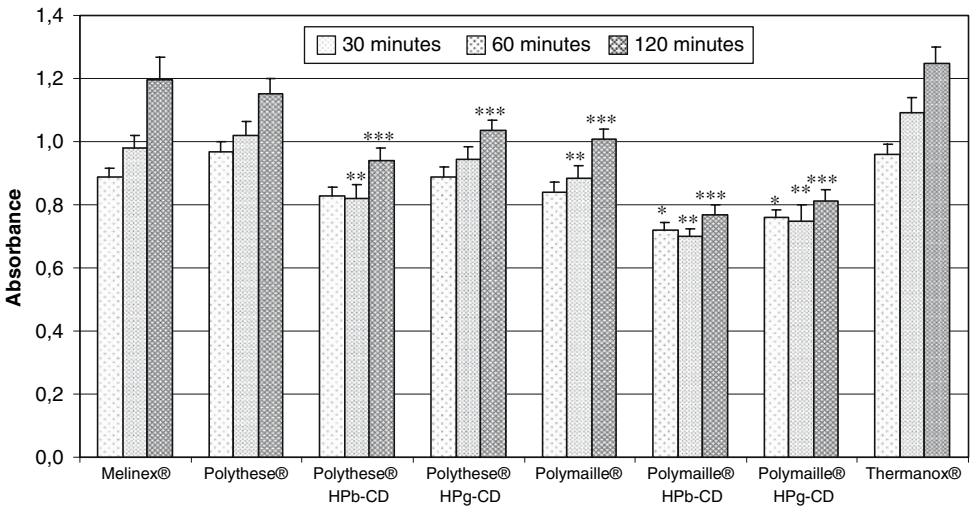

Fig. 5 Kinetic of adhesion of HPMEC cells on Polythese® and Polythese® Cyclo-D. Statistical significance was assessed relative to Thermonox for all other groups with denoting * (for 30 min), ** (for 60 min) and *** (for 120 min) ($P < 0.05$)

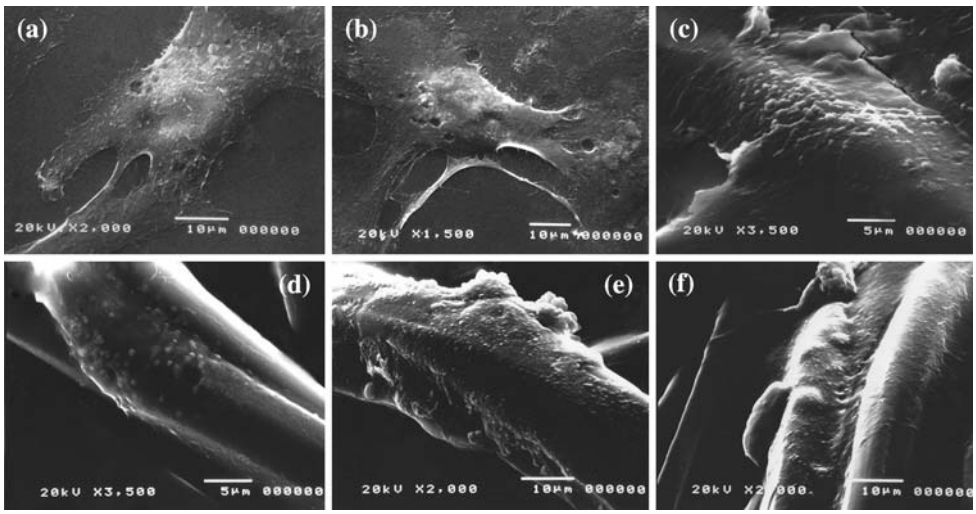

Fig. 6 Morphology of HPMEC control cells (**a**) and of cells grown on Melinex® (**b**), on Polythese® (**c**), Polymaille® (**d**), Polythese® HP-CD (**e**) and Polymaille® HP-CD (**f**)

and on Polymaille® HP-CD had a similar aspect enveloping the fibrils (Fig. 6d, f). Also here, we observed only very few cytoplasmic prolongations. No differences could be detected between cells grown on HPβ-CD and those grown on HPγ-CD. The weak adhesion of endothelial cells on virgin and grafted prostheses shown by these micrographs completed the results of adhesion kinetic assessments.

3.6 Immunocytochemistry

The observation of antibody-labelled HPMEC cells confirmed the polygonal, mostly elongated form. The cells grown on Thermanox® (Fig. 7a) and on Melinex® (Fig. 7b) showed a well-developed cytoskeleton with active spreading of multiple actin stress fibres. Cells cultured on Thermanox® also exhibited numerous filopodia and interconnections between cells demonstrating a good adhesion behaviour. These filopodia are less remarkable on cells grown on Melinex® probably due to the more hydrophobic state of this material.

An irregular cytoskeleton was observed in the endothelial cells grown on the test samples of virgin and CD-grafted woven Polythese® (Fig. 7c, d) and a very low amount of actin filaments could be detected on the virgin and CD-grafted knitted Polymaille® (Fig. 7 e, f). The form of these cells is mostly determined by the yarns and the grooves of the woven or knitted PET tissue.

A similar adhesion behaviour was observed by the labelling of focal adhesion contacts with anti-vinculin

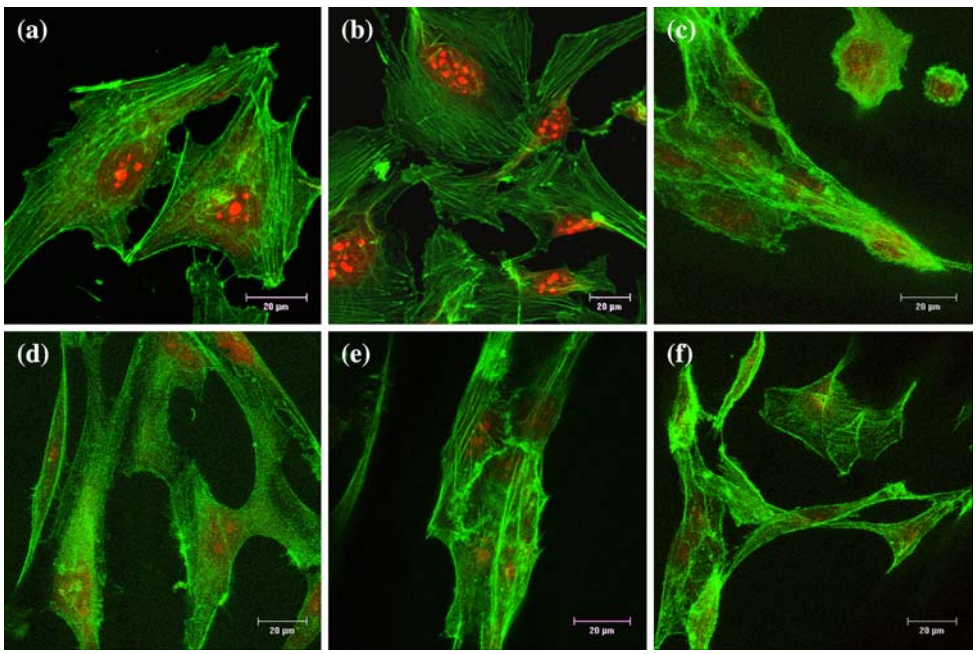

Fig. 7 Cytoskeleton assessment in HPMEC control cells (**a**) and of cells grown on Melinex® (**b**), on Polythese® (**c**), Polymaille® (**d**), Polythese® HP-CD (**e**) and Polymaille® HP-CD (**f**)

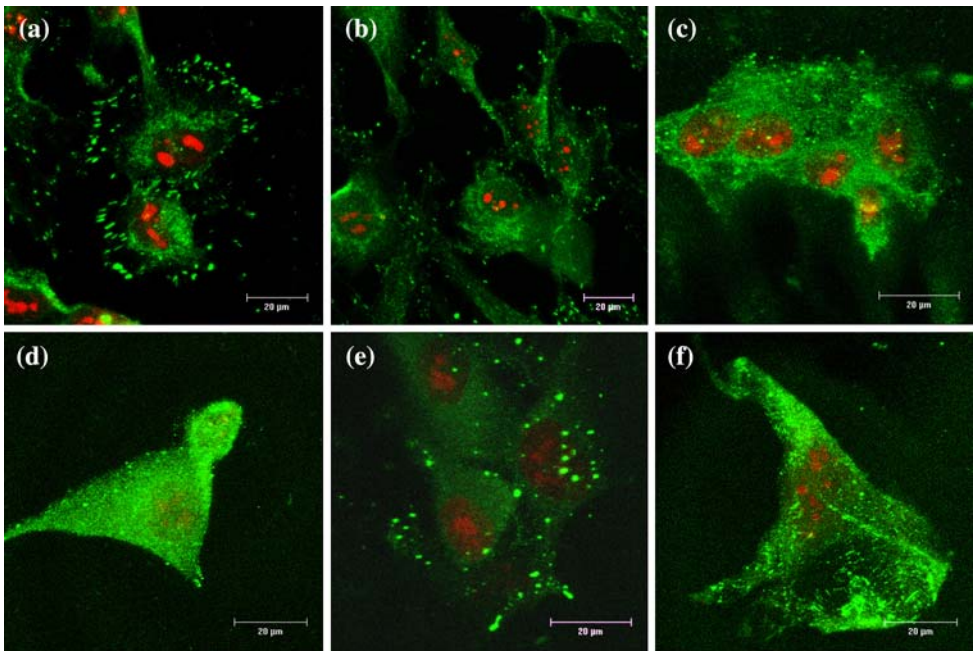

Fig. 8 Immuno-cytochemical labelling of vinculin in focal adhesion contacts in HPMEC control cells (**a**) and of cells grown on Melinex® (**b**), on Polythese® (**c**), Polymaile® (**d**), Polythese® HP-CD (**e**) and Polymaille® HP-CD (f)

(Fig. 8). The cells grown on Thermanox® (Fig. 8a) and in particular on Melinex® (Fig. 8b) have a spread form with regular distribution of focal adhesion contacts of similar size all over the area covered by the cells. A large amount of focal contacts is also observed in the endothelial cells grown on the test samples of virgin and CD-grafted woven Polythese® (Fig. 8c, d). The size of these focal contacts, however, is much larger than in control and reference cultures, and in some areas the overall labelling of vinculin suggests a disassembling of the focal contacts. This latter aspect becomes more evident in cells grown on the virgin and CD-grafted knitted Polymaille® (Fig. 8e, f), where only a few well-defined focal contacts can be seen. These findings are in keeping with the weak adhesion of HPMEC cells on PET tissue already observed in the adhesion kinetics assays, the morphological assessment and the cytoskeletal distribution.

4 Discussion

Cytocompatibility tests are performed to determine the integration of an implant (prostheses, cement, etc.) in the human organism. For all tests presented in this paper, Thermanox® was used as control and Melinex® as reference. Melinex® is a PET film with exactly the same composition as the woven PET used in vascular prostheses (Polythese® and Polymaille®). Using proliferation tests we observed a significant difference between the three forms of PET (film, woven, knitted). An excellent cytocompatibility was observed on these three PET, but the proliferation increased on Melinex® after 6 days, whereas it decreased on Polythese® and Polymaille®. The CD grafting (Polythese® HP-CDs and Polymaille® HP-CDs) induced a further decrease of HPMEC proliferation compared to virgin prostheses. In fact, after a 6-day incubation, a proliferation of 35% on Polymaille® HP-CD was observed compared to 55% for Polymaille®. A lower proliferation decrease was observed with Polythese® and Polythese® HP-CDs. These differences with the virgin PET tissues are probably due to the texture of the tissues, which is much rougher for Polymaille® than for Polythese®, i.e. the knitted tissue provides much more irregularities than the woven tissue. The regular surface tracks of this latter may give to the cells an orientation along the microfilaments of the PET yarns [26–30], which may help the cells to compensate the hydrophobic nature of the surface, unfavourable for an optimal cell adhesion and cell proliferation [31–34].

The viability test determines quantitatively only one criterion of toxicity, which is cell death or cell survival, and is easily reproducible. This test allows ranking of the cytotoxicity effect of any chemical substance by the comparison of the 50% lethal concentrations (LC50) [18, 20, 21]. Previous investigations revealed no toxicity either of Polythese® and Polymaille® [16] or of HP-CD and PolyHP-CD even at extremely high concentrations: The values were 96 and 80% survival rates for HPβ-CD and PolyHPγ-CD at 4,000 mg/l, respectively; and 92 and 79% survival rates for HPβ-CD and PolyHP γ-CD at 4000 mg/l, respectively. With respect to these excellent results of cell viability, the lower proliferation rate revealed on grafted prostheses cannot be explained by a cytotoxic effect.

The vitality test revealed a high cell function on treated and untreated prostheses with HP-CD. The metabolic activity of endothelial cells was slightly higher compared to proliferation. The measurements of cell vitality are based on the oxidation/reduction level of Alamar Blue. The one-step Alamar Blue assay involves the addition of a fluorogenic redox indicator to growing cells in culture. Healthy and well proliferating cells produce intracellular reducing compounds such as $FADH_2$, NADH, NADPH, and have also a preserved cytochrome activity contributing to the reduction of Alamar Blue [22]. The oxidized form of Alamar Blue is a dark blue colour and has little intrinsic fluorescence. When taken into cells, the dye becomes reduced and turns red. This reduced form of Alamar Blue is highly fluorescent. The extent of this conversion, which is a reflection of cell vitality, can be quantified by its optical density or fluorescence for greater sensitivity [35]. In addition, the Alamar Blue assay has the advantage compared to other cytocompatibility tests of being an easy one-step and non-toxic test and can thus be used for screening purposes for drugs [36]. This means that the endothelial cells studied here with a higher vitality than proliferation rate have a normal if not stimulated cell vitality without any decrease of important cellular functions. In addition, these results clearly show that the Alamar Blue assay is a test for cell vitality and cannot be generally be taken as a measure of proliferation and viability assessment [37].

For a better understanding of this discrepancy between excellent viability, good vitality and decreased proliferation, we performed different approaches to assess the cell adhesion behaviour, i.e. by adhesion kinetics, morphology and cytochemical examination of intracellular processes. Thus, on the Melinex® and TCPS controls similar adhesion kinetics were observed, and morphology was characterized by spread cells with many cytoplasmic prolongations, actin stress fibres and well-defined focal contacts. This corresponds to a normal adhesion behaviour of endothelial cells on these supports [38], and confirms the non-cytotoxicity of PET.

On Polythese®, Polythese® HP-CDs, Polymaille®, Polymaille® HP-CDs, HPMEC exhibited mostly a normal wide spread form and were aligned in the tracks of PET microfibres. This normal cell form reflects the normal cell vitality, and the numerous spherical structures on the cell surface show an increased exchange between cells and the surrounding milieu, that what further explain the increased cell vitality with respect to the cell proliferation. The cells showed also a reduced number of cytoplasmic prolongation, which strongly suggested their weak adhesion on these substrates. Although a similar profile of adhesion kinetics was observed with respect to the control or Melinex®, the number of adherent cells was lower. This disturbed adhesion behaviour was confirmed by the immunocytochemical staining of actin and vinculin in cells on all grafted or ungrafted PET samples, revealing a reduced number of actin stress fibres and imperfect organization of the fibre framework, and disturbed assembling of focal adhesion contacts. The reduced organization of the cytoskeleton is a consequence of the molecular processes during adhesion.

It is important to stress the difference between the early adhesion process, when a cell commences interaction with the substrate, and the continuous adherence strength, once the cell is attached on the substrate. This differentiation is necessary to understand better certain phenomena of the interactions at the material-biosystem interface generating and controlling further cell growth and subsequent tissue integration of an implant or prosthesis [39–41]. The material (alloy, ceramic, polymer), the structure and the surface treatment may have a direct influence on the production of the extra-cellular matrix and on the tissue integration. An unsuitable treatment, however, may lead to the loosening of a prosthesis [28, 30, 42].

The weak adhesion of HPMEC cell on Polythese®, due to its woven form, and Polythese® Cyclo-D, due to the woven form and roughness of grafting, explain the low proliferation of HPMEC on Polythese® and the very low proliferation of Polythese® Cyclo-D compared to Melinex®. For Polymaille®, the same explanation could hold true, with the knitted form giving weaker adhesion than the woven form and CD grafting yielding an increased adhesion. Important roughness of the order of 5–10 μm depth has been shown to induce weaker cell adherence and lower cell proliferation even on highly cytocompatible materials [24, 42, 43], whereas roughness in the nanoscale domain can markedly improve cell proliferation on nearly all cytocompatible materials, mostly accompanied by a decrease of adherence strength at the material/biosystem interface [25, 29, 44, 45].

The lower cytocompatibility of HPMEC cells on grafted prostheses could be explained by a weak adhesion of cells due to two parameters: woven or knitted form of prostheses and the CD coating of grafted prostheses [42]. However, no significant difference could be observed between HPβ-CD and HPγ-CD coating on vascular PET prostheses.

5 Conclusion

Cytocompatibility tests revealed the non-toxicity of HP-CD and PolyHP-CD. The low proliferation of HPMEC cells on Polythese® and Polymaile® compared to Melinex® was explained by a weak adhesion of cells on the woven or knitted form of PET. The very low proliferation of HPMEC cells on Polythese® HP-CD and Polymaille® HP-CD compared to Melinex® was explain by the woven form of

PET and CD grafting (roughness of PET fibres). Nevertheless, the CD grafting was not toxic, although the proliferation was less on grafted prostheses compared to virgin prostheses.

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