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Vascular PET Prostheses Surface Modification with Cyclodextrin Coating: Development of a New Drug Delivery System

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Purpose. *Cyclodextrins (CDs) are torus shaped cyclic oligosaccharides with a hydrophobic internal cavity and a hydrophilic external surface. We performed and analysed an antibiotic binding on Dacron (polyethyleneterephtalate, PET) vascular grafts, previously coated with CDs based polymers.*
Methods. *The CDs coating process was based on the pad-dry-cure method patented in our laboratory. The Dacron prostheses were immersed into a solution containing a polycarboxylic acid, a cyclodextrin and a catalyst, and placed into a thermofixation oven before impregnation with an antibiotic solution (Vancomycin). Biocompatibility tests were performed with L132 human epithelial cells. The antibiotic release in an aqueous medium was assessed by batch type experiments using UV spectroscopy.*
Results. *Viability tests confirmed that the CDs polymers coating the Dacron fibers were not toxic towards L132 cell. Cell proliferation was similar on coated and uncoated grafts.*
A linear release of Vancomycin was observed over 50 days.
Conclusion. *Our results demonstrate the feasibility of coating CDs onto vascular Dacron grafts. Biological tests show no toxicity of the different cyclodextrins coated. A linear release of antibiotics was depicted over 50 days, demonstrating that cyclodextrin grafting was an efficient drug delivery system.*

Keywords: Drug delivery system; Polyethyleneterephtalate; Vascular grafts; Graft coating.

Various modifications have been applied to Dacron (polyethyleneterephtalate, PET) and ePTFE grafts to improve their thrombogenicity and durability. One option is to develop a drug-delivery-system vascular graft, with attachment of active (e.g. anticoagulant, antithrombotic, antibiotics, growth factors) agents to the graft. A major concern is the duration of the agents' function on the graft surface. Various methods are described to attach the 'drug' to the vascular graft and progressively release it: including heparin ionic binding with a cationic agent (tridodecil-methyl-ammonium-chloride),[1] silver ions incorporated to the collagen sealant coating,[2] Rifampicin ionically bound to modified gelatin,[3] fibroblast growth factor incorporated in fibrin glue impregnated on the vascular graft,[4] plasma polymerization (deposition of polymers under the influence of partially ionized gas),[5] tissue engineering.[6]

Cyclodextrins (CDs) are truncated torus shaped cyclic oligosaccharides, issued from enzymatic degradation of starch. They are made of 6–8 glucopyranosic units (α, β, and γ-CD, respectively), with a hydrophobic internal cavity and a hydrophilic external wall.[7–9] This configuration allows the CDs to capture various active molecules (e.g. biocides, fragrances, dyes, drugs) and progressively release them unmodified.[10] The coating technique of CDs onto textile Dacron was developed in our laboratory and previously reported.[11,12] The Dacron fibers are coated by a polymer network of cross-linked CDs. It has already been reported that CDs have the ability to interact with antiseptics and antibiotics, such as Chlorhexidine,

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Vancomycine or Rifampicin,[13] with a delayed drug delivery action.[8]

We performed and analysed the binding of an antibiotic onto a Dacron vascular graft, previously coated with cyclodextrins.

Material and Methods

The Dacron graft was a polythese vascular graft provided by Laboratoires Perouse (Ivry-Le-Temple, France), with the following characteristics: woven PET, 2 yarns of 100 dTex, 24 mm diameter, crimped, surface weight $=133\ g/m^2$. β-CDs and γ-CD were gifts from Roquette (Lestrem, France) and Wacker Chemie GmbH (Burhausen, Germany), respectively. Citric acid (CTR) (used as crosslinking agent) and sodium dihydrogen hypophosphite (catalyst) were provided by Aldrich chemicals (Milwaukee, WI, USA).

Cyclodextrin grafting

The coating process was based on the pad-dry-cure method, currently applied in the textile industry.[14] CDs, catalyst and CTR were solubilized in water. Prostheses were impregnated by this solution, roll-squeezed and dried at 90 °C. Coating was performed in a thermofixation oven. Polymerisation by polycondensation between CTR and CDs occurred during this step and resulted in a crosslinked CD polymer that physically adhered to the Dacron fibers. The modified prostheses were finally rinsed with warm water and were then submitted to successive extractions with hexane, ethanol, and water, in order to eliminate unreacted products. The rate of CDs coated onto the Dacron grafts was related to the temperature and the time of the thermofixation reaction. It was evaluated according to the weight increase of the prostheses. Prostheses with 5%-wt and 10%-wt were used in the following experiments.

Biological tests

Biological tests were carried out to observe if the polymerized CDs located on the surface of the graft modified the Dacron biocompatibility. Human epithelial embryonic cells (cell-line L132, ATCC-CCL5) were used for all biological tests. Cells were incubated at 37 °C in a 5% CO_2 atmosphere with a 100% relative humidity. Earl's minimum essential medium (MEM) supplemented with 5% foetal calf serum containing L-glutamine (Gribco BRL), streptomycin (0.1 g/l) and penicillin (100 UI/ml) was used. To assess cell proliferation, a disk of each textile sample (untreated Dacron, 5% wt-CDs and 10% wt-CDs coated Dacron, and Nickel) was placed in the bottom of a 15.5 mm Multiwell plate (COSTAR 24 wells) with sterile forceps.[15] For each sample, four replicates were performed. On each disk, 10,000 cells in 1 ml of culture medium supplemented with 5% foetal calf serum (Eurobio) were placed. Cell proliferation in empty culture chambers was also assessed (control group). The culture chambers were separately harvested with trypsin 3 and 6 days after the beginning of the experiment. Cell counting was performed (three times for each sample) using a Coulter ZI cell counter. Cell proliferation rate was calculated as a ratio between the number of cells on the textile and the number of cells in the empty control culture chambers. Viability tests assessed the relative plating efficiency (RPE) and subsequently, the 50% lethal concentration (LC50), using the colony forming method on culture of human epithelial cells (L132).[15] Cells were incubated for 9 days in MEM medium supplemented with 10% foetal bovine serum under 5% CO_2 atmosphere. Cells were continuously exposed to increasing concentrations (0, 25, 50, 100, 200, and 400 μg/ml) of polymer of CDs, untreated Dacron (negative control), and Nickel (positive control), without renewal of the growth medium during the experiments. Eight similar experiments were performed for each concentration. After the incubation period, the medium was withdrawn and the colonies were coloured with crystal violet dye. After drying, the clones were counted with a binocular microscope.

Antibiotic binding and release quantification

γCDs-coated (5 and 10%-wt grafting rates) grafts were immersed into a 5 g/l solution of Vancomycin (Vancomycin chlorydrate, Merck, Darmstadt, Germany) for 4 h. For each sample, four replicates were performed. They were dried for 12 h at 37 °C. The samples were placed in physiologic medium for 80 days. Every day, an aliquot of physiologic medium was withdrawn and analysed by UV spectroscopy (Fluorocount—Pakard) at 280 nm to quantify the amount of Vancomycin released by the CD-coated graft.

Results

Biological tests

Viability tests (Fig. 1) revealed no toxicity of β-CD and γ-CD polymer. A minimum of 50% survival rate was

always observed. CD-coated samples showed similar viability tests results compared to uncoated Dacron. Proliferation tests (Fig. 2) confirmed these results. L132 cells did not proliferate on woven Dacron (12% proliferation after 3 days, decreasing at 3% after 6 days). On CD-coated Dacron samples, the results were similar (19% proliferation after 3 days decreasing at 5% after 6 days).

Antibiotic release

The release of Vancomycin (Fig. 3) was linear during more than 50 days at a rate of 0.30 mg/l/day and 0.13 mg/l/day, respectively, for 10%-wt and 5%-wt coated γ-CD prostheses. Untreated Dacron grafts released the total amount of antibiotic within 1 h into the batch aqueous medium.

Discussion

This study demonstrated the feasibility of cyclodextrin coating on vascular Dacron grafts using a process previously developed[12] by our laboratory. It also demonstrated that the Dacron–cyclodextrin association was an efficient drug delivery system. The degree of polymerization and the crosslinking rate of the CD-polymer fashioned in the Dacron fiber network allowed formation of a stable three dimensional CD-polymer network. A different process is currently being developed to coat expended PTFE with CDs. We, therefore, focused our investigations on the development of a drug delivery system on Dacron prostheses. Some authors report that ePTFE, with its microporous structure, offers a better resistance to graft infection compared to Dacron.[16] However, most clinical series report no significant difference in infection rate when comparing Dacron and ePTFE vascular grafts.[17]

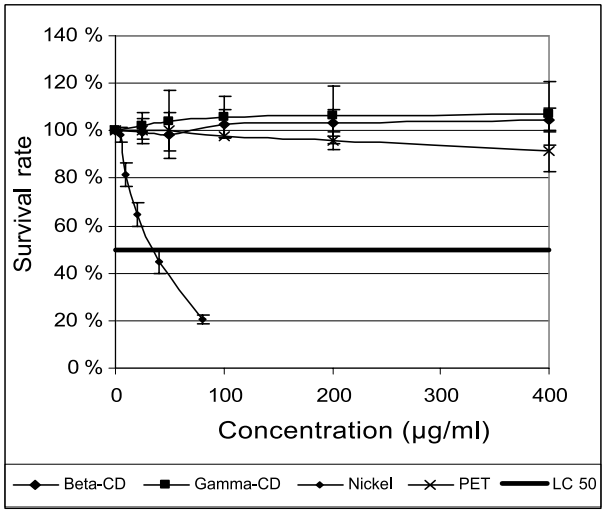

Fig. 1. Viability of human embryonic epithelial cells L132 after a 9-day exposure to increasing concentrations of beta-cyclodextrin, gamma-cyclodextrin, Nickel and pure untreated PET (LC50 = 50% lethal concentration).

CDs are considered 'high performance pharmaceutical excipients'.[18] Their toxicity has been evaluated, depending on the route of administration, the cavity size (α, β or γ-CD), and chemical modification (hydroxypropyl-CD, sulfobutylether-CD, CD sulphate, methylated CD). Therefore, it was important in the present project to verify the innocuous nature of the polymerized CD present on the surface of the modified Dacron fibers. The biological tests we performed did not demonstrate any toxicity of the CD-polymers on the Dacron surface. Cell proliferation tests showed a low proliferation and activity of human embryonic epithelial cells L132. CD-coating did not modify cell response compared to untreated Dacron. The external component of the cyclodextrins, which contains many hydrophilic hydroxide groups, could improve the interaction between the PET and the cells.

Infection of vascular grafts occurs in 1–5% of patients. Most early (<4 months) and late infections are secondary to graft contamination during the initial procedure. Late infections can also be secondary to bowel fistula, redo procedures and false aneurysms. The site of the arterial reconstruction has a major incidence on the rate of infection and the bacteria involved. Graft infection treatment is associated with a high rate of morbidity and mortality, with significant time and cost implications. It was previously demonstrated in an experimental animal study that systemic antibiotic prophylaxis associated with local antibiotic prophylaxis significantly decreases the incidence of graft infection.[19] We, therefore, focused the development of our vascular graft drug delivery system towards antibiotic release. Vancomycin, the antibiotic used in the present study, was selected because of its stability in physiological medium. The release of antibiotic was, therefore, easily quantified by UV spectroscopy. A linear release of Vancomycin in physiological medium was depicted over a 50-day period. Our study aim was to demonstrate the feasibility of CD grafting and the linear *in vitro* release of an antibiotic. We do not recommend the use of Vancomycin for the treatment of primary or secondary grafts infections. Experimental and clinical data with Rifampicin-bounded Dacron grafts have reported inhibition of bacterial growth and reduction in graft infection rate.[20–23] Rifampicin binding on Dacron grafts previously coated with cyclodextrin could be

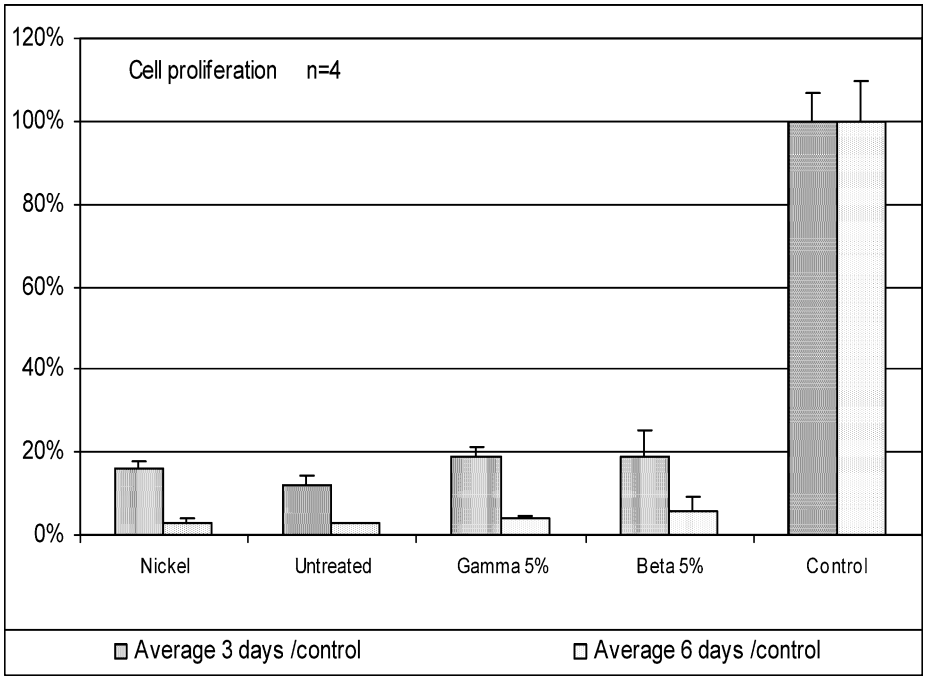

Fig. 2. Proliferation of human embryonic epithelial cells L132 (at 3 and 6 days) on Nickel, untreated PET, and beta and gamma CD coated Dacron.

an alternative to modified gelatin binding to prevent early graft infection. The release of Rifampicin by CDs-Dacron grafts is currently under investigation in our laboratory. Other antibiotics, depending on the type of infection, could also be bound to CDs-Dacron grafts in the setting of late infection.

This study confirmed that cyclodextrins coating was an efficient drug delivery system adapted to

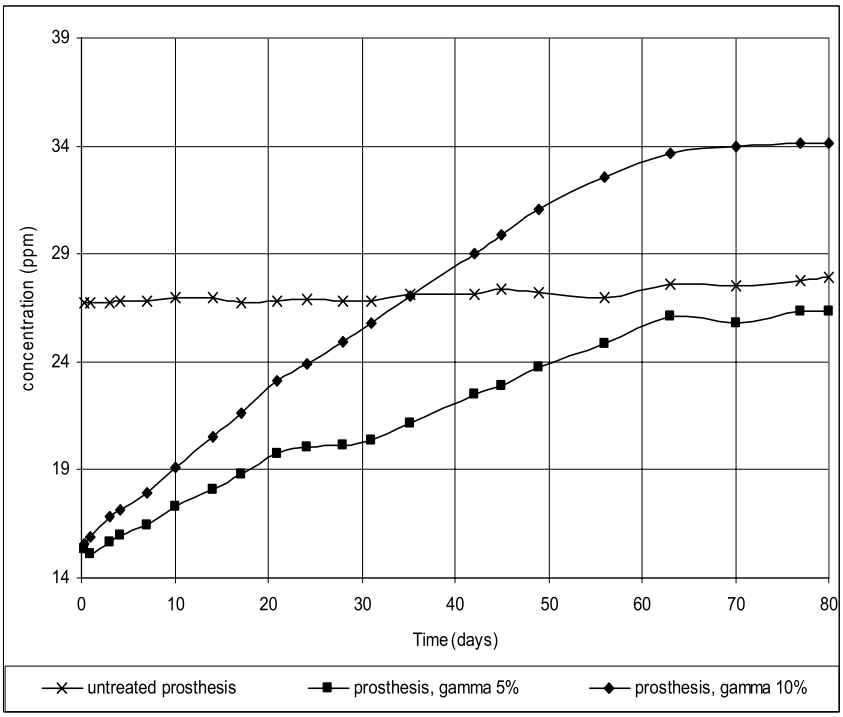

Fig. 3. Release of Vancomycin in physiologic medium during 80 days. Results with uncoated and gamma cyclodextrin coated Dacron prosthesis (coating rate of 5 and 10%).

antibiotics. Many other active molecules could be attached on the vascular prostheses: antithrombotic agents, cell growth or inhibitor factors, extra-cellular matrix. Their evaluation is also scheduled in the present research program.

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