HAL
open science

Heparin and non-heparin-like dextrans differentially modulate endothelial cell proliferation: In vitro evaluation with soluble and crosslinked polysaccharide matrices

Didier Letourneur, Delphine Machy, Anne Pellé, Eva Marcon-Bachari, Gisela d'Angelo, Magali Vogel, Frederic Chaubet, Jean-Baptiste Michel

To cite this version:

Didier Letourneur, Delphine Machy, Anne Pellé, Eva Marcon-Bachari, Gisela d'Angelo, et al.. Heparin and non-heparin-like dextrans differentially modulate endothelial cell proliferation: In vitro evaluation with soluble and crosslinked polysaccharide matrices. Journal of Biomedical Materials Research, 2002, 60 (1), pp.94-100. 10.1002/jbm.10072 . hal-03034029

HAL Id: hal-03034029

https://hal.science/hal-03034029v1

Submitted on 8 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Heparin and non-heparin-like dextrans differentially modulate endothelial cell proliferation: *In vitro* evaluation with soluble and crosslinked polysaccharide matrices

Didier Letourneur,[1,2]* Delphine Machy,[3] Anne Pellé,[4] Eva Marcon-Bachari,[3] Gisela D'Angelo,[4] Magali Vogel,[3] Frédéric Chaubet,[1] Jean-Baptiste Michel[3]
[1]*INVIMAT, X. Bichat Medical School, 75018 Paris, France*
[2]*CNRS—University Paris 13, 93430 Villetaneuse, France*
[3]*LRM, Institut Galilee, University Paris 13, 93430 Villetaneuse, France*
[4]*INSERM U460, X. Bichat Medical School, 75018 Paris, France*

Received 26 June 2001; accepted 4 September 2001

Abstract: Proliferation of endothelial cells (ECs) is a cellular step of particular importance for implanted cardiovascular biomaterials. Heparin and some synthetic water-soluble non-anticoagulant polysaccharides derived from dextran and bearing anionic carboxymethyl and hydrophobic benzylamine groups were first investigated for their effects on EC proliferation *in vitro*. The results assessed by cell counting, ^{3}H-thymidine uptake, and flow cytometry analysis, showed that the derivatized dextran-bearing hydrophobic groups stimulated the EC growth in the presence of serum, whereas native dextran or dextran-bearing anionic carboxymethyl groups were inactive and heparin was slightly inhibitory. Then, we showed that the derivatized dextran enhanced EC proliferation by potentiation of the mitogenic activities of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2), two potent EC growth factors. In the presence of 2 nM of derivatized dextran, a 3-fold and 13-fold increase of ^{3}H-thymidine uptake was obtained with VEGF and FGF-2, respectively. Finally, proliferation of ECs was investigated on crosslinked gels made of polysaccharides. It is of interest that EC proliferation was higher on gels containing the derivatized dextran than on plain hydrogels, and heparinized gels inhibited cell proliferation. From the obtained results, we propose that the synthetic non-heparin-like dextran may be of interest as a coating for the endothelialization of cardiovascular biomaterials. © 2002 John Wiley & Sons, Inc. J Biomed Mater Res 60: 94–100, 2002

Key words: dextran; endothelial cell proliferation; heparin; synthetic polysaccharides; vascular prostheses

INTRODUCTION

Reendothelialization is a physiological phenomenon ending the successful scarring process of an injured vascular wall. Endothelial cell (EC) proliferation is involved in therapeutic angiogenesis, with the induction of new blood vessels clinically relevant after stroke or heart attack, whereas inhibition of vascularization is likely to block tumor growth or retinopathy disease.[1] The use of angiogenic mitogens, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2), have been shown to promote angiogenesis, accelerate endothelial repaving, and attenuate intimal hyperplasia after *in vivo* arterial injury.[2,3] It is of interest that heparin, a sulfated anticoagulant glycosaminoglycan and heparan sulfates, physiological heparin analogs present within the vascular walls, interacted with these growth factors.[4,5]

For the compatibility of synthetic vascular grafts, endothelial cell adhesion and proliferation remain the current goals. Thus, heparin and synthetic or natural analogs are of potential interest for the development of matrices for vascular grafts.[6–9] Among such potential polymers for this application, an increasing number of polysaccharides are studied including native dextran,[10] non-sulfated poly(4-hydroxyphenoxy acetic acid),[11] or sulfated polysaccharides such as suramin, pentosan polysulfate, laminarin sulfate, fucoidan, and dextran sulfate.[10,12–14] In this context, a previous study has shown that some water-soluble sulfated dextrans can mimic heparin in the protection, stabilization, and potentiating effects of FGF-1 and FGF-2 on fibroblastic

Correspondence to: D. Letourneur; e-mail : didier.letourneur@galilee.univ-paris13.fr

Contract grant sponsor: Ministère de l'Education National de la Recherche et de la Technologie; contract grant numbers: 98C253 and 98C254 (D.L. and J.B.M.)
Contract grant sponsor: GIP-Therapeutiques Substitutives doctoral fellowships (D.M. and E.M.B.)
Contract grant sponsor: Société des Amis des Sciences postdoctoral fellowship (G.D.)

© 2002 John Wiley & Sons, Inc.
DOI 10.1002/jbm.10072

cells.[15] These sulfated dextrans were also capable of modulating EC growth.[16] More recently, non-sulfated derivatized dextrans were shown to exhibit direct interactions with FGF-2.[17–19] In the present work, we investigated whether soluble and immobilized derivatized dextrans devoid of sulfate groups, and hence without anticoagulant activity, were able to modulate endothelial cell growth. This work showed that hydrogels containing derivatized dextrans, in contrast to heparin, greatly enhanced endothelial cell growth, providing a new type of synthetic matrix for the coating of vascular prostheses.

MATERIALS AND METHODS

Derivatized dextrans

Derivatized dextrans were prepared from 40-kDa dextran (T40; Pharmacia), as previously described.[16,20,21] Briefly, carboxymethylation of hydroxyl groups on glucose units of native dextran (D) was performed with monochloracetic acid in alkaline medium at 60°C for 1 h. Coupling of benzylamine to carboxylic groups of carboxymethylated dextran (CM) was obtained by using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoleine as coupling agent in water-:ethanol (1:1 v/v) mixture at room temperature. The resulting derivatized dextrans (CM, B) were washed with water and equilibrated at pH 7 with buffer by using a Diaflo ultrafilter (molecular cutoff 5000) from Amicon (Paris, France) and finally lyophilized. Chemical characterizations were performed by acidimetric titrations and elemental analysis as described,[20] and the compositions are reported in Table I. None of the tested derivatized dextrans exhibited a significant anticoagulant activity.[22] Heparin (batch H108; 20 kDa) was from Sanofi Recherche (Gentilly, France). Before use in cell culture, stock solutions of polymers (5 mg/mL) were prepared and filtered on 0.2-μm sterile filters.

TABLE I
Chemical Characterization of Dextran Polymers and EA.Hy 926 Cell Growth[a]

Polymers	Composition (% of dextran units)		EC Growth (%)
	Carboxylate	Benzylamide	
Native dextran (D)	0	0	+2 ± 1
Polymer CM	61	0	+8 ± 4
Polymer B	60	45	+35 ± 5*
Heparin	—	—	−18 ± 5*

[a]Derivatized dextrans were prepared from dextran (D), after carboxymethylation (CM) of hydroxyl groups on glucose units of dextran, and conversion of carboxylate groups to benzylamide groups (B). The main substitution occurred on hydroxyls in position C2. Chemical characterizations were performed by acidimetric titrations and elemental analysis. EC growth stimulation in one representative experiment was evaluated by cell counting on EA.Hy 926 cells after 4 days' incubation with 10 μg/mL of polysaccharides.

*Statistically different compared with the absence of polysaccharide, $P < 0.01$.

Crosslinked hydrogels

Crosslinked gels made of dextran were obtained with sodium trimethaphosphate (STMP). Dextran solutions containing or not 3 wt % of heparin or derivatized dextrans were mixed for 1 h at 50°C with STMP in aqueous NaOH solutions as previously described.[23,24] The tested hydrogels contain only dextran, dextran and heparin, or dextran and the derivatized dextran B. The resulting hydrogels were poured before gelification into 24-well tissue culture plates (0.25 mL/well) and were extensively washed with phosphate buffer saline (PBS) and culture medium.

Endothelial cell cultures

EA.Hy 926 human endothelial cell line was kindly provided by Dr. C.-J. Edgell (University of North Carolina). They resulted from the fusion between human endothelial cells and human epithelial cells-derived carcinoma. They were routinely cultured as previously described[25,26] in Dulbecco's minimal essential medium (DMEM; Gibco) supplemented with 10% fetal calf serum (FCS; Gibco), 2 mM L-glutamine (Gibco), and HAT (100 mM hypoxanthine, 0.4 mM aminopterine, 16 mM thymidine; Gibco). Human umbilical vein endothelial cells (HUVEC) were obtained from fresh human umbilical veins by collagenase digestion (0.1%; Boehringer Mannheim). Cells used at passage 1 or 2 were grown in M199/RPMI v/v (Gibco), supplemented with 20% FCS, 2 mM L-glutamine, penicillin (100 U/mL; Gibco), streptomycin (100 μg/mL; Gibco), and fungizone (2.5 μg/mL; Gibco).

ECs grew to confluent monolayers after 5–7 days in culture at 37°C in 5% CO_2 incubator. Cell morphology was examined under conventional light microscope (Axiovert 100; Zeiss). Identity of ECs was confirmed by a positive staining with a monoclonal antibody for von Willebrand factor. Absence of mycoplasma contamination was checked by using the enzymatic ADOP assay.[27]

DNA synthesis

ECs were seeded at 2×10^4 cells in 24-well culture plates with 1 mL of culture medium. For experiments in presence of serum, cells were incubated 24 h after seeding in 2% serum. Cells were then left untreated or stimulated with various concentrations of heparin or derivatized dextrans in media containing 2% serum. For experiments with growth factors, ECs after plating were serum-starved in DMEM containing 0.5% serum for 24 h. Cells were then stimulated for 24 h with different concentrations of human recombinant FGF-2 (generous gift of H. Prats, INSERM U397, Toulouse, France) or human recombinant VEGF 165 (Genentech, San

Francisco, CA) in the presence or absence of various concentrations of derivatized dextrans. DNA synthesis was evaluated by addition of 0.6 μCi ^{3}H-thymidine/well (20.1 Ci/mmol; Dupont NEN) to ECs for the last 4 h of incubation.[28] The reactions were ended after removal of supernatant and addition of 5% trichloroacetic acid. ^{3}H-thymidine incorporation was measured by liquid scintillation counting after solubilization with 0.25*N* NaOH of the cell precipitates.

Cell growth measurement

For cell growth measurement, ECs were seeded at 2×10^4 cells in 24-well culture plates with 1 mL of culture medium. Twenty-four hours after seeding, cells were incubated in 2% serum for 20 h. Cells were either untreated or stimulated with various concentrations of polysaccharides in media containing 2% serum and left without medium change for the indicated time. Similarly, cells were seeded and grown onto the gels containing or not heparin and the derivatized dextran. For all experiments, quadruplicate plates per sample were trypsinized and counted by using a Coulter Counter (ZM; Coultronics). Percent of stimulation was determined as followed: % stimulation = [growth in the presence of polysaccharides/growth in control dishes] × 100. The EC_{50}, concentrations to achieve 50% of the maximum growth stimulation, were obtained from the log dose-response curves.

Flow cytometry analysis

Cells were seeded in 75-cm^2 flasks in culture medium. After 24 h, the cells were incubated in 12 mL of incubation medium in the presence or absence of polysaccharides. Cell cycle distributions were given 20 h after the addition of polysaccharides, by flow cytofluorimetric analysis of propidium iodide-stained cells.[28] Briefly, cells were trypsinized, washed twice with PBS, fixed with ethanol/PBS/water (70:15:15, v/v/v) for 1 h at −20°C, and then resuspended in PBS containing 1% glucose. Cells (10^6) were treated at 4°C with 1 mL of staining solution: 15 μg/mL propidium iodide, 100 μg/mL RNase, 500 μg/mL sodium citrate, and 0.015% Triton X-100 (Fluka). After gentle pipetting, nuclei were analyzed on a Becton-Dickinson FacScan II.

Statistical analysis

Results are expressed as means ± standard deviation. Comparisons between groups were made by the unpaired t test. A p value of <0.01 was considered to be statistically significant.

RESULTS

Soluble derivatized dextrans stimulate the growth of ECs

The proliferation of ECs was first screened by using the human endothelial cell line, EA.Hy 926 and various water-soluble polysaccharides (Table I). On the dose-response curves after 4 days, we observed that the non-sulfated B dextran stimulated the serum-induced cell proliferation with a maximum between 1 and 10 μg/mL, that is, 3–40 nM. B at 10 μg/mL significantly stimulated the EC proliferation, whereas heparin slightly inhibited it, and native dextran and the anionic CM polymer had no effect on cell growth (Table I).

Similarly, heparin and the derivatized dextrans differentially affected the growth of primary culture of HUVEC (Fig. 1). B at 10 μg/mL increased ^{3}H-thymidine uptake (50% stimulation), whereas native dextran and the anionic CM had no effect on HUVEC DNA synthesis, and heparin inhibited it (30% inhibition). B stimulated HUVEC growth with doubling times of 43 h versus 55 h for control cells in serum alone.

We also determined by flow microcytofluorimetry the distribution of cells in the different phases of the cell cycle after 1 day of incubation with the polysaccharides. As indicated in Table II, treatment with the derivatized dextran B induced a twofold increase of cells in the synthesis S phase and a decrease in the quiescent G0/G1 phase. In contrast, a decrease in S phase and increase in G0/G1 was observed with heparin. No effect was obtained with native dextran or the anionic CM dextran (Table II).

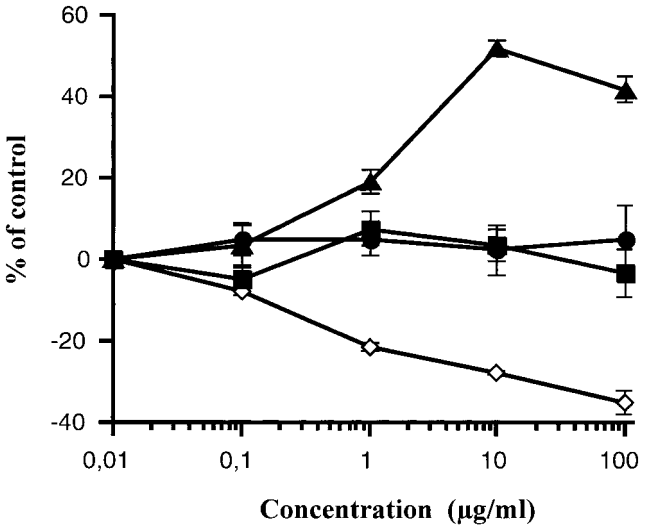

Figure 1. Polysaccharides modulate serum-induced EC proliferation. Cells were seeded at 2×10^4/well in culture medium supplemented with 2% serum. The following day the medium was renewed and supplemented with 2% serum and the indicated concentrations of derivatized dextrans, D (native dextran, ●), CM (■), B (▲), or heparin (◇). Cell numbers were then determined after 4 days with a Coulter Counter. Percent of stimulation was determined by the ratio of the growth in the presence of polysaccharides/growth in control dishes. Results are means ± standard deviations for four determinations.

TABLE II
Cell Cycle Distribution of ECs After Stimulation With Polysaccharides[a]

Polymers	Phases (%)		
	G0/G1	S	G2/M
Control	75	21	4
Native dextran	76	21	3
Polymer CM	77	19	4
Polymer B	48	46	6
Heparin	81	16	3

[a]Flow cytofluorimetry analysis of cell cycle distribution of ECs treated with native dextran, derivatized dextrans CM or B, and heparin. ECs were exposed to 2% serum (control) or 2% serum + 10 μg/mL of polysaccharides. After 20 h, cells were harvested and processed for flow cytofluorimetry. Values of the proportion of cells in the three phases are representative of one experiment. Similar results were obtained in two separate experiments.

Derivatized dextran B potentiate the effect of VEGF and FGF-2 on EC proliferation

Heparin has been already shown to potentiate the mitogenic activity of FGFs and VEGF on EC proliferation.[4,29,30] We investigated if derivatized dextrans could modify the EC response to specific endothelial mitogens, FGF-2 and VEGF. Low-density ECs responded in our conditions to both FGF-2 and VEGF by increased DNA synthesis. Indeed, increasing concentrations of FGF-2 stimulated EC proliferation, with an EC_{50} equal to 1 pM. A maximal fivefold stimulation of the ^{3}H-thymidine incorporation into DNA was obtained with 25 pM (0.45 ng/mL) of FGF-2. VEGF also stimulated ECs in a dose-dependent fashion, with an EC_{50} equal to 200 pM. A maximal twofold stimulation was observed with 500 pM (22.5 ng/mL) of VEGF.

To analyze the effects of derivatized dextrans on growth factor-induced EC proliferation, experiments were carried out at growth factor concentrations lower than their respective EC_{50}. A 0.1 pM concentration of FGF-2 induced a twofold stimulation of basal proliferation [Fig. 2(A)]. When B was added at 0.5 μg/mL (2 nM) together with 0.1 pM FGF-2, it potentiated the FGF-2 mitogenic effect by 13-fold [Fig. 2(A)]. The stimulation of DNA synthesis on ECs by B with only 0.1 pM FGF-2 was more than twice of the maximum level obtained with the highest dose of FGF-2 (25 pM).

As shown in Figure 2(B), a 50 pM concentration of VEGF had a weak but significant mitogenic effect (1.5-fold). The addition of B (2 nM) increased the proliferative effect of VEGF of 3 times [Fig. 2(B)]. Again, the observed stimulation with low doses of B and VEGF was higher than with any dose of VEGF alone.

Hydrogels containing the derivatized dextran B potentiate EC proliferation

Hydrogels made of dextran alone or co-crosslinked with heparin or the derivatized dextran B were studied for their effects on EC proliferation. Attachment of cells reached a plateau on all hydrogels 3 h after cell seeding. Cell counting indicated a higher cell density on gels containing the B polymer (+15 ± 4% compared to plain gels; n = 4). Heparinized gels contained less cells (−46 ± 2% compared to plain gels; n = 4). When following cell proliferation over 6 days, we always observed the highest cell growth on gels containing the derivatized dextran B (Fig. 3). In contrast, gels containing heparin were less efficient to promote EC proliferation than control gels (Fig. 3). At day 6, cell numbers were +33 ± 5% and −20 ± 3% (n = 6) for B gels and heparinized gels, respectively, compared to plain gels. Examination of wells through all the cell growth period showed the persistence of the gels *in vitro*, suggesting a possible sufficient stability of the matrix for the coating of biomaterial devices.

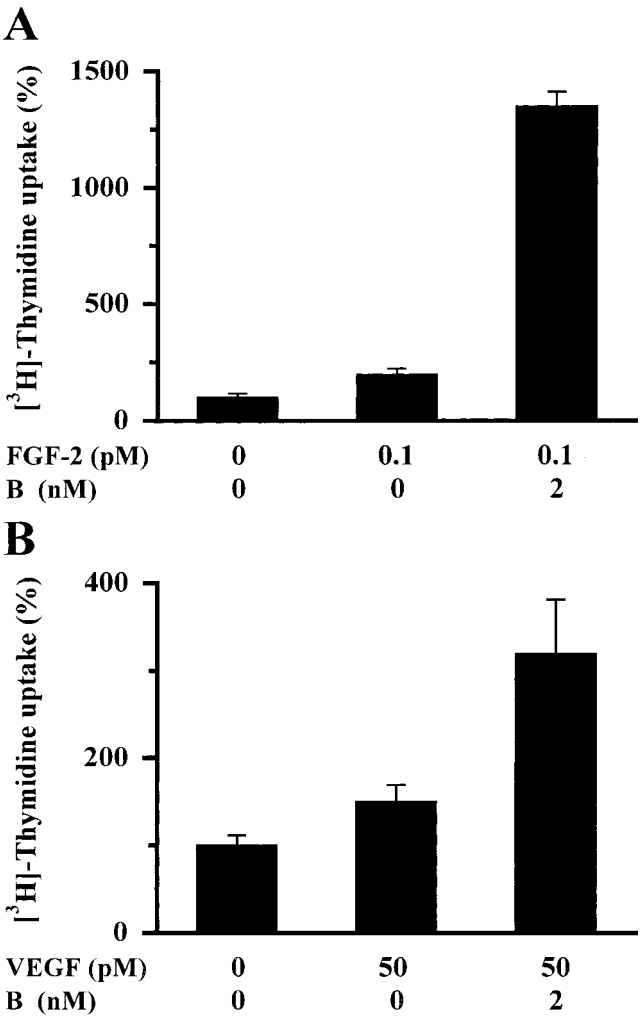

Figure 2. Derivatized dextran B potentiates (A) FGF2- and (B) VEGF-induced EC DNA synthesis. DNA synthesis was evaluated by ^{3}H-thymidine addition to ECs for the last 4 h of 24 h incubation in the presence of (A) 0.1 pM FGF-2 alone or with 2 nM of polymer B, or (B) 50 pM VEGF alone or with 2 nM of polymer B. Results normalized to the values in the absence of growth factor are means ± standard deviations for three determinations.

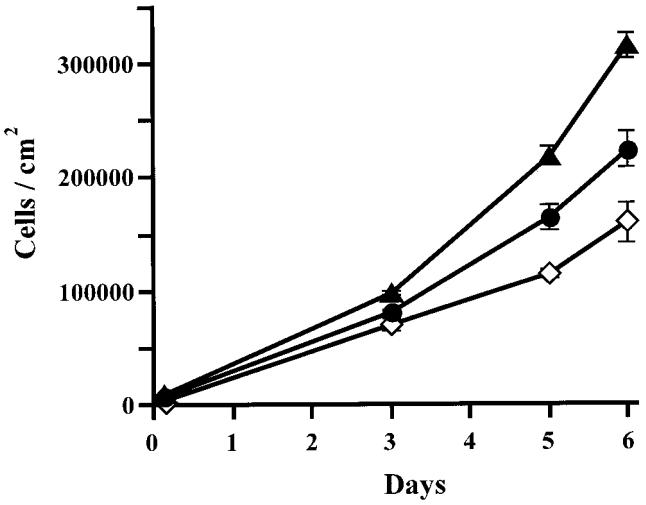

Figure 3. Derivatized dextran B immobilized on dextran hydrogels potentiates EC proliferation. Crosslinked gels made of dextran (●) were obtained with STMP and contained or not 3 wt % of heparin (◇) or derivatized dextran B (▲). Cell proliferation over a 6-day period was followed by cell counting after trypsination. Results are means ± standard deviations for four determinations in one representative experiment.

DISCUSSION

EC growth control is of particular importance in wound healing, angiogenesis, and after vascular graft implantation. To develop a biocompatible coating of vascular prostheses, we first investigated on ECs the structure-mitogenic activity relationship with polysaccharides in the presence of serum and mitogenic growth factors. From studies reported here, we showed that non-sulfated derivatized dextrans were able *in vitro* to stimulate EC growth in serum containing media. Similar results were obtained by using either a human EC line (EA.Hy 926) or human primary ECs (HUVEC). In contrast to derivatized dextrans, we observed that heparin inhibited HUVEC and EA.Hy 926 cell growth in the presence of serum.

Heparin modulates the mitogenic activities of growth factors, but the structural mechanisms of EC growth regulation by natural heparin and synthetic heparin-like polysaccharides are still not completely elucidated.[31] Interactions are assumed to involve specific chemical sequences in sulfated polysaccharides and cell membrane receptors or growth factors within the extracellular matrix, thereby activating mitogenic signals or repressing growth-inhibitory signals.[13,32–34] For this purpose, we further decided to study the combination of non-sulfated derivatized dextrans with two growth factors known to stimulate EC proliferation: VEGF and FGF-2. The addition of the derivatized dextran B in the culture medium significantly potentiated the proliferative action of both growth factors (Fig. 2). Indeed, a 13-fold induction of DNA synthesis was observed in the presence of low doses of polymer B and FGF-2. A 3.5-fold stimulation was obtained with B on VEGF-stimulated cells. It is worth noting that such levels of stimulation could never be obtained even with the highest concentrations of growth factors. Direct interactions between FGF-2, TGFβ1, and PDGF-BB but not EGF or IGF-1 and derivatized dextrans bearing benzylamide groups were already demonstrated.[17–19] Thus, derivatized dextrans could either stabilize FGF-2 and VEGF to increase their respective half-life or act on the translocation of the mitogens from the extracellular matrix on ECs. It is likely that these compounds trapped in a synthetic or natural extracellular matrix could protect surrounding mitogenic factors enhancing EC proliferation, whereas a higher affinity of growth factors with, for instance heparin, can prevent by sequestration their bioavailablity. Our future investigations of the fate of complexes between various polysaccharides and growth factors or extracellular matrix proteins[35] could provide clues of the intracellular phosphorylation events leading to the regulation of EC proliferation.

The adjuvant effect of the water-soluble derivatized dextran to serum or growth-factor-induced EC proliferation is associated to a chemical structure that implies a distribution of substituted glucosyl units. In previous studies, the structural determinants responsible for the anticoagulant activity were shown to be related to the sulfate content in the dextran units.[22,36] However, synthetic dextran without sulfate units, such as B, are completely devoid of anticoagulant activity. We show here that derivatized dextran B, which contain anionic carboxyl groups and hydrophobic benzylamine moieties, have a stimulatory effect on serum-induced EC growth. This non-sulfated derivatized dextran also increases the EC distribution in S phase of the cycle cell (Table II). In contrast, carboxymethyl dextran CM, bearing only anionic carboxyl units, has no effect on EC growth, ruling out a simple charge effect mechanism. Our results also indicated that the grafting of benzylamine groups on this non-heparin-like dextran appeared to be crucial to significantly enhance FGF-2 and VEGF mitogenic properties on ECs (Fig. 2). Because chemical groups as benzylamine distributed along the macromolecular chain endowed the non-anticoagulant derivatized dextran with a pro-proliferative activity on ECs, we conclude that the anticoagulant and proliferative effects of polysaccharides on ECs were dissociated. Conversely, the presence of sulfate groups on the dextrans was not required for the EC growth stimulation and can even inhibit it as for heparin. This result is consistent with the work with other molecules such as sucrose-octasulfate,[33] aurintricarboxylic acid,[37] poly(2-acrylamido-2-methyl propanesulfonic acid),[38] and the non-sulfated poly(4-hydroxyphenoxy acetic acid) containing carboxyl and aromatic groups,[11] revealing a

general effect of polymers substituted with both aromatic rings and negatively charged residues.

The presented results suggested that these non-anticoagulant synthetic dextrans hold promise for the coating of vascular biomaterials as well as tissue engineering applications requiring neovascularization. It is of interest that the data indicated that the immobilization of such polymers on a hydrogel promote EC proliferation (Fig. 3). In contrast, heparin immobilization on our matrices gave an inhibitory effect to EC growth, in agreement with other reports for immobilized heparin on different matrices.[7,39] In fact, enhancement of EC proliferation with the anticoagulant heparin was only reported with the addition of growth factors within the scaffolds.[8,40–42] In contrast, our method to obtain a stable, inexpensive, and biocompatible surface able to enhance EC growth required only synthetic compounds and a non-toxic crosslinking procedure. The dextran coating by mimicking the glycocalyx of cell surfaces,[43] and the simple method for incorporation of potent bioactive molecules may open the way to broad applications. In ongoing experiments, we are thus investigating the *in vitro* and *in vivo* effects of polysaccharides coated on different vascular prostheses.

We thank F. Savoie (Inserm U460, Paris) for technical assistance with HUVEC cultures, J. Champion and P. Zussi (Université Paris 13, Villetaneuse) for EA.Hy 926 cell cultures, and P. Planchon (Université Paris 13, Bobigny) for FACS analysis. We thank R. Gaertner (U460 INSERM) for the critical reading, T. Avramoglou (Université Paris 13, Villetaneuse) for the preparation of the derivatized dextrans, H. Prats (INSERM U397, Toulouse) for its generous gift of hr-FGF-2, and C-J. S. Edgell (U. North Carolina, Chapel Hill, NC, USA) for providing the EA.hy 926 cells.

References

1. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995;1:27–30.
2. Asahara T, Bauters C, Pastore C, Kearny M, Rossow S, Bunting S, Ferrara N, Symes J, Isner J. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in ballon-injured rat carotid artery. Circulation 1995;91:2793–2801.
3. Lindner V, Reidy MA. Expression of VEGF receptors in arteries after endothelial injury and lack of increased endothelial regrowth in response to VEGF. Arterioscler Thromb Vasc Biol 1996;16:1399–1405.
4. Bikfalvi A, Klein S, Pintucci G, Rifkin D. Biological roles of fibroblast growth factor-2. Endocr Rev 1997;13:18–32.
5. Klagsbrun M, Baird A. A dual receptor system is required for basic fibroblast growth factor activity. Cell 1991;67:229–231.
6. Edelman E, Nathan A, Katada M, Gates J, Karnovsky M. Perivascular graft heparin delivery using biodegradable polymer wraps. Biomaterials 2000;21:2279–2286.
7. Chupa JM, Foster AM, Sumner SR, Madihally SV, Matthew HW. Vascular cell responses to polysaccharide materials: in vitro and in vivo evaluations. Biomaterials 2000;21:2315–2322.
8. Wissink MJ, Beernink R, Poot AA, Engbers GH, Beugeling T, van Aken WG, Feijen J. Improved endothelialization of vascular grafts by local release of growth factor from heparinized collagen matrices. J Control Release 2000;64:103–114.
9. Wissink MJ, Beernink R, Pieper JS, Poot AA, Engbers GH, Beugeling T, van Aken WG, Feijen J. Immobilization of heparin to EDC/NHS-crosslinked collagen. Characterization and in vitro evaluation. Biomaterials 2001;22:151–163.
10. Massia SP, Stark J, Letbetter DS. Surface-immobilized dextran limits cell adhesion and spreading. Biomaterials 2000;21:2253–2261.
11. Miao HQ, Ornitz DM, Aingorn E, Ben-Sasson SA, Vlodavsky I. Modulation of fibroblast growth factor-2 receptor binding, dimerization, signaling, and angiogenic activity by a synthetic heparin-mimicking polyanionic compound. J Clin Invest 1997; 99:1565–1575.
12. Miao HQ, Ishai-Michaeli R, Peretz T, Vlodavsky I. Laminarin sulfate mimics the effects of heparin on smooth muscle cell proliferation and basic fibroblast growth factor-receptor binding and mitogenic activity. J Cell Physiol 1995;164:482–490.
13. Zugmaier G, Favoni R, Jaeger R, Rosen N, Knabbe C. Polysulfated heparinoids selectively inactivate heparin-binding angiogenesis factors. Ann NY Acad Sci 1999;886:243–248.
14. Urasawa K, Kaneta S, Nakano N, Saito T, Sakai H, Takagi C, Takagi Y, Sugiki H, Kitabatake A. Vascular smooth muscle cell proliferation is effectively suppressed by the non-specific growth factor inhibitor suramin. Jpn Heart J 2001;42:221–233.
15. Tardieu M, Gamby C, Avramoglou T, Josefonvicz J, Barritault D. Derivatized dextrans mimic heparin as stabilizers, potentiators and protectors of acidic or basic FGFs. J Cell Physiol 1992; 150:194–203.
16. Letourneur D, Champion J, Slaoui F, Jozefonvicz J. In vitro stimulation of human endothelial cells by derivatized dextrans. In Vitro Cell Dev Biol 1993;29A:67–72.
17. Bagheri Yarmand R, Liu JF, Ledoux D, Morère JF, Crépin M. Inhibition of human breast epithelial HBL 100 cell proliferation by dextran derivative (CMDB7) with the FGF2 autocrine loop. Biochem Biophys Res Commun 1997;239:424–428.
18. Bagheri-Yarmand R, Kourbali Y, Morère JF, Jozefinvicz J, Crépin M. Inhibition of MCF-7ras tumor growth by carboxymethyl benzylamide dextran: blockage of the paracrine effect and receptor binding of transforming growth factor β1 and platelet-derived growth factor-BB. Cell Growth Differ 1998;9: 497–504.
19. Bittoun P, Bagheri-Yarmand R, Chaubet F, Crépin M, Jozefonvicz J, Fermandjian S. Effects of the binding of a dextran derivative on fibroblast growth factor 2: secondary structure and receptor-binding studies. Biochem Pharmacol 1999;57:1399–1406.
20. Chaubet F, Champion J, Maïga O, Mauray S, Jozefonvicz J. Synthesis and structure-anticoagulant property relationships of functionalized dextrans: CMDBS. Carbohyd Polym 1995;28: 145–152.
21. Prigent Richard S, Cansell M, Vassy J, Viron A, Puvion E, Jozefonvicz J, Letourneur D. Fluorescent and radiolabeling of polysaccharides: binding and internalization experiments on vascular cells. J Biomed Mater Res 1998;40:275–281.
22. Maaroufi RM, Jozefowicz M, Tapon-Bretaudiere J, Jozefonvicz J, Fischer AM. Mechanism of thrombin inhibition by heparin cofactor II in the presence of dermatan sulphates, native or oversulphated, and a heparin-like dextran derivative. Biomaterials 1997;18:359–366.
23. Gliko-Kabir I, Yagen B, Penhasi A, Rubinstein A. Phosphated crosslinked guar for colon-specific drug delivery. I. Preparation and physicochemical characterization. J Control Release 2000;63:121–127.

24. Le Bail P, Morin FG, Marchessault RH. Characterization of a crosslinked high amylose starch excipient. Int J Biol Macromol 1999;26:193–200.
25. Edgell CJS, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci USA 1983;80:3734–3737.
26. Cansell M, Gouygou JP, Jozefonvicz J, Letourneur D. Lipid composition of cultured endothelial cells in relation to their growth state. Lipids 1997;32:39–44.
27. Bonissol C, Stoiljkovic B. AdoP assay detection of mycoplasmal contamination in biological media. Res Virol 1989;140:241–251.
28. Logeart D, Prigent Richard S, Boisson Vidal C, Chaubet F, Durand P, Jozefonvicz J, Letourneur D. Fucans, sulfated polysaccharides extracted from brown seaweeds, inhibit vascular smooth muscle cell proliferation. II. Degradation and molecular weight effect. Eur J Cell Biol 1997;74:385–390.
29. Gospodarowicz D, Cheng J. Heparin protects basic and acidic FGF from inactivation. J Cell Physiol 1986;128:475–484.
30. Gitay-Goren H, Soker S, Vlodavsky I, Neufeld G. The binding of vascular endothelial growth factor to its receptors is dependent on cell surface-associated heparin-like molecules. J Biol Chem 1992;267:6093–6098.
31. DiGabriele AD, Lax I, Chen DI, Svahn CM, Jaye M, Schlessinger J, Hendrickson WA. Structure of a heparin-linked biologically active dimer of fibroblast growth factor. Nature 1998; 393:812–817.
32. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 1991;64:841–848.
33. Spivak-Kroizman T, Lemmon MA, Dikic I, Ladbury JE, Pinchasi D, Huang J, Jaye M, Crumley G, Schlessinger J, Lax I. Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. Cell 1994;79:1015–1024.
34. Liekens S, De Clercq E, Neyts J. Angiogenesis: regulators and clinical applications. Biochem Pharmacol 2001;61:253–270.
35. Yu WH, Woessner JF Jr. Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7). J Biol Chem 2000;275:4183–4191.
36. Mauzac M, Jozefonvicz J. Anticoagulant activity of dextran derivatives. Part I: Synthesis and characterization. Biomaterials 1984;5:301–304.
37. Gagliardi AR, Collins DC. Inhibition of angiogenesis by aurintricarboxylic acid. Anticancer Res 1994;14:475–479.
38. Liekens S, Neyts J, Degreve B, De Clercq E. The sulfonic acid polymers PAMPS [poly(2-acrylamido-2-methyl-1-propanesulfonic acid)] and related analogues are highly potent inhibitors of angiogenesis. Oncol Res 1997;9:173–181.
39. Nojiri C, Noishiki Y, Koyanagi H. Aorta-coronary bypass grafting with heparinized vascular grafts in dogs: a preliminary study. J Thorac Cardiovasc Surg 1987;93:867–877.
40. Kang SS, Gosselin C, Ren D, Greisler HP. Selective stimulation of endothelial cell proliferation with inhibition of smooth muscle cell proliferation by fibroblast growth factor-1 plus heparin delivered from fibrin glue suspensions. Surgery 1995; 118:280–286.
41. Weatherford DA, Sackman JE, Reddick TT, Freeman MB, Stevens SL, Goldman MH. Vascular endothelial growth factor and heparin in a biologic glue promotes human aortic endothelial cell proliferation with aortic smooth muscle cell inhibition. Surgery 1996;120:433–439.
42. Wissink MJ, Beernink R, Scharenborg NM, Poot AA, Engbers GH, Beugeling T, van Aken WG, Feijen J. Endothelial cell seeding of (heparinized) collagen matrices: effects of bFGF preloading on proliferation (after low density seeding) and procoagulant factors. J Control Release 2000;67:141–155.
43. Holland NB, Qiu Y, Ruegsegger M, Marchant RE. Biomimetic engineering of non-adhesive glycocalyx-like surfaces using oligosaccharide surfactant polymers. Nature 1998;392:799–801.