Bellon, J.M. et al. (1997) 'Mesothelial versus endothelial cell seeding: Evaluation of cell adherence to a fibroblastic matrix using In oxine', European journal of vascular and endovascular surgery, 13(2), pp. 142–148. doi:10.1016/S1078-5884(97)80010-X.

Available at https://doi.org/10.1016/S1078-5884(97)80010-X

Mesothelial Versus Endothelial Cell Seeding: Evaluation of Cell Adherence to a Fibroblastic Matrix Using ^{111}In Oxine

J.M. Bellön* [I], N. Garcia-Honduvilla [I], C. Escuder0[2], M-J„ Gimeno [I], L. Contreras [I], J. de Haro2 and J. Bujån [I]

Department of Morphological Sciences and Surgery, University of Alcala de Henares, Madrid and [2]Clinica Puerta de Flierro, Madrid, Spain

Objectives: The aim of this study was to compare the behaviour of mesothelial cells (MC) to that of endothelial cells (EC) when seeded onto a PTFE prosthesis coated with a fibroblastic matrix.

Design,onaterial and methods: Three study voups were examined: a control group (Control) of PTFE prostheses with a fibroblast matrix (n = 8); Group EC, PTFE prostheses seeded with EC on a fibroblastic matrix (n = 8); and Group MC, PTFE prostheses seeded with MC on a fibroblastic matrix (n = 8). All cell types were labelled with ^{III}In (100 uCi/mI) 24 h after seeding, when the cells had formed a monolayer on the prosthetic surface. Radioactive levels were measured at 2, 4, 6, and 24 h.

Results: Both EC and MC showed optimal adherence. The MC had a better radioactive uptake and retention than the EC. The number of EC and MC cells that remained adherent to the matrix was large enough to ensure complete covering of the prosthetic surface.

Conclusion: The use of MC is therefore feasible as an optimal alternative for achieving a natural covering on vascular prostheses prepared with a fibroblastic matrix.

Key Words: Endothelial cell seeding; Mesothelial cell seeding; Vascular prostheses.

Introduction

The problems involved in seeding cells on mediumsized and small-sized vascular prostheses concern the optimal source for cells and the optimal substrate for ensuring their fixation to the prosthetic material. In the laboratory, human umbilical cord vein has been found to be an ideal source of endothelial cells (EC) for studies in vitro. Jugular vein has also been used for this purpose in experimental animals. In human beings it is preferable to conserve the main leg veins because they are still the best option for bypass and vascular replacement surgery. Nonetheless, saphenous vein and even varicose veins have been used as a source of EC. However, these sources are clearly insufficient,5 particularly when large vascular prosthetic surfaces have to be covered.

*Please address all correspondence to: Prof. Juan M. Bellön, Dept. of Morphological Sciences and Surgery, Garret. N.II, Km 33,6. Alcalå dc Henares, 28871 Madrid, Spain

An alternative to EC may be mesothelial cells (MC) obtained from the omentum. The identity of mesothelial cells is disputed. Some authors5-8 claim they are endothelial because they come from microcirculation vessels, whereas others9—11 classify them as mesothelial, and a third group of authors state that criteria are insufficient for classifying them as either endothelial or mesothelial. In an earlier study, -14 we found that MC are positive for EC antibodies and for antibodies specific to smooth muscle cells (anti-alpha actin and anti^{w}total actin). We also confirmed[915] the presence of optimal prostacyclin production.

A no less important problem is to ensure the adherence of the cells seeded on prosthetic materials. In an earlier study by our group using a fibroblastic matrix, 16 cellular adherence improved because the resulting monolayer of seeded EC was more stable. Based on the findings of previous studies, this study compared labelled EC and MC seeded on a fibroblastic matrix positioned between the cells and the prosthetic material.

1078-5884/97/020142 + 07 $12.00/0 0 1997 W: B. Saunders Company Ltd.

Materials and Methods

Cell extraction and culture

(a) Mesothelial cells. The MC were obtained from 30-40 g fragments of human omentum obtained from donors in the course of elective abdominal surgery. The omental fragments were transported to the

laboratory immersed in minimum essential medium (MEM) (Gibco BRL) supplemented with antibiotics (100 000 IU / ml penicillin/ 10 000 ug/ ml streptomycin)
(Gibco BRL) and antimycotics (25 ug/ml fungizone) (Gibco BRL). Fragments were processed following the methods described by Kern et al. [17] with some modifications.

In the laboratory, the omental fragments were incubated in a solution of 0.1% type I collagenase (Worthington) in MEM, with agitation (100 oscillations/ min) for 20 min. Surplus omental fragments were removed and the cell suspension was centrifuged at 200 g for 7 min. The supernatant was eliminated and the resulting cellular precipitate was resuspended in 5 ml of Medium 199 (M-199) (Gibco BRL) supplemented with 20% fetal bovine serum (Gibco BRL), antibiotics (100000 IU / ml penicillin/ 10000 ,ug/ml streptomycin) (Gibco BRL), 10 mM Hepes (Gibco BRL), 2 mM L-glutamine (Gibco BRL), and sodium heparin (90 ug/ml) (Roche). The cell suspension was transferred to Nunclon 25 cm culture flasks and incubated in a culture oven (5% C02) at 37° c until a confluent monolayer had formed on the culture surface.

(b) Endothelial cells. The EC were obtained from human umbilical vein and placed in culture following the methods described by Gimbrone. [18] In the laboratory, the inner vein was washed several times with MEM under sterile conditions. The vein was then filled with a 0.1% solution of type I collagenase (Worthington) in MEM and incubated for 15 min at 37^0 C. The cell suspension was collected in a 50ml conical centrifuge tube (Falcon) and centrifuged at 2()() g for 7 min. The precipitate was resuspended in 5 ml MEM and centrifuged again under the same conditions. This last step was repeated three times to eliminate any blood components that may have been released during enzymatic digestion. After the final centrifugation, the EC were resuspended in 5 ml of M-199 (Gibco BRL) supplemented with 20% bovine fetal serum (Gibco BRL), antibiotics (100 000 IU/m1 penicillin/l() 000 ug/ml streptomycin) (Gibco BRL), 10 Hepes (Gibco BRL), 2 mtvl L-glutamine (Gibco BRL), endothelial cell growth _factor (20 ug/ml) (ECGF) (Sigma), and sodium heparin (9() ug/ml) (Roche), placed in a 25 cm culture flask (Nunclon), and incubated at 37° c in a C02 oven (5% C02).

(c) Fibroblasts. A small skin sample (1 cm >< 0.5 cm) was obtained from donor patients in the same operation that yielded MC and EC. This small skin fragment was submerged immediately in MEM supplemented as described, then transported to the laboratory. Under sterile conditions the explants were placed in 25 cm culture flasks containing 5 ml of McCoy's medium (Gibco BRL) supplemented with 10% bovine fetal serum (Gibco BRL), antibiotics (100 000 IU/ml penicillin/ 10 000 {Lg/ml streptomycin) (Gibco BRL), 10 mM Hepes (Gibco BRL), and 2 mM L-glutamine (Gibco BRL). The culture flasks containing the explants were incubated at 37° c in a 5% C02 oven.

In all cases, the culture medium was changed every 2 days until a confluent monolayer had formed on the culture surface. Once the cellular monolayer had formed, cellular subcultures were made to obtain enough cells to completely cover the prosthetic surface by seeding. These subcultures were made by treating the cellular monolayer with a suspension of 1% trypsin-EDTA (Gibco BRL) in HBSS free of ca^2 + and Mg^2 + (Gibco BRL) for 5 min at 37^0 C. After incubation, the proteolytic enzyme was inactivated by adding medium (M-199 for EC and MC cultures; McCoy's medium for fibroblast cultures) supplemented with fetal calf serum (Gibco BRL). The cell suspension was divided into culture flasks at a ratio of 1:2 to 1:3. All the cells used in this study came from the first subculture.

Cell count identification and prostacyclin production

The number of viable cells was determined using trypan blue and counted in a Neubauer chamber. Cells were identified using immunohistochemical tech.niques: anti-CD34 antibodies (Serotec), anti-factor VIII (Biomeda) (specific for EC), anti-cytokeratin 18 (Biogenesis) (normally used for MC identification), antivimentin (Biomeda), anti-desmin, anti-actin and antitotal actin (all courtesy of Professor Gabiani). Immunohistochemical reactions were detected with commercial kits using FITC (immunofluorescence) (Nordic Immunology), ABC (StreptoAvidin-Biotin Complex) (Sigma), and PAP (Peroxidase-Anti-Peroxidase) (Dako) techniques.

MC and EC production of prostacyclin was determined using radioimmunoassay techniques to measure the level of 6-keo-prostaglandin Flu, a stable intermediate in the prostacyclin synthesis pathway:
This intermediate compound was detected using the method described in the commercial radioimmunoassay kit (Amersham).

Cell seeding and labelling

The seeding chamber was constructed following the methods reported by Budd et al. [19]

Fibroblasts from the first subculture were lifted from the surface of the culture flask using an enzymatic treatment with trypsin-EDTA solution following the method indicated for subcultures. A small aliquot of the cell suspension was separated for a viable cell count to determine the number of cells to be seeded in each seeding chamber. Before seeding, all prostheses were incubated for 1 h at 37° c with 100 of a solution containing 20 ug/ml of fibronectin (Sigma) in MEM. Excess fibronectin was eliminated and the prosthesis surface was seeded with a cell suspension containing 2 x 10^5 fibroblasts/ ml. Once seeded, the prostheses were incubated at 37° c in a C02 oven (5% C02) for 24 h. When the fibroblastic monolayer had formed on the prosthetic surface, it was fixed with 5% glycerol. This produced a fibroblastic matrix for EC and MC seeding.

After the fibroblastic matrix was created on PTFE, MC and EC from the first subculture were raised from the surface of the culture flask as described earlier. The resulting cell suspensions were centrifuged at 200 g for 7 min, resuspended in complete M-199, and seeded on the fibroblastic matrix after it was incubated with fibronectin (20 æg/ml) at 37° c for 1 h. A small aliquot was separated to make the cell count and the EC and MC were seeded at a density of 1.5—2 >< 10^5 cells/ ml.

In the 24 h after seeding, when the cells had formed a monolayer on the surface of the prosthesis, cells

^{111}In-oxine (100 μCi/ml).

radioactive substance was added to each well, then incubated for 15 min at room temperature. The surplus radioactive substance then was removed by washing three times with MEM.

Measurement of the surface covering

The specimens obtained were fixed in 3% glutaraldehyde for 2 h then introduced into Milloning buffer (pH 7.3). The samples were dehydrated in a graduated acetone series, brought to critical point in a Polaron E-300() with C02, metallised in palladium gold* and studied under a Zeiss 950 DSM scanning electron microscope. Measurements of the surface covered were taken using 16 microphotographs (100) of each sample observed by scanning electron microscopy. Each sample was divided into four quadrants and four microphotographs were taken of each quadrant at random. The microphotographs were then subjected to image analysis (Microm) to determine the surface covered cells in proportion to the total surface. Results were expressed as percentages.

Experimental design

Three study groups were set up to compare the behaviour of MCs to that of ECS when seeded onto a PTFE prosthesis coated with a fibroblastic matrix:

(a) Control group: PTFE vascular prostheses with an unseeded fibroblastic matrix

(b) EC group: PTFE vascular prostheses seeded with EC on a fibroblastic matrix

(c) MC group: PTFE vascular prostheses seeded with MC on a fibroblastic matrix

In all the groups radioactive substance uptake was measured under the same conditions. The study times were 2, 4, 6, and 24 h after the formation of a monolayer on the prosthetic surface. At each study time the culture medium was removed and the prostheses were washed three times with PBS buffer to eliminate cells that had separated from the prosthetic surface.

Statistical analysis was made using the MannWhitney U-test for comparisons between groups and the Wilcoxon test for intragroup comparisons.

Results

Cell cultures

Fibroblasts. The fibroblasts in culture had a morphology that clearly differed from the EC and MC, characterised by a spindle shape and concentric colony growth. Cellular secretion in culture was manifested by intracytoplasmatic vacuolised clumps and extracellular secretion in culture by a material that was discreetly refringent on reverse microscopy:

Endothelial cells. The EC in culture had an elongated morphology with large, round or oval nuclei and one, two, or three mucleoli. The EC formed a monolayer that showed a characteristic cobblestone appearance after 7—10 days of culture.

Mesothelial cells. The MC behaved well in culture, forming a confluent monolayer on the flask surface a few days after seeding. The monolayer consisted of polygonal cells with a rounded nucleus. This cells were surrounded by an abundant glycocalyx.

All the cell types had the same behaviour and morphologic characteristics after the first subculture. In every case, the trypan blue test revealed a viability rate that was sufficient to guarantee effective seeding on the prosthetic material after the first subculture.

Cell identification and prostacyclin production

The EC were clearly positive for anti-CD34 and antifactor VIll antibodies, and the fibroblasts were positive for anti-vimentin antibodies. The MC were positive for all antibodies assayed. MC positivity was strong for anti-cytokeratin 18, anti-desmin, anti-actin, and anti-total actin antibodies. Both EC and MC produced high prostacyclin levels in the primary cultures and in the first subcultures (> 5 ng/ ml); prostacyclin activity was not detected in the fibroblast cultures.

Cell seeding and labelling

Fibroblasts. The fibroblasts seeded on PTFE had an initial morphology that was rounded with numerous spiked prolongations that contacted each other and formed a typical cellular reticulum. After cytoplasmatic organisation, the fibroblasts formed a cell covering and the cells acquired a typical spindleshaped morphology. They soon began an intense secretory activity that gave the cell covering a filamentous, fibrillar aspect on its apical face and produced an abundant glycocalyx on the fibroblastic layer. The fibroblasts covered the entire prosthesis with a stable monolayer 18—24 h after seeding. Once the monolayer had formed, it was fixed to the prosthetic surface with 5% glycerol. This produced a rough surface suitable as a matrix for seeding the other cell types.

Endothelial cells. The EC had good adherence to the fibroblastic matrix 24 h after seeding. At this time, the prosthetic surface was covered totally by a confluent endothelial layer (Fig. la).

Mesothelial cells. The MC formed a stable cell layer on the fibroblastic matrix 24 h after seeding. The cells of the layer were extended on the matrix. Some had a spherical morphology and there was a typical appearance of cells in different stages of division (Fig. 1b).

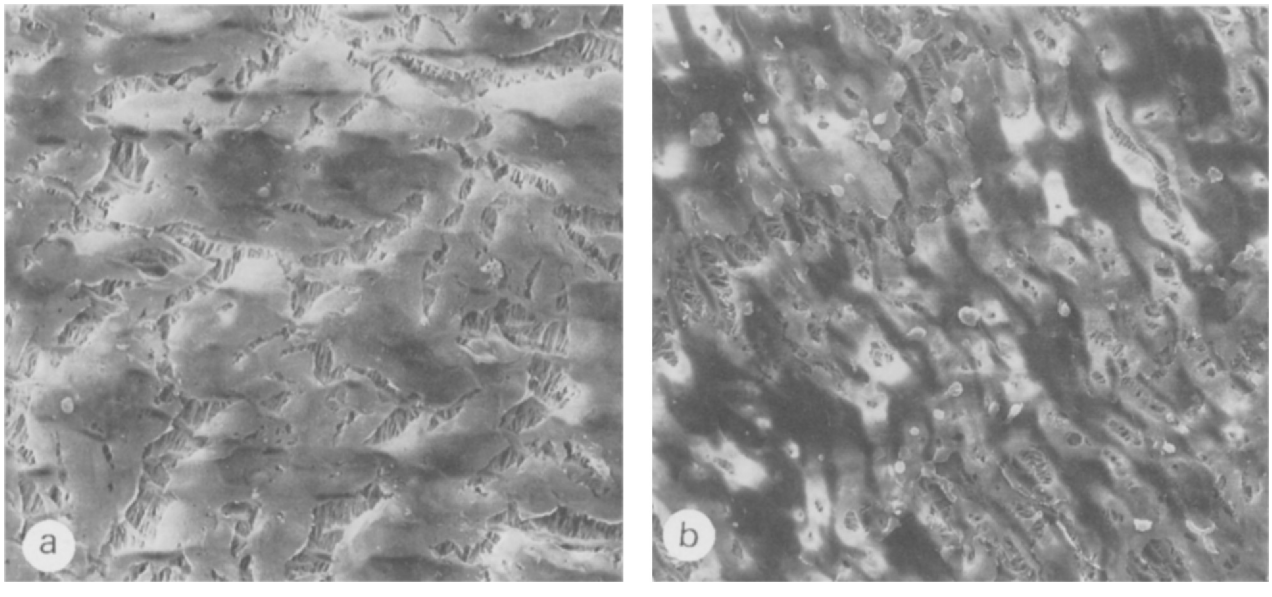

Fig. 1. Cell covering of the prosthetic surface before ^{Tlf}ln labelling. (a) EC adhered to the fibroblastic matrix form a confluent monolayer on the PTFF surface 24 h after seeding (>< 200). (b) MC seeded on the fibroblastic matrix form a confluent monolayer 24 h after seeding. (X 20()). Reproduced here at 7()%.

When the control specimens were incubated with 111In-oxine, the fibroblasts at first (t = 0) incorporated large amounts of ^{111}In, attaining 99.48% effectiveness in labelling. After the first wash (t = 2 h), the amount of radioactive substance remaining on the surface of the matrix decreased (82% retention) and continued to decrease throughout the study (53% at t = 24 h). Radioactive Substance retention by the seeded surface decreased over time in every study group (Table 1). In

the EC seeded on the fibroblastic matrix, ^{111}In uptake of 72% and radioactive substance retention decreased progressively to 29% at the end of the study. These results are shown in Table 1. The differences in ^{111}In retention were significant ($p < 0.01$) at all study times except between 4 and 6 h. The EC seeded on the fibroblastic matrix had a significantly lower ($p < 0.01$) ^{111}In retention at all study times than the control group (Fig. 2). The MC seeded on the fibroblastic matrix also had a lower ^{111}In retention than the controls, but not as low as the EC ($p < 0.01$) (Fig. 2). Comparing ^{111}In retention by the EC and MC, the MC had a larger initial radioactive substance uptake and lesser release throughout the study time than the EC. Therefore, ^{111}In retention was significantly greater ($p < 0.05$) in the MC group than in the EC group throughout the study (Fig. 2).

Table 1. Retention of ^{111}In-oxine by the different groups of prostheses in the first 24 h after formation of a monolayer on the prosthetic surface (Mean±s.D.).

	Control (%)	Group EC (70)	Group MC
	99.5±9.7	41.9±3.4	71.9±8.1
2	82.2±7.3	19.3±1.9	62.9±7.2
4	76.5±7.1	15.2±1.3	38.1±4.0
6	74.3±6.4	13.9±2.9	32.4±3.9
24	52.7±4.3	10.5±2.7	29.2±2.5

Surface covering

Forty-eight hours after seeding, both the EC and MC seeded prostheses showed a large loss of ^{Tll}In labelled cells, but the prosthetic surface with fibroblastic matrix was covered totally by cells which formed a typical confluence monolayer that appeared to be very stable (Fig. 3). No significant differences were found between EC and MC in terms of the prosthetic surface covered (100% in both cases). .

Discussion

Sources of cells for seeding prostheses have been widely reviewed in the literature.[4] The possibility of using cell types that have characteristics similar to EC, such as MC, has strong practical advantages.

Earlier studies20-22 demonstrated a better endothelial function when the cells on which the endothelium formed were similar to natural subendothelial cells. Although the use of fibronectin (20 pg/ml) has given good results,23 the interposition of a fibroblastic matrix yielded our best results for EC seeding.16 Nonetheless, the fibroblast matrix was found in earlier studies to be stable for only the first 24 h after seeding.[24] This lead us to fix the fibroblast layer

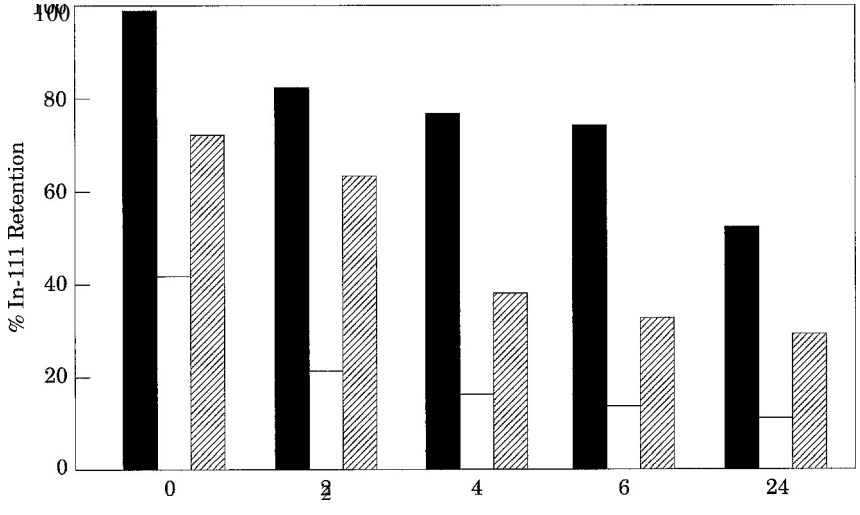

Fig. 2. Retention of ^{111}In over time: Significant differences ($p < 0.01$) were observed between the control prostheses and the EC-seeded on the fibroblastic matrix (Fb + EC). Significant differences ($p < 0.01$) were observed between the control prostheses and the M&seeded on the fibroblastic matrix (Fb + MC). The MC seeded on th_e fibroblastic matrix (Fb + MC) retained more radioa_ctive substance ($p < 0.05$) than the EC seeded on the fibroblastic matrix. control; (D) Fb + F,C,• (4) Fb + MC.

with ethanol, but ethanol modifies the permeability of the prosthesis,25 so it was later replaced by glycerol, which fixes the fibroblast layer to the surface of the biomaterial without modifying its structure.

In the current study, MC and EC adherence to the fibroblastic matrix was optimal. The MC formed a stable monolayer on the fibroblast matrix in the first 24 h after seeding and the monolayer remained on the prosthetic surface throughout the study time. ITI Inoxine labelling showed that the fibroblastic matrix had a high radioactive substance uptake, superior to that of EC and MC. The sustained high radioactive substance retention levels observed throughout the study confirmed the stability of the fibroblastic matrix on PTFE. Although 111In uptake by the seeded EC was good, 19,26 MC uptake was better. The superior synthetic capacity of MC compared with EC may explain not only their greater initial radioactive substance uptake, but also their better retention throughout the study after successive washings. Nonetheless, scanning electron microscopy revealed that although both the EC- and MC-seeded prostheses lost radioactive substance-labelled cells, the cell population that remained adherent to the biomaterial was sufficient to cover almost the entire surface.

We conclude that:

(a) MC had a greater III ln uptake and retention than EC after seeding on the fibroblastic matrix.

(b) In spite of successive washings, a large enough number of EC and MC remained adherent to the matrix to ensure complete covering of the prosthetic surface.

(c) MC can be considered a good alternative to EC for seeding prostheses in experimental animals.

Acknowledgements

The work was supported by a grant from the CICYT SAF 92/0875. We thank WD. Gore-Ass Sarl for supplying the prostheses.

References

1 GRAHAM LM, VINTER DW, FORD JW et al. Endothelial cell seeding of prosthetic vascular grafts. Arch Surg 1980; 115: 929—933.

2 HAEGERSTRAND A, GILLrs C, BENGTSSON L. Serial cultivation of adult human endothelium from the great saphenous vein. J Vasc surg 1992; 16: 280-285.

3 LESFCHE G, BII<FALVI A, DUPUY E. et al. Prelining of polytetrafluoroethylene grafts with cultured human endothelial cells isolated from varicose veins. Surgery 1989; 105: 36-45.

4 MOSQUERA DA, GOLDMAN M. Endothelial cell seeding. Br J Surg 1991; 78: 656-660.

5 PEARCE WH, RUTHERFORD RB, WITEHILL TA et al. Successful endothelial seeding with omentally derived microvascular endotheiial cells. J vasc surg 1987; 5: 203-206.

6 LACHAT M, BAITELLA-EBERLE G, BAY U et al. Endothelialization of 6 mm vascular prostheses with microvascular endothelial cells from fatty tissue of the omentums. Helv Chir Acta 1990; 57: 343-346.

7 STERPETTI AV, HUNTER WJ, SCHULTZ RD et al. Seeding with endothelial cells derived from the microvessels of the omentum and from the jugular vein: A Comparative study. J Vasc Surg 1988; 7: 677-684.

8 WANG ZG, DU W, Ll GD et al. Rapid cellular luminal coverage of Dacron inferior vena cava prostheses in dogs by immediate seeding of autogenous endothelial cells derived from omental tissue: results of a preliminary trial. J Vasc Surg 1990; 12: 168-179.

9 VAN HINSBERGH VWM, KOOISTRA T, SCHEFFFR MA et al. Characterization and fibrinolytic properties of human omental tissue mesothelial cells. Comparison with endothelial cells. Blood 1990; 75: 1490-1497.

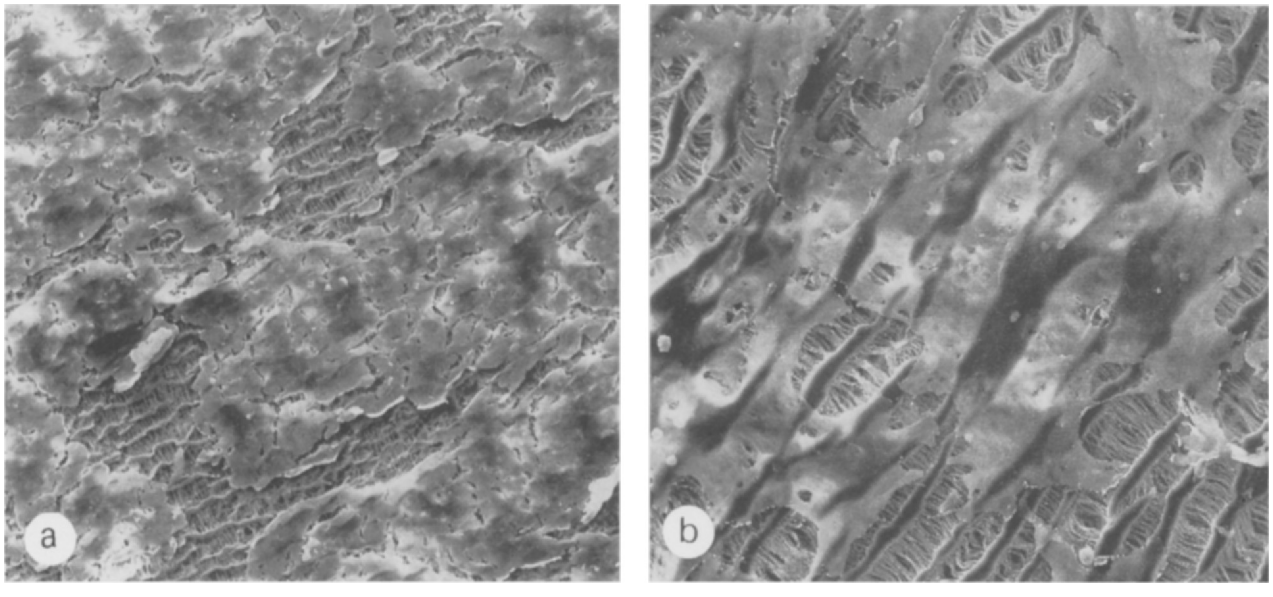

Fig. 3. A stable monolayer remains on the fibroblastic matrix fixed to the PTFE prosthesis after successive washings and in spite of the decrease in radioactive substance levels. (a) EC (x 10()). (b) MC (X 2()0). Reproduced here at 7()%.

10 TOPLEY N, BROWN Z, JC)RRES A et al. Human peritoneal mesothelial cells synthesize interleukin-8. Synergistic induction by interleukin-1 beta and tumour necrosis factor-alpha. Am J Pathol 1993; 142: 1876-1886.

11 BETJFS MG, TUK CW, SHJIJK DG et al. Interleukin-8 production by human peritoneal mesothelial cells in response to tumour necrosis factor-alpha, interleukin-1, and medium. conditioned by macrophages cocultured with Staphylococcus epidermidis. J Infect Dis 1993; 168: 1202-1210.

12 TAKAHASHI K, GOTO T, MUI<AI K ei al. Cobblestone monolayer cells from human omental adipose tissue are possibly mesothelial, not endothelial. In Vitro Cell Dev Biol 1989; 5: 109—111.

13 STANSBY G, FULLER B, HAMILTON G. Human omental microvascular endothelial cells: Are they endothelial? In: Zilla P, Fasol R, Callow A, eds. Applied cardiovascular biology 1990—91. Int Soc Appl Cardiovasc Biol. Basel, Karger 1992; 2: 120—123.

14 HERNANDO A, GARCIA-HONDUVILLA N, BELLÖN JM et al. Coafings for vascular prostheses: Mesothelial cells expess specific markers for muscle cells and have biological activity similar to that of endothelial cells. Eur J vasc surg 1994; 8: 531-536.

15 BEARN PE, SEDDON AM, MCCOLLUM CM et al. Mesothelial seeding of knitted Dacron. Br J Surg 1993; 80: 587—591.

16 BELLÖN JM, BUJÅN J, C.;[a] HONDUVILLA N et al. Endothelial cell seeding of polytetrafluoroethylene vascular prostheses coated with a fibroblastic matrix. Ann Vasc Surg 1993; 7: 549—555.

17 KERN PA, KNEDLER A, ECKFL RH. Isolation and culture of microvascular endothelium from human adipose tissue. J Clin Invest 1983; 71: 1822-1829.

18 GIMBRONE MA. Culture of vascular endothelium. Prog Hemost Thromb 1976; 31: 1-28.

19 BUDD JS, BELL PRF, JAMES REL. Attachment or Indium[w] lll labelled endothelial cells to pretreated polytetrafluoroethylene vascular graft. Br J Surg 1989; 76: 1259—1261.

20 BAKER KS, WILLIAMS SK, JARRELL BE et al. Endothelialization of human collagen surface with human adult endothelial cells. Am J surg 1985; 150: 197-200.

21 WEINBERG CB, BELL E. A blood vessel model constructed from collagen and cultured vascular cells. Science 1986; 231: 397-400.

22 JAUREGUI HO. Cell adhesion to biomaterials: The role of several extracellular matrix components in the attachment of nontransformed fibroblast and parenchymal cells. Trans 11m Soc Artif Intern Organs 1987; 33: 66—74.

23 GARCIA-HONDUVILLA N, BI.JJÅN J, LIZARBF MA et (11. Adhesion and stability of fibronectin on PTFE before and after seeding with normal and synchronized endothelial cells. Artif Organs 1995; 19: 144-153.

24 BUJÅN J, BELLÖN JM, NAVLET J et al. Seeding of expanded polytetrafluoroethylene (ePTFE) vascular grafts. A morphological study of porcine endothelial and fibroblast cells. Histol Histopathol 1992; 7: 635-642.

25 VAN DER LEI B, STRONCK JW, WILDEVUUR CR. Improved healing of small-caliber polytetrafluoroethylene prostheses by induction of a clot layer: a review of experimental studies in rats. Int Angiol 1991; 10: 202-208.

26 SHAREFI<IN JB, LATHER C, SMITH M et al. Endothelial cell labelling with Indium-Ill oxine as a marker of cell attachment to bioprosthetic surfaces. J Biomed Mater Res 1983; 17: 345—357.