Comparative behaviour of L-929 fibroblastic and human endothelial cells onto crosslinked protein substrates

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Abstract

Studies were carried out to compare the behaviour of human umbilical vein endothelial cells (HUVEC) and L-929 fibroblastic cells towards proteins crosslinked by glutaraldehyde (GTA) or carbodiimide (CDI) proposed for coating of vascular prostheses. CDI crosslinking of bovine serum albumin used alone, or mixed with gelatin, allowed higher rates of cell growth and DNA synthesis than GTA crosslinking independent of cells. Assessment of the plating efficiency revealed a similar behaviour of both cells towards membranes and reference plastic surface in terms of percentages of bound cells. HUVEC proliferation onto CDI crosslinked gelatin and/or albumin membranes did not differ significantly whereas the growth of L-929 was enhanced onto gelatin albumin membranes in comparison with both gelatin membranes and the reference surface. The analysis of DNA synthesis corroborated the results of the growth curves and elicited a delay of the growth phases in HUVEC cultured onto CDI crosslinked membranes, unlike the L-929 fibroblast.

Introduction

The L-929 fibroblastic cell line is routinely used in laboratories for in vitro assessment of the biocompatibility of implantable material. The response given in terms of cell growth and attachment may not be extrapolated to in vivo conditions without further investigation, since transformed cells have lost their original specific functions. For this reason, they cannot be compared to differentiated cells grown in direct contact with biomaterial. Therefore, it was of interest to analyse and to compare the behaviour of L-929 fibroblastic cells and human umbilical vein endothelial cells (HUVEC) grown on crosslinked protein membranes previously designed for coating Dacron® vascular prostheses. HUVEC, at the first passage following primary culture, behaved as differentiated endothelial cells retaining specific functions such as the secretion of Von Willebrand factor [9], and the synthesis of prostacyclin [18]. They could thus be considered a more reliable model than dedifferentiated cell lines such as L-929.

Our aim in these studies was to compare the behaviour of HUVEC and L-929 cells cultured onto albumin and/or gelatin membranes crosslinked by carbodiimide (CDI) or glutaraldehyde.
(GTA), the stability of which having been previously controlled in fast protein liquid chromatography [17].

**Material and methods**

**Cell cultures**

HUVEC were harvested from freshly collected human umbilical vein by collagenase treatment according to Jaffe [10]. Primary culture of cells plated in T-25 plastic flasks were established in 199 medium (Boehringer France) supplemented with 20% fetal calf serum (Boehringer France) and antibiotics. After 4–6 days, confluent cultures were briefly treated by 0.125% trypsin, 0.10% EDTA and seeded at a density of 2 x 10^4 cells/cm^2 into each well of 4-well plates (Nunc-lon) coated with crosslinked proteins as described below. Medium was then added with endothelial cell growth supplement (ECGS, 50 μg/ml, Collaborative Research) and heparin (50 μg/ml, Sigma). Harvesting of cells from confluent primary cultures provided about 2 x 10^6 cells which allowed comparative growth analysis on 4 different substrates. Cell counts were performed in a hemocytometer at 3-day intervals. Data are presented as the mean values of 4 counts ± standard error.

L-929 fibroblasts (Eurobio, approximate number of cell passage: 570) were routinely grown in MEM medium (Boehringer France) supplemented with 5% fetal calf serum, non-essential amino-acids and antibiotics.

**Protein coating of growth surface**

Fraction V bovine albumin (SIGMA) and pigskin gelatin (ROUSSELOT) were covalently crosslinked according to techniques using CDI (SIGMA) or GTA (MERCK).

**Crosslinking by CDI.** Albumin and gelatin were dissolved 20% and 10% respectively in 0.02 M PBS pH 6.8. Albumin membranes M(A)C were crosslinked by mixing successively 2 vol. albumin, 1 vol. CDI. 0.2 ml of this mixture were poured into each well of 4-well-plates.

Gelatin membranes M(G)C and mixed albumin and gelatin membranes M(AG)C were performed by pouring respectively 0.2 ml gelatin or 0.2 ml of the mixture 2 vol. albumin, 1 vol. gelatin into each well. Plates were kept at least 3 h. in a laminar flow hood to allow solutions to gel and to dry. M(G)C and M(AG)C were postreticulated by adding 0.2 ml of CDI solution, allowed to stand 3–4 min.

All the membranes were thoroughly rinsed at least 3 times with PBS pH 7.2 and kept at 4°C for 4 days, rinsed again 3 times before seeding. All the solutions were sterilized by filtration on 0.45 μm filters (Sartorius).

**Crosslinking by GTA.** Albumin and gelatin were dissolved 20% and 10% respectively in 0.02 M PBS pH 4.75. A solution of 0.2 M 1-ethyl-3(dimethylaminopropyl) carbodiimide in PBS pH 4.75 was prepared extemporaneously. Albumin membranes M(A)C were crosslinked by mixing successively 2 vol. albumin, 1 vol. CDI. 0.2 ml of this mixture were poured into each well of 4-well-plates.

Gelatin membranes M(G)C and mixed albumin and gelatin membranes M(AG)C were performed by pouring respectively 0.2 ml gelatin or 0.2 ml of the mixture 2 vol. albumin, 1 vol. gelatin into each well. Plates were kept at least 3 h. in a laminar flow hood to allow solutions to gel and to dry. M(G)C and M(AG)C were postreticulated by adding 0.2 ml of CDI solution, allowed to stand 3–4 min.

All the membranes were thoroughly rinsed at least 3 times with PBS pH 7.2 and kept at 4°C for 4 days, rinsed again 3 times before seeding. All the solutions were sterilized by filtration on 0.45 μm filters (Sartorius)

**Cell adhesion assay**

Subconfluent cultures plated in Falcon T-25 plastic flasks were labeled with 2 μCi/ml [6-3H] thymidine (25 Ci/mmole, CEA France). After 24 h