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En face Organ Cultures of Rabbit Aortic Segments after a Single Dilatation Trauma in vivo

A New Model in the Study of Endothelial Regeneration

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Summary. Following an in vivo lesion of the rabbit thoracic aorta, endothelial regeneration was studied in silver-stained specimens from en face organ cultures of aortic segments with and without intercostal arteries. The in vivo lesion had been inflicted by an embolectomy catheter.

Our results confirmed that endothelium regenerates from preexisting endothelium around the mounths of the intercostal arteries. A conspicuous observation was the orientation pattern of endothelial cells during regeneration. Transmission electron microscopy suggests that the cell cytoskeleton plays a role in the regulation of regeneration. The organ culture technique described, complementary to in vivo and in vitro investigations with cell cultures, seems to be a valuable tool in further investigations on factors involved in the repair of arterial tissue.

Key words: Rabbit aorta – Organ culture – Endothelial regeneration – Electron microscopy.

Introduction

Previous in vivo investigations on intimal repair have shown that endothelium develops from pre-existing endothelium in the mouths of intercostal arteries, and that pseudoendothelium is probably derived from smooth muscle cells in neointima (Collatz Christensen and Garbarsch, 1973; Schwartz et al., 1975; Collatz Christensen et al., 1977).

De Bono (1975) has described a simple in vitro technique for maintaining wholemount arterial sections with intact endothelium for up to 10 days.

The present report describes an experimental model for investigation of endothelial regeneration in vitro following a standardized mechanical lesion in vivo (Helin et al., 1971). The studies confirmed that endothelium regenerated

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from pre-existing endothelium around the mouths of intercostal arteries. The feasibility of this organ culture technique for further investigations on the repair in arterial tissue is discussed.

Materials and Methods

Animals and Aortic Lesion. Male albino rabbits of the Danish country strain (5 months old, weighing approximately 3 kg) were used. The aortic lesion was produced by an embolectomy catheter (12-080-5F) introduced to the level of the aortic arch via the left femoral artery and retracted with an inflated balloon till resistance was felt at the diaphragm (Helin et al., 1971; Collatz Christensen and Garbarsch, 1973). Twenty-four h after the lesion the aorta was used for en face organ cultures.

Organ Cultures. Under general anaesthesia the thoracic aorta was exposed through a thoracotomy. After applying clamps at the ends of the vessel and injecting a small quantity of heparin (1000 U/ml) into the lumen, the thoracic aorta was atraumatically excised. It was placed in a bath of oxygen saturated Minimal Essential Medium (MEM-Fll) with antibiotics (penicillin 100 IU/ml and streptomycin 100 µg/ml) at room temperature, the adventitial tissue was excised, and the wall slit open along the ventral side. Segments (5 × 5 mm) with and without intercostal arteries were excised, placed with the endothelial side downwards over 4 mm diameter holes punched in stainless steel grids, which were placed in Falcon organ culture dishes filled to the level of the grid with culture medium (approximately 2 ml). The adventitial side of the specimens were covered with pieces of sterile Millipore filters (1.2 µm pore size) in order to support and flatten the specimens. Culture medium: Waymouth's medium (GIBCO 705/1) supplemented with 10% inactivated fetal calf serum (GIBCO) and antibiotics (penicillin 100 IU/ml and streptomycin 100 g/ml), buffered with bicarbonate/5% CO_2. Within 45 min all organ culture dishes were placed in a gastight chamber. The chamber was then flushed with 95% O_2 and 5% CO_2, sealed, and kept in an incubator at 37° C. The gas phase was renewed daily, and the medium was changed every other day.

Wholemount Specimens for Surface Light Microscopy (Surface LM). Segments with and without intercostal arteries excised at autopsy one day after the lesion, as well as specimens from organ cultures (4 and 8 days after the lesion, i.e. cultivated 3 and 7 days as organ cultures) were stained with 0.25% $AgNO_3$ for 1 min, exposed to light and photographed in order to estimate the rate

Fig. 1. Silver-stained aorta segment with two intercostal arteries one day after the lesion. Preserved endothelium around the mouth of intercostal arteries is demonstrated. × 12

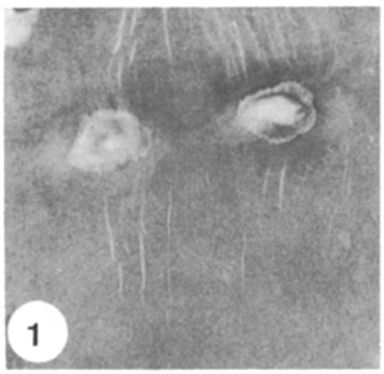
Fig. 2. Silvver-stained aorta segment with two intercostal arteries 8 days after the lesion, 7-day culture. Considerable outgrowth of endothelial cells from the mouths of intercostal arteries is seen. Note the elongated form of this endothelial outgrowth. × 12

Fig. 3. Surface light microscopy of preserved silver-stained endothelium around an intercostal artery. The border of the endothelial rim is distinct, no endothelial outgrowth is seen at this time. × 160

Fig. 4. Surface light microscopy of silver-stained newformed endothelium from pre-existing endothelium around an intercostal artery. The border of the outgrowth of endothelium is indistinct. Several large irregular cells are visible. Cell contacts are incomplete. × 160

Fig. 5. Electron micrograph of the re-endothelialization around the intercostal arteries. No intimate contact with the underlying elastic lamella is visible. × 4300

Fig. 6. Electron micrograph of the contact between two endothelial cells. There is not yet the characteristic endothelial flap. Note the abundance of contractile microfilaments with spotty appearance of condensed streaks. Endocytotic vesicles are seen. × 25,000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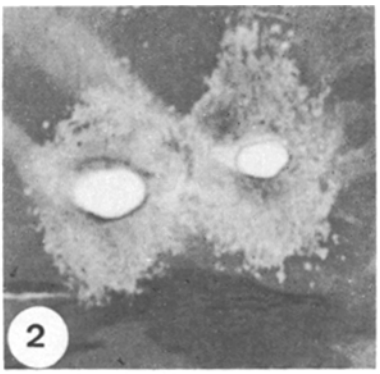
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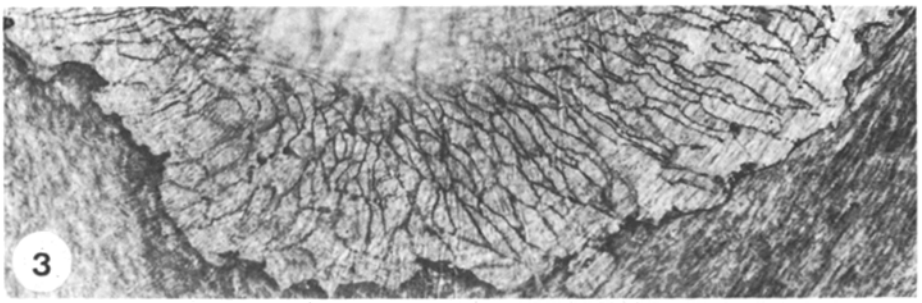
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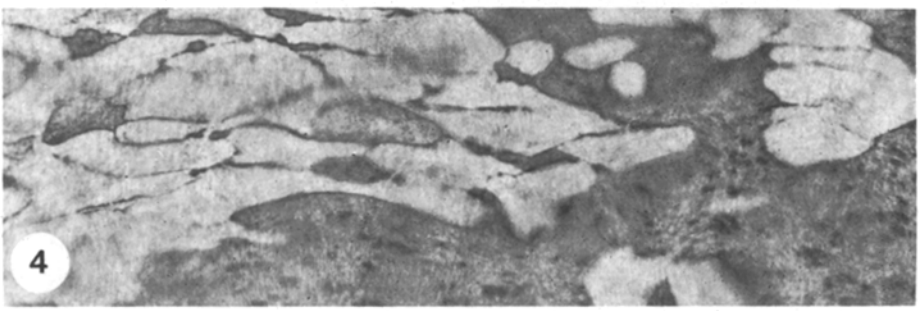
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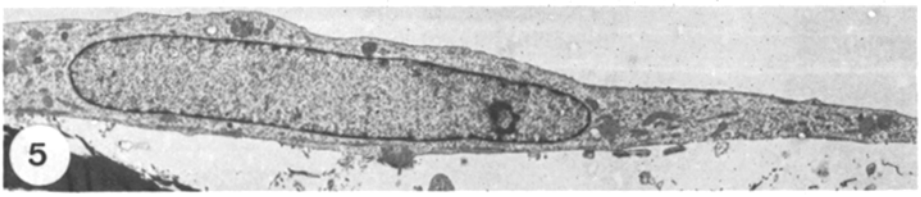
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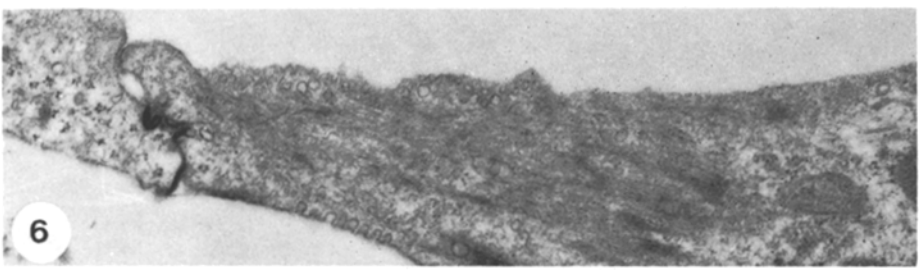
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of endothelial growth. Afterwards the tissue was prepared as wholemount specimens for surface LM (Collatz Christensen and Garbarsch, 1973).

Transmission Electron Microscopy (TEM). Segments cultivated for 7 days as organ cultures (i.e. 8 days after the lesion) were fixed in 1.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer at 37° C, pH 7.2 (total osmolality 550 mOsm). After postfixation in 1% OsO_4 the segments were exposed to a solution of 1% low molecular weight galloylglucoses (LMGG) in cacodylate buffer (Simionescu and Simionescu, 1976), dehydration in acetone, embedding in Araldite. Ultrathin sections were contrasted with zinc-uranyl acetate and with lead citrate.

Results

Surface LM of Control Specimens

Photographs of the silver-stained specimens presented a narrow rim of preserved endothelial cells around the intercostal arteries (Fig. 1). Surface LM (Fig. 3) revealed patterns of elongated endothelial cells oriented with their long axis parallel to the long axis of the vessel, and the border of the endothelial rim was very sharp and distinct. Specimens without intercostal arteries presented fenestrated elastic membranes and smooth muscle cells, but only sporadically were flakes of endothelial cells visible. The endothelial lesion apparently was almost perfect.

Surface LM of Silver-Stained Specimens Cultivated as Organ Cultures

After 4 days specimens with intercostal arteries showed an incipient outgrowth of endothelial cells around the mouths of intercostal arteries. After 8 days a considerable expansion of endothelium was observed (Fig. 2). The endothelial outgrowth was elongated, resembling in vivo changes 8 days after the lesion (Collatz Christensen et al., 1977), and the borders were indistinct. The majority of cells were arranged with their long axes parallel to the long axis of the vessel. Near the border of expanding endothelium the cell contacts were incomplete, and at the boundary between endothelium and denuded surface irregular, flattened cells dominated (Fig. 4).

In segments without intercostal arteries no significant proliferative activity had apparently taken place compared to the control specimens.

TEM of Segments Cultivated as Organ Cultures for 7 Days

TEM examinations showed that the cells in the endothelial outgrowth had no intimate contact with the underlying elastic lamellae (Fig. 5). The endothelial cells looked immature with prominent endocytotic vesicles and secretory appa-

ratus. Cell contacts were established, but in general they were not fully developed, and endothelial flaps were missing (Fig. 6). A conspicuous feature was an abundance of microfilaments, with spotty appearance of condensed streaks in the cytoplasmic rim near cellular contacts (Fig. 6). In the elongated cells microtubules running in the long axis of the cell were observed, while no organization of the microtubular system was evident in the cytoplasm of flattened cells at the rim of expanding endothelium.

Discussion

Organ culture-systems for cultivation of aortic segments for up to 10 days have previously been described (Clair and Lofland, 1971; De Bono, 1975). Clair and Lofland (1971) succeeded in maintaining arterial segments in a metabolically active state for up to 9 days. They did not perform a detailed morphologic study of arterial segments from organ culture. In en face organ cultures of vascular endothelium De Bono (1975) was able to maintain intact sheets of endothelium for up to 10 days. He showed that endothelium tended to grow out in continuity after 4 or 5 days over areas accidentically or purposely denuded of endothelium when the culture was set up.

The method described for maintaining organ cultures of aortic segments with and without intercostal arteries provides the opportunity to study endothelial regeneration under well defined in vitro conditions.

Our preliminary surface LM examinations revealed a surprising resemblance to endothelial regeneration in vivo: elongated endothelial islands centered around the intercostal arteries, and a longitudinal orientation of endothelial cells. This evidence from in vitro experiments indicates that haemodynamic forces are not decisive in orientation of regenerating endothelial cells. The mechanisms involved remain obscure, however, preliminary investigations by TEM suggest that changes in systems of microtubules and microfilaments may be involved in polarizing the endothelial cells, as well as in establishment of cell contacts.

The organ culture method, based on an in vivo lesion studied under in vitro conditions, may be a valuable tool in further investigations on factors involved in repair of arterial tissue, and thus add new information to the understanding of the atherosclerotic processes.

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References

Clair, R.W., Lofland, H.B.: Uptake and esterification of exogenous cholesterol by organ cultures of normal and atherosclerotic pigeon aorta. Proc. Soc. exp. Biol. Med. **138**, 632–637 (1971)

Collatz Christensen, B., Chemnitz, J., Tkocz, I., Blaabjerg, O.: Repair in aterial tissue. The role of endothelium in the permeability of a healing intimal surface. Vital staining with Evans

blue and silver-staining of the aortic intima after a single dilatation trauma. Acta path. microbiol. scand. Sect. A **85**, 297–310 (1977)

Collatz Christensen, B., Garbarsch, C.: Repair in arterial tissue. A scanning electron microscopic (SEM) and light microscopic study on endothelium of rabbit thoracic aorta following a single dilatation injury. Virchows Arch. Abt. A Path. Anat. **360**, 93–106 (1973)

De Bono, D.: En face organ culture of vascular endothelium. Brit. J. exp. Path. **56**, 8–13 (1975)

Helin, P., Lorenzen, I., Garbarsch, C., Matthiessen, M.E.: Repair in arterial tissue. Morphological and biochemical changes in rabbit aorta after a single dilatation injury. Circulat. Res. **29**, 542–554 (1971)

Schwartz, S.M., Stemerman, M.B., Bendit, E.P.: The aortic intima. II. Repair of the aortic lining after mechanical denudation. Amer. J. Path. **81**, 15–42 (1975)

Simionescu, N., Simionescu, M.: Galloylglucoses of low molecular weight as mordant in electron microscopy. I. Procedure and evidence for mordanting effect. J. Cell Biol. **70**, 608–621 (1976)

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