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Proliferation and attachment of endothelial cells on a collagen surface

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Introduction

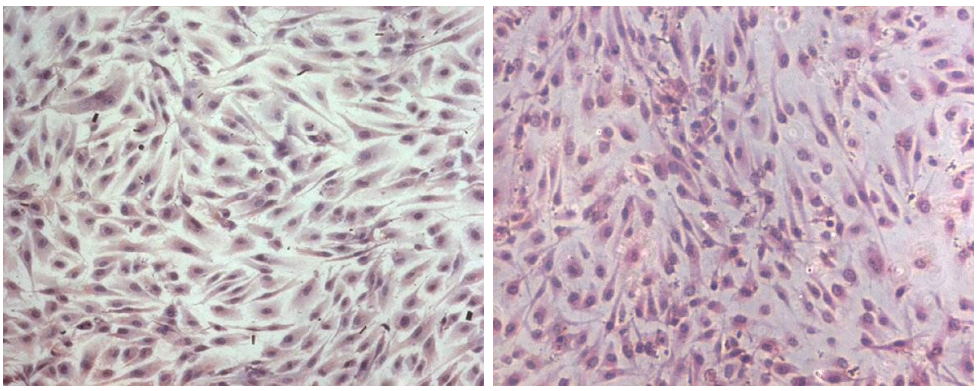
Besides proliferation and maturation of osteoblasts, mineralization of the collagenous matrix alongside with vascularization plays a crucial role in bone regeneration. Endothelial cells are critically involved in bone development, remodelling and can influence the bone cell recruitment, formation and activity. The microvascularization and circulation are basis for bone regeneration and this is dependent on interaction between osteoblasts and endothelial cells (EC). Successful clinical application of compound biomaterials should involve detailed investigations on cell adhesion and differentiation, giving insights to guided angiogenesis leading to tissue repair. The contacts of EC to extracellular matrix play a crucial role in angiogensis during tissue remodelling and β3 subunit containing integrins are the major matrix receptors expressed by EC. Not only cell-matrix adhesion is vital for physiological role of endothelium, but also cell-cell contact. These junctions are ubiquitous along the vascular tree and are formed by transmembrane proteins belonging to cadherin superfamily. EC express the cell specific vascular endothelial (VE)-cadherin, that plays a morphogenic role in vascular development. With the present investigation, we examined the behaviour of EC on a collagen surface, in order to optimise osteogenesis with a cytokin-cell-carrier complex.

Material und Methods

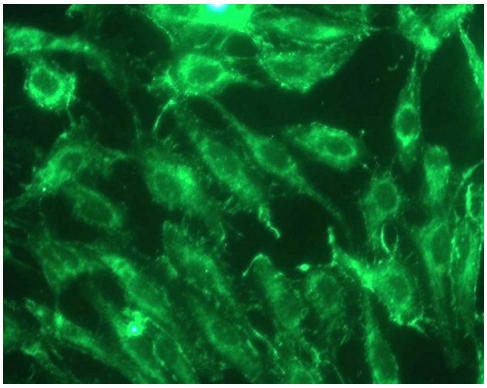
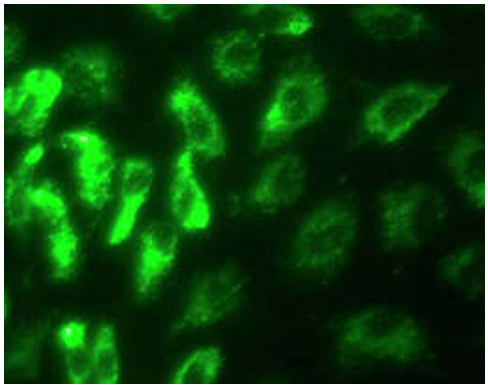
Primary human umbilical vein endothelial cells (HUVEC) were isolated according to the collagenase dispersion method (1). After isolation, cells were cultured and maintained to passage 2 in M199 medium (Gibco-BRL Products) containing 20% of inactivated fetal bovine serum (FBS), 1% of penicillin-streptomycin (PS), 1% of EC growth supplement (ECGS-Sigma) and 0.1% heparin at 37°C in a humidified atmosphere with 5% CO_2. Purity of cultures on passage 2 was demonstrated by immunocytochemistry with a panel of monoclonal antibodies (anti-von Willebrand Factor (vWF), anti-PECAM-1 (CD31) and anti-α-smooth muscle cells (α-SMA)). For studies, EC were seeded on circular sections (13 mm diameter) of commercial available type-I collagen membranes (Lycoll, Resorba, Nürmberg) placed in 24 well plates, at density of 80.000 cells/cm^2, maintained for 1 to 7 days. Haematoxyline/eosin staining was performed on paraffin sections. Probes were also fixed and prepared for immunohistochemical studies and scanning electron microscopy (SEM). Primary mouse monoclonal antibodies specific for human vascular endothelial (VE)-cadherin (secondary fluorescence) and integrin aVb3 (primary fluorescence) were used.

Results

Many in vitro studies on the interaction of cells with naturally produced matrix have demonstrated significant effects on cell shape and growth characteristics in that cell adhesion, spreading and migration are each related to interactions with the matrix. (2,3). Our analyses revealed proliferation and a confluent monolayer of EC's as well as attachment of the EC to the collagen matrix. On day 1 cell-cell contacts could be visualized as well as cell-matrix contacts. There was no sign of apoptosis or cell death during observation.

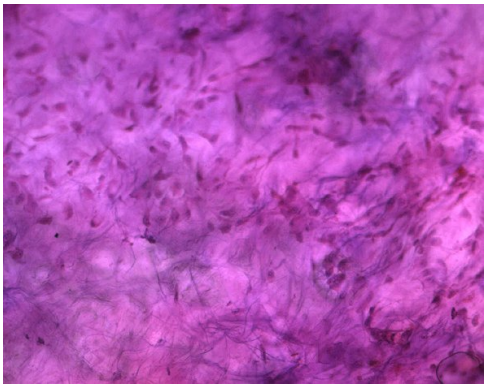
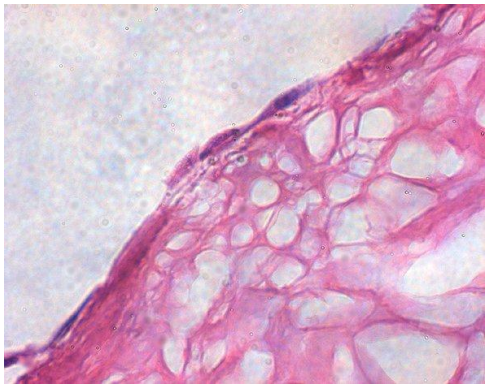


Normal endothelial cells on surface of thermanox after 1 and 7 days

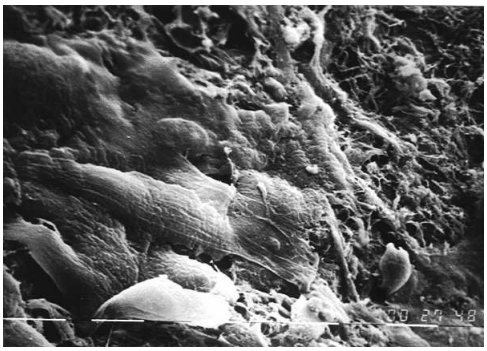
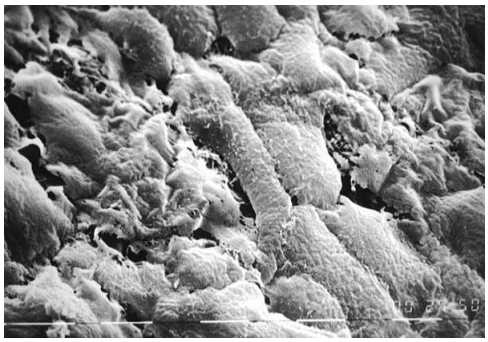
Immunological Identification of endothelial cells

CD31 (PECAM 1) VWF(Factor VIII)

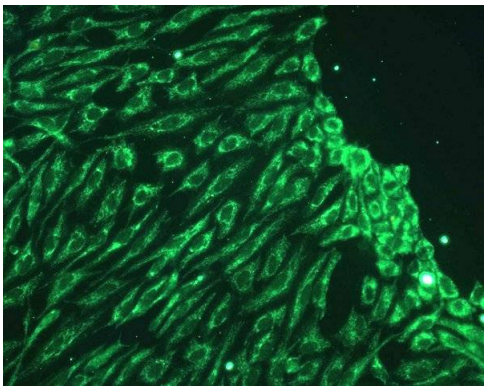
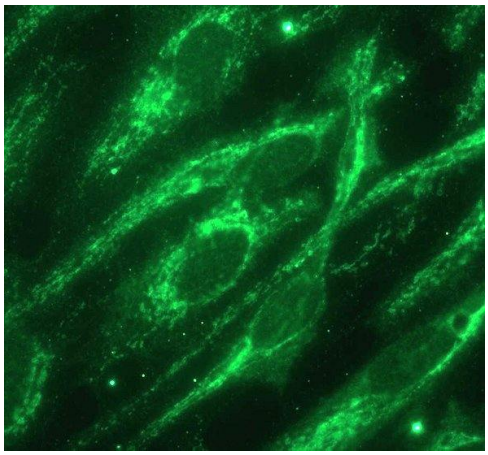
Haematoxylin/Eosin staining on collagen membrane

1 day(10x) 7 days(40X)

SEM of the EC on collagen membrane

1 day 7 days

Immunological staining for cell adhesion molecule

VE-Cadherin

Conclusions

Endothelial cells not only attach but also proliferate on a collagen type-I matrix. Therefore it might be another step towards a tissue engineered bone substitute by combining matrix with osteoblasts, endothelial cells and growth factors.

Bibliography

1. Marin V, Kaplanski G, Grès S, Farnarier C, Bongrand P. Endothelial cell culture: protocol to obtain and cultivate human umbilical endothelial cells. J Immunol Methods 254:183-90, 2001.
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This poster was submitted by Priv.-Doz. Dr. Dr. Johannes Kleinheinz.

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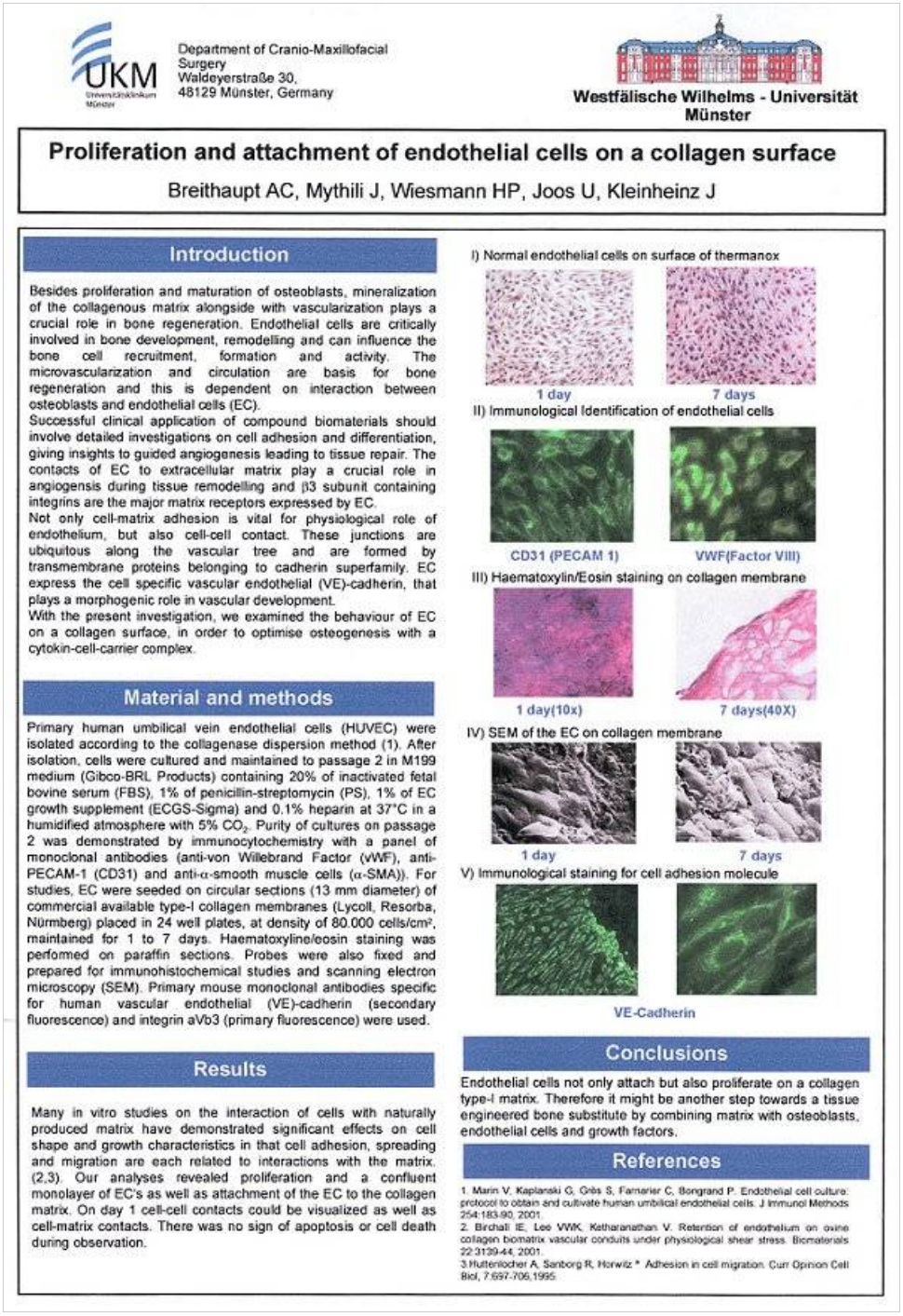

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