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Direct Magnetic Tubular Cell Seeding: A Novel Approach for Vascular Tissue Engineering

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Key Words
Blood vessels · Endothelium · Tissue engineering · Vascular development · Magnetic tubular cell seeding

Abstract
Optimizing seeding efficiency, reducing delayed culture periods and mimicking native tissue architecture are crucial requirements for the development of seeding procedures in tissue engineering. In vascular applications, the tubular geometry of the grafts further hampers the efficient delivery of cells onto the scaffold. To overcome these limitations, a novel technology based upon the use of magnetic fields is presented in this study: a radial magnetic force drives the cells immediately onto the luminal surface of a tubular scaffold and immobilizes the cells on the substrate's surface promoting cell attachment. Human smooth muscle cells (SMCs) labeled with CD44 magnetic Dynabeads® were successively seeded onto the luminal surface of a tubular shaped collagen membrane. After 5 h, one additional layer of human umbilical vein endothelial cells (HUVECs) labeled with CD31 magnetic Dynabeads® was seeded onto the luminal SMCs. The co-culture was incubated during 5 days prior to analysis. Cell viability and expression profiles were preserved during the entire seeding process. Histological examination of the constructs highlighted densely packed multilayers of SMCs covered by a monolayer of endothelial cells. SEM inspection confirmed a heterotypic multilayer assembly formed by multiple layers of elongated SMCs covered by a single layer of endothelial cells. Seeding kinetics of HUVECs and SMCs showed over 90% seeding efficiency after 20 and 40 min magnetic exposure respectively. Magnetically induced cell seeding provides a valuable tool for rapid seeding procedures of tubular scaffolds while complying with the histological architecture of tissue.

This work was presented at the 4th Annual Meeting of the European Tissue Engineering Society, Munich, September 2005 and at the 8th Annual Meeting of the Tissue Engineering Society International, Shanghai, October 2005, and awarded the Young Investigator Award at the International Conference on Biomedical Engineering, Singapore, December 2005.

Abbreviations used in this paper

CLSM	confocal laser scanning microscope
EGF	epidermal growth factor
FCS	fetal calf serum
HE	eosin-hematoxylin
HUVECs	human umbilical vein endothelial cells
MRI	magnetic resonance imaging
SEM	scanning electron microscope
SMCs	smooth muscle cells

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Introduction

Over half a million coronary artery bypass procedures are performed annually alone in the USA [American Heart Association, 2005]. Autologous veins are the material of choice in bypass surgery; however, major complications arise when no suitable vessels are available. The use of synthetic grafts has been successfully introduced for large diameter vessel replacement ($D > 10$ mm) but for artery bypass grafting, lower extremity bypass procedures or hemodialysis access, the use of small caliber synthetic grafts ($D < 6$ mm) is not acceptable due to their low long-term patency rates [Tiwari et al., 2001; Kakisis et al., 2005]. Endothelial cell seeding of prosthetic grafts prior to implantation [Parikh and Edelman, 2000] and vascular tissue engineering [Hoerstrup et al., 2001; Vara et al., 2005] have emerged as promising alternatives to overcome the lack of suitable autologous small diameter grafts.

In the last years, significant progress has been achieved in vascular tissue engineering [L'Heureux et al., 2006; Meinhart et al., 2001; Matsumura et al., 2003] and recent advances in stem cell research harbor promising outcomes in this field [Schmidt et al., 2004; Sales et al., 2005]. However, considering clinical applications, some key limitations still have to be addressed [Griffith and Naughton, 2002; Nerem, 2004]. The scarce number of differentiated cells available, the poor mechanical compliance and the long culture periods hamper the widespread use of tissue-engineered vascular grafts in surgical procedures.

Cell seeding onto adequate scaffolds is the first step in the cultivation of engineered tissues. Seeding density, homogeneity and cell integrity are key parameters for the subsequent patency and functionality of the tissue in vivo [Vunjak-Novakovic et al., 1998; Martin et al., 2004]. Moreover, optimizing seeding efficiency, promoting rapid cell attachment to the scaffold and mimicking tissue histology may contribute to mitigate current constraints related to cell availability, prolonged culture protocols and mechanical and physiological performance, respectively. Numerous seeding concepts have been proposed for vascular tissue engineering [Bowlin and Rittgers, 1999; Dunkern et al., 1999; Sutherland et al., 2002; Barron et al., 2003; Nasseri et al., 2003; Mironov et al., 2005; L'Heureux et al., 2006]. However, to the date, none of these techniques has prevailed for standardized applications.

Magnetic particles ranging from the nanometer to micrometer scale are gaining increasing interest due to their potential biomedical applications [Pankhurst et al., 2003; Neuberger et al., 2005]. In tissue engineering, magnetic nanoparticles provide a valuable tool to perform target cell delivery into the scaffold. Recently, investigators have used permanent magnets to gather cells on planar substrates and promote tissue formation [Ito et al., 2005].

This study explores a novel seeding approach based on the feasibility to guide magnetically labeled cells with magnetic field gradients for direct seeding of tubular structures. A radial magnetic force acting as a remotely controlled driving force is used to deliver cells directly onto the luminal surface of a tubular scaffold. Once the cells reach the surface of the scaffold, the magnetic force provides for the necessary mechanical support to avoid undesired settling effects due to gravity. As soon as cell attachment to the surface occurs, the magnetic field is no longer required and the culture may continue according to customary physiological conditions. Through successive seeding steps, cells are assembled into organized multilayers mimicking vessel architecture (fig. 1).

The main goal of this study is to present a novel magnetic-based seeding technology to enable rapid multilayered assembly of defined co-cultures in vitro and to discuss the advantages and future perspectives.

Materials and Methods

Cell Cultivation

Human umbilical vein endothelial cells (HUVECs) obtained from Cascade Biologics Inc. (Mansfield, UK) were cultured in endothelial growth medium (Promocell GmbH, Germany) supplemented with 10% fetal calf serum (FCS), 0.004 ml/ml endothelial cell growth supplement/heparin, 0.1 ng/ml epidermal growth factor (EGF), 1 ng/ml basic fibroblast growth factor and 1 μg/ml hydrocortisone at 37°C in a humidified atmosphere with 5% CO_2.

Primary human smooth muscle cells (SMCs) isolated as previously described [Laflamme et al., 2005] were maintained in Ham's F12/DMEM medium (Promocell GmbH) supplemented with 15% FCS in a humidified environment at 37°C with 5% CO_2.

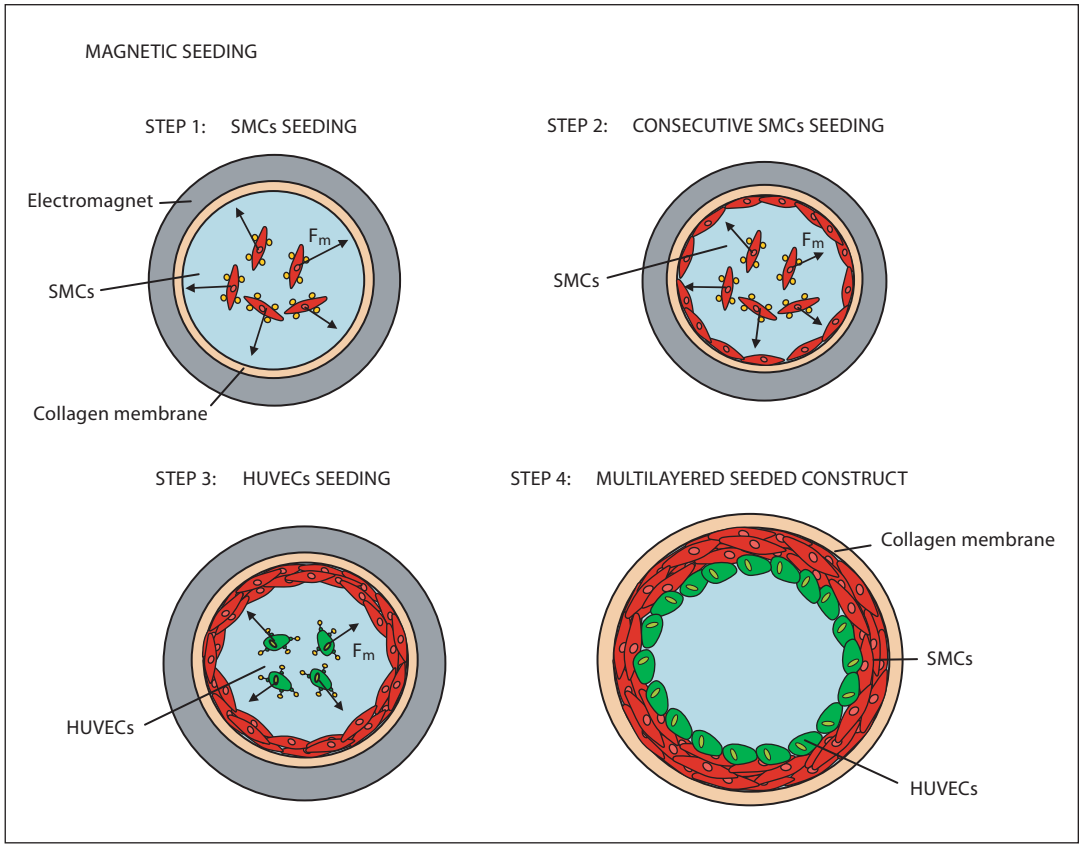
Immunomagnetic Labeling

HUVECs (passage 2–4) were labeled with anti-human CD31 Dynabeads® (Dynal Biotech, Norway) according to the standard protocol provided by the manufacturer with an average number of 6 beads per cell to avoid toxic effects of magnetic beads at high concentrations [Tiwari et al., 2003]. Dynabeads® are 4.4-μm-diameter spheres consisting of an agglomeration of sub-micron-sized iron oxide particles incorporated in a polymeric binder.

SMCs (passage 4–8) were labeled with a primary mouse anti-human CD44 antibody (Dianova GmbH, Germany) and coupled to a secondary Pan Mouse IgG Dynabeads® antibody (Dynal Biotech).

Bioabsorbable Collagen Scaffolds

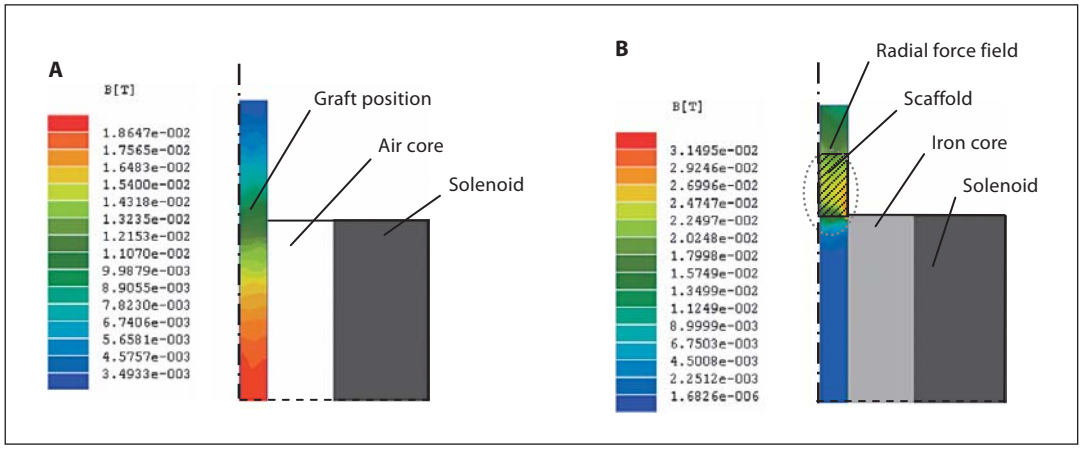
A resorbable equine collagen membrane kindly provided by Resorba Clinicare GmbH (Germany) was cut into square sheets


MAGNETIC SEEDING
STEP 1: SMCs SEEDING
Electromagnet
SMCs
Collagen membrane
Fm
STEP 2: CONSECUTIVE SMCs SEEDING
SMCs
Fm
STEP 3: HUVECs SEEDING
HUVECs
Fm
STEP 4: MULTILAYERED SEEDED CONSTRUCT
Collagen membrane
SMCs
HUVECs

1


A
B
C
*

2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B[T]
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9.9879e-003
8.9055e-003
7.8230e-003
6.7406e-003
5.6581e-003
4.5757e-003
3.4933e-003
Graft position
Air core
Solenoid
B
B[T]
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2.9246e-002
2.6996e-002
2.4747e-002
2.2497e-002
2.0248e-002
1.7998e-002
1.5749e-002
1.3499e-002
1.1249e-002
8.9999e-003
6.7503e-003
4.5008e-003
2.2512e-003
1.6826e-006
Radial force field
Scaffold
Iron core
Solenoid

3

of 10 × 10 mm, rolled, carefully introduced into a glass capillary (3.5 mm inner diameter) (Neolab, Germany) and adapted to the inner cylindrical shape of the tube (fig. 2).

Magnetic System

The magnetic force, F_m, acting on a magnetic particle is given by:

$$\overrightarrow{F_m} = \mu_0 V \chi_m \left(\vec{H}\nabla\right)\vec{H}$$

where μ_0 is the permeability in vacuum, V is the volume of the particle, χ_m is the magnetic susceptibility of the particle, H the magnetic field, and ∇H the magnetic field gradient. Thus, to drive cells with magnetic forces, high non-uniform magnetic fields are required. A solenoid with 1,370 turns, 8 cm length and 4 cm inner diameter was designed to provide the required magnetic field due to its cylindrical geometry. The radial magnetic field gradient was increased by a tubular soft iron core. Finite element analysis was performed to map the magnetic field and picture the adequate region for seeding purposes. Radial magnetic force fields were observed beyond the extremes of the coil. Accordingly, the glass capillaries were allocated vertically inside the iron core positioning the collagen membrane just above the upper extreme of the coil (fig. 3). The magnetic field in the region of interest at a current of 1 A ranges from 20 to 30 mT. Assuming a linear increase of the magnetic field from the axis to the lumen of the tubular scaffold, the average magnetic force, F_m, acting on a single magnetic bead (χ_m = 16 × 10^{-5} m^3/kg and d = 4.4 μm) is about 2 pN. It should be noted that the settling force in the medium of a single bead (density 1.5 g/cm^3) is 0.07 pN, clearly below the driving magnetic force.

Fig. 1. Schematic view of the novel magnetic seeding concept. Upon application of a radial magnetic force, SMCs are successively deposited onto the luminal surface of a tubular collagen membrane (step 1). Further seeding steps provide a multilayered cell assembly of SMCs (step 2). Finally, the lumen is seeded with a single layer of HUVECs (step 3). Once the seeding process is completed, the magnetic field is removed and the construct may be cultured under customary conditions (step 4).

Fig. 2. Scaffold preparation. **A** Stretched collagen membrane in a Petri dish. **B** Membrane is cut, rolled and introduced into the glass capillary. **C** The capillary (*) is placed inside the solenoid to allocate the scaffold above the upper extreme of the coil.

Fig. 3. Finite element analysis of the magnetic field of the solenoid. For symmetry reasons, only the upper half of the solenoid is displayed. **A** Air core solenoid. Radial component of the magnetic field is nearly constant, thus low magnetic radial forces are induced. **B** Solenoid with soft iron magnetic core. The iron core alters the magnetic field. Radial magnetic field gradients appear above the upper extreme of the coil offering the optimal force field for tubular cell seeding.

Magnetic Seeding Procedure – Multilayer Seeding

The glass capillaries were prefilled with medium reaching the lower margin of the scaffold. A current of 1 A was applied to the solenoid to induce the radial magnetic force. At this point, 200 μl suspension containing 200,000 cells were gently pipetted into the test tube. The magnetic field exposures for HUVECs and SMCs were 20 and 40 min respectively.

To achieve a multilayered cell assembly, 5 successive seeding steps with SMCs were carried out at a rate of one single seeding step per hour. One additional seeding step was performed with HUVECs in order to deposit one single layer of endothelial cells. The co-culture was incubated for 5 further days at 37°C in a humidified atmosphere with 5% CO_2 prior to analysis.

Seeding Kinetic

Seeding efficiencies at different field exposure intervals were measured to optimize the seeding procedures. Seeding kinetic was determined separately for SMCs and HUVECs. Scaffolds were seeded in a single step with 200,000 cells according to the following magnetic field exposure intervals: 10, 20, 30, 40 and 60 min for SMCs and 5, 10, 20, 40 and 60 min for HUVECs. The samples were cultured at 37°C in a humidified atmosphere with 5% CO_2 for 12 h. Thereafter, scaffolds were removed from the tubes and cell concentrations were determined by measuring the mitochondrial activity through a WST-1 assay.

Histology and Immunohistology

Immunohistology was performed after fixation of the seeded collagen membranes with 70% ethanol. To display endothelial cell phenotype, primary mouse anti-human CD31 (Dianova GmbH) antibody was combined with a secondary goat anti-mouse Alexa 488 (Molecular Probes, USA). Counterstaining of the nuclei was carried out with ethidium bromide. Von Willebrand factor expression on endothelial cells was assessed by using a primary polyclonal von Willebrand factor (Lab Vision Corp., USA) detected via a secondary goat anti-rabbit FITC-conjugated antibody (Medac, Germany).

For histological analysis, the samples were prepared by fixation in 4% phosphate-buffered formalin and paraffin-embedded. Sections of 10 μm were prepared and stained with hematoxylin and eosin (HE). The samples were investigated using a confocal laser scanning microscope (CLSM) LSM 510 Meta (Zeiss, Germany).

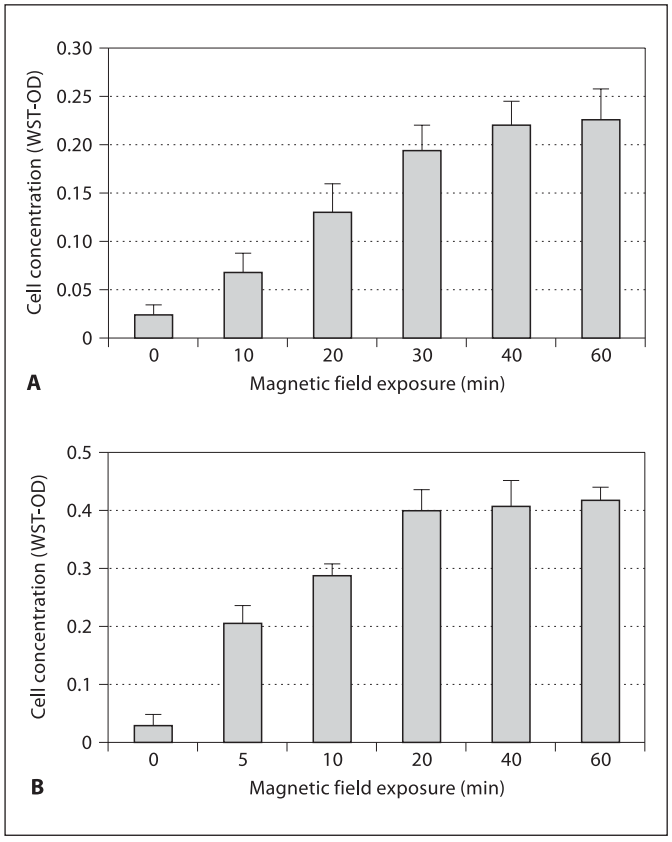
Scanning Electron Microscopy

Specimens fixed in glutaraldehyde were gradually dehydrated in an ethanol series, passed through a critical point drying and finally sputter-coated with gold. Preparations were viewed under a field emission scanning electron microscope (Hitachi 3500N, Japan) operated at 5 kV.

Results

Optimization of Magnetic Field Exposure

The magnetic field exposure intervals were determined to maximize the number of cells attached to the scaffold. SMCs showed an increasing proclivity to adhere during the first 40-min field exposure (fig. 4A), whereas



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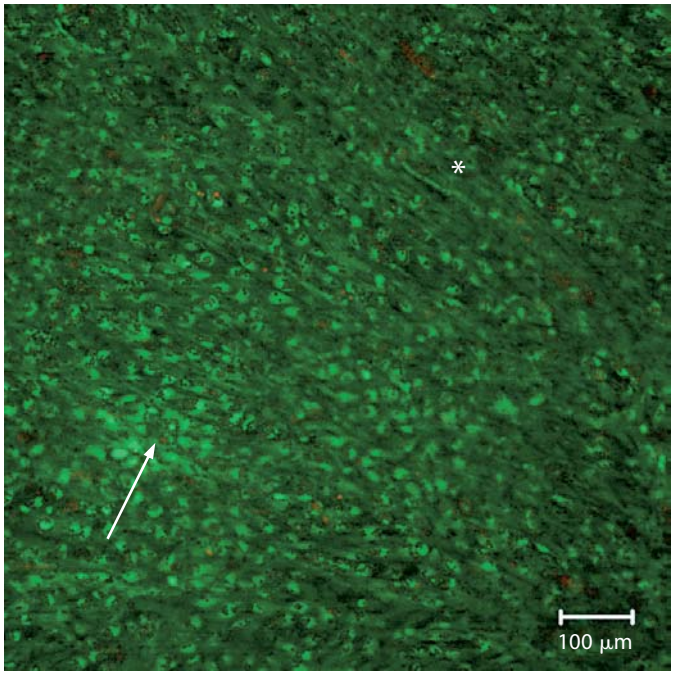

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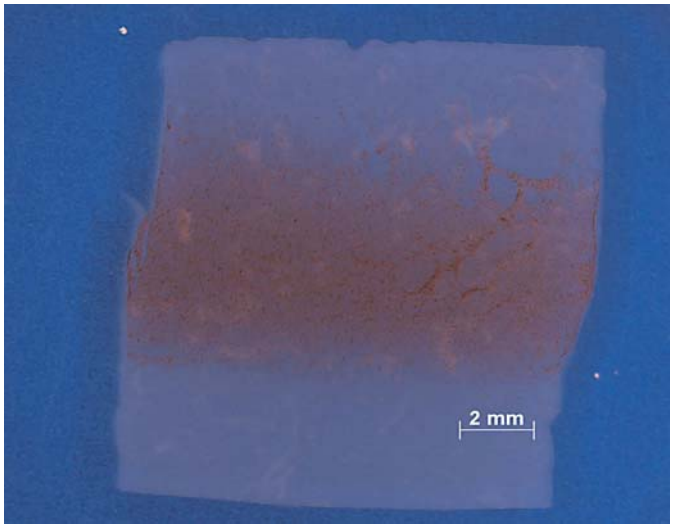

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Fig. 4. Seeding kinetics for SMCs (**A**) and HUVECs (**B**) upon magnetic field exposure. WST after 12 hours' cultivation. SMCs and HUVECs adhere mainly after 40 and 20 min respectively. Longer field exposures do not increase significantly the seeding density.
Fig. 5. Cell viability assay of SMCs and HUVECs reveal over 98% cell viability. Green luminescent rounded HUVECs (→) and green luminescent elongated SMCs in deeper layers (*) display viability in co-culture.
Fig. 6. Macroscopic view of the densely seeded collagen membrane after removal from the glass capillary.

HUVECs were observed to adhere mostly after 20 min (fig. 4B). Longer exposure periods did not result in significant increase of cell attachment. Thus, the field exposure for SMCs and HUVECs seeding was set at 40 and 20 min for all subsequent experiments.

Cell Viability

Cell viability assay was performed using a LIVE/DEAD viability/cytotoxicity kit (Molecular Probes) to assess cell survival during the entire seeding process. Inspection under CLSM revealed overall densely packed vital cells. Closer observation of the image displays two overlapping cellular morphologies: green luminescent cobblestoned endothelium on the one hand, and green luminescent elongated SMCs on the other, evidencing cell viability of HUVECs and SMCs in co-culture (fig. 5).

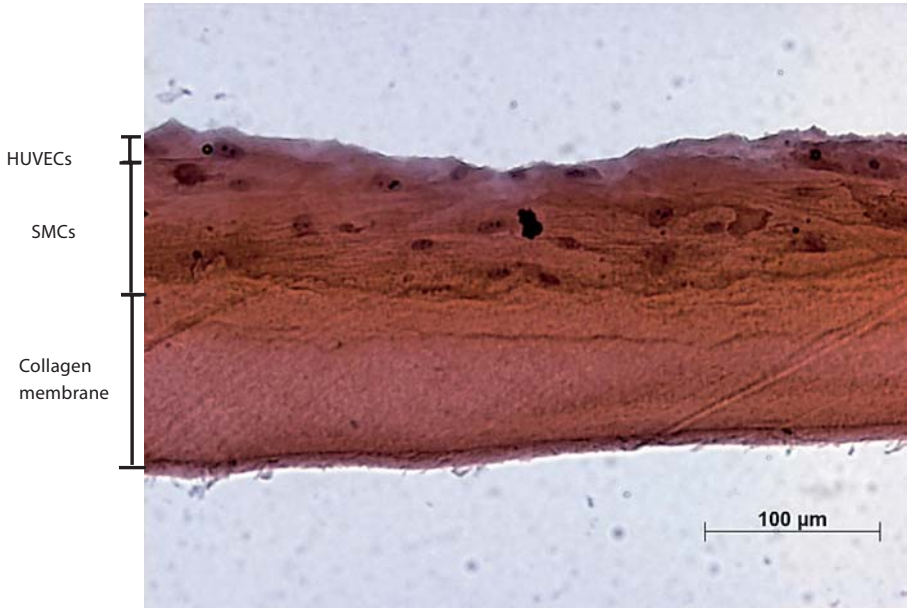

Fig. 7. Histological appearance (HE) of seeded collagen membrane. SMCs assembled into multiple layers covered by a monolayer of HUVECs.

Histology and Macroscopic Appearance

Macroscopic inspection of the uncoiled collagen membrane displayed a clearly visible stripe of densely seeded cells (fig. 6). The cell stripe corresponds to the area exposed to the radial magnetic force above the upper extreme of the coil. The axial symmetry of the magnetic force provided for a homogenous cell distribution across the entire perimeter of the tubular scaffold.

HE staining of paraffin-embedded sections displayed an organized multilayered cell assembly (fig. 7). The upper layer corresponds to the luminal surface of the tubular shaped collagen membrane during the seeding process and consists of a single monolayer of HUVECs. Endothelial cell lining shows good integration into the underlying SMCs layers. Densely packed layers of SMCs highlight good cell-cell interaction. The interface between cells and collagen membrane reveals a cumbered profile suggesting advanced scaffold-substrate integration. The multilayered cell assembly excluding the scaffold reveals a thickness of approximately 100 µm.

Immunohistology

Immunohistochemistry by using CD31 antibody confirmed the endothelial phenotype of the luminal layer. Inspection under CLSM revealed a densely seeded monolayer of endothelial cells (fig. 8A). HUVECs appear assembled into a confluent cobblestoned monolayer signalizing excellent cell-cell aggregation. Overlapping cell nuclei highlight multiple underlying cell layers (fig. 8B, C).

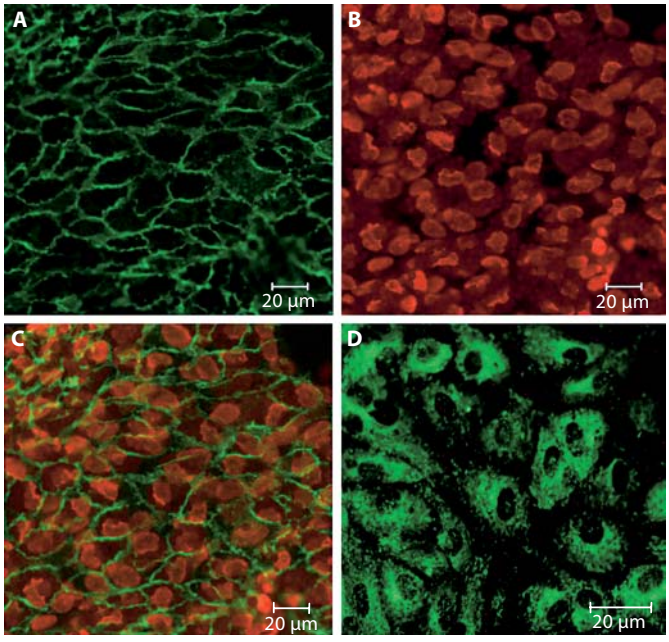

Fig. 8. Confocal laser scanning microscope imaging of SMCs and HUVECs in co-culture. **A** CD31 staining reveals confluent endothelium. **B**, **C** Overlapping cell nuclei reveal multiple cell layers. **D** Von Willebrand expression was separately assessed supporting the endothelial phenotype. Bars: 20 µm.

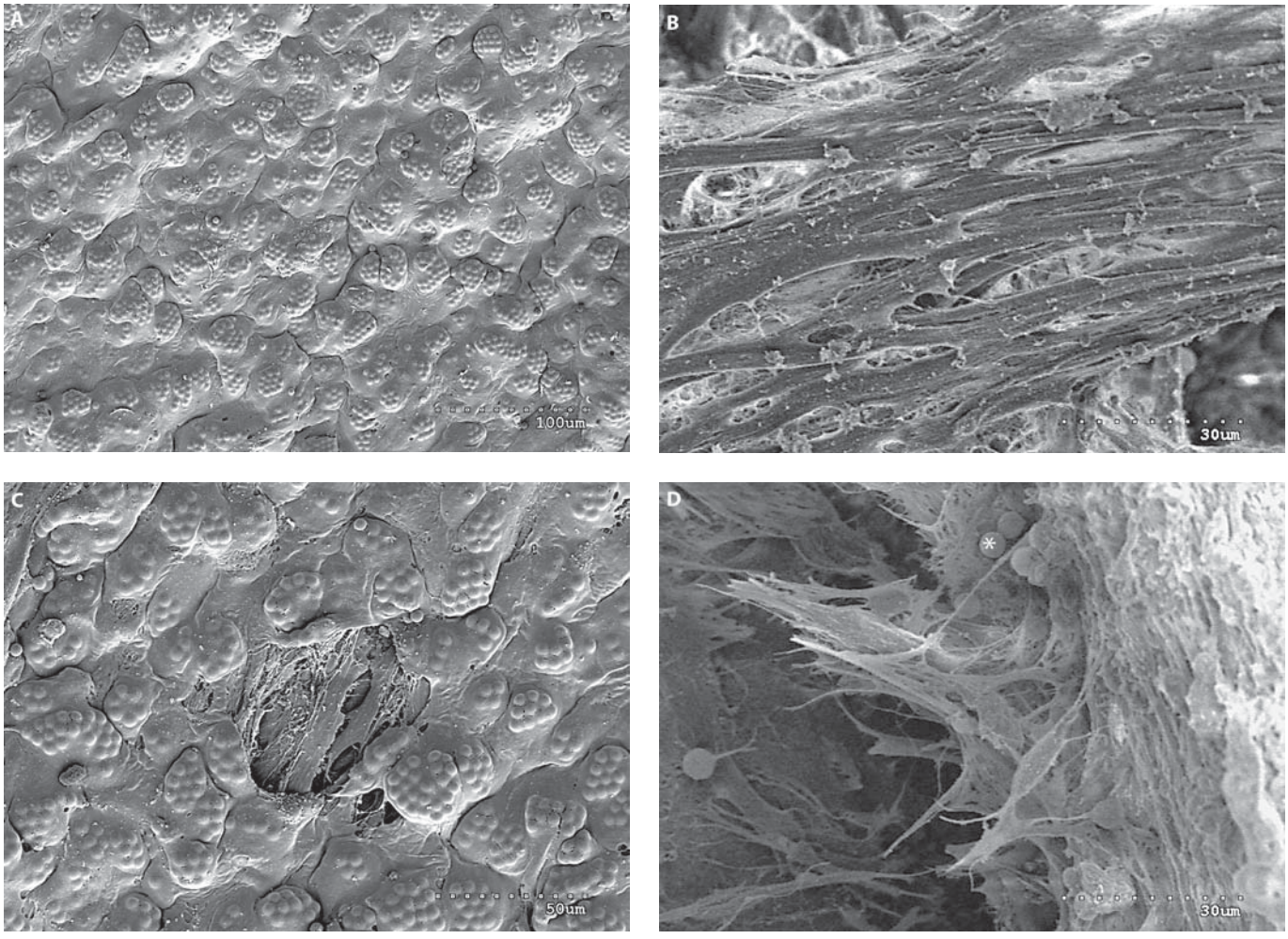

Fig. 9. Scanning electron microscopy of magnetically seeded collagen membrane. **A** Confluent endothelium. Magnetic beads are visible on the cell membrane. **B** Elongated SMCs building densely assembled multilayers. **C** Underlying multiple layers of SMCs are covered by a dense cobblestoned monolayer of HUVECs. **D** Multiple layers of SMCs. Magnetic beads (*) can be observed among the cell layers. Bars: **A** 100 μm; **B**, **D** 30 μm; **C** 50 μm.

Von Willebrand expression was separately assessed supporting the endothelial phenotype (fig. 8D).

Morphology Study

SEM inspection assessed multilayered heterotypic cell assembly. The luminal layer is formed by a confluent monolayer of endothelial cells. HUVECs display a rounded morphology and a compact cobblestoned packing as to be expected from static culture conditions (fig. 9A). Beneath the endothelium, SMCs appear aligned in multiple layers, revealing elongated, flattened morphology (fig. 9B, C). Overall, high cell density highlights good tissue assembly. Magnetic beads are still visible among cell layers (fig. 9D). Dynabeads® remain attached to the cell membrane during the culture period. However, note that the initial bead/cell ratio decreases in the course of the cultivation period as cell proliferation occurs.

Discussion

The delivery of cells into the scaffold is one of the key steps in tissue engineering procedures. Optimizing seeding efficiencies, mimicking tissue architecture and preserving morphological integrity of the cells are crucial factors for the growth and conditioning of functional tis-

sue substitutes in vitro. Considering these requisites, this study addresses a novel seeding technology based upon the application of magnetic fields for rapid endothelial cell seeding of vascular grafts or for multilayered cell seeding for vascular tissue engineering.

Magnetic Cell Seeding Promotes Cell Adhesion

Off-the-shelf availability has been identified as the ultimate goal for long-term success of tissue-engineered products [Ahsan and Nerem, 2005]. Hence, one of the major challenges in tissue engineering is to reduce prolonged culture periods. To comply with this ambitious goal, seeding strategies should ideally favor rapid cell adhesion to the scaffold optimizing the use of available cells.

To this date, dynamic rotational seeding seems to be an efficient approach to deliver cells onto tubular scaffolds [Sodian et al., 2002; Sutherland et al., 2002]. Briefly, a graft or tubular scaffold containing a cell suspension is rotated along a longitudinal axis to enhance uniform distribution of the cells. However, the continuous relative movement between the graft surface and the cell suspension hampers the attachment of the cells to the substrate thus resulting in prolonged seeding protocols. Nasseri et al. [2003] suggest 10 days' rotational seeding at 5 rpm to seed a polymer graft with myofibroblasts. Dunkern et al. [1999] propose 4 hours' rotation at 0.3 rpm to endothelialize a PTFE graft, whereas Hsu et al. [2005] reported optimal seeding efficiency of a polyurethane vascular graft with HUVECs by rotating the graft at 0.16 rpm for 12 h.

Optimally, cells should be immediately allocated and immobilized on the target surface to promote cell adhesion and proliferation. In our study, a gentle radial magnetic force drives the cells immediately towards the surface of the graft. Once the cell reaches the target surface, the magnetic radial force provides for the necessary mechanical support to avoid settling effects and cell-substrate adhesion takes place. This concept leads to rapid seeding procedures. In our experiments, we observed that HUVECs required only 20 min magnetic field exposure to adhere to a tubular collagen membrane and SMCs 40 min. This result shows a clear improvement compared to rotational methods. It should be remarked that no fibrin glue or further adhesive coatings were applied onto the scaffold's surface to promote cell adhesion.

Magnetic Cell Seeding Preserves Cell Integrity

Morphological integrity of the cells must be preserved during the entire seeding process to ensure viability and functionality of the tissue. Some seeding techniques have been reported to cause undesired cell damage. Shan-Hui et al. [2005] have recently demonstrated that rotational seeding vessels expose cells to undesired wall shear stress. Cell morphology examined after rotational culture revealed cell shrinkage, especially at high speeds 5–20 rpm. Turbulences generated from the rotational motion promote the continuous collapse of unattached cells against the graft structure, resulting in cell shrinkage, a sign of apoptosis.

During the magnetic seeding procedure, stress arises during the displacement of the cells within the medium upon application of the magnetic force. However, the maximal magnetic driving force acting on the cell results in being about 100 times higher than its own weight, which still remains low compared to the forces exerted on cells during customary centrifuging procedures. Thus, forces used in our technique do not endanger cell morphology or compromise cell viability, as assessed through viability assays and SEM inspection.

Magnetic Attractive Forces Promote Cell-Cell Interaction

Another key factor to accelerate cell expansion and tissue growth is the initial cell density. Densely packed cell distributions promote cell-cell adhesion as well as the diffusion of growth factors [Vunjak-Novakovic et al., 1998; Li et al., 2001]. A recent study by Ito et al. [2004] demonstrates that magnetically labeled mesenchymal stem cells gathered with a permanent magnet showed a proliferation rate fivefold greater than cells cultured under customary conditions. Upon magnetic field exposure, magnetic attractive forces arise due to magnetic dipole interactions among single particles leading to concentrated cellular interactions. Thus, magnetic seeding allows for high initial seeding densities promoting cell proliferation and tissue growth.

Multilayered Seeding: Mimicking Tissue Architecture

Mimicking tissue architecture is paramount to achieve desired mechanical performance and functionality of the tissue. Consecutive seeding steps allow deposition of successive cell layers onto the tubular scaffolds, promoting stable multilayered tissue assembly. Other investigators recently created a tubular structure by wrapping planar cell sheets around a cylindrical permanent magnet [Ito et al. 2005]. Direct magnetic seeding enables a controlled deposition of cells onto the scaffolds. It further promotes rapid and reproducible seeding protocols without tedious specimen handling. In this way we produced a graft consisting of multiple layers of SMCs and a monolayer of endothelial cells, thus complying with tissue architecture.

The choice of the scaffold material in our procedure is exemplary. Collagen is a well-known matrix to support the development of vascular tissue [Boccafoschi et al., 2005; Huyhn et al. 1999]. However, the technique is not limited to the use of collagen, but may be applied to any other adequate scaffolds for vascular engineering. Similarly, the cells chosen for this experiment (autologous HUVECs and SMCs) are only one approach among many others. Further cell sources may be suitable to obtain vascular tissue substitutes as well [Riha et al., 2005].

Magnetic Beads

A major issue considering clinical applications based on magnetic cell seeding arises from the use of magnetic particles in vivo. Currently, magnetic particles ranging from nanometer to micrometer scale are commonly used in a wide range of biological and clinical applications [Pankhurst et al., 2003]. Dynabeads® (1–4 μm) have a long successful history for in vitro diagnostic purposes and cell-sorting procedures [Fonnum et al., 2005]. A certain toxicity has been observed for Dynabeads® at high bead concentrations (50 beads per cell) [Tiwari et al., 2003]. However, during our experiments the average number of beads per cell was set at 6 to avoid toxic effects. In MRI, magnetic beads are applied intravenously as contrast agents to enhance the sensitivity and specificity [Wang et al., 2001]. Standard superparamagnetic iron oxide agents are easily sequestered by cells of the reticuloendothelial system in the liver or spleen and enable the detection of metastatic lesions in these tissues [Bulte and Kraitchman, 2004]. Further therapeutic applications of magnetic particles are being investigated, such as drug delivery carriers or their use for the catabolism of tumors by hyperthermia [Neuberger et al., 2005]. Magnetic beads in tissue engineering provide not only a valuable tool for seeding purposes, but could also serve as contrast agents to monitor tissue development in situ, or deliver chemicals if required. The optimal magnetic carrier for tissue engineering procedures, considering future clinical applications, still has to be investigated. In our study we used microbeads due to their high magnetic susceptibility enabling the use of moderate magnetic fields (up to 30 mT). However, the use of nanoparticles in combination with stronger fields is feasible.

Clinical Outlook

Since the pioneering study of Weinberg and Bell in 1986 describing the first blood vessel model constructed in vitro, numerous research groups have been attempting to find an adequate strategy to obtain a clinically viable autologous vascular implant. Despite significant progress in in vitro and animal studies, the translation of this early success into the clinical practice is still hampered by the extensive culture time, the limited cell availability and the highly-demanding infrastructure required for these approaches [Nerem, 2004]. Recent studies in stem cell research are exploring novel cell sources for clinical applications [Riha et al., 2005]. In the scope of this flourishing field, clinical institutions worldwide are being widely equipped with cell culture facilities. The field of bioreactor design will certainly play a key role as advances in basic science trigger new clinical applications. Highly reliable and reproducible procedures will be required to widespread the use of cellular based therapies in the clinical setup.

The use of magnetic particles in tissue engineering addresses the need for rapid, efficient and reliable procedures in a clinical framework. The technology is applicable to any scaffold material, any cell source and any scaffold size. It is valid for single step seeding procedures, such as endothelialization of synthetic vascular prostheses, or for complex approaches such as the generation of completely biological blood vessels. It also provides a valuable platform for high throughput screening for research purposes in the short term and in order to face clinical challenges in the near future. Finally, the feasibility to visualize magnetically labeled cells non-invasively in a clinical MRI bridges a crucial safety gap between tissue culture in vitro and tissue implantation in vivo.

Conclusions

Magnetically induced cell seeding provides a novel tool to seed tubular scaffolds with cells. A radial magnetic force drives magnetically labeled cells onto the luminal surface of the graft and provides for the necessary mechanical support, avoiding settling effects and promoting cell-scaffold adhesion. Successive seeding steps enable multilayered cell assembly to mimic tissue architecture of native vessel wall. Magnetic seeding opens new perspectives for cell delivery purposes in tissue engineering, providing rapid seeding procedures and complying with tissue histology in native tissue.

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