J Biomed Mater Res A. 2009; 88(4):1104-21. Review.

Danni (sistema vascolare)

- Diffusi
- Localizzati (biforcazioni, diramazioni)
- Ateroma → ischemia downstream e trombo → embolo
- Se stenosi parziale → angioplastica endoluminale e stent vascolare
- Se stenosi completa → bypass (ramo in parallelo; vena safena o arteria mammaria o protesi artificiale)

Needs

- patients needing replacementsfor small-diameter (<4 mm) blood vessels(SDBVs).
- Patients requiring coronary artery bypass, peripheral vascular surgery, or arteriovenous shuntsurgently need replacement SDBVs
- Big market

MUST

- Proprietà elastiche
- Rivestiti da endotelio, che impedisce coagulazione
- Devono modificare dimensione lume, secondo necessità fisiologiche (risposta vasomotoria, rimodellamento)

Characteristics of ideal SDBV replacement

- Anti-thombotic (endothelial cell role!!

  → given transmural), but relatively slow in vitro growth
- Mechanical properties such as compliance and burst pressure comparable to the native vessel in order to sustain anastomotic pressure without rupture (composition and orientation of ECM and cells: collagen, elastin, …) *tunica media*
- stiffness, compressibility, elasticity and viscoelasticity

Protesi artificiali

- Protesi artificiali in Dacron ® (polietilenftalato) o GoreTex ® (politetrafluoroetiline espanso)
- → rischio trombosi
- 1-2 anni: 50% occlusione
- Limite dimensione: solo > 5 mm
- Patologie arti inferiori richiedono vasi di ca. 4 mm diametro (SDBV)

Vessel replacement

- three main types of replacement vessels:
  - biologic (predominantly autologous): saphena, **mamm**
  - Synthetic: problematic → endothelized, somehow rigid, a-thrombotic therapy necessary, cannot grow
  - tissue engineered (TE) biological grafts: HOPES!
    - collagen, fibrin, decellularized vessels, or other hydrogels, in sheets
    - without any matrix or scaffold,
    - in porous scaffold supports
  - problems include thrombogenicity, inappropriate mechanical properties and vasoactivity, and excessively long culturing time → inflammation, degradation

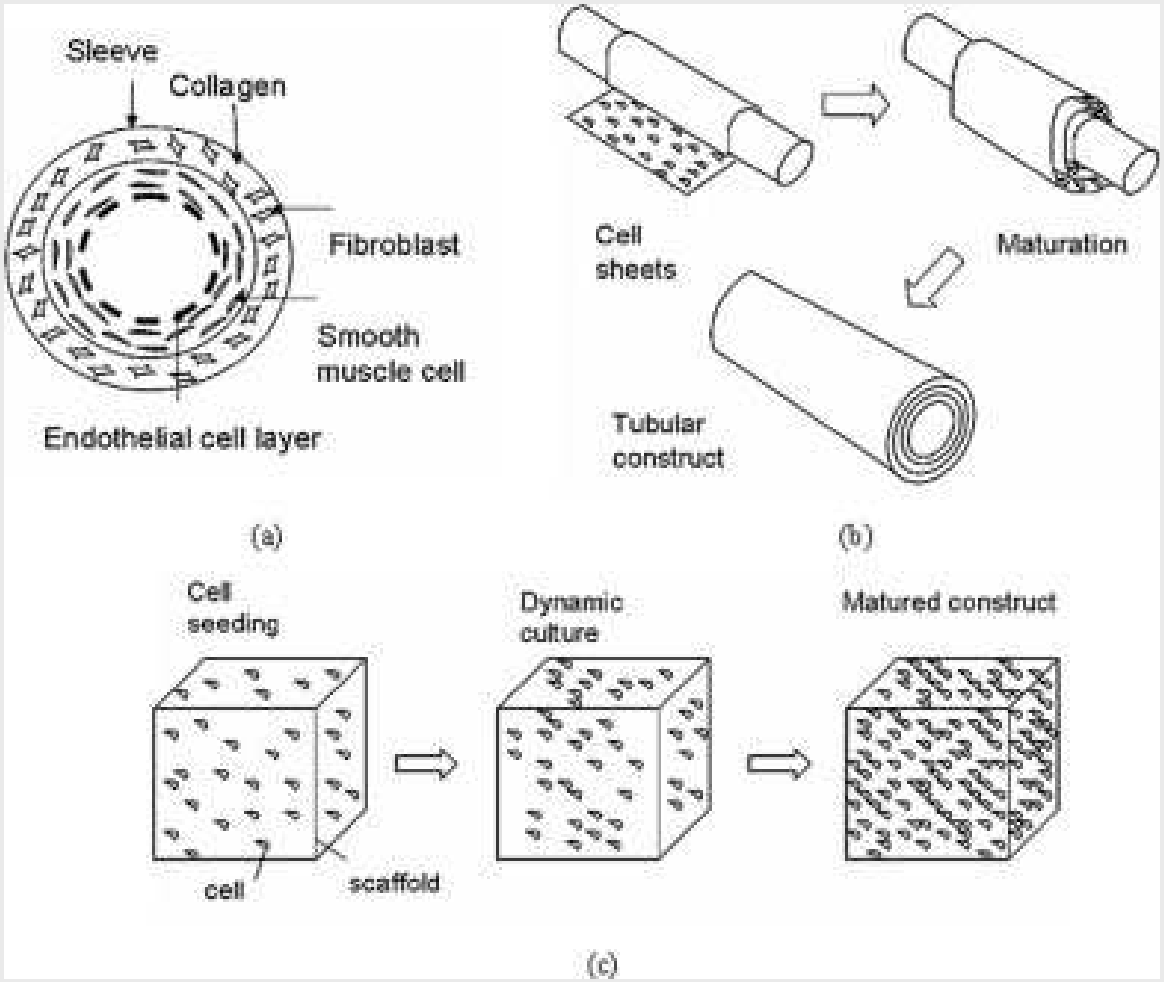
scaffolds

- Tess vascolare decellularizzato
  - Limiti: disponibilità, alta densità ECM, x cui scarsa penetraz cells
- Biomateriali naturali
  - collagene glicosilato, elettrofilato
  - Fibrina aggiunta a cells e poi gelificata in forma tubulare → induce elastina. rischi
- Biomateriali sintetici
  - PGA, PGA/PLLA. Possibile acidificazione e risposta infiammatoria
  - HYAFF

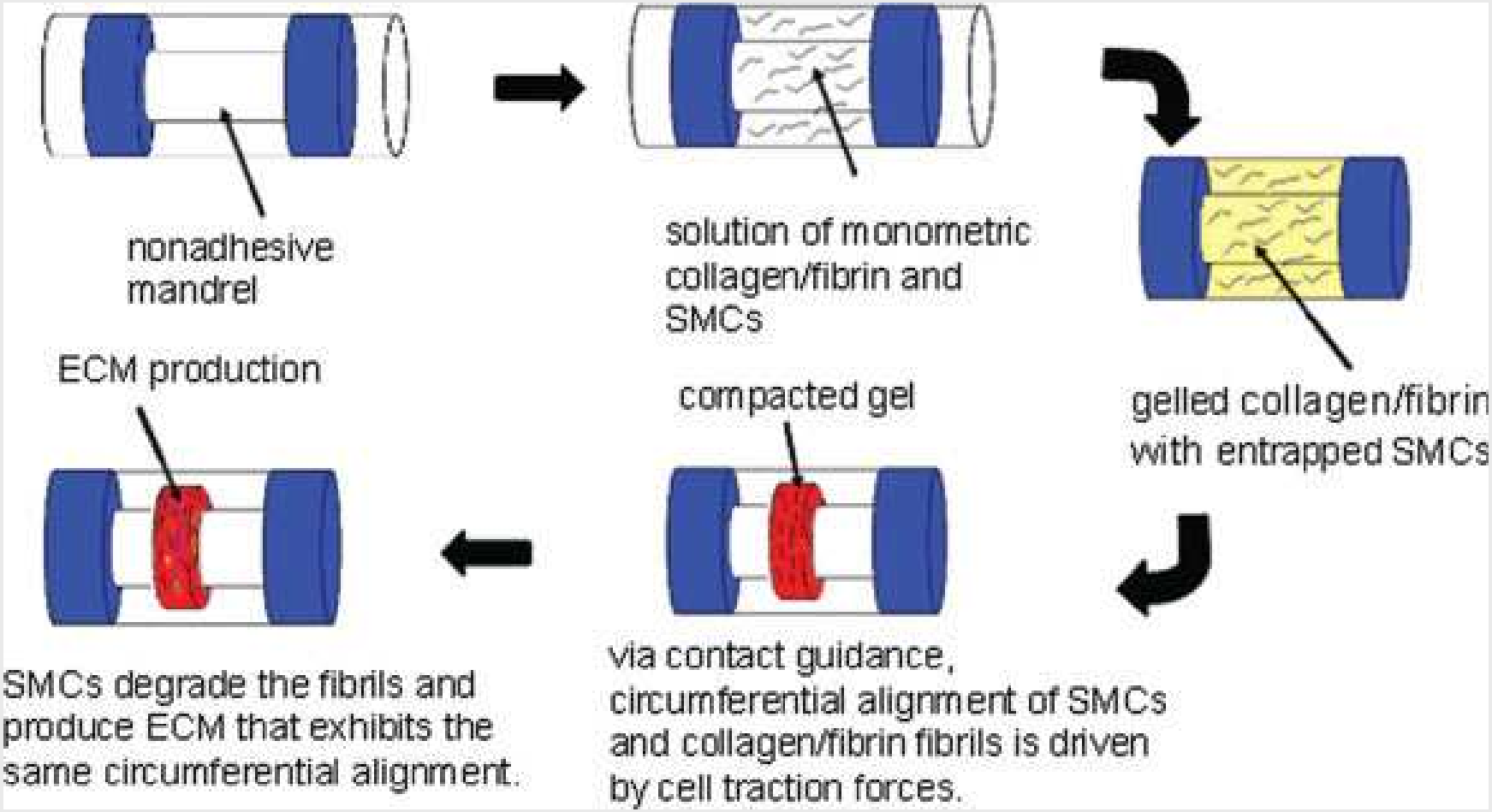
Limits of the scaffolds

- collagen-based small-diameter TE vascular grafts: poor mechanical properties because of the poor chemical integrity of the reconstituted collagen, low SMC density, and largely longitudinal orientation of ECM and SMCs
- PGA scaffolds: undesirably long culture time to obtain desirable histological organization and burst strength and the acidic environment from rapid degradation

Schematic representation of (a) collagen-based, (b) cell sheet-based, and (c) scaffold-based vascular grafts



Free collagen gel compaction over a nonadhesive central rod to induce circumferential alignment of SMCs and fibrils (below-knee substitute)



- Using a series of PEG and fibrinogen hydrogels, Seliktar and coworkers have shown that the matrix stiffness and proteolytic resistance of the matrix are important for promoting cell spreading and remodeling

TE vascolare

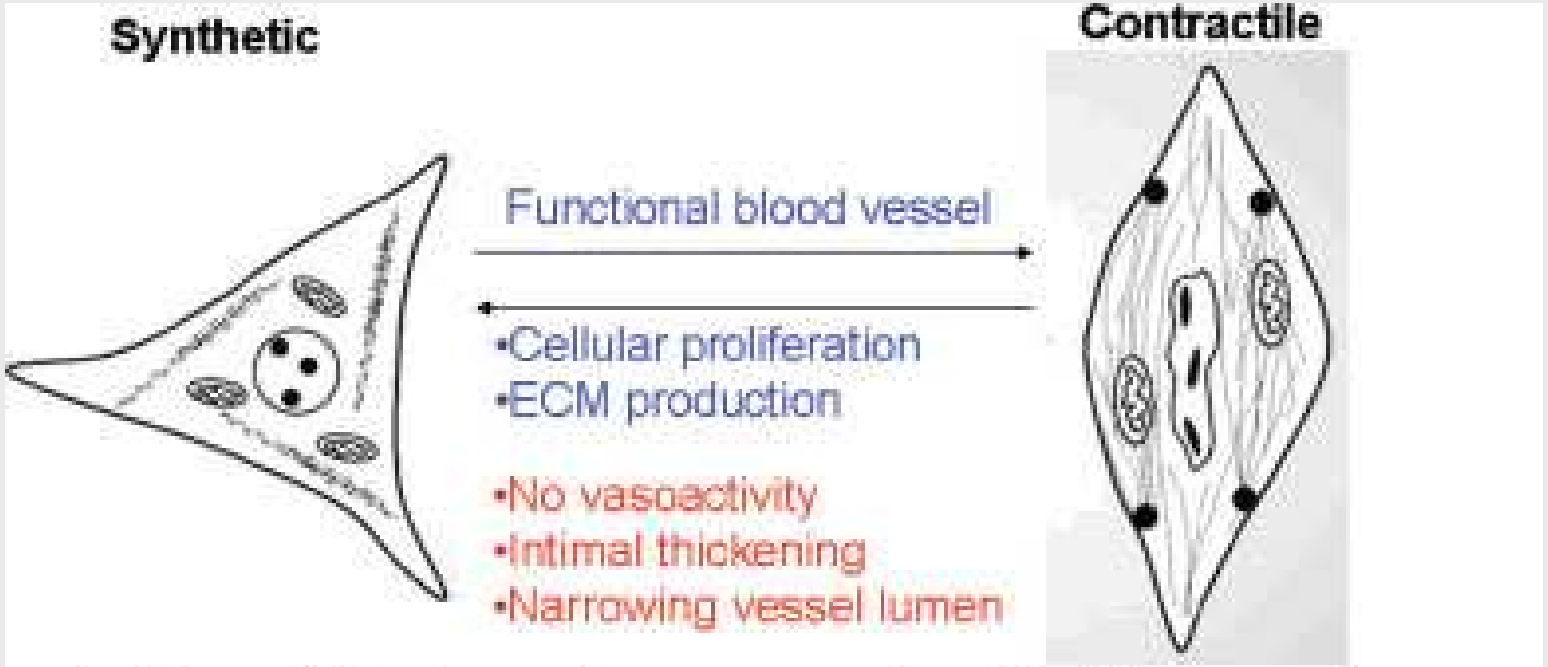
- Sviluppo materiali x scaffold biodegradabili
- Fonte cellulare
- Ottimizzazione colture cellulari x TE in vitro

Characteristics of ideal SDBV replacement

- Anti-thombotic (endothelial cell role!!

  → given transmural), but relatively slow in vitro growth
- Mechanical properties such as compliance and burst pressure comparable to the native vessel in order to sustain anastomotic pressure without rupture (composition and orientation of ECM and cells: collagen, elastin, …) *tunica media*
- stiffness, compressibility, elasticity and viscoelasticity

Morphology of contractile and synthetic SMCs



Effects of ECM on SMC

- Structure
- Function
- Cell senses the environment
- fibronectin and collagen type I have been found to induce a shift toward a **synthetic phenotype**, whereas laminin, elastin, heparin, and collagen type IV can revert this phenotype transition and maintain cells in a quiescent and **contractile state** in vitro
- Heparan sulfate bind GFs

Different effects of GFs on SMC & ECM

- PDGF-BB generally stimulates SMC migration, proliferation, and ECM production, which indicates a shift to a synthetic phenotype. PDGF-BB is relatively more chemotactic than mitogenic.
- TGF-b1 usually inhibits proliferation but promotes migration of vascular SMCs and increases ECM production
- FGF, CTGF, VEGF, ..
- Signal transduction

Effects of mechanical stimulation

- Vessel walls are dynamic environments. They are subjected to radial and longitudinal forces arising from cyclic pressure variations in the fluid-filled lumen, to shear stress due to longitudinal fluid flow, and to forces arising from the vasoactive response of the wall itself
- cyclic mechanical strain applied to cultured SMCs induces increased production of growth factors, cell proliferation, ECM production, and contractile protein expression
- Cell alignment perpendicular to strain gradient

Effects of scaffold geometry

- Randomly porous morphology
- Micropatterning of cell culture substrates (plasma lithography → spindle-like form cells)
- Collagen lines
- SMCs could be aligned in grooves (20–80 μm wide and 5 μm deep) in nonbiodegradable polydimethylsiloxane can control the vascular SMC aspect ratio, alignment, and oriented remodeling of the ECM, but nondegradable scaffolds affect vasoactivity (synthetic rather than contractile)

Effects of scaffold geometry

- Wide microchannels (80–160 lm wide) regulate the SMC morphology and orientation only nearing confluence →, switch to a more contractile phenotype (α-actin protein-positive).

3D tissue

- layer-by-layer (LBL) process has also been developed to build 3D tissue with thin layer of collagen gel (type I collagen from rat tail) between each SMC layer to provide a substrate for the seeding of the next SMC layer (1 day for cell confluence/alignment and 0.5 day for collagen gelation and further contraction)
- A typical human coronary artery replacement may need about 10 layers.

3D microgrooved scaffolds

- Wide 3D microchannels for SMC alignment, elongation, and phenotype switch are relatively unexplored but have shown significant promise in early studies; in particular, they promote cell proliferation at low cell density and modulate the cells toward a contractile phenotype at high cell density.
- Combination of scaffold patterning and biochemical/ mechanical stimulation might be needed to achieve optimal control of SMCs to produce a functional vascular media for tissue engineered SDBVs.

DIFFERENTIATION OF STEM CELLS TO SMCs

- TGF-b1 is critical for adherent, spindle-shaped adult human MSCs to express SM a-actin-positive microfilaments and the SM-specific proteins, that is metavinculin, SM myosin heavy chain and calponin.
- The progenitor cells destined to become SMCs expressed platelet-derived growth factor-B receptor (PDGFR-B) which has been shown to play a role in the differentiation of mesenchyme-derived SMCs.

- cyclic strain to a cell-laden membrane to emulate in vivo conditions → contractile phenotype, alignment perpendicular
- human SMCs or MSCs were seeded onto aligned nanofibrous membranes: well organized layers of ECs & SMCs (little platelet aggregation)
- cells seeded into decellularized canine carotid arteries

SUMMARY AND FUTURE PROSPECTS

- A successful small-diameter TE vascular graft must meet certain requirements, which include non thrombogenicity, sufficient burst pressure and suture retention strength, native-like viscoelasticity, appropriate remodeling responses, and vasoactivity
- → so far relatively unsatisfactory

Comments

- None of the currently available tissue engineering approaches have resulted in vascular media with a clinically useful combination of durability, desirable mechanical properties, vasoactive function, remodeling response, etc, Due to the instability of the SMC contractile phenotype
- Mechanical and biochemical stimulations and micropatterned scaffolds applied to regulate the phenotype, orientation, and ECM production of SMCs, BUT long period of stimulation (on the order of several weeks) needed

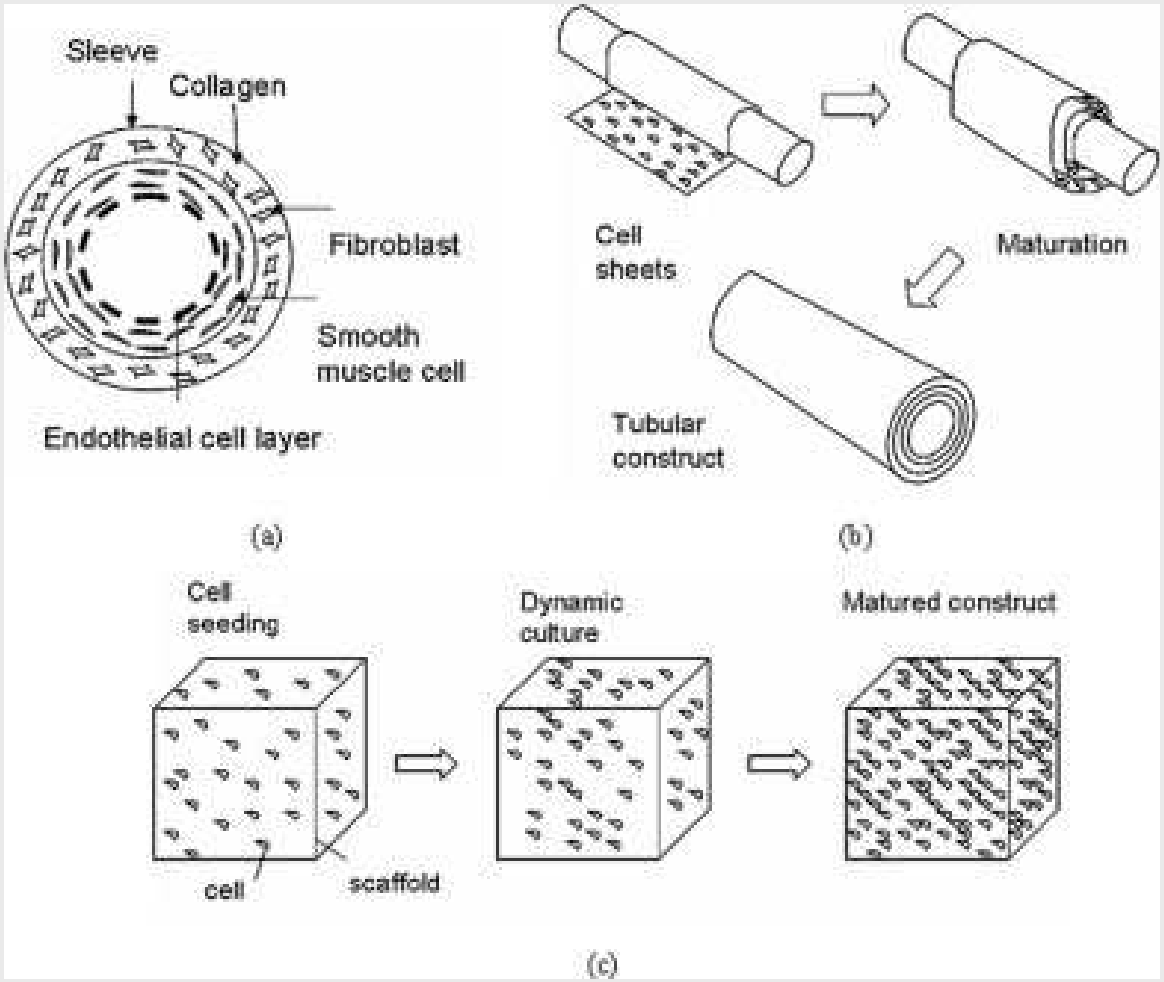
Fonte cellulare

- Poiché disponibile # limitato
  - terapia genica con m-TERT (rischiosa!!) /cellule differenziate di altro tessuto: foglietti fibroblasti da biopsie cutanee avvolte su mandrino cilindrico e rivestiti internam da strato endoteliale: tempi lunghi (settimane)
  - ESC indotte a diff (CD31, VE-cadherin) → strutt tubulari in matrigel 3D → in topo: microvasi

Fonte cellulare

- Poiché disponibile # limitato
  - Stem cell adulte autologhe: EPC circolanti in segmenti arteriosi decellularizzati → topo: funzionali x 130 gg
  - MSC → cellule muscolari lisce (SMC)

Schematic representation of (a) collagen-based, (b) cell sheet-based, and (c) scaffold-based vascular grafts



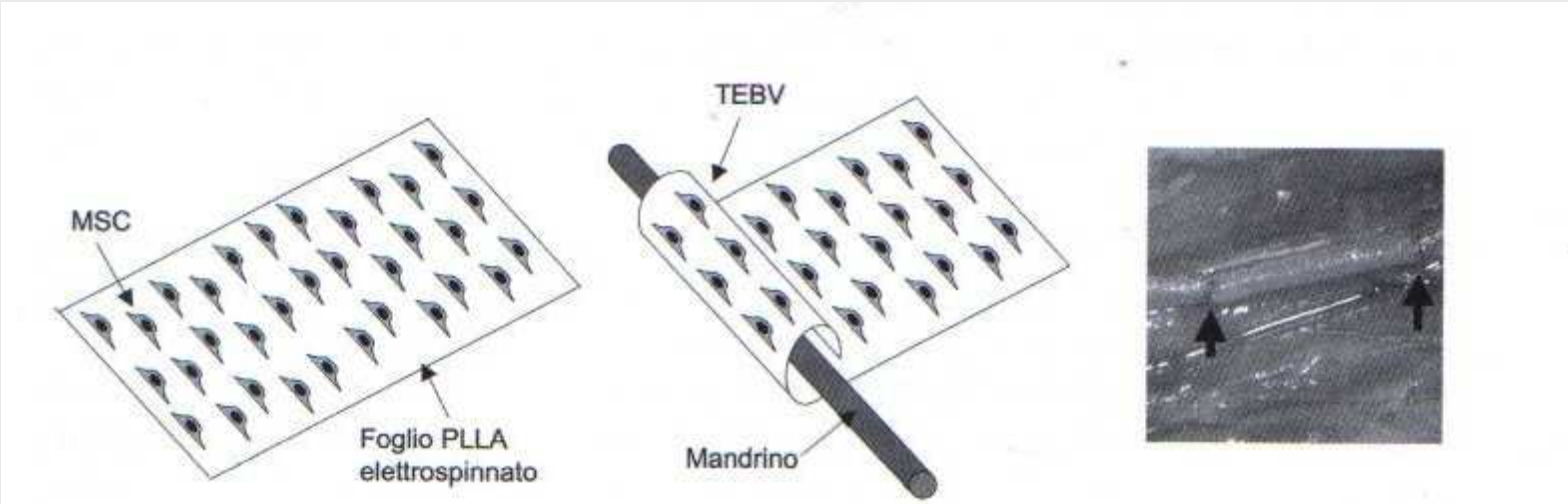
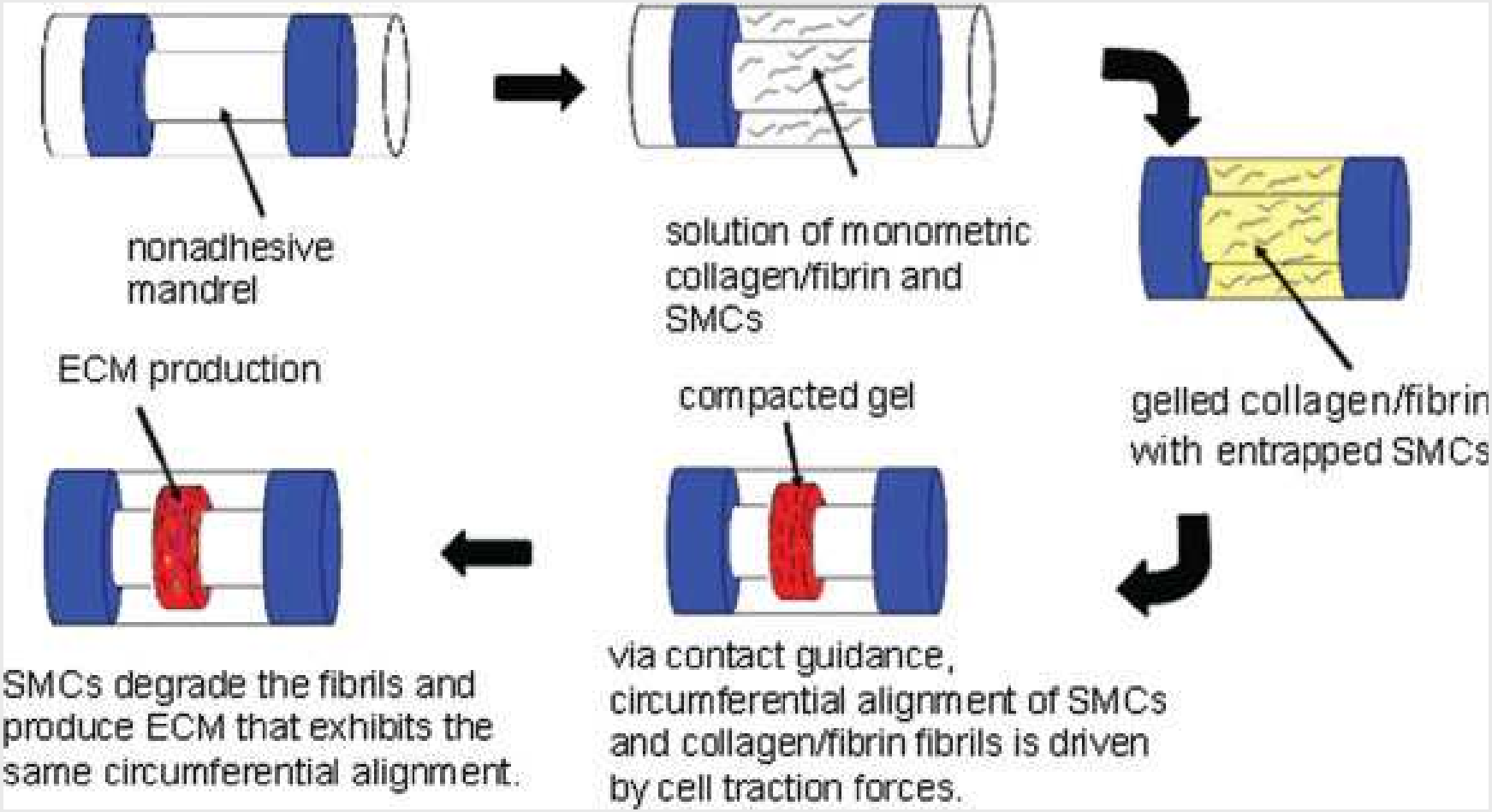


Fig. 5.22. Schema della tecnica utilizzata da Hashi et al. 2007 per la ricostruzione di un vaso ingegnerizzato ottenuto con cellule mesenchimali del midollo osseo. Particolare del vaso impiantato.

Free collagen gel compaction over a nonadhesive central rod to induce circumferential alignment of SMCs and fibrils (below-knee substitute)



Ottimizzazione colture cellulari x TE in vitro

- Necessari
  - Ossigenazione e nutrienti
  - Stimoli meccanici: pressione radiale (shearing)
    → bioreattori x colture dinamiche

Studi clinici

- 2001: pz pediatrico (prima connessione art polmonare-vena cava)
- Cell vascolari da vena periferica → in vitro su scaffold tubulare policaprolattone/PGA
- OK ai controlli
- Altri: Bone Marrow Mesenchymal Stem C
- NB: circolazione polmonare: << pressione idraulica e di assoluta necessità

Studi clinici (L’Heureux 2007)

- Accesso vascolare in sostituzione graft sintetici poco funzionanti e non rischioso x pz in emodialisi
  - Fibroblasti da biospia cutanea
  - Coltivati foglietti (settimane)
  - Avvolti su cilindri e lasciati maturare (settimane)
  - Ricoperti da endotelio da vena (autologo)(gg)
  - Impiantato: consistenza e suturabilità tipo safena
  - Buon risultato a > 1 anno, ma trattamento classico è ancora più vantaggioso

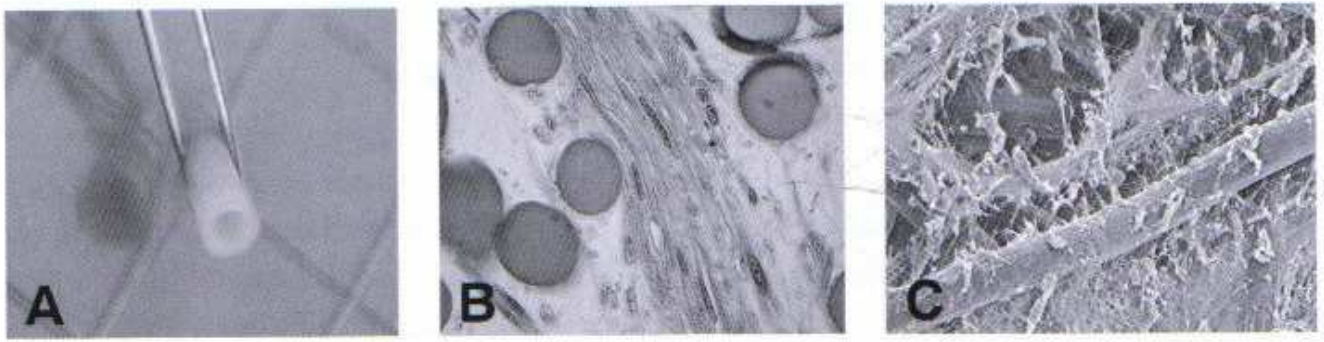


Fig. 5.23 A) Costrutto vascolare ingegnerizzato a base di HYAFF e cellule muscolari lisce. B) Particolare della parete del costrutto colorato con ematossilina ed eosina. B) Immagine SEM della superficie del costrutto. Modificato da: Arrigoni C et al. Vascular Tissue Engineering 2006 Cell Transplantation 15 suppl.1: S119-25 Review.

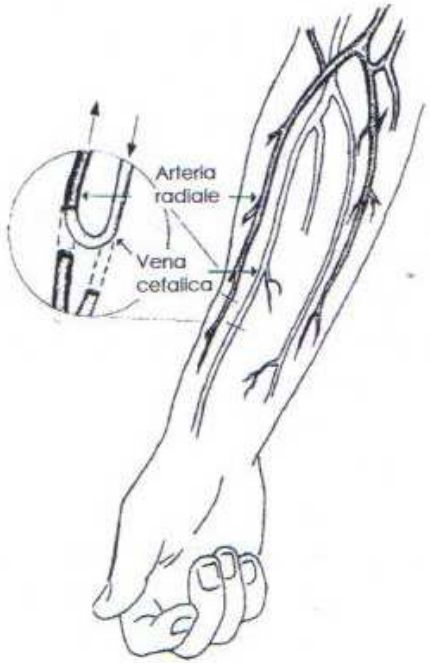


Fig. 5.25 Schema dell'intervento chirurgico per la realizzazione della fistola arterovenosa per l'accesso vascolare in pazienti in trattamento emodialitico.

TISSUE ENGINEERING: Part A
Volume 16, Number 1, 2010

Inosculation

- formation of anastomoses between graft and recipient vessels.
- As inosculation occurs rapidly, it guarantees survival of the graft early after transplantation.
- three approaches have been attempted for vascularization of bioengineered tissue:
  - incorporation of soluble angiogenic factors,
  - gene transfer approaches,
  - seeding of endothelial cells onto or into matrices and scaffolds

in vitro engineering of prevascularized interstitial matrices

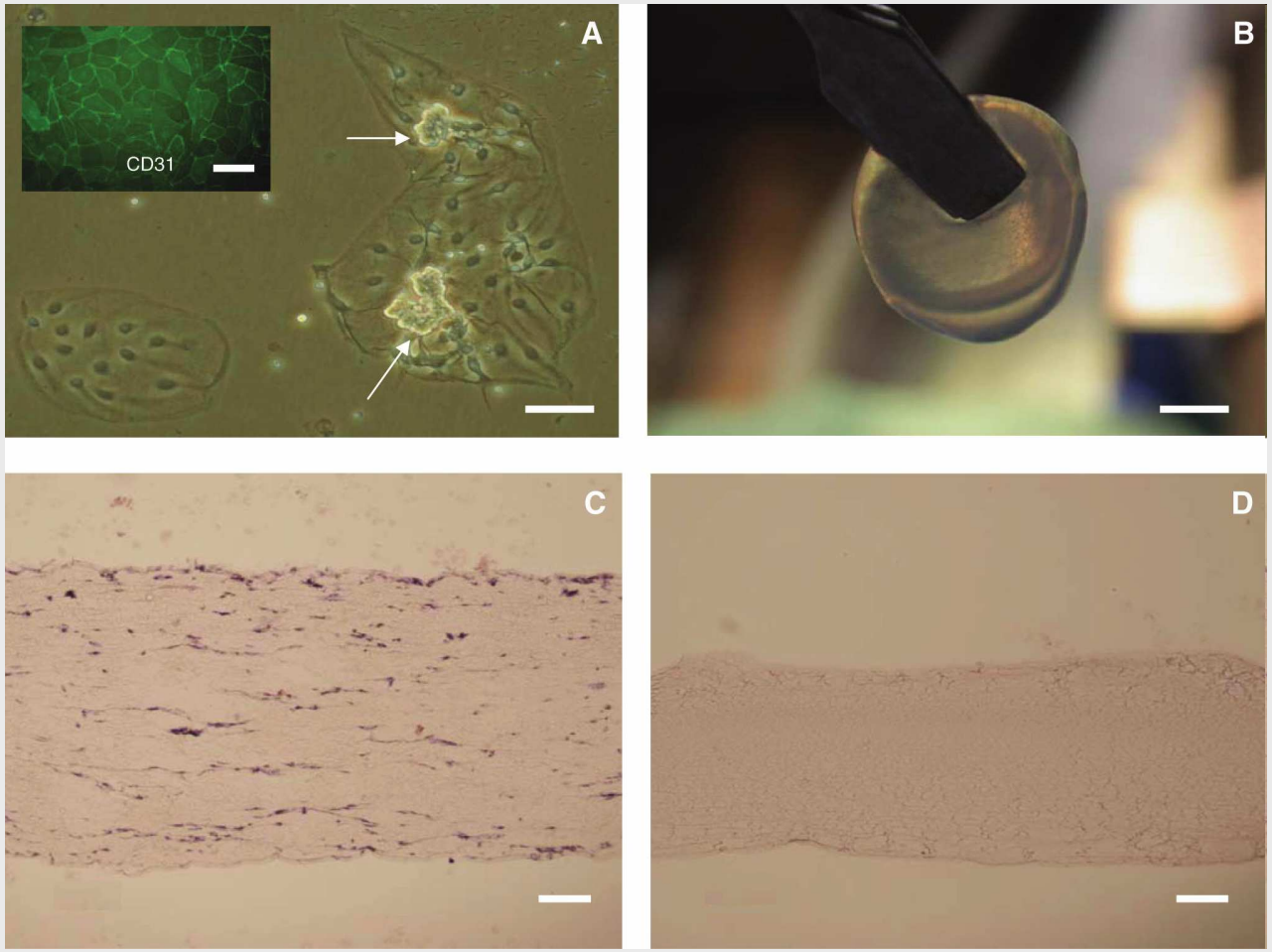
- human dermis is a perfect source of microvascular endothelial cells, can be expanded in a reproducible manner in culture.
- vasculogenesis and angiogenesis can occur in vitro underappropriate conditions and that the construction of true three-dimensional (3D) capillary networks is possible.
- transplanted these prevascularized matrices onto the backs of immunoincompetent rats and showed that mural cells recruited from the underlying recipient mesenchyme stabilized the engineered vessels.

Materials & Methods

- HuMEC = microvasc endot cell from hu foreskin (fibroblasts)
- prevascularized fibrin matrices
- collagen type I hydrogels
- Athymic rats
- Histological and Immunohistochemical analyses

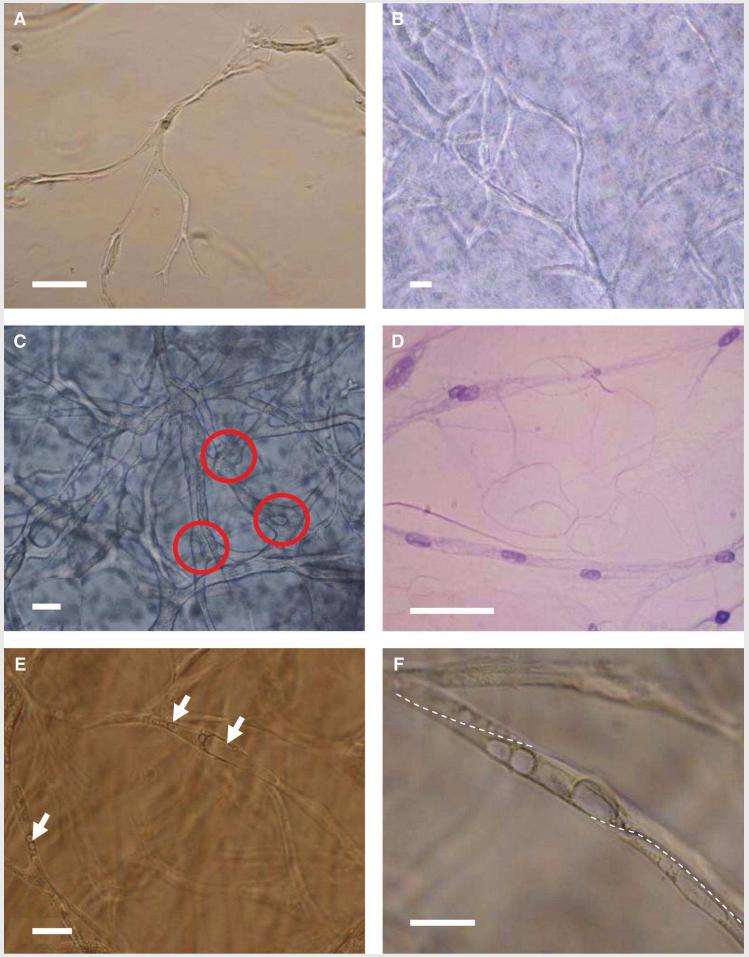
- HuMECs, CD31 (PECAM1)+, can develop into lumenized vascular structures within fibrin hydrogels.
- collagen and fibrin-based biodegradable matricesto screen for optimal 3D organotypic structure formation.

HuMECs stabilize the biodegradable matrix



- the in vitro development of branching and lumenized vascular structures was only possible in a limited density (1.1 mg/ml) of reconstituted fibrin matrix (1 mm thick)
- Rapid fibrin polymerization – in which cells were added- was crucial x their even distribution
- within 5 days branching organotypic structures consisting of single, elongated cells
- By d 7–10: the vascular structures became longer and stronger and developed into solid branching cords
- arranged into a network of solid cords but lumen formation not detected in any of the structures

capillary formation in vitro

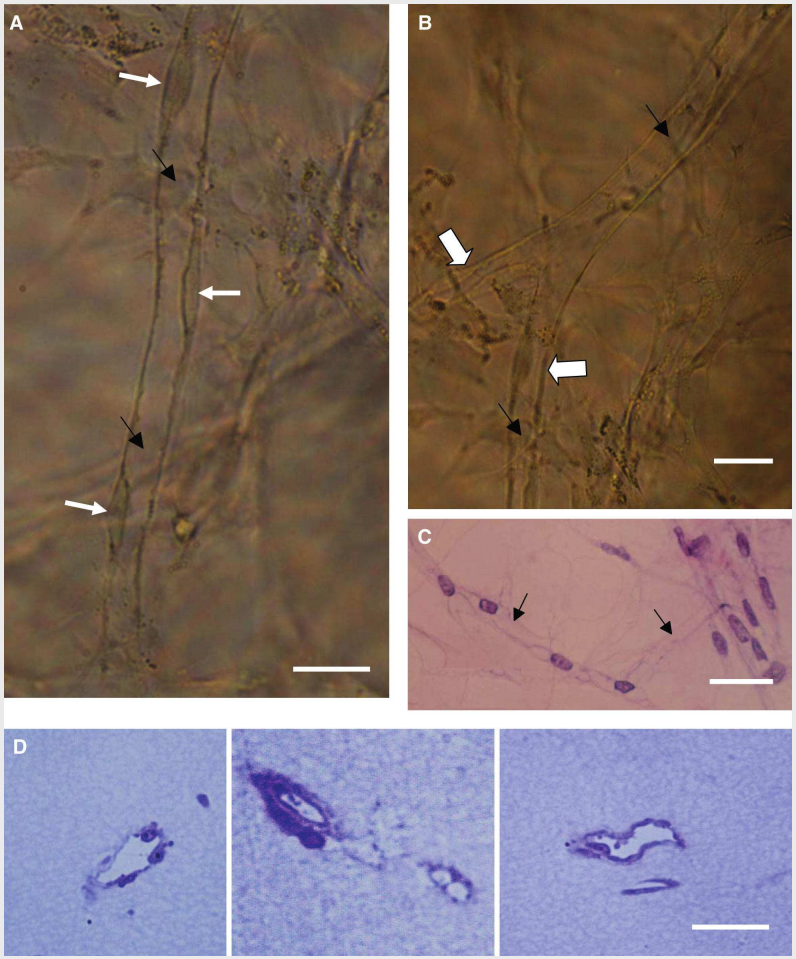


d 7

d 10

vacuole formation

branching, completely lumenized capillaries developing in hydrogels in vitro

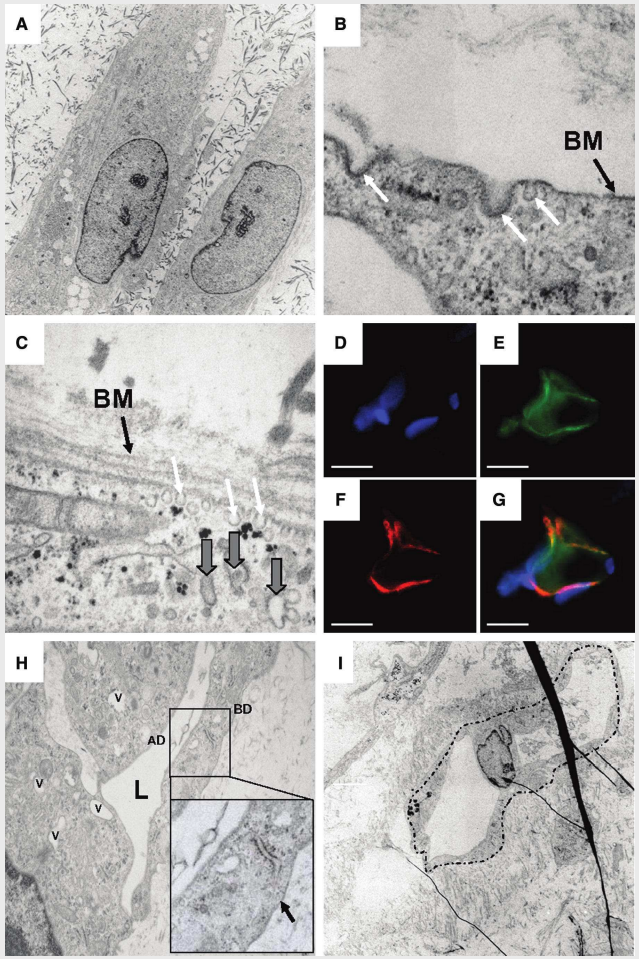


- cell–cell and cell–matrix adhesion in the establishment of cell polarization
- intercellular lumen formation after submersion in a fibrin gel.

d 2

d 10

polarization

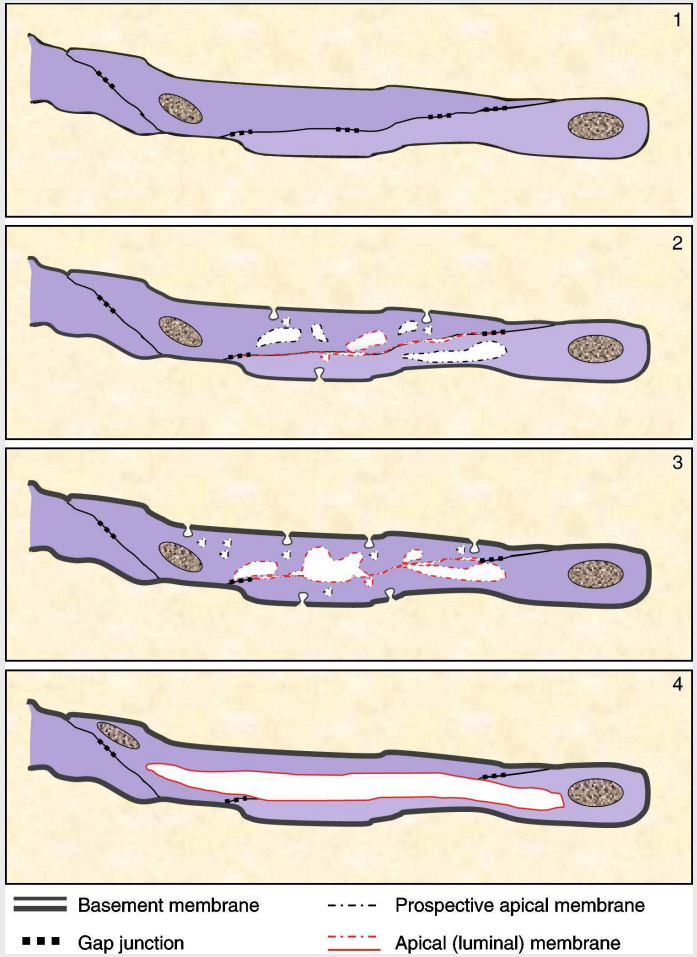


d 7

Lamini
CD31
Hoechs

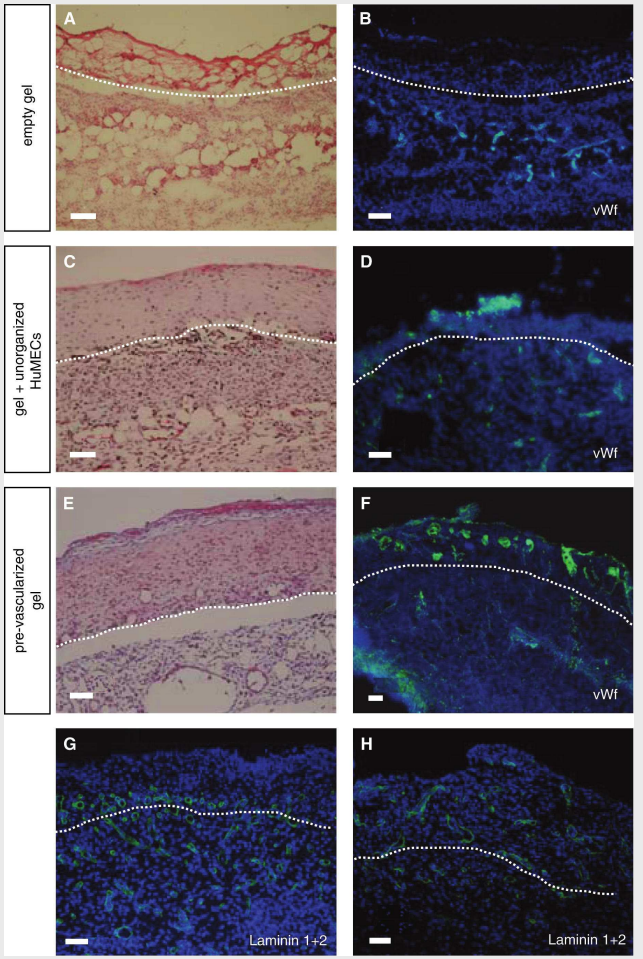
lumen

model depicting the distinct steps of endothelial lumen formation



three different types of hydrogels onto immunoincompetent rats

- 5 days after transplantation

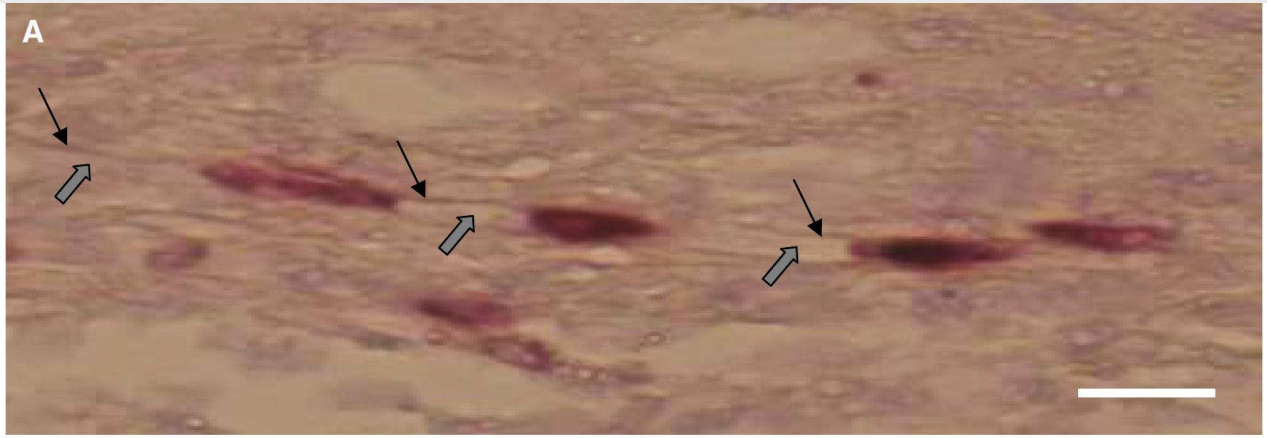


Cells as C-D

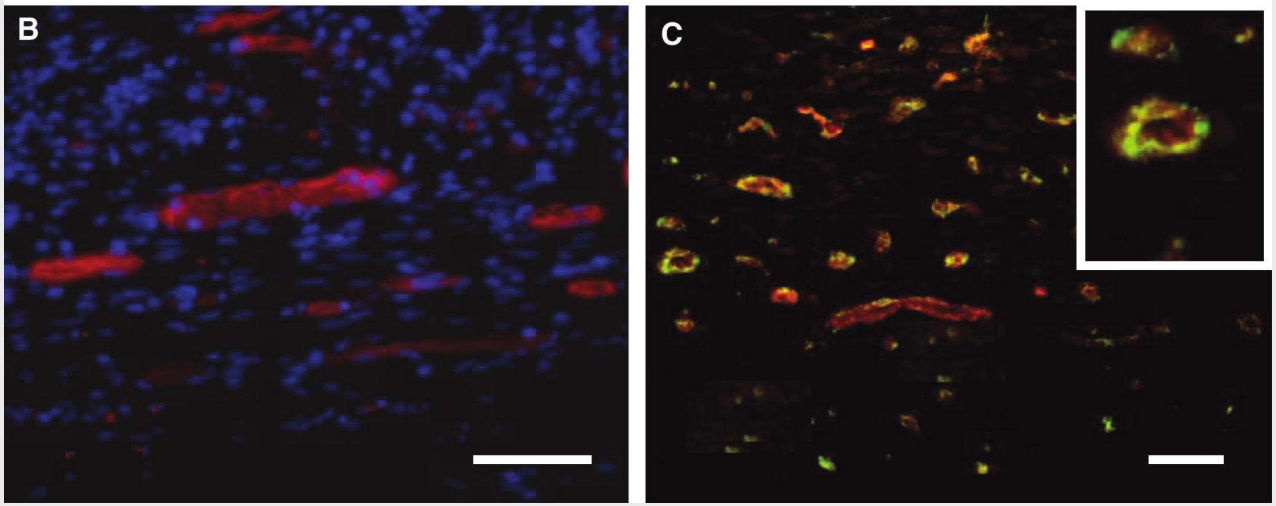
Cells as E-F

Engineered human capillaries are stabilized by mural cells of rat origin

d 19



Hu CD31



CD31
+
Anti-actin

Results

- Endothelial cells arrange into networks of lumenized capillaries within 15 to 21 days
- likely that endothelial progenitor cells (EPCs), present in the initial cell preparations, give rise to de novo developing vascular structures
- angiogenesis, and also vasculogenesis, may be involved

Suggested papers

- Yazdani et al. Smooth muscle cell seeding of decellularized scaffolds: the importance of bioreactor preconditioning to development of a more native architecture for tissue-engineered blood vessels. Tissue Eng Part A. 2009;15:827-40
- Gui et al. Development of decellularized human umbilical arteries as small-diameter vascular grafts. Tissue Eng Part A. 2009;15:2665-76
- Stevens et al. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proc Natl Acad SciU S A. 2009 Sep 29;106(39):16568-73