ABSTRACTS

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CARDIAC PRECURSORS IN HUMAN BONE MARROW: IN VITRO CELL CARDIOGENESIS

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INTRODUCTION: Cardiac precursors are present in mouse bone marrow and can be used to repair the infarcted myocardium in mice. We searched for these precursors in human bone marrow and analyzed gene expression patterns in cells induced to differentiate in vitro.
METHODS: Cells from human bone marrow were isolated and cultured in medium supplemented with autologous serum and 5% CO_2. Cell characterization was performed by immunocytochemical analysis. mRNA was isolated and retrotranscribed. The active genes were detected by PCR using specific oligonucleotides.
RESULTS: Some inducers pushed the cells through different stages of cardiogenesis, with expression of cardiac transcriptional activators and structural proteins. Some combinations of stimuli were able to drive cells to advanced stages of cardiogenesis. These studies lead to an exact description of in vitro cardiogenesis in humans. Our aim is also to assess the residual proliferative capacity of cells and to enhance differentiation efficiency, thus maximizing their repair capacity and their likelihood to integrate functionally with the surrounding cardiac tissue.

COMPUTER GUIDED PERCUTANOUS INTRAMYOCARDIAL DELIVERY OF AZACYTIDINE STIMULATED MESENCHYMAL STEM CELLS

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Transplantation of mesenchymal stem cells (hMSC) may improve heart function in ischemic heart disease. hMSC were isolated according to Caplan's method. After the second passage adherent hMSC were incubated with 5-azacytidine (10 μmol/mL) for 24 hours. After 2 weeks in vitro expansion immunhistochemical analysis showed 80% smooth muscle actin (SMA)-positive cells with fiber-like intracytoplasmic morphology. These cells were negative for troponin T-C, alpha-actinin, myosin heavy chain, CD31, ACC133, and fibroblast-specific antibodies. For percutanous cell delivery we used the computer guided NOGA system with a transendocardial injection catheter (Myostar, Cordis). Precise injections of 2 to 6 million hMSC into the pig heart after mapping the left ventricle could be identified at necropsy. Ten percent of delivered cells were identified in the injection channels histologically. Passaging hMSC through the Myostar catheter had no disadvantageous effects on viability and proliferation in vitro.
CONCLUSIONS: (1) 5-azacytidine induces a myogenic phenotype of hMSC in vitro. (2) Percutanous intramyocardial MCS delivery is a valuable tool for transplantation strategies in ischemic heart disease.

GENE EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS MEASURED BY REAL-TIME RT-PCR

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Tissue engineering of 3-dimensional (3-D) cardiovascular structures requires an extracellular matrix to contain the living cells. During the cell derived remodelling of this matrix, little is known about gene expression under conditions that imitate the circulation. Fibroblasts were seeded in collagen gels and exposed to pulsatile hydrostatic pressure. The expression of fibronectin and collagen were quantified relative to a housekeeping gene HPRT on a Lightcycler PCR machine using real-time RT-PCR. The initial 8 hours of culture of fibroblasts in a 3-D matrix significantly decreased expression of collagen by 50% as compared to cells grown on a surface. This inhibitory effect was lost at 24 hours but pressure induced a 50% increase in expression. 24 hours of growth in a 3-D matrix resulted in a significant 2-fold increase in fibronectin expression. Real-time RT-PCR is a feasible tool for measuring gene expression of cells cultured in 3-dimensional matrices. Complex regulation of extracellular matrix genes by the environment can be quantified. These data should prove useful in the optimization of tissue construction.

EMBRYONIC STEM CELL–DERIVED CARDIOMYOCYTE TRANSPLANTATION INTO THE INFARCTED MYOCARDIUM

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PURPOSE: Investigation into the survival of embryonic stem cell (ES cell)–derived cardiomyocytes transplanted into the infarcted myocardium.
MATERIALS & METHODS: Donor cells: mouse ES cells transfected with GFP were cultivated in hanging drop and plated onto dish, and then beating regions were dissected. These cells were identified as cardiomyocytes by RT-PCR, recording of action potential, and immunostaining. Myocardial infarction: the left coronary artery of a rat was ligated. Transplantation: donor cells were injected into the border zone between the infarcted myocardium and normal myocardium, and ciclosporin was administered. Histological studies: 30 after transplantation, GFP expression and immunostaining were investigated.
RESULTS: In vitro MLC-2v genes, action potential like a ventricular myocyte, and positivity of anti-sarcomeric myosin stain were confirmed. GFP-expressed cells were detected after transplantation, but only a small number of them were positive of anti-sarcomeric myosin stain.
CONCLUSIONS: We confirmed that ES cell–derived cardiomyocytes could be transplanted into the infarcted myocardium and survive, but for clinical application it would be necessary to separate cardiomyocytes from ES cell-derived cells more strictly.

EXPERIMENTAL EVALUATION OF TISSUE ENGINEERING HEART VALVES USING DECELLULARIZED CRYOPRESERVED ALLOGRAFTS

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PURPOSE: The purpose of this study is to investigate the integrity of cryopreserved allograft valves as scaffold of tissue engineering valves.
METHODS: A porcine pulmonary valve was cryopreserved for 1 month and decellularized with Triton-X after thawing. Biomechanical characteristics of decellularized valve were tested using uniaxial tensile testing of circumferential and radial specimens and compared with those of non-cryopreserved decellularized valve. Pulmonary valve replacement was performed in the pig (n = 3) using decellularized cryopreserved pulmonary allografts. After 4 weeks of operation, characteristics of implanted valve were evaluated by echocardiography, direct pressure measurement, and histological examination.
RESULT: Biomechanical characteristics of decellularized/cryopreserved valves were similar to those of decellularized/non-cryopreserved valves. Echocardiography showed good valve function, and no pressure gradient across the valve was detected. Histological examination confirmed the

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endothelial coverage of the valve surface at 4 weeks after implantation.
CONCLUSION: Decellularized cryopreserved allograft valves showed excellent mechanical properties. In vivo valve function was satisfactory in the short term. Decellularized cryopreserved allograft valves have the possibility to be scaffold of tissue engineering valves.

DECELLULARIZED ALLOGRAFT VALVE FOR TISSUE ENGINEERING: EXPERIMENTAL STUDY OF HEART VALVES USING DECELLULARIZED CRYOPRESERVED ALLOGRAFTS

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OBJECTIVE: Existence of donor cells on allograft valves has been considered as one of the major causes of allograft degeneration. Decellularization and autologous endothelialization process will provide viable heart valves. This study investigated the possibility of allograft valves as scaffold of tissue engineered valves.
METHODS: A porcine pulmonary valve was procured and decellularized with TRITON X. Pulmonary valve replacement (PVR) was performed in the pig (n = 5), of which vascular endothelial cells were cultured from a piece of femoral artery for seeding on the surface of a decellularized valve. After 4 weeks (n = 3) and 12 weeks (n = 2) of operation, implanted valve characteristics were evaluated by echocardiography and histological examination.
RESULT: No obvious pulmonary insufficiency was detected. Histological examination confirmed the endothelial coverage at 4 weeks after the operation and some interstitial cell invasion of the valve tissue at 12 weeks after the operation.
CONCLUSION: Decellularized allograft valves with autologous endothelial cell seeding showed good valve function in the short term. Endothelialization will provide better anti-thrombogenicity at the time of implantation.

IN VITRO AND IN VIVO BIOMECHANICAL PROPERTIES OF DECELLULARIZED HEART VALVES

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Decellularized heart valves with autologous cells keeping intact structure and biomechanical properties may provide more durability with potential growth and invisible immunogenicity than homograft valves. Our recent study on biomechanical properties of decellularized valves was reported both in vitro and in vivo. Minipig pulmonary valves were immersed in Triton X-100 with EDTA, RNase, and DNase and maintained in a 37°C incubator under gentle shaking. They were then subjected to light and electron microscopy and mechanical property measurement. For an in vivo study, decellularized valves were replaced by native valves and examined histologically and biomechanically 1 month after transplantation. The leaflets treated were completely cell free. The biomechanical measurement showed increases in both of tensile strength and Young's modulus of the leaflets; however, they had no significant effects on the valve replacement. The grafts were well functioning and their surfaces were recellularized by endothelial cells. The leaflets transplanted had no significant changes of biomechanical properties. Heart valves decellularized by detergent have intact biomechanical properties and may provide more durable bioprostheses.

INCREASED PLASMA LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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The VEGF is a mitogenic and angiogenic cytokine for endothelial cells. It can also stimulate the synthesis of endothelin-1 (ET-1) by activation of endothelin-converting enzyme. Increased plasma levels of VEGF were found out in patients with CAD, peripheral artery disease, and hyperlipidaemia. We hypothesized that the plasma levels of VEGF will be elevated in patients with an acute myocardial infarction (AMI) in comparison to stable angina (SA) and correlated with endothelial injury marker ET-1. We studied 35 patients with AMI and 30 with SA. The concentrations of VEGF and ET-1 were assayed using ELISA kits. The levels of VEGF were significantly higher in AMI patients (mean ± SEM 312 ± 67 pg/mL) in comparison to SA patients (221 ± 67 pg/mL) ($P < .0001$); higher AMI patients with angiographically documented multivessel versus 1-vessel disease; ejection fraction <40% versus >40% and Killip class I-II versus III-IV ($P < .05$). In AMI group the ET-1 levels were higher than in SA patients ($P < .05$). There was no significant correlation between the VEGF and ET-1 levels. The VEGF levels in the top quartile (313 pg/mL) were associated with 2.3-fold higher OR for AMI in comparison to lower quartile (198 pg/mL) (OR, 2.35; 95% CI, 1.23-3.56). In conclusion: patients with AMI have significantly higher levels than patients with chronic stable angina.

CONSTRUCTION OF A TRILEAFLET VALVE SCAFFOLD USING THE INVOLUTED CYLINDER METHOD

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BACKGROUND: Previous attempts to design a tissue engineering scaffold to create a functional trileaflet valve have been hindered by central orifice regurgitation due to insufficient leaflet coaptation. A scaffold is constructed using a new method in an effort to increase leaflet coaptation and preserve the native sinuses of Valsalva.
MATERIALS AND METHODS: An unwoven polyglycolic acid (PGA) mesh sheet 24 mm × 75 mm and 1.5 mm thick is prepared and rolled into a cylinder. Three equidistant longitudinal 10-mm incisions are used to create 3 flaps which are involuted inside the cylinder and secured 120° apart to form commissures. Scallop-shaped segments of the outermost wall of the cylinder are removed between the commissures.
RESULTS: The PGA scaffold demonstrated a coaptation surface of at least 50% the leaflet height. The scaffold maintained commissural height following scalloping the outermost wall.
DISCUSSION: A novel method of preparing a scaffold for a tissue engineered trileaflet valve enabled leaflet coaptation and potential for maintaining the native sinuses of Valsalva. Further studies of seeding the scaffold with autologous cells and in vivo implantation are necessary to assess valve function and potential for human use.

CHARACTERIZATION AND IN VITRO TESTING OF HUMAN UMBILICAL CORD CELLS FOR CARDIOVASCULAR TISSUE ENGINEERING

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BACKGROUND: Cardiovascular tissue engineering attempts to create viable heart valve and vascular replacements with the potential to grow, repair, and remodel. This study characterized and evaluated a potential new cell source for cardiovascular tissue engineering—human umbilical cord cells (UCC).
MATERIAL AND METHODS: Umbilical cords and isolated UCC were characterized by immunohistochemistry, flow cytometry, SDS-gel electrophoresis, proliferation assays. Copolymer scaffolds were seeded with UCC and grown in-vitro. Morphological characterization of the scaffolds included immunohisto- chemistry, electron microscopy (EM), biochemical assays.
RESULTS: Umbilical cords and UCC stained positive for vimentin, ASMA, MHC, and flow cytometry showed comparable expression levels between UCC and vein-derived cells (VC). UCC demonstrated superior growth compared to VC. EM showed good cell-polymer adhesion of myofibroblasts and collagen fibrils. Analysis of scaffolds revealed beginning tissue and extracellular matrix formation consisting of collagen I, III, and glycoaminoglycan. SDS-gel electrophoresis revealed identical collagen banding patterns of UCC and VC.
CONCLUSIONS: UCC showed characteristics of immune-competent myofibroblasts with excellent growth properties. Tissue formation on copolymer scaffolds was feasible with extracellular matrix formation typical for cardiovascular structures. It appears that UCC represent a promising new cell source for cardiovascular tissue engineering. Autologous UCC seeding seems to be required to avoid immunological responses following construct implantation.

ICE-FREE CRYOPRESERVATION REDUCES BIOPROSTHETIC HEART VALVE CALCIFICATION

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We have proposed the hypothesis that the rapid deterioration observed in some allograft heart valve recipients is due to disruptive interstitial ice damage that occurs during cryopreservation and subsequently leads to accelerated valve degeneration upon implantation. We have compared a standard commercial heart valve freezing method of cryopreservation with a novel ice-free (vitrification) method and fresh controls in a rat model of calcification. Statistically significant calcification ($P < .05$) was observed in both syngeneic and allogeneic cryopreserved valves relative to vitrified and fresh valves. Syngeneic valves that were not cryopreserved demonstrated essentially no calcification. Vitrified syngeneic valves showed higher calcium levels compared to fresh controls (71 mg/g versus 24 mg/g at 21 days and 80 mg/g versus 33 mg/g at 42 days). However, the calcium content in vitrified samples were lower than that in frozen syngeneic valves (108 mg/g at 21 days and 121 mg/g at 42 days, respectively). Preliminary results using the ice-free cryopreservation method show changes in the time course of heart valve calcification in the rat model. These observations demonstrate that ice-free cryopreservation methods may improve the in vivo performance of cryopreserved heart valves. We anticipate that tissue engineered cardiovascular constructs in development may similarly require alternative cryopreservation methods.

MOBILIZED BONE MARROW CELLS AUGMENT NEOVASCULARIZATION IN A 3-D COLLAGEN SCAFFOLD ENGRAFTED ONTO INFARCTED HEART

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Treatment with granulocyte colony-stimulating factor (G-SCF) 5 days before and 3 more days after MI results in regeneration of cardiac muscle. Clinically, this technique is not feasible because of the need to start treatment before the incidence of MI. An alternative approach is to graft a 3-D collagen-1 scaffold onto the myocardium. Three weeks after grafting, we found: the scaffold prevented LV dilation (LV lumen diameter was decreased ($P < .05$) from 11041 ± 212 to 9144 ± 135 mm, n = 4); it shifted off the LV pressure-volume curve to the left toward control; and it induced neoangiogenesis within the graft (700 ± 25 neovessels/mm^2, n = 5); $75\% \pm 11\%$ of these vessels were arterioles/arteries ranging in diameter from (25-110 mm). These new vessels were connected to the coronary vasculature in the survived myocardium. Treatment with G-CSF (50 mg/kg IV for 5 days) increased ($P < .05$) the vascular density within the scaffold to 978 ± 57 neovessels/mm^2, n = 5). Thus, this study shows that grafting 3-D collagen-1 scaffold onto an injured myocardium induces mature neovessels, which was enhanced by mobilizing bone marrow cells using G-CSF.

VASCULARIZATION OF ECM SCAFFOLDS WITH CARDIAC DERIVED PRIMARY ENDOTHELIAL CELLS

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Xenogeneic, porcine-derived extracellular matrix (ECM) has been shown to be an effective scaffold for promoting the constructive remodeling of damaged or missing myocardial tissue. Vascularization of these ECM scaffolds has been a prominent feature during the remodeling process. The present study investigated selected components of ECM scaffolds for their ability to attract primary cardiac endothelial cells in an in vitro Boyden chamber system. Primary cardiac endothelial cells were isolated from mature Balb/c mice. The efficacy of a 5- to 16-kDa fraction of porcine-derived extracellular matrix for the ability to attract endothelial cells was determined. In addition, these cardiac derived endothelial cells were seeded onto sheets of porcine-derived ECM in vitro. Confluent sheets and tube-like structures lined by EC were observed to invade the ECM scaffold within 3 to 7 days. We conclude that porcine-derived xenogeneic ECM scaffolds contain chemotactic peptides for mature cardiac EC and that intact ECM scaffolds support the growth and differentiation of cardiac derived EC in vitro.

DEVELOPMENT OF A HYBRID VASCULAR GRAFT HIERARCHICALLY LAYERED WITH THREE CELL TYPES

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We developed hybrid grafts hierarchically incorporated with endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts (FCs) and investigated the cellular behaviors and ultrastructure of regenerated extracellular matrix (ECM) of those grafts in vivo. Hybrid grafts were constructed on knitted Dacron grafts (ID, 4mm; length, 6 cm) in vitro. Hybrid grafts were hierarchically structured with a confluent monolayer of ECs, a middle layer of SMCs, and an outer layer of FCs. The designed artificial ECM was a mixed gel of collagen and dermatan sulfate. Hybrid grafts were implanted in canine carotid arteries of 17 dogs for up to 23 weeks. Patent grafts (n = 15 out of 17 implanted grafts) were completely endothelialized. Observation of grafts at 12 weeks showed that hybrid grafts should be achieved similar to those of natural arteries structured at both cellular and biomolecular levels in many parts. At 23 weeks, hierarchically structured hybrid grafts completely resembled natural vessels. Thus, it can be said that hybrid grafts incorporating intima-, media-, and adventitia-type cell layers can significantly enhance the process of arterial wall reconstruction.

A TISSUE ENGINEERED CONTRACTILE CARDIAC GRAFT IMPROVES THE CARDIAC PERFORMANCE IN INFARCT RAT HEART

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Recent progress in tissue engineering will likely provide implantable and functional tissue. In the present study, we have exploited engineered cardiac grafts and implanted these grafts to infarct myocardium. Poly(N-isopropylacrylamide), temperature-responsive domain, was grafted on polystyrene cell culture surfaces. Neonatal rat cardiomyocytes were cultured on these dishes and detached as a square cell sheet under 20°C. Then, contractile cardiac grafts (T group, n = 7) were implanted to syngeneic infarct rat heart after 2 weeks of LAD ligation. The infarct hearts (Control group, n = 10) were not implanted. After 2 weeks of implantation, cardiac performance was significantly improved in the T group. Cardiac grafts attached on the infarct myocardium with gap junction containing Connexin 43 and seemed to be homogeneous tissue in the myocardium. In conclusion, cardiac grafts attached to the infarct myocardium with improvement of cardiac performance in infarct myocardium. Thus, tissue engineered contractile cardiac grafts may have potential as a cellular cardiomyoplasty for clinical application in patients with severe heart failure.

SKELETAL MYOBLAST TRANSPLANTATION DURING BYPASS GRAFTING IN THE TREATMENT OF HEART FAILURE—PHASE I CLINICAL TRIAL FEASIBILITY STUDY AND EARLY RESULTS

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Cardiomyocyte loss as a result of myocardial infarction leads to ventricular remodeling and subsequent heart failure. Experimental data suggest that myogenic cell grafting into the area of postinfarction injury is a promising approach to the treatment of heart failure, but transplantation of fetal cardiomyocytes raises availability and ethical issues. Transplantation of autologous skeletal myoblasts (ASM) has been evidenced by experimental data and suggested by initial clinical observations to improve both systolic and diastolic function after MI. Therefore we have established a phase I clinical trial to evaluate the use of ASM transplantation performed during coronary artery bypass grafting (CABG) by nonprofit research and academic institutions. Survivors from MI scheduled for CABG, with an akinetic area of the left ventricle, were screened by means of dobutamine stress echocardiography and included into the study when no viable myocardium was detected. In 7 patients to date, the skeletal myocardial biopsy was obtained from vastus lateralis. After isolation the cells were cultured for 3 weeks and up to 2×10^7 myoblasts per patient were grown. Myoblast injection into the aki-

netic area was done after constriction of the anastomoses during CABG procedure. No major perioperative complications were observed, and an increase in segmental contractility was seen during initial 4 and 8 week visits. We conclude that ASM transplantation for treatment of postinfarction heart failure is feasible, and initial observations justify further research to validate the method and define its role in clinical practice.

EVIDENCE FOR HUMAN HEART REGENERATION BY BONE MARROW–DERIVED CELLS

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Recent reports of experimental studies indicate that bone marrow–derived cells can regenerate cardiomyocytes and blood vessels in damaged hearts. In this study we examined the human female recipient heart following opposite-sex bone marrow transplantation by using fluorescence in situ hybridization (FISH) to determine the presence of Y chromosome containing cardiomyocytes derived from donor bone marrow cells. A 22-year-old woman with advanced Hodgkin's lymphoma received allogeneic bone marrow transplantation from her HLA-identical brother in January 1999. During the clinical course, the patient had received 550 mg/m^2 of doxorubicin and 4000 cGy of mediastinal irradiation. When the patient died from relapse 26 months after the bone marrow transplantation, specimens of her heart muscle were obtained from autopsy for this research. FISH using sex-chromosome–specific DNA probe CEP X SpectrumOrange/Y SpectrumGreen (Vysis) was performed on 4 μm-thick sections. FISH analysis identified Y chromosome–specific green signals within cardiomyocytes located on the periphery of the damaged myocardium. Y-positive myocytes were characterized by little lipofuscin accumulation, a single nucleus, and intense autofluorescence of myofibrils. These results provide previously undemonstrated proof that bone marrow–derived cells can regenerate the damaged human heart.

AUTOLOGOUS MYOBLAST IMPLANTATION RESULTS IN IMPROVEMENTS IN CARDIAC FUNCTION—PRECLINICAL TO CLINICAL STUDIES

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Autologous myoblast implantation for the restoration of deficits in cardiac function has evolved from the laboratory to the patient. Preclinical studies have provided a basis for developing procedures for initial human clinical trials. Skeletal muscle biopsy and expansion results in highly enriched populations of myoblasts that are characterized by desmin staining and their ability to form myotubes. Preclinical studies of stable models of heart failure in dogs have displayed evidence of functional improvements in dP/dtmax (approximately 15% increase) and LVEDP (approximately 4 mm Hg decrease) relative to control (saline injected animals) and baseline heart failure. Initial human clinical experience with 13 patients using the endovascular route appears to show evidence of progressive improvement in ejection fraction and wall thickness in a time-dependent fashion. Preliminary data suggest that the therapy appears to be safe and offers the potential for enhanced cardiac function.

LONG SURVIVAL OF TISSUE-ENGINEERED PULSATILE CARDIAC TISSUE GRAFTS

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To repair impaired hearts, cardiac tissue engineering is currently pursued utilizing the technology to fabricate 3-D biodegradable scaffolds as a temporary extracellular matrix. By contrast, we have exploited new technique, "Cell sheet engineering," that layers cell sheets to construct 3-D tissues. We apply novel cell culture surfaces grafted with temperature-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm), from which confluent cells detach as a cell sheet simply by reducing temperature without any enzymatic treatments. Neonatal rat cardiomyocyte sheets detached from PIPAAm-grafted surfaces were overlaid to construct cardiac grafts. In vitro, layered cardiomyocyte sheets were macroscopically observed to pulse simultaneously in a few days. In vivo, 4-layer cardiomyocyte sheets were transplanted into subcutaneous tissues of nude rats. Surface electrograms originating from transplanted grafts were detected at 2 weeks after the transplantation in the earliest case. When transplantation sites were opened at 3 weeks, transplanted grafts were pulsating in macroscopic views. Both surface electrogram and macroscopic pulsation were preserved at least up to 6 months posttransplantation. Histological studies showed characteristic structures of heart tissue and multiple neovascularization. These results demonstrate long survival of 3-D functional cardiac tissue grafts fabricated by cell sheet engineering. Cardiac tissue engineering based on this technology may prove useful for cardiovascular tissue repair.

MYOBLAST TRANSPLANTATION IMPARTS REGENERATIVE ABILITY TO THE MYOCARDIUM?

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PURPOSE: The absence of satellite cells and lack of DNA telomeric repeats in cardiomyocytes render the heart muscle less capable to regenerate in the event of injury. Our study involves heart cell transplantation using human myoblasts (HM) for myogenesis and restoration of normal cardiac function.
MATERIALS AND METHODS: Myoblasts were generated from human donor skeletal muscle biopsies. Purity of the myoblasts was assessed by immunostaining for desmin expression. Myoblasts (3×10^8 cells/5 mL) were labeled with lac-z gene and xenotransplanted into a porcine heart model of chronic ischemia. The animals were maintained on cyclosporine for 6 weeks post–cell transplantation. Three months later, animals were sacrificed and heart was processed for immunohistochemical studies.
RESULTS: HM were >95% desmin positive with >98% viability. Histochemical studies showed presence of lac-z–expressing donor nuclei in the host tissue at 3 months post–myoblast transplantation. Immunostaining for proliferating cell nuclear antigen showed mitotically active nuclei in host cardiac tissue. Proliferative cell index was up to 30% in the lac-z–positive region of the cardiac tissue. Ultrastructure analysis revealed multinucleation of cardiac fibers and the presence of satellite cells in the host cardiac tissue.
CONCLUSION: Our findings show that myoblast transplantation leads to a "regenerative heart."

ROUTINE CLINICAL CELL TRANSPLANTATION IN VASCULAR SURGERY

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PURPOSE: We report on our long-term routine clinical experience with endothelial cell transplantation of femoro-popliteal bypass grafts.
PATIENTS AND METHODS: PTFE grafts were precoated with fibrinolytically inhibited fibrin glue and confluently lined with cultured autologous endothelial cells prior to implantation. Based on the successful 3-year results of a pilot study with 49 patients, the authors offered in vitro endothelialized grafts to all patients who did not have a suitable saphenous vein available from June 1993 onward. Another 169 patients received 190 successfully endothelialized ePTFE grafts in this second phase of the transplantation program.
RESULTS: The routine clinical implantation of endothelialized ePTFE grafts showed a primary patency rate of 70.5% after 3, 61.0% after 6, and 55.2% after 8 years.
CONCLUSION: We conclude that in vitro endothelialization of ePTFE grafts results in a patency rate of arterial prostheses which is comparable to that of vein grafts. It further proves that methods employing cell transplantation can be transferred to surgical routine.

RAPID ENDOTHELIALIZATION OF SYNTHETIC VASCULAR GRAFTS WITH STEM CELLS

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PURPOSE: In this work we assessed the possibility to seed bone marrow–derived CD34$^+$ cells in a single stage procedure onto small-diameter vascular grafts.

METHODS: Human bone marrrow–derived $CD34^+$ cells were seeded on small-diameter PTFE vascular grafts by using a rotation device for 5 hours. After rotation grafts were further incubated in cell culture medium for 1 day. Specimens were examined immediately after rotation and after 1 day with a vital fluorescence dye, electron microscopy, and immunohistochemistry.
RESULTS: Immediately after seeding, a multilayer of cells was present on the grafts. Only a fraction of these cells stained positive for CD34. After 1 day in culture, the multilayered structures had disappeared and a preconfluent monolayer of cells was visible. Some cells still exhibited a rounded shape whereas others appeared to be fully spread. These cells stained positive for CD34 and resembled endothelial cells by morphology.
CONCLUSION: A preconfluent monolyer of endothelial-like cells can be created on small-diameter PTFE grafts within 1 day in a single-stage procedure. This procedure might be a last alternative for an increasing number of patients, when no adequate autologous grafts are available.

MORPHOLOGICAL CHANGES OF IMPLANTED IN VITRO ENDOTHELIALIZED VASCULAR GRAFTS

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PURPOSE: Segments of in vitro endothelialized grafts from three patients were obtained after 41, 26, and 14 months of implantation.
METHODS: Prior to implantation the grafts were confluently lined with autologous first passage mass cultures of cephalic-vein endothelial cells. Re-operation became necessary because of symptomatic atherosclerotic lesions in the grafts. Excised graft segments were examined by histology, immunohistochemistry, and electron microscopy.
RESULTS: All three grafts were lined with an endothelial monolayer. In areas of severe atherosclerotic changes the endothelial layer was partially disrupted. In one graft vessel, wall formation occurred with a tunica intima and a tunica media, divided by an internal elastic membrane. All three patients suffered from hyperlipidemia and diabetes.
CONCLUSIONS: We conclude that the confluent in vitro lining of synthetic vascular grafts with autologous endothelial cells facilitates graft healing which may result in a hybrid structure with features of a native vessel. These structures are prone to atherosclerotic changes.

TISSUE ENGINEERING OF AUTOLOGOUS HUMAN HEART VALVES USING ENDOTHELIAL PROGENITOR CELLS

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OBJECTIVES: An important factor in allograft valve failure is related to imunological deterioration. To overcome this limitation we converted homograft valves into autologous tissue by removal of allogeneic cells and repopulation of the scaffold with autologous differentiated endothelial progenitor cells (EPCs).
MATERIAL AND METHODS: Human pulmonary and aortic allografts were connected to a specially designed bioreactor and treated with trypsin/EDTA for decellularization. Peripheral mononuclear cells (40×10^6 to 300×10^6) were isolated from human blood and reseeded on the luminal valve surface. The EPCs were cultivated and differentiated on the acellular matrix under the flow conditions in culture medium supplemented with endothelial growth factors and 15% human serum up to 21 days.
RESULTS: Trypsinization of the allografts resulted in formation of cell-free scaffolds (H-E staining, DNA-assay) with preserved extracellular matrix (confocal microscopy). Recellularization under flow conditions resulted in formation of confluent cell monolayer on both surfaces of the valve leaflets (fluorescent microscopy). The cells on the matrix expressed endothelial cell specific proteins (vonWillebrand factor, PECAM1) and were positively stained for Flk-1 a receptor of vascular endothelial growth factor.
CONCLUSION: These results represent first experience in creation of autologous heart valves based on an allogenic scaffold reseeded with EPCs isolated from peripheral human blood.

STRUCTURAL FEATURES AND CELL COLONIZATION OF INTACT ACELLULAR AORTIC VALVE SCAFFOLDS

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Morphological features of decellularized pig aortic valve scaffolds devised for preparation of heart valve bioprostheses by tissue engineering have been assessed by histological and ultrastructural investigation before and after in vitro colonization with valve interstitial cells (VIC) from porcine aortic leaflets. Study has been carried out by SEM, TEM, light microscopy, and immunohistochemical investigation of cell differentiation markers. Basal lamina, underlying the fibrillar network and the whole body of extracellular matrix (ECM) scaffold, as obtained by this new decellularization procedure, appeared intact and closely corresponded to that observed in untreated control valve samples. Cells did adhere to the surface and progressively migrated within the ECM scaffold. After 2 weeks, grafted cells found within the leaflet's bioscaffold expressed endothelial cell markers in addition to those of VIC. On the whole, our data indicate that interstitial cells following cultivation on a matrix conserving both original architecture and epitopes could express their potential to make the whole repertoire of valve cells.

PERIVASCULAR DELIVERY OF THERAPEUTIC COMPOUNDS WITH SURGICAL FIBRIN GLUE

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Revascularization procedures for treating coronary artery lesions can fail as a result of restenosis, a reduction in vascular lumen area at the site of intervention due to intimal hyperplasia. While restenosis is amenable to inhibition with anti-proliferative compounds, satisfactory delivery of these agents has limited their successful application. In this study, we evaluated the use of fibrin glue (Tisseel) as a method of local delivery for the prevention of restenosis. Following balloon angioplasty of the porcine superficial femoral artery, Tisseel containing a potential anti-restenotic agent (losartan) was applied directly to the adventitial surface of the injured blood vessel. It was assumed the Tisseel coagulum would slowly elute the drug, thus extending the delivery period. Furthermore, Tisseel could accommodate a losartan concentration (25 mM) significantly greater than could be delivered systemically. Morphometric evaluation of the degree of restenosis after 14 days revealed a statistically significant decrease of 82% (n = 5) in the neointimal index. The inability to obtain 100% inhibition was attributable to cell migration, since cell proliferation was completely blocked. These data demonstrate that a fibrin glue such as Tisseel is an appropriate medium for the delivery of water soluble drugs.

HYPOXIA INDUCES MYOCYTE-DEPENDENT COX-2 GENE REGULATION IN HUMAN VASCULAR ENDOTHELIAL CELLS

Gui Fu Wu

To investigate the role of myocytes in induction of COX-2 expression in endothelial cells, human umbilical vein endothelial cells (HUVEC) were exposed to myoblast hypoxia-conditioned medium (MB-HCM). Myoblasts were incubated in a hypoxia chamber ($O_2\% < 1$) for 24 hours followed by treatment of HUVEC with MB-HCM. A 3-fold increase of COX-2 mRNA was detected as early as 1 hour, which returned to normal after 6 hours with MB-HCM treatment. When HUVEC were stimulated with a variety of cytokines and growth factors to determine the specificity of COX-2 mRNA induction, the level of COX-2 mRNA increased in HUVEC stimulated with VEGF or IL-1b, but other factors including IGF-1, FGF2, PDGF, and TNFa did not. Moreover, an approximately 7-fold increase of VEGF was expressed in myoblasts under hypoxia for 24 and 48 hours, while expression of COX-2 in HUVEC induced by VEGF (25 ng/mL) was time-dependent and comparable with COX-2 expression in MB-HCM. NOS inhibitor, L-NAME, could block COX-2 expression induced by MB-HCM but not by myoblast itself during hypoxia. PCK inhibitor, staurosporine, could inhibit COX-2 expression in HUVEC in myoblasts both during hypoxia and in MB-HCM. Our data show that myocytes may play important role in COX-2 expression in endothelial cells during hypoxia.

LONG-TERM SURVIVAL OF XENOTRANSPLANTED HUMAN MYOBLASTS IN A PORCINE HEART MODEL OF CHRONIC ISCHEMIA

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BACKGROUND: In vivo behavior of human myoblasts (HM) for heart transplantation remains incompletely characterized. Considering the similarity of porcine and human hearts, we have studied the survival of HM in chronic myocardial ischemia using transient immunosupression.
METHODS: Human myoblasts were transduced with lac-z gene. A porcine heart model of chronic ischemia in 9 pigs (control group, n = 3 and cell transplanted group, n = 6) was created by clamping an ameroid ring around the left circumflex coronary artery. Four weeks later, 20 injections (0.25 mL each) of 5 mL basal medium containing 3×10^8 cells or without cells were injected into the left ventricle endocardially. Immunosupression was achieved with 5 mg/kg cyclosporine for 6 weeks after cell transplantation. Animals were euthanized between 6 weeks to 30 weeks after cell transplantation; heart was explanted and processed for histological studies.
RESULTS: The donor biopsies generated >95% desmin-positive HM population with >98% viability. Histological examination showed lac-z–positive donor cells in host cardiac tissue up to 30 weeks posttransplantation and expressed human skeletal myosin heavy chain in the pig heart. Discontinuation of immunosupression after 6 weeks of cell transplantation showed no signs of donor cell rejection.
CONCLUSION: HM showed long-term survival in the ischemic cardiac environment using transient immunosupression.

ANGIOMYOGENESIS USING HUMAN MYOBLASTS CARRYING HUMAN VEGF165 FOR THE TREATMENT OF CRYOINJURED RODENT HEART

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PURPOSE: We hypothesized that a combinative approach of angiomyogenesis will restore global myocardial function in a failing heart.
METHODS: Human myoblasts were transduced with lac-z and human VEGF165 genes and characterized for VEGF165 expression by immunostaining, ELISA, and RT-PCR. A cryoinjured heart model was created in 26 female Wistar rats (control: n = 12; cell transplanted: n = 14). Ten days later, 150 μL basal medium containing 3×10^6 HM or without HM was injected endocardially in the infarct. Animals were maintained on cyclosporine (5 mg/kg) for 6 weeks post–cell transplantation. Heart function was assessed by echocardiography. The animals were sacrificed at 6 weeks post–cell transplantation and hearts were processed for histological studies.
RESULTS: The transduced HM continued to secrete VEGF165 for longer than 18 days in vitro, significantly higher (37 ± 3 ng/mL) than the control HM. Histological examination showed lac-z expressing HM in host cardiac tissue at 6 weeks posttransplantation. Echocardiography showed 14.2% improvement in ejection fraction in cell-transplanted animals compared to control animals ($P < .05$). Immunostaining for vWF-VIII showed increased vascular density in low power field (17.5 ± 1.05) in transplanted animals as compared to control group (3.6 ± 0.7; $P < .01$).
CONCLUSION: HM are potential therapeutic transgene carriers for the repair of damaged heart muscle.

COMPARATIVE STUDY ON THE ANGIOGENIC EFFECTS OF ANGIOPOIETIN-1 AND VEGF165 IN A PORCINE MODEL OF CHRONIC MYOCARDIAL ISCHEMIA

Zhang Li

The potential effects of angiopoietin-1 (Ang-1), a newly identified angiogenic factor, are less well defined. This study compared angiogenic effects of adenovirus-mediated gene transfer of Ang-1 (AdAng-1) and vascular endothelial growth factor 165 (AdVEGF165) in a porcine model of chronic myocardial ischemia. Animals were randomized to 4 groups: ischemic control (without further interventions) (n = 4), AdNull (null adenoviral vector) (n = 5), AdAng-1 (n = 6), and AdVEGF165 (n = 6). Adenoviral vectors were injected directly into the ischemic myocardium along the free wall of left ventricle. After 3 months, there was marked angiogenesis in the AdAng-1– and AdVEGF165-treated myocardium, as revealed by anti–von Willebrand factor and anti–smooth muscle actin staining. The average capillary density of AdAng-1 group was significantly higher than that of AdNull group and ischemic control group, while there was no statistical difference between AdAng-1 group and AdVEGF group. Myocardial blood flow measured with fluorescent microspheres increased significantly in AdAng-1 group 3 months following treatment. Ang-1 and VEGF expression could not be detected by RT-PCR 3 months after treatment. In conclusion, Ang-1 functions as an angiogenic inducer in vivo, similar to VEGF. Adenovirus-mediated gene transfer of Ang-1 may be more potent than that of VEGF in improving blood flow in chronic ischemic myocardium.

OPIOID CYTOPROTECTION BY INTRACELLULAR SIGNALING

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Opioids protect the ischemic myoctye and enhance mitochondrial activity, presumably by G protein intracellular signaling. We examined the effect of the delta opioid receptor agonist, DADLE, on cytoplasmic lactate dehydrogenase (LDH) as well as the integrity of the mitochondria by the MTS assay. Using an immortalized murine myocyte line, cells were either maintained at normoxia or underwent 1 hour of ischemia followed by 2 hours of reperfusion. Viability was determined by trypan blue exclusion. Control (CM) myocytes were compared to myocytes pretreated with DADLE or naltrindole, a delta opioid antagonist. Normoxic maintained cells retained $89\% \pm 2\%$ viability. However, following 1 hour of ischemia, CM viability fell to $60\% \pm 2\%$ ($P < .001$), DADLE treated cells were unchanged at $82\% \pm 2\%$ (P = NS), and naltrindole treated cells were not viable $1\% \pm 1\%$ ($P < .001$). Results of LDH and MTS assays showed $P < .05$ for beneficial effects and $P < .05$ for adverse effects. DADLE-treated cells had significantly lower LDH and higher mitochondrial activity, and this protective effect was reversed by delta antagonism. These data demonstrate that delta opioid agonists decrease cellular injury and death while maintaining mitochondrial metabolic activity. Additionally, the adverse effects of delta antagonists indicate an endogenous anti-ischemic delta opioid regulatory activity.

A STENT-MOUNTED AUTOLOGOUS PERICARDIAL CORONARY ARTERY BYPASS GRAFT

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Rapidgraft (Ramus Medical Technologies, Santa Barbara, CA, USA) is a conduit constructed from autologous pericardium that has been briefly treated in 0.6% glutaraldehyde. Preclinical animal studies demonstrated that autologous pericardial conduits remain viable, developing a complete endothelium with an organized smooth-muscle component consistent with normal artery. Rapidgraft has been evaluated as a coronary bypass graft in 24 patients. A total of 26 grafts with mean length of 13.1 cm were used in the study, 3 mm (n = 21) and 4 mm (n = 5). Predischarged patency was 80%. At 6 months, 0 of 6 were patent. A 3-mm Rapidgraft was explanted at 17 months postoperatively. The graft was patent at discharge and occluded at 6 months. The lumen was completely free of adherent thrombus, but a thin pannus at the proximal anastomosis obstructed flow. Rapidgraft may be susceptible to pannus formation at the proximal anastomosis. Design modifications or use of a proximal connector may eliminate pannus formation. Further clinical evaluation is required to assess the long-term implications of an autologous pericardial conduit, but the preliminary data showing a viable, thrombus-free conduit raise the possibility that Rapidgraft could be the solution to the search for a long-term viable graft.

A NOVEL METHOD FOR LINING A BLOOD VESSEL WALL PRIOR TO STENTING

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Improving the outcomes with the use of arterial stents remains a clinical challenge. Lining stents has been suggested as a method for improving the incidence of in-stent restenosis. Others have suggested that stent-grafts may be useful in repairing perforations or excluding aneurysms. Small arterial stent-grafts constructed with synthetic materials such as ePTFE have failed to improve clinical outcomes in the coronary arteries. Several investigators have previously reported results using segments of saphenous vein to cover an arterial stent. These methods typically involve the removal and replacement of the stent on the catheter, and can add significant bulk to the stent. The authors present novel methods for predeploying a thin lining of autologous tissue, or other biocompatible material, over an arterial lesion prior to deployment of the stent. A temporary stent is used to precisely position an open-end cylinder of autologous tissue over the targeted lesion. The stent is deployed within the tissue cylinder, trapping the tissue lining between the stent and vessel wall. The temporary retaining stent is removed along with the guide wire. Previously reported results with saphenous vein–lined arterial stents suggest that a biocompatible lining may improve the utility and clinical outcomes associated with arterial stenting.

DEVELOPMENT OF BIOCOMPATIBLE ARTIFICIAL GRAFTS WITH AUTOLOGOUS VASCULAR

Yoshifumi Chida

The artificial vascular graft has potential disadvantages that relate to use of a conduit, including lack of growth potential and thromboembolism for children. A biocompatibly structured vascular graft, resembling the multilayered structure of a natural vessel, may accelerate to regenerate physiological function and growth potential similar to those of a natural vessel. In this study, we developed biocompatible vascular grafts incorporated with autologous endothelial cells (ECs), smooth muscle cells (SMCs,) and fibroblasts (FCs) on a biodegradable polymer. Biocompatible vascular grafts were constructed on tubular scaffolds (internal diameter, 5 mm; length; 6 cm) in vitro. Tubular scaffolds were made of biodegradable polymers composed of a polyglycolic acid (PGA), and a copolymer of L-lactide and aprolactone. Canine vascular cells were isolated from their jugular veins, and grown in cell cultures. The grafts consisted of ECs confluent monolayer and homogeneously mixed SMCs and labeled FCs layer. The designed artificial ECM was a mixed gel of fibrin glue. Pre-implanted grafts were completely endothelialized by scanning electron microscopy (SEM). Grafts have been implanted bilaterally in canine carotid arteries without anticoagulant. These grafts were achieved at physiological perfusion pressures. Vascular cells incorporated into biodegradable artificial grafts may contribute to enhanced tissue regeneration process including antithrombogenicity and growth potential.

TISSUE ENGINEERING A LIVING HEART VALVE: BIOCOMPATIBILITY OF AN ACELLULAR PORCINE VALVE MATRIX

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OBJECTIVE: To assess the biocompatibility and reseeding potential of an acellular porcine valve matrix.
METHODS: Porcine cells (smooth muscle cells, fibroblasts and valve interstitial cells) were cultured with SDS-decellularized porcine valve leaflets and extracted soluble components. Changes in cell morphology and viability were then determined. Following subdermal implantation in mice, inflammation and tissue integration were assessed using standard histological and imunohistological techniques. Leaflets were cultured with porcine cells and migration was assessed using standard histological techniques.
RESULTS: No significant morphological change or loss in cell viability was observed following contact with the leaflets or the soluble components. There was limited inflammatory cell infiltration, good tissue integration and the porcine cells migrated into the leaflet matrix.
CONCLUSION: The SDS-decellularized porcine valve matrix was not cytotoxic or inflammatory and had good tissue integration potential. Cultured porcine cells readily attached and migrated into the leaflet matrix.

LONG-TERM SURVIVAL OF NOVEL MUSCLE-DERIVED STEM CELLS AFTER TRANSPLANTATION INTO MYOCARDIUM

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Cellular cardiomyoplasty has suggested its feasibility as an alternative therapy for heart failure. To date, however, the survival of transplanted cells over 6 months has not been reported. This study demonstrates the ability of a novel muscle-derived stem cell, mc13, to survive for at least 9 months after transplantation into hearts. The mc13 cells were isolated from skeletal muscle of mdx mice by the modified preplate technique and were transfected with a plasmid containing mini-dystrophin, LacZ, and neomycin-resistance genes. The mc13 cells expressed stem cell markers, Sca-1 and Flk-1, as well as myogenic markers (desmin, c-met, MNF). The mc13 cells ($1\text{-}5 \times 10^5$) were injected into the left ventricular free wall of SCID mice. At 36 weeks posttransplantation, histological examination showed that the transplanted cells survived and formed LacZ-positive myotubes within the host myocardium. Immunohistochemistry revealed the β-galactosidase–expressing myotubes were colocalized with a cardiac specific–myosin heavy chain. Future experiments will be performed to assess the marker and the potential therapeutic benefit of mc13 on cellular cardiomyoplasty for damaged hearts.

NOVEL MUSCLE-DERIVED STEM CELLS DELIVER DYSTROPHIN INTO A DYSTROPHIN-DEFICIENT MURINE HEART

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Patients that suffer from X-linked cardiomyopathy, Duchenne and Becker muscular dystrophy, progressively develop dilated cardiomyopathy. To date, little attempt has been made to correct the dystrophin deficiency in the heart of these conditions. In this study, we hypothesize that the transplantation of a novel population of muscle-derived stem cells (MDSCs) would result in dystrophin delivery in a dystrophin-deficient murine heart. MDSCs, isolated from skeletal muscle of normal newborn mice and transduced with a LacZ reporter gene, were injected into the heart of SCID/mdx mice, an immune- and dystrophin-deficient model. At 2, 4, and 8 weeks posttransplantation, histological and immunohistochemical stainings showed that the injected MDSCs survived and differentiated into a large number of LacZ- and dystrophin-positive myotubes and myofibers. Colocalization of dystrophin and myosin heavy chain, a cardiac-specific marker, was evident at 4, 8 weeks postinjection. This study demonstrates the ability of MDSCs to survive and deliver dystrophin within the heart for at least 8 weeks postinjection. Further experiments will be performed to study the effect of dystrophin delivery via cell transplantation on cardiac function.

THE HUMAN AORTIC ENDOTHELIUM AS THE IDEAL SOURCE FOR CARDIAC VALVE ENGINEERED PROSTHESES

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To identify the most suitable human EC for bioengineered matrix endothelization of cardiac valve prostheses, endothelia were purified from human aorta, aortic vasa vasorum, and right atrial appendage from 20 patients undergoing cardiac surgery. The functional behavior of EC was evaluated in terms of spontaneous and mitogene-induced proliferation, capillarylike structure formation, migration properties, resistance to inflammatory cytokines, adhesion to extracellular matrix proteins, and nitric oxide production. Aortic endothelium showed a significantly higher proliferation under mitogen stimulation (growth increment, 0.3), higher resistance to inflammatory cytokines, and a superior capability to migrate and to repair vascular injury under inflammatory mediator treatments. The analysis of NO production failed to demonstrate significant differences of functional behavior in terms of biochemical synthesis. Aor-

tic endothelium seems to be the most suitable for engineered cardiac valves because of its favorable characteristics in terms of growth, cytokines resistance, adhesion capillary-like structure formation, and migration capabilities.

DEVELOPMENT OF A NEW TECHNIQUE TO CULTURE SKELETAL MUSCLE–DERIVED STEM CELLS FOR MYOCARDIAL REGENERATION

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A technique devised to generate multipotent adult stem cells from the dermis of mammalian skin was adapted in order to isolate and expand human skeletal-muscle–derived stem cells (SkmSC) from brachioradialis muscle specimens obtained from patients undergoing coronary surgery. Tissue specimens (100 mg) were digested with trypsin, and the resulting cells were cultured in collagen coated flasks with EGF and FGF in absence of fetal calf serum. Adhering cells showed a typical spindle morphology of satellite cells; after 5 to 7 days we observed rounded floating cells forming microspheroids of 4 to 10 cells, that could be passed and maintained in vitro for more than 6 months. After induction of cell differentiation, the immunohystochemical analysis revealed that both the adherent and the floating cells were positive to smooth muscle actin, striatum muscle actin, and desmin, thus generating cells committed to a myogenic lineage. This procedure provided an alternative approach to expand in a short period autologous SkmSC for myocardial regeneration.

A NEW OUTSIDE STENT—DOES IT PREVENT VEIN GRAFT INTIMAL PROLIFERATION?

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OBJECTIVE: Sheathing of the vein graft with a pressure-resistant tubing should potentially prolong vein graft patency rates.
METHODS: 21 sheep received a radial vein and hybrid dacron mesh grafts into carotid artery position. Light and electron microscopy and proliferating cell antigen (Ki-67) stains were used to assess proliferation.
RESULTS: 5 days from implantation the mean intimal wall thickness in hybrid grafts was 19.2 ± 10.7 μm versus 24.4 ± 6.5 μm in unsheathed grafts; after 9 day it was 22.3 ± 5.8 versus 26.2 ± 10; 4 weeks, 22.6 ± 7.6 versus 52.2 ± 15; 6 weeks, 36.6 ± 20.7 versus 89.9 ± 31; 8 weeks, 56.6 ± 30.8 versus 103.6 ± 28; 10 weeks, 58.3 ± 20.6 versus 132.5 ± 31.6; 12 weeks, 72 ± 21.5 versus 244 ± 99.8 ($P < .001$ in 7 time points). Cell proliferation after 5 days in hybrid grafts was 6% ± 2.3% versus 8% ± 2.4% in unsheathed grafts; 9 days, 8.7% ± 2.9% versus 8.1% ± 2.2% (NS); 4 weeks, 8.1% ± 2.4% versus 7.8% ± 1.9% (NS); 6 weeks, 5.6% ± 3.8% versus 7.5% ± 2.6%; 8 weeks, 5.1% ± 3.4% versus 6.9% ± 2%; 10 weeks, 1.72% ± 1% versus 6.3% ± 2.3%; 12 weeks, 1.7% ± 0.9% versus 5.4% ± 1.9% ($P < .001$).
CONCLUSION: The external vein graft support with a mesh tubing reduces intimal thickening and cell proliferation in composite vein grafts.

MYOCARDIAL TISSUE REPLACEMENT WITH EXTRACELLULAR MATRIX SCAFFOLDS

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BACKGROUND: Dilated cardiomyopathy and heart failure resulting from myocardial infarction contribute to substantial mortality and morbidity worldwide. Recent evidence suggests that repair of the infarcted region and recovery of left ventricular contractile function might be possible through tissue engineering approaches such as autologous skeletal myoblast transplantation. An alternative strategy is the replacement or repair of infarcted regions with biological materials or matrix scaffolds that would support growth of viable cardiac tissue.
PURPOSE AND METHODS: To test the hypothesis that a type of extracellular matrix scaffold might enable myocardial repair, we implanted peracetic acid-treated and buffer-rinsed porcine urinary bladder extracellular matrix scaffolds approximately 80 to 100 μm thick and approximately 2 to 3 cm diameter (UBM; CorMatrix Inc, Atlanta, GA, USA) as full-thickness right ventricular (RV) outflow tract free wall replacement patches at sites of excision in 4 juvenile Yorkshire hybrid pigs and 1 adult mongrel dog; 1 pig received a glutaraldehyde-treated bovine pericardial patch (GP) approximately 1 mm thick and approximately 2.5 cm diameter as a control. Hearts were harvested at 6 weeks and the sites of patch implant were examined grossly; histopathologic and immunohistochemical studies of tissue sections were performed to assess the morphologic appearance and cellular responses to the UBM implants.
RESULTS: Pigs and dogs underwent uncomplicated recovery from UBM and control implant surgery, and exhibited no clinical signs of cardiac insufficiency. At harvest all hearts showed pericardial adhesions, and patch implant sites were pearly white at the epicardial aspect. Endocardial surfaces of UBM-implanted areas showed pearly white tissue interdigitated with extensive brown-colored tissue suggestive of trabeculated myocardium whereas the GP-implanted region showed primarily pearly white tissue with only sparse brown-colored tissue. Histopathologic examination of tissue sections from the GP implants showed the material as a discrete region of eosinophilic material surrounding by a fibrotic capsule incorporating numerous inflammatory cells including foreign-body giant cells. UBM implants were not discernible and the RV wall at the implant was a mixed tissue including myocardium and fibrocellular areas interdigitated in the body of the implant site; extensive neovascularization was present and in one instance, Purkinje cells were observed within the suture line. Immunohistochemical studies revealed a population of myeloid cells (MAC-387 clone, Dako, myeloid/histiocyte antigen) at the endocardial aspect and in isolated regions within the implant site of UBM. In counterpoint, cells expressing muscle actin (HHF-35 clone, Dako, all muscle actin) were present in 2 morphologies within the body of the UBM implant sites: those with stellate and spindle-shaped morphology resembling smooth muscle cells or myofibroblasts, and striated cells resembling cardiomyocytes. Numerous profiles of actin-positive blood vessels including small arteries, veins, and microvessels were present, often juxtaposed with hemosiderin deposits. There was extensive replicative activity in various cells types evidenced by positive proliferating cell–nuclear antigen staining. The endocardial surface was covered by vWf-positive endothelial or endothelial-like cells.
CONCLUSIONS: UBM was acceptable as a single-thickness sheet to replace an RV free wall defect, and animals so treated did not suffer ventricular aneurysm, pericardial effusion with tamponade, or clinical signs suggestive of cardiac insufficiency. UBM implants appeared to be incorporated into the RV free wall by a tissue replacement process; this may involve in part recruitment of circulating cells as well as appearance of muscle cells, which may derive either from the circulation or adjacent myocardium. In contrast, GP was incorporated into a fibrotic capsule in the absence of such a replacement process. Further research into the potential utility of UBM and similar materials for myocardial tissue replacement, and the mechanisms of the replacement process, appears warranted.

VASCULAR ENDOTHELIAL GROWTH FACTOR-1 GENE TRANSFER EFFECT OF ANGIOGENESIS IN TRANSMYOCARDIAL LASER REVASCULARISATON TREATED PORCINE MYOCARDIUM

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INTRODUCTION: Transmyocardial laser revascularisation (TMLR) results in symptomatic improvement in patients with chronic ischemic heart disease through the enhancement of collateral vessel formation by laser channels and also by an angiogenic response to TMLR. We hypothesized that direct myocardial injection of plasmid DNA of VEGF-1 will significantly improve neoangiogenesis on the TMLR-treated myocardium without fibrotic activity.
METHODS: 10 male Yorkshire pigs (weight: 52 ± 12 kg) underwent TMLR treatment via left thoracotomy under Propofol + Ketamine anesthesia. Group I (baseline controls, n = 5) underwent TMLR treatment (8-10 channels around the marginal branch) and no further intervention. Group II (n = 5) underwent TMLR treatment (8-10 channels around the marginal branch) with equidistant intramyocardial g promotor (pCI factor) + cDNA of VEGF-1 (165 kD) surrounding the injection of 10 to each TMLR site. All animals were followed to 12 weeks and harvested. Histologic evaluation of each injection site was performed with hematoxylin-eosin and Azan staining, and the effect of VEGF-1 on angiogenesis was evaluated by determining

the number of capillaries in each area observed by immunostaining with antigrowthfactor VIII. antibody and binding of the avidin-biotin PAP complex. VEGF-1 expression in infected myocytes and in endothelial cells, respectively, was proved histologically by an anti–VEGF-1 antibody reaction.
RESULTS: The number of capillaries at the TMLR treated baseline group was 10.6 ± 1.35 (mean ± SEM). Delivery of expression plasmid DNA of VEGF-1 into the TMLR treated sites significally increased the number of arterioles (mean ± SEM, 16 ± 5.74) ($P < .01$). Histological data showed a definitive decrease of fibrotic activity in the combined treated myocardium compared to the TMLR treatment alone. It was also proved histologically that all laser channels showed neoendothelization.
CONCLUSION: These results suggest that the combined use of TMLR treatment with direct injection of an expression plasmid DNA encoding VEGF-1 significantly enhanced the collateral capillary neoangiogenesis at the surrounding area of the laser channels. It might be an improvement of wall motion in the chronic ischemic and cardiomyopathic myocardium.

AUTOLOGOUS STEM CELLS INJECTION IN A PATIENT AFTER ACUTE

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Experimental data suggest that injection of adult bone marrow (BM) stem cells into the border zones of infarcted heart muscle may reduce myocardial infarction (MI)-size by affecting myocardial remodeling. A 57-year-old male with acute anterolateral MI was initially treated with thrombolysis. Coronary angiography demonstrated severe 1-vessel LAD disease with diffuse lesions not suitable for PCI. Six weeks postinfarction BM stem cells were harvested by aspiration from the iliac crest hipbone and injected intramyocardially. Injections were guided and performed with NOGA-mapping and -injection needle (Biosense Webster, Diamond Bar, CA, USA). A total of 2×10^9 BM stem cells (5.2 mL) were implanted at 12 different sites into the transition zone between scar and viable myocardium into the anterolateral and –septal region. Myocardial perfusion imaging (MPI) was performed before and 3 and 6 months after stem cell implantation. Compared to initial MPI, polar map analysis showed decrease in rest extent (34% to 25%) of perfusion-defect 6 months after stem cell transplantation. In addition left ventricular ejection fraction (EF) obtained by gated SPECT improved from 33% to 41%. NOGA unipolar map showed an increase of 2.6 and 1.7 mV and 1.5 and 0.3 mV in the middle and basal portion of the septal and lateral segments, respectively. Intramyocardial injection of BM stem cells was safe and feasible in a patient with recent acute MI. The decrease in MPI rest extent after 6 months and improvement of EF may indicate beneficial remodelling.

DECELLULARIZATION PROCEDURES FOR PORCINE HEART VALVES AND THEIR SUSCEPTIBILITY FOR SEEDING WITH HUMAN CELLS

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The aim of this study was to compare different methods to decellularize porcine heart-valves and their susceptibility for seeding with human endothelial-cells and myofibroblasts. Porcine aortic roots were treated with either Trypsin, sodium-dodecyl-sulphate (SDS), or Triton-X 100 and sodium-deoxycholate and seeded with cultured human saphenous-vein cells. Specimens were processed for scanning electron-microscopy (SEM). Cryostat sections were stained for vonWillebrand Factor (vWF), collagen types I and III, and elastin. After Trypsin treatment SEM demonstrated a confluent cell-monolayer on the porcine heart-valve matrix, and vWF staining revealed a viable endothelial cell layer. However, cell remnants were still detectable in the matrix. Unexpectedly, when human cells were seeded onto SDS-treated specimens massive cell lysis was observed; beyond that a strong disintegration of the matrix fibers was seen. Triton-X 100 and sodium-deoxycholate completely removed porcine cells, induced no visible structural alterations, and enabled host recellularization. SDS shows no susceptibility for reseeding and has a destructuring effect. Only the porcine matrix treated with Triton-X 100 and sodium-deoxycholate presents an excellent scaffold for recellularization with human cells.