FALL/WINTER 2003

ISACB

CIRCULATOR

INTERNATIONAL SOCIETY FOR APPLIED CARDIOVASCULAR BIOLOGY

ISACB's 9th Biennial Meeting in *Savannah*

MARCH 10-13

The International Society for Applied Cardiovascular Biology's 9th Biennial Meeting, entitled, "Towards Biofunctional Cardiovascular Implants", will be held March 10-13, 2004 at the Westin Savannah Harbor Resort in Savannah, Georgia. The 9th Biennial Meeting will follow and be coordinated with the 8th Annual Hilton Head Workshop: "Cardiovascular Tissue Engineering: From Basic Biology to Cell-Based Therapies" sponsored by Georgia Tech/Emory Center for the Engineering of Living Tissues. Both the venue and the science promise to lure you to Savannah.

The Venue

Savannah, the sultry and mysterious "Belle" of the Southeastern coast, captivates the suitors that come to call with her natural beauty, eccentric charm and traditional Southern Hospitality — because Savannah is genteel, gracious and captivating. Savannah is the beautifully preserved hidden treasure of the Low Country. Come unlock the history, romance and beauty that lie within. Explore every nook and cranny because you are her guest and Savannah loves sharing her treasures with your.

Savannah is surrounded by natural beauty, charm, architecture and history of the Old South. She offers a number of unique architectural features including a unique city layout with an abundance of squares, parks and open public spaces. Savannah boasts the largest National Historic Landmark District and captivates one's interest with hidden gardens with southern floral beauty: Spanish moss, live oaks, magnolias and azaleas. Restored cotton warehouses, restaurants, art galleries, shops and activities surround the Savannah River Street Area.

The ISACB meeting will be held at the beautiful Westin Savannah Harbor Resort. Poised above Hutchinson Island, overlooking the Savannah River, The Westin Savannah Harbor Resort & Spa, offers a unique combination of magnificent meeting facilities, classic Westin Service and a resort atmosphere, and is just a water taxi ride away from the southern charm of Savannah. A two-minute water taxi ride ushers you to the wonderful restaurants and nightlife along the Savannah River.

Savannahians like to take their love for food outdoors when the weather turns cool. Some highly enjoyed casual meals are outdoor bar-b-ques, oyster roasts on the beach and a coastal favorite known as the Low Country Boil. This popular concoction consists of boiled shrimp, corn on the cob, onions, sausage and new potatoes all boiled together and dumped on long tables were partygoers stand and devour the feast. A "Low Country Boil" will be the highlight of the ISACB themed dinner next year, following a trolley tour of this elegant and interesting city.

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WE'RE NOW ONLINE!

ISACB now has its own home page at
www.isacb.org

The internet site includes information about the goals and organization of ISACB, a copy of the latest edition of the ISACB *Circulator* and updated information regarding our biennial meetings.

PRESIDENTIAL ADDRESS

"What we have here is a failure to communicate."

— Paul Newman in Cool Hand Luke

Howard Greisler, ISACB President

The ISACB was established sixteen years ago primarily as a direct reaction to the above general sentiment stated so eloquently by Paul Newman. The biomedical sciences had become a Tower of Babel. The Tower was rising but its blueprint was misplaced. The sheer volume of important information was rapidly increasing, but at a rate far less than the exponential increase in unimportant information. Reductionism is an essential tool of modern science, but reductionists have difficulty communicating with each other. This "frustrated communication" was keenly sensed by those at the organizing meeting of the ISACB in Cape Town. Consequently, the stated goals of the Society prominently included the encouragement of inter-disciplinary communication.

The upcoming 9th Biennial Meeting of the ISACB in Savannah will be held in coordination with the 8th Annual Hilton Head Workshop, this year on Cardiovascular Tissue Engineering, immediately preceding it. The planning for both meetings, including location, dates, programs and integrated events, has been a joint effort and represents a further step toward facilitating communication among the disparate disciplines with science, engineering and clinical medicine. Both programs will be very strong and will, by intent, firmly complement each other. Participation in both is encouraged.

Admittedly, and proudly, the ISACB is not a "mainstream" society of an isolated subspecialty. One joins the Society and participates in the meetings out of a keen interest in the field, not as an obligatory function of the "guild" or "union" of the discipline of the individual. Again, Paul Newman had it right when, allowed by his police captors to relieve himself behind a bush (attend his official conference), he was required to keep the bushes rustling to assure his presence. As he said continually "I'm shakin' it Boss", he moved on to another place, a place of mutual communication and productivity.

While the ISACB represents a coming together by chance of like minded individuals from complementary yet disparate disciplines, its independence comes at a price. No one feels obligated to belong or to participate. That is good and that is bad. Once a society becomes obligatory, it loses its flexibility and the spontaneity. On the other hand, it often gains financially. The ISACB has persisted uninterruptedly on a shoestring budget because of the dedication of its members. Its lack of secure long term financial stability presents ongoing challenges to the Society's leadership and members and suggestions are very deeply encouraged. However, the commitment of the membership and supporters is extraordinarily strong because of the intellectual underpinning the Society and its freedom to explore multidisciplinary and often unconventional approaches developing from true communication. From communication, we have everything to gain. We can be innovative and challenging because we have nothing to lose.

As Paul Newman also said in Cool Hand Luke, "Nothing can be a pretty cool hand."

And for those who are eager to celebrate St. Patrick's Day, Savannah has been celebrating her Irish heritage for more than a century. Traditional Irish values and customs of old are mingled with the vibrancy and charm of a city whose quirky personality comes out in those who call Savannah home. Today nearly 500,000 celebrants from almost 50 states and many countries descend on Savannah for the celebration.

Participants of the ISACB's 9th Biennial Meeting will have an opportunity to partake in many of the festivities that will be offered prior to St. Patrick's Day. You should be prepared for green streetlights, pets adorned in green costumes, green food and beverages. St. Patrick's Day is even a day off of school for Savannah area children! Although the ISACB Meeting ends a few days before the official "Day", festivities for the world famous event begin in mid-February with the Irish festival.

The ISACB's 9th Biennial Meeting offers you a venue that will allow you to experience the history, wonder, elegance and mannerisms of the Old South along with an excellent scientific meeting. We look forward to welcoming you to Savannah in March 2004!

The Science

The ISACB Program Committee for the 9th Biennial Meeting includes Howard Greisler, M.D., Peter Zilla, M.D., Ph.D., Bob Nerem, Ph.D., Frederick Schoen, M.D., Ph.D., Ivan Vesely, Ph.D. and John Mayer, M.D. Collectively this Committee defined a stimulating roster of thematic topics for the meeting and thoughtfully assembled invited speakers for each session. The program was designed in cognizance of the fact that the 9th Biennial Meeting was being coordinated with the 8th Annual Hilton Head Conference and Workshop which just precedes the ISACB meeting in Hilton Head and that the theme of the Hilton Head meeting this year is *"Cardiovascular Tissue Engineering: From Basic Biology to Cell Based Therapies."* The Committee was faced with the challenge of complementing and not-overlapping the scientific intent of the Hilton Head meeting and yet recognizing that not all ISACB Meeting attendees were Tissue Engineering oriented. The Committee chose two of the most important issues in Cardiovascular Tissue Engineering to structure two scientific sessions for the ISACB meeting - namely Scaffolds and Stem Cells. One of the invited speakers on the ISACB program, David Mooney from the University of Michigan was recently quoted in *The Scientist* as saying "the biggest thing that has changed the [tissue-engineering] landscape [is] the very significant shift to the emphasis on stem cells". The hope of the Program Committee is that investigators interested in Cardiovascular Tissue Engineering will make Hilton Head and Savannah the place to be in March, 2004!

In addition to the dedicated sessions on Tissue Engineering, the Program Committee selected three additional session topics of traditional interest to attendees at former ISACB meetings: Therapeutic Angiogenesis, Heart Valves and Cardiovascular Development, Adaptation and Remodeling. Noted speakers have been confirmed to present current data of significance regarding each of these topics.

The thematic topics of the scientific program and roster of confirmed speakers includes the following:

CARDIOVASCULAR TISSUE ENGINEERING I: STEM CELLS

Chaired by: Howard P. Greisler and Robert M. Nerem

Mechanisms of Cardiac Development and Regeneration
Silviu Itescu, New York, NewYork, USA

Adult Stem Cells for Cardiovascular Tissue Engineering
Karen K. Hirschi, Houston, Texas, USA

Stem Cell Sources for Tissue Engineering
Anthony Atala, Boston, Massachusetts, USA

Embryonic Stem Cells for Cardiovascular Tissue Engineering
Steven L. Stice, Athens, Georgia, USA

CARDIOVASCULAR TISSUE ENGINEERING II: SCAFFOLDS

Chaired by: Howard P. Greisler and Robert M. Nerem

Embedding Biological Signals into Biomaterials
Andreas Zisch, Zurich, Switzerland

Intelligent Materials for Tissue Engineering
Teruo Okano, Tokyo Japan

Biological Scaffolds for Tissue Engineering
Stephen F. Badylak, Pittsburgh, Pennsylvania, USA

Issues and Progress in Vascular Tissue Engineering
Jennifer L. West, Houston, Texas, USA

HEART VALVES

Chaired by: Ivan Vesely and John Mayer

Development of a Composite, Tissue-Engineered Aortic Valve
Ivan Vesely, Cleveland, Ohio

Tissue Engineered Pulmonary Valve
John E. Mayer, Jr., Boston, Massachusetts

Cardiac Valvulogenesis
Roger Markwald, Charleston, South Carolina, USA

Cell/Matrix Interactions
Donald Ingber, Boston Massachusetts, USA

TISSUE REGENERATION: THERAPEUTIC ANGIOGENESIS

Chaired by: Peter Zilla

Kinins In Therapeutic Angiogenesis And The Mechanisms Behind Their Biological Effects
Costanza Emanueli, Sassari, Italy.

Polymer Based Delivery Systems For Therapeutic Angiogenesis
David J. Mooney, Ann Arbor, Michigan, USA

Progress In Clinical Trials Of Therapeutic Angiogenesis
Michael Simons, Lebanon, NH, USA

CARDIOVASCULAR DEVELOPMENT, ADAPTATION AND REMODELING

Chaired by: Frederick J. Schoen

The Mystery of Cardiovascular Remodeling: Collagenase, the Prime Suspect
Masanori Aikawa, Boston, Massachusetts, USA

Vascular Development and Remodeling
Stephen M. Schwartz, Seattle, Washington, USA

Biology and Therapeutic Potential of Circulating Progenitor & Stem Cells
Shahin Rafi, New York, New York, USA

Relevance to Tissue Engineering
Frederick J. Schoen, Boston, Massachusetts

I S A C B

ESSAYS

The Essay section of the ISACB Circulator contains invited and submitted manuscripts. The essays may summarize the state of development of new technology in applied cardiovascular biology or highlight recent important research results. The editor of the ISACB Circulator invites your submission. Manuscripts may be sent to the ISACB business office at the address on page 2.

Ventricular Sourcing An Alternative to Traditional Coronary Artery Bypass

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"If at first an idea does not seem absurd, it is not worth pursuing". —Albert Einstein

This quotation was used by Dr. Daniel Van Heeckeren of Case Western Reserve's University Hospital, Cleveland, OH in his first presentation to the scientific advisory board of Percardia, Inc., a venture capital backed company located in New England. Dr. Van Heeckeren was reporting on the chronic survival of pigs after implantation of a ventricle to coronary artery stent (VSTENT™) distal to an acute ligation of the coronary artery.

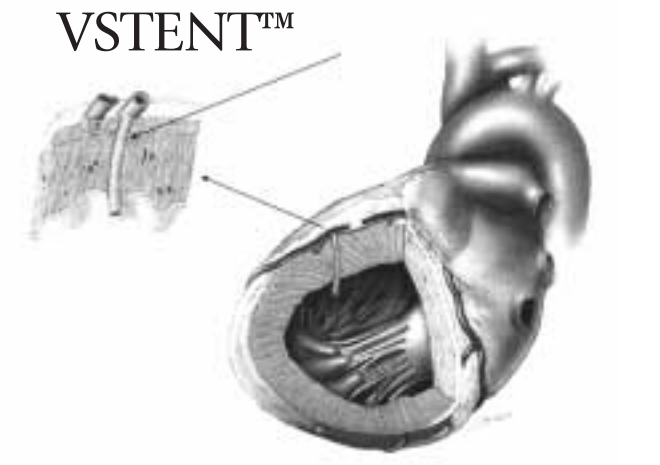
There are a number of reasons why the idea of direct ventricle to coronary artery bypass (VCAB™) seems absurd (See figure 1 — VSTENT™). These reasons center on the undeniable fact that this approach lacked important components present in traditional coronary artery bypass; namely, a blood source with a valve closed during diastole, and with the compliance of the aorta.

Figure 2 is from a canine study performed by Daniel Burkhoff's group at Columbia University, New York. The flow through the left anterior descending coronary artery (LAD) was measured (in ml/minute), as well as the left ventricular pressure (LVP), EKG, and regional myocardial function (segment length in mm) using sonomicrometry. In the first column normal baseline values are seen, and based on the EKG maximum LVP is obtained at the end of systole, and LAD flow is during diastole. Regional myocardial movement has a regular and repeatable pattern. When a direct channel is placed between the left ventricle (LV) and the LAD, and the vessel is occluded proximal to the channel the flow through the channel peaks now during systole, and there is a strong backwards flow during diastole. The net flow past the probe is approximately 50% of the normal baseline flows, and the regional myocardial function also decreases approximately 50%. The third and fourth columns represent different flow biasing of the backward component by use of a Starling like resistor. Net flow and function increased in proportion to reduction of the backwards flow component. Finally in the fifth column total closure without blood from the VCAB channel or LAD resulted in paradoxical wall motion of the myocardium acutely and could lead to fibrillation if left in this state.(1)

These initial findings were considerably better than earlier studies of redirected flow from the LV to the LAD, where as little as 25% of baseline flow was reported, and the animals in these studies were showing acute ischemia. (2) The conclusion from Munro's studies was that the concept could not support the myocardium and severe ischemia would result. While in Suehiro's study acute ischemia was avoided, the question remained as to how well the animal could survive chronically and with stress, or in other words what was the impact on coronary reserve. Historical evidence would suggest that a 50% reduction in resting (non stress) flow could represent a constriction or stenosis of about 80-90%. (3) The distal vascular bed would dilate to compensate and attempt to maintain flow at the expense of coronary reserve.

Also in Suehiro (1) it was shown that improvement of baseline flow from 50% to 70% by restriction of the backward component could improve perfusion of the endocardium significantly, and suggested possible ability to prevent ischemia chronically even with episodes of stress. This optimism was greatly held in check by the concern that systolic flow in VCAB would not be able to support the endocardium as perfusion to the endocardium had been shown to be mainly achieved during diastole in the LV. (4)

FIGURE 1

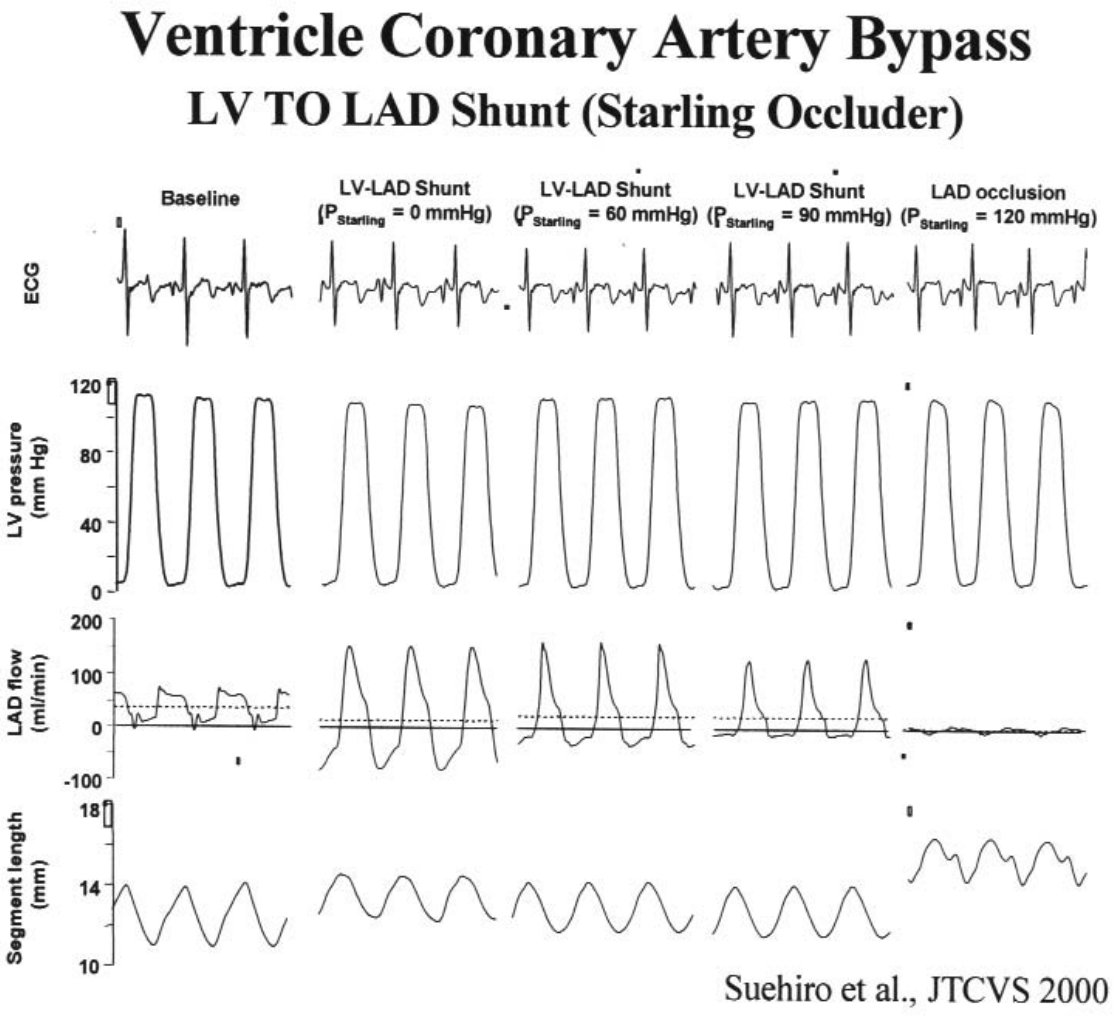


The absurdity of the idea of ventricular sourcing was reduced even more when after one month of chronic implant in a porcine model the heart was able to survive a dobutamine stress test. Traditionally animal models of ischemic hearts had shown a biphasic response in a dobutamine stress echo (DSE) test. (5) At higher heart rates the animals with the VCAB channel were showing normal wall motion. At this point feelings of absurdity were changing into a determination to understand why all the predicted failure modes were not functioning.

An early hypothesis for this unexpected ability to perfuse the heart sufficiently under stress with a totally ligated LAD was unusual recruitment and arteriogenesis in pre-existing collaterals. The first indications were seen in comparisons of angiographic baselines versus one month post VCAB channel placement. High shear rates from the unique flow pattern in the channel were thought to be a possible stimulus for remodeling, and specific studies to look at remodeling were conducted by Keith March's group at IUPUI, in Indianapolis, and this work showed convincing positive remodeling in the large coronary vessels. (6)

While interesting this remodeling was taking place in healthy animals that were treated with a significant surgical procedure. The obvious clinical question was how the channel would affect an ischemic heart that had sufficient collateral recruitment to function normally at rest, but not enough to maintain normal function under periods of stress. At this point the hard core skeptics were certain that the channel would steal the collateral flow and normal function would not even be possible at rest. To address this concern an ischemic model was developed. The model consisted of an ameroid occluder placed on the LAD for one month followed by either traditional CABG or implantation of the VCAB device. One month past the placement of the ameroid the LAD was completely occluded, and flow in the distal vessel was very slight (< 2ml/min) and due to collateral filling. Net forward flow was increased immediately to greater than 10 ml/min after placement of the device. In addition to answering question concerning steal, this experiment measured myocardial perfusion using microspheres, and showed very similar behavior in perfusion between the VCAB and CABG animals. (7) Experiments continue at Columbia University looking at VCAB and the unique arteriogenesis seen. Preliminary results have shown unusually large collaterals staining positive for BrdU in the areas of the myocardium affected by the stent flow. (8)

FIGURE 2



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Since the majority of patients presenting symptoms in the clinic are not total occlusions; but rather high grade stenosis, the question stilled remained as to whether a VCAB stent could be placed distal to a high grade stenosis and not result in a steal phenomenon. Peter Boekstegers of Klinikum Grosshadern in Munich who actually implanted the first acute VCAB stent supplied the important experiments in animals to answer this question. (9) In these experiments stenoses of varying degrees were created, and a LV-CA stent placed distally. Intra-coronary artery pressure and flow, regional myocardial function (sonomicrometry), and coronary reserve were all measured under conditions of stress (pacing and adenosine). In these acute studies not only was there no steal phenomenon, but the stenosis actually contributed to better net forward flow and function. It was concluded that a partial stenosis proximal to the stent contributed to a higher average intra-coronary artery pressure which in turn reduced the backward flow component of the channel. These critical experiments paved the way for the first human clinical cases the initial results of which were presented recently. (10)

"whoever exalts himself shall be humbled, and whoever humbles himself will be exalted" (Matt 23:11-12)

This quote is always appropriate when one has the delusion of scientific proof of principle. Considerable time and resources have gone into research and development efforts to prove that the concept of ventricular sourcing as an alternative to traditional coronary artery bypass is viable. Initial results are very encouraging if only because the device is performing in a manner that many said was not possible. More importantly the continuing effort to answer all constructive criticisms of the concept not only results in a safer clinical product and procedure, but is generating additional questions that identify new indications and potential platforms for arteriogenesis and beyond.

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I S A C B
ESSAYS

Heart Valve Tissue Engineering - Taking a "Biomimetic" Approach

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The ideal heart valve substitute: Dwight E. Harken's "Ten Commandments"

The essential characteristics of ideal heart valve substitutes have been described already in the 1950's by Dwight E. Harken, a pioneer in heart valve surgery, and summarized as the so-called "Ten Commandments" [1]. These include durability, absence of thrombogenicity, resistance to infections, lack of antigenicity, and the potential of growth. In principle, he stated the fundamental properties of natural, living, and autologous tissues. Unfortunately, these requirements are still not met by today's heart valve prostheses. For treatment of heart valve disease, mechanical or biological valves are currently in use. The drawbacks of mechanical valves include the need for life-long anticoagulation, the risk of thromboembolic events, prosthesis failure, and the inability of the device to grow. Biological valves (homograft, xenograft, fixed by cryopreseration or chemical treatment) have a limited durability due to their immunogenic potential and the fact that they represent non-living tissues without regeneration capacities. All types of contemporary valve prostheses basically consist of non-living, foreign materials posing specific problems to pediatric applications when devices with growth potential are required for optimal treatment.

Tissue engineering of heart valves — where do we stand?

In an attempt to overcome these limitations, tissue engineering represents an experimental approach aiming at living, autologous cardiovascular replacement structures. The basic concept currently used for tissue engineering of heart valves is to transplant autologous cells onto a biodegradable scaffold, to grow and to condition the cell-seeded scaffold device in vitro and finally to implant the tissue-like construct into the donor patient.

The heart valve scaffold may be based either on biological or synthetic materials. Donor heart valves or animal derived valves depleted of cells can be used as a scaffold material. Removing the cellular components results in a material essentially composed of extracellular matrix proteins that can serve as an intrinsic template for cell attachment [2]. In general, non-fixed acellularized valve leaflets have shown recellularization by the host, as demonstrated in dogs [3] and sheep [4]. However, first clinical applications of this concept in children resulted in rapid failure of the heart valves due to severe foreign body type reactions associated with a 75% mortality [5]. In a further approach, specific biological matrix constituents can be used as scaffold material including collagens and fibrins [6, 7]. These materials have the disadvantage that they are difficult to obtain from the patients in sufficient quantities. Therefore, most of these scaffolds are of animal origin. In this context, identification of retroviruses and prionic diseases has given rise to great concern as to the risk of zoonoses. Recently, epidemiological data strongly indicates transfer of Creutzfeldt-Jakob disease from cattle onto humans via infected meat, surgical materials derived from bovine gut and drugs or vaccines prepared using fetal calf serum [8]. Porcine endogenous retroviruses (PERV) can be present in many tissues as multiple copies of PERV can be integrated into germ-line DNA. New and more infectious groups of PERV are being identified [9], as well as their capacity to infect various types of human cells in vitro [10].

The use of synthetic materials as scaffolds has already been broadly demonstrated for cardiovascular tissue engineering. Initial attempts to create single heart valve leaflets were based on synthetic scaffolds, such as polyglactin, PGA (polyglycolic acid), PLA (polylactic acid), PLGA (copolymer of PGA and PLA). To create complete trileaflet heart valve conduits, PHA based materials (polyhydroxyalkanoates) were used [11]. These materials are thermoplastic and can therefore easily be molded into any desired three dimensional shape. Recently, we introduced a combined polymer scaffold consisting of non-woven PGA and P4HB (poly-4-hydroxybutyrate) showing promising in vivo results [12].

In most cardiovascular tissue engineering approaches, cells are obtained from donor tissues, e.g. from peripheral arteries, and mixed vascular cell populations consisting of myofibroblasts and endothelial cells are grown. Out of these, pure viable cell lines can be easily isolated by cell sorters [13] and the subsequent seeding onto the biodegradable scaffold is undertaken in two steps: First the myofibroblasts are seeded and grown in vitro. Second the endothelial cells are seeded on top of the generated neo-tissue leading to the formation of a native leaflet-analogous histological structure [14].

With regard to clinical applications, several human cell sources have been investigated [15]. Recently, cells derived from bone marrow or umbilical cord have been successfully utilized to generate heart valves and conduits in vitro [16, 17]. In contrast to vascular cells, these cells can be obtained without surgical interventions representing an easy-to-access cell source in a possible routine clinical scenario. Due to their good proliferation and progenitor potential, these cells are expected to be an attractive alternative for cardiovascular tissue engineering applications.

Where to go? Considering the "golden" standard

The ultimate rationale of the tissue engineering concept is the creation of living neo-tissues identical or at least very close to native heart valves. So far, the majority of work has been focused on valves of the semilunar type, such as aortic or pulmonary valves; mainly because of their less complex design in comparison to the atrio-ventricular valves (i.e. absence of chordae tendineae etc.). A prerequisite to a sophisticated prospective development of native-analogous tissue engineered valves is an accurate understanding of the fundamentals of normal heart valves representing the "golden standard". A description of its composition, structure and function as exemplified by the normal aortic valve is given in the following section. Interestingly, it is the tissue engineering research of recent years which has stipulated a novel interest in heart valve anatomy, biomechanics, embryology, cell biology and pathology with many important implications to the whole field of heart valve diseases.

How does nature do it?

When the first heart contraction takes place during embryonic development, the heart is not more than a tube consisting of a single lumen. This tube is transformed into an H-shaped outflow channel with large tissue cushions in the right and left outflow tract. These will each divide into three equal mounds of cushion material and form the origins of the aortic and pulmonary valve [18]. Endothelial cells lining these cushions appear to be able to differentiate into leaflet interstitial cells, regulated by local growth factors [19].

The hemodynamic environment during development of the valve cusps determines the cell shape, proliferation and fiber formation [18]. The cells on the ventricular side of the leaflets are flattened, due to the shearing effect of the blood flow during ventricular ejection, whereas the cells at the arterial side stay more cuboidal. The cusps grow by proliferation of cells in the downstream end, the region with low pressure and low shearing force. Cell proliferation seems to stop when the leaflets are long enough to contact the arterial wall above the sinuses during opening. Elastic fibers become prominent at the ventricular side of the leaflet, which is exposed to intermittent flexural stresses during systole. At the arterial side, exposed to the predominantly static stresses during diastole, collagenous fibers develop. At the line of closure, the leaflets consist of solely collagenous fibers, which correlate to the tensions at both sides of the leaflets.

The development of the aortic valve takes place under pressure values below 10 mm Hg, at heart rates ranging from 65-160 beats per minute, and hypoxic conditions [20].

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The acceleration of the heart rate might be a compensatory phenomenon in the absence of the Frank-Starling mechanism, as the immature fetal myocardium does not possess the ability to increase the ejection fraction in response to increasing preload. By increasing hematocrit and a shift of the hemoglobin-oxygen dissociation curve towards optimized oxygen binding characteristics the fetus compensates for the hypoxic conditions.

Architecture: Matrix and cells

The load-bearing part of adult aortic valve leaflets shows a layered architecture within the endothelial coverings, enabling the extraordinary changes in shape and dimension. The ventricularis, the layer at the inflow surface, is predominantly composed of radially aligned elastin fibres. The central layer, the spongiosa, consists of loosely arranged collagen fibres and an abundant amount of proteoglycans. The layer at the outflow surface, the fibrosa, comprises mainly circumferentially aligned collagen fibres. All collagen bundles diverge into the aortic wall, thereby transferring the gross load from the leaflet to the aortic wall. The individual layers can easily compress and shear during opening and closing of the valve. The fibrosa and ventricularis are inherently preloaded due to their attachment to each other, the fibrosa under compression and the ventricularis under tension [21].

Among the valvular interstitial cells three cellular phenotypes can be identified: smooth muscle cells, arranged in bundles or just as single cells [22], fibroblasts maintaining the extracellular matrix, and 60 % [23] of them are myofibroblasts, cells that have phenotypic features of both fibroblasts and smooth muscle cells [24]. These cellular phenotypes are situated depending on their biological and mechanical microenvironment. Myofibroblasts and fibroblasts are able to convert from one to another, triggered by either a lack of mechanical tension or the presence of continuous mechanical tension [25]. The idea of passively functioning aortic valve leaflets was refuted by identifying a smooth muscle cell system in the leaflets 22], contractile properties of valvular interstitial cells [24, 26] and sensory nerve elements in the leaflets [27]. Contraction within the leaflets might help to sustain the hemodynamic forces that are exerted on the leaflet during systole and diastole [26] and represents a reactive cytoskeleton that can anchor collagen fibrils during valve closure [24].

Structure and function — The anisotropy of life

The individual layers of valve leaflets show different mechanical characteristics due to their difference in composition [28-30]. The fibrosa is considered to be the main load-bearing layer of the leaflet and prevents excessive stretching [31]. The difference in radial and circumferential extensibility, a phenomenon known as anisotropy, is not as large in this layer as it is in the ventricularis, where the radial extensibility is much larger than the circumferential extensibility [28-30]. The overall mechanical response of the leaflet is the summation of the mechanical properties of the individual layers. Lo and Vesely [32] measured a maximal extensibility of porcine aortic valve leaflets of 24 % in radial direction and 11 % in circumferential direction by whole-valve biaxial testing, a reliable way to test natural biaxial loading environment in the valve is reflected. In circumferential direction the mechanical behavior exhibits the properties of collagen bundles and in radial direction the elastin mesh is the predominant factor. The leaflet shows an extremely low compressive modulus, which is most likely influenced by the spongiosa [28].

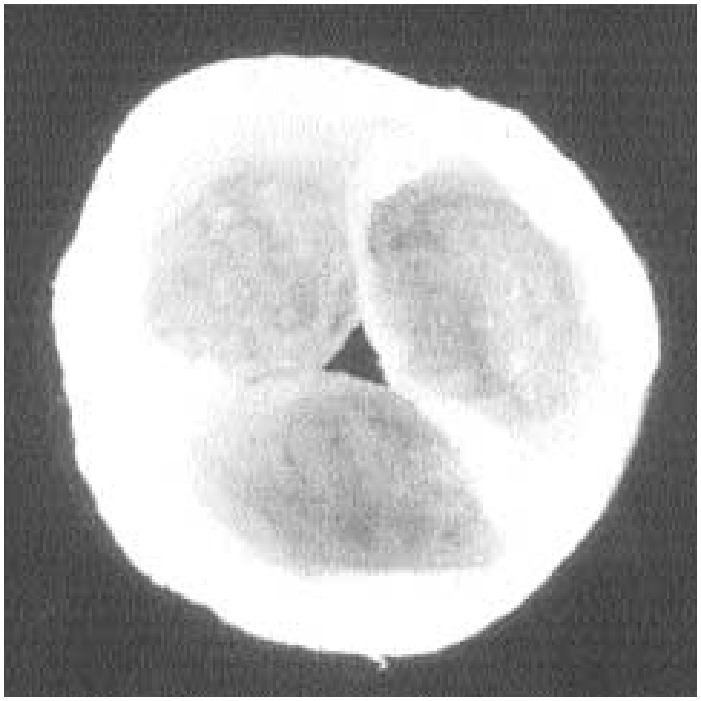
Schoen and Levy [33] summarized the biomechanics of the aortic valve as follows. When the valve is nearly closed and the collagen bundles in the fibrosa are fully unfolded, collagen is the load-bearing element, enabling a stress increase while preventing a prolapse of the leaflets. The loose spongiosa layer is able to dissipate the shock of closure of the leaflets, as the hydrophilic proteoglycans in this layer readily absorb water and swell. Due to deformation of the sinus walls, resulting in an increase in volume, the pressure difference across the valve decreases. During opening of the valve, elastin extends at minimal load in the ventricularis to return the fibrosa in its original corrugated state, facilitated by the spongiosa that dissipates the arising shear stresses.

Lessons from the "golden" standard — taking a "biomimetic" approach

It is the fascinating perfection of architecture and function of a native, living heart valve which enables the enormous life-time performance with billions of cycles without malfunctions. The above described structural and functional complexity of normal heart valves obviously is a "conditio sine qua non" for this performance. Therefore, the concept of tissue engineering should be close to what has been termed a "biomimetic" approach. This approach is based on the hypothesis, that in vitro exposure of the developing tissue to physical signals similar to those encountered in vivo may result in more mature tissue engineered heart valves. The utilization of bioreactors for physiological in vitro preconditioning of tissue engineered structures has been introduced to the field by us and others and stipulated a considerable progress in cardiovascular tissue engineering [12, 34]. We have demonstrated that using a pulse duplicator bioreactor prior to in vivo implantation resulted in living, autologous heart valves with good functional performance. The in vitro conditioning protocol was elaborated to mimick the hemodynamic conditions heart valves experiences during embryological development. Native-analogous tissue formation was observed in vitro with regard to morphological and biomechanical features. Interestingly, the ultimate refinement and maturation of the tissue engineered heart valves occurred during the subsequent in vivo period of this experiments, showing an evolution of cell phenotype and extracellular matrix towards native valve tissue. This remodelling has been investigated in collaboration with Frederick Schoen and co-workers providing a more accurate understanding of the neo-tissue changes prior and after implantation [35].

In summary, it appears that the biomimetic approach is a key to tissue engineering of functional heart valves. The cells need to be placed into the appropriate "environmental niche" to produce the "right tissue". Our work demonstrates that this process can be initiated by bioreactors. However, so far the refinement is done by nature. Future research focused on improved bioreactor protocol may realize this refinement already in vitro. This would be the next important step towards generation of more ideal substitutes. Taking all this into account and remembering Dwight E. Harken's "Ten Commandments" as to ideal heart valve substitutes, in contrast to the usual saying "Do not imitate - innovate" the motto as to heart valve tissue engineering rather should be "Imitate (nature) to innovate (valve substitutes)!"

FIGURE



The biomimetic approach to heart valve tissue engineering: Living, autologous tri-leaflet valves generated in vitro in a pulse duplicator system (Hoerstrup et al. Circulation 2000, 102 : III44-49).

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I S A C B
ESSAYS

A Bioengineer's Challenge and Dream

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My biomaterial research life begun almost a quarter century ago when I joined the newly established National Cardiovascular Center (NCVC) Research Institute in Osaka, Japan. My initial assignment there was to actively participate in the government-directed project entitled "Artificial Heart and Ventricular Assist Device". During the course of the project, I learned considerably from implantation experiments using goats as an animal model how potent and complex the foreign-material-activated body defense mechanism is, and how the hydrodynamic wash-out effect minimizes the nature and magnitude of blood/material interactions. An analogy of Virchow's triad concept of homeostasis of vessels (flow, nature of blood and pathophysiological changes in the vascular wall), which was proposed by the famous German pathologist, Dr. R. Virchow, more than a century ago, was also realized on the blood compatibility of blood pumps, which is called Akutsu's trinity concept (pump design determining flow pattern and the degree of wash-out, surface fabrication related to smoothness without any voids or pits, and blood-contacting biomaterial related to inherent bioinertness) [Note that Dr. T. Akutsu, the pioneer of artificial heart, began his research with Dr. Kolff at The Cleveland Clinic in 1956, and headed the NCVC project team when I joined].

After seven years active participation in the blood pump project, my research interest has shifted to the development of artificial and tissue-engineered vascular grafts. At the present, the development of vitally functioning small-diameter vascular substitutes has been a more than a half-century endeavor. Despite many years of effort, numerous hypotheses and various approaches, small-diameter artificial grafts with the inner diameter of less than 5~6 mm have not yet been realized in clinical settings, compared with medium- to large-diameter artificial grafts, which do not have serious problems of patency in clinical situations. The general consensus is that stenoses derived from the thrombus formed on the luminal surface of artificial grafts in the early phase of implantation and from an excess tissue ingrowth at anastomotic sites (intimal hyperplasia) in the later phase are the major causes of the failure of small-diameter grafts.

The search for small-diameter vascular graft substitute materials, biological modulators and fabrication process technologies for providing an extracellular milieu similar to the natural physiological vessel environment has a long history. Although the search for materials that are completely nonreactive materials with blood and the surrounding tissue is likely unrealistic, the precision extracellular space design triggering "minimal" foreign body reactions and enhancing normal vascular tissue morphogenesis is essential. Various concepts and hypotheses have been individually proposed and tested *in vivo*. These include 1) porosity, 2) compliance matching, 3) biologically active coatings and 4) natural nonthrombogenic lining of endothelial cells.

The first two issues mentioned above have been discussed over four decades. The generally accepted knowledge is that the higher the porosity is, the faster the tissue regeneration is, and the higher the patency. As for compliance matching, clinically used artificial grafts made of plastics are noncompliant, that is, they do not respond to periodically loaded stress derived from pulsatile blood flow. The difference in pressure-dependent mechanical properties between native arteries and artificial grafts induces hydrodynamic flow disturbances and stress concentration, thereby causing tissue damage as well as impairing cellular functions driven by a mechano transduction mechanism.

Based on the above experimental as well as computer-simulation studies of biomechanical failure, my colleagues and I initiated studies of 1) the development of a "mechano-active and tissue permeable" scaffold design and biologically active coatings for artificial and tissue-engineered vascular grafts, and 2) enhanced tissue morphogenesis technology using new cell sources as a nonthrombogenic cell lining.

Compliant Scaffold: The first compliant graft scaffold tested was a segmented polyurethane foam (SPU: highly durable synthetic elastomer which is the essential material for the diaphragm of blood pumps)-based tube. In addition to the inherent elastomeric property, microporosity markedly increased the compliance which became closer to that of native arteries. Although the implanted grafts (inner diameter: 3 mm) performed very well for up to almost one year of implantation in a canine model, a gradual dilation resulting from the accelerated biodegradation of SPU foam due to considerably high surface-to-volume ratio inherent in microporous forms was observed.

The second approach, aimed at the minimal surface-to-volume ratio that could eliminate surface degradation, was a laser-ablated micropored SPU-film-type tube with well-controlled porosity (pore size and pore-to-pore distance or pore arrangement). The pulsed UV irradiation of KrF excimer laser (wavelengh; 248nm) ablated tubular SPU films to generate pores with very high dimensional accuracy, which was manipulated using computer-assisted design (CAD) and computer-assisted manufacturing (CAM) technologies[1,2]. Multiply generated micropored SPU tubes (wall thickness, 100 μm; micropore size, 50 μm; pore-to-pore distance, 200 μm; and length, 2 cm) also exhibited compliance matching with native arteries. A mixed solution of photoreactive-group (benzophenone or phenylazide)-derivatized gelatin, heparin and growth factors such as the

basic fibroblast growth factor (bFGF) and/or vascular endotherial growth factor (VEGF) was coated and photoirradiated to form a biologically active substance-immobilized gelatinous gel layer covalently fixed on an SPU surface. Upon implantation into the rat abdominal aorta, minimal thrombus formation probably due to a sustained release of heparin and rapid tissue ingrowth was noted. Although the transmural endothelialization accompanied by capillary ingrowth in micropores was enhanced by immobilized bFGF and VEGF, the transanastomatic endothelialization was still predominant[3-6].

Although the second-generation graft appeared to be promising as a compliant, nonbiodegradable small diameter graft, a biomechanical issue that remains to be solved or improved is the design of a more mechano-active scaffold in which the intraluminal pressure-diameter relation resembled that for native arteries in the entire physiological pressure range (60~150 mmHg): large inflation in low-pressure regions, and gradually reduced inflation in physiological pressure regions and little inflation in high-pressure regions are observed, which is termed the "J" curve in the intraluminal pressure-diameter plot (Figure 1). However, a single SPU tube, regardless of microporous foam or film used, as mentioned above, exhibits an "inverse J" curve which is derived from the creep characteristics inherent to synthetic elastomers.

The third approach to the development of a "mechano-active" scaffold was to use a coaxial double tubular graft which is assembled using a high-compliance inner tube and a low-compliance outer tube (Figure 2). By increasing intraluminal hydrodynamic pressure, the inner tube inflates markedly in low-pressure regions, and after the inner tube comes in contact with the outer tube, both tubes inflate together gradually in high-pressure regions. Wall thickness, pore diameter and density (relative area of micropores), which are the principal parameters determining the pressure-dependent diameter change, were adjusted according to the design criteria based on the native arteries. This fabricated coaxial double-tubular graft exhibited a "J" curve mimicking that for target canine carotid arteries. Surface processing aimed at reducing thrombus formation on the luminal surface of the inner tube in the early stage of implantation and preventing tissue-mediated adhesion between the inner and outer tubes and also between the outer tube and the surrounding tissues, was conducted by photochemical grafting of hydrophilic polymers[7]. Upon implantation into canine carotid arteries, the implanted grafts pulsated in response to pulsatile blood flow, and vascular morphogenesis proceeded with implantation period. Tissue adhesion gradually occurred with implantation period, resulting in a steeper J curve with longer implantation period[8]. Therefore, a higher performance tissue-adhesion-preventing hydrophilic material is essentially required for realization of the working principle of a coaxial double tubular graft. Thus, a coaxial double-tubular graft was theoretically realized, but the shortcoming of the current technology mentioned above should be eliminated in the near future.

Tissue-Engineered Grafts: The major issue in vascular tissue engineering is cell source; how to harvest or obtain a sufficient amount of nonthrombogenic luminal lining cells. Although the harvesting of vascular cells from patients' veins has been carried out by Zilla's group in Austria/South Africa, such a tissue-engineered vascular graft has not been routinely performed in clinical situations probably due to difficulties in autologous harvesting. Inspired by Weinberg and Bell's model proposed in the mid-1980's[9], we extensively conducted experimental studies of tissue-

FIGURE 1

Pressure-diameter relationships of native vascular graft, noncompliant graft, compliant elastomeric graft (single tube) and coaxial double tubular graft mimicking native artery's mechanical behaviors.

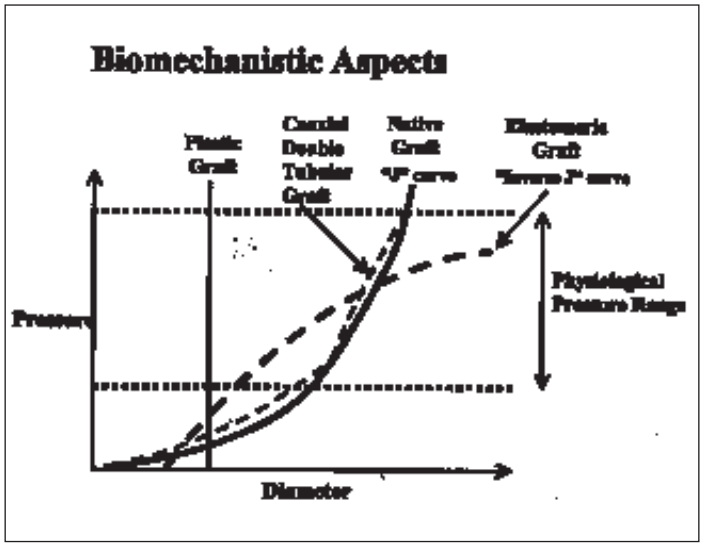
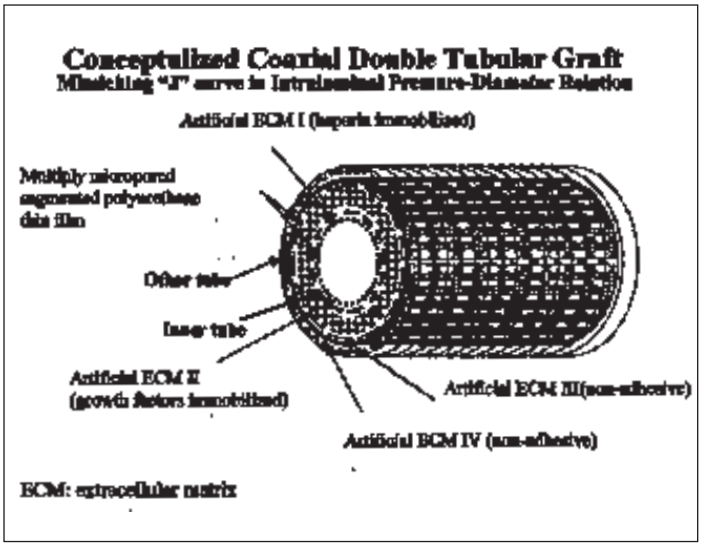


FIGURE 2

Schematics of coaxial double tubular graft under development.



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engineered arterial and venous replacements using autologous vascular cells including endothelial cells (ECs), smooth muscle cells (SMCs) and fibroblasts with laser-micropored SPU tubes as a mechano-active scaffold. The hierarchically structured trilayer model with monolayered ECs as an intimal layer, SMC-innoculated medial layer, and fibroblast-innoculated adventitial layer exhibited the highest regeneration potential, followed by the bilayer model composed of intimal and medial layers[10-14]. The lowest potential was observed in the EC-monolayered one, although all the grafts, regardless of the type of model used, were patent. With implantation period, various morphogenic events were observed, which included the cell sorting or segregation, subtissue-specific localization of each cell type[15], cellular orientation, SMC phenotype reversion from synthetic to contractile, supramolecular assembly of extracellular matrices (ECMs) such as collagen and elastin and neoarterial tissue formation, in which tissue thickening occurred in the early phase of implantation, followed by thinning in the later phase[11,12]. With implantation-period-dependent morphogenic events at ECM, cellular and tissue levels appear to depend on the type of model used, as mentioned above[15-20].

Natural nonthrombogenic ECs are an essential cell component. Havesting them from patients' veins is clinically difficult. As an alternative to ECs, we attempted to use two other cells: endothelial progenitor cells (EPCs), harvested from peripheral blood or bone marrow and then monoclonally cultured, and genetically engineered fibroblasts that transiently express antithrombogenic potentials. The antithrombogenic potentials of EPCs were found to be slightly lower than those of ECs[23,24]. However, the patency rate and neoarterial tissue formation process for EPC-seeded grafts are almost the same as those for EC-seeded grafts. Thus, EPCs are promising alternative to ECs, but the drawback is the low harvest yield. Further improvement of clonal cultures will be needed for the realization of EPC-based engineered grafts in clinical settings. The intraluminal EPC "paving" via stent technology was also explored and its effectiveness was verified[25,26].

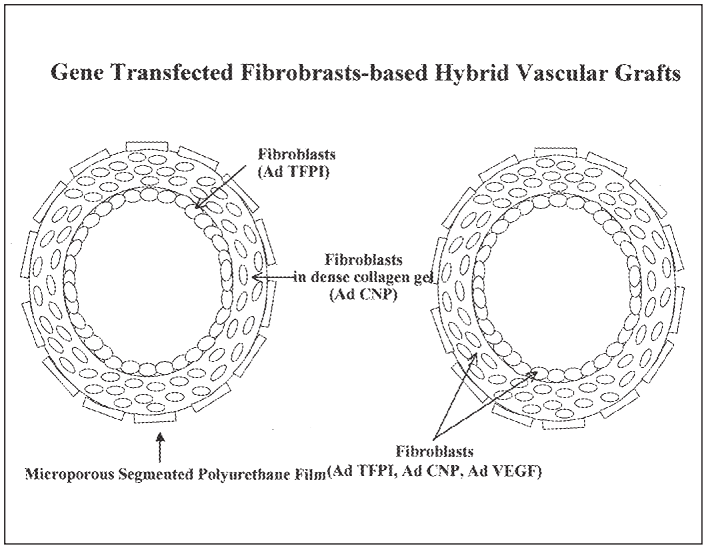
If provisional or transient pseudoendothelial cells that secrete anticoagulant molecules and cytokines that specifically enhance EC recruitment and proliferation are generically engineered, such engineered cells may be used for tissue-engineered grafts (Figure 3). To this end, fibroblasts harvested from skin tissue were transfected by gene-encoding adenovirus to express various bioactive proteins including the tissue factor pathway inhibitor (TFPI) which potently neutralizes the tissue factor (a very potent coagulation enzyme activating the extrinsic pathway of the coagulation system) secreted by fibroblasts, C-type natriuretic peptide (CNP) which is a multipotent tissue modulator, and VEGF[27]. Such three gene-transfacted fibroblast-innoculated collagenous hybrid tissue gel wrapped in a micropored SPU film was implanted into canine carotid arteries for up to three months postoperativively. In contrast to the result that all the nontransfacted fibroblast-innoculated grafts occluded within two days after implantation, the three-gene-transfected fibroblast-based graft exhibited high patency: 100 % at one month, 70 % at three months postoperatively. Thus, fibroblasts that are highly thrombogenic due to TF secretion can be used as transient endothelial-like cells.

In conclusion, the search for small-diameter vascular graft substitute materials, biological modulators and fabrication process technologies for providing extracellular milieu in portions of diseased vessels similar to the natural physiological environment has a long, frustrating history. When an appropriate cell source, a "mechano-active" and "tissue-permeable" scaffold design, and biologically active artificial ECMs are maximally incorporated into the designed small-diameter vascular graft, such a graft may be applicable in clinical settings. Mechanobiological, genetic and progenitor/stem cell engineerings will help in realization of "real" vascular tissue morphogenesis for small-diameter grafts. I envisage that these disciplines and various field of engineering, once combined, should give the ultimate solution for the long-awaited, functionally viable vascular substitute.

To this end, a minimal foreign body reaction on material surface is essential for small diameter grafts. The most difficult task to design "true" biocompatible surface often recalls me the following message from the German Nobelist, Dr. Pauli: The Solid is created by God, but the Surface is made by Devil (Dr. Pauli was awarded with Nobel prize in physics in 1955). My scientific thinking and practical approach is that the complex phenomena is factorized to essential components and then reconstruct them with the simplest way as Hamlet says "Simplicity is the soul of wit" (Shakespeare). However, such attempts are behind Nature's strategy, and my frustrating and endless odyssey still goes on.

FIGURE 3

Cross-sectional gene-transfected fibroblasts-based hybrid grafts: Thrombogenic fibroblasts were gene-transfected to express tissue factor pathway inhibitor (TFPI), multipotent tissue modulator (C-NP) and vascular endothelial growth factor (VEGF).



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POSITION AVAILABLE

POST-DOCTORAL RESEARCH POSITION IN VASCULAR TISSUE ENGINEERING

A post-doctoral research associate is sought for an NIH-funded vascular tissue engineering project. This position will integrate with ongoing research, including studies on the effects of biophysical forces on stem cell differentiation (and subsequent physiology and function of differentiated cells) and on the fabrication and functional assessment of a tissue engineered blood vessel construct. The candidate will interface with cell biologists, molecular biologists, vascular surgeons and bioengineers. The candidate will supervise and perform experiments on cultured cells using parallel plate flow chambers and cyclic strain devices, and on 3D constructs, including tissue-engineered blood vessels. The candidate should have extensive experience in experimental techniques. Desirable skills include sterile technique, surgical dissection, handling of blood vessels, tissue culture, cell culture, immunohistochemistry, protein and molecular biology assays, etc. A background (PhD) in cell/molecular biology, or bioengineering/tissue engineering is preferred. The ability and willingness to write manuscripts and grant proposals is also necessary.

Candidates must already have (or be in the position to easily obtain) authorization to work in the United States. The position will remain open until a suitable candidate is found.

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POSITION AVAILABLE

MOLECULAR BIOLOGIST

The Cardiovascular and Metabolic Diseases Discovery Research Department at Wyeth is seeking a highly qualified and motivated scientist to become a member of a laboratory dedicated to identifying novel molecular mechanisms involved in the development and progression of cardiovascular disease. We are specifically seeking an individual interested in vascular biology with a molecular biology background to perform experiments focused on the interrelationship between diabetes, obesity and atherosclerosis. Target experiments could include the impact of adipose tissue-specific molecules on vascular remodeling as a means to provide insight on the progression of atherosclerosis in obese diabetics. The signal transduction pathways involved in vascular disease progression will also be investigated using appropriate transgenic models. Data generated from these studies will provide a better understanding of the etiology of vascular disease associated with diabetes and obesity, as well!

The position would be for a molecular biologist with experience in cell biology and signal transduction pathways, and the projects could begin immediately. Independent scientific thought, attendance at scientific meetings, and publication of experimental results in peer review journals are encouraged.

Applicants must hold a PhD in an appropriate field, and be eligible to work in the United States. Qualified candidates please send current curriculum vitae to: kornhas@wyeth.com and reference source code "OIAMPS" in the subject line.

Wyeth offers competitive compensation and benefits programs including flex-time, business casual attire and professional development programs.

For more information, visit our website at:
http://www.wyeth.com

POSITION AVAILABLE

RESEARCH POSITIONS IN THE ENGINEERED TISSUE MECHANICS LABORATORY

Department of Bioengineering and the McGowan Institute for Regenerative MedicineUniversity of Pittsburgh

The Engineered Tissue Mechanics Laboratory (ETML) has immediate openings for the following positions:

POST-DOCTORAL:

Numerical simulation of the mechanics of native and engineered valvular tissues. This is a 2-4 year Post-Doctoral research position funded by a new NIH R01 grant. The focus of this position is the development and implementation of computational models of native and engineered soft tissues, based on structural constitutive models. The primary application is simulation of aortic valve tissues under quasi-static and dynamically loaded states. Other potential applications include the urinary bladder, muscle-derived cell-seeded scaffolds for urological repair, engineered tissue design, and cell/tissue mechanical interactions. Candidates with doctoral degrees in Biomechanics, Mechanical Engineering/Applied Mechanics, and related engineering disciplines with strong computational skills are encouraged to apply. Salary level will be commensurate with previous experience.

Micromechanics of engineered cardiac muscle. This is a 2-4 year Post-Doctoral research position to head a Biomechanics Core of a Bioengineering Research Partnership grant on Cardiopulmonary Organ Engineering funded to the McGowan Center for Regenerative Medicine (Dr. William Wagner, PI.). The focus of this position is the investigation of the effects of mechanical conditioning on the micromechanics of engineered cardiac muscle and functional comparisons to native myocardium under multi-axial stress states. Candidates with doctoral degrees in Biomechanics, Mechanical Engineering/Applied Mechanics, and related engineering disciplines with strong experimental and experimental device development skills, with an interest in and experience with soft tissues are encouraged to apply. Salary level will be commensurate with previous experience.

GRADUATE RESEARCH ASSISTANTS:

Native and engineered valvular tissues and related areas. We have three NIH R01 funded doctoral-level student research positions involving the study of 1) the micromechanics of native and engineered valvular tissues, 2) valve tissue growth using engineered valve tissue bioreactor , 3) 3D geometric characterization of valve-related cardiac structures. These positions are part of our ongoing efforts in aortic valve mechanics and multi-scale modeling. Students with Master degrees in Biomedical engineering (biomechanics), Mechanical Engineering/ Applied Mechanics, and related engineering disciplines are particularly encouraged to apply.

If interested, please contact:
Dr. Michael S. Sacks
Website: http://www.pitt.edu/~msacks/etml.html
msacks@pitt.edu
McGowan Institute for Regenerative Medicine
100 Technology Drive, Room 234
University of Pittsburgh
Pittsburgh, PA 15219
Tel: 412-235-5146, Fax: 412-235-5160

POSITION AVAILABLE

POSTDOCTORAL POSITION

POSTDOCTORAL POSITION is available in a vascular biology laboratory at the University of Arizona to study the biological mechanisms regulating vessel differentiation and vascular network maturation following angiogenesis. The laboratory employs a multidisciplinary approach that includes genomic, molecular/cellular, physiological and imaging techniques. The successful applicant should have proven experience in an area related to vascular biology. Familiarity with mouse models is preferred.

To apply, forward a CV and names of three references to: James B. Hoying, Ph.D.; University of Arizona, PO Box 245084, Tucson, AZ 85724; jhoying@u.arizona.edu.

The University of Arizona is an Affirmative Action/Equal Opportunity/ ADA Employer.

POSITION AVAILABLE

RESEARCH SCIENTIST OPENINGS

I have two research scientist openings for recent (0-4 years) Ph.D. recipients with experience in tissue engineering to participate in tissue engineering projects on cardiac myocytes and vascular grafts. The work will be performed at the Charleston Research Center of Organ Recovery Systems in Charleston, SC. Background information on the company can be obtained at www.organ-recovery.com and CVs of interested candidates can be submitted to the attention of Dr. Brockbank, Chief Science Officer, by e-mail (kkbrockbank@organ-recovery.com).

Kelvin GM Brockbank, Ph.D.
Chief Science Officer,
Organ Recovery Systems

ISACB Membership Application

Name .....

Mailing Address .....

Phone number.....E-mail .....

Fax number.....Citizenship .....

Signature of applicant .....Date .....

Please submit a current curriculum vita and abbreviated bibliography.
Your curriculum vita should include the following information:

- Educational background, including postdoctoral/ fellowship research experiences
- Current hospital and/ or academic appointment
- Professional societies
- Awards and honors
- Statement describing your areas of research interest

Send all of the above information to:

Steven Schmidt, Ph.D.
Falor Division of Surgical Research
Summa Health System
525 East Market Street
Akron, Ohio 44304, USA
Phone: 1 (330) 375-3693
Fax: 1 (330) 375-4648
www.isacb.org
E-Mail: schmidts@summa-health.org