FALL/WINTER 2002

ISACB CIRCULATOR

INTERNATIONAL SOCIETY FOR APPLIED CARDIOVASCULAR BIOLOGY

MARK YOUR CALENDAR NOW!

ISACB'S IXTH BIENNIAL MEETING

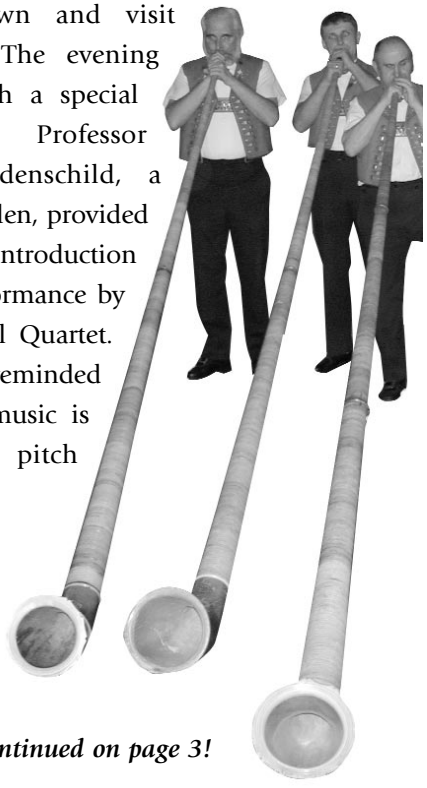
SAVANNAH GEORGIA, USA MARCH 10-14 2004

SCIENCE & LEISURE EVIDENT AT THE 8TH BIENNIAL MEETING IN ST. GALLEN

Alpine foothills, chocolate, Alpenbitters, Alphorn players, Nauer Yodel, Appenzellerland, Appenzell Cheese, Romantik Hotel Säntis, a wealth of hospitality...these are just some of the memories that participants took with them from the 8th Biennial Meeting in St. Gallen, Switzerland in March.

The success of the 8th Biennial Meeting was not only due to the unique surroundings and meeting facilities offered by the Abbey of St. Gall, a UNESCO global heritage site, but also to the excellent scientific program and poster session which highlighted a spirited exchange of ideas and information. The ISACB meeting kicked off with an excellent International Workshop on Tissue Engineering hosted by Jeff Hubbell, Ph.D., from the Institute for Biomedical Engineering in Zurich. The meeting continued with scientific presentations by a distinguished group of researchers in Tissue Engineering. Participants also had a wonderful opportunity to participate in the interactive poster session. Jeff Hubbell and his team from ETH in Zurich provided the "local flavor" and ensured that everyone experienced the best that St. Gallen had to offer.

Moving to charming Appenzell on Friday evening, members and guests were greeted by Alphorn players, (real musicians, forget the Ricola commercials) at the beautiful Hotel Säntis. From there everyone got to sample flavorful Appenzell Bitters, meander around the lovely town and visit the Museum. The evening culminated with a special treat when Professor Christian Haudenschild, a native of St. Gallen, provided an informative introduction to a lively performance by the Nauer Yodel Quartet. Everyone was reminded that beautiful music is just a perfect pitch away.



IN THIS EDITION

Continued on page 3!

ISACB Circulator is published as an information service for the members of the International Society for Applied Cardiovascular Biology.
Address correspondence to:
Steven Schmidt, Ph.D.
ISACB Circulator
Falor Division of Surgical Research
Summa Health System
525 East Market Street
Akron, Ohio 44304, U.S.A.
Phone: 1-330-375-3695
Fax: 1-330-375-4648
www.isacb.org
schmidts@summa-health.org

ISACB Circulator Editor: Peter Zilla, M.D., Ph.D.

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.

letter from the pres. here

VIIIth Biennial Meeting of ISACB

ISACB 2002
ST. GALLEN, SWITZERLAND
FEBRUARY 27 – MARCH 2

St. Gallen, Switzerland

The ISACB will continue its tradition of biennial meeting in Savannah, Georgia, March 10-14, 2004. We look forward to seeing you there.

ISACB'S BIENNIAL MEETING • SAVANNAH, GEORGIA, USA • MARCH 10-14, 2004

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.

Does Success Stifle Innovation?

Ivan Vesely, Ph.D.
Department of Biomedical Engineering
The Cleveland Clinic Foundation
Cleveland, Ohio, USA 44195

There is a thesis that I have been thinking about for a number of years, and it goes something like this:

If we could replace the entire vasculature of a patient with synthetic grafts that would last for 15 to 20 years, would there be any vascular biology research?

I feel that this has been the case for my field of artificial heart valves – we now have valves that can last a lifetime, so no one is interested in the mechanisms of heart valve disease. Indeed, besides my lab and those of Fred Schoen and Alain Carpentier, I cannot think of any other concerted effort to investigate the etiology of myxomatous mitral valve prolapse, one of the key reasons for mitral valve repair surgery.

For the aortic valve, the situation is even more bleak. I cannot think of a single person working on solving the mechanism of calcific aortic stenosis. Indeed, after a key word search on Medline, I found that during the past 40 years, there were only 2600 publications on the topic of heart valve disease, and most of those reported on the clinical experience with prostheses. Contrast that with the 1.1 million publications on cardiovascular disease over the same time frame. This represents an amount 400 times greater than that for heart valves. It can be argued, of course, that vascular disease represents a far larger case load in hospitals and a greater prevalence in the general population than heart valve disease, hence warranting the greater interest in solving the disease.

Disease Statistics

A recent survey of the 2002 Heart Stroke Statistics update, available from the American Heart Association web site, reports that 1 million Americans die from Cardiovascular Disease (CVD) each year, compared to 500,000 from cancer, 100,000 from accidents and 15,000 from HIV. Interestingly, the U.S. death rate from CVD is not nearly the highest in the world. The Russian republics have a death rate of 1167 per 100,000 per year. The lowest is Japan with 186 deaths per 100,000, and the U.S. is roughly in the middle of the rankings, at 360 deaths per 100,000. Treatment, or lack thereof, surely contributes to the death rate rankings. Prevalence also contributes, but statistics for that outside the US are more difficult to find. In the U.S., coronary heart disease has a prevalence of 6.9% in men and 5.4% in women aged 20 years or older.

By comparison, the total mortality resulting directly from heart valve disease was 19,612 in 1999, a factor 50 times lower than that for CVD. This is not because the prevalence of heart valve disease is 50 times lower than that for CVD. Indeed, the prevalence of valvular disease is 1 to 2% of the population, or about a quarter of all CVDs. This means that there are only 3 times more non-valvular CVDs affecting patients than there are valvular diseases, yet they produce a death rate that is 50 times greater. Clearly, we have made major progress towards relieving death from valvular disease, but not from other cardiovascular diseases. This indeed may explain why there are 400 times more publications on vascular disease than on valvular disease.

Have we solved heart valve disease?

Before we congratulate ourselves for having a mortality 50 times lower than that for other CVDs, we need to recognize what remains – mortality may be low, but morbidity is, in my opinion, still intolerable. Since mechanical prosthetic valves induce blood clotting, patients who receive these valves need to be on chronic anticoagulant therapy. Chronic anticoagulation is associated with cumulative morbidity and mortality, often as high as 4% per year [1, 2]. This essentially guarantees some serious adverse event within a 25-year implantation period. Indeed, long-term studies have shown that the incidence of thromboembolism alone is 41% at 34 years [3]. The primary disadvantage of bioprosthetic valves, on the other hand, is their relatively poor long-term durability – they eventually need to be replaced. While the mortality for the first valve replacement surgery is lower than 1%, the reoperation to replace a failed bioprosthetic valve is much more risky (7% - 20% mortality) [4-6]. Because of considerable adhesions within the thorax resulting from the first operation, opening up the chest to expose the heart and establish cardiopulmonary bypass risks tearing of critical tissues, vessels and even the heart itself. Artificial heart valves are therefore meant to be inserted only once, and are expected to last the life of the patient. Because they last only 10 to 12 years [1, 3, 7, 8], they are typically used in the elderly, who are not expected to outlive their bioprosthetic valve. Younger patients therefore need to live with a mechanical valve and put up with the morbidity associated with anticoagulation therapy.

Has innovation been stifled?

I believe it has. A very nice poster I received from Sulzer Carbomedics several years ago shows photos of 30 mechanical valves developed between 1953 and 1994. The Edwards Lifesciences museum has over 200 **different** valve designs (or variants) that it has collected over the years. This is not atypical of any industry that aims to improve its products. But has anything happened recently? Remember the Medtronic Intact and Mosaic - those were innovative valves! Well, the Intact has been around since 1984 [9] and the Mosaic has been in clinical trials since 1994 [10]. This means that the Mosaic, what I consider the latest-generation valve, was developed essentially 10 years ago! And what have we had since? There was the St. Jude Silzone fiasco and the venture into tissue engineering by CryoLife when it received CE mark approval in October 16, 2000. That is essentially all that we have had in tissue valves. In mechanical valves, we have the MCRI On-X and the Tri-Flow. The On-X has taken an evolutionary approach to mechanical valves, optimizing the flow characteristics; and the Tri-Flow is an innovative, three-leaflet carbon valve. Both of these valves, however, have been in the news for almost 10 years. It would seem that in the first 20 years of the industry, we've had well over a 100 new valve types, whereas in the last 20 years, we've had fewer than a dozen.

Why the lack of interest in innovation?

First of all, we must give credit for most of the devices that we are familiar with to the valve manufacturers. By far, most of the realizable advances in bioprosthetic valve technologies have come from industry. The valve industry, however, is operating under relatively difficult market conditions. Because of competitive and regulatory pressures, new products are introduced into the market only if the risk of failure can be minimized. Many of the speculative anticalcification approaches, published routinely in the biomaterials literature, have never been implemented in a commercial product. Because the prosthetic valve industry is so mature, valve manufacturers have carved out their respective market share by selling proven designs with very predictable performance characteristics. Most manufacturers have been reluctant to introduce new valves to the U.S. market because of uncertainty about their long-term durability. Over the years, valve manufacturers have introduced different stents, different anticalcification pretreatments, but all of these changes have been "safe" and evolutionary. In the only recent example of a "revolutionary" change, Sulzer Carbomedics introduced a completely new method of chemical stabilization, which unfortunately failed in clinical trials. Carbomedics has suffered considerably as a result of this incident. The Carbomedics Photofix-a pericardial valve was crosslinked, not with conventional glutaraldehyde, but with a dye-catalyzed photooxidization process [11]. This approach appeared to work very well in vitro and in animal trials, but failed during clinical trials due to unusual abrasions that were not detected during pre-clinical testing. Although it happened over 10 years ago, this

failure has left Carbomedics struggling to find new products and establish a foothold in a very competitive marketplace. Valve manufacturers have therefore become very wary of introducing a potentially less durable valve on the market, and innovation has been very slow.

Is this typical of other areas of technology?

The above analysis therefore begs the question – "Is this type of stagnation typical of other areas of technology?" This is certainly not the case with the computer industry or the automotive world. Both have been extremely successful over the past decade, and both are racing ahead to develop better and better technologies. For example, PCs and workstation computers that used to run at 16 MHz in 1992 are now running at nearly 4 GHz. I remember buying my first research computer for $6000 in 1988 – it was a 4 MHz '286 with 1 Mbyte of memory. My latest acquisition (which, by the way, will be totally obsolete by the time you read this) is a 2.4 GHz machine with 1 Gbyte of memory and two flat-screen monitors, and it cost me less than $4,000. If we assume another nearly 3 orders of magnitude increase in the next 10 years, we should see computers running at up to 1 Terahertz. We will be able to do complex computational simulations - like fully coupled solid-fluid interactions of valves opening and closing in real time - on our desk top.

In the automotive world, manufacturers have incorporated racing engine technologies into regular sedans (my bottom-of-the-line Audi has 5 valves per cylinder – a technology originated in Formula 1 motorcycle racing), which has produced huge increases in horsepower and minimal emissions. Cars have heated and air-conditioned seats, satellite navigation technologies, self-inflating tires, and completely electronic linkages between the driver and the throttle. This, for example, allows the car to learn the "aggressiveness" of the driver and adjust throttle response accordingly. Indeed, electronics have replaced most, if not all, mechanical systems in automobiles, and we are on the cusp of having electronic-only linkages between the brake pedal and steering wheel, and the wheels themselves. In

continued on page 6

1984, the state-of-the-art luxury sedan was just beginning to have catalytic converters and was getting the worst gas mileage of the last century. In 1984, the original Apple Macintosh commercial was aired during the Super Bowl. The same year Medtronic introduced the Intact valve into clinicals. Look at what has happened since in these three market segments.

The stagnation in the valve industry, however, is not unique. The same can be said of the total artificial hip joint. In the early days of hip joint surgery, a wide range of techniques were tried, ranging from partial femoral head replacement to porous ingrowth devices. The state-of-the-art in this field is the 40-year-old cemented total hip joint, which lasts 15 years or more. Individuals with these joints can resume many of their normal activities, but they cannot play football or go skiing. A total hip joint prosthesis for a 40-year-old is not something to be taken lightly, since revision surgery at age 60 carries considerable operative risk. Heart valves are not much different. Having a mechanical valve at 40 means you shouldn't engage in activities that risk injury, and you should expect the valve implant to give you a stroke before you turn 80. Having a prosthetic valve is no different from having a metal hip, in the way it affects your life – you know it is there, every minute of your life.

What about basic science?

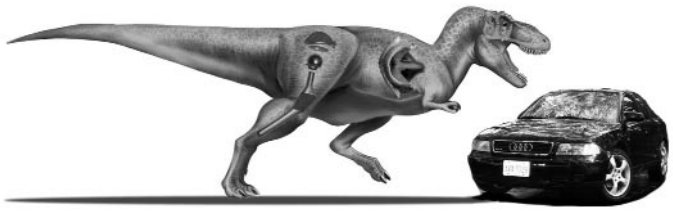
While the business-related arguments described above may characterize the nature of the valve industry, they do not explain the lack of research on the basic mechanisms of heart valve disease. While I have argued above that the justification for a lack of research is the relative success of the prosthetic valve, clearly we have a long way to go. Personally, I don't see many breakthroughs on the horizon for the heart valve world, and I am particularly skeptical of tissue engineering as a vehicle for "curing" a heart valve patient. Just look at what happened in the treatment of coronary artery disease. First we had balloons; that didn't work, then we had stents which led to in-stent restenosis - now we have drugs and radiation to treat the disease that we have created by putting the stent in the vessel in the first place!

Advances in the treatment of CVD patients have clearly come from understanding the biology of the system and the etiology of disease. We have made great advances in helping people by realizing that one of the major risk factors for coronary artery disease is abnormal cholesterol metabolism. Changes in lifestyle, as well as lipid-lowering drugs, have saved many lives. The same needs to be done in the field of heart valve disease! We need to know what causes heart valve disease, both aortic and mitral; who is at risk; how do we manage that risk; and ultimately how do we intervene in the patients' lives before they need to have their valves cut out and replaced with 1980s technology.

Maybe the true benefit of the tissue-engineered "revolution" is that researchers will finally look at the native tissue and the native cells and begin to study the system in the way that vascular biologists have studied the coronary arteries. Maybe the therapy of the future will not be the replacement of diseased valves with tissue-engineered prostheses, but rather the treatment of valvular disease with cells and molecules to slow down and potentially even reverse the course of the disease itself, thus avoiding a prosthesis altogether.

By that time, however, I hope to be dictating my next research proposal to the computer embedded in my shirt sleeve, as my hydrogen-powered automobile drives me to work.

Acknowledgements: The author would like to aknowledge the help of Drs. T. Doehring, J. Grande-Allen and A. Ramamurthi for their valuable input, as well as that of Ms. Kassuba for help in compiling the publication statistics and in editing this essay.



References:

1. Hammermeister, K.E., et al., *A comparison of outcomes in men 11 years after heart-valve replacement with a mechanical valve or bioprosthesis. Veterans Affairs Cooperative Study on Valvular Heart Disease.* New England Journal of Medicine, 1993. **328**(18): p. 1289-96.
2. Jamieson, W.R., et al., *Multiple mechanical valve replacement surgery comparison of St. Jude Medical and CarboMedics prostheses.* Eur J Cardiothorac Surg, 1998. **13**(2): p. 151-9.
3. Grunkemeier, G.L. and E. Bodnar, *Comparative assessment of bioprosthesis durability in the aortic position.* J Heart Valve Dis, 1995. **4**(1): p. 49-55.
4. Otaki, M. and N. Kitamura, *Reoperations on prosthetic heart valves: An analysis of outcome. Artificial Organs*, 1993. **17**(9): p. 791-6.
5. Biglioli, P., et al., *Reoperative cardiac valve surgery: A multivariable analysis of risk factors.* Cardiovasc Surg, 1994. **2**(2): p. 216-22.
6. McGrath, L.B., et al., *Perioperative events in patients with failed mechanical and bioprosthetic valves.* Ann Thorac Surg, 1995. **60**(2 Suppl): p. S475-8.
7. Bernal, J.M., et al., *Valve-related complications with the Hancock I porcine bioprosthesis. A twelve- to fourteen-year follow-up study.* Journal of Thoracic & Cardiovascular Surgery, 1991. **101**(5): p. 871-80.
8. Burdon, T.A., et al., *Durability of porcine valves at fifteen years in a representative North American patient population.* Journal of Thoracic & Cardiovascular Surgery, 1992. **103**(2): p. 238-51; discussion 251-2.
9. Corbineau, H., et al., *Medtronic intact porcine bioprosthesis in the aortic position: 13-year results. J Heart Valve Dis,* 2002. **11**(4): p. 537-41; discussion 541-2.
10. Eichinger, W.B., et al., *European experience with the Mosaic bioprosthesis.* J Thorac Cardiovasc Surg, 2002. **124**(2): p. 333-9.
11. Schoen, F.J., *Pathologic findings in explanted clinical bioprosthetic valves fabricated from photooxidized bovine pericardium.* J Heart Valve Dis, 1998. **7**(2): p. 174-9.

ISACB ESSAYS

Electrospinning of Collagen: Finally the "Ideal" Tissue Engineering Scaffolds?

Gary L. Bowlin, Ph.D.
Associate Professor, Biomedical Engineering
Virginia Commonwealth University
Richmond, VA 23298-0694

The overall goal of tissue engineering is to apply the foundations and innovations of biology, medicine, and engineering to develop and manipulate viable, three-dimensional physiologic substitutes that are capable of reinstating, sustaining, or recovering the function of tissues and organs. Tissue engineers historically have used some formulation of cells, scaffolds, and chemical and/or mechanical conditioning in attempts to fabricate the desired product. Since its inception, the field of tissue engineering has been pursuing the holy grail of an "ideal" scaffold in terms of production method, composition, and performance. Scaffold selection with regard to biomaterial choice and structure is an essential instrument and template utilized by a tissue engineer. It is the author's opinion that this "ideal" scaffold for any product will not be generic, fabricated utilizing a synthetic, biodegradable polymer and traditional production methods. Thus, the challenge is upon us to develop the materials and processing technique needed to create the next generation of advanced tissue engineering scaffolds. This advanced scaffold will allow cells to be placed in a three-dimensional environment containing the necessary molecular cues in a physiologic temporal and spatial manner to allow tissue/organ regeneration. In addition, this must be accomplished in a manner that is reproducible, economical, and capable of a full-scale production level [1].

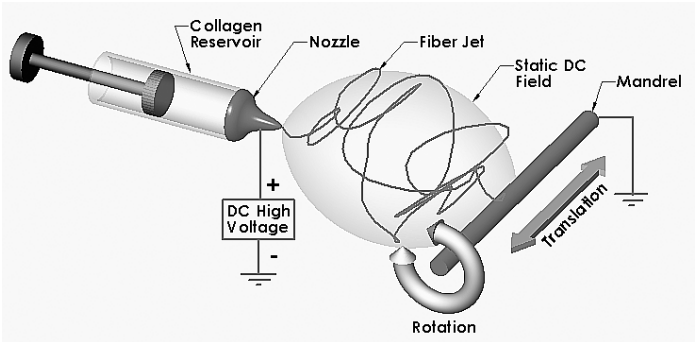
What is the "ideal" scaffold? One word comes to mind as a tissue engineer when this question is raised: biomimicking. To create a truly biomimicking scaffold, one must be able to reproduce in vitro the complexity of the physiologic extracellular matrix in terms of structure, composition, and function for each specific tissue or organ. Replication of Mother Nature, then, is the key to creation of the "ideal" tissue engineering scaffolding.

In the field of tissue engineering, the general consensus is that the "ideal" biomaterial for use in fabricating scaffolds is collagen. Types I, II, and III collagens are the most abundant component of physiological scaffolds throughout the interstitial spaces. These scaffolds function to impart overall structural integrity and strength to tissues and organs. The collagen structure is extremely important also in that it provides the cells with the appropriate physiological environment for embryologic development, organogenesis, cell growth, and wound repair.

Collagen has been utilized in the field of tissue engineering since its inception in the form of gels, fibers, decellularized extracellular matrices, and lyophilized, porous structures. Some additional characteristics of collagen that have made it an extremely attractive scaffold material are high tensile strength, low antigenicity, biodegradability, biocompatibility, enhanced ability for tissue regeneration when compared to synthetic polymers, and the ability to control the mechanical properties and degradation rates by the method and degree of crosslinking [2].

Until recently, there was no technique available for processing of raw collagen into truly biomimicking scaffolds. This has now changed with the pioneering work of the author and his collaborators and the introduction of collagen electrospinning to the field of tissue engineering [3].

Historically, the technique of electrospinning has been utilized to process a variety of synthetic polymers for many applications. In this process, a high energy electric field is the driving force for production of fine fibers that are at least one to two orders of magnitude smaller than traditional techniques that employ mechanical forces to process fibers. A basic electrospinning system consists of a charge-injected collagen solution that is delivered through a small nozzle or orifice opposite a grounded target or mandrel that is some distance away. The charge-injected collagen solution is drawn toward the grounded mandrel as a liquid jet when the applied electric field strength overcomes the surface tension of the fluid. During the liquid jet travel, the solvent gradually evaporates, and a collagen fiber is formed and collected on the target. Over the processing period of several minutes, the resulting product collected on the grounded target is a non-woven fibrous mat that is composed of fibers with diameters on the order of nanometers to microns. This fibrous mat can be created on mandrels of various geometries to serve as tissue engineering scaffolds for any implant site.



continued on page 8

To date, the electrospinning of collagen has been completed with type I (rat tail, bovine, and human placenta), type II (chicken sternal cartilage), and type III (human placenta). In all the pioneering work, the collagens were electrospun from the solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) at concentrations varying from 0.03 to 0.16 g/ml to produce fiber diameters ranging from 80 nanometers to 5 microns. The electrospinning process and scaffold production parameters are presented in a recent manuscript [3].

As an example, after electrospinning collagen type I, the scaffolds produced were characterized by scanning (SEM) and transmission (TEM) electron microscopy. In the case of electrospinning from a 30 mg/ml solution, the scaffolds produced were composed of 100 nm diameter collagen fibers with a uniform size distribution. It is important to note that this value falls in the physiologic range for collagen type I fibers. The TEM evaluation demonstrated that the fibers also exhibited the 67 nm banding pattern that is characteristic of the native polymerized collagen. Evidence of this banding pattern in addition to the very fine fiber diameter implies the creation of a truly biomimetic fiber and potentially the "ideal" tissue engineering scaffold. Similar results have been obtained with collagen types II and III.

Polymer concentration is a key variable in the electrospinning process [4]. For example, collagen type I electrospun from HFP at concentrations ranging from 30 to 100 mg/ml with all other parameters held constant produced scaffolds composed of fibers with diameters that ranged from 100 nm to 5 microns, respectively, with a linear relationship between collagen concentration and scaffold fiber diameter. Thus, varying concentration allows one to have dramatic flexibility in the fabrication of collagen scaffolds composed of a range of fiber diameters.

In a more elaborate electrospinning system, one has control over fiber orientation within the scaffold. This is an important factor necessary to induce proper interaction with the cellular environment and provide adequate strength and function to the engineered tissue. During scaffold electrospinning, this level of control is achieved with rotational and translational motion of the mandrel [3]. This now provides the opportunity to manufacture any structure required to mimic the extracellular matrix of the tissue of interest.

There are many other attractive aspects of electrospinning as a process for fabricating tissue engineering scaffolds. First, the thickness of the scaffolds and individual scaffold layers, of which the composition can be tailored as well, can be controlled. This is accomplished by simply adjusting the electrospinning time to result in a scaffold thickness ranging from a single fiber diameter to several millimeters. Second, the mandrels can be virtually any geometry from simple tubes to complex, sculptured substrates. Another important aspect is that the scaffolds are three-dimensional and seamless which will decrease the potential for mechanical failure. Lastly, the scaffolds can be tailored to a specific site and application by creation of blended fibers composed of multiple materials such as collagen types I and III or by co-electrospinning with various additives such as other matrix proteins (i.e., elastin) and/or growth factors.

The inclusion of various additives is an aspect that leads one to theorize that electrospinning is the optimal process for the creation of the "ideal" tissue engineering scaffold. We must not forget that the extracellular matrix not only functions as a structural entity but also contains specific binding sites for cell receptors and serves as a sequestration site for a variety of molecules important to cell/tissue/organ function and viability under a dynamic equilibrium. It is thought that collagen must be in a physiologic state, which includes collagen molecule stacking (represented by the 67 nm banding) and specific overall fiber diameters (typical fiber diameters range from 50 - 150 nm), to present many of these binding sites to the cells. Electrospinning, to date, meets these requirements which are considered necessary of an "ideal" or truly biomimicking scaffolding.

In summary, electrospinning provides a multitude of possibilities for the construction of scaffolds of various shapes and sizes with precise control over fiber dimensions, composition, and orientation. Thus, this process has great potential for the creation of "ideal" scaffolds that replicate the fibrous architecture of the extracellular matrix found in native tissue to achieve the regeneration of any tissue of interest by the use of a specific collagen or blend of components. The long-awaited and much anticipated construction of a truly biomimicking tissue engineering environment for every tissue and organ using the process of electrospinning is now feasible at a reproducible, economical, and full-scale production level.

Now that a technology that has the potential to revolutionize the field of tissue engineering has been developed, the author's laboratory along with his collaborators are now embarking on the massive endeavor to determine if electrospinning of collagen and other components is indeed the "ideal" scaffold for all the various tissues and organs. At this early stage, only time and Mother Nature's acceptance and maintenance of the products will determine if electrospinning of collagen leads to the "ideal" scaffolds.

The author would like to thank the Whitaker Foundation (RG-98-0465) for financial support of the research on electrospinning of collagen. It should also be noted that the technology portfolio representing information presented in this essay is patent pending, U.S. and International, and has been licensed to NanoMatrix, Inc., of which the author has a financial interest.

References:

1. Hutmacher, D.W. "Scaffold Design and Fabrication Technologies for Engineering Tissues-State of the Art and Future Perspectives." *J. Biomater. Sci. Polymer Edn.* 12 (1): 107-24, 2001.
2. Miyata, T., Taira, T., and Y. Noishiki. "Collagen Engineering for Biomaterial Use." *Clinical Materials* 9: 139-48, 1992.
3. Matthews, J.A., Simpson, D.G., Wnek, G.E., and G.L. Bowlin. "Electrospinning of Collagen Nanofibers." *Biomacromolecules* 3 (2): 232-238, 2002.
4. Boland, E.D., Wnek, G.E., Simpson, D.G., Pawlowski, K.J., and G.L. Bowlin. "Tailoring Tissue Engineering Scaffolds Using Electrostatic Processing Techniques: A Study of Poly(Glycolic Acid)." *J. Macromol. Sci.* 38: 1231-43, 2001.
5. Nimni, M. and R. Harkness. "Molecular Structures and Functions of Collagen." In Collagen Volume I: Biochemistry. M.E. Nimni, Ed., CRC Press, 1-78, 1988.

I S A C B
ESSAYS

A Natural Bioreactor to Produce Artifical Arteries From Cells of the Bone Marrow

Julie H. Campbell and Gordon R. Campbell,
Centre for Research in Vascular Biology,
University of Queensland, QLD 4072
Australia

In patients with coronary heart disease a common surgical treatment is to bypass regions of artery blocked or narrowed by plaque with mammary artery or saphenous vein from the patient him/herself (autografts). However, the supply of mammary artery may not be sufficient for multiple bypass or repeat procedures, and saphenous vein in elderly patients often has varicose degenerative alterations that lead to atheroma or aneurysm when transplanted into high pressure arterial sites. In the peripheral vasculature, supply of undiseased autologous vessels as bypass, replacement or access vessels is even more problematic. In particular, lack of healthy saphenous vein as arteriovenous access fistulae for haemodialysis is a major cause of morbidity and cost for patients with end stage renal failure. While synthetic vascular prostheses such as Dacron (fabric grafts) and expanded polytetrafluoroethylene (ePTFE) perform reasonably satisfactorily in high flow low resistance conditions such as the large peripheral arteries, they are not suitable for small caliber arterial reconstructions (eg. coronary circulation) where they are associated with graft thrombogenicity, poor healing, lack of compliance and excessive intimal hyperplasia. It is therefore desirable to develop a low-cost artificial blood vessel with no biocompatibility problems in order to overcome these problems.

The ideal characteristics of an 'artificial artery' are as follows:

1) It must be compatible with the blood flowing through it ie. non-thrombogenic and non-immunogenic. Both of these characteristics are normally provided within an artery by an intact endothelium, which in addition acts as a selective permeability barrier and a metabolic-secretory tissue.
2) It must possess appropriate mechanical properties - strength and compliance. The vessel must be strong enough to withstand considerable internal pressure before bursting and allow sutures to hold under longitudinal/circumferential tension but still retain compliance.
3) It should possess appropriate physiological properties - the media to constrict/relax in response to neural or chemical stimuli.
4) It should be able to be manufactured in a relatively short space of time, cheaply and in sufficient numbers with differing specifications (diameter, length etc) to meet commercial demand.

Vascular cells cannot remodel ePTFE or Dacron (in the same way as they remodel normal extracellular matrix components such as collagen and elastin, hence the lack of success of these substances as substitutes for small diameter arteries. Consequently, recent attempts to produce an 'artificial artery' involve growing vascular cells in culture to produce the structural (extracellular) components of the artificial vessel matrix before grafting, or encouraging cells (from the host or transplant) to migrate into a scaffold in situ. In both cases the aim is to remodel the starting structure to create a biocompatible tissue analog. The three main approaches using the natural remodelling paradigm are:

1) Grafting into arteries of acellular tubes of extracellular matrix (such as collagen) that become colonized by host cells [1].
2) Cell-seeded polymeric biodegradable scaffolds in which constructs of biodegradable mesh are seeded *in vitro* with vascular smooth muscle and cultured for several weeks under pulsatile radial stresses in a bioreactor [2].
3) The 'cell self-assembly model' in which intact layers of human vascular cells are grown in culture to over-confluence to form sheets of cells and extracellular matrix. These sheets are then rolled over a mandril to form a hollow tube [3].

To date, none of these approaches has resulted in an appropriate artery media structure with circumferentially aligned smooth muscle cells between elastic lamellae in what has been described by Wolinsky and Glagov [4] as a "lamellar unit" - the functional unit of the artery. Also, a major difficulty with any organ developed in an ex vivo culture situation is that with time the cells alter their phenotype as well as their immunogenic properties.

The approach we have taken to develop an 'artificial artery' is to adapt the body's natural wound healing response to produce a hollow tube [5]. When this tube is autologously grafted into an artery the cells respond to the local environmental cues and produce structures similar to those of the native vessel. We begin by inserting a length (up to 250mm long and 1-7 mm in diameter) of biocompatible tubing into the peritoneal cavity of dogs, rabbits, rats or mice. This induces the formation of a granulation tissue capsule around the tubing over 2-3 weeks [6,7]. The capsule consists of myofibroblasts and the collagen matrix they have produced covered by a single layer of mesothelial cells. The mesothelial cells are derived from the lining of the peritoneal cavity and possess anti-thrombotic properties similar to endothelial cells. The capsule can be removed from the tubing mould and everted by cutting one end then pulling it back over itself such that the mesothelium now lines the lumen. The resulting tube of living tissue (minus

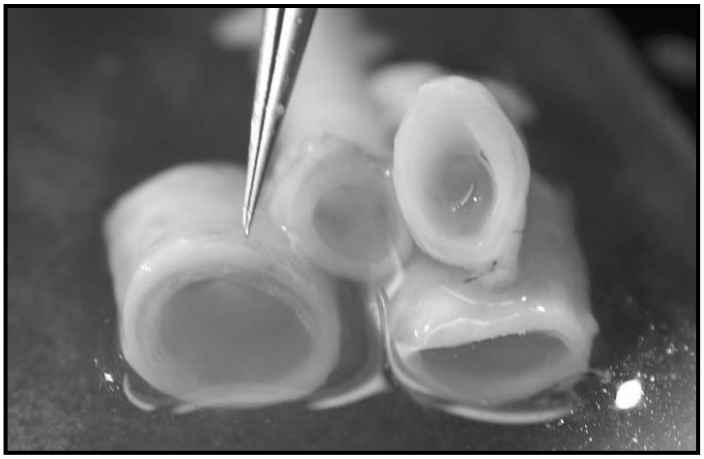
continued on page 10

tubing) now resembles a blood vessel with an inner lining of mesothelial cells (the 'intima'), and a 'media' consisting of multiple layers of tightly packed myofibroblasts (smooth muscle-like cells) and collagen matrix. For dog tissue the bursting strength of the tube of living tissue is in excess of 2500mmHg and suture holding strength is 11.5 Newtons.

Lengths of the tube of living tissue have been successfully grafted by end to end anastomoses into the carotid artery (rabbit), abdominal aorta (rat) [6, 7] or femoral artery (dog) [8] of the animal in whose peritoneal cavity it was grown, replacing segments of excised natural artery. To date, patency (normal blood flow) through the artificial blood vessel has been demonstrated by Doppler ultrasound for up to 16 months in rabbits and 6.5 months in dogs before the animals were sacrificed for histological examination. By 3-4 months the artificial arteries in the high pressure sites have doubled in thickness and an 'adventitia' containing vasa vasorum has developed on their outer surface. Elastic fibres have formed within the 'media' and the myofibroblasts have differentiated further into smooth muscle-like cells with a volume fraction of myofilaments not significantly different from cells of the nearby artery. Stretch studies *in vitro* demonstrated that this remarkable differentiation is due, at least partly, to the intermittent stretching of the wall from the pulsatile flow of blood [9]. Functionally, by 6 weeks the artificial blood vessels respond to vasoconstrictor agents and undergo endothelium-dependent relaxation in response to acetylcholine in organ bath studies indicating that humoral agents can regulate their luminal diameters in the same way as natural vessels.

While the source of the 'endothelium' (mesothelial cells) in the artificial arteries was clear, the origin of the granulation tissue myofibroblasts that subsequently become 'smooth muscle-like' cells was not readily apparent. To address this question we inserted foreign bodies into the peritoneal cavity of rats, rabbits and mice then harvested the capsule at different times [10]. In the first 2 or 3 days, rounded cells were seen attached to the surface. These cells stained with labelled antibodies to Ly5 (CD45) showing that they were derived from haemopoietic cells of the bone marrow. Ultrastructurally they resembled resident peritoneal macrophages or less differentiated cells of bone marrow origin. By day 6, the capsule was already quite thick and composed of the same rounded cells plus matrix covered by a continuous layer of mesothelium. By this time, some of the cells deep in the capsule had begun to take on the appearance of fibroblasts and only a few cells stained with antibodies to Ly5. Cells resembling macrophages and fibroblasts were both present alongside others with features of both cell types. By 2 weeks nearly all cells in the capsule were spindle-shaped, multi-layered as in the media of a blood vessel, and contained peripheral bundles of myofilaments. They stained with labelled antibodies to (-smooth muscle actin but none of these cells stained for Ly5. It was not clear whether the Ly5 antigen disappeared as the cells of haemopoietic origin differentiated into myofibroblasts or whether the original cells were gradually replaced by a 'wave' of cells entering the tissue from a mesenchymal source.

To conclusively test the bone marrow origin of these cells, female C57BL/6 mice expressing the Ly5.2 variant on the



surface of their haemopoietic cells were X-irradiated to destroy bone marrow, then immediately transfused with 106 nucleated bone marrow cells taken from the femur and tibia of a congenic strain of male mice expressing the Ly5.1 variant. Four weeks later, flow cytometry of female mouse blood with Ly5.1 antibodies confirmed successful engraftment (80-99%) by male marrow. Tubing was then placed into the peritoneal cavity of these female mice and the capsule harvested 14 days later. In situ hybridization with a Y-chromosome probe confirmed the male donor, and thus bone marrow origin of the elongated cells that formed the capsule and thence differentiated into smooth-muscle-like cells.

This vessel, if it can be successfully grown in humans, may open new options in the field of arterial reconstructive surgery for replacing or bypassing diseased arteries or as an access arteriovenous fistula for haemodialysis. A graft that is grown entirely from the patient's own cells within the peritoneal cavity ensures no tissue rejection and limited graft complication. Since it forms around a tubular mould, it can be grown with a predetermined diameter and length as a form of 'designer' artery. Since several tubes of tissue can be grown at the same or different times, it allows for multiple bypass grafting or repeat procedures. Its biocompatibility, together with tensile strength, elasticity, vascular reactivity and non-thrombogenic surface, would make it superior to existing synthetic grafts.

References:

1. Huynh T, Abraham G, Murray J, Brockbank K, Hagen PO, Sullivan S (1999). Remodeling of an acellular collagen graft into a physiologically responsive neovessel. Nature Biotechnology 17: 1083-1086.

2. Niklason LE, Gao J, Abbott WM, Hirshi KK, Houser S, Marini R, Langer R (1999). Functional arteries grown in vitro. Science 284: 489-493.

3. L'Heureux N, Stoclet JC, Auger FA, Lagaud GJ, Germain L, Andriantsitohaina R. (2001). A human tissue-engineered vascular media: a new model for pharmacological studies of contractile responses. FASEB J 15: 515-524.

4. Wolinsky H, Glagov S (1967). A lamellar unit of aortic medial structure and function in mammals. Circ Res 20: 99-111.

5. Patent No. PCT/AU99/00670 "Implant Material" registered in the name of the University of Queensland, inventors J.H. and G.R. Campbell and is in the international phase. Publication date is March 2, 2000(WO 00\10620), and priority date is August 21, 1998. A second, related patent application has recently been applied for ("Bioreactors," August 2, 2002).

6. Campbell JH, Efendy JL, Campbell GR (1999). Novel vascular graft grown within recipient's own peritoneal cavity. Circ Res 85: 1173-1178.

7. Campbell JH, Efendy JL, Campbell GR (1999). Blood vessels from bone marrow. Annals NY Acad Sci 902: 224-229.

8. Chue W-L, Campbell GR, Caplice N, Muhammed A, Berry CL, Thomas AC, Campbell JH (2002). The dog peritoneal and pleural cavities as bioreactors to grow autologous artificial blood vessels. Submitted for publication.

9. Efendy JL, Campbell JH, Campbell GR (2000). The effect of environmental cues on the differentiation of myofibroblasts in peritoneal granulation tissue. J Pathol 192:257-262.

10. Campbell JH, Efendy JL, Han CL, Girjes A, Campbell GR (2000). Haemopoietic origin of myofibroblasts formed in the peritoneal cavity in response to a foreign body. J Vasc Res 37:364-371.

I S A C B
ESSAYS

Arterial geometrical remodeling: an undervalued determinant of arterial luminal narrowing

Gerard Pasterkamp
Experimental Cardiology Lab
The University of Utrecht
Utrecht, the Netherlands

Atherosclerotic plaque formation has long been considered the only determinant of arterial occlusive disease. Although it was recognized that arteries can adapt their geometry in response to blood flow increase, it took more time to appreciate the effect of this geometrical remodeling response on atherosclerosis induced luminal narrowing. Just seventeen years ago it was described in human primates and human post mortem studies that radial enlargement of vessels can occur in response to progressive plaque growth [1,2]. The last decade, scientific interest in the role of arterial remodeling in occlusive arterial disease boomed with the upcoming use of the visualization technique of intravascular ultrasound (IVUS) [3-5]. At first it was assumed that arteries were only capable to undergo expansive remodeling in response to local plaque formation. This enlargement temporarily prevented lumen loss by compensating for plaque growth [2]. However, the observation that arteries may also fail to enlarge or, even worse, may shrink when plaque accumulates turned the geometrical remodeling response into an important determinant of lumen loss [5-8]. With identical plaque formation, the local remodeling response will influence the lumen size varying from luminal enlargement when overcompensation takes place to total occlusion when constrictive remodeling is prevalent.

How strong is the effect of this segmental change in vessel size, e.g. remodeling, compared to local changes in plaque size on the diameter of the lumen? In previous studies we already demonstrated that only a weak relation exists between arterial plaque load and lumen area [5]. In a more recent IVUS study in 609 patients we measured the lumen, plaque and vessel areas (= plaque + lumen area) at the site with most severe luminal obstruction and compared those with the lumen, plaque and vessel areas at a proximal and distal reference site [9]. For each lesion we calculated the axial changes in lumen, plaque and vessel area (for example: axial change in lumen area = lumen area reference – lumen area lesion). In this large patient group the frequency distribution revealed a comparable width of the 95% confidence intervals for segmental variation in plaque area and vessel area [–1.02 mm^2, +11.56 mm^2] and [–6.44 mm^2, +5.04 mm^2], respectively. Thus, surprisingly, in a large population suffering from coronary atherosclerotic disease, average segmental axial variation in vessel area equaled that of plaque area (see confidence intervals) This observation supports the concept that arterial remodeling is a major determinant of luminal narrowing in *de novo* atherosclerosis.

A similar study was also performed in 125 atherosclerotic femoral arteries in which the lumen, plaque and vessel areas were measured in a total of 3266 segments. In these femoral arteries we observed that when, on average, lumen area decreased (from 21.8 mm^2 to 7.0 mm^2), the average increase in plaque area could only explain minor part of the decrease in lumen (plaque increased from 10.3 to 16.5 mm^2). On the other hand, segmental decrease in vessel area appeared to be the main determinant of lumen decrease (32.1 to 23.5 mm^2) [10].

Thus, chronically lumen area is influenced by both plaque area and the local changes in vessel size (remodeling). However, the mode of arterial remodeling is also associated with sudden decreases in lumen area. Cross-sectional as well as follow up studies revealed that expansive remodeling is associated with adverse cardiovascular events and a vulnerable plaque phenotype [11-12]. Histological features that have been associated with plaque rupture and acute thrombotic occlusion is more frequently observed in expansively enlarged lesions [13]. This makes remodeling a paradoxical phenomenon: while expansion prevents encroachment of the plaque upon the lumen, it hides a plaque that may lead to acute clinical events. Although the relation between expansive remodeling and plaque vulnerability is associative rather than causal, expansively remodeled plaques should be considered as a wolf in sheep's clothes [9].

It should be emphasized, however, that the definition of plaque vulnerability merits careful consideration. The current histopathological definition of 'the vulnerable plaque' (large atheroma and thin fibrous cap with inflammatory cells) is an heritage of *post mortem* studies in which a selection of ruptured plaques have been studied. One has to realize, however, that the often used histopathological criteria for vulnerable plaques are not specific for ruptured lesions. Newly developed visualization techniques that are capable of visualizing plaque components have great potential to provide us the knowledge that leads to further understanding of the processes of plaque destabilization and rupture by a prospective study design.

continued on page 12

In summary, the role of arterial geometric remodeling is undervalued. Question remains why so little research is aimed to develop intervention strategies to influence the remodeling mode. The answer is not that difficult: knowledge on biological and mechanical triggers that induce these segmental remodeling responses is lacking. To gain more insight in shared mechanisms of plaque vulnerability and expansive remodeling, animal models reflecting human like atherosclerotic disease are needed. In contrast to expansive remodeling, constrictive remodeling has not been described in atherosclerotic animal models.

References.

1. Armstrong ML, Heistad DD, Marcus ML, Megan MB, Piegors DJ. Structural and hemodynamic responses of peripheral arteries of macaque monkeys to atherogenic diet. Arteriosclerosis 1985;5:336-346.
2. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis G. Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med 1987;316:1371-1375.
3. Hermiller JB, Tenaglia AN, Kisslo KB, Phillips HR, Bashore TM, Stack RS, Davidson CJ. In vivo validation of compensatory enlargement of atherosclerotic coronary arteries. Am J Cardiol 1993;71:665-668.
4. Alfonso F, Macaya C, Goicolea J, Iniguez A, Hernandez R, Zamorano J, Perez-Vizcayne MJ, Zarco P. Intravascular ultrasound imaging of angiographically normal coronary segments in patients with coronary artery disease. Am Heart J 1994;127:536-544.
5. Pasterkamp G, Wensing PJW, Post MJ, Hillen B, Mali WPTM, Borst C. Paradoxical arterial wall shrinkage contributes to luminal narrowing of human atherosclerotic femoral arteries. Circulation 1995;91:1444-1449.
6. Pasterkamp G, Borst C, Post MJ, Mali WPTM, Wensing PJW, Gussenhoven EJ, Hillen B. Atherosclerotic arterial remodeling in the superficial femoral artery: individual variation in local compensatory enlargement response. Circulation 1996;93:1818-1825
7. Nishioka T, Luo H, Eigler NL, Berglund H, Kim CJ, Siegel RJ. Contribution of inadequate compensatory enlargement to development of human coronary artery stenosis: an in vivo intravascular ultrasound study. J Am Coll Cardiol 1996;27:1571-1576.
8. Mintz GS, Kent KM, Pichard AD, Satler LF, Popma JJ, Leon MB. Contribution of inadequate arterial remodeling to the development of focal coronary artery stenoses: an intravascular ultrasound study. Circulation 1997;95:1791-1798.
9. Pasterkamp G, Fitzgerald P, de Kleijn DPV. Expansive arterial remodeling: a sheep in wolf's clothes. J Vasc Research, 2002 in press.
10. Vink A, Schoneveld AH, Borst C, Pasterkamp G. The contribution of plaque and constrictive remodeling to de novo atherosclerotic luminal narrowing in the femoral artery. J Vasc Surgery, 2002, in press.
11. Smits PC, Pasterkamp G, de Jaegere PJ, Eefting FD, Stella PR, Borst C. Coronary artery disease: arterial remodeling and clinical presentation. Heart 1999;82:461-464.
12. Dangas G, Mintz GS, Mehran R, Lansky AJ, Kornowski R, Pichard AD, Satler LF, Kent KM, Stone GW, Leon MB. Preintervention arterial remodeling as an independent predictor of target lesion revascularization after non stent coronary intervention. Circulation 1999;99:3149-3154.
13. Pasterkamp G, Schoneveld AH, van der Wal AC, Haudenschild CC, Clarijs RJG, Becker AE, Hillen B, Borst C. The relation of arterial geometry with luminal narrowing and plaque vulnerability: the remodeling paradox. J Am Coll of Cardiol 1998;32:655-662.

I S A C B
ESSAYS

Common Pathway for Environment-Mediated Remodeling of Heart Valves

Elena Rabkin
Brigham and Women's Hospital
Harvard Medical School, Boston, MA

Sophisticated prospective development of tissue valve substitutes depends on an understanding of the mechanisms limiting the durability of previous and current tissue valves.[1] This in turn necessitates an understanding of the fundamental structure of the normal aortic valve ("gold standard"), the biology of its cells and extracellular matrix (ECM), and the changes which occur in tissue during fabrication and following implantation. Accurate descriptions of normal heart valve composition and properties, including both cells and ECM, are critical in the establishment of the ideal end points for *in vivo* remodeling of tissue-engineered valves. The development of tissue-engineered heart valves would be aided by a thorough understanding of how different environmental stimuli, including mechanical stress or injury impact the cells within valve leaflets.[2] Although fibroblast-like valvular interstitial cells (VIC) form the major cell population of normal cardiac valves, and these cells synthesize collagen, elastin and proteoglycans, their physiology in health, disease and valve substitution is largely unknown. Two other types of cells have also been cultured from heart valve leaflets: smooth muscle cells and myofibroblasts. Myofibroblasts may well represent an intermediate state between fibroblasts and smooth muscle cells. Knowledge of the phenotypes of leaflet VIC is important; however, the distinction between fibroblasts, myofibroblasts and smooth muscle cells is not clear and continues to be debated. Detection of myofibroblasts is difficult due to their heterogeneity and absence of particular immunohistochemical markers. Characterization of myofibroblasts is based on antibody reaction to two of the three cellular filaments: 1) actin, a component of the microfilament; 2) vimentin, desmin, laminin, members of the intermediate filament system; and 3) the tubulins of the microtubules. A classification of myofibroblasts has been proposed based on immunohistochemical staining of these filaments.[3] Myofibroblasts that express vimentin and desmin are called VD-type, those that express vimentin and a-smooth muscle actin are called VA-type, and those that express vimentin, a-smooth muscle actin and desmin are called VAD-type.

Since degradation of the extracellular matrix scaffold enables reshaping of tissue, participation of specialized enzymes called matrix metalloproteinases (MMPs) has recently become the object of intense interest in relation to physiological cardiovascular remodeling, including adaptation and repair, and pathological remodeling, which underlies the pathogenesis of major cardiovascular diseases. The interplay of MMPs, tissue inhibitors of MMPs and their regulators are especially important in cardiac and vascular remodeling. Dysregulation of matrix metabolism modulates major features of myocardial, arterial, and possibly valvular pathology, which include atherosclerosis, myocardial infarction, aortic aneurysm, aortic valves from patients with Marfan's syndrome and valves with myxomatous degeneration.[4-9] MMPs are a family of enzymes including interstitial collagenases and gelatinases involved in the degradation and remodeling of connective tissue. Interstitial collagenases mediate the initial step of collagen degradation by breakdown of the native helix of the fibrillar collagen network (type I is the most abundant, comprising about 70% of the total collagen in valves). These fragments then become accessible to the other proteases, such as gelatinases which further catabolize collagen.[10] Recent study demonstrates that cysteine endoproteases (cathepsin S and K) are also involved in remodeling of ECM, particularly elastin.[11] Cathepsin K is the most potent elastase yet described, and also possesses collagenolytic activity.[12] Several studies have suggested that ECM degradation in many degenerative diseases may be mediated by the cells residing in those tissues. This evidence suggests that these cells are being stimulated in some way to produce and secrete soluble extracellular messengers, which in turn interact with indigenous cells inducing them to initiate matrix degradation. Such a factor as cardiac catabolic factor, derived from porcine heart valves, was found to stimulate collagen and proteoglycan degradation i*n vitro*.[13-15] We recently demonstrated that excessive levels of proteolytic enzymes such as collagenase-1 (MMP-1) and collagenase-3 (MMP-13) or gelatinase-A (MMP-2) and gelatinase-B (MMP-9) and cysteine endoproteases (cathepsin S and K) elaborated by valvular VIC may contribute to collagen and elastin degradation leading to structural morphological changes resulting in expansion and weakness of leaflets of heart valves with myxomatous degeneration, suggesting an important role of proteolytic enzymes in matrix remodeling in myxomatous valve disease[9] as well as in tissue-engineered heart valves[16] and in clinical pulmonary autograft aortic valve substitutes.[17] ECM degrading proteases have been identified in pericardial heart valves.[18,19]

Pathological studies done by our group and others over the past decade have demonstrated that the quality of the ECM, particularly the collagenous skeleton, and its resistance to deterioration are critical to valve performance. Moreover, the quality of valvular ECM depends on interstitial cell viability and function. However, the ability of valvular interstitial cells (VIC) to remodel and renew ECM, and to adapt to different environments is largely unknown. We hypothesize that VIC respond to different environmental stimuli (e. g., abnormal mechanical stress, greater systemic pressure, trauma, genetic abnormalities) by phenotypic modulation and mediate ECM remodeling by secretion of proteolytic enzymes.

We studied four groups of valves: 20 normal human mitral, aortic and pulmonary valves; 15 mitral valves with myxomatous degeneration; 10 clinical pulmonary autograft aortic valve substitutes (PA) in place for 15 days to 6 years; and 10 tissue engineered heart valves (TEHV) fabricated dynamically *in vitro* for 14 days, implanted in lambs and explanted at 4-20 weeks. We examined ECM composition and collagen architecture by histology, using Movat pentachrome stain (distinguish ECM components: proteoglycans-green, collagen-yellow, elastin-black). We used polarized light microscopy to determine collagen type composition following staining by picrosirius red, a strong anionic dye, which enhances birefringence of collagen and permits assessment of fiber maturity/type (type I red or orange, and type III green). We have examined VIC phenotypes, and distinguished smooth muscle cells from myofibroblasts using immunohistochemical markers for differentiation and maturation. We assessed cell immunophenotype by antibodies to a-actin (microfilaments), vimentin (intermediate filaments), and SM1, SM2 (differentiated smooth muscle cells).[20] We used SMemb (nonmuscle myosin heavy chain) antibody known to identify activated mesenchymal cells.[21] Proteolytic enzymes were detected by MMP-1, MMP-2, MMP-9 and MMP-13, and cysteine endoproteases by cathepsin S and K antibodies.

Normal valves had three well-defined layers each containing cells and characteristic ECM composition and configuration: the fibrosa, spongiosa, and ventricularis. Myxomatous valves showed: expansion of the spongiosa by loose amorphous ECM stained strongly positive for proteoglycans, diminished staining for collagen fibers, and fragmentation of elastin. Overall, myxomatous valves had less birefringence compared to normal.

VIC in normal valves were immunoreactive to vimentin, but not a-actin, SM1, SM2 or SMemb, MMP-1, MMP-13 and cathepsins (quiescent fibroblasts). However, many vimentin-positive/SM1-negative cells in myxomatous valves expressed a-actin and SMemb (activated myofibroblasts). Quantitative analysis showed that levels of MMPs, especially collagenases in myxomatous valves, were significantly increased compared to normal suggesting an important role of the proteolytic enzymes in ECM deterioration accompanying myxomatous degeneration. VIC of short-term PA explants were mostly myofibroblasts of vimentin/a-actin phenotype and showed strong MMP activity indicative of collagen remodeling, while long-term explants were stained predominantly for vimentin, resembling the fibroblast-like cells of normal aortic valve, and had a weaker expression of MMPs. Cells in TEHV constructs grown *in vitro* for 14 days were activated myofibroblasts by strong co-expression of vimentin, a-actin and SMemb. VIC from in vivo explants at 16-20 weeks expressed mostly vimentin and were characterized as fibroblast-like cells (similar to normal valve). In TEHV ***(Figure 1)***, ECM remodeling was evident by collagen production in vitro at 14 days that was augmented

continued on page 14

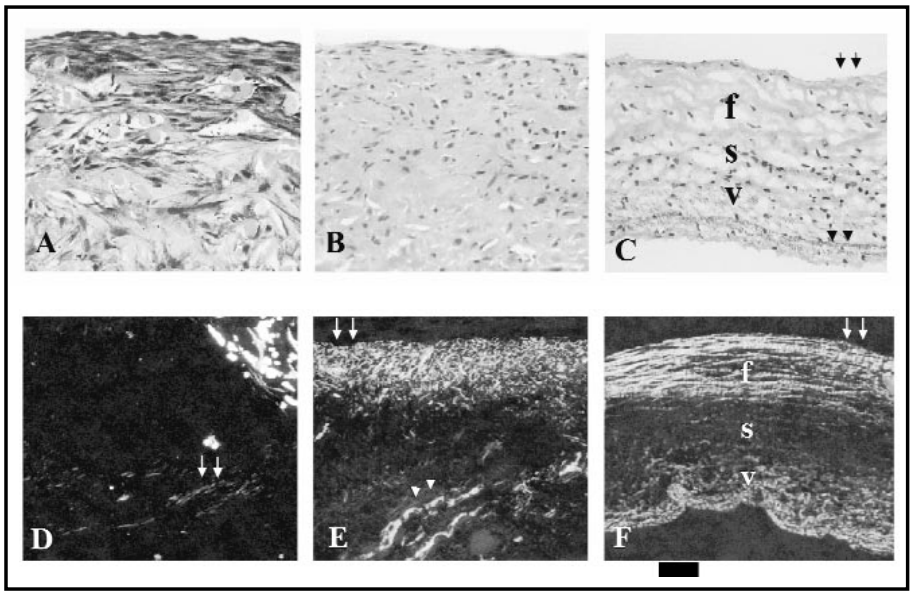


Figure 1 **Evolution of tissue toward three-layered valve structure in TE heart valves *in vitro* and *in vivo*.**

(A) Formation of ECM *in vitro* after 14 days in bioreactor and (B) in cusp at 4 weeks implants as predominant proteoglycan accumulation. Elastin was not detected. (C) 20 weeks explant demonstrates three-layered structure: collagen in the fibrosa (f), proteoglycans in the spongiosa (s), and elastin (arrowheads) near the ventricular side (v), similar to native pulmonary valve. Movat pentachrome stain.

(D). In 14 days *in vitro* construct collagen was evident as a few fibrils of weak birefringence (arrows); (E) 4 weeks implants show disorganized and disoriented accumulation of fibrillar collagen on the free edge of the leaflet (arrows). Polymer residue indicated by arrowheads. (F) 20 weeks explant demonstrates three-layered collagenous architecture reminiscent of native valve (f-fibrosa, s-spongiosa, v-ventricularis). Outflow surface indicated by arrows. Picrosirius red under polarized light. Circumferential sections. Original magnification X100. Modified and reproduced from Rabkin E. J Heart Valve Dis 11:308-314, 2002.

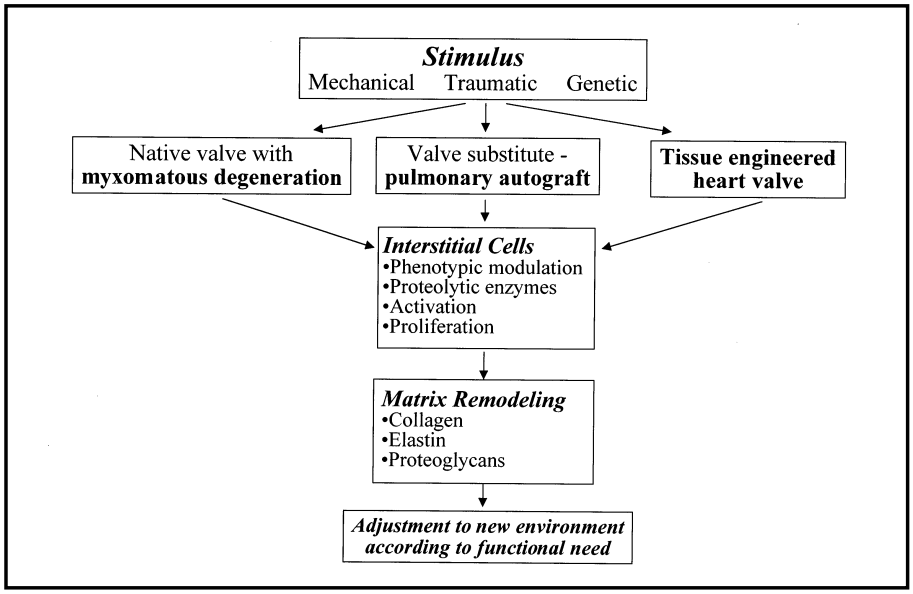


Figure 2 **Common pathway for environment-mediated remodeling of valvular structure.**

In response to different environmental stimuli (abnormal mechanical stress, trauma or genetic disorder) interstitial valvular cells in native, biological substitute and tissue-engineered valves undergo activation, phenotypic modulation, and secrete proteolytic enzymes which mediate ECM remodeling according to functional need.

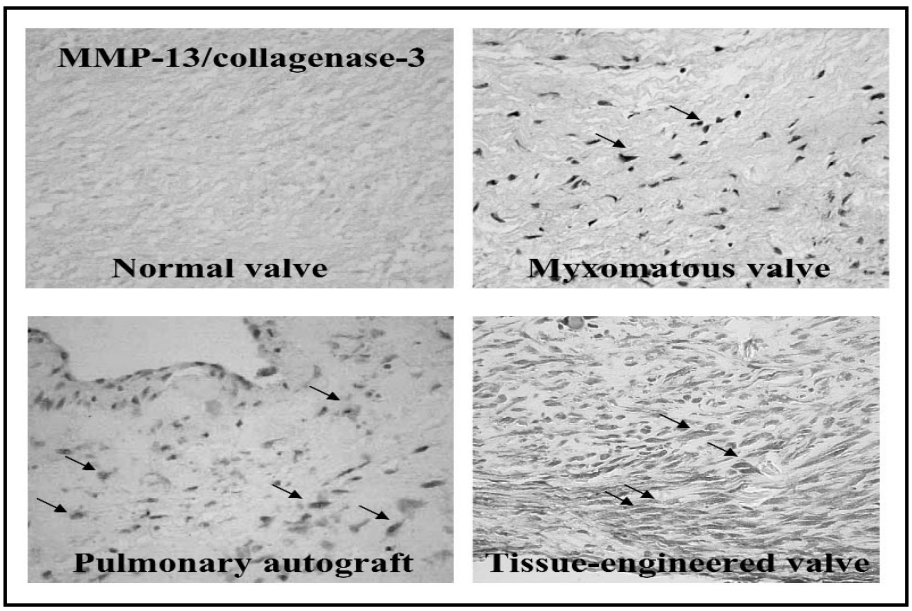


Figure 3 MMP-13/collagenase-3 expression in normal valves, valves with myxomatous degeneration, clinical pulmonary transplants (Ross procedure), and tissue-engineered heart valves. Original magnification X400.

in vivo initially as disorganized collagen with strong MMP-13 expression of VIC at 4 weeks, followed by organization of collagen architecture and diminished MMP-13 staining apparent at 20 weeks (similar to normal valve).

Recently it has been demonstrated that the application of different pressure forces[22] or injury[23] are associated with the phenotypic changes of cells in the leaflet. Therefore, our results suggest that VIC undergo phenotypic modulation from resting fibroblast-like cells in normal valves to activated myofibroblasts in myxomatous valves, short-term pulmonary autograft valve explants, and tissue-engineered heart valves. Our data are summarized schematically in ***Figure 2*** that illustrates a common pathway associated with physiological/adaptive and pathological remodeling of valvular structure. This pathway involves a phenotypic modulation of valvular interstitial cells that mediate ECM remodeling by secretion of proteolytic enzymes ***(Figure 3)***. Dysregulation of this pathway may contribute to a variety of diseases, including myxomatous degeneration. The evolution of cell phenotype (fibroblast > myofibroblast > fibroblast) and ECM in tissue-engineered and long-term pulmonary autograft valves may reflect the ability of viable tissue to repair and remodel *in vivo* according to dynamic functional need, ultimately to recapitulate the architectural features of normal valves and potentially grow.

References

1. Schoen FJ, Levy RJ. Tissue heart valves: current challenges and future research perspectives. J Biomed Mater Res. 1999;47:439-465
2. Durbin AD, Gotlieb AI. Advances towards understanding heart valve response to injury. Cardiovascular Path. 2002;11:69-77.
3. Schurch W, Seemayer TA, Gabbiani G. The myofibroblast: a quarter century after its discovery. Am J Surg Pathol. 1998;22:141-7.
4. Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, Fallon JT, Regnstrom J, Fuster V. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. Circulation. 1995;92:1565-9.
5. Sukhova GK, Schonbeck U, Rabkin E, Schoen FJ, Poole AR, Billinghurst RC, Libby P. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. Circulation. 1999;99:2503-9.
6. Aikawa M, Rabkin E, Okada Y, Voglic SJ, Clinton SK, Brinckerhoff CE, Sukhova GK, Libby P. Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content of rabbit atheroma: a potential mechanism of lesion stabilization. Circulation. 1998;97:2433-44.
7. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, Lee RT. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest. 2000;106:55-62.
8. Segura AM, Luna RE, Horiba K, Stetler-Stevenson WG, McAllister HA, Jr., Willerson JT, Ferrans VJ. Immunohistochemistry of matrix metalloproteinases and their inhibitors in thoracic aortic aneurysms and aortic valves of patients with Marfan's syndrome. Circulation. 1998;98:II331-7; discussion II337-8.
9. Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P, Schoen FJ. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. *Circulation*. 2001;104:2525-2532.
10. Krane SM, Byrne MH, Lemaitre V, Henriet P, Jeffrey JJ, Witter JP, Liu X, Wu H, Jaenisch R, Eeckhout Y. Different collagenase gene products have different roles in degradation of type I collagen. J Biol Chem. 1996;271:28509-15.
11. Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. J Clin Invest. 1998;102:576-83.
12. Chapman HA, Riese RJ, Shi GP. Emerging roles for cysteine proteases in human biology. Annu Rev Physiol. 1997;59:63-88.
13. Decker RS, Dingle JT. Cardiac catabolic factors: the degradation of heart valve intercellular matrix. Science. 1982;215:987-9.
14. Dingle JT, Saklatvala J, Hembry R, Tyler J, Fell HB, Jubb R. A cartilage catabolic factor from synovium. Biochem J. 1979;184:177-80.
15. Henney AM, Decker RS. Production of a factor by cultured human heart valves that is immunologically related to interleukin 1. Cardiovasc Res. 1987;21:21-7.
16. Rabkin E, Hoerstrup SP, Aikawa M, Mayer JE, Schoen FJ. Evolution of cell phenotype and ECM in tissue-engineered heart valves during in vitro culture and in vivo remodeling. *J Heart Valve Dis.* 2002;11:308-314.
17. Rabkin E, Kouchoukos NT, Mitchell MB, Jonas RA, Schoen FJ. Clinical pulmonary autografts undergo initial remodeling but maintain cell viability and intact collagen architecture to 6 years. Cardiovascular Path 2002;11:31.
18. Simionescu D, Simionescu A, Deac R. Detection of remnant proteolytic activities in unimplanted gluteraldehyde-treated pericardium and explanted cardiac bioprostheses. J Biomed Mater Res. 1993;27:821-826.
19. Simionescu A, Simionescu D, Deac R. Biochemical pathways of tissue degeneration in bioprosthetic cardiac valves. The role of matrix metalloproteinases. Asaio J. 1996;42:M561-7.
20. Aikawa M, Rabkin E, Voglic SJ, Shing H, Nagai R, Schoen FJ, Libby P. Lipid lowering promotes accumulation of mature smooth muscle cells expressing smooth muscle myosin heavy chain isoforms in rabbit atheroma. Circ Res. 1998;83:1015-26.
21. Aikawa M, Sivam PN, Kuro-o M, Kimura K, Nakahara K, Takewaki S, Ueda M, Yamaguchi H, Yazaki Y, Periasamy M, et al. Human smooth muscle myosin heavy chain isoforms as molecular markers for vascular development and atherosclerosis. Circ Res. 1993;73:1000-12.
22. Weston MW, Yoganathan AP. Biosynthetic activity in heart valve leaflets in response to in vitro flow environments. Ann Biomed Engineering. 2001;29:752-763.
23. Tamura K, Jones M, Yamada I, Ferrans VJ. Wound healing in the mitral valve. J Heart Valve Dis. 2000;9:53-63.

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