FALL/WINTER 2000

ISACB

CIRCULATOR

INTERNATIONAL SOCIETY FOR APPLIED CARDIOVASCULAR BIOLOGY

MARK YOUR CALENDARS NOW!

VIIIth Biennial Meeting of ISACB

ISACB 2002

ST. GALLEN, SWITZERLAND

FEBRUARY 27 – MARCH 2

Here we go again! Organizational plans are well underway for the VIIIth Biennial Meeting of ISACB in 2002. The meeting is scheduled for February 27 – March 2, 2002 and will be held in St. Gallen, Switzerland. Jeffrey Hubbell, Ph.D., from Zurich is the local organizer for this meeting.

The city of St. Gallen is the metropolitan center of eastern Switzerland. St. Gallen is in close geographic proximity to Lake Constance and is a city full of cultural and leisure attractions. St. Gallen is a quick 40 minute train ride directly from the Zurich airport. The meeting will be held in a new, attractive conference center located in the heart of the old town of St. Gallen, adjacent to the Abbey of St. Gall.

Distinguished researchers, and clinician scientists are currently being invited to participate in the program that will be organized into the following sections:

Minimally Invasive Cardiovascular Therapies
- Stu Williams and Elliot Chaikof, Section Chairs

Mechano-transduction
- Fred Schoen and Bauer Sumpio, Section Chairs

Cardiovascular Tissue Engineering
- Jeffrey Hubbell, Section Chair

Bioprosthetic Heart Valves
- Fred Schoen and Peter Zilla, Section Chairs

Pathology of Vascular Injury and Repair
- Jointly sponsored with the Society for Cardiovascular Pathology
- Jagdish Butany and Howard Greisler, Section Chairs

As in the past, the program will include presentations from contributed abstracts and an interactive poster session.

Additional details regarding ISACB 2002 will be mailed in January, 2001.

IN THIS EDITION

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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.

ISACB NOTES

Notes from the Business Office

We are pleased to announce the registration of our own domain name – the Society's web site is now found at www.isacb.org. We are also phasing in some changes to our site. The membership list has been updated, with additional names added as renewal dues are paid (and new members are added). The roster of Tucson registrants has been posted, along with some photos from the event. Ultimately we would like to see the web site expand to include research-related pages for citations of member publications, commentary, patent announcements, and meeting/conference/ workshop announcements (with web links). A separate page is planned for post-doc, research and employment opportunities. We hope to implement convenient and secure on-line payment and meeting registration in time for the St. Gallen 2002 meeting. Finally, we are looking into listserv capabilities for members to exchange viewpoints, information and questions in a forum. As always, we solicit the members for information of potential interest to the membership as a whole, and offer the web site as a venue for exchange of questions and ideas. Contributions and suggestions are always welcome.

Distribution of announcements via email is a new service available to members at no cost. Some announcements have already been distributed on behalf of members, to ISACB members, Tucson attendees, and to biomed/eng graduate programs, as appropriate. Suggested contacts for the latter will be very much appreciated. Announcements will be posted to the appropriate web pages as well.

Email is the most efficient and cost effective communication tool at our disposal. However, approximately 1/3 of our members do not have a good email address on file. If you are not receiving mail or email from us, please email, fax or send us your correct address information.

Reminder: the membership year runs from June through May. Membership certificates for the current 00-01 year will be sent out within the next few weeks.

We would like to thank outgoing Executive Council members Harvey Borovetz and Peter Lelkes for their many services to the Society, and extend a warm welcome to new members Dan Simionescu and Mark Torrianni.

Best regards,

Karla M. Funk
Office of Steven P. Schmidt,
Secretary/Treasurer
Email: funkk@summa-health.org

I S A C B
ADDRESS

PRESIDENTIAL ADDRESS:
A Life of its Own

Howard Greisler, President, ISACB
Loyola University, Medical Center
Maywood, Illinois, USA

This is an explosive period in the allied fields that comprise applied cardiovascular biology and a time of both maturation and expansion in the International Society for Applied Cardiovascular Biology. Our recent Tucson meeting was a great success scientifically and socially and was sound financially. Our Society is founded on the premise that we can enable bridges to be built to minimize the chasms often separating science and clinical practice, academia and industry, and biology and engineering. We have been remarkably successful! Our Society has taken on a life of its own. Financially, although still hovering at the fulcrum, we are now able to engage a professional organization, BostonBased, to assist the able office of our Secretary/Treasurer (whose efforts have been Herculean) in the massive responsibilities required to effectively organize our meetings and maintain the administrative stability of our Society. While control of all aspects will remain with the officers and the Executive Council, members will soon note that correspondence will come from both sources. Our arrangements for the upcoming two year period leaves all decision making processes unchanged but the group at BostonBased will greatly facilitate mailings, program announcements, abstract handling, organizational aspects for the 2002 St. Gallen international meeting and fundraising.

The ISACB is no longer a fledgling society. Our biennial meetings are strong with predictably excellent and cutting edge science. Our members and guest participants now anticipate upcoming meetings with enthusiasm and the number and quality of abstract submissions has grown. Invited speakers have uniformly given our Society praise and many have become active members. We again published our accepted abstracts in the Cardiovascular Pathology journal. We have reached the point at which a hitherto fledgling society enters the age of maturation.

However along with maturation, we are at a time of expansion. The concept of bridging the chasms must not be confined to a single biennial meeting. To that end we have nurtured appropriate selected interactions with sister societies. ISACB cosponsored a session with the Society for Cardiovascular Pathology at its recent annual meeting in New Orleans, and that Society successfully cosponsored a session at our Tucson meeting. Similar plans are underway with the SCVP for future meetings. I'm happy to announce that our two Societies are cosponsoring a major session at the 2001 Experimental Biology meeting in Orlando under the auspices of the ASIP and the EB2001 leadership has approved the ISACB's status as an official Participating Guest Society, a status which will appear prominently on all EB2001 mailings.

I view the successful interactions with the SCVP rather as a template. Both Societies benefit and interaction and communication among members is facilitated. The ISACB is focused on the big picture of applied cardiovascular biology and as such represents vascular biologists, pathologists, tissue engineers, chemical and mechanical engineers, clinicians and experts in the imaging sciences. Along with our Executive Council, I have been engaged in a series of discussions with leaders of the rapidly exploding tissue engineering organizations about potential interactions and am excited to announce that the plans for the ISACB 2004 conference are well underway in conjunction with the Engineering Tissues Workshop meeting. The 2004 conference will be held at Hilton Head's new and state of the art conference facilities between March 10-14. This meeting is traditionally organized by Bob Nerem from Georgia Tech University. While the details are not yet finalized, be assured that all aspects within the "big picture" of applied cardiovascular biology will continue to be well represented. Both our meeting and the Engineering Tissues Workshop are similar in attendance and duration and consequently scientific discussion and future membership opportunities will be similarly enhanced. Given that the two organizations have overlapping but not identical scopes, one option may be a combined 2-3 day conference extended at either end for an additional day focusing on issues more (but not exclusively) associated with one of the two groups. The details are still at a highly formative stage and all suggestions will be greatly appreciated.

Thus, maturation plus expansion. We cannot rest on our laurels and become complacent. The field of applied cardiovascular biology too is just reaching the same stage of maturation and expansion, integration and separation. There remains too much potential benefit for science and for our Society to not continue to build bridges between the shifting and often provincial disciplines which comprise our field. We must continue to facilitate interaction and collaboration among all the scientific and clinical specialities within our "big picture". The ISACB has indeed taken on a life of its own. That life was borne of the need to communicate and collaborate and we must continue to guide and enable the ISACB to fulfill that need as the scientific achievement and clinical potential of applied cardiovascular biology matures and expands.

ISACB Tucson
ARIZONA, USA
7TH BIENNIAL MEETING
MARCH 9-11 • 2000

THE LEGENDS

Our VIIth biennial meeting in Tucson, while just a memory now, has certainly created new legends of ISACB. It was once said of the great Apache Indian warrior Geronimo that "He had magical powers. He could see into the future, walk without creating footprints and even hold off the dawn to protect his own". Many attendees seemed to have gleaned some of these powers. The science presented and discussed proved our society is indeed seeing into the future (with some appropriately reminding us to never forget the past!). Saturday morning's early start after a night of "Old Tucson" celebration caused many to attempt to hold off the dawn. On the other hand, the quantity of food at our society meetings makes walking without footprints nearly impossible.

Each of the major sessions provided an outstanding opportunity to learn the current status of the field. Applied cardiovascular biologists - represented by clinicians, engineers, as well as applied and basic scientists - presented the hard facts and the controversies that exist in our field. The discussions of results continued throughout the breaks. The poster facilities were available and frequented by attendees throughout the meeting. The formal poster viewing session never seems to be long enough! These discussions are an integral part of our meeting, and continue to provide an opportunity for new collaborations to emerge and old long-standing friends to be re-united.

…Great job in Tucson – the Society continues to grow and fills a great need at present.

– Rod White, UCLA Harbor Medical Center Division of Vascular Surgery (Invited Speaker)

THE ORGANIZING CREW

IS THIS MY DOOR PRIZE?

AND YOU SAID THAT I COULDN'T PULL THIS CONFERENCE OFF.

ADVANCED JOB HUNTING

STU AND SPIELBERG - I'M READY FOR MY SCREEN TEST.

"SO AS A TISSUE ENGINEER CAN YOU GROW NEW FINGERS FOR ME?"

BEING PRESIDENT IS GOOD. ARE THESE MY INTERNS?

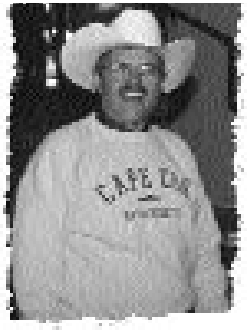
Just a short message to say thank you for a fantastic conference.

…I am going over the notes from the conference, it continues to impress me the amount of relevant material (to mine) that was presented which I'm sure will have a positive impact on the final outcome of my Ph.D.. Another bonus was the four potential Post Doc. positions offered.

In short, thanks for your efforts, if I'm still in the industry in 2 yrs time I'll be at the next meeting.

– Pete McFetridge, Attendee

OF ISACB 2000



YEEE HAAAA

Our plan to identify a budding western movie star was anticipated with great expectations. While the set and costumes were award winning, and the casting director performed with brilliance, the overall result is still under active editing. The administration of Old Tucson Studios is currently considering a future Halloween release of our efforts since many scenes, upon review, are considered some of the most frightening things ever filmed at these film studios.

While our movie making may not have produced an Oscar, this unbiased observer believes our Society truly shined with the help of Miss Lil and friends. The photographic documentation establishes we should spend more time in the Saloon. The banquet was equally successful with a supporting cast of young Mariachi musicians. Clearly our membership seems at its best when music is playing.

I'M THE NEW SHERIFF IN TOWN.

The success of our biennial meetings is based on the efforts of many individuals and groups. Sponsors, organizers and an unbelievably dedicated staff are to be congratulated for a memorable occasion. The legends born in Tucson will live on with the society for years to come.

Stuart Williams, Ph.D.
University of Arizona
Div. Biomedical Engineering Sciences
Tucson, Arizona

KEEP SMILING, IT'S ALMOST OVER.... FOR THIS YEAR.

Tucson was very interesting and great fun indeed. Thanks a lot for the organization.

– Gunnar Riepe, Allemeines Krankenhaus Harburg, Hamburg, Germany, (Invited Speaker)

THIS IS ONE GROUP THAT DOES NOT TAKE DIRECTION WELL.

It was a pleasure to meet you in Tucson this past week. I very much enjoyed the Tucson meeting as this was my first ISACB meeting. I am particularly grateful for your efforts with the Young Investigator Award program. This recognition is very meaningful for a young scientist and I am very happy to have received it. Congratulations on an excellent conference.

– Dror Seliktar, Young Investigator

WERE WE SUPPOSED TO SHARE?

JUST CHECKING

…The ISACB meeting was excellent. There were terrific presentations and discussion, and it all took place in a marvelous spot. I congratulate you on putting on a wonderful meeting and running it flawlessly!

– Alexander Clowes, MD, Professor of Surgery, University of Washington School of Medicine (Invited Speaker)

HAVE I TOLD YOU THE HISTORY OF ENDOTHELIAL CELL SEEDING?

YOU KNOW STEVE, I HAVE A SURFACE MODIFICATION JUST FOR YOU.

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.

Where to Now...That We Have Tissue Engineered Blood Vessels? Tissue Engineered Blood Vessels! Heavens, What Will They Think of Next!

F.A. Auger, M. Rémy-Zolghadri, G. Grenier, L. Germain

Laboratoire d'Organogénèse Expérimentale/LOEX, Hôpital du Saint-Sacrement and Department of Surgery, Faculty of Medicine Laval University, Québec city, Québec G1S 4L8.
www.loex.ulaval.ca

My father would have wished me to be more frequently his assistant as he was an active vascular surgeon. We did only " operate " together three times : but these surgeries and my intimate relationship with vascular and cardiothoracic surgeons has instilled into me a good dose of respect for this art. Such surgical prowess has not gone unnoticed in the medical history of the XXth century.

The advent of vascular grafts has saved countless lives and paved the way to audacious surgical heart and limb salvages. Synthetic prosthesis for vascular implantation are one of the most fascinating story in medicine and many great names come to mind : Carrel, Blakemore and Voorhees, Sauvage, DeBaquey. However, as we are now entering into the XXIst century, a critical appraisal of these approaches is unavoidable. These prosthesis have limitations and the most blatant is their complete lack of biological function. This renders them unsuitable for small diameter grafting. The few attempts to transplant vessels less than 5 mm of internal diameter have mostly led to dismal results on a long term basis. Thus, the stage is set for a new approach in this clinical niche, such as tissue engineered blood vessels.

One must never forget that tissue engineering was first introduced as a life saving procedure for burn patients [(1)]. The successful engraftment of autologous epidermal sheets was the initial and seminal proof of concept of the powerful technology that we know today.

The subsequent efforts in this biotechnological field were developed according to essentially three types of approaches. The first approach consists in the seeding of cells into various gels, which are then reorganized by the incorporated cells.[2-8)] Alternatively, a second approach is to deposit cells into a scaffold where they will thrive and secrete an extracellular matrix.[9-11] The scaffold materials are at times bioresorbable over a wide range of time periods depending on their chemical nature.[12-14] A third approach is different since it uses the principle of a tissue template that allows, after implantation, the ingress of cells into the appropriately organized scaffold. Thus, these grafts are acellular and must stimulate the regenerative potential of the tissue, in vivo, wherever they are implanted.[15-17]

However, our group has initiated a different and original method for the reconstruction of soft tissues. It takes full advantage of the various intrinsic properties of cells when appropriately cultured. This entails particular media composition and appropriate mechanical straining of these three-dimensional structures. We call it the **self-assembly approach.**

Our own experience with the culture of autologous epidermal sheets gave us some insight in the properties of cells, in order to recreate in vitro human living tissue substitutes.[26] Furthermore our various adaptations of the gel construct approach has shown that cells could be aligned along with their extracellular matrix if the mechanical forces generated were appropriately harnessed: either passively, by matrix anchorage,[18] or actively, by cyclic traction of these constructs.[6]

The self-assembly approach is a combination of all our previous experiences. Basically, we coax the cells into secreting their own extracellular matrix in a sheet form. Thereafter, we either roll or stack these sheets to create a three-dimensional organ substitutes. Our concept has been applied with impressive results to the reconstruction of blood vessel and skin.[19, 20]

The first attempt to produce a reconstructed blood vessel by tissue-engineering methods appeared in 1986 with the model published by Weinberg and Bell.[3] The method devised by these researchers was based on collagen gels seeded with bovine vascular cells. Such a technique was the basis for subsequent research conducted by other teams.[4,21,22] But the resulting structures were not resistant enough to sustain normal blood pressure[3,4] and some of these prostheses had to be reinforced with a synthetic mesh[3,23] making them hybrid artificial substitutes (composed of living cells in association with a synthetic support) with all the untoward properties associated with such constructions.

Since the previous approaches did not seem to be conducive to an appropriate clinical result, we developed a tissue-engineered blood vessel (TEBV) based exclusively on the use of human cells in the absence of any synthetic or

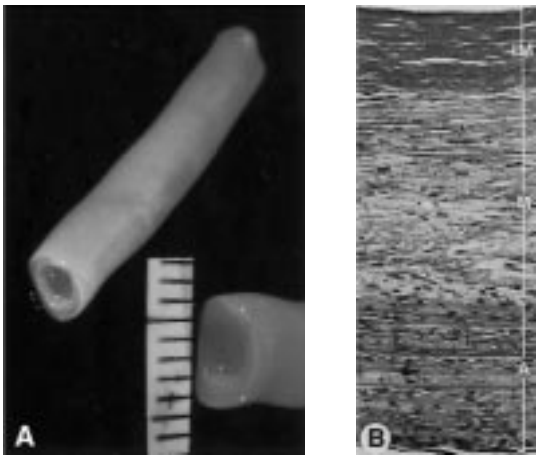


FIGURE 1

Macroscopic (A) and microscopic (B) views of the mature TEBV.

(A) When removed from the tubular mandrel, the TEBV is self-supporting with an open lumen (3 mm internal diameter).

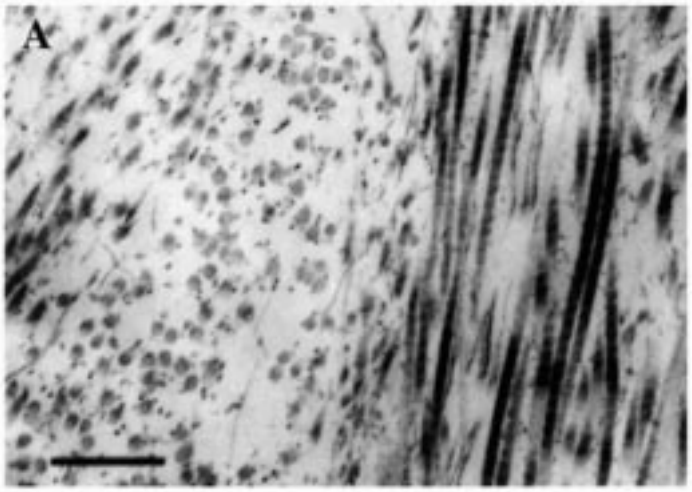
(B) Paraffin cross section of the TEBV wall stained with Masson's Trichrome. Collagen fibers are stained in blue-green and cells in dark purple. Inner membrane (IM)=125 mm, media (M)=320 mm and adventitia (A)=235 mm. Note the endothelium covering the luminal surface of the TEBV.

Reprinted by permission from L'Heureux et al. (19).

exogenous material[19] (figure 1). This prosthesis was shown to have a supra-physiological blood pressure resistance (figure 2) and a histological organization comparable to that of a native artery (figure 1).

The cells used for such a TEBV were endothelial cells (EC), smooth muscle cells (SMC) isolated from human umbilical cord vein using an enzymatic method for EC[24] and the method of Ross for SMC isolation.[25] The fibroblasts were provided by the enzymatic treatment of a small biopsy of human skin [(26)]. Each layer of the vascular wall was thus reconstructed : the intima (composed of EC), the media and the adventitia. In order to obtain an abundant extracellular matrix production, fibroblasts and SMC were cultured in media supplemented with ascorbic acid until they self-assembled into sheet that could be detached from the culture support and then be wrapped around a tubular mandrel.

The mechanical, histological and physiological properties of the TEBV were very interesting. Although completely biologic, this reconstructed blood vessel was highly resistant with a burst strength of over 2500 mm Hg (figure 2B). This resistance is significantly higher than that of the human saphenous vein, considered to be the best biological material for lower limb vascular reconstruction.[27] This impressive resistance is due to the well-organized extracellular matrix composed of collagen fibrils that were oriented in perpendicular directions to



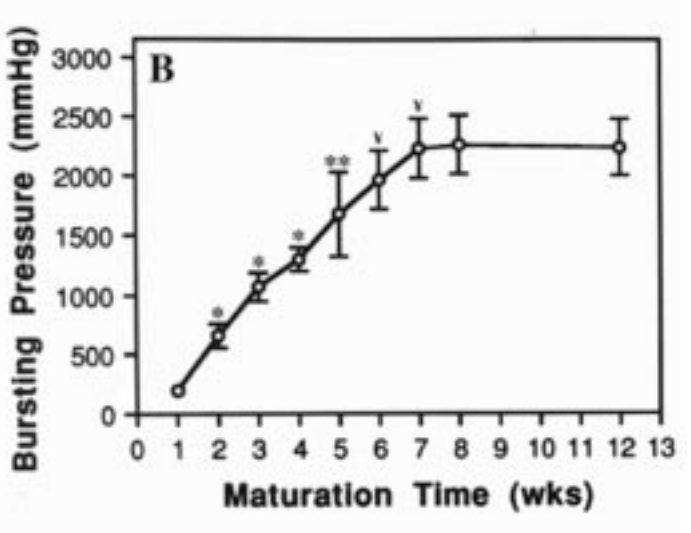


FIGURE 2

(A) Adventitial extracellular matrix ultrastructure observed by transmission electron microscopy. Uranyl acetate and lead citrate staining (scale bar=500 nm).

(B) Burst strength of the adventitia over time of maturation in vitro.

*Significantly different than the precedent point (*p<0.001, **p<0.005, ¥p<0.05) with the Student's t test (n=8 to 13).

Reprinted by permission from L'Heureux et al. (19).

one another in the concentric layers of the adventitia (figure 2A). Moreover, a constant gelatinase activity was measured starting at 2 weeks of adventitia maturation.

Histological and immunohistological analyses of the TEBV showed cells surrounded by a dense extracellular matrix composed of collagen and elastin. Interestingly, desmin, a protein component of the cellular intermediate filaments, known to be lost in culture, is reexpressed in SMC of the TEBV. This result indicates how close to its physiological counterpart our model is. Only quiescent SMC can produce such a molecule.

continued on page 8

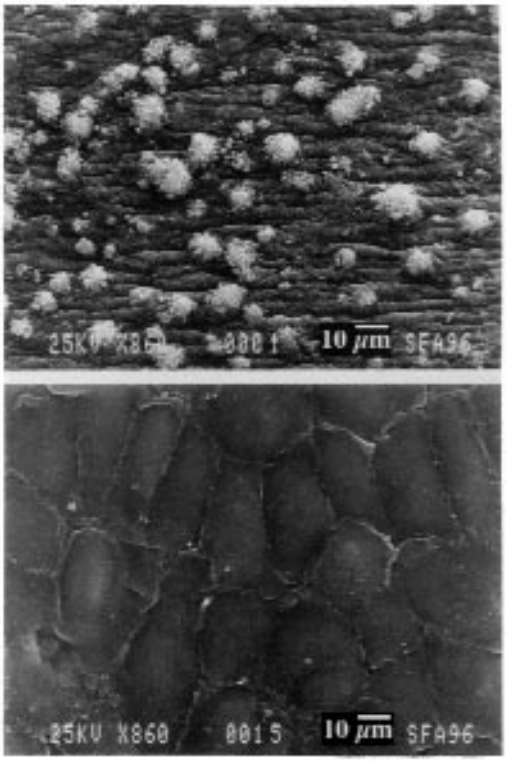


FIGURE 3

Inhibition of platelet adhesion by the endothelium. Scanning electron micrographs of unendothelialized IM (A) promoted platelet adhesion and activation whereas endothelialized IM (B) almost completely inhibited the process.

Reprinted by permission from L'Heureux et al. (19).

The EC seeded on the inner membrane formed a confluent monolayer (figure 3) expressing von Willebrand factor and the cells were able to incorporate acetylated low-density lipoproteins. This endothelium inhibited platelet adhesion in contrast to the non-endothelialized acellular membrane (figure 3). Thus, this endothelium was functional and provided an anti-thrombotic surface.[19]

These human TEBVs were implanted for one week in femoro-femoral interposition in the dog. Because of the xenogeneic situation, the prostheses did not contain EC. The implanted prostheses demonstrated that they could be easily handled and sutured by the use of conventional surgical techniques. A patency level of fifty percent was obtained after one week of implantation and the patent implants were exempt of early tearing or dilatation.[19]

We are convinced in our research group, the LOEX, that this self-assembly approach may offer great opportunities in the field of soft tissue reconstruction. Thus, the next generation of small diameter blood vessel should be of a tissue engineered nature.

We feel that we presently have the most advanced technology in the field of cardiovascular reconstruction. The auto-assembly approach could be easily translated into other areas of this field in order to produce cardiac valves, cardiac muscle, etc. The future of cardiovascular surgery could then be brighter for clinician and patients alike.

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I S A C B
ESSAYS

Degradation of Stent-Grafts after Implantation in Human Beings

Gunnar Riepe, Carsten Heintz
Nabil Chakfé, Herbert Imig

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Albert Einstein died in 1955 because of the rupture of an AAA.[18] One year later in 1956 MEADOX (USA) introduced the first commercially available polyester vascular graft. Autologous graft material with the diameter of the aorta is not available. Artificial vascular grafts are essential for the exclusion of AAA. Two polymers, polyester (Dacron®) and polytetrafluorethylene (PTFE) have proven their biocompatibility and long-term stability in conventional grafts since 1956 and 1975. Open surgery still needs a large abdominal incision for graft interposition. Excessive abdominal scars, which disabled an abdominal approach, inspired Parodi to choose an endoluminal access in the groin for the implantation of a graft in an AAA.[12,13] The success of this operation in 1990 initiated an euphoric development of endovascular devices. Increasing experience in the implantation procedure and numerous improvements of the devices has today lead to acceptable technical success rates with a reasonably low morbidity and mortality.[11]

The primary end point in the treatment of AAA is the prevention of rupture.[23] Considering the risk of re-operations[4,15,20,] the success of the AAA treatment has to last for the rest of the patient's life. Compared with conventional polyester and PTFE grafts, today's "gold standard" for long-term durability of artificial vascular substitutes, none of the modern endovascular devices have been able to prove their long-term durability. Little is known about the long-term duration of stent grafts. This is mainly due to their shorter history and the rapid alternation of device generations within the last 10 years. Loss of the device stability, loosening of the fixation and holes in the cover lead to graft dislocation and endoleakage. These major failures have to be detected by follow-up examinations.[4,23] The retrieval of explants and their examination is necessary to find an explanation for these failures and to learn about changes of the device material which have not been visible to follow-up diagnostic.[1]

The Vascular Surgeons' Laboratory of the General Hospital of Hamburg-Harburg (GPL Gefäßchirurgisches Prüflabor) and the GEPROVAS (Groupe Européen de Recherche sur les Prothèses appliquées à la Chirurgie Vasculaire, Université Louis Pasteur, Strasbourg, France) together have acquired an archive of over 1000 explanted vascular substitutes from many vascular surgeons in Germany, France, Austria and Switzerland. Among these are over 50 endovascular grafts. The majority of the explants were MinTec devices. These 24 Stentor® and 1 Cragg® (MinTec, Bahamas) devices have been examined closely. Their mean duration of implantation was 30±14 months (range 5 to 50 months). After inspection with the bare eye the explants were cleaned and the polyester coating was removed. The frame was examined completely by stereomicroscopy, irregularities were assayed by scanning electron microscopy (SEM) and energy dispersive x-ray microanalysis (EDAX).

The polyester cover

The Stentor® is covered by 0.16mm thin, polyfilamentosous woven polyester, which is fixed to the internal stent by single polypropylene ligatures at both ends of the graft. In early Stentor® devices the woven textile was sewn to a tube using a polypropylene suture. This created a thick, longitudinal seam, which was easily subject to holes due to internal radial force. The holes were commonly covered by chunks of tissue. The occurrence of an endoleakage due to these holes in two cases after 27 and 41 months duration suggests a progression of the separation of the warp yarns. Occasionally small holes of up to 2mm diameter could be seen on the surface in kinked sections of the graft. These were the results of wear of the polyester textile in-between disconnected stent wires. A similar mechanism of damage causing endoleak in a 9 month-old Vanguard® device was recently described in literature. The reperfused AAA ruptured.[8]

The stent

The self-expanding, internal, full-body stent of the Stentor® device is made of Nitinol®. It is a zig-zag shaped approx. 300µm thick wire spiral, held together by multiple 90µm thick polypropylene ligatures, wound 2-3 times around the wires and closed by hand-made, heat-fused knots. The gaps between the zig-zag wire are approx. 6x10mm on the body upper and lower ring as well as on the limbs and approx. 11x22mm on the body middle ring. The proximal anastomosis has small, short barbs for fixation in the arterial wall.

The movement of the stent wires within the polypropylene ligatures lead to breakage of the ligatures.

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The examination of explants showed that many of the broken ligatures were lost. Peripheral embolisation was not noticed. Some ligatures were found caught within the pseudointima. This observation and follow-up x-rays showing a disconnection of the stent frame in-vivo prove that the ligatures do break before the surgical graft explantation. The highest frequency of ligature breakage was observed on the connection zone of the body upper ring and the body middle ring. Here 17% to 44% of the ligatures of the body middle ring were loose.[15] Further zones of ligature rupture were kinks of the graft legs.

The fractures of the stent wire were unexpected. Complete fractures were seen on 7 explants. Corrosive damage of the surface, possibly preliminary to the fracture, was seen on all the examined 24 Stentor® grafts. We have characterized 4 different expressions of corrosive damage: pits, bizarre craters, large deficiencies and cracks or fractures.[5,16]

pits

Circular holes, 10-25 µm in diameter were seen on all examined explants, occurring in single form or multiple, in groups. (Fig. 1a).

bizarre, map-shaped craters

These were approx. 100-180 µm large in diameter and occurred occasionally in 15 of our 22 examined explants after at least 15 months. All grafts of more than 33 months of implantation showed one or more areas with this kind of damage. (Fig. 1b).

large surface deficiencies

Large damages of approx. more than 200 µm size in diameter were seen in 3 cases on explants of more than 32 months of age. Stress crack lines, oblique to the wire axis, can be seen in these defects. All these grafts also showed fractures of the wire in other locations (Fig. 1c).

fractures

Complete fractures of the wire occurred occasionally in 7 explants. One graft showed 4, another 6 fractures. Due to the few cases and the statistical distribution, it was not possible to define a preferred location. The greatest damage was seen on a 9 months old Stentor® graft. A section of the wire was completely disintegrated and fractured. A severe damage of this kind was only seen in one of the 25 examined cases. This explant is therefore regarded separately (Fig. 1d).

The energy dispersive x-ray microanalysis (EDAX) revealed a reduction of the nickel concentration in all 4 types of corrosive damage. In all examined defects the nickel peak vanishes in the corrosion product.

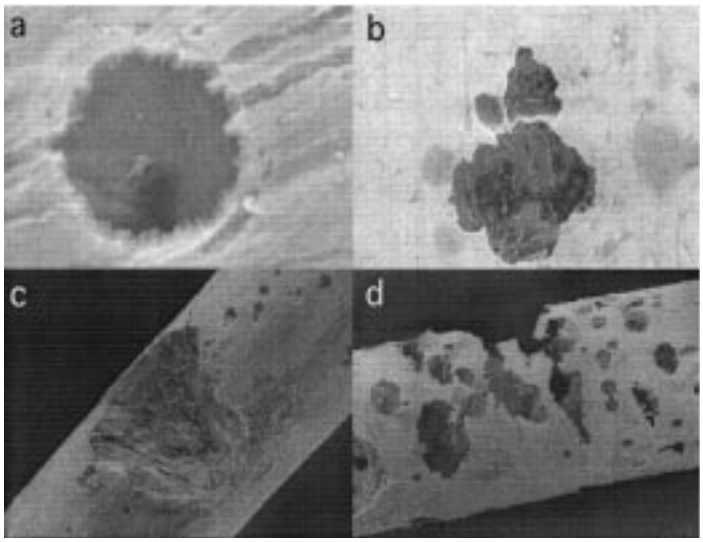

FIGURE 1

Summary and classification of the types of corrosion seen on the stent wire of explanted Stentor® devices: (a) a pit, 10µm in diameter, (b) a bizarre, map-shaped corrosion , (c) a large surface deficiency with a crack beginning on the upper edge, (d) a fracture of the stent wire, in this case with unusually severe corrosion of the surrounding.

Discussion

The Stentor® device (MinTec®, Bahamas), introduced in 1994, was an early endovascular device with a very high, world-wide distribution. The Stentor® device is not available anymore since it's modification to the Vanguard® device by Boston Scientific in 1996. Although the examinations of explanted Stentor® devices therefore have an "archaeological" character, the unexpected findings must be known and regarded during follow-up and the development of future devices. Although the structure of the device is rather unique and not comparable with other stent grafts, the component materials **polyester** and **Nitinol®** are wide spread among endovascular devices.

The materials for the components of endovascular grafts are in general no unique developments. The cover materials (polyester, PTFE) are identical to or derived from those used in conventional grafts. The stent metals (316L stainless steel, Nitinol®) are known from cardiac and peripheral endovascular interventions. The use of conventional graft materials in endovascular devices is due to many years of material experience and the very high standards as well as time-intensive trials required for the introduction of new components so far not used in human beings. Despite over forty years of very good experience in development and clinical use of conventional polyester grafts, our explant retrieval studies have revealed

alterations, which have not been described in literature.[17] These alterations have lead to the rupture of conventional grafts after 10 to 20 years of implant duration requiring re-operation and occasionally causing a patient's death (in our explant archive 3 cases of 41 ruptures). Considering the common origin of the components, the findings made in explant retrieval of conventional vascular grafts may be applicable for endovascular devices too. Regarding our findings on explants we assume the following mechanisms of failure of Stentor® devices. Each material component, the polyester cover, the ligatures and the stent wire plays its particular role in the failure of the device.

The polyester cover

The presence of type III[22] endoleakage is a failure of the graft cover. The polyester cover needs to be thinner than in conventional grafts and must have a low porosity to be blood-proof. These demands are fulfilled by woven fabrics. The fraying of the ends, a problem known in conventional surgery, does not occur in endovascular therapy. The grafts are of predefined length and neither cut intraoperatively by surgeons nor harmed by sutures. A high porosity of the wall is not necessary as external ingrowth on the inside of the excluded, thrombus filled aneurysm in not probable. Histological examinations confirm this.[9] An entirely new problem concerning woven fabrics in endovascular grafts is the missing ability of stiff woven textiles to tolerate over-dimensioned internal pressure. Points of weakness are the longitudinal sutures known from first generation Stentor® devices. The care taken by experienced teams during the implantation is neglected during re-interventions with the intention of repairing endoleaks. The longitudinal suture line and ligatures that fix the cover to the stent are subject to distortion of the warp yarns of the fabric. The resulting holes 1 to 2 mm in diameter allow leakage. A further cause of cover damage is due to the constant movement of the internal stent frame leading to local damage by wear.

The ligatures

The broken ligatures play a role in type I and III[22] graft failure. The pulsatile blood circulation (approx. 35 million beats/year) causes micro-movements of the stent wires. The most movement takes place in the large frames of body middle ring and in sharp bends of the limbs. The twisting of the wires within the ligatures leads to wear and finally fatigue with rupture of the ligatures. The longitudinal strength of the graft body is weakened and further kinking of the main body and limbs is possible. The instability allows the distal anastomosis to slip up causing a secondary leakage. This kinking effects tube grafts and the limbs and the modular connection site of bifurcations. The stent kinking and dislocation can be observed in conventional x-ray examinations during follow-up (Fig. 2). More or less all Stentor grafts show kinking by the time with or without suture break. Distal secondary leakage of tube grafts is also a problem related to improper length of the graft and its fixation in a frequently conical neck. Among 115 Stentor™ grafts implanted in Nürnberg this was observed in 23 patients during follow-up. At least five of these patients already had to be re-operated because of graft leakage.[15]

Theoretically this failure mechanism could be prevented by a better fixation of the distal anastomosis. Furthermore the security of the distal anastomosis depends on the suitability of the distal neck of the infrarenal aneurysm. This has to be regarded by the applying surgeon. An unsuitable, short and conic neck implies a high risk of dislocation. In general bifurcated grafts should be preferred for endovascular grafting. Tube grafts remain important for the repair of arterial injury.

FIGURE 2

X-ray of an explanted Stentor® bifurcation device with distortion of the frame.

The stent wire

The observed corrosion of the Nitinol® wire can be regarded as the beginning of material failure. The stability of the thin, approx. 300µm thick Nitinol® wire is significantly reduced by numerous pits (10-25µm in diameter) and bizarre craters (100-180µm in diameter). The influence of the pulsatile oscillation on the corrosion of the wire must be also regarded. Pietsch could show that the application of cyclic stress on Nitinol SE508 lead to the growth of stress cracks after 90 % of the metals experimental lifetime.[14] We observed stress cracks on 3 explants with large surface deficiencies and a complete fracture of the wire in 8 cases. The importance of the occasional wire fractures for the stability loss of the stent frame is unknown. The effect of severe corrosion and the release of nickel ions within the body is not completely understood.

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Which is the cause of the corrosion?

Pitting corrosion is known as an electrochemical process. An analogy can be found on harbour quay walls. Here adherent bacteria create an electrochemical element with a current flow of metal ions.[2,7] A similar process, activated by adherent fibroblasts, is possible. The bizarre craters posses a morphologic analogy in the destruction of bone by osteoclasts. A local destruction by macrophages, close relatives of the osteoclasts, is assumable.

Does the quality of the surface layer reduce corrosion and is there a difference in the corrosion resistance of the currently used stent metals Nitinol®, Elgiloy® or stainless steel?

In-vitro, the importance of the quality of the titanium-dioxide surface finish is known to have a major influence on the corrosion resistance of Nitinol®.[19] The ceramic-like titanium-oxide layer must resist cracking in the pulsatile oscillation and wear on contact points with other materials. The influence of the thickness of the surface oxide layer is not clear. In-vitro the corrosion resistance of stainless steel is far less than of Elgiloy®, the resistance of Elgiloy® slightly less that Nitinol®. [19]

Does the location of the stent in the body and the polyester coating have an influence on the corrosion?

The ingrowth of polyester in the human body is associated with an inflammatory reaction.[10] It is possible, that a cellular reaction, induced by the polyester, initiates the corrosion of Nitinol® too. As endoscopic examinations of the explants showed, the internal tissue covering (pseudo-intima) was missing in several regions of the graft. Especially in kinked limbs the stent wire, ligatures and textile coating were totally free of tissue covering. The complete ingrowth of uncovered stents in other, less pulsatile locations of the body (e.g. coronaries, peripheral arteries) could be a protection against cellular attack.

Conclusion

The missing ingrowth of the cover implicates the lifetime necessity of the stent as fixation of the cover. Stability loss of the stent sooner or later leads to graft migration. The unexpected corrosion of the Nitinol® wire reminds us of how little medical professionals know about this sophisticated alloy (intermetal). The superelastic and shape-memory characteristics are highly desired for stents in the pulsating aorta. For an acceptable long-term durability the quality of the surface passivation is essential. A close co-operation with very experienced Nitinol® producers has to be established to guarantee high quality standards. Nitinol is not Nitinol!

It is obvious, that an experimental character of the endovascular AAA treatment is still present. Routine follow-ups by CT, colour-duplex sonography and conventional x-ray have to be demanded and continued beyond the first years, possibly for a patient's lifetime.[4,20] Continuous assessment of the pressure in the aneurysm sack is desirable but not yet possible. Therefore the threat of endotension remains present.[3] In our opinion the minimal invasive character is a benefit for high-risk patients (ASA III-IV). Furthermore the option of having a "bridging technique" for symptomatic or even ruptured aneurysms is promising.[6,21] Continuous explant examinations are inevitable to review whether the routine deployment of endovascular AAA devices is defensible.

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I S A C B

DEBATE

An Introduction to a Scientific Debate

Immunological processes are important in tissue valve failure - pro and con.

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The following two pieces were stimulated by a lively discussion of potential immunological mechanisms of tissue heart valve failure initiated by Peter Zilla following a paper entitled "Cellular viability vs. extracellular matrix in the preservation of prosthetic valve function: Comparison of explanted cryopreserved allograft valves, Ross procedure pulmonary autograft valves, and aortic valves from heart transplants" presented by Richard Mitchell at the 1998 ISACB meeting at Wilbad Kreuth. A debate involving these two scientists was arranged at the 2000 ISACB Tucson meeting entitled, "Immunological processes are important in tissue valve failure - pro and con". Dr. Zilla took the pro view that immune mechanisms are important and Dr. Mitchell argued con. The papers were filled with ample data supporting their respective positions and the discussion was active, with proponents of both viewpoints adding interpretation and evidence. The following two pieces summarize the data for and against there being an important immunological component to tissue heart valve failure.

Dr. Zilla and Paul Human advance the following experimental results to conclude that "proof exists for the involvement of immune mechanisms in bioprosthetic heart valve degeneration":

- Conventional cross-linking (with 0.2% glutaraldehyde [GA]) leads to a macrophage response and pannus; higher tissue cross-linking (3.0% GA) is associated with reduced inflammation, including giant cells, and reduced pannus overgrowth.
- Higher cross-linking (3.0% GA) reduces bioprosthetic tissue calcification (relative to that fixed in 0.2% GA), in a subcutaneous rat model of calcification.
- Conventionally-fixed tissue elicits a strong antibody response; this is suppressed by better cross-linking.
- Rabbits immunized with Freund's incomplete adjuvant - containing homogenates of porcine aortic wall tissue fixed in 0.2% but not 3.0% -showed high quantities of circulating specific antibodies.
- Subcutaneously implanted 0.2% GA-fixed aortic wall tissue incubated with immune serum had higher calcification than those treated with pre-immune sera.

Dr. Mitchell acknowledges that "valve tissues are indeed antigenic and capable of eliciting an immune response including antibodies and antigen-specific T cells", and counters with the following observations:

- Degeneration of cryopreserved (but not fixed) allograft valves is not associated with inflammatory cells, and has features that are typical of ischemic/autolytic injury.
- Macrophages are unusual within the cusps of bioprosthetic valves.
- Immune injury in other systems does not typically result in matrix degradation or calcification, especially in the absence of substantial macrophages.
- Immune-mediated valvular injury has morphological features different than from associated with tissue valve failure.
- Heart valves from clinical and experimental heart transplants that have undergone massive cellular myocardial rejection have minimal inflammation and are free of injury.

Dr. Mitchell postulates two potential reasons for the lack of observed rejection in heart valves: 1) excessive shear forces that overwhelm the leukocyte-endothelial adhesion binding forces, and 2) a unique pattern of antigenic expression on valve endothelium (including the absence of ABO blood group markers) that is different from expression in the remainder of the circulation. Valves are also different in their relative or complete lack of a deep vascular network. Presumably, they are sufficiently thin to be adequately perfused by the surrounding oxygenated blood.

What is my point of view? I firmly believe that "valve tissues are indeed antigenic and capable of eliciting an immune response including antibodies and antigen-specific T cells". Moreover, I do not summarily dismiss a possible role for immune responses in the failure of bioprosthetic and other tissue heart valves. However, I am unaware of direct clinical or experimental evidence implicating immune mechanisms in the failure of tissue heart valves. This would take the form of appropriate morphologies in failed specimens with evidence that valve destruction or functional loss is immune mediated. As suggested by Rick Mitchell, a carefully designed and executed set of experiments that satisfied (or refuted) the immunological variant of Koch's postulates (the conditions that proved the role of microbiologic organisms in infectious disease in the 19th century) would be a key step in resolving this debate. Clearly, this is an important and fertile area of future investigation.

PRO

Immunological processes are important in tissue valve failure.

The Possible Role of Immune Responses in Bioprosthetic Heart Valve Failure

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From the beginning of bioprosthetic heart valve research, crosslinking aimed at the suppression of immunogenicity. All other effects of fixation are less essential. Tissue degradation, for instance, is less pronounced in native homografts than in fixed xenografts, demonstrating that tissue does not need to be "stabilised" against physiological degradation processes. Similarly, sterilization was easily achievable by antibiotic cocktails rather than fixatives. Thus, the initial choice of formaldehyde as a tanning agent of bioprosthetic heart valves had one single goal: the masking of antigens through crosslinking. When these early heart valves failed[1,2] [Yarbrough, 1973 #1578] [Rosenberg, 1956 #1579; Rosenberg, 1957 #1580; Rosenberg, 1958 #1581; Rosenberg, 1961 #1582; Rosenberg, 1962 #1583] mainly due to inflammatory degradation,[3,4] it was concluded that formalin was incapable of sufficiently masking xenograft antigenicity. As a consequence, glutaraldehyde was adopted as a fixative because of its perceived ability to irreversibly cross-link proteins[5] [Cheung, 1982 #1586; Cheung, 1982 #1587] and thus render them non-immunogenic. This perception of thoroughly suppressed antigenicity in xenograft heart valves remains prevalent in the clinical literature, although scientific proof has never been provided. One reason for this may well lie in our clinically driven experience of the immune response. As cardiac surgeons, we were not only the main force behind the early development of bioprosthetic heart valves, but were also naturally pre-conditioned to the vigorous rejection patterns of transplanted hearts or lungs. Therefore, we readily disregarded the rather low levels of antigen expression and the mild inflammatory infiltration of prosthetic tissue as irrelevant. On top of this came an almost emotional bias against the use of glutaraldehyde in the 1980s, when its ability to intrinsically augment bioprosthesis calcification[6] [Weissenstein, 2000 #1731-Citation 1=Golomb] distracted from the main purpose of glutaraldehyde treatment, namely the suppression of an immune response. What we should have kept in mind throughout was the fact that the failure of bioprosthetic heart valves is a protracted process occurring over many years rather than days. Thus, we should have looked at even the mildest of all immune responses with the magnifying glass of time.

With the beginning of the 1990s awareness towards a role for low-level immune processes in the degeneration of tissue valves began to increase, but has only recently gained momentum.[7-10] It certainly also helped that a similar debate began to change the perceptions regarding homograft failure.[11-13] All these studies emphasized that immune mechanisms contributing to bioprosthetic heart valve failure may rather act through mild and protracted mechanisms augmented by time than through acute and dramatic events. It is exactly this "smoking gun" of dramatic events, however, which the opponents of a role of immune mechanisms in xenograft failure keep asking for. In their eyes, there should be a direct proof of a distinct rejection-type inflammatory graft infiltration in degenerated valve explants. As justified as this demand would be in living organ transplants, it ignores the limitations that crosslinking imposes on conventional rejection patterns, without concomitantly eliminating the possibility of rejection all together. Crosslinking preserves collagen in its intact fibrillar conformation[14] Peter pse check this ref – Mona 1 which is only susceptible to degradation by the interstitial collagenases (MMP-1 and MMP-8)[15] [Jeffrey, 1988 #1733]. While activated T lymphocytes have been shown to induce contact-dependent expression of MMP-1 by macrophages, lymphocytes themselves preferentially secrete MMP-2, MMP-9 and MMP-14.[16,17] Since the lymphocyte-derived MMPs have a limited capacity to degrade collagen,[16,17] macrophages (secreting MMP-1) are the obvious mononuclear cell type capable of infiltrating crosslinked tissue. Nevertheless, as degradation studies with collagenase which cleaves fibrillar collagen demonstrate, crosslinking with higher glutaraldehyde concentrations provides increasing protection from degradation. Therefore, it is easily conceivable that incomplete fixation at the low glutaraldehyde concentrations used in contemporary bioprosthetic fixation further facilitates tissue degradation by macrophages. Since the interaction of macrophages with foreign material needs to result in degraded molecules which are presentable through the MHC class II receptors on the macrophage surface, the low-grade crosslinking of tissue valves augments their immunogenicity on two fronts - once through incomplete antigen masking and once through facilitated antigen presentation.

Therefore, in view of the central role macrophages would need to play in the immune recognition of bioprosthetic heart valves, macrophage adherence to the prosthetic surface is an obvious prerequisite for all these subsequent events. In order for macrophages to adhere to blood surfaces, however, low shear stresses are required[Sprague, 1999 #1736]. One argument brought forward against a crucial role of macrophages in tissue valve failure was the wide-spread belief that turbulencesturbulence occurs at the outflow surface of heart valves which causes particularly high shear forces. Bellhouse and Talbot[18] demonstrated that the flow across an aortic valve is laminar, and used a quasi-steady laminar model to describe the pulsatile vortex motion

within the sinus. From their calculations the shear stresses within the sinuses and on the fibrosa side of the leaflets are substantially lower than those experienced on the ventricularis side. This theoretical consideration correlates precisely with histological observations of our own explants and those of others: . Infiltrating Infiltrating macrophages are found on the outflow, surface rather than the inflow surface of leaflets, the protected aortic wall portion of stented valves as well as the lower sinus portion and adventitial surface of root prostheses. In contrast, macrophages are practically always absent on the blood surface of the aortic wall of root prostheses[19] where shear forces are high. Concomitantly, it is not surprising that the highest macrophage density is regularly found in completely flow-protected areas such as the interface between the prosthesis and surface thrombi[19] [Maxwell, 1989 #1738].

All these arguments for a crucial role of macrophages in bioprosthetic heart valve failure may be countered by the insistence that we are merely dealing with a foreign-body type reaction. It is undisputed, that such a reaction may well represent the initial event in an immune response against bioprosthetic tissue. In contrast to inert foreign bodies such as synthetic implants, however, macrophages of such a foreign body reaction are principally capable of presenting bioprosthetic antigens to the immune system of the host, even more so in under-crosslinked tissue. It would therefore be a gross underestimation of the potential impact of a macrophage reaction on bioprosthetic heart valve degeneration if one simply explained the phenomenon as a persistent foreign body reaction.

All these arguments would remain in the realm of speculation without supporting evidence for such a proposed macrophage-driven immune response effect on actual valve failure. Therefore, if bioprosthetic tissue is indeed insufficiently crosslinked as we and othersothers and we suspected and, moreover, if there iswere a link between poor antigen masking and bioprosthetic degeneration, the following phenomena would need to be observed in optimally crosslinked tissue:

- *Reduced recruitment of inflammatory cells and the mitigation of tissue erosion*
- *The alleviation of pannus outgrowth*
- *The detection of specific circulating antibodies* in response to mildly crosslinked tissue and the ability to suppress this specific response through higher crosslink density
- Last not least, *a link between a specific immune response and bioprosthetic calcification would need to be established.*

1) SUPRESSION OF INFLAMMATORY INFILTRATION AND TISSUE EROSION THROUGH HIGHER CROSSLINK DENSITY

In spite of the regular observations of both macrophage dominated tissue inflammation and insufficiently suppressed immunogenicity, a direct link between the two was suspected rather than proven [Geha, 1979 #1745; Magilligan, 1980 #1729;(10). Other than the brief observation of mitigation of inflammation through higher GA concentrations [Sherman, 1984 #1713] no study has yet provided evidence yet, that better crosslinking is capable of preventing inflammatory infiltration of bioprosthetic heart valves. In order to prove the previously suspected link between immunogenicity and tissue inflammation, our group correlated the effect of enhanced crosslinking with inflammation. On the tissue side, both aspects affecting a macrophage-dominated immune response were addressed, namely antigen masking and tissue degradability. In analogy to Nimni's work in the 1980s, enhanced crosslinking was achieved through long-range diamine extensions on top of increased GA concentrations. When GA concentrations were increased from 0.2 to 3.0% without additional long-range diamine extensions, shrinkage temperature (SrT°), resistance to protease digestion and the tensile modulus increased significantly[20] (PZ: JBMR: submitted). The introduction of the additional diamine step to 3.0% GA fixation resulted in a further significant and more distinct increase in these crosslink parameters. When whole root prostheses were implanted in the sheep model, crosslink density and resistance to tissue degradation correlated with inflammatory infiltration and tissue erosion (20) (PZ:JBMR: submitted). In conventionally fixed, low-glutaraldehyde-treated porcine valves, in which we had demonstrated a low crosslink density, the inflammatory reaction was distinct, particularly in the fibrosa of leaflets. Infiltrating cells were primarily identified as macrophages. This preference for the fibrosa of leaflets was in accordance with other studies which also found collagen-destructing macrophages primarily in the fibrosa.[21] In contrast, inflammation and tissue erosion could practically be eradicated when GA concentrations were increased and additional long-range bonds introduced (Figure 1). While spots of foreign body giant cells were occasionally still found in the adventitial area of this highly crosslinked tissue, leaflets were completely free of inflammation.[20] Thus, better crosslinked tissue indeed led to a reduced recruitment of inflammatory cells and a mitigation of tissue erosion.

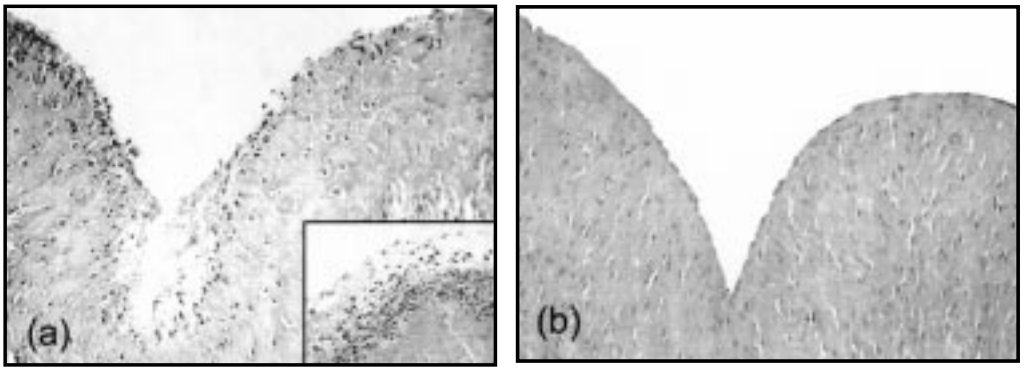

FIGURE 1

Histological sections of porcine aortic root leaflet following 6 weeks of implantation in the sheep descending aorta. (a) Fibrosa surface of leaflet fixed with 0.2%GA showing distinct inflammatory infiltration and erosion. (b) Fibrosa surface of leaflet fixed with 3.0%GA and enhanced with L-Lysine free of infiltration and/or erosion.

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2) MITIGATION OF PANNUS OUTGROWTH THROUGH SUPPRESSED INFLAMMATION IN WITH HIGHER CROSSLINK DENSITY THROUGH SUPPRESSED INFLAMMATION

Although the outgrowth of anastomotic intimal tissue onto the prosthetic surface is a multifactorial event, inflammation certainly contributes towards sustaining the growth signal for the myointimal tissue. With the emergence of stentless valve prostheses, myointimal host tissue-overgrowth became a significant reason for concern, due to the fact that the suture line is closer to the cusps than in stented valves. Yet, overgrowth of this so-called pannus tissue is also a feared complication of stented heart valve prostheses. In the mitral position, for instance, 20% of failed tissue valves showed pannus overgrowth on the inflow aspect.[14]

In order to tie insufficient masking of antigens in bioprosthetic heart valves to myointimal pannus growth, however, we needed to correlate crosslink density with macrophage activation and pannus outgrowth. Since spreading of macrophages on prosthetic surfaces is not only linked to a higher ability to degrade the prosthetic material, but also with an increased secretion of IL-1ß, TNF-a and IL-6, it seemed reasonable to first assess macrophage spreading in relation to crosslink density. Just Simply by increasing the glutaraldehyde concentration from 0.2% to 3.0%, we observed a significant reduction of the spreading area of macrophages from 243.2 +± 10.8mm2 to 144.3 +± 54.5mm2 (p = 0.023)[22] on thoroughly detoxified tissue [Zilla 1987]. Therefore, this phenomenon can certainly not be attributed to the cytotoxic effect of glutaraldehyde. When entire porcine roots were subsequently implanted into sheep, the pannus length after 6 six weeks and 6 six months correlated with both crosslink density and foreign body giant cell density accumulated at the interface of pannus tissue and prosthetic material (Figure 2). When the correlation between giant cells and cross-sectional pannus area or pannus length at this interface was examined, a direct correlation was confirmed between crosslink density and giant cell index. Although the density of macrophages correlated directly with that of giant cells, the clear delineation of giant cells as well as their equal ability to degrade and present bioprosthetic antigen made them easier targets for assessment.

In view of the dramatic mitigation of pannus outgrowth through better crosslinking of tissue, it seems important to extend the alert-list of possible immune mechanisms contributing to bioprosthetic heart valve failure to anastomotic pannus outgrowth, which has the potential of immobilising entire leaflets.

3) PROOF OF SPECIFIC CIRCULATING ANTIBODIES AND THEIR SUPPRESSION THROUGH BETTER CROSSLINKING

It has been known for a long time that the low-dose glutaraldehyde treatment applied to the fixation of bioprosthetic heart valves only reduces immunogenicity but does not abolish it [Bajpai, 1983 #1709; Salgaller, 1985 #1710; Lowe, 1993 #1711]. The low-grade fixation used in commercial valve preparation fails to significantly alter

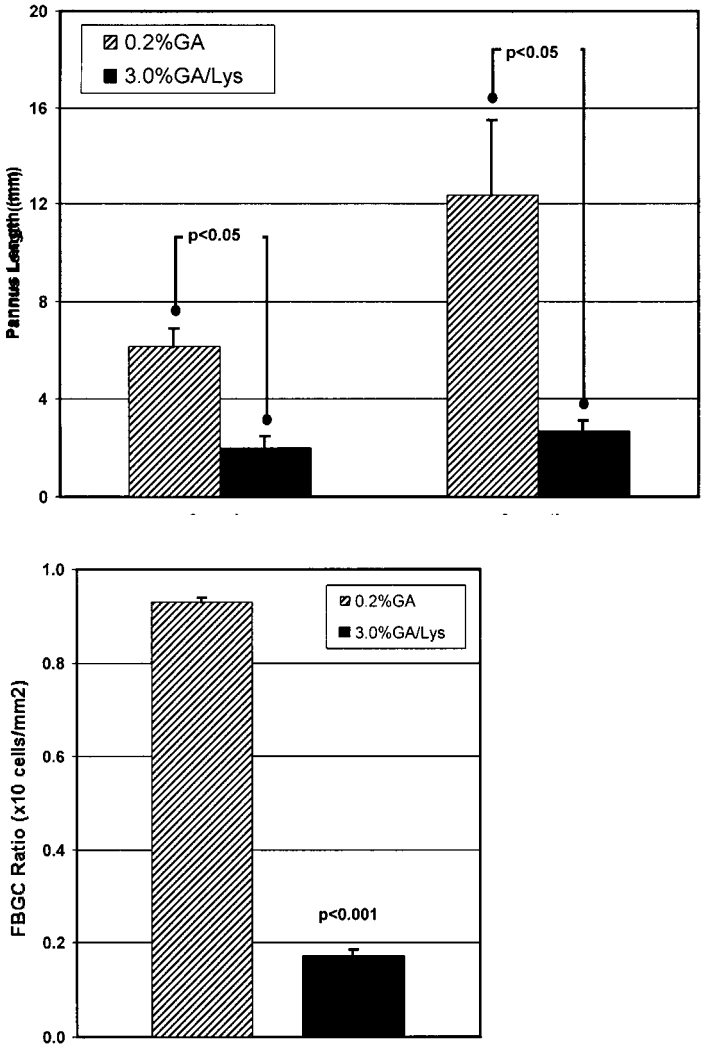

FIGURE 2

Pannus overgrowth associated with the distal anastomosis of porcine aortic roots implanted in the sheep descending aorta. Shaded bars represent tissue fixed with 0.2%GA and solid bars represent tissue fixed with 3.0%GA and enhanced with L-Lysine. (a) Pannus length after 6 weeks and 6 months of implantation. (b) Ratio of foreign body giant cells to pannus area after six weeks of implantation (means ± std error).

membrane bound receptors or structural glycoproteins[23] [Carpentier, 1969 #1740], thus eliciting both T-cell and humoral immune responses.[24] Various groups have demonstrated the generation of specific antibodies against pericardium fixed in 0.2% glutaraldehyde (24, 25) [Nimni, 1988 #1685; Dahm, 1990 #1585]. The main criticism against these studies was the fact that subcutaneous administration of immunogens hardly reflected the low-grade exposure to the immune system of intracardially placed bioprosthetic tissue. Yet, circulating antibodies were regularly also detected in human recipients of bioprosthetic heart valves. In patients with porcine valves, specific antibodies were detected in as many as 58%.[26] In homograft patients, 82% of recipients demonstrated a specific antibody response, 92% of which produced IgG against HLA class I antigens.[11] In view of the role preformed antibodies play in

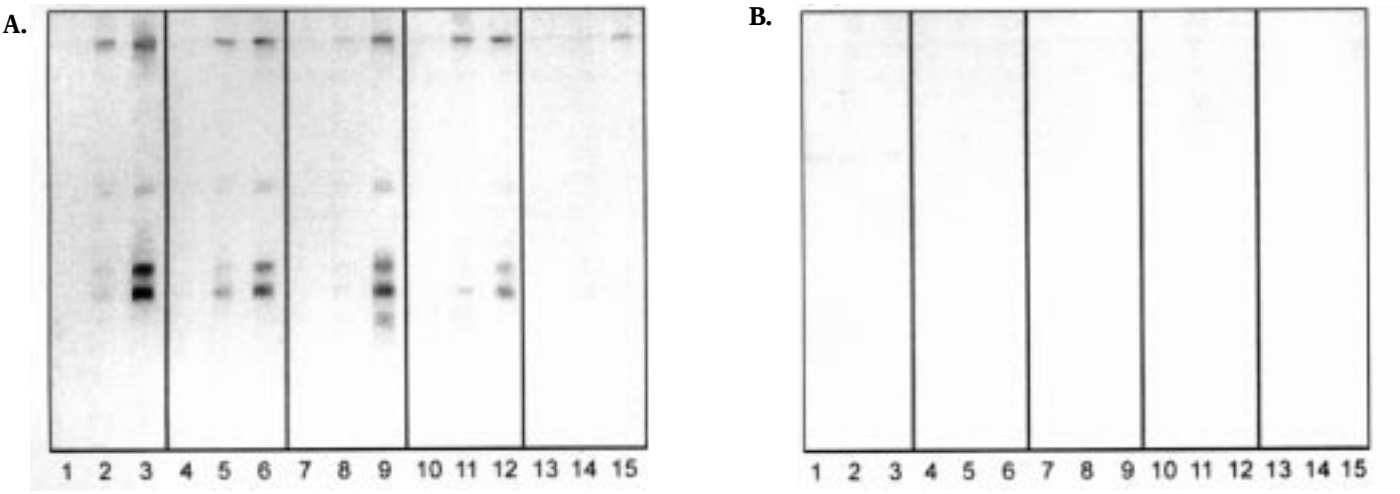

FIGURE 3

Western blots demonstrating circulating IgG with specificity for porcine aortic wall extract in immunised New Zealand White rabbits (n = 5 per group). Lanes 1, 4, 7, 10, 13 are control sera from individual animals prior to immunisation; Lanes 2, 5, 8, 11, 14 are sera two weeks after immunisation and prior to boostering; Lanes 3, 6, 9, 12, 15 are sera one week after boostering. (a) Sera from animals immunised and boostered with 0.2%GA porcine aortic wall homogenates. (b) Sera from animals immunised with 3.0%GA plus L-Lysine porcine aortic wall homogenates. Positive controls are not shown but were confirmed to be identical on both blots.

the opsonization of antigen, the detection of preformed IgG antibodies in 67% of patients may certainly also have significance.[9] Together, these IgG antibodies bind to the poorly masked tissue thereby inducing monocytes to both take up residence and differentiate into macrophages.[27] Although it is increasingly likely that a broad range of immune mechanisms contributes to the failure of biological heart valve prostheses, the ability to augment inflammation through antibodies alone is a strong argument for the suppression of a specific immune response.

Since better crosslinking had previously resulted in mitigated inflammation, we investigated the ability of better crosslinked tissue to suppress a specific antibody response. The immunisation was done in New Zealand White rabbits were immunised, using emulsions of Freund's incomplete adjuvant and homogenates of porcine aortic wall tissue which was either fixed in 0.2% GA, or 3.0% GA followed by enhancement with long-range diamine bridges (L-Lysine). Rabbits were subsequently boostered with the same antigens, and the presence of specific immunoglobulin examined by Western blot analysis against extracted native porcine aortic wall tissue. While rabbits immunised with 0.2% GA-fixed tissue showed a significant antibody response, there were practically no detectable specific circulating antibodies in the enhanced fixation group (Figure 3). Although the blots specifically examined the IgG response rather than an IgM response, minor bands were seen in pre-immunisation sera in both groups. Some of these bands were seen to amplify upon immunisation and boostering, although the bulk of the response was limited to antigen without associated preformed IgG. The dramatic amplification of the primary response subsequent to boostering clearly denotes a secondary response, suggesting acquired immunity with memory.

4) PROOF OF CAUSATIVE CONNECTION BETWEEN SPECIFIC, CIRCULATING ANTIBODIES AND TISSUE CALCIFICATION/ DEGENERATION

The most crucial step in proving an involvement of immune responses in tissue valve failure that goes beyond inflammatory destruction would be the demonstration that specific antibodies facilitate calcification. In the past few years, both Vincentelli's and our own group have provided this proof independently. Vincentelli et al demonstrated in 1998 (7) that xenogeneic tissue fixed in 0.65% GA calcified 35 times more in the same animal model compared to fixed autologous tissue. Our own group found a direct correlation between crosslink density, inflammation and calcification in both the rat.[28] and the sheep model.[19] After six weeks of implantation of entire root prostheses in the sheep model, overall, bioprosthetic calcification of 0.2% GA-fixed aortic wall tissue was reduced by 90.0% ($p = 0.004$) and 53.7% ($p < 0.001$) after 6 weeks and 24 weeks, respectively,, if crosslinking was increased through fixation in diamine-enhanced 3% glutaraldehyde. There was a high correlation between tissue calcification and the four crosslink density parameters - shrinkage temperature, digestion resistance, tensile modulus and free amines, - after both six weeks (correlation coefficients: -0.9767, -0.9460, -0.9820, and 0.9964 respectively) and 24 weeks of implantation (-0.8954, -0.8390, -0.9066, and 0.9871). of implantation. However, as much as these data strongly support the notion that calcification correspondingly decreases with decreasing inflammation and increasing masking of antigenicity, the multifactorial nature of tissue mineralisation makes it likely that higher crosslinking itself, rather than the mitigation of an immune reaction alone, may contribute to the lower calcification. Therefore, a more direct connection between an immune response and tissue calcification was necessary. By demonstrating threefold higher calcification in aortic wall tissue, which was incubated in serum containing high

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levels of antibodies against the bioprosthetic tissue, (P3-27) we were eventually able to prove a direct immune-mediated link to calcification. In this experiment aortic wall coupons were fixed with 0.2% glutaraldehyde and detoxified with urazol. After perforation in order to increase their surface area, they were incubated with either immune serum or the corresponding control pre-immune sera obtained prior to immunisation of the same animals. Immunisation was achieved by subcutaneous injection of emulsions of tissue homogenates and Freund's incomplete adjuvant. Following serum incubation, Tthe tissue was then implanted subdermally on the back of unrelated New Zealand White rabbits (n = 8) for a period of 3 weeks. After the coupons were explanted, tissue calcium levels were determined by atomic absorption spectroscopy. Tissue calcium was increased in all five immune serum-treated replicates (range 61.8% to 431.2%, mean 225.9% ± 73.2) when compared to control samples treated with pre-immune sera. Overall, the mean calcium level was significantly increased ($p << 0.0001$) when tissue was treated with immune sera (66.0µg/mg ± 10.0 versus 22.6µg/mg ± 4.8 in controls – Figure 4). Graft specificity of immune sera was confirmed by Western blot analysis.

Conclusion

- Better cross-linking resulted in significantly reduced inflammation and tissue erosion
- Conventional crosslinking leads to both a strong macrophage/foreign body giant cell response underneath pannus tissue and distinct pannus outgrowth. Higher cross-link density significantly mitigates both the mononuclear reaction and the anastomotic tissue overgrowth.

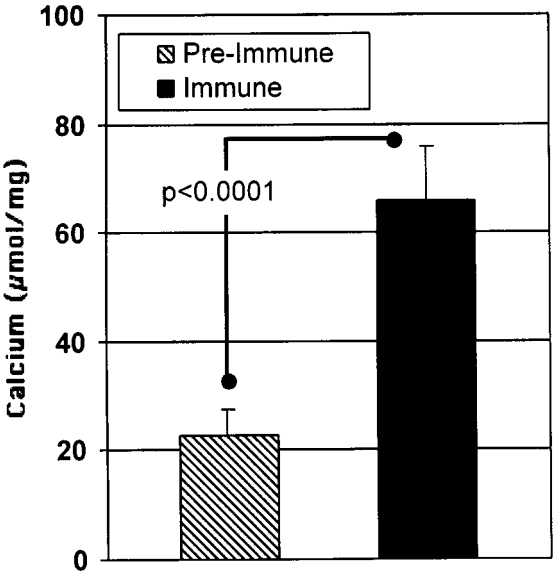

FIGURE 4

Porcine aortic wall calcification as determined by atomic absorption spectroscopy in samples implanted subdermally into New Zealand White rabbits following preincubation with either non-immune control sera (Shaded bar) or sera from animals immunised with 0.2%GA fixed porcine aortic wall tissue and shown to contain graft specific antibody (means ± std error, one way analysis of variance).

- Cross-link density correlates inversely with calcification: - the higher the cross-link density, the lower the mineralisation.
- Conventionally fixed tissue elicits a strong, specific antibody response which can be suppressed by better cross-linking
- Tissue exposure to specific antibodies facilitates calcification.

In summary, we are still in a phase of observation rather than elucidation. However, although the mechanisms of immune-degeneration of heart valve prostheses are far from being established, sufficient proof exists for the involvement of immune mechanisms in bioprosthetic heart valve degeneration to date, to break the existing taboo.

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CON

Immunological processes are important in tissue valve failure.

Don't Blame the Lymphocyte: Immunologic Processes are not Important in Tissue Valve Failure

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Glutaraldehyde-fixed bioprosthetic valves are mainstays of modern valve replacement surgery (40% of valve replacements world-wide); cryopreserved allografts are also being increasingly utilized, particularly in the setting of surgery for endocarditis.[1] The promise of cryopreserved valves is the potential for valve interstitial cell viability with long-term matrix production and remodeling. However, it is increasingly apparent that while cryopreserved valves do exhibit somewhat better durability than fixed non-viable valves, both types of bioprostheses nevertheless eventually undergo degeneration and failure (<50% fixed valve survival at 10-15 years; 50-90% allograft survival at 10-15 years).[2-4]

Fixed valves, either of porcine valve or bovine pericardial origin, typically fail due to intrinsic calcification and/or non-calcific degeneration of the extracellular matrix.[5] In the absence of physiologic repair mechanisms in these non-living valves, it is not difficult to understand how progressive matrix degeneration leads to bioprosthesis failure. Moreover, calcification in these valves is most likely the direct consequence of calcium-phosphate nucleation on matrix elements or phospholipid-rich membranes of cell remnants in the absence of normal calcium homeostatic pathways.[5] Although occasional recipient macrophages may be identified on these valves, calcification notably occurs independently of mononuclear cell infiltrates.[5]

Pathologic changes in failed cryopreserved valves characteristically include a loss of cellularity (endothelial and interstitial cells), homogenization of the valvular trilaminar architecture, focal calcification of matrix elements or cellular remnants, and a generally sparse mononuclear inflammatory cell infiltrate (predominantly macrophages) 6. The changes are evident as early as 2-8 days following implantation, with loss of cell viability likely due to the vagaries of cryopreservation with subsequent autolysis; the histopathologic changes are well established by 2 months.[6] Long-term allograft valves (up to 9 years) show progressive deterioration with calcification, cuspal hematomas, and pannus overgrowth, but notably no neovascularization or increased matrix production.[6]

It seems reasonable to assume that the pathologic changes experienced by fixed or cryopreserved valves are largely attributable to primary degenerative processes in non-viable tissues 5,6. However, immune-mediated injury (rejection) has also been invoked to explain the pathologic features, as well as the eventual failure of fixed and cryopreserved bioprosthetic valves; an obvious corollary to this proposal is that immunosuppressive agents may help to ameliorate valve failure.[7-9] Hopefully, this essay and its companion piece will help shed some light on the salient issues, and engender a more informed discussion.

First, it isn't at all clear what is meant to "reject" fixed, dead valves; rejection denotes an inflammatory process leading to tissue death and is attributable to a specific immune response.[10] Certainly, matrix elements can be degraded by proteolytic enzymes released by antigen non-specific cells such as neutrophils or macrophages; however, the requisite cells are not characteristically associated with failing valves to an extent sufficient to explain the matrix degradation. Moreover, neither antibody- nor lymphocyte-mediated immune injury can induce such matrix degradation, at least not without the contribution of macrophage or neutrophil effector cells. In fact, fixed tissue - lacking necessary co-stimulator molecules - is incapable of maintaining a helper T cell response.[10] While calcification has been postulated to have an immune-mediated mechanism,[9] this would constitute a new and previously unrecognized form of immunologic injury. Rather, calcification is much more likely to be a consequence of mineralization of dead and degenerating tissues.[5] Indeed, the most common form of valvular calcification is senile calcific degeneration of the native aortic valve - which is certainly not an auto-immune disease. The well-recognized greater incidence of calcific failure in valves implanted in the pediatric population[11] is probably a consequence either of the increased calcium metabolism in that group or perhaps an increased frequency of circulating osteogenic precursor cells,[12] rather than any intrinsic differences in immune reactivity.

Secondly, and as will be discussed in greater depth below, there is absolutely no evidence that the observed loss of cellularity and architectural degeneration of cryopreserved allograft valves can be attributed to immunologic responses. Since cryopreserved valves are at least potentially viable at the time of implantation, and the concept of rejection therefore has some theoretical foundation, the bulk of the remaining comments will be focused on the pathways of cryopreserved allograft valve failure.

It is clear from a number of excellent papers that valve tissues are indeed antigenic and capable of eliciting an immune response including antibodies and antigen-specific T cells.[13-17] Indeed, it would be unusual if tissue expressing foreign histocompatibility antigens did not elicit a detectable response. Nevertheless, it is important to understand that:

i) tissue immunogenicity is not equivalent to immunologically mediated dysfunction

ii) although mononuclear inflammatory cell infiltrates are characteristically associated with rejection, the presence of such cells does not necessarily denote a rejection pathogenesis.

Indeed, with regards to the first point, some sort of immunologic variant of Koch's postulates should be satisfied before one jumps to the conclusion that immune injury is the basis for valve failure.[18] Essentially:

1) Antigen-specific elements (antibodies or cells) should be directly associated with failing valves. Moreover, isograft control experiments should be performed to demonstrate that any antibodies or cells on implanted valves are not simply present because of surgical manipulation or aberrant flow conditions.
2) Antibodies or cells from animals that have dysfunctional transplanted valves should cause valve failure by transfer into an appropriate second host (i.e., haplotype-matched to the original valve donor).
3) Adoptively transferred cells or antibodies should be recoverable from the failed valve in the second host.

Animal models provide an excellent way to satisfy such postulates. However, the appropriate experiments have not yet been reported. In fact, in animal and human studies,[13-17] workers have demonstrated only that allograft valves are immunogenic and that immunosuppressants (e.g., cyclosporine) may modulate the ability to generate an immunologic response.[13] *There exists no evidence that valve destruction or loss of function is mediated by immune elements, or that blockade of immune mechanisms by immunosuppression prevents that outcome.*

The second point, i.e., that mononuclear cells do not equate to rejection, is particularly germane in regards to the conclusions reached by a number of workers; mononuclear inflammatory cells in association with an allograft aortic valve have been interpreted to represent cell-mediated "rejection".[7] The criteria that permit the presence of mononuclear cell infiltrates in valves to be interpreted as immunologically specific rejection are not stated. It is not even possible to unequivocally conclude that these valvular "infiltrates" are above background levels; as we demonstrated[6], non-implanted human valves have a low-level, diffuse population of macrophages and T cells present as normal cellular constituents. In all papers thus far purporting to demonstrate immune-mediated injury, no evidence is presented to suggest that the mononuclear inflammatory cells are causing functional valve degeneration, or again that immunosuppression might be efficacious in modulating the inflammatory response.

Another significant point is that well-characterized examples of valvular injury -universally accepted as immune in origin - exhibit morphologies markedly different than that seen in failed cryopreserved allografts. These include *rheumatic valvular disease* and *Libman-Sacks endocarditis,* where tissue-specific antibodies or antigen-antibody complexes, respectively, deposit and lead to the accumulation of Fc-receptor-bearing *antigen-non-specific* inflammatory cells (neutrophils and macrophages).[19,20] The inflammatory cells then engender valve destruction by release of proteases. With endothelial cell injury and dysfunction, there is also platelet-fibrin deposition, but death of the interstitial connective tissue cells is not a hallmark in these diseases. The long-term sequelae of these pathologies are post-inflammatory transmural scarring and neovascularization, findings that are likewise not seen in long-term failed cryopreserved valves. Carcinoid heart disease is yet another valvular pathology with characteristic histologic changes (intimal smooth muscle cell proliferation and matrix production) attributable to toxic mediators and/or cytokine growth factors.[21] In none of the above entities do we see the pathology described in failing cryopreserved allograft valves, i.e., the loss of valve cellularity and architectural degeneration.

Rather, examination of explanted cryopreserved allograft valves days to years following implantation suggests that factors related to harvesting, handling, ischemic time, freezing, and thawing are most responsible for the loss of cellular viability.[6] The absence of significant neutrophilic or mononuclear cell infiltrates in explanted cryopreserved valves, *even at time points where clear-cut architectural changes and loss of cellular staining occur*, leads to the inescapable conclusion that immunologic phenomena cannot be causally implicated in most allograft degeneration. Moreover, in heart transplant patients where immunologic phenomena unequivocally caused cardiac allograft failure (overwhelming rejection or allograft arteriopathy), evidence of immunologic injury to the valves was not seen (i.e., no valvular scarring or loss of cellularity).[6] The argument that immunosuppression in these transplanted hearts has somehow modulated the immune injury does not make sense since the hearts nevertheless suffered overwhelming rejection pathology. Rather, it is likely that the long-term viability of the valves in transplanted hearts is attributable to the brief ischemic time from cardiac harvest to implantation (without cryopreservation), and perhaps to some intrinsic resistance to typical allograft rejection mechanisms.

Why doesn't rejection occur in tissue heart valve substitutes?

The results from orthotopic heart transplants are provocative and worth revisiting. The lack of valve destruction in failing heart transplants clearly suggests that allogeneic valves are in some way resistant to allogeneic injury, at least those mechanisms involved in cellular rejection and arteriopathy. However, it is not clear whether this resistance is attributable to some combination of high flows over the valve surface, the normal lack of valvular microvasculature, low alloantigen expression, and/or lower expression of relevant adhesion or co-stimulator molecules.[6] Basically, are valves protected by local hemodynamic forces, or are they somehow intrinsically immunopriveleged?

Adhesion molecules normally confer leukocyte-endothelial adhesion in post-capillary venules, where the shear forces are typically 1-3 dynes/cm.^{2} Experimental models demonstrate that mononuclear cell adhesion can occur in up to shear forces of 5-15 dynes/cm,^{2} while neutrophils can bind under shear forces up to 40 dynes/cm;^{2}

firm adhesion requires several seconds to minutes.[22] However, the shear stress at the aortic valve (maximum = 79 dynes/cm^2) exceeds the forces that T cells, macrophages, and neutrophils can overcome to bind via their adhesion molecules.[23] Moreover, with valves beating 60-80 times per minute, the forces on most of an aortic valve surface are pulsatile and chaotic, with shear stress gradients significantly greater than typical laminar shear forces.

Recent work with heterotopic heart xenografts also demonstrates that although pre-formed circulating antibodies bind to microvascular endothelium and causes complement-mediated hyperacute graft failure, antibodies did not bind to the xenograft valve endothelium.[24] What is more remarkable is that this occurred in the heterotopic transplant model (donor aorta anastomosed end-to-side of the recipient aorta) where the aortic valve cusps initially maintain competency against the aortic flow and do not appreciably move. Work from the same group also demonstrates that valves do not express many of the endothelial cell markers—including ABO blood group antigens - present on other parts of the cardiovascular tree.[25] Preliminary data demonstrate that removal of pig valve cusps from the circulation and culture under static conditions does not induce expression of the carbohydrate antigens responsible for hyperacute rejection, despite continuous expression by myocardial capillaries (Kadner, et al., unpublished observations). The differences in antigenic expression on valve endothelium are therefore not exclusively attributable to valve flow conditions.

Perhaps there is something unique about the valve endothelium. Certainly, the isolated loss of valve architecture in certain mice genetically deficient in an isoform of the nuclear factor of activated T cells (NFATc) suggests that there is a unique valvular anlage which might distinguish valve endothelia from brethren endothelia elsewhere in the cardiovascular tree.[26] This potential uniqueness of valve endothelium has significance for the design of bioartificial valves, as well as for the use of fresh, non-fixed porcine valves in human patients. Instead of using generic microvascular endothelium to coat vascular prostheses, perhaps we should consider using endothelium derived from valves. Instead of fixing pig valve prostheses, perhaps we should also consider the possibility of harvesting fresh, viable valves from pathogen-free pigs on demand at the time of surgery.

In summary, there is no substantive evidence to implicate an immune pathogenesis in the failure of cryopreserved valves. Rather, the loss of cell viability is more likely attributable to ischemia, freezing, and thawing; the subsequent valvular degeneration occurs because there are no viable cells capable of synthesizing new matrix. Indeed, as with fixed porcine bioprosthetic valves, it is remarkable how well and how long non-viable matrix material can function as a valve.

At this point, it is extremely premature to advocate the use of immunosuppression (itself associated with well-known and substantial morbidity) to prevent valvular degeneration in cryopreserved allografts. Before subjecting patients to long-term immunosuppression, we must perform experiments in suitable animal models to at least:

- demonstrate that comparably cryopreserved isografts do not show the same pathology.
- adoptive transfer of cells and/or antibodies can cause valve failure.
- demonstrate that immune blockade prevents the observed outcome of non-viable, degenerating valves.

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ISACB BULLETIN BOARD

5th Annual Hilton Head Workshop & Annual Engineering Tissues Workshop

February 21-25, 2001
Sea Pines Plantation
Hilton Head Island, S.C USA

The Engineering Tissues Workshop focuses on engineering tissues, gene delivery, and tissue repair and regeneration. There will be a total of six sessions over four days. Each day will start with a keynote speaker, and is devoted to the fundamental science, specific application areas, and modeling methodologies.

The deadline for submission of abstracts is November 15, 2000. For updated information and a complete schedule, please visit our website, **www.gtec.gatech.edu.**

There will also be a one-day short course entitled "The New Biology Toolbox for Tissue Engineers." This will be held on Wednesday, February 21, prior to the workshop. The short course will cover three topics. These include nuclear transfer, embryonic stem cell technology, and genetic engineering and its importance to tissue engineering.

Early registration is $395 for the workshop and $595 with the short course. Registration forms and housing information are available on the conference web site.

RESEARCH POSITION AVAILABLE

BIOCHEMISTRY/BIOLOGY OF ELASTIN

Department of Biomedical Engineering
Lerner Research Institute,
The Cleveland Clinic Foundation

We are seeking a candidate for a full-time position (postdoctoral fellow or research associate) in the Heart Valve Laboratory http://www.lerner.ccf.org/bme/valve/index.html. Required qualifications are a Ph.D.-degree with background in basic biochemistry/biology or matrix biology. The position is available immediately. The candidate would be funded under a grant from NIH (HL57780-0182; Ivan Vesely, Ph.D., Principal Investigator).

Candidates should submit a curriculum vitae with a cover letter indicating areas of expertise and career interest to:

Ivan Vesely, Ph.D.
Dept. of Biomedical Engineering/ND20
The Cleveland Clinic Foundation
9500 Euclid Avenue
Cleveland, OH 44195
Fax: 216/444-9198
e-mail: vesely@bme.ri.ccf.org

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POSTDOCTORAL POSITION(S)

The newly established Laboratory for Cellular Tissue Engineering, Drexel University in Philadelphia has immediate vacancies for several postdoctoral positions as well as for outstanding graduate students to work on diverse aspects of cardiovascular and neuronal/neuroendocrine tissue engineering.

Postdoctoral Position(s) in Vascular Biology: Study signal transduction mechanisms involved in the expression of adhesion molecules in cultured endothelial cells exposed to hemodynamic forces. Successful candidates will have a strong background and training in cell and molecular biology. Thorough, proven expertise in isolation and maintenance of human vascular endothelial cells is required as is demonstrated proficiency in molecular biological techniques (promoter deletion analysis, EMSA, DNA foot-printing, quantitative RT-PCR, Northern Blotting, RNAse protection assay), immunochemical assays (ELISA, Western Blotting, flow cytometry)and intracellular signal transduction pathways (protein phosphorylation cascades, kinase assays). Expertise in the analysis of differential gene expression (microchip array technology, differential display PCR, subtraction cloning,etc.), as well as demonstrated, familiarity with theory and praxis of endothelial cell mechanoactivation are definite advantages.

Postdoctoral Position(s) in Cellular/Molecular Tissue Engineering: Study signal transduction pathways and genomics involved in the tissue-like assembly and differentiation of PC12 pheochromocytoma cells cultured in Rotating Wall Vessel Bioreactors. Successful candidates will have a strong background and training in cell and molecular biology. Demonstrated expertise in cell culture and biochemical/immunological techniques (HPLC, radioenzyme assays, ELISA, Western blotting, flow-cytometry, immunohisto-chemistry) is required, as is thorough familiarity with molecular biological methods (RT-PCR, RNAse protection assay, EMSA, "promoter-bashing", microchip-based genomic analysis). Expertise in signal transduction research will be of advantage.

Send inquiries and applications (including names, telephone numbers and e-mail addresses of three referees to:

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Experimental Biology 2001 Meeting Announcement

The meeting will be held in Orlando, Florida from 31 March to 4 April 2001. ISACB will be a Participating Guest Society and a co-sponsor of the Cardiovascular Tissue Engineering session. The published deadline for abstract submission has unfortunately passed (6 November). For more information, visit the ASIP web site at http://asip.uthscsa.edu/ . You may also email inquiries to eb@faseb.org/eb2001, or call (301) 530-7010, or fax to (301) 530-7014.

EDITOR-IN-CHIEF

The Society for Cardiovascular Pathology seeks a new Editor-in-Chief for its official journal Cardiovascular Pathology, the leading journal in its field.

Published for a decade by Elsevier Scientific Publishers, **Cardiovascular Pathology** is a bimonthly journal that publishes original articles by clinicians and scientists on topics covering the entire spectrum of the prevention, mechanism, diagnosis, and treatment of cardiovascular disease. Subjects include (but are not limited to) disease-orientated morphology and pathogenesis, basic and applied myocardial and vascular biology, molecular cardiology, clinical cardiac disease, heart failure, sudden cardiac death, animal models, and cardiovascular interventions and prosthetic devices. Elsevier also publishes the Journal of the American College of Cardiology, Annals of Thoracic Surgery, and other leading journals in the fields of cardiovascular medicine, surgery and scientific investigation.

The Editor shall serve for 5 years beginning January 1, 2002. Re-nomination for one additional 5 year term is permitted.

Prospective candidates should submit an application that includes 1) an outline of their plan for running the journal, 2) documentation of a suitable administrative support infrastructure for the editorial office, 3) 2 letters of reference and 4) a copy of their curriculum vitae to:

Dr. Peter Anderson
Department of Pathology, VH G-046
University of Alabama at Birmingham
1670 University Blvd.
Birmingham, AL 35294-0019
Phone (205) 934-2414
Fax (205) 934-1775

Application deadline is Jan. 15, 2001. Selection will be made in March 2001. An applicant need not presently be a member of the Society for Cardiovascular Pathology but will be expected to become a member following selection.

VIIIth Biennial Meeting of ISACB

ISACB 2002

ST. GALLEN, SWITZERLAND

FEBRUARY 27 – MARCH 2

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

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

Send all of the above information to:

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