FALL/WINTER 2005

ISACB CIRCULATOR

INTERNATIONAL SOCIETY FOR APPLIED CARDIOVASCULAR BIOLOGY

ISACB's 10th Biennial Meeting

Abstract Submissions Due: Nov. 18, 2005

TOWARDS REGENERATIVE THERAPIES:

STEM CELLS, INTELLIGENT MATRICES AND MOLECULAR APPROACHES

Never has the timing been more optimal nor the venue more appropriate than for the 10th Biennial meeting of the International Society for Applied Cardiovascular Biology (ISACB) which will be held March 8 – 11, 2006 at the Torrey Pines Hilton Hotel, La Jolla (San Diego) CA.

This meeting is designed to examine critical issues in the translation of the cutting edge cardiovascular research into the clinic and focuses upon opportunities for translational therapies based upon stem cell and tissue engineering technologies. California is an ideal setting for this meeting because of the passage of Proposition 71- the Stem Cell Research and Cures Act. As a result, California-based investigators have a unique opportunity to take advantage of funds to support human embryonic stem cell research and we all have opportunities to network and communicate. .

Because ISACB believes that both scientists and the public will benefit from cooperation and communication we decided to host our meeting in this forward thinking community of La Jolla, CA.

Continued on page 4!

All of the details related to the program of activities, session topics and so forth are available on line at:

www.isacb.org

IN THIS EDITION

ISACB Circulator is published as an information service for the members of the International Society for Applied Cardiovascular Biology.
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WE'RE NOW ONLINE!

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

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

PRESIDENTIAL ADDRESS

Thoughts on the Future, or Dances with Faust

As the ISACB enters its third decade with the 10th Biennial meeting in La Jolla on March 8-11, 2006, it is time we evaluated our strengths and our limitations and re-defined our identity and where we may want to go from here. When the Society was founded, the central concept was to facilitate communication and collaboration between clinicians and basic scientists and among academia, industry and regulatory agencies. It was recognized that free communication among these disparate groups would be best enabled by maintaining a relatively small but sophisticated and dedicated membership and informal, spontaneous conferences based on state of the art science. This in essence defined the soul of the ISACB. To a great extent, I believe this has all been achieved. However, no glass half full isn't half empty. While the founding goals have largely been achieved and the soul and vision of the Society maintained, there remains considerable opportunity for improvement and for growth, a need to reach out to both new scientific disciplines and to new younger members of the scientific community, a persistent need to enhance our fundraising capabilities and a need to do all of this while maintaining and not selling the soul of the ISACB.

Below I list, in no particular order, a far from complete group of issues for your consideration and hopefully discussion when we meet in La Jolla. Risking my neck, I will commit to my own position on each issue, although resolution of any single issue likely affects decisions on the others.

1. Size of membership.

Our membership has been relatively unchanged in size over the years. The advantage of this relatively small membership is the close interaction among members and the spontaneity and forthrightness it promotes in our meetings. The disadvantage is in collection of dues and in furthering the expertise and diversity of our membership.

My position – membership should be increased (without a reduction in qualifications required for membership).

2. Size of meetings.

Our meeting size has varied very little over the years. The scientific quality has universally been praised as has been the discussion sessions, in large part a result of the size and informality of the conferences. The relatively small size has greatly enhanced the opportunity for personal interactions among investigators and between trainees and speakers. On the other hand, greater attendance would generate much needed income and potentially could further enhance the discussions and interactions. Larger membership could result in larger meeting size, a dilemma whose resolution might require altering meeting structure to include parallel

"While the founding goals have largely been achieved and the soul and vision of the Society maintained, there remains considerable opportunity for improvement and for growth..."

Howard Greisler, ISACB President

sessions, a format which could potentially diminish cross-disciplinary fertilization.

My position – the optimal meeting size is probably slightly, not greatly, larger than our current size. However, this depends on whether we retain the current meeting themes and meeting frequency (both addressed below).

3. Meeting frequency.

We have to date always held biennial meetings. This has in large part been dictated by finances. More frequent meetings of the same nature would be financially difficult! However, those infrequent meetings lead to an "out of sight, out of mind" dilemma for the ISACB as relates to both membership accrual and fundraising. We have in the past successfully co-sponsored sessions at meetings of other societies and have recently begun "off year" smaller meetings focused on "para-scientific" issues such as scientific ethics.

My position – the meeting frequency should be increased to annually if finances permit. Co-sponsorship of sessions at both ISACB meetings and those of other compatible societies should be encouraged.

4. Conference session themes.

As the ISACB represents a broad spectrum of scientific disciplines and clinical foci, we have historically tried to include sessions covering broad themes. The obvious advantage is that most attendees find topics of interest while the obvious disadvantage is that no single theme is fully covered. Given the biennial conference frequency, this seems the lesser of two evils. Were we to focus on a single theme, members with different interests would find little relevance to ISACB meetings for four year periods.

My position – move to an annual meeting frequency. Every other meeting should maintain this broad thematic structure and the alternating meetings should be focused on more specific topics. If the meetings were to grow in attendance, parallel sessions should be considered for the broader biennial meetings, with the caveat above.

5. Need for enhanced fundraising.

It's no secret that the ISACB has functioned in "near bankruptcy" throughout its years. This remains the case. This in part is due to the broad nature of our scientific interests, our core membership of individuals not deemed likely to purchase equipment or devices in large quantities and our relatively small size. We have been fortunate that several industrial sponsors have been repeatedly willing to provide some support and that the NIH has often added support for our meetings when held in the U.S. For the La Jolla meeting, I believe that we are ahead of our past efforts in generating funding and I am optimistic about our immediate financial future. However, of concern, 100% of the funds raised as of this date (10/31/05) have been provided by the same sponsors (and the NHLBI), solicited by the same ISACB members, as in past years.

My position – we VERY MUCH need new energy, ideas and efforts at improving our financial situation. However, under NO circumstances can we do this at the Faustian price of losing our independence, our peer review process, our soul.

6. Peer review.

To date, all speakers at ISACB meetings, both invited speakers and those selected based on abstracts submitted, have been selected by peer review. We have always included industry scientists and on occasion have had "industry speaks" sessions but these too have been developed through the peer review process. On the other hand, the ISACB could conceivably benefit financially from opening the program to speakers based not on peer review, but on financial contribution.

My position – the peer review process is absolutely essential to the integrity of the Society, without which we have no reason to exist.

7. Organizational structure.

The ISACB was essentially founded by a small group of compatible individuals and the bylaws were written in a somewhat cursory fashion. Over the two decades the leadership of the ISACB has changed little and the membership not much more. However, both the leadership and the membership are in need of new blood. This transition is essential, new ideas are needed, but change must be structured so as to preserve the soul and vision of the ISACB.

My position – we need to establish more defined bylaws regulating officer responsibility and succession, Executive Council membership selection processes and responsibilities, responsibilities of existing committees (Fundraising Committee, Local Organizing Committee) and establishment of appropriate new committees as needed (eg. Publication Committee, Nominating Committee, etc.) and their responsibilities as well as a host of related bylaws governing the functioning of the ISACB.

I have been proud to serve the ISACB and I am very optimistic about our near term finances and about our near and long term continuing scientific excellence. The issues above are an indisputably incomplete list of possibilities for future organizational development. I strongly encourage all to consider these and other such issues and to engage in their discussion when we meet in La Jolla.

The upcoming 10th biennial meeting is shaping up to be excellent indeed. I look forward to it and to seeing everyone there.

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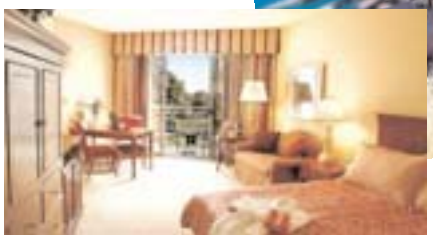
ISACB's 10th Biennial Meeting

TOWARDS REGENERATIVE THERAPIES:

STEM CELLS, INTELLIGENT MATRICES AND MOLECULAR APPROACHES



In addition, the organizers for the 10th Biennial meeting have added many new features to enhance the educational objectives of the meeting.

- A **satellite meeting** that will be presented on Wednesday, March 8th prior to the Welcome Reception and formal opening of the ISACB meeting. The satellite meeting will be organized by Shu Chien and his colleagues from the Department of Biomedical Engineering at the University of California, San Diego and will feature the research activities of Dr. Chien, his colleagues and students at UCSD as well as those of local biotech companies. Further details of the satellite meeting are available on line at www.isacb.org.

- The organizers have recruited an expert team of speakers to **discuss relationships that develop in the translation of cardiovascular therapies**. This session will be held on Saturday morning March 11th as part of the regular ISACB program and will feature speakers that will talk about funding, academic ventures, relationships that develop between academia and small companies, relationships that develop between small companies and large companies, start-up legal issues and relationships that develop between large companies and other large companies all in the context of the translation of cardiovascular technologies. This highly stimulating session should appeal to all meeting attendees.

- On March 8th, ISACB will host a **tech expo** that presents local bio-tech exhibitors from the California area with products and services related to stem cell and tissue engineering technologies.

- As usual, ISACB will host a **manned poster session** and reception;

- The special banquet dinner venue will be the Birch Aquarium on the campus of UCSD.

This is an especially well organized and timely meeting. We look forward to a lively exchange of information that has been the hallmark of prior ISACB meetings.

We'll see you in La Jolla in March, 2006.

See page 12 for ISACB's 10th Biennial Meeting Sponsors.

The session topics and chairs for those topics include the following:

Cell Sourcing for Cardiovascular Tissue Engineering

Co-chaired by: Robert Nerem, Ph.D. and Howard Greisler, M.D.

Multi-disciplinary Approaches to Cardiovascular Repair

Co-chaired by: Doris Taylor, Ph.D. and Ivan Vesely, Ph.D.

Recapitulating Development: A Template for Tissue Engineering?

Chaired by: Julie Campbell, Ph.D.

Inflammatory Processes in Healing, Remodeling and Failure

Chaired by: James Anderson, M.D., Ph.D.

Novel Molecular Approaches in Translational Cardiovascular Research

Chaired by: Frederick Schoen, M.D., Ph.D.

The Novel Molecular Approaches session is co-sponsored by the Society for Cardiovascular Pathology.

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.

HUMAN TISSUE ENGINEERING AND BEYOND: The Upcoming Regulation in the European Union

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In March, 2004, the European Parliament passed the Directive 2004/23/EC entitled "On setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells". This Directive was passed to prevent the transmission of infectious diseases when grafting heart valves, cornea, bone and other tissues derived from cadavers into patients. Thus the directive focuses on microbiological testing, sterility and traceability from donor to recipient. The directive made it especially clear that all tissue establishments must comply with GMP guidelines.

Although the directive also mentions living somatic cells, it did not further discuss them. That is because during the law making process it came quite obvious that advancements in biomedical research will make therapies with living cells alone or combined with biomaterials possible which are currently unknown. These discoveries will have a major impact on public health, by improving the quality of life of patients and changing medical practice significantly. Therefore, the European Commission, together with the European Counsel, decided to regulate therapeutic approaches such as genetic engineering, tissue engineering and somatic cell therapy, which together are called now Advanced Therapies.

In initial meetings the EU drafted first milestones and decided that the regulations shall fulfill the following key objectives:

1. *to guarantee a high level of health protection for European patients*
2. *to harmonise market access for advanced therapies by establishing a tailored and comprehensive regulatory framework for their authorization, supervision and post authorization vigilance*
3. *to foster the competitiveness of European undertakings operating in this field*
4. *to provide overall legal certainty, while allowing for sufficient flexibility at technical levels, in order to keep the pace with the evolution of science and technology.*

No doubt, the implementation of these key objectives is desired and justified. However, in the upcoming months lawyers, public officials and experts will be confronted with a tremendous number of questions regarding advanced therapies. The debates are already starting with how to define the matter itself: What are advanced therapies altogether? Tissue engineering might fall under this term. But what exactly is a tissue engineered product? A first draft on this issue states, that tissue engineering products are produced by an industrial process. According to this definition, a skin substitute made of cultured allogeneic neonatal cells showing all morphological layers may easily be identified as a tissue engineered product. But is the simple cell culture procedure producing an autologous keratinocyte sheet already an industrial process? So it was proposed, that the extent of the engineering process should be incorporated into the definition. Tissue Engineering uses *"Engineered human cells or tissues, which are cells or tissues removed from a human donor and manipulated via a manufacturing process, so that their normal biological characteristics, physiological functions or structural properties are substantially altered"*. Many uncertainties reside in this sentence - especially, because this is exactly the opposite what Tissue Engineers try normally to achieve. They do everything to restore and mimic the normal biological, physiological and structural properties of a given tissue. It remains to be seen if, and how, this important paragraph will survive the law making process. An additional key issue in the first proposal is "autologous versus heterologous" treatments. Some countries want autologous procedures to be regulated like heterologous procedures. Others do not.

Thus, many terms and processes have to be defined. And, it is quite clear that the accuracy of discriminations is important and will have a profound effect on biomedical research and the therapies deriving therefrom.

One major subject of the new regulation will be the marketing authorization procedure. Although the EU acknowledges that Advanced Therapies are highly specific and differ significantly from classical pharmaceuticals, they will nevertheless be treated like other biotechnology derived medicines. For example, it may not be possible to perform conventional clinical trials, but some proof of efficacy, both

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preclinical and clinical, will be necessary in order to gain application authorization. Most likely, the authorization will be granted from EMEA, the European counterpart to the FDA.

By their nature, advance therapy products can stay in the human body much longer than classical pharmaceuticals, sometimes even during the entire lifetime of a patient. Therefore, long-term follow-up and monitoring will be necessary. The regulation will ensure that any producer of advanced therapy products puts in place a suitable risk management system. This means nothing less than the producer establishing a system allowing complete traceability of the patient, the product and all starting materials for thirty years. This means more work during the preclinical and clinical test phases and even more work during routine application of advanced therapies. The costs of the end product will therefore significantly rise. Considering the strained reimbursement situation for medical treatments in Europe in general, one can foresee how hard it will become to invent, test and sell advanced therapy products. This will threaten especially research in public hospitals and small and medium-sized research companies. The European Commission itself has recently recognized this problem and promised to grant some relief for the latter group.

The regulation of advanced therapies in Europe was, beyond doubt, overdue. Today, every member state, even local provinces, have their own rules which makes cooperative research challenging and marketing of products even more difficult. The new regulations will provide a common ground giving all players in the field legal certainty and the same opportunities. Most importantly, the new regulations should grant all patients in Europe the same access to new therapies which are equally safe and efficient. To really guarantee this, clinicians and researchers in Europe must get strongly involved in the law making process. I therefore call to all colleagues in Europe to get in touch with their competent national authority and work together with them, providing them with expertise and insight on this matter.

The Regulation on Advanced Therapies will mainly be negotiated under the Austrian Presidency during the first half of 2006 and shall be finished at the end of 2006. Once it has been passed by the European Parliament, it will be binding for all Member States.

ISACB ESSAYS

To Engineer is to Create: The Link between Engineering and Regeneration

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The reason why there is the word 'engineering' in the term 'tissue engineering' is not intuitively obvious. Leaving aside the more trivial suggestions that engineering is that part of an organisation that deals with machines, and engineers are train drivers, the vast majority of definitions of engineering invoke the use of scientific knowledge to solve practical problems and/or the systematic analysis of physical data to yield tangible end products. Although not entirely unconnected, neither of these concepts is readily translated into the paradigms that are now represented by tissue engineering. Tissue engineering does have practical end products but the underlying science is far more related to cell, molecular and developmental biology and to pharmacology than to the physical sciences that normally underpin classical engineering. There is, however, another meaning of engineering, appreciated best when we consider that the origin of the term is the Latin ingenium, from which we can see that it is ingenuity or creativeness that is really at the heart of the subject. This is not a matter of semantics but of immense importance in both the philosophy and practical development of tissue engineering, and the broader area of regenerative medicine. We should bear in mind that tissue engineering, after some 15 years, has yet to really make its mark either clinically or commercially, and it could be argued that this is related to a misunderstanding of what it actually is.

Tissue Repair, Replacement and Regeneration

Tissues and organs suffer from a wide variety of diseases and injuries, as a result of which they lose some degree of function. Primarily these conditions are associated with acute injury or chronic degenerative changes. Without any medical intervention, the response of the body is quite limited and is mainly restricted to repair processes. Repair

may lead to the restoration of continuity in the affected part by the synthesis of scar tissue, which is essentially collagenous and not reminiscent of the indigenous damaged tissue. This may be an effective front line response to injury but does not lead to the restoration of normal structure and function and may, if uncontrolled, lead to detrimental effects in the patient.

During the last fifty years, it has become the accepted mode of treatment for many of these conditions to excise the affected part, either a tissue or an organ, and replace it with some form of structure that could replicate part or all of the function that has been lost. This replacement or augmentation of tissues could be achieved through the use of synthetic implanted devices, which clearly do not lead to restoration of structure since they are non-viable, or through the use of grafts or transplants, which should restore both structure and function. We need not rehearse the difficulties with both the logistics of supply and the immunological responses with respect to transplantation here. With replacement devices, referred to as implants or prostheses, there have been excellent clinical performances but as well as failing to restore structure, the nature of the function they can replace is largely limited to the simple mechanical and physical, whilst problems of biocompatibility lead to restricted longevity in most cases.

The logical conclusion to the discussions that emphasise that repair is not an effective outcome, and that replacement has serious limitations with respect to logistics and lack of biological functionality, is to consider tissue regeneration as the only possible alternative, which is aimed at restoring normal structure and function through the production of new tissue that does replicate exactly that which has been lost. The only problem is that adult mammals do not spontaneously regenerate their organs that are damaged and have only limited ability to regenerate certain tissues. If we wish to persuade the human adult to regenerate whole organs or tissues that do not spontaneously regenerate, then we have to give them some cues or signals, and superimpose on them a mechanism that is not the natural response to those conditions. Induced regeneration is the essence of tissue engineering, which is, of course, very different to either repair or replacement of tissues. Tissue engineering is, therefore, a matter of the creation of new tissue and to engineer here is, quite simply, to create. The creative process has to be achieved by cells, and they are stimulated into this unnatural regenerative mode by a variety of factors, which may be collectively described as either biomolecules or supporting structures, the former providing molecular signals and the latter mechanical signals. Although there are several very general definitions of tissue engineering, my preference, in line with this concept, has been that 'tissue engineering is the persuasion of the body to heal itself, through the delivery to the appropriate site, of cells, biomolecules and / or supporting structures'.

The Central Tissue Engineering Paradigm

Clearly it is not a trivial process persuading cells to produce new tissue under circumstances in which they do not normally do so. Moreover, it is of the utmost importance that, during this process, exactly the right type of tissue is generated, that the signals given to the cells can be switched off when the process is complete, and that the resulting tissue is fully functional. The process of tissue engineering starts with the sourcing of the relevant cells and ends with the full incorporation of the functional regenerated tissue into the host. The pathway between these two points can take many forms. The types of cells include those derived from autologous, allogeneic or, possibly, xenogeneic sources, and they may be fully differentiated cells or stem / progenitor cells. The degree of cell manipulation will depend on the origin of the cells and the complexity of the tissue and may be dependent on gene transfer in order to optimise processes of, for example, cell expansion, or to control phenotype under these abnormal circumstances. Normally the cells will require some supporting structure, either a scaffold, a matrix or a membrane, within or on which they will express the new tissue and will be persuaded to do so by molecular signals provided by relevant cytokines, growth factors or other molecules, and by mechanical signals, transmitted via the support and the fluid medium. The environment in which this takes place is usually described as a bioreactor . The tissue that forms, often referred to as a construct, will, if generated *ex vivo*, have to be placed within the host, where it has to be fully and functionally incorporated, taking into account the responses that should be avoided, such as excessive inflammation, an immune response and carcinogenicity or teratogenicity, and the responses that may be required, such as vascularisation and innervation, and indeed the further development and maturation of the tissue itself. It should be borne in mind that this paradigm does not have to be rigidly followed, and many tissue engineering processes are evolving with, for example, much of the regeneration actually occurring *in vivo* rather than *ex vivo*, as we shall see below.

Critical Barriers to Progress

Having set out the framework of the generic tissue engineering approach, we have to identify the scientific and infrastructure factors that have so far held back progress. There are several prime candidates but probably the most important is the difficulty of integrating all of these components into a coherent system that is able to accommodate the requirements and specifications for each phase of this paradigm into an efficient and cost effective process within a quality-validated, clinically-oriented environment, that takes into account the impositions of regulatory, ethical and reimbursement schemes. A systems engineering approach to regenerative medicine appears to be an essential element of future developments with respect to this integration, and it is possible that this will also require some elements of systems biology with respect to the underlying science.

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When considering the individual components of this new paradigm, there are a number of critical issues, a few of which are discussed below in the context of complexity and the challenges of engineering new tissue.

The first concerns the role of stem cells. Embryonic stem cells are still associated with logistics issues based on the ethical dilemma and safety concerns related to the possibility of teratogenicity. Many now believe that adult stem cells provide the most relevant source of cells for tissue engineering. The most significant questions facing this use, however, concern the ability of current knowledge of stem cell science to direct the tissue engineers into the optimal processes to precisely control the expansion and differentiation of these cells, from wherever sourced, such that they provide the right phenotype with the right activity in order to generate the right tissue. The key here will be the transition from purely stochastic control of stem cell behaviour to that of environmental or extrinsic regulation, and the application of those factors known to influence this regulation, including growth factors, cytokines, morphogens and adhesion factors, in robust biomanufacturing processes. Secondly there is the role of gene transfer in tissue engineering. It is clear that gene transfer can be employed several scenarios to enhance cell performance within *in vitro* systems and small animal models, but it is far from clear whether existing gene transfer materials and techniques can be applied efficiently and safely in human patients.

The *in vivo* use of growth factors in tissue engineering is an important consideration but is very problematic in view of their short biological half lives and the potential for systemic toxicity. This has led to the search for methods to immobilise or protect growth factors on or within the materials used for the supporting matrices and then arrange for their sustained and controlled release. One of the main tenets of the now classical tissue engineering process is the need for a supporting structure for the control of cell behaviour, and scaffold biomaterials have played a major role in developments so far. The majority of scaffolds are porous polymers, usually synthetic biodegradable polyesters prepared by solvent casting (with porosity achieved by porogen or leaching technology), by fibre spinning processes, or by solid free form fabrication methods. However these materials and architectures hardly mimic the extracellular matrix that a cell normally expects, and neither the physico-chemical supporting role nor the provision of active mechanical signalling can be considered at all optimal, even if the cells are provided with adhesive peptides immobilised on the material surface in order to encourage adhesion. Two issues are of considerable importance, the need to distance the design of tissue engineering matrices and scaffolds, from the classical development of biomaterials for implantable devices and the need to know more about the extracellular matrix that forms within the scaffold, including events of cell adhesion and migration, growth factor distribution and interactions, and molecular recognition events. Finally there is the question of mechanotransduction, the science of which is rapidly developing but, apart from recognising its potential importance in the regulation of cell behaviour in the pre-conditioning of cells prior to implantation in patients, little has been done to actually target the molecules that could effect mechanotransduction within a tissue engineering construct, including cell-cell adhesion molecules, cytoskeletal filaments and signal transduction molecules.

Reassessing the Future of Tissue Engineering

The above examples point very clearly to the generic difficulties facing the engineering of new tissues. There are too many scientific and technological components of the paradigm and, although relevant data and knowledge are emerging fast, there has been no way of incorporating these individual components into a complete system. The paradigm discussed here is not hierarchical but temporal, based on the practical transition from cell derivation to tissue construct integration. If we do not understand how the physical and genetic components within a cell that we have sourced for a tissue engineering process operate together within their system, how can we hope to provide the optimal conditions for them to create the right tissue. Here we have to turn to systems engineering since it is essential that these disparate components are integrated so that the interactions between them are factored into the process. There is, therefore, a profound link between engineering systems and regenerative medicine. One of the consequences of this evaluation of the current status of tissue engineering is the realisation that not only may the paradigms be wrong, but also some of the concepts.

I S A C B
ESSAYS

Elastin in Blood Contacting Applications: Friend or Foe

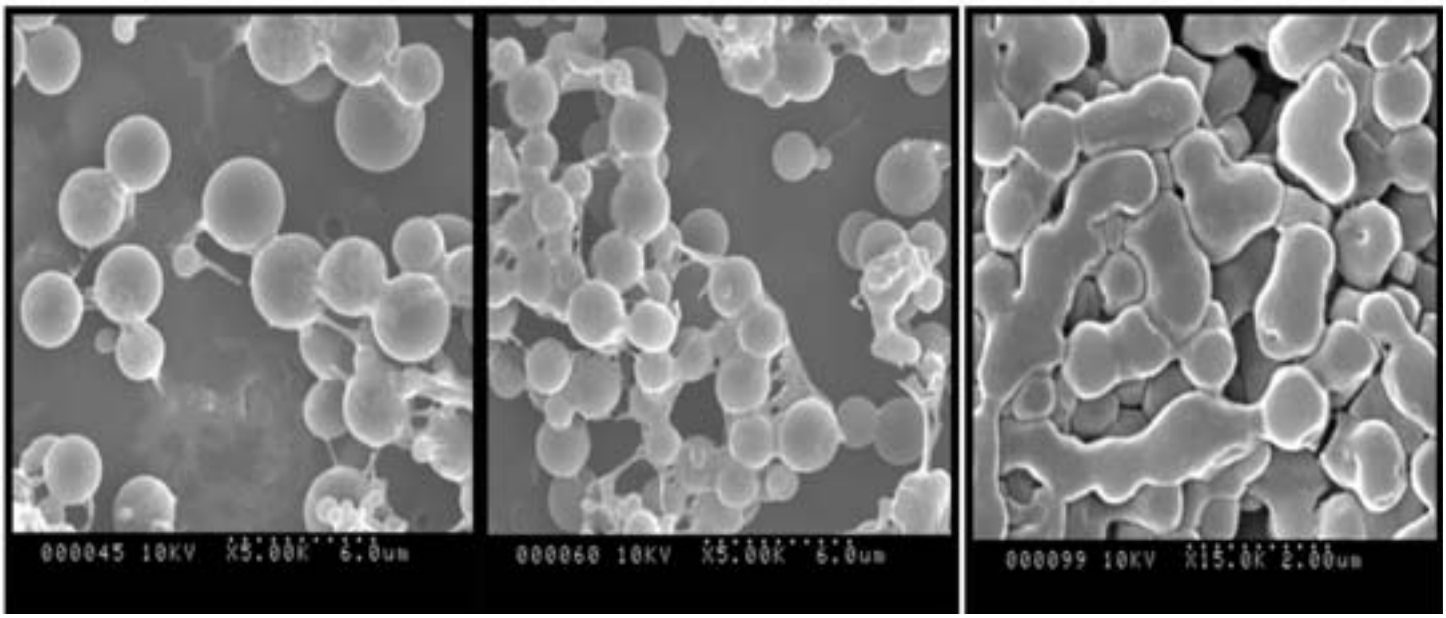
Kimberly A. Woodhouse, Ph.D., P.Eng, Professor Chemical Engineering and Applied Chemistry Institute of Biomaterials and Biomedical Engineering University of Toronto, Scientist Sunnybrook and Women's Health Science Centre

In the past several years, elastin and elastin-like polypeptide based materials are being considered for a multitude of applications including those in the cardiovascular system, for example in tissue engineered vascular grafts and in graft-stents. What are the issues and opportunities in using this unique molecule?

Elastin is an extracellular matrix protein found in extensible tissues. It constitutes approximately 50% of the dry mass of arterial walls[1]. In addition to providing structural support and elastomeric properties, elastin is an important autocrine factor that helps to maintain vascular homeostasis[1]. The elastin fibre found in tissues is composed of two components, the elastin protein, formed from the elastin protein precursor, tropoelastin and microfibrils, 10 nm fibrillin-containing fibrils [2, 3]. These components are assembled *in vivo* in a complex process thought to include molecular chaperones, elastin binding proteins, and the microfibrillar network [3]. Tropoelastin is a 67 kDa protein, which is composed of alternating hydrophobic and cross-linking regions[2]. These alternating domains are thought to play a role in the self-assembly that occurs during elastin formation[3,4]. Once cross-linked by lysyl oxidase *(in vivo)* or other cross-linkers *(in vitro)*, the elastin fibre becomes highly insoluble[5]. Lysyl oxidase cross-links elastin via oxidative de-amination and condensation of the lysine side chains[2, 6]. Insoluble (cross-linked) elastin has a very slow turnover in normal tissues *in vivo* and is considered highly resistant to proteolytic cleavage[5,6]. The elastin protein is characterised not only by its stability *in vivo* but its ability to self assemble *in vitro*, undergoing the phenomena of coacervation. Coacervation is a phase separation, generally believed to be reversible, in which the protein forms aggregates when the temperature of the solution is raised [7, 8]. The temperature at which coacervation occurs can be influenced by pH, ionic strength of the solution, protein or peptide concentration and peptide sequence [4]. Generally it is the tropoelastin [9] or portions of the elastin protein that are utilised in biomedical and pharmaceutical applications [10-14]. A variety of different peptides have been studied as both coatings and incorporated into materials including those containing the glycine, valine, and alanine rich sequences associated with elastin such as the GVGVAP hydrophobic domains [4,10-14], or polypeptides of alternating hydrophobic/hydrophilic block structure [4].

Why consider the elastin protein and its derivatives for blood contacting applications? Elastin protein and elastin peptide based materials have been shown to have elastomeric mechanical properties conducive to their use in the vascular applications [4,15]and in addition, investigations have shown that unlike collagen, elastin is not a strong agonist of platelet degranulation or platelet

FIGURE 1



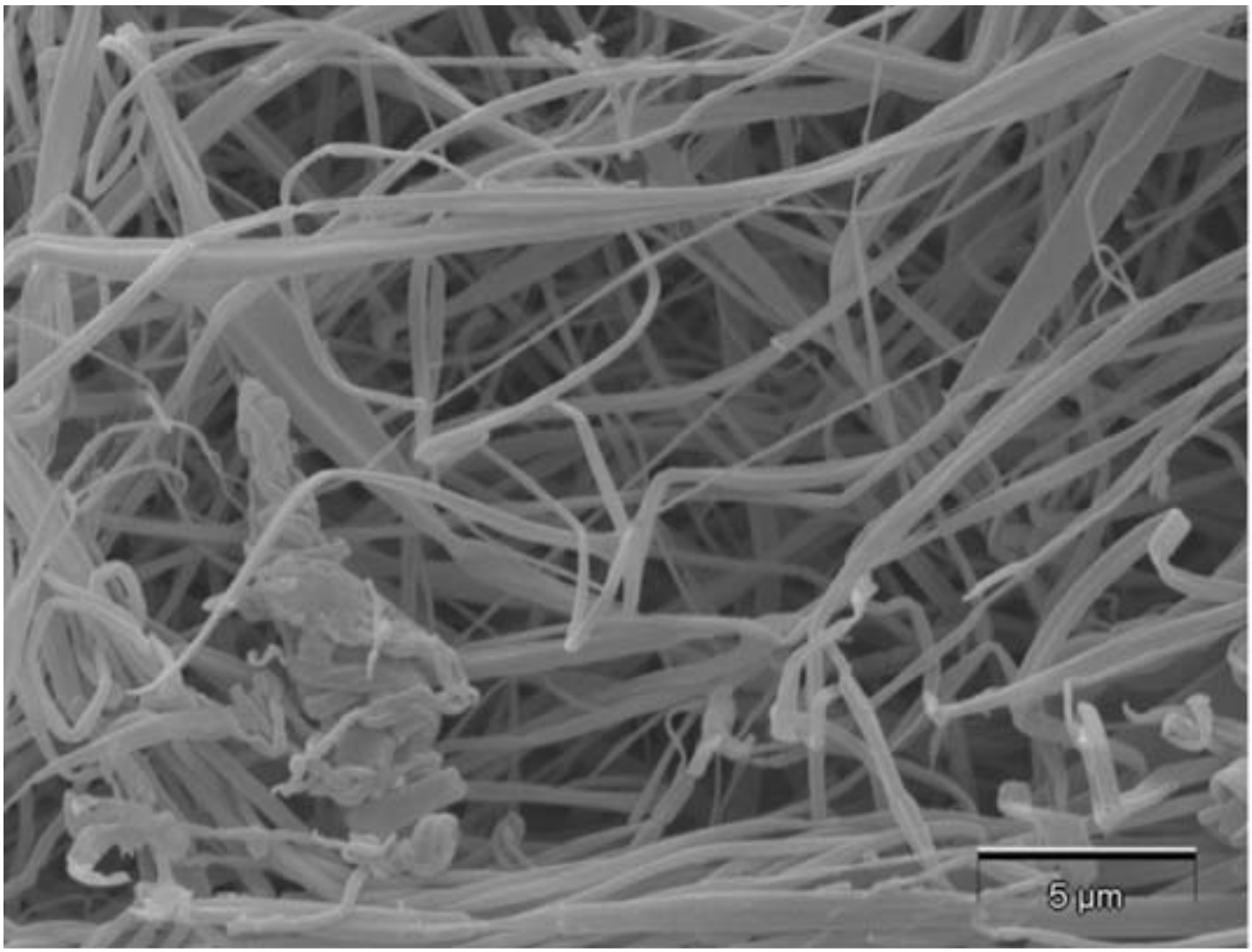
Stages in fibre formation of human elastin polypeptides with alternating hydrophobic and hydrophilic domains.

activation[16]. Spaet and Erichson[17] found limited platelet adhesion to isolated human elastin. Similarly, using isolated pig aorta elastin in an EDTA-containing platelet rich plasma, Barnes and MacIntyre[18] showed modest platelet adhesion but no aggregation of platelets on elastin as compared to type I collagen. Specifically, 7.2 % of the available platelets adhered to the elastin protein, and 1% adhered to the microfibril component of the elastic fibre. When the same tests were performed on various collagens, anywhere from 24 to 77% of platelets became attached. Dutoya et al. have also demonstrated reduced platelet activation using adsorbed α-elastin[19]. More recently, in work with recombinantly expressed elastin polypeptides, we have shown that the polypeptides passively adsorbed onto commercially available synthetic materials significantly reduced platelet activation *in vitro*, as measured by p-selectin expression and microparticle release. In an *in vivo* study in an acute rabbit model, the same polypeptides coated onto catheters enhanced patency, decreased fibrin accretion, and decreased emboli to the lungs compared to uncoated controls [20]. Interestingly, the reason for elastin's relatively low reactivity to platelets is still unclear.

Investigators have shown that elastin affects the response of both endothelial and smooth muscle cells (SMC). Ito et al. showed that alpha-elastin incorporated into a type 1 collagen gel inhibited proliferation and migration of SMCs, but not of endothelial cells[21]. In a second study, they showed that cross-linked alpha-elastin peptide coated directly onto a tissue culture dish inhibited SMC proliferation [22]. Gobin and West utilised polyethylene glycol materials grafted with VAPG to evaluate smooth muscle cell adhesion and found that VAPG is specific for adhesion of smooth muscle cells [23]. Finally, Karnik et al found *in vitro* that elastin inhibits the proliferation of vascular smooth muscle cells by inducing a contractile smooth muscle phenotype and found in a porcine model of restenosis found that an elastin sheath around a coronary stent reduced inflammation, the thrombotic response, and neointimal formation in comparison to the uncovered stents [24].

Recent work in tissue engineering has shown elastin polypeptide based polymers can be electrospun [9,12, Rabolt and Woodhouse, unpublished data, shown below] and Berglund et al have shown that isolated elastin can

FIGURE 2



Electrospun elastin polypeptide material

be combined with collagen type 1 to produce tissue–engineered blood vessels with mechanical properties closer to the natural vessel than collagen constructs alone [15]. The ability to process the elastin-like materials using commercially available processes and the appropriate mechanical properties will be important for the long term use of these materials in biomedical applications.

One of the main concerns with using elastin or elastin like materials in the vasculature is calcification. When considering calcification, it is important to discuss separate phenomena associated with elastin based materials; calcification of the elastin fibre, calcification of the purified elastin protein, and calcification of the elastin-like polypeptides. Calcification of atherosclerotic vessels and in bovine or porcine heart valves has been associated with the degradation or "damage" of the elastin fibre, with calcium found in close association with the fibres, frequently in areas of mechanical stress [25-27] although it is not totally clear that both vessels and heart valves calcify via the same mechanism [27]. There is evidence that calcification is frequently associated with the fibrillin component of the elastin fibre and not the elastin protein itself [28,]. More recently, investigators have looked at elastolysis itself as a mechanism for calcification. There is data that supports that elastolysis leads to upregulation of MMP's particularly MMP 2 which results in calcification [29]. Originally observed in damaged vessels and valve implants, the evaluation of the mechanism of calcification *in vivo* has been controversial because of discrepancies in results based on the animal models. Investigators have found that purified elastin protein does not calcify in adult mice but does calcify moderately in a juvenile model [28]. The elastin fibre or impure elastin preparations appear to calcify more readily than pure elastin protein.

The information on calcification of elastin-like polypeptides is limited, with most of the work on calcification of the PGVG repeat sequences undertaken in the late 70's and 80's principally by Urry and colleagues, who had developed elastin-like sequences based on variations of this hydrophobic sequence. Many of the studies were undertaken specifically with a view to causing calcification [30, 31]. The polypeptides investigated where essentially repeats of the PGVG sequence. He found that only a polypentapeptide of sequence (VPGVG)n where n was greater than 100, calcified and that calcification was molecular weight and sequence dependent. Many researchers are working with much smaller sequences than those found to calcify. In addition, the impact of hydrophilic repeat units that alter the hydrophobicity of the material is not clear. Urry also speculated that organised fibre structure might impact calcification.

Elastin is a deceptively simple protein, and it seems that elastin and materials and peptides based on both its structure and its function, will keep many of us busy as we unravel both its strengths and weakness in blood contacting applications (and we haven't even mentioned the degradation products yet!)

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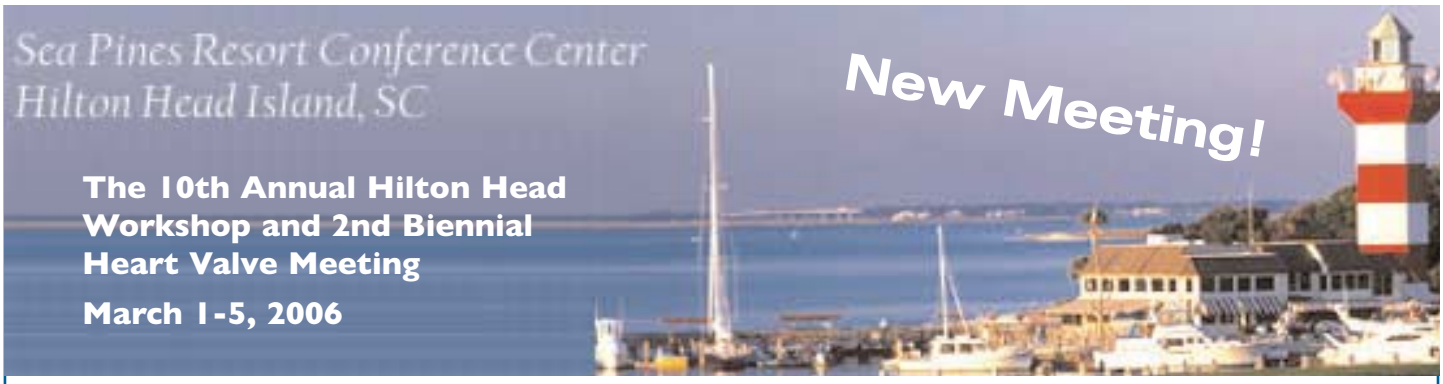

Advances in Tissue Engineering and Innovative Technologies For the Treatment of Heart Valve Disease

Presented by the Parker H. Petit Institute for Bioengineering and Bioscience at Georgia Institute of Technology, Imperial College London and the University of Florence. The goal of the workshop is to allow both academic and corporate researchers an opportunity to gain insight into innovative and imaginative work in laboratories around the world as the field of "tissue engineering" continues to move from benchtop science to products.

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