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Biotechnology Meets Biomaterials

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Four principal themes percolated through the presented papers and exhibited posters at The Workshop on Biotechnology Applications in Biomaterials, held April 26, 1993, in Birmingham. The first was the relationship of cells to the *extracellular matrix* (ECM). In thinking about old and new materials for use in biotechnology, their constitution and design may be dictated to a large degree by how tissue cells see them. M.D. Pierschbacher (this issue) provided a good opening example, citing the Arg-Gly-Asp (RGD) sequence, a ligand found in many extracellular proteins which is recognized by the pericellular receptors, the integrins. What is becoming clear is that the three dimensional arrangement of the RGD ligands in the matrix, and not just the sequence itself, stamps the individuality of the different matrix proteins, underscoring the dictum that microarchitecture as well as molecular diversity is part of the message. There is growing evidence that the ECM generates the signals that cells need to carry out their programs of division, morphogenensis and differentiation. If there is any hope of inducing cells to regenerate a missing part by installing a prosthesis, the material of which it is made, at least, must be permissive, and at best, instructive. In reviewing progress with the scanning probe microscope, V. Pizziconi et al. (Arizona State University), pointed to the value of using that tool in conjuction with others to learn more about the three dimensional topography of the ECM.

The second theme, closely related to the first, had the ring of tissue engineering, the leitmotif being a variety of materials capable of the *induction of regeneration* of body parts damaged by injury, disease or genetic disorder. Schema for inducing regeneration divided into five materials approaches: (1) natural biomaterials without cells, (2) natural biomaterials with cells, (3) unnatural materials with cells, (4) mixed materials with cells, and (5) natural or unnatural materials having potential as extracellular matrix materials with or without cells.

In the first categories, A.H. Reddi (page 192, this issue) presented his now classic story of bone induction with collagen and bone morphogenetic protein (BMP). It has emerged as the most reliable bioassay for bone growth factors. The use of collagen and proteoglycans in the form of "regeneration templates" was described by I.V. Yannas (page 188, this issue) reporting on work with skin, peripheral nerve and knee meniscus replacements. Comments prompted by the presentations suggest that animal biopolymers are angiogenic, and that cells which populate implanted matrix grafts are mobilized from contiguous tissues, from the circulation or from stem cell reservoirs. Examples of other prostheses possible without cells include vascular, ligament, tendon, cartilage, and many body tubes and ducts. The basic question that begs an answer is: what information must a prosthesis have to direct regeneration of a functional replacement?

The second category included a report by T. Ziegler and R.M. Nerem (page 204, this issue) on the use of a smooth muscle cell tissue equivalent in which smooth muscle cells were cast in a collagen lattice. After contraction the lattice was overplated with vascular endothelial cells (EC). The model will be used to study the influence of flow on EC proliferation. The system has been used to reconstitute vascular prostheses, [Weinberg and Bell, 1986] but it is too compliant, without reinforcement, for an arterial replacement. Building cartilage tissues was described by C. Frondoza et al. (John Hopkins/Good Samaritan Hospital), who seeded a collagen sponge with chondrocytes, but did not see much increase in the biosynthesis of type II collagen over that observed in chondrocyte monolayers.

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In the third category, L.G. Cima (page 155, this issue) described polyethylene hydrogels embroidered with ECM ligands as a substrate for growing hepatocytes. It will be of interest to assess how the three-dimensional disposition of these ligands resembles that typical of the ECM that hepatocytes would normally see.

The fourth category dealing with a mix of natural and unnatural material to which cells were applied included a presentation by R. Young et al. (Case Western Reserve University), on a prosthetic device designed to promote tendon regeneration. Polyglactan 910 in the form of a sheath was seeded with collagen fibers, or 4-0 poly-polyglycolic acid sutures (both resorbable) collagen and mesenchymal stem cells (from bone marrow). While the histology, 12 weeks after implantation, was tendon-like, mechanical properties of the implants made to the Achilles tendon and restoration of function were not yet studied. In a similar approach, E.E. Stableman (VA Medical Center, Palo Alto, CA) and colleagues fill a glycolide trimethylene carbonaic conduit with Schwann cells and a collagen gel. The conduits were installed in gaps created in rat peroneal nerve with good gap bridging by regenerating axones reported when cells were present in the graft; conduits with collagen alone repaired nerve in 57% of operated animals. This points to variables in collagen preparation as vital to success. It is worth commenting that testing materials in vitro with cells they encounter in vivo, as implanted prostheses without cells, is a basic requirement for determining expression and maintenance of phenotype and possibly histiotypic organization, but biocompatibility in vitro may not be predictive for the reception materials get in vivo. In reviewing these reports on materials, we seem to be witnessing a trend toward the use of what may be considered bioabsorbable materials. It is known that naturally occurring biopolymers are, in general, bioabsorbable and remodelable. Now, synthetic materials that are self-dissipating are coming into vogue. The effects of their degradation products need to be carefully examined in experimental transplant systems. For example, poly-L-lactate breakdown products are known to interfere with bone regeneration.

In category five, we witness the introduction of new materials such as cross linked peptides containing unnatural amino acids (H. Blanch, University of California, Berkeley), recombinant proteins (D.A. Tirrell, University of Massachusetts, Amhrest; J.F. Ramalho-Ortigao et al., Albert Einstein Universitat, Ulm, Germany) and new acrylates, spiro-orthocarbonates, and calcium phosphate cements for dental applications (J.A. Tesk et al., National Institute of Standards & Technology). That polymers containing unnatural amino acids mimic the physical and structural properties of proteins makes them especially interesting as possible components of bioabsorbable reconstituted three-dimensional ECMs. We await experiments that combine them with tissue cells.

To sum up theme two, the notion that regeneration of missing body parts can be induced is attractive and challenging and invites experiment and discussion. The information for organogenesis is present in the genome and expressed during development, so it is appropriate to suggest that tissue building may be recapitulated. Sabelman et al. suggest that positional and migratory information needed by cells for regeneration may be imparted by an artificial but not entirely synthetic extracellular matrix. The secret lies in getting cells able to do so, to turn back their clocks, or finding stem cells ready to dance when given the right cue. There is already plenty of precedent for provoking regenerative responses in the adult. It is a matter of faith that much more is possible.

The third theme of The Workshop, *membranes for biotechnology*, is divided into three categories based on the kinds of materials used for creating the membranes and on their properties in membrane assembly. These catagories are membranes constituted from (1) natural self-assembling materials, (2) non-self-assembling natural materials, and (3) non-self-assembling unnatural materials.

In the first category, H. Bayley (page 177, this issue) reported on the α-hemolysin (αHL) peptide secreted by *Staphylococcus aureus,* which assembles into a hexameric pore of 1,1–1.2 nm in lipid bilayers. The hexamers can be induced to form crystalline monolayers. The protein pores have applications in membrane separation systems, and potentially as a device for targeting malignant cells for lysis by conjugation with antibodies to tumor cell antigens. Fortunately, wild-type αHL is not lytic for human blood cells because it cannot bind to them. U.B. Sleytr (page 171, this issue) also uses a microbial source of materials for assembling membranes. S-layers which make up the crystalline surface layers of the outermost component of the cell

envelope of *Archaea* are formed from self assemblies of a single protein or glycoprotein. The S-layer subunits can be isolated and induced to form two dimensional crystalline arrays. The arrays have uses for immobilizing functional molecules for biosensors, as an adjuvant for weak antigens, and for ultrafiltration. A.L. Plant (National Institute of Standards and Technology), working with phospholipid bilayer membranes, is also studying their permeability characteristics for their potential application to signal transduction in biosensors. A. Rudolph (page 183, this issue), evaluated liposomes and other lipid self-assemblies, including lipid microcyclinders, cubic phase lipids, and lipid-based microemulsions. The microcyclinders have been examined for controlled release properties, and liposomes were investigated as a means of encapsulating hemoglobin to produce a resuscitative fluid shown to deliver oxygen to tissues in animal models of hemorrhagic shock.

In category two, D. Fink et al., (Case Western Reserve University) presented work on the avian egg shell membranes that are laid down as the egg passes through the uterine canal. Analysis of the process of egg shell assembly should throw new light on mechanisms of biomineralization.

The last category included work by M. Lysaght (page 196, this issue) and M.S. Shoichet et al., dealing with a technology for encapsulating cells for transplantation. The approach is based on the development of thermoplastic membranes onto which poly(ethylene oxide) is grafted. Finding the right cells to encapsulate is still a major problem, as it is still not possible to cultivate human pancreatic islet cells, or human dopamine producing cells, for example. Although porcine cells may be useful when encapsulated, they too have been refractory to cultivation.

The fourth theme of the meeting focused on *therapeutic delivery systems*. Liposomes, porous membranes, and crosslinked peptides with unnatural amino acids as delivery systems were mentioned above, falling also into the theme of membranes for biotechnology. Regretfully, a scheduled presentation on vehicles for direct gene transfer was not delivered. A new technology was described by N. Kossovsky et al. (UCLA), who created a novel colloidal particle using various small solids made up of a small number of atoms coated with a carbohydrate. The particle forms a cradle for attachment of a wide variety of molecules which confer specific properties. Using the approach, carrier or delivery systems for decoy viruses can be created for use in vaccines.

A final comment is called for: There is a need for standard model systems for in vitro and in vivo testing of implantable, natural, unnatural and hybrid materials to be used in transplantation or as delivery vehicles. Standardized tests for measuring the immunogenicity and other reactions to materials used in prostheses or in delivery vehicles would help support new material development efforts.

REFERENCE

Weinberg C, Bell E (1986): A blood vessel model constructed from collagen and vascular cells. Science 231:397.