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TRANSIENT BIOCOMPATIBLE SCAFFOLDS FOR REGENERATION OF THE ARTERIAL WALL

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ABSTRACT The elements of a normal arterial wall may be reorganized through natural repair processes if the scaffold of a vascular graft is made of a slowly disappearing, biocompatible mesh. The challenge is to design tubular fabrics which at the time of implantation display the mechanical strength, suturability and limited thrombogenicity of the standard arterial grafts, but lead, after the disappearance of the synthetic polymers, to the formation of a regenerated tissue structure of appropriate strength and hemocompatibility, without at any time allowing the rupture of the arterial wall. Standard dimension aortic and carotid grafts made entirely of bioresorbable polymers function effectively in canine, bovine and porcine models, to a point where the mechanical and biological properties of the conduit are due entirely to newly formed tissues, which can adapt to the growth of the recipient.

Several investigators have formulated the research hypothesis that the elements of a normal arterial wall may be reorganized through natural repair processes if the scaffold of a vascular graft is made of a slowly disappearing, non-toxic mesh (1,2,3,4). The challenge is to design tubular fabrics which at the time of surgical implantation display the mechanical strength, ease of handling, suturability, limited blood loss and low thrombogenicity of the standard arterial grafts, yet lead, after the disappearance of the synthetic polymers, to the formation of a regenerated tissue structure of appropriate strength and hemocompatibility. The development of the new blood conduit involves the simultaneous decay of the synthetic polymer and growth of the constitutive elements of an arterial wall. The acid test for appropriate matching of these two processes is the absence of aneurysm formation or

rupture of the prosthesis under the stress of transmural hydrostatic pressure (5).

A similar approach has been proposed for the replacement of other duct structures in the body (e.g. trachea, esophagus) or entubulation of regenerating tissue (e.g. nerve guidance channel), but much of the initial experience in controlling the production of new tissue in vivo has been derived from arterial replacement.

A distinction can be made between biodegradation and bioresorption as related to tissue engineering. Biodegradation is an in vivo depolymerization process where changes in implant shape, dimensions or mechanical integrity result from a breakdown which liberates macromolecular or particulate fragments. Whereas the original material may have been quite biocompatible at its interface with living tissue, debris and split products cannot be easily eliminated by the body, and are likely to be recognized as "non-self" by the host organism. In contrast, bioresorption implies that the living recipient of the implant is not only capable of depolymerizing the synthetic material, but will also eliminate it by phagocytosis, intermediate metabolism, consumption as a fuel substance, or excretion of the decomposition products. In other words, to demonstrate bioresorbability, the products of depolymerization must either be transformed along standard biochemical pathways, and then consumed locally without harmful effects to tissues, or they must be eliminated through standard excretion mechanisms, which typically involve coupling with water-soluble transport molecules.

Historically, the first step in the design of bioresorbable vascular grafts has been the search for polymer yarns which would depolymerize at the appropriate rate in the warm, watery environment of a mammalian body. The standard bioresorbable suture materials (polyglycolides and polylactides) which lose their mechanical strength within two or three weeks, often lead to catastrophic failure of the implants. Materials which last over six months often elicit an intense giant cell reaction. Yet there is no clear cut relationship between the stability of the polymer and the thickness of the tissue layer formed on the luminal side of a vascular graft, perhaps because the intensity of the tissue reaction is not only related to the chemical nature of the polymer, but also to the mass of material involved. Encouraging results have been obtained with polymers which degrade over a period of two to more than four months. Such materials can be novel bioresorbable homopolymers (e.g. polydioxanone) or copolymers, composites of polyurethanes (6) with polylactides (4) or fibrin (7), or fast resorbing polyglycolide yarns coated with "retardant"

polymers (5), e.g. a mixture of polylactide and poly (2,3 butylene succinate).

In our own experience an 8mm internal diameter, 8-9 cm long aortic graft made entirely of bioresorbable materials has functioned effectively as an arterial conduit in dogs for as long as six months. Thus far the best results, measured in terms of patency, absence of aneurysmal dilation, and formation of a new blood interface devoid of mural thrombosis and intimal hyperplasia, have been observed with polymers which have largely decayed at three months, as ascertained by standard and polarized light microscopy, as well as determinations of molecular weight by gel permeation chromatography (4,8). Since at that point the synthetic fabric has lost its structural strength completely, the newly-formed tissues provide the mechanical integrity of the conduits. However the healing process has not yet stabilized and a true endpoint has not been reached from a cell biology standpoint. Interestingly enough, we have never observed the incidence of infection with fully bioresorbable prostheses. Stress-strain studies performed on the tissue formed on the inside of the original implant indicate that this element of the conduit, which morphologically appears to be made of fibroblasts, myofibroblasts and collagen fibrils, has about one quarter to one half the mechanical strength of a control specimen from the same animal (3). The experience of other investigators suggests that the compliance of the original prosthesis is a critical factor in obtaining the development of a circularly-oriented smooth muscle layer and the deposition of elastin (4). Aortic implants placed in young growing animals have shown the ability of the newly-formed conduit to double in size to match the growing diameter of the native artery (9). It seems also that the use of bioresorbable suture material in matching the anastomoses is a critical factor in preventing the formation of stenosis at the point of attachment of a biodegradable prosthesis with the aorta in a fast-growing animal such as the miniature pig. We have observed calcium salt deposition in the interstices of a non-woven composite fabric made of polyurethane and fibrin, implanted as an aortic graft in the rat, but neither our group, nor others have yet reported calcification with prostheses made of a woven or knitted bioresorbable fabric.

TISSUE ENGINEERING

Now that the research hypothesis of controlled in vivo synthesis of living tissue has received its initial validation, what can be the contribution of "tissue

engineering?" I will define tissue engineering as the creative concept of locating at the level of tissues - meaning assemblages of different cell types and their ground substances or secretion products - the interface between engineering/materials science with modern biology.

The issue, as I see it, is how to reconcile the engineering analysis and systems approach to life processes, both of which adequately describe the phenomena, but have yet no obvious link with the underlying cellular and molecular processes, with the pathobiology approach to organ dysfunction, which is increasingly served by a cellular and molecular approach, and focuses on the resolution of clinically relevant problems, but features a number of unquantifiable "black boxes" which defy engineering analysis.

If tissue engineering is to be the meeting ground of engineering and materials science with cell biology, both disciplines need to develop new methodologies to effect a fruitful encounter. For engineering, the challenge is first one of instrumentation to measure the physical properties of tissues on very small samples and under conditions which do not lead to post-mortem alterations. Soft tissue micromechanics (3) must address the isotropy and uniformity (or absence thereof) of the components of regenerated tissues, as compared to normal tissues. The next challenge is to develop computer models of these structures which may help to predict failure mode and the role of individual building blocks. For materials science, the question is to relate surface and bulk characteristics, including the microarchitectonics of a bioresorbable tubular fabric, with the intensity and duration of the cellular response to the implanted material. For cell biology, the challenge is two-fold: to address the various components of the tissue reaction in quantitative terms, using the methods of computer-assisted histological and histochemical analysis; and to find ways to influence actively the individual steps of tissue regeneration (10), using either cell seeding with endothelial cells (11) or smooth muscle cells (12), drug release systems, immobilized enzymes, growth activators or growth inhibitors attached to the bioresorbable polymer scaffold. Throughout this effort, it will be critical to express design objectives in biologically meaningful terms.

For vascular wall regeneration, the issue of animal model deserves serious consideration. Long term experiments in large adult animals are prohibitively expensive. Therefore the number of prostheses in any cohort is of necessity small. Explant study methods which consume all or large parts of a specimen cannot be statistically correlated with data from morphology, histology or biochemistry except in large scale studies. Species differences also leave

unresolved questions as experimentalists diversely use rabbits, dogs, sheep, baboons and miniature pigs. Our group has recently elected the microsurgical implantation of aortic prostheses in the rat as a screening method for new materials or new doping substances on existing materials.

We subscribe to the view that the formation of a new arterial wall in relation to a bioresorbable polymer scaffold represents a special case of the wound healing process. Contrary to our initial hypotheses, the core of the wound healing process does not take place within the interstices of the porous, bioresorbable fabric, but inside and outside of it, with the result that the disappearing polymer strands are progressively squeezed between a neointima and neo-media on the luminal side, and a neo-adventitia on the outer side. The various phases in the cellular reactions to a bioresorbable implant are qualitatively the same as with a biodurable material. To control the process and guide it toward a structure as closely resembling the natural arterial wall as possible, it appears important to dampen the intensity of the inflammatory reaction, to avoid the retention of a permanent irritant, to minimize the proliferation of fibroblasts and the deposition of collagen fibrils, to favor the differentiation of myocytes and their spatial organization in circular and longitudinal bands, and lastly to achieve a continuous endothelial lining on the luminal side. Competing with nature is a tall order, but given time and ingenuity, the goal of a biological replacement for a diseased artery no longer appears totally unrealistic.

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