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Reducing capsular thickness and enhancing angiogenesis around implant drug release systems

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Abstract

Biological encapsulation and the foreign body reaction can impair the performance of implanted drug release devices. In this article, the classic definition of biocompatibility is questioned. Examples are presented of biomaterials showing unique healing behavior. A new paradigm for biomaterials healing is proposed in which non-specific protein adsorption is inhibited and matricellular proteins are controlled at the surfaces of implants. © 2002 Elsevier Science B.V. All rights reserved.

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Professor James M. Anderson has led the field of biocompatibility research for over 20 years. His ideas and teachings have strongly influenced multiple generations of biomaterials scientists. I have been strongly influenced by his ideas on the foreign body reaction and the role of the macrophage. This article, and the perspective presented here, are in recognition of Jim Anderson's contributions to biomaterials science and to the UWEB effort to engineer biomaterials that heal.

1. Introduction

Controlled release devices have grown from a research curiosity in the late 1960s to an important component of clinical medicine and a successful industry. However, long-term, implanted controlled release devices are relatively rare, and where there has been in vivo application, there have also been problems associated with performance. For example, the difficulties in accurately controlling steroid release in Norplant™ controlled delivery devices may be associated with the inability to accurately predict the thickness of the foreign body capsule that forms around the device. This article will suggest that biocompatibility, and our present definition of biocompatibility, are the roots of this impediment to the further development of implanted controlled release devices. A strategy that should lead to improved performance of future implanted drug delivery systems will be presented.

Criteria for a successful, long-term, implanted controlled release device include appropriate release rates (often zero order), drug delivery to the body paralleling the release rate from the device, stability (of the device itself and the drug inside), reasonable size consistent with the anatomy, sterilizability and

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biocompatibility. Controlled release scientists have learned to engineer and optimize most of these criteria. However, the last, biocompatibility, has largely not been addressed with success or precision.

2. Biocompatibility

Since millions of medical devices are implanted into humans each year with reasonable levels of success, one would assume that biocompatibility is well understood. The FDA and other regulatory agencies 'stamp' our medical devices as biocompatible. In fact, a central word in the biomaterials field that distinguishes biomaterials from other materials is biocompatibility. Surprisingly, given the clinical impact of biomaterials, biocompatibility is poorly defined. The widely accepted definition of this word is:

> ''*the ability of a material to perform with an appropriate host response in a specific application*'' [1]

This definition, though accurate, offers no insights into how to evaluate biocompatibility or to enhance it.

Researchers, over some 40 years, have suggested that biocompatibility may be related to surface energy, negative charges, hydrogels, heparin, titanium, phosphatidyl choline, polysulfones, roughness, hardness, etc. To this day, there are no clear rules that can be used to design a material for biocompatibility — good evidence that we do not yet understand biocompatibility. What is biocompatibility and what route might we take exploiting surfaces to obtain a precise definition of biocompatibility? Why is this word poorly defined? Consider the following two ideas:

1. Smooth materials that do not leach biologically reactive substances will heal in the body in a manner now considered biocompatible. Are all materials equally biocompatible?
2. The body reacts similarly to nearly all materials that we call biocompatible and walls them off in an avascular, collagenous bag, 50–200 μm thick. This reaction is referred to as the foreign body reaction. The foreign body reaction is illustrated schematically in Fig. 1. The accepted regulatory definition of biocompatibility revolves around this reaction of the body to isolate itself from 'biocompatible' biomaterials.

If all materials heal similarly, and the regulatory agencies have declared this reaction to materials acceptable, what are the concerns with today's biomaterials and how they heal? Uncontrolled biological encapsulation impedes the performance of many implanted devices. For example, consider drug delivery systems, implant electrodes, and breast implants. All are seriously degraded in performance by this capsule that prevents intimate contact between device and tissue. The foreign body reaction (long-term, low level inflammation and macrophage activation) may also inhibit the luminal healing of vascular grafts, trigger capsular opacification found with intraocular lenses, extrude percutaneous devices, exacerbate device calcification and generally lead to less than desirable outcomes associated with today's medical devices.

The human body has an excellent capacity to heal wounds and injuries with healthy, vascularized tissue. Could normal healing be wrong? Why do 'biocompatible' implants shut off normal wound healing?

3. Toward a new paradigm for biocompatibility

Since we can obviously heal devices within a foreign body capsule, what's next? Can we bypass this aberrant healing and achieve normal, vascularized tissue? These questions and comments require clarification and justification.

Let us examine a list of 10 common materials used in medicine: Teflon polyurethane, silicone rubber, polyethylene, PMMA, polyHEMA, Dacron, gold, titanium, alumina. These materials are hydrophilic, hydrophobic, hard, soft, polymeric, metallic and ceramic. After a 1-month implantation in mammals, they are all found to heal essentially identically. On the other hand, each material will be found, in vitro, to adsorb different proteins, and to show substantially different cell attachment and cell growth behavior. An experiment similar to this has been performed

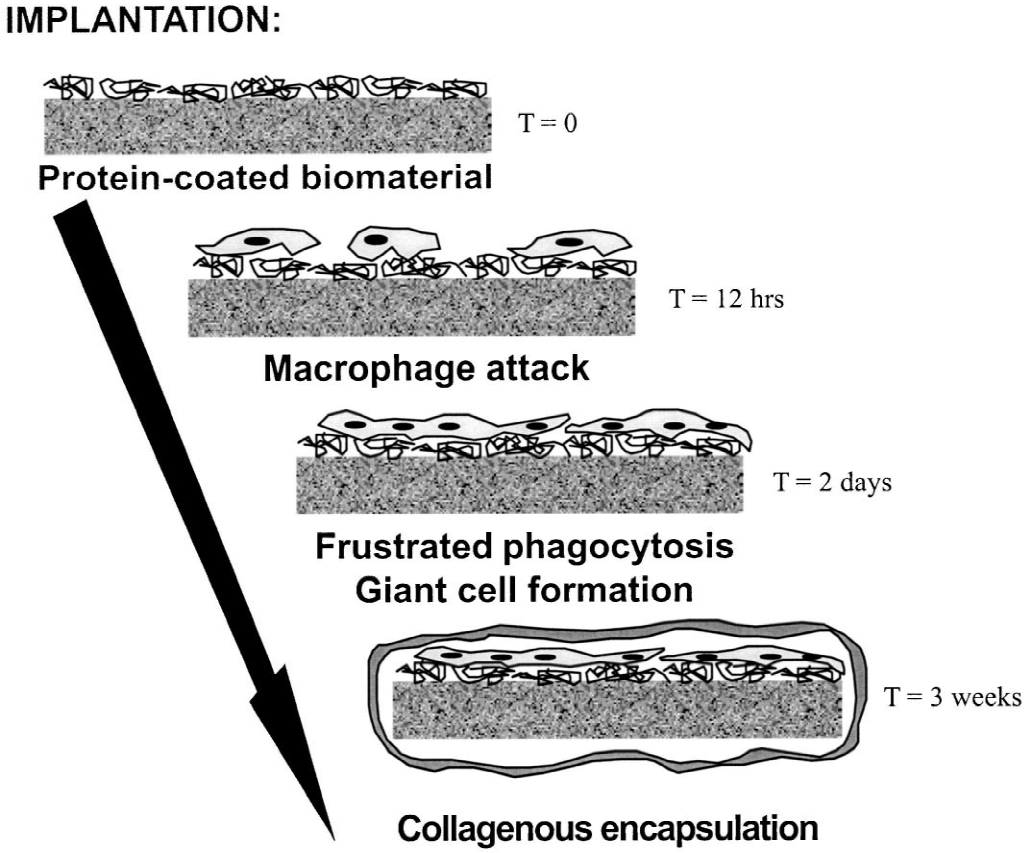

Fig. 1. The time course and generalized reactions that comprise the foreign body reaction.

with results consistent with the 'thought experiment' described above [2]. This perplexing difference between in vivo and in vitro represents a pervasive problem in biomaterials science.

The 10 materials in the previous paragraph have a commonality — they adsorb a complex, non-specific layer of proteins. Each will have a different surface protein mixture, but all the materials will quickly acquire a layer that contains many proteins (possibly comprised of 200 or more proteins) in many orientations (up, down, sideways) and conformational states (native to highly denatured). Nature *never* uses such non-specific layers of protein. Nature uses one (or a few) specific proteins in fixed conformations and orientations for optimal biosignal delivery. I hypothesize that the body interrogates this non-physiologic, proteinaceous layer, finds it to be something with which it has no experience and reacts to it as an unrecognized foreign invader to be walled off. These non-specific layers are, in my opinion, 'the enemy.' For progress to be made, we must go beyond this ill-controlled reaction, i.e. defeat the enemy. Hence, surfaces must be developed that control the conformation and orientation of proteins with precision so that the body will specifically recognize them.

In a normal wound, the macrophage, responsible for 'orchestrating' wound healing, is activated. In the presence of an uncomplicated wound, the macrophage turns on the pathways leading to normal healing by first cleaning the wound site and then secreting appropriate cytokine messengers. These soluble molecules activate processes in the cells needed for healing (keratinocyte, fibroblast, osteoblast, etc.).

The surfaces of synthetic implants modulate the normal wound healing process. Macrophages adhere

to the biomaterial, do not specifically recognize it and spread on its surface as they try to phagocytose it. They cannot digest or engulf a large mass of biomaterial — to increase their effectiveness, they fuse to form multinucleated giant cells. These giant cells still cannot engulf a macroscopic medical device. The multinucleated giant cells send out signals indicating a large foreign body to be walled off. Fibroblasts arrive and synthesize the collagen capsule, most likely guided by the signals from the macrophages.

To achieve improved healing of controlled drug delivery devices (i.e. no capsule or greatly reduced capsule formation) it seems reasonable to interfere with initiating events in the foreign body reaction. There are distinct approaches and strategies that should be applicable to realize 'biomaterials that heal.'

1. The study of the basic biology of normal wound healing must be central. This is in contrast to two themes that dominate today's biomaterials studies, i.e. 'inventing' biocompatible materials without a full knowledge of the biological reactions that must be controlled, and wound healing with a biomaterial present (in contrast to normal wound healing). The basic study of normal wound healing biology will tell us what molecular and cellular pathways to turn on, what pathways to turn off and what are the triggers (and blockers) for those pathways.

2. Non-specific adsorption of proteins and other biomolecules must be inhibited.

3. The surfaces of biomaterials should be synthesized to present to the body the same signaling groups (protein, extracellular matrix) as, for example, the surface of a fresh wound. Evolution has developed recognition-activated pathways and it is probably futile to try to bypass this system optimized over millions of years.

This hypothesis on healing and the foreign body reaction opens myriad opportunities. Professional biological researchers can best undertake the study of the basic biology. However, when biological discoveries are made, the ability to inhibit non-specific interactive events on surfaces and the intellectual challenges of delivering the specific biological signals, opens exciting frontiers for engineers and materials scientists.

4. Non-fouling surfaces: eliminate the non-specific

Considering point 2, above, many strategies to inhibit non-specific protein adsorption (non-fouling surfaces) have been developed (Table 1). How resistant to protein pick-up can such surfaces be made? Why are they resistant to protein adsorption? How long can they remain resistant to protein fouling? Can they be functionalized with organic groups permitting the immobilization of active biomolecules on a bland background? These questions drive research in this area. A number of recent issues of *Journal of Biomaterials Science: Polymer Edition* (Volume 11, 2000) and an issue of *Colloids and Surfaces B: Biointerfaces* (Volume 18(3,4), 2000) have focused on these points.

Surfaces made by the RF-plasma deposition of tetraethyleneglycol dimethylether (tetraglyme) have been explored in our group [3,4]. These surfaces have been characterized by modern surface techniques revealing a crosslinked PEG-like structure and have been shown to have extremely low protein pick-up (Table 2). Furthermore, this low protein pick-up translates to low cell adhesiveness as seen with *Pseudomonas aeruginosa*, blood platelets, monocytes and endothelial cells. Thus, such surfaces may have utility on implant drug delivery devices to inhibit the build-up of undesirable biological materials that could impede release. However, they may not be sufficiently bland to turn off the foreign body reaction and they certainly do not transmit signals to the body that might encourage normal healing.

5. Turning on and off healing

There are a number of materials, most discovered fortuitously, that do promote healing more in the direction of normal wound repair or regeneration and less in the direction of foreign body encapsulation. It is worthwhile considering these since their understanding may lead to strategies to design the normally healing biomaterials needed for advanced controlled delivery systems. A number of these materials only show good biological integration in bone. But, their influence on the healing process is dramatic and

Table 1
Protein-resistant (non-fouling or stealth) surfaces

Surface strategy	Comment
Poly(ethylene glycol) (PEG)[a]	Effective, but dependent on surface chain density. Damaged by oxidants
PEG-like surfaces by plasma deposition	Applicable for the treatment of many substrates and geometries. Highly non-fouling
PEG oligomers in self-assembled monolayers	Highly protein-resistant. Applicable for precision molecularly engineered surfaces
PEG-containing surfactants adsorbed to the surface	A simple method for achieving non-fouling surfaces. Durability may be low and high surface densities are hard to reach.
PEG block copolymers coated on the surface	A relatively low density of surface PEG groups
Saccharides	Nature's route to non-fouling surfaces. Some synthetic successes but much remains to be explored.
Choline groups (phosphatidyl choline)	Has shown good success in many applications
Hydrogen bond acceptor surfaces	This principle may explain the non-fouling properties of PEG surfaces and is leading to new discoveries of surface functional groups exhibiting non-fouling behavior.
Adsorbed protein layer	A pre-adsorbed protein layer resists further adsorption. This approach, long used by biologists, is simple but of low durability.
Hydrogels, in general	PEG is in this class. Many other hydrogels have shown non-fouling behavior.

[a] Also called poly(ethylene oxide) (PEO).

worthy of observation. It is also worth noting that in bone, healing begins with macrophages, just as in soft tissue, and can proceed to an implant encapsulated in collagen and separated from the bone tissue.

5.1. Surface-treated titanium

A treatment of titanium surfaces with strong base and high temperature strikingly alters the healing characteristics of the material in bone [5]. Untreated titanium heals in bone with close apposition of bone and metal. However, there is a thin (50–200 Å), organic layer separating the bone and metal, and no bonding between them. The treated titanium, on the other hand, fuses to the bone with the absence of sharp, definable interfaces.

Table 2
Protein adsorption to TEGDME plasma-treated surfaces

Surface	Protein adsorption (ng/cm^2)
Teflon	93.6±5.3
Tetraglyme-treated Teflon	1.7±1.0
Polyethylene	91.0±10.3
Tetraglyme-treated polyethylene	1.7±1.5

5.2. Tyrosine polycarbonates

Tyrosine polycarbonates are a family of biodegradable polymers that are well tolerated by the body upon implantation. One member of this series, an ethyl ester, shows an excellent ability to heal in bone [6]. The mechanism may be related to an appropriate hydrolysis rate for the ester (not too fast or slow).

5.3. Hydroxyapatites

Hydroxyapatite, a form of calcium phosphate, comprises the mineral phase of bone. Hydroxyapatites and probably some other forms of calcium phosphate readily heal into bone and fuse with it. Hydroxyapatites are widely used in clinical medicine and represent a subject of intense research exploring both the materials science and the biological reactivity of this important biomaterial.

5.4. Tissue-derived materials

A number of biologically-derived, processed materials have been found to heal into the body in an integrated fashion. Foremost among these is a membrane material derived from porcine small intestinal submucosa (SIS) [7]. This material is isolated primarily as an acellular extracellular matrix (ECM) material. However, it has been shown by extraction studies to be rich in various growth factors [8]. SIS has been shown to heal in humans with little or no scarring in sites such as wounds, arteries, internal organs and eyes. The implant seems to be broken down and replaced by normal tissue when implanted.

5.5. Porous materials

In 1995, Brauker et al. reported that certain porous structures could heal into soft tissue with a unique biological response [9]. The experiments described were part of a program to identify a suitable immunoisolation membrane for cell-based implants. A large number of commercially available, porous membranes were evaluated by implantation into rats for 4 weeks. After harvesting the implants, fixation and histological analysis, the majority of the implanted membranes were found to be encapsulated into avascular, collagenous sacs, the classic foreign body reaction. However, a different healing was noted with a small fraction of the samples. These had reduced collagen formation, an open structure to the collagen, and blood vessels in close proximity to the implanted membrane. The common aspects of the porous materials that induced this special response, a response much closer to normal wound healing than the classic foreign body reaction, were pores with sizes in the range 5–15 μm, interconnectivity for the pores and the absence of expanses of flat surface on to which the inflammatory cells could spread. The special healing reaction was seen with a number of different types of porous materials (Teflon, mixed esters of cellulose, etc.) and seemed independent of material type. Thus, an improved healing could be generated by simply manipulating the porosity of the material. The Brauker experiments were preceded by porous implant experiments in the 1970s led by Brand and Brand that hinted at these observations [10] and by modern experiments in our laboratories and by others [11] validating these ideas.

5.6. Fine fibers

Recent experiments by J. Saunders and students using fine, electrostatically spun fibers have revealed a new path that might potentially lead to healing biomaterials [12]. Fibers with diameters greater than 5 μm induced a classic foreign body reaction. Fibers with diameters less than 5 μm generated little or no observable encapsulation. No fibrous capsule was seen for 1-μm fibers. The cells of the body seemed to lose the ability to recognize and respond to the fibers when they became thin enough.

6. The molecules that turn on and off healing

Within the University of Washington Engineered Biomaterials (UWEB) program (an NSF Engineering Research Center), and in a number of other research groups around the world, key molecules that turn on and off normal healing are being explored. UWEB has focused on a class of proteins sometimes referred to as matricellular proteins that are always found in healing wounds and are largely absent in healed wounds. The name ‘matricellular’ refers to the fact that these molecules exist between the extracellular matrix (ECM) molecules such as collagen and cells. Matricellular proteins of interest to UWEB include thrombospondin, SPARC and osteopontin — all have been shown to be important for the foreign body reaction and for normal healing.

6.1. Osteopontin

Osteopontin, an approximately 43 kDa phosphorylated, acidic glycoprotein, has often been associated with calcification, healing, inflammation and integrin-mediated cell adhesion [13,14]. It down-regulates inducible NO synthase (iNOS) and reduces NO in macrophages and other cells. It has been shown to enhance cell survival, inhibit calcification and quiet inflammation. UWEB is using osteopontin at surfaces to test its ability to reduce the foreign body response, and also to inhibit calcification. A

study of osteopontin on titanium suggests its ability to enhance healing in bone [15].

6.2. Thrombospondin 2

Thrombospondin (TSP) 2, a trimer protein with a chain molecular weight around 145 kDa, is a member of a family of secreted glycoproteins including TSP 1, TSP 3, TSP 4 and TSP 5 [16]. TSP-2 is up-regulated in wounds. TSP-2 knockout mice were found to heal dermal wounds more rapidly and with enhanced angiogenesis. When silicone elastomer implants in TSP-2 knockout mice were examined after 4 weeks, they were found to have a higher blood vessel density in their vicinity and an open, unoriented collagen structure surrounding the implant [17]. In contrast, the control, wild-type mice mounted a classic foreign body reaction characterized by a tough, dense foreign body capsule with little vascularity. Recently, Kyriakides et al. have explored the delivery of anti-sense DNA to wipe out TSP 2 production and enhance healing [18]. Some success was noted with this approach.

6.3. SPARC

SPARC (Secreted Protein Acidic and Rich in Cysteine), also called osteonectin, binds to hydroxyapatite, collagen, vitronectin and thrombospondin 2, to name just a few of its interactions. SPARC inhibits cellular proliferation and also regulates growth factors, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF)-2, and vascular endothelial growth factor (VEGF) [19]. SPARC is associated with healing wounds and has demonstrated anti-angiogenic and pro-angiogenic activity.

These matricellular proteins are clearly important to normal wound healing. The application of these proteins for enhancing the healing of implant biomaterials and controlled release devices raises a number of questions. Can these be effective on surfaces? What strategies might be used to immobilize them in a precise manner? Are one or many of these proteins necessary for normal healing? How can we effectively implement inhibitory strategies for proteins driving the foreign body reaction? How can we address high cost, stability and sterilizability of such proteins? These are challenging questions, but if we are to ever hope to emulate nature's own mechanisms and apply them to heal implants, we as bioengineers must come to grips with these issues.

7. Concluding remarks

The surface structure of biomaterials and drug delivery systems that interact with precision with biological systems will be complex — multicomponent, multilayer, orientated, patterned. These will emulate the ECM to appropriately deliver signals to turn on normal healing. Given the complexity of the molecular structures that make up the individual biomolecules comprising these surfaces coupled with the multicomponent nature of such surfaces, fabrication and characterization of such surfaces will push the skills of surface scientists and bioengineers. New developments in surface science applied to biology will make the analysis of such complex surfaces feasible [20]. Ideas from materials science and nanotechnology such as self-assembly, supramolecular structure and nanofabrication will also contribute to the fabrication of these surfaces.

In the future, tissue engineering (coupled with truly biocompatible scaffolds), stem cell technology, control of regeneration and the knowledge of the human genome will completely change biomaterials and drug delivery devices. But, before these revolutionary technologies replace today's biomaterials, we still probably have 30 years during which biomaterials as we know them today will be of increasing importance. Thus, there is strong impetus to evolve the surface strategies needed to control biological interactions.

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