MOLECULAR DESIGN STRATEGIES FOR BIOMATERIALS THAT HEAL

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Abstract: Biomaterials are widely used in medical devices with good success. However, the surface chemistries of our present generation of biomaterials are not specifically recognized by living organisms. Thus, biological reactions to biomaterials in use today are primarily influenced by non-specific interactions occurring at their surfaces. This paper develops a hypothesis for the development of a future generation of biomaterials. A discussion of the meaning of biocompatibility is followed by a strategy for developing biomaterials that actively induce healing and reconstruction by turning on specific biologic pathways. Materials strategies for encouraging specific reactions and inhibiting non-specific bioreaction are presented.

INTRODUCTION

Biodegradable and biostable biomaterials are widely used in medicine and in other applications interfacing with biological systems. Many reviews that offer broad descriptions of biomaterials science are readily available [1-6]. Biomaterials are found in vascular prostheses, heart valves, pacemakers, sutures, intraocular implants, hip joint prostheses, contact lenses and drug delivery systems, to mention a few common examples. Millions of implants are used in humans each year, saving lives and improving the quality of life. Yet, there are problems with how these implants heal, and they are never as good as the part they are intended to replace. This article highlights important principles guiding the application of materials in medicine and biology and the opportunities to advance biomaterials and control healing. In particular, the potential for the molecular design of materials is emphasized. Ideas to be exploited in molecular design of materials, in contrast to more traditional materials science approaches, are suggested in Table 1.

A special characteristic that sets a biomaterial apart from materials that might be used in a tennis racket, automobile, airplane or computer is that a biomaterial should be "biocompatible."

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Biocompatibility, defined at a conference on definitions in biomaterials [7], is "the ability of a material to perform with an appropriate host response in a specific application." Let us examine this definition. First, the definition directs us to some "specific application." Table 2 lists a few of these specific applications.

Table 1 Traditional Materials Design versus Molecular Design

Two Approaches to Materials Design ...	
design based on	design based on
• **mechanical properties** • **permeability** • **refractive index** • **conductivity** • **biodegradability**	• **molecules** • **specificity** • **orientation** • **assembly** • **epitaxy**
design with the "bulk"	*design with the "surface"*

TABLE 2. Biomedical Devices and Biomaterials

Application	Material	Special Property
dialysis membrane	cellulose	permeability
vascular prosthesis	Teflon, PET*	burst strength, porosity
heart valve	carbon, metal, Dacron	durability, hydrodynamics
artificial heart	polyurethane	flex-fatigue resistance
pacemaker lead	polyurethane	insulator, durability
contact lens	PHEMA*	clarity, wettability
intraocular lens	PMMA*	clarity, refractive index
hip prosthesis acetabular cup	UHMWPE*	lubricity
bone cement	PMMA*	quick setting, strength
hydrocephalus shunt	silicone rubber	flexibility
tendon prosthesis	Dacron	strength, flexibility
cochlear electrode	platinum	electrical characteristics
tissue engineering template	poly(lactic acid)	biodegradability

*PET = poly(ethylene terephthalate); PHEMA = poly(2-hydroxyethyl methacrylate); PMMA = poly(methyl methacrylate); UHMWPE = ultrahigh molecular weight polyethylene

A specific application imposes demands on the properties of materials. Thus, a membrane in an artificial kidney requires a certain permeability and permselectivity, a hip prosthesis requires a defined strength and wear resistance, and, for an intraocular lens, there are clarity and refractive index requirements. The other major idea in the definition, "host response," is more complex and will be dealt with in subsections in the Biocompatibility section, below. Each subsection contributes an important idea to the words, "host response."

BIOCOMPATIBILITY

Factors that contribute to our understanding of the term "biocompatibility," and further help to clarify the unique materials aspects of biomaterials are discussed in this section.

Toxicology

A biomaterial should not be toxic, unless specifically engineered to be toxic (e.g., a "smart bomb" drug release system that seeks via a recognition mechanism only cancer cells and destroys them by releasing an agent specifically toxic to those cells). Since the absence of toxicity is accepted as an unquestioned requirement for biomaterials, the field of toxicology for biomaterials has evolved into a sophisticated science. Toxicology measures the substances that migrate out of biomaterials. For example, polymers, often have low molecular weight "leachables" (additives, low molecular weight fractions, initiator fragments, etc.) that can exhibit physiologic activity and cell toxicity. A biomaterial should not release anything from its mass unless specifically designed to do so. Fortunately, we can synthesize and purify polymers so they readily pass sensitive toxicology assays.

Tissue and Blood Compatibility

The term "biocomptibility" is widely used in describing biomaterials, and is clearly related to the interfacial interactions between materials and the contacting biology. The study of the interactions of synthetic materials with blood and tissue (soft tissue, bone, nerve, eye, etc.) is a subject unique to biomaterials science. The science driving these interactions must be appreciated and methods developed to study and quantify the interfacial biology. Unfortunately, we lack precise definitions or accurate measurements of blood and tissue compatibility. More often than not, blood and tissue compatibility are defined in terms of medical device performance or success at a specific task. Thus, for a patient who is alive and thriving with a properly functioning heart valve (unoccluded and free of other complications), few would argue that this heart valve is, in this case, "blood compatible." However, this operational definition

offers us little to use in the design of new or improved prostheses for use in the blood stream. It is probable that tissue and blood compatibility may have to be specifically defined for applications in hard tissue, nerve, skin and the cardiovascular system. In fact, tissue and blood compatibility may have to be uniquely defined for each application. Ideas on this subject that extend this operational description of biocompatibility are offered in the Perspectives section of this article.

Healing

When a material or device is implanted into the body, and healing occurs around the surgical site, special aspects of this process induced by the implanted material can be observed. Injury to vascularized tissue (the surgical incision) will trigger the well-defined inflammatory reaction sequence that underlies healing and reconstruction. This physiological system, involving many cell types and biomolecules, is designed to clean up the debris at the injury site, and then heal the wound. Where a foreign body (*e.g.*, an implant) is present, the reaction sequence is referred to as the "foreign body reaction." The normal response of the organism to the wound will be modulated because of the solid implant. Furthermore, this reaction will differ in duration and intensity, depending upon the precise implant anatomical site, implant mechanical properties, abrasive irritation and toxicological considerations. The time course of the healing process, and the cells and molecules involved are suggested by Figure 1.

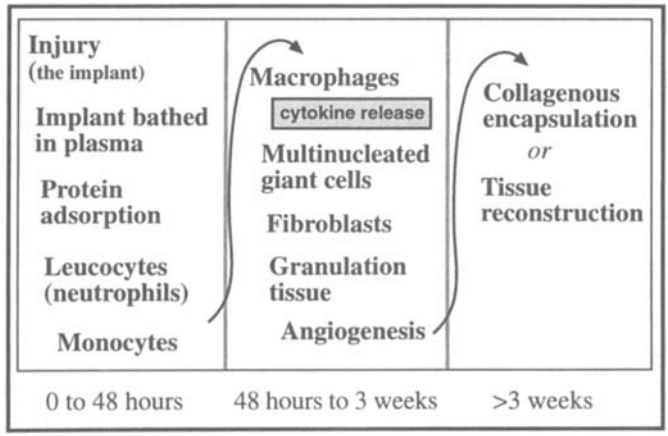

Figure 1. The time course of the inflammatory and healing processes, and some of the cells and molecules involved.

Anatomical Sites for Implantation

The anatomical site of an implant device or material can have a profound effect on its performance. Consider four examples. An intraocular lens may be surgically inserted into the lens capsule or the anterior chamber. A joint prosthesis (typically hip or knee) will be implanted in bone and span an articulating joint space. A heart valve will be sutured into cardiac muscle. A catheter may be placed in a vein, artery or body cavity. This range of sites challenges the biomedical device designer with special requirements for geometry and size, mechanical properties, and bioreaction.

Mechanical and Functional Performance Requirements

In addition to biointeraction considerations, demanding mechanical and physical performance requirements are intrinsically superimposed on biomaterials and medical devices. Such physical properties largely originate from the physical (bulk) properties of the material, although some, for example lubricity, are surface properties. These requirements can be categorized as follows: mechanical performance (*e.g.*, modulus, toughness, hardness), mechanical durability (*e.g.*, strain to break, flex fatigue life), and physical properties (e.g., permeability, transparency, electrical conductivity). A material that does not perform with the required physical and mechanical properties might appear as non-biocompatible.

In summary, the understanding of biomaterials and biocompatibility has convoluted within it many subjects that must be mastered, or at least appreciated. Some important themes are listed in Table 3.

Table 3: Some Important Themes in Biomaterials Science

THEME	PRACTITIONER
new or modified biomaterials	polymer chemist, materials scientist
mechanics of materials, defects	materials scientist, mechanical engineer
surface modification, surface analysis	surface scientist
proteins at interfaces, immobilization of biomolecules	biochemist
assessing the toxicologic potential of materials	toxicologist
design of biological reactions at the material interface	molecular biologist, computational chemist
understanding cellular reaction to biomaterials	cell biologist
testing biologic reaction in animals	veterinary surgeon
evaluation and implantation in humans	physician
evaluation and implantation in the oral environment	dentist
compliance with legislation to protect the consumer	regulatory and legislative specialist
final packaging	sterilization and packaging specialist

PERSPECTIVES AND OPPORTUNITIES IN BIOMATERIALS

Although the case has been made that biomaterials are useful for many medical and biological applications, consider this perspective the biomaterials field today, and where it must go:

After 35 or more years of the systematic study of biomaterials, we have a number of materials that we commonly use to fabricate implanted medical devices. We frequently use the term "biocompatible" when we describe these materials. These synthetic materials, medicine's biomaterials *circa* 1997, typically elicit a non-specific response from biological systems. For example, consider this set of biomaterials: silicone rubber, poly(glycolic acid), titanium, polyurethane, hydrogel, stainless steel, alumina, Teflon and plasma-deposited siloxane on titanium. The materials we compare in this list are metals, ceramics, polymers, soft, hard, wettable, non-wettable, chemically reactive, inert, biodegradable, permeable, impermeable, etc. If we implant them, all will heal similarly (as surmised from many previously published studies and studies in our group), and the implants will all be surrounded by a thin, almost avascular collagenous capsule. If we followed the healing process for this list of materials over time, we would observe the classical inflammatory sequence at and around the implant: neutrophils -> macrophages -> foreign body giant cells, fibroblasts, capsule formation. Furthermore, similarities in healing would be seen in this set of materials if they are implanted in soft tissue, the peritoneum, nerve, the eye or in bone. Yet, if we had studied these samples *in vitro*, we would find that they adsorb different proteins in different conformations and they adhere and activate cells differently. Clearly, biological systems can interrogate the surfaces of these different materials and respond differently to them. Yet, *in vivo*, the differences in reaction seen *in vitro* are not observed. So, if all materials heal similarly, why do we need different biomaterials, and why do we continue to develop new biomaterials? Further, we have no guidance or directions for designing "more biocompatible" biomaterials. This article will now elaborate upon this conundrum, present a hypothesis that addresses the problem, and briefly discuss materials approaches that may lead us to biomaterials that heal in a more physiologically normal manner and might truly be called "biocompatible."

To explain the similarities observed in healing of almost all of the medical implant materials, consider the protein layer that adsorbs to them. The surfaces of materials typically adsorb a monolayer of protein within seconds of being placed in proteinaceous media. This protein layer is comprised of many different proteins. Furthermore, these proteins are in a spectrum of conformational states ranging from almost native to highly denatured, and also many orientations (facing up, down, sideways, etc) with respect to the surface. Each materials will have its own unique mixture of proteins on it, but, in common, all materials adsorb complex mixtures of proteins. Cells (first neutrophils, soon after, macrophages) that arrive to interrogate a material upon implantation detect a complex surface of proteins offering many possible sites for receptor interactions. This multiprotein surface, almost chaotic in its complexity, is a

reasonable model for the appearance of this surface that is consistent with our knowledge about adsorbed proteins [8]. Thus, I hypothesize that on such a complex surface (offering a broad range of possible interactive sites), a number of receptors on the cell membrane are simultaneously activated. This is in contrast to normal biological reactions where one ligand accurately and *specifically* triggers a particular process. Evidence supporting this idea of a heterogeneously triggered reaction is the "shower" of many different cytokines released from surface-adherent macrophages [9]. Thus, to expand the hypothesis, this cascade of mixed cytokines, a consequence of the mixture of proteins on the surface, is aberrant from normal physiological cytokine release. The body responds to these complex, non-physiological signaling processes by initiating the biological reaction to wall off the obviously foreign object, the implant.

How can we avoid this non-specific and unphysiologic signaling that occurs? The final part of my hypothesis is that for biomaterials to heal in tissue in a normal manner, they must switch on precise healing pathways via recognition (lock and key) events which, in turn, activate signaling pathways. The body has the ability to heal complex wound sites and rebuilt many tissues. We must tap into those evolutionarily optimized biological pathways. Examples do exist where biomaterials can turn on normal reconstructive pathways, in contrast to simply walling off the implant. For example, hydroxyapatite, the mineral phase of bone, heals in most tissues without encapsulation. Also, certain porous structures have been found to heal without the foreign body capsule and with new blood vessel formation (angiogenesis) [10].

Also, advanced biomaterials must not activate non-specific reactions (non-specific protein adsorption). By designing receptor sites onto the materials used in medicine and biology, cell and tissue reactions to those materials might be accurately controlled -- we, the biomaterials' designers, should be in charge of telling the body what event should be triggered. Areas without receptor sites should be engineered to inhibit non-specific protein adsorption. Such materials will demand precision in design and synthesis. Is it possible to synthesize such materials? I believe the technology is now available to do so.

In order to synthesize surfaces that demonstrate specificity will turn on reactions with precision and control, we need the appropriate surface analytical tools to guide our synthetic efforts. Methods such as electron spectroscopy for chemical analysis (ESCA), static secondary ion mass spectrometry (SIMS) , infrared reflection absorption spectroscopy (IRAS), extended x-ray absorption fine structure (EXAFS) and atomic force microscopy (AFM) can analyze surface composition, order and organization. Review articles outlining these methods for the surface characterization of biomaterials are available [11-13].

A general principle in the design of recognition biomaterial surfaces is that non-specific interactions and especially non-specific protein adsorption, should be inhibited. Surfaces that

are resistant to the adsorption of proteins may serve this purpose. Many research groups have investigated poly(ethylene oxide) surfaces for non-adsorptive interfaces. Such surfaces are made in our laboratory by the gas-phase RF-plasma deposition of ethylene glycol oligomers to form poly(ethylene oxide)-like thin films [14]. Reactive groups suitable for immobilization of recognition moieties can be incorporated into these non-adsorptive films.

Alternately, molecular self-assembly can be used to make surfaces with high degrees of crystalline perfection and relative freedom from defects or irregularities which may trigger non-specific interactions [15,16]. Small degrees of molecular mobility can be incorporated in such surface structures to reduce the demanding geometric requirements associated with a too precise molecular fit. A modified AFM tip contain recognition moieties can be used to specifically characterize such surfaces [17]. Finally, surfaces can be manipulated with the AFM to create patterns and nanoscale features that can be used to control proteins and cells [18].

When we can create biomaterials that can turn on specific reactions and inhibit non-specific reactions, we must ask which reactions need to be turned on (and turned off). Exploration of the basic biology of normal healing and tissue regeneration should provide us with specific mechanisms and pathways so we will know which biological reactions must be controlled.

CONCLUSIONS

This article focuses on a hypothesis for the design of a new generation of biomaterials. And, although it is indeed only a hypothesis, it is consistent with well accepted ideas in contemporary cell biology and modern materials science. Furthermore, I will argue that, although we've done a fine job with biomaterials for medical applications, we have gone as far as we can with "biocompatibility" and *in vivo* bioreaction of existing biomaterials. To advance the field and provide improved materials for health care and biotechnology, we must begin exploring new ideas and approaches. In particular, we must couple ideas from modern receptor biology and cell biology with materials that are molecularly engineered to turn on precise reactions.

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