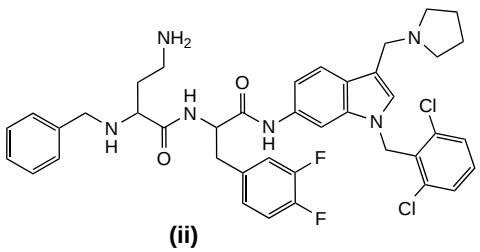
Thrombin receptor (PAR1) antagonists

Thrombin receptors are attractive drug discovery targets because they mediate a variety of cellular actions of thrombin, such as platelet aggregation, lymphocyte mitosis, monocyte chemotaxis and endothelial cell proliferation. The first example of a thrombin receptor (protease-activated receptor 1; PAR1) was identified as a member of the superfamily of seven-transmembrane, G-protein-coupled receptors. Thrombin activates PAR1 by proteolytically cleaving the N-terminal extracellular domain between Agr41 and Ser42 to reveal a new N-terminus containing the activated motif SFLLRN, which serves as a 'tethered' peptide ligand. Because PAR1 mediates many cellular actions of thrombin, a PAR1 antagonist could have therapeutic potential for treating various disorders, such as thrombosis, restenosis and stroke. The identification of potent and selective PAR1 antagonists has proved to be challenging. This situation is partially a result of the unfavourable entropy for an external ligand to compete with a tethered ligand. Of the PAR1 antagonists reported, several have notable deficiencies, such as weak potency, inability to inhibit thrombin-induced platelet aggregation consistently, mixed agonist–antagonist activity, and/or lack of PAR selectivity. Through a *de novo* design approach, a series of novel indole-based SFLLR peptide mimetics as potent and selective PAR1 antagonists have been discovered [2].

A library of >200 single compounds was synthesized on a Sieber amide solid-phase resin. The library compounds were screened for their ability to inhibit platelet aggregation induced by thrombin, SFLLRN-NH_2, collagen and a thromboxane mimetic (U46619). One of the most potent compounds isolated was **ii**, which possessed an IC_{50} value of 340 nM against thrombin and was also inactive against collagen and U46619. This work has generated rapid SAR data and the identification of potent and selective indole-based peptide mimetics as PAR1 antagonists; this could provide the basis for further development of PAR1 antagonists.



(ii)

2 Zhang, H-C. *et. al.* (2001) Thrombin receptor (PAR1) antagonists. Solid-phase synthesis of indole-based peptide mimetics by anchoring to a secondary amide. *Bioorg. Med. Chem. Lett.* 11, 2105–2109

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Drug delivery

Thoughts on the future of biocompatible devices

Implantable devices, including controlled release devices, have become an important component of clinical medicine. All of these devices must receive approval as biocompatible by the US Food and Drug Administration (FDA) before they are put into clinical use.

Most of these biocompatible devices are subject to the foreign body reaction, in which the immune defenses encapsulate the device in an avascular, collagenous bag that is 50–200 μm thick. After implantation, a layer of proteins accumulates on the surface of the device. This proteinaceous layer consists of many different proteins, in many orientations and conformational states. Nature never uses such non-specific layers of protein, and this is the signal to the immune system that the device is an unrecognized foreign invader that must be encapsulated.

The encapsulation problem

Uncontrolled encapsulation impedes the performance of many implanted devices. Drug delivery systems, implant electrodes, and silicone breast implants are all seriously degraded in performance by this capsule, which prevents intimate contact between the device and tissue. In one specific example, it has been proposed that the difficulties in controlling steroid release from NORPLANT® controlled delivery devices (The Population Council; http://www.popcouncil.org) might have been associated with the inability to accurately predict the thickness of the foreign body capsule that forms around the device.

To circumvent these problems, it would be advantageous if the next generation of medical devices could be made from biocompatible materials that do not promote the foreign body reaction. If normal wound healing took place around the site of an implanted device, the device would be surrounded by normal, vascularized tissue instead of the collagenous bag. In a thought-provoking review of work in this area, including several original references, B.D. Ratner presents some developments toward the next generation of biocompatible materials [1]. He proposes that engineers and material scientists capitalize on developments in the study of normal wound healing, and the inhibition of non-specific adsorption of proteins. The ultimate goal is to engineer future implantable devices such that the surface of the device presents the same signaling groups to the body as a fresh wound. Ratner then presents some of the initial findings toward this end, and these are summarized here. The article is quite thought-provoking, as he lays out the questions that currently drive the research, and some of these are also presented here.

New strategies

Various strategies to inhibit non-specific protein adsorption have been developed; the resulting materials are referred to as

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non-fouling surfaces. How resistant to protein adsorption can such surfaces be made? Why are they resistant to protein adsorption? How long can they remain resistant to protein fouling? These are the questions that drive this area of research. Many of the materials currently being explored are surface-modified polyethylene glycols (PEGs).

Another recent example uses radio frequency-plasma deposition of tetraethyleneglycol dimethylether onto the surface of materials such as Teflon™ and polyethylene. Untreated Teflon and polyethylene adsorb a layer of protein to an extent of 93.6 ng cm^{-2} and 91.0 ng cm^{-2}, respectively. By comparison, the corresponding tetraglyme-treated materials both adsorb a layer of protein ~1.7 ng cm^{-2} thick. However, these *in vitro* studies give no indication as to whether these surfaces will turn off the foreign body reaction, and they certainly do not transmit signals that promote normal healing.

Promoting wound healing

Several materials have been discovered, many fortuitously, that promote healing in a way that is more similar to normal wound repair and less toward collagenous encapsulation. So far, most of these materials have only shown good integration into bone. However, the healing process in bone, as in other tissues, begins with macrophages, and so these materials could serve as good starting points.

Titanium is one example; untreated titanium heals in bone, but there is a thin (50–200 Å) organic layer separating the bone and metal, and no bonding between them. By comparison, titanium treated with a strong base and high temperature fuses to the bone. Other materials that promote normal healing include tyrosine polycarbonates and hydroxyapatite. Hydroxyapatite, a form of calcium phosphate, comprises the mineral portion of bone and, therefore, is less prone to attack by the immune system. However, as a material it is not applicable to the fabrication of all medical devices.

Porous materials

Biocompatible porous materials have also been explored for their ability to promote normal healing. When implanted, most were encapsulated by avascular, collagenous sacs, the classic foreign body reaction. However, some of these materials promoted healing that was closer to normal, with reduced collagen formation, an open structure to the collagen and blood vessels in close proximity to the implanted membrane.

The materials that promoted this special healing response had several characteristics in common, including pores of 5–15 μm, interconnectivity between the pores and the absence of expanses of flat surface onto which the inflammatory cells could spread. This healing reaction was seen with several different kinds of materials as long as they possessed the listed characteristics, independent of material type. Similarly, it has been found that fine, electrostatically spun fibers with diameters <5 μm generate little or no collagen encapsulation.

Matricellular proteins

The matricellular proteins that are always found in healing wounds are currently being explored for their potential uses in biomaterial research. These proteins have a role in the foreign body reaction and in normal wound healing; they effectively turn healing on and off. Included in this class of proteins are osteopontin, thrombospondin-2 (TSP-2) and osteonectin.

Osteopontin downregulates inducible nitrogen oxide (NO) synthase and reduces NO in macrophages and other cells. It has been shown to enhance cell survival, inhibit calcification and calm inflammation. A study of titanium coated with osteopontin suggests that it can enhance healing in bone.

TSP-2 is a member of a family of secreted glycoproteins that is upregulated in wounds. When silicone elastomer implants were placed in TSP-2 knockout mice and examined after four weeks, they were found to have a higher blood-vessel density in their vicinity and an open, unoriented collagen structure surrounding the implant. Osteonectin binds to many other biological compounds, including hydroxyapatite, collagen, vitronectin and TSP-2. It has also been associated with wound healing and angiogenic activity.

The future

The real question regarding matricellular proteins is whether their properties can be applied successfully to the surfaces of medical devices. What strategies might be used to immobilize them in a precise manner? Will one of these proteins be sufficient, or will several be required to promote normal wound healing around an implanted device? Can issues of cost, stability and sterilizability be addressed?

In the near future, researchers at the interface of biology, in particular wound healing, and biomaterials research will be faced with these challenges. Over time, ideas from other areas will also make contributions to this area, such as self-assembly, supramolecular structure, genomics and nanofabrication. In the meantime, the complexity of the problem will push the skills of surface scientists and bioengineers to the limits.

1 Ratner, B.D. (2002) Reducing capsular thickness and enhancing angiogenesis around implant drug release systems. *J. Control. Release* 78, 211–218

A novel nasal nicotine formulation for smoking cessation

Smoking is the most prevalent preventable cause of death in modern society. It causes one in five deaths in the UK and accounts for nearly half a million deaths in the USA every year. Despite this well-known fact, it proves to be difficult for a smoker to quit. When smoking, a dose of nicotine is rapidly delivered to the nicotine receptors in the brain, dopamine levels increase and a pleasurable

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