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54	TITLE OF INVENTION
	Delivery of antimicrobial toxins

57	ABSTRACT (NOT MORE THAN 150 WORDS)

NUMBER OF SHEETS 79

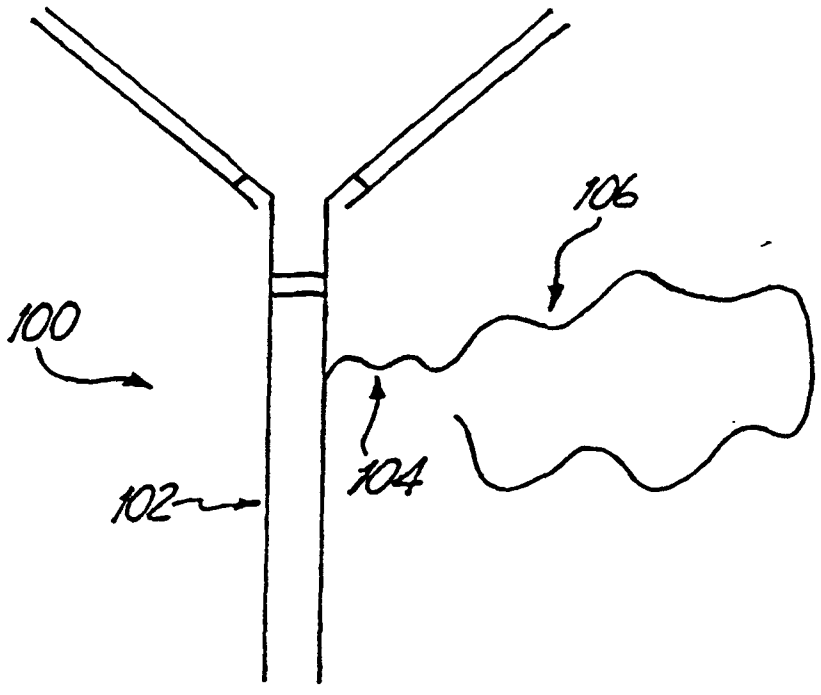
The sheet(s) containing the abstract is/are attached.

If no classification is furnished, Form P.9 should accompany this form.
The figure of the drawing to which the abstract refers is attached.

A&A P208

(57) Abstract

An antimicrobial conjugate (100, 120, 154) can be formed that includes an antibody (100, 122) or ligand bonded to an antimicrobial agent (106, 124). The antibody (102, 122, 154) or ligand has an affinity for microbial antigens or receptors. The antimicrobial conjugate (100, 120, 154) can be used alone or associated with biocompatible material (152) incorporated into a medical device (150). An antimicrobial conjugate (100, 120, 154) can be placed in contact with a solution to eliminate viable microorganisms from the solution. In particular, the antimicrobial conjugate (100, 120, 154) can be used to reduce the risk of infection associated with the contact of a medical device with patient's bodily fluids or tissues.



DELIVERY OF ANTIMICROBIAL TOXINS

BACKGROUND OF THE INVENTION

The invention relates to the delivery of antimicrobial toxins using antimicrobial antibodies/ligands conjugated with the toxins. In particular, antibodies/ligands having affinities for microorganisms are conjugated with toxins that kill or inhibit at least some forms of microorganisms. The invention further relates to medical devices associated with toxin-conjugated antimicrobial antibodies/ligands.

A variety of microorganisms can be associated with disease in animals. The diseases can be due to a systemic infection with microorganisms or due to proliferation of microorganisms in a localized area of the body, such as sinuses or gastrointestinal track. Even with the advent of antibiotics, many microbial infections can be difficult to treat and remain potentially fatal. In addition, serious microbial infections can be associated with the invasive use of medical articles. Studies indicate that over one half of hospital-acquired infections are associated with implants or indwelling medical articles. Thus, there remains a great need for more effective delivery of antimicrobial agents and improved delivery approaches for antimicrobial agents.

Bacteria that adhere to medical devices may begin to secrete both extracellular mucopolysaccharides and molecules that attract additional bacteria. Highly organized multicellular bacterial colonies within a tenacious matrix of extracellular polymers may rapidly form. These systems are called biofilms and are particularly resistant to both host defense mechanisms and standard antibiotic therapy. The best protection from biofilms is to prevent formation by inhibiting

bacterial adhesion or killing bacteria before they can synthesize and secrete bioactive molecules.

Another serious concern is the increasing frequency of infections that are resistant to standard antibiotic therapy. Unfortunately, hospitals have become a site for the proliferation of antibiotic resistant organisms, and it is estimated that approximately fifty percent of all hospital infections are resistant to common antibiotics. Of further concern, recent evidence indicates the communication of antibiotic resistance between distinct strains of bacteria.

A variety of medical articles are designed particularly for contact with a patient's bodily fluids or tissues. The duration of this contact may be relatively short, as is typical with wound dressings, or may be long term, as is typical with prosthetic heart valves implanted into the body of a recipient. Some articles, such as catheters, can have either short term or relatively long term contact. Other articles typically having relatively short term contact with the patient include, without limitation, burn dressings and contact lenses. Articles typically having long term contact with a patient include, without limitation, implanted prostheses.

Contact of medical articles with bodily fluids or tissue creates a risk of infection which can be very serious and even life threatening. In addition, considerable costs, and longer or additional hospital stays may result due to infection. For example, infections associated with dressings can increase the seriousness of the injury for burn victims. Also, infection associated with an implanted prosthesis can necessitate replacement of the device.

Infections are a particularly common complication resulting from the use of percutaneous devices such as catheters. Infections related to catheter use can result from invasion of microbial organisms during catheter insertion or from invasion by way of the exit site during use. Adherence of bacteria to the catheter surface may complicate treatment of the infection.

Medical articles are being developed that incorporate standard antibiotics on the surface of the device. The in vitro efficacy of these modified articles has yet to be determined. Furthermore, their use on a permanently indwelling device such as a heart valve prosthesis may be contraindicated in certain embodiments due to the increased risk of developing an antibiotic resistant infection.

Prostheses, i.e., prosthetic articles, are used to repair or replace damaged or diseased organs, tissues and other structures in humans and animals. Prostheses generally must be biocompatible since they are typically implanted for extended periods of time. Examples of prostheses include, without limitation, prosthetic hearts, prosthetic heart valves, ligament and bone repair materials, vessel repair and replacement materials, stents, and surgical patches. A variety of prostheses may incorporate tissue as at least a component of the prosthesis.

Physicians use a variety of prostheses to correct problems associated with the cardiovascular system, especially the heart. For example, the ability to replace or repair diseased heart valves with prosthetic devices has provided surgeons with a method of treating heart valve deficiencies due to disease and congenital defects. A typical procedure involves

removal of the native valve and surgical replacement with a mechanical or bioprosthetic valve. Another technique uses an annuloplasty ring to provide structural support to the natural annulus of the native valve.

Prosthetic Valve Endocarditis (PVE) is an infection that can be associated with a heart valve prosthesis. Bacteria can form colonies at the surgical site associated with the implant and in the fabric of the sewing cuff of the valve prosthesis. For this reason, heart valve recipients are cautioned regarding activities that may introduce bacteria into the bloodstream, such as dental work. For bioprosthetic replacement valves, PVE also is associated with the leaflet portion of the valve as well as the sewing cuff portion of the valve.

PVE can be caused by gram-negative bacteria, gram-positive bacteria, fungi or yeast. PVE caused by gram-positive bacteria is particularly prevalent. Diagnosis is based generally on two positive blood cultures for the same organism along with compatible clinical symptoms. Certain organisms are difficult to culture, however, which can complicate diagnosis.

With respect to replacement heart valves, care must be taken to ensure sterility during production and to prevent contamination during the replacement valve implantation process. For example, to ensure sterility or to reduce perioperative contamination, some surgeons apply antibiotics to the prosthesis prior to implantation. These techniques, however, have relatively short-term effectiveness. In spite of these efforts, PVE occurs in about 4 percent to 10 percent of patients.

Typically, infections occurring within the first 60 days after valve replacement are termed early onset PVE while infections occurring more than 60 days after valve implantation are termed late onset PVE. Mortality rates for early onset PVE may range from 30 percent to 80 percent. Mortality rates for late onset PVE can be greater than 20 percent. These high mortality rates emphasize the seriousness of these infections. Similar infections are associated with prostheses and other medical articles that contact bodily fluids of a patient.

SUMMARY OF THE INVENTION

In a first aspect, the invention pertains to an antimicrobial conjugate comprising an antibody or ligand associated with an antimicrobial agent. The antibody or ligand has an affinity for microbial antigens or receptors.

In another aspect, the invention pertains to a medical article comprising a biocompatible material associated with an antimicrobial conjugate. The antimicrobial conjugate comprises an antibody or ligand bonded to an antimicrobial agent. The antibody or ligand has an affinity for microbial antigens or receptors.

In a further aspect, the invention pertains to a method of eliminating viable microorganisms comprising placing an antimicrobial conjugate in contact with a solution from which viable microorganisms are to be eliminated. The antimicrobial conjugate preferably comprises an antibody or ligand associated with an antimicrobial agent. The antibody or ligand has an affinity for microbial antigens or receptors.

In addition, the invention pertains to a method of forming an antimicrobial conjugate, the method

comprising associating an antimicrobial agent with an antibody or ligand having an affinity for antimicrobial antigens or receptors.

Furthermore, the invention pertains to a method of forming an antimicrobial conjugate, the method comprising generation of an antimicrobial conjugate by expressing a DNA construct containing both DNA coding for the antimicrobial agent and an antibody or ligand.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic illustration of an antibody/ligand conjugated with toxin molecules/ complexes.

Fig. 2 is a schematic illustration of a toxin-conjugated antibody/ligand involving a chimera.

Fig. 3 is a schematic illustration of a polypeptide chimera involving an antimicrobial toxin, a polypeptide of an antibody/ligand, and an optional linker.

Fig. 4 is a schematic illustration of a medical device with associated toxin-conjugated antimicrobial antibodies/ligands.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Antimicrobial antibodies/ligands are used to direct toxins/antimicrobial agents (hereinafter referred to as "toxin") to kill microorganisms or to inhibit proliferation of microorganisms that are infecting a patient. Microorganisms/microbes, as described herein, include, without limitation, bacteria, fungi, and protozoa, i.e., all single cell organisms, whether eukaryotic or prokaryotic. The toxins are conjugated to the antimicrobial antibodies/ligands. There is a definite need for a new class of antibiotic therapy. The approaches described herein provide additional tools to use against problematic infections.

The toxins can have specific toxicity against microbes, broad spectrum toxicity against all or many cell types or limited toxicity against all or many cell types with enhanced toxicity against some or all microorganisms relative to mammalian cells. Since the antibodies/ligands, i.e., specific binding targeting moieties, direct the toxins to microorganisms with antigens/receptors recognized by the antibody/ligand, effective toxicity against the patient's cells can be reduced significantly, even if the toxin in unconjugated form is toxic to the patient's cells. The toxin-conjugated antimicrobial antibodies/ ligands, i.e., antimicrobial conjugate, can be delivered directly into the patient, or they can be associated with a medical article or device that contacts the bodily fluids or tissues of the patient.

A toxin is conjugated, i.e., physically associated, with an antibody/ligand having an affinity for microorganisms, as shown schematically in Fig. 1. The toxin conjugated antibody/ligand 100 includes antimicrobial antibody (or ligand) 102, shown in the figure with standard antibody structure for convenience, an optional linker 104, and toxin molecule/complex 106. A plurality of toxin molecules/ complexes 104 can be bound to each antibody/ligand 102. In preferred embodiments, the toxins are chemically linked with the antibodies/ligands. The chemical linkage can be performed by <u>in vitro</u> chemical bonding or by genetic engineering where the chemical linkages are formed as a natural part of protein synthesis.

When genetic engineering is used to form the toxin-conjugated antibody/ligand, a chimera 120 is formed, as shown in one embodiment in Fig. 2. A polypeptide of the antibody/linker is extended to

include a polypeptide antimicrobial toxin 124 with or without an optional peptide linker 126. Thus, chimera 120 is a polypeptide chain, as shown schematically in Fig. 3 with the toxin 124, an antibody/ligand polypeptide 128 and an optional linker 126 between them.

The antimicrobial antibodies/ligands preferably target microbial antigens/receptors and has little, if any, affinity for the patient's cells. Because of the antibody/ligand specificity, the toxin is brought into physical proximity with a microorganism. The microbial cell that attaches to the antibody/ligand becomes the target of the toxin associated with the antibody/ligand. Since the antibodies/ligands direct the toxin to a microorganism, any potential toxicity to the patient's cells is reduced or eliminated. Furthermore, due to synergism resulting from targeting with the antibody/ ligand, the effectiveness of the toxin against the microorganisms should increase significantly for a particular amount of toxin. The antibodies/ligands can be directed broadly against common microbial cell surface antigens/receptors or, alternatively, against microbial cell surface antigens/receptors of specific microorganisms such that the toxins are only directed to the specific microorganisms rather than all microorganisms.

The toxin can be any toxin that is effective against at least some microorganisms. A wide variety of antimicrobial toxins are suitable for use. In some preferred embodiments, the toxin is cytotoxic against all or most cells, i.e., effective to efficiently kill or inhibit all or most cells, both prokaryotes and eukaryotes. In other preferred embodiments, the toxin kills or inhibits most cells, but the toxin has increased effectiveness against microorganisms. In

still other preferred embodiments, the toxin effectively has exclusive toxicity against microorganisms, with little or no toxicity against the patient's cells. Furthermore, the toxin can be broad based with toxicity toward a wide range of microorganisms, or the toxin can have more specific toxicity against selected microorganisms.

In certain embodiments, the toxin-conjugated antibodies/ligands are delivered by direct introduction into the patient. For direct delivery, the toxin-conjugated antibodies/ligands can be delivered systemically by injecting the toxin-conjugated antibody/ligand into the blood stream of a patient. Systemic delivery can be particularly effective in the treatment of systemic microbial infections. In lieu of injection for delivery into the body's entire circulatory system, specially designed catheters may be used for local drug delivery into an isolated portion of the vasculature. In other embodiments for direct delivery, the toxin-conjugated antibodies/ligands are injected into specific tissue. Approaches based on direct delivery of toxin-conjugated antibodies/ligands may be particularly effective for treating entrenched infections located in tissue of the patient's body with a limited blood supply, which under traditional treatments may require the use of large quantities of antibiotics over long periods.

In alternative embodiments, the toxin conjugated antibodies/ligands are associated with a medical article. A variety of medical articles are used in contact with bodily fluids or tissues of a patient. Relevant biocompatible medical articles generally incorporate a biocompatible material which is intended to contact the patient's bodily fluids and/or tissues.

Bodily fluids include, for example, blood, plasma, serum, interstitial fluids, saliva and urine. The patient can be an animal, especially a mammal, and preferably is a human. Referring to a schematic depiction in Fig. 4, medical device 150, such as a heart valve prosthesis, includes a biocompatible material 152 having associated toxin-conjugated antimicrobial antibodies/ligands 154. As shown in Fig. 4, the toxin/conjugated antibodies/ligands 154 associate with only a portion of the medical article. Alternatively, the toxin-conjugated antibodies/ligands can be associated with the entire device.

The antibodies/ligands can be associated with the medical article using direct association, chemical bonding, specific binding interactions, adhesives or incorporation into the matrix of a biocompatible material used in the formation of the medical article. Medical articles that contact a patient's bodily fluids or tissue can provide sites for colonization by microorganisms. Microorganisms in the vicinity of the medical article can be bound by the antibody/ligand and subsequently killed or inhibited by the conjugated toxin. Thus, the association of toxin-conjugated antimicrobial antibodies/ligands with a medical article can reduce the risk of infection associated with contacting a patient's bodily fluids/ tissue with the medical article. Generally, the association of the toxin-conjugated antibody/ligand is reversible, such that the toxin-conjugated antibody/ligand gradually disassociates from the medical article. Due to the reversibility of the association, disassociated toxin-conjugated antibodies/ligands are available in the vicinity of the medical article, which in some

embodiments may be required for the efficacy of the toxin in the vicinity proximate the medical article.

Furthermore, a plurality of different toxin-conjugated antibodies/ligands can be used simultaneously by direct introduction into the patient and/or by association with a medical article. For example, two or more different toxins can be conjugated to one or more types of antibodies/ligands. The toxins can be bound to the antibodies so that a single antibody/ligand can include more than one type of toxin, or the toxins can be applied so that they are physically bound to different antibodies/ligands. Similarly, one or more toxins can be bound to a plurality of types of antibodies/ligands so that the antibodies/ligands direct the toxin(s) to the desired type of microorganisms. The approaches based on toxin conjugates described herein can be combined with other approaches for reducing the risk of microbial infection associated with contacting medical articles with a patient's bodily fluids or tissues, as described further below.

Antibodies/ligands Specific for Microbes

The toxins are conjugated to an antibody or ligand, either natural or synthetic, to direct the toxin to target microorganisms. The term antibody is intended for broad interpretation as any type of immunoglobulin, modified immunoglobulins and/or fragments thereof. The specific binding portion of an appropriate antibody recognizes a cell surface feature, generally a cell surface antigen, of target microorganisms. Similarly, the term ligand is intended for broad interpretation as any specific binding agent, other than an antibody, that has specificity for a cell receptor, generally a cell surface receptor. Through the use of an appropriate antibody and/or ligand, toxins can be specifically

targeted at potentially harmful microorganisms rather than eukaryotic cells of the patient.

Cell antigens/receptors, which can be recognized by an antibody or a ligand, include cell surface compounds, for example, carbohydrates, glycoproteins, glycolipids, proteins, lipids, lipoproteins and fragments and derivatives thereof. Derivatives of organic compounds are intended to include covalent modified compounds as well as compounds with noncovalently associated molecules and/or ions. Proteins are intended to include all polypeptides which may be covalently or noncovalently bound to other chemical species/compounds.

Eukaryotic cells and prokaryotic cells, e.g., bacteria, have some cell surface antigens/receptors in common and other cell surface antigens/receptors that are different. The antibodies/ligands of interest have relatively specific affinity for prokaryotes, fungi and/or protozoa. In particular, preferred antibodies/ligands have an affinity for microorganisms at least a factor of ten greater than their affinity for eukaryotic cells of the patient, more preferably at least a factor of one hundred greater and even more preferably at least a factor of one thousand greater than their affinity for the patient's eukaryotic cells. This specificity for microorganisms results in reduced toxicity to the patient's cells.

In order for the antibody/ligand to be appropriately specific for at least some microorganisms, the corresponding antigen/receptor must have a corresponding unique identification with the microorganisms. Any analogous antigens/receptors on eukaryotic cells of the patient preferably have sufficiently different structures such that a desired

level of specificity of the antibody/ligand can be achieved. One general class of antimicrobial antibodies is the antiadhesins. Adhesins are viral, bacterial or fungal surface receptors that specifically bind extracellular matrix proteins or cell surface ligands. Bacterial adhesins can be divided into two major groups: pili or fimbriae adhesins and non-pilus or afimbrial adhesins. A single pathogen may express many different adhesins, and some of these organisms have a highly evolved capacity for genetic rearrangement that allows for antigenic variation.

Antibodies directed against adhesins have been used to combat a number of different types of infections. For example, vaccination with bacterial adhesion FimA can prevent adhesion of Streptococcus parasanguis to damaged heart tissue and reduce the incidence of endocarditis. FimA is an adhesin present on both streptococci and enterococci. Thus, antibodies against the FimA adhesion can protect a patient from infection by either pathogen. See Viscount et al., "Immunization with FimA protects against Streptococcus parasanguis endocarditis in rats," Infect. & Immun., 65(3), 994-1002 (1997), incorporated herein by reference. Vaccination against a similar adhesin, FimH, has been used to protect mucosal surfaces from infection by Escherichia coli. See Langermann et al., "Prevention of mucosal Escherichia coli infection by FimH-adhesin-based systemic vaccination," Science 276:607-611 (1997), incorporated herein by reference. Additionally, vaccination with a fragment of a collagen adhesin can protect against Staphylococcus aureus-mediated sepsis.

Although a single adhesin that is conserved among all microbial pathogens has not been described, there are common elements or domains in many adhesins.

An example of such a domain is in sequences coding for the adhesin filamentous hemagglutinin (FHA) which is homologous to a number of both bacterial and eukaryotic adherence molecules. Antibodies to these homologous domains may cross-react with a number of different bacterial strains.

Molecular biology techniques are now used to gather abundant nucleotide sequence data from many of the medically relevant microbial strains. As this data becomes increasingly available, it may be possible to evaluate more extensively homologous sequences between strains. For example, a conserved six amino acid sequence is a common motif in many gram-positive organisms. See Finlay et al., "Common themes in microbial pathogenicity revisited," Microbiol. and Molecularbiol. Rev. 61(2):136-169 (1997), incorporated herein by reference. When identified sequences encode cell surface receptors, antibodies or ligands can be developed to selectively bind the protein or peptide domains that these sequences encode, analogous to the FHA sequences described above. Some common themes in the sequences are already under investigation.

It is no longer necessary to identify specific sequences before designing a selective antibody or ligand. Powerful new techniques, such as phage display or phage-antibody display, can be used to find appropriate targeting molecules. These techniques rely on production of a library of phage each with a specific protein, peptide, or antibody region displayed on its surface. The phage are then allowed to interact with the cells of interest. Those phage that bind to the appropriate (microbial) cells and do not bind to inappropriate (mammalian) cells are analyzed to determine the protein that coats their surface. This

protein can then be used as a selective microbial binding agent. Phage display approaches are described further, for example, in Griffiths et al., "Strategies for selection of antibodies by phage display," Current Opinion in Biotechnology, 9:102-108 (1998).

Antibodies may be particularly effective to direct toxins to prokaryotic cells because they can have affinity constants higher than about 10^8 moles/liter, and preferably higher than about 10^{10} moles/liter, and more preferably higher than about 10^{12} moles/liter. In addition, antibodies have the ability to form multiple bonds, increasing avidity for the receptor by a thousand-fold or greater. The affinity of an antibody/ligand for a antigen/receptor can be determined by standard approaches, such as competitive binding assays.

For use in association with a medical article, the antibodies/ligands preferably are broadly directed against bacterial or microbial antigens/receptors. With broad binding of the antibodies/ligand conjugated with toxin, the medical article can be guarded against serving as an incubation point for microbial colonies. In associating antibodies/ligands with medical devices, it is important that the antibody/ligand does not serve as an adhesion molecule that binds a viable microbial pathogen to the medical device. Binding of the microorganism with the toxin-conjugated antibody/ligand may affect the disassociation rate of the toxin-conjugated antibody/ligand from the medical device. Also, disassociation of the toxin-conjugated antibody/ligand from the medical device may increase effectiveness of the toxin against the microorganisms.

In preferred embodiments for direct introduction into the patient, the antibodies/ligands

can be broadly directed against bacterial or other microbial antigens/receptors. Alternatively, the antibodies/ligands can be specific for antigens/ receptors for a relatively narrow range of microorganisms or, in particular, for a specific genus or species of microorganisms. Using antibodies/ligands directed against a narrow range of microorganisms provides for treatment of a specific infection without necessarily destroying beneficial microorganisms in the patient's body, which can lead to subsequent opportunistic infection by less desirable bacteria and fungi/yeast.

Suitable antibodies include monoclonal antibodies and polyclonal antibodies. Preferred antimicrobial antibodies include antibody fragments with the Fab, Fab', $F(ab')_2$, single chain, Fv and/or single variable domain portion(s) of the antibody. Preferred antibodies include humanized antibodies. Generally, antibodies are generated against microbial antigens in non-human animals including, for example, chickens, mice and rabbits.

Antibodies from non-human animals can be humanized by integrating the antigen binding surface into the framework of a human antibody variable region. Humanized antibodies are less likely to create an immune response in the recipient patient. Antibodies can be humanized according to procedures described, for example, in C. Rader, et al., "A phage display approach for rapid antibody humanization: designed combinatorial V gene libraries," Proc. Natl. Acad. Sci USA 95(15): 8910-8915 (1998), incorporated herein by reference. Using recombinant DNA technology, humanized antibodies also can be made by expression of human genes in mammalian cell systems, where the variable region of the

antibody is engineered to produce the desired specific binding activity. A suitable procedure for constructing such humanized/human engineered antibodies is described in U.S. Patent 5,756,699 to Better et al., entitled "Immunotoxins Comprising Ribosome-Inactivating Proteins," incorporated herein by reference.

Both monoclonal and polyclonal antibodies directed against a wide variety of infectious agents are commercially available. Many of these sources and antibodies that they provide are listed in the annually updated Linscott's Directory of Immunological and Biological Reagents (Santa Rosa, CA), 10th edition for 1998 and 1999, incorporated herein by reference.

In addition to the use of antibodies, ligands specific for bacterial adhesins can be used to target toxins to microbial pathogens. As adhesins bind to selective sites on the extracellular matrix or surface of eukaryotic cells, the domains to which these adhesins bind can be used as specific ligands. For example, the P pili adhesin expressed by a number of E. coli strains binds to the α-D-galactopyranosyl-(1-4)-ß-D-galactopyranoside moiety on the surface of many cells lining the urinary tract.

Furthermore, many eukaryotic cells that bind extracellular matrix proteins have an affinity to the arginine-lysine-aspartic acid (RGD) domain on these adhesin receptors. The filamentous hemagglutinin adhesin contains an RGD binding domain that may allow pathogens expressing this adhesin to mimic host cell RGD binding. Microbial pathogens often have much greater affinity for these RGD binding sites than do the host cells. It is possible to take advantage of the attraction between adhesins and naturally occurring ligands by using recurring motifs, such as the RGD

domains, in these ligands as targeting moieties, i.e., targeting ligands.

Another class of potentially useful ligands is the group of sigma-factors that are secreted by bacteria, such as Staphylococcus epidermidis, and used to attract other Staphylococcus epidermidis bacteria. It has recently been shown that these factors specifically bind a 140 KDa receptor on the bacterial surface. Thus, these sigma-factors may also be used as ligands to target toxins to microbial pathogens.

Antimicrobial Toxins

Suitable antimicrobial toxins generally can be divided into toxins that have significant specific toxicity toward microorganisms and those that are broadly toxic to many or all cell types. Since the antibodies/ligands direct the toxins to microbial targets, toxins with general toxicity can be used without introducing significant unwanted toxicity toward a patient. In this way, powerful toxins can be used with potent and rapid effectiveness against cells, preferentially killing undesirable microorganisms or inhibiting their proliferation. Toxins specific against microorganisms can be used even more effectively, such that potential side-effects to the patient are diminished.

Regardless of the specificity of the antimicrobial toxin, the toxin preferably is highly toxic to microorganisms. Binding of the antibody/ligand to the toxin provides for the specific delivery of the toxin to the microbes. The targeting by the antibody/ligand provides a synergistic effect that significantly decreases the toxicity to cells that are not targeted by the antibody/ligand while significantly increasing the efficacy of a particular toxin

concentration with respect to cells that are targeted by the antibody/ligand. Thus, with preferred toxins, a significant fraction of microorganisms contacting the antibody/ligand are killed, or their proliferation is inhibited.

Preferred types of particularly potent toxins with broad toxicity include the genus of ribosome-inactivating proteins (RIPs). RIPs are ubiquitous in higher plants and have been identified in some bacteria. RIPs are potent inhibitors of protein synthesis in eukaryotic cells. RIPs from plants have been divided into two types. Type I plant RIPs have a single polypeptide chain that inhibits ribosome function. Type II plant RIPs have an A-chain and one or more B-chains possibly with a disulfide linkage(s) between the polypeptide chains. The A-chain inhibits ribosome function and is analogous with the type I polypeptide. The B-chain has cell binding properties and facilitates cell uptake.

Bacterial RIPs, like type II plant RIPs, have multiple polypeptide chains. Bacterial RIPs are also called Shiga toxins. One of the polypeptides is an inactive proenzyme that is activated upon proteolysis of the chain and reduction of disulfide bonds.

Studies with rat liver ribosomes indicated that in these rat liver cells, RIPs act as a N-glycosidase that remove a single adenine base from a 28S rRNA section within the rat ribosome. The adenine base is part of GAGA sequence of an RNA loop that is conserved within most species from bacteria to man. Plant RIPs are effective generally against eukaryotic ribosomes.

Some plant RIPs have minimal ability to inactivate bacterial ribosomes. However, several plant

RIPs, including Mirabilis Antiviral Protein, a single chain RIP, have measurable activity for inactivating bacterial ribosomes. Plant RIPs with activity against bacterial ribosomes are generally about 100 times more effective against eukaryotic ribosomes. At least some bacterial RIPs (Stx1A1 protein fragment from select strains of Escherichia coli) have nearly equivalent effectiveness against both bacterial ribosomes and eukaryotic ribosomes. The native bacteria that produce Shiga toxins secrete the toxins in inactive form to prevent autotoxicity. Proteolysis of the toxin after uptake into infected host cells activates the toxin.

The expression and purification of recombinant bacterial RIPs is described in detail in J. K. Suh et al., "Shiga Toxin Attacks Bacterial Ribosomes as Effectively as Eukaryotic Ribosomes," Biochemistry 37(26):9394-9398 (1998), incorporated herein by reference. In particular, the Suh et al. reference provides a strategy for producing recombinant Shiga toxins. Appropriate polymerase chain reaction (PCR) primers are described with which to amplify the Stx1A1 coding sequence. The Suh et al. reference describes the placement of the coding sequence into an expression vector. A tightly controlled promoter is used such that little or no expressions of the Stx1A1 toxin takes place before an inducer is added. In this way, the E. coli colonies can grow to reasonable densities before the activation of the gene by the addition of the inducer 1mM IPTG, isopropyl thio-ß-D-galactoside, to produce the Stx1A1 toxin which then kills the bacteria. A histidine tag on the C-terminus of the recombinant toxin is used to purify the toxin protein on a nickel column.

Nearly identical Shiga toxins (ShxA1) from other strains of bacteria are likely to be equally

effective at killing bacteria. These Shiga-like toxins can also be produced through recombinant re-engineering using a similar mechanism, as described in the Suh et al. reference, supra.

There are several advantages to the use of a RIP immunotoxin as an antimicrobial toxin. First, these are very potent toxins with an enzymatic mechanism and IC_{50} (concentration to achieve 50 percent protein synthesis inhibition) values in the nanomolar range. A procedure for the measurement of IC_{50} values for ricin A-chain activity is described by Press et al., Immunol. Letters, 14:37-41 (1986), incorporated herein by reference. Only low levels of antibody/ligand binding are needed to produce toxicity. The implication is that antibodies/ligands binding even minor components of the bacterial cell surface can be used as targets. This increases the likelihood of finding appropriate cross-strain antigens for broad spectrum toxicity.

Second, the mechanism of RIP toxicity prevents protein synthesis and secretion without causing cell lysis. Inhibition of protein synthesis prevents bacterial signalling, recuitment, secretion of adhesion molecules, and biofilm production. Preferred embodiments avoid bacterial lysis, as this could trigger an inflammatory response that is potentially as destructive as the bacterial infection itself.

Third, the mechanism for toxin mediated cell death is separate from the binding component of the complex. Therefore, if resistance is acquired, it is likely to be against the binding component of the immunotoxin rather than against the toxin. This preserves antimicrobial function. Bacterial resistance traits have been correlated more significantly with

failure of therapy than with a pathogen's susceptibility to a particular antimicrobial agent.

Generally, a variety of inconsequential mutations can be made to the toxin proteins without altering the proteins' toxicity toward bacteria. For example, a histidine tag at the end of the protein does not alter the protein's effectiveness. However, other mutations may destroy or significantly reduce the activity of the RIP toxin. The effectiveness of modified toxins can be evaluated routinely based on the procedure of Suh et al., supra. As used herein, the term ribosome inactivating proteins (RIPs) refers to all ribosome-inactivating proteins and mutations and modifications thereof, and Shiga toxin refers to all RIPs derived from bacteria and mutations and modifications thereof. Active RIPs and active Shiga toxins refer to mutated or modified forms that retain measurable toxicity toward microorganisms. Preferred mutated or modified forms of the toxins have at least about 50 percent of the toxicity of the native forms and more preferably at least about 75 percent of the toxicity, as measured by IC_{50} values.

An alternative strategy for engineering a toxin to more selectively kill microbial cells is to modify the domain separating the binding (B) and catalytic, i.e., toxic, (A) domains to produce a domain cleaved specifically by microbial enzymes, such as proteases. Cleavage to separate the (A) and (B) domains generally is required for activation of the toxin. Furin or furin-like enzymes are the proteases most often responsible for toxin cleavage and activation in eukaryotic cells. Furin requires a four amino acid sequence with two arginine amino acids spaced apart by

two amino acids. By altering this cleavage site, the toxin may no longer be activated by mammalian cells.

The cleavage site can be altered to a sequence that is recognized by specific prokaryotic proteases. Then, the toxin may be transformed into a selective antimicrobial agent. For example, the cleavage site can be altered to a sequence recognized by bacterial proteases, such as subtilisin, or fungal proteases, such as proteinase K. The Staphylococcus aureus strain V8 protease specifically cleaves peptide bonds on the carboxyl side of aspartic and glutamic acid residues. Placement of these residues in the A-B linker region of the proenzyme may allow for the selective killing or inhibition of this strain of S. aureus. To generate toxins effective against a broader spectrum of bacterial cells, the A-B linker region may be engineered to contain sites for the more ubiquitous Lon or Clp cellular proteases.

In eukaryotic cells, the toxin must often undergo elaborate intracellular trafficking to reach and inactivate ribosomes. Attempted use of these toxins as anti-cancer agents has led to the discovery that a variety of toxin mutations can significantly reduce toxicity by interfering with proper trafficking of the toxin after infection of mammalian cells. See Fitzgerald, "Why Toxins!", Cancer Biology 7:87-95 (1996). As prokaryotes lack most intracellular organelles and the elaborate trafficking pathways that are associated with the eukaryotic organelles, toxin mutations that interfere with eukaryotic cell toxicity may not affect prokaryotic cell toxicity. Thus, toxins may be re-engineered to reduce patient toxicity without significantly altering toxicity to microbial pathogens.

In other preferred embodiments, the toxin has significant specificity for microorganisms, i.e., prokaryotes, yeasts or Protista, to promote preferential destruction of microorganisms over cells of the patient. In preferred versions of these embodiments, antimicrobial toxins have at least a factor of 10 greater IC_{50} value for microbial cells relative to mammalian cells, and preferably a factor of 100 and even more preferably a factor of 1000 greater IC_{50} value for microbial cells of some type than for any mammalian cells. Thus, with specific antimicrobial toxins, the antigen/ligand targets the toxin preferentially to microorganisms, and, furthermore, the toxins are preferentially toxic to microorganisms.

A wide variety of specific antimicrobial agents have been discovered and the search for additional compositions continues. Some preferred toxins also have specific effectiveness against particular types of microorganisms, such as a particular species of bacteria. For example, proton pump inhibitors, such as omeprazole and lansoprazole, are bacteriocidal against Helicobacter pylori. See Figura et al., "In vitro activity of lansoprazole and Helicobacter pylori," J. Antimicrobial Chemotherapy, 39:585-590 (1997), incorporated herein by reference.

Pathovars of Pseudomonas syringae can produce antimicrobial phytotoxins. Some of these phytotoxins, such as tabtoxins, are weakly antibacterial against most gram negative strains of bacteria. Tabtoxin inhibits glutamine synthase. Other phytotoxins, such as phaseotoxin (an ornithine carbamoyltransferase inhibitor), can be very effective, but only against a few microbial strains including, for example, Clavibacter michiganese. Syringomycins are

lipodepsipeptide toxins produced by other pathovars that have both broad spectrum antibacterial activity and antifungal activity. See Volksch et al., "Toxin production by pathovars of Pseudomonas syringae and their antagonistic activities against epiphytic microorganisms," J. Basic Microbiol., 38:135-145 (1998), incorporated herein by reference.

Thennarasu and Nagaraj generated designer antibacterial peptides by modifying the N-terminal region of the pore forming toxin, paraxin. See Thennarasu et al., "Specific antimicrobial and hemolytic activities of 18-residue peptides derived from the amino terminal region of the toxin pardaxin," Protein Engineering, 9(12):1219-1224 (1996), incorporated herein by reference. They describe an 18 amino acid peptide that is no longer humolytic but retains antibacterial activity against Escherichia coli. The amino acid sequence can be found in the Thennarasu reference above. Recombinant DNA technology may enable production of numerous highly selective antimicrobials. For example, it may be possible to modify other toxins to prefer prokaryotic substrates, for example, diphtheria toxin may be modified to preferentially ADP-ribosylate elongation factor-Tu rather than elongation factor 2.

Suitable antimicrobial agents also include traditional antibiotics. Some antibiotics are polypeptides, such as actinomycin, bacitracin, circulin, fungisporin, gramicidin S, malformin, mycobacilin, polymyxin, tyrocidine and valinomycin.

Generally, specific antibiotics block cellular activity specific for microbial function. For example, several antibiotics, such as penicillin, phosphonomycin, bacitracin and vancomycin, interfere with cell wall synthesis. In particular, penicillin inhibits a

crosslinking reaction in cell wall synthesis. Other antibiotics, such as streptomycin, tetracycline, chloramphenicol and erythromycin, act by inhibiting protein synthesis by binding to bacterial ribosomes.

Examples of toxins that are toxic to a relatively narrow range of microorganisms include bacteriocins. Bacteriocins are proteins that are produced and secreted by bacteria. Bacteriocins kill and may lyse bacteria related to the bacteria that produced the particular bacteriocin. For example, lysostaphin is produced by a single known strain of Staphylococcus, S. simulans. Lysostaphin is effective to kill and lyse practically all known species of Staphylococcus while being inactive against bacteria of different genera. The production of lysostaphin is described in U.S. Patent 5,853,962, entitled "Composition for Treating Mastitis and Other Staphylococcal Infections," incorporated herein by reference.

Conjugating Toxins With Antibodies/Ligands

As noted above, the toxins are conjugated to antibodies or ligands. The conjugation involves chemical bonding of the toxin to the antibody/ ligand. The chemical bonding can involve peptide bonds that are formed in vivo, for example, in a bacterial fermentation system using a gene engineered to form the conjugate/chimera and normal cellular protein production. If desired, a linker can be used between the toxin and the antibody/ligand. For example, additional amino acids can serve as a linker in a chimera with the toxin added to an antibody/ligand polypeptide. The conjugation of a toxin to an antibody/ligand can involve chemical binding initiated by a selected chemical reagent and/or a chemical binding

agent. The chemical binding agent can act as a linker and/or an activator that activates functional groups in the toxin and/or antibody/ligand to provide for chemical bonding. In other words, reactants and/or binding agents are used to form a chemical association between the toxin and the antibody/ligand. A toxin conjugated with an antibody/ligand with or without a linker between the toxin and antibody/ligand is considered a toxin-conjugated antibody/ligand for the purposes of the present application.

Chemical binding involves specific molecular interactions between the toxin and the antibody/ligand. The chemical binding of compounds to antibodies/ligands as well as the development of chimeras is well established, especially where the compound is a protein. Chemical binding can involve covalent bonding, noncovalent chemical interactions, or a combination of both covalent and noncovalent interactions. Noncovalent chemical interactions include, for example, hydrogen bonding, van der Waals interactions, ionic interactions and/or molecular rearrangements, which characterize specific binding interactions, such as antibody-antigen interactions, protein-receptor binding and enzyme-substrate associations.

In one approach, a recombinant antimicrobial protein, such as a recombinant RIP protein, is engineered to have cystine residues at the carboxyl terminus. Cystine residues have reactive sulfhydryl groups. The antibody or a protein-based ligand can be modified at lysine residues with a linker, such as 5-methyl-2-iminothiolane, to introduce reactive sulfhydryl groups at modified lysine residues. The modified recombinant proteins can be bonded with the modified

antibody by sulphur-sulphur bonds between the reactive sulfhydryl groups.

In alternative embodiments, the chemical binding of the toxin with the antibody/ligand can involve covalent bonding involving polyfunctional reactive agents such as glutaraldehyde and other suitable crosslinking agents. This is an especially suitable procedure for binding polypeptide toxins with antibodies or polypeptide ligands. A typical procedure for the crosslinking of two proteins or other polypeptides makes use of glutaraldehyde, which crosslinks proteins by way of two aldehyde groups. Other polyfunctional chemical reagents for covalent bonding of polypeptide toxins with polypeptide antibodies/ligands include, for example, epoxies and other difunctional aldehydes, such as glyoxal.

Generally, polyfunctional linkers can be used that target for binding specific functional groups in the toxin and in the antibody/ligand. Thc approaches described above using either disulfide bridges with a 5-methyl-2-iminothiolane linker or glutaraldehyde crosslinking are specific examples of the general approach of using polyfunctional linkers. Other linkers can be designed with one or more functional groups to bind with specific functional groups of the toxin and one or more functional groups to bind with specific functional groups of the antibody/ligand. Thus, the linker covalently links the toxin to the antibody/ligand.

In other embodiments, the linker associates with the toxin and/or the antibody/ligand by a plurality of non-covalent specific binding interactions. For example, if the antibody/ligand is a humanized antibody, the linker can be an anti-human IgG antibody with

covalently attached toxin. Similarly, the linker can be an anti-toxin antibody, preferably monoclonal, covalently bonded to the antimicrobial antibody/linker.

In an alternative embodiment, photochemical coupling can be used for covalent coupling. Photochemical coupling is based on the use of high energy light, e.g., ultraviolet light, to form reactive intermediates of certain functional groups. These reactive intermediates can form carbon-carbon bonds between two compositions. Aryl ketone functional groups are particularly useful in this respect.

Photochemical coupling can be used for attachment of toxins to antibodies/ligands, especially if the ligands are proteins. See, for example, Dunkirk et al., J. Biomaterials Applications 6:131-156 (1991), incorporated herein by reference. Alternatively, photochemical coupling can be used to attach a linker to the antibody/ligand either before, after, or during binding of the linker to the toxin.

Empirical adjustments can be made to ensure that the activity of the toxin molecule is not significantly impaired. When using antibody linkers, monoclonal antibodies may provide more consistent binding without the loss of toxin activity. Activity of the toxin conjugated antibodies can be verified in vitro. The use of in vitro assays to establish the toxicity of a toxin-conjugated antibody against human cells is described in U.S. Patent 5,756,699, entitled "Immunotoxins Comprising Ribosome-Inactivating Proteins," incorporated herein by reference. This procedure can be adapted to the evaluation of effectiveness of the toxin-conjugated antibodies/ligands against microorganisms, and for the evaluation of IC_{50} values.

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Toxin-conjugated antibody/ligand can also be produced by recombinant genetic engineering. An example of this type of gene fusion construction is described in Murphy et al., "Genetic construction, expression and melanoma-selective cytotoxicity of a diphtheria toxin-related alpha melanocyte-stimulating hormone fusion protein," Proc. Nat'l Acad. Sci USA 83:8258-8262 (1986), incorporated herein by reference. An advantage of this approach for generation of an antimicrobial toxin conjugate is the ease with which portions of the molecule can be reengineered to provide added selectivity. As discussed above, it may be desirable to produce point mutations in a linker region of a proenzyme molecule to allow for cleavage by prokaryotic proteases preferentially over eukaryotic proteases. Alternatively, it might be desirable to provide for specific sequences between the binding and catalytic regions to allow for selective cleavage by microbial proteases. As noted above, another alternative is to engineer a gene such that a peptide toxin is synthesized as part of an antibody or ligand peptide. Engineering of the appropriate gene is straightforward based on the sequence of the antibody/ ligand and of the toxin.

Medical Articles and Biocompatible Materials

Toxin conjugated antibodies/ligands can be used alone or in combination with a medical device formed with biocompatible material. Relevant biocompatible medical articles or devices include all medical devices that contact bodily fluids/tissues. These articles can be organized roughly into three groups: implanted devices, percutaneous devices and cutaneous devices. Implanted devices broadly include articles that are fully implanted in a patient, i.e., are completely internal. Percutaneous devices include

items that penetrate the skin or enter the body through a cavity, thereby extending from outside the body into the body. Cutaneous devices are used superficially, for example, at a wound site or at a moist membrane. Regardless of the placement of the medical device, the medical device can simply be a substrate to provide for the delivery of a toxin-conjugated antibody/ligand, as described further below.

Implanted devices include, without limitation, prostheses such as pacemakers, electrical leads such as pacing leads, defibrillators, artificial hearts, ventricular assist devices, anatomical reconstruction prostheses such as breast implants, artificial heart valves, pericardial patches, surgical patches, coronary stents, vascular grafts, vascular and structural stents, vascular or cardiovascular shunts, biological conduits, pledgets, suture, annuloplasty rings, stents, staples, valved grafts, dermal grafts for wound healing, orthopedic spinal implants, orthopedic pins, intrauterine devices (IUDs), maxial facial reconstruction plating, dental implants, intraocular lenses, clips, sternal wires, bone, skin, ligaments, tendons, and combinations thereof.

Percutaneous devices include, without limitation, catheters of various types, cannulas, bronchial tubes, intratracheal tubes, Foley catheters, drainage tubes such as chest tubes, surgical instruments such as forceps, retractors, needles, and gloves, and catheter cuffs. Catheters can be used for accessing various bodily systems such as the vascular system, the gastrointestinal tract, or the urinary system. Some catheters, such as Hickman catheters, are designed to dwell for extended periods of time within a patient. Cutaneous devices include, without limitation, skin

grafts, burn dressings, wound dressings of all types, dental braces and bridges, and contact lenses.

These biocompatible medical devices can be made from one or more biocompatible materials described below. Biocompatible materials are suitable for contact with a patient's bodily fluids and tissues. Appropriate biocompatible materials include natural materials, synthetic materials and combinations thereof.

Natural, i.e., biological, material for use in the invention includes relatively intact (cellular) tissue as well as decellularized tissue. These tissues may be obtained from, for example, natural heart valves, portions of natural heart valves such as roots, walls and leaflets, pericardial tissues such as pericardial patches, connective tissues, bypass grafts, tendons, ligaments, skin patches, blood vessels, cartilage, dura matter, skin, bone, fascia, submucosa, umbilical tissues, and the like.

Natural tissues are derived from a particular animal species, typically mammalian, such as human, bovine, porcine, canine, seal, kangaroo or transgenic mammals. Suitable natural tissues generally include collagen-containing material. Natural tissue is typically, but not necessarily, soft tissue. Appropriate tissues also include tissue equivalents such as tissue-engineered material involving a cell-repopulated matrix, which can be formed from a polymer or from a decellularized natural tissue. Tissue materials are particularly useful for the formation of tissue heart valve prostheses.

Tissues can be fixed by crosslinking. This provides mechanical stabilization, for example, by preventing enzymatic degradation of the tissue. Glutaraldehyde is typically used for fixation, but

other difunctional aldehydes or epoxides can be used. Tissues can be used in either crosslinked or uncrosslinked form, depending on the type of tissue, the use and other factors.

Relevant synthetic materials include, for example, polymers, metals and ceramics. Appropriate ceramics include, without limitation, hydroxyapatite, alumina and pyrolytic carbon. Appropriate metals include medals approved for medical use including, for example, stainless steel and titanium. Appropriate synthetic materials include hydrogels and other synthetic materials that cannot withstand severe dehydration.

Polymeric materials can be fabricated from synthetic polymers as well as purified biological polymers. The polymeric materials can be woven into a mesh to form a matrix or substrate. Alternatively, the polymer materials can be molded or cast into appropriate forms.

Appropriate synthetic polymers include, without limitation, polyamines (e.g., nylon), polyesters, polystyrenes, polyacrylates, vinyl polymers (e.g., polyethylene, polytetrafluoroethylene, polypropylene and poly vinyl chloride), polycarbonates, polyurethanes, poly dimethyl siloxanes, cellulose acetates, polymethyl methacrylates, ethylene vinyl acetates, polysulfones, nitrocelluloses and similar copolymers. Synthetic conductive polymers include, for example, doped polymers of poly(sulfur nitride), polyacetylene, poly(p-phenylene), poly(phenylene sulfide) and polypyrrole.

Biological polymers can be naturally occurring or produced *in vitro* by, for example, fermentation and the like. Purified biological polymers can be

appropriately formed into a substrate by techniques such as weaving, knitting, casting, molding, extrusion, cellular alignment and magnetic alignment. For a description of magnetic alignments see, for example, R. T. Tranquillo et al., Biomaterials 17:349-357 (1996). Suitable biological polymers include, without limitation, collagen, elastin, silk, keratin, gelatin, polyamino acids, cat gut sutures, polysaccharides (e.g., cellulose and starch) and copolymers thereof.

Other suitable polymers include natural or synthetic resorbable polymers such as dextran, hydroethyl starch, gelatin, derivatives of gelatin, polyvinylpyrrolidone, polyvinylalcohol, poly[N-(2-hydroxylpropyl) methacrylamide], polyglycols, polyesters, poly (orthoesters), poly(ester amides), polyanhydrides. Resorbable polyesters include, for example, poly (hydroxy acids) and copolymers thereof, poly(ε-caprolactone), poly (dimethyl glycolic acid), and poly (hydroxy butyrate). Preferred resorbable polymers include, for example, D, L-polylactic acid, L-polylactic acid, poly(glycolic acid), and copolymers of L-lactic acid, D-lactic acid and glycolic acid.

Biocompatible materials can form the entire medical device or it can form portions of the medical device. Biocompatible materials can include a combination of the various natural materials and synthetic materials described above. For example, some prosthesis are made entirely from tissue, or tissue with fabric such as sewing rings, other polymer components and/or metal components. Other relevant prostheses are made completely from metal, ceramics or a combination of metal, ceramics and, optionally, additional natural or synthetic materials. Mechanical heart valves, artificial hearts, ventricular assist devices, coronary

stents, annuloplasty rings, pacing leads and defibrillators are relevant products, which generally are made from metallic, polymeric and/or ceramic components.

The toxin-conjugated antibodies/ligands can be associated with an entire biocompatible material or a portion of the biocompatible material. Similarly, if the medical article includes more than one biocompatible material, a toxin-conjugated antibody/ligand can be associated with one or more of the biocompatible materials. For example, a tissue heart valve can be combined with a fabric sewing cuff to form a heart valve prosthesis, where the tissue and/or the fabric can be associated with a toxin-conjugated antimicrobial antibody/ligand.

A medical article can include one or more associated toxin-conjugated antimicrobial antibodies/ligands. If a plurality of toxin-conjugated antibodies/ligands are used, the different conjugates can be associated with the same biocompatible material(s) or portions thereof, or with different biocompatible material(s) or portions thereof. For example, one toxin-conjugated antibody/ligand can be associated with the tissue portion of a tissue heart valve prosthesis while a second toxin-conjugated antibody/ligand is associated with the sewing cuff. Similarly, a first toxin-conjugated antibody/ligand can be associated with the entire medical article, such as both the tissue portion and the sewing cuff portion of a tissue heart valve prosthesis, while a second toxin-conjugated antibody/ligand is only associated with the sewing cuff portion. Other variations can be used.

The toxin conjugated antibodies/ligands can be associated with the biocompatible material before or

after the various components are combined into the medical device. The selected approaches for association of the toxin conjugated antibody/ligand with the biocompatible material may influence the order of construction of the medical device.

Associating Toxin Conjugated Antibodies/Ligands With Biocompatible Materials

In some embodiments, the toxin conjugated antibodies/ligands are associated with a biocompatible material of a medical device. The toxin-conjugated antibodies/ligands can be effective to reduce or eliminate the colonization of the biocompatible material by microorganisms that are vulnerable to the toxin. The toxin conjugated antibodies/ligands can be associated with the biocompatible material by direct association, chemical bonding, adhesive binding and/or incorporation into the matrix of the biocompatible material.

Suitable binding approaches may depend on the mechanism by which the toxin is effective against the microorganism. In particular, for some embodiments, the toxin may have to penetrate significantly into the microbial cell. For these embodiments, it may be desirable that the binding of the toxin-conjugated antibody/ligand to the medical article is relatively weak such that an ultimately unbound toxin-conjugated antibody/ligand can interact with the microorganism. The toxin-conjugated antibody/ligand can be initially bound to the medical device if the antimicrobial antibody/ligand can readily dissociate from the device after binding to the microorganism. Furthermore, weak binding of the toxin-conjugated antibody/ligand to the medical device results in the dissociation of the toxin from the medical article over a relatively short time frame such that the development of toxin-resistant

strains of microorganisms are less likely to result. The strength of the association of the toxin-conjugated antibody/ligand with the medical device can be adjusted, as desired.

As an alternative to relatively weak binding of the toxin-conjugated antibody/ligand to the medical device, the bond between the toxin-conjugated antibody/ligand to the medical device can include a linkage that is sensitive to metabolic or enzymatic activity within the patient. For example, the linkage between the toxin-conjugated antibody and the medical device can include peptide sequences that are sensitive to proteolysis by enzymes to which the medical device is expected to be exposed in vivo. Thus, over a time frame determined by the efficiency of the metabolic or enzymatic activity, the toxin-conjugated antibody/ligand will gradually be released from the medical device. This type of linkage to the device may be particularly useful if the peptide sequence binding the toxin-conjugated antibody/ligand to the device is sensitive to microbial proteases rather than common mammalian proteases. Such a binding approach assures release of the toxin conjugate in the presence of microbes and limits release when these cells are absent. As described above with respect to the sequences linking binding and catalytic domains of A-B toxins, these linker sequences may be engineered to provide for cleavage by common proteases if broad spectrum antimicrobial activity is desired or by specific proteases if targeting to a particular strain is preferred.

To the extent that the toxin-conjugate is released from the medical device in a particular embodiment, the medical device functions as a toxin-

conjugated antibody/ligand delivery system. In this role, the medical device can be a simple biocompatible substrate to provide for toxin-conjugated antibody/ligand delivery to the patient or the medical device can serve any other useful purpose, such as prostheses described above. Depending on the location of the medical device, the release of the toxin-conjugated antibody/ligand can involve systemic or localized delivery.

Direct association entails combining the biocompatible material with a solution of the toxin-conjugated antibody/ligand, without the use of an additional chemical binder. Due to direct contact, the toxin-conjugated antibody/ligand can bind with the biocompatible material, possibly due to chemical bonding. For direct association of the toxin-conjugated antibody/ligand to the biocompatible material, the biocompatible material or a portion thereof is combined with a solution of one or more toxin conjugated antibodies/ligands at a concentration generally from about 0.1 nanomolar (nM) to about 1 micromolar (μM) and preferably from about 0.5 nM to about 10 nM. If a plurality of toxin-conjugated antibodies/ligands are placed in solution simultaneously, the solution can have individual toxin-conjugated antibodies/ligands at different concentrations, preferably each within the concentration range noted above.

In preferred embodiments, the solution containing the toxin-conjugated antibodies/ligands preferably is buffered at a near physiological pH ranging from about 6.0 to about 8.5, and more preferably ranging from about 6.9 to about 7.5. Suitable buffers can be based on, for example, the following compounds: phosphate, borate, bicarbonate, carbonate, cacodylate,

citrate, and other organic buffers such as tris(hydroxymethyl) aminomethane (TRIS), N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES), and morpholine propanesulphonic acid (MOPS).

Alternatively, the toxin-conjugated antibody/ligand can be associated with the biocompatible material through the use of a binder or adhesive. The toxin conjugated antibody/ligand associates with the biocompatible material due to incorporation into the structure of the adhesive when the adhesive cures. The toxin conjugated antibody/ligand and the adhesive form a coating on the biocompatible material, generally with some penetration into the biocompatible material. Preferred adhesives include, for example, biologic glues such as fibrin glue, and the like. Fibrin glue can be formed from the polymerization of fibrinogen and thrombin. Suitable fibrin glues are available from, for example, Immuno AG, Austria and Zymogenetics, Seattle, WA. Other preferred adhesives include, for example, polyurethanes. Suitable polyurethane adhesives are described in U.S. Patent 4,740,534 to Matsuda et al., incorporated herein by reference.

To apply the toxin conjugated antibody/ligand with a fibrin glue to a biocompatible material, a small amount of thrombin can be absorbed into the biocompatible material. The toxin conjugated antibody/ligand can be mixed with a solution containing fibrinogen to yield a fibrinogen-toxin conjugated antibody/ligand solution with a concentration of the toxin conjugated antibody/ligand preferably ranging from about 0.1 nM - 1 μM. Then, the fibrinogen-toxin conjugated antibody/ligand mixture can be brushed or sprayed over the surface of the biocompatible material with absorbed thrombin, or the biocompatible material

with absorbed thrombin can be dipped into the fibrinogen-toxin conjugated antibody/ligand solution. The coating can be applied to all or just a portion of the biocompatible material.

With synthetic biocompatible materials, the toxin-conjugated antibody/ligand also can be incorporated into the material when the biocompatible material is formed. When incorporated into the matrix of the biocompatible material, the toxin-conjugated antibody/ligand can be located in interstitial spaces within the biocompatible material. In particular, at an appropriate time in the preparation of the biocompatible material, an amount of the toxin-conjugated antibody/ligand can be mixed with the components of the biocompatible material to form a blend. When the biocompatible material is formed, the biocompatible material includes within its structure the toxin-conjugated antibody/ligand. The biocompatible substrate can include several regions and/or layers such that the toxin-conjugated antibody/ligand is only distributed within a subset of the regions and/or layers of the biocompatible material. Additional toxin-conjugated antibodies/ligands can also be applied to the finished medical article, if desired.

In other embodiments, the association of a toxin conjugated antibody/ligand with a biocompatible material involves chemical binding initiated by a selected chemical reagent and/or a chemical binding agent. In contrast with the use of an adhesive, chemical binding involves specific molecular interactions with biocompatible material compositions, rather than a collective adhesion. Chemical binding can involve covalent bonding, noncovalent chemical interactions, or a combination of both covalent and

noncovalent interactions. Noncovalent chemical interactions include, for example, hydrogen bonds, van der Waals interactions, ionic interactions and/or molecular rearrangements, which characterize specific binding interactions, such as antibody-antigen interactions, protein-receptor binding and enzyme-substrate associations. In other words, reactants or binding agents are used to form a chemical association between the toxin-conjugated antibody/ligand and the biocompatible material, possibly involving a linker molecule. The chemical binding of the toxin-conjugated antibody/ligand preferably takes place at or near physiological pH values, preferably ranging from a pH of about 6 to about 8.5 and more preferably from about 6.9 to about 7.5.

As noted above, in some embodiments it may be desirable for the bonding of the toxin-conjugated antibody/ligand to be relatively weak such that the bonding is reversible on some desired time frame. The length of the desired time frame depends on the mechanism of the toxin activity, the binding strength of the antimicrobial antibody/ligand, the amount of toxin-conjugated antibody/ligand present, the rate of inactivation by degradative enzymes, and possibly other factors. The chemical bonding of the toxin-conjugated antibody/ligand to the medical device can be adjusted by selection of the binding approach and/or binding compositions to yield the desired equilibrium constants with respect to unbound toxin-conjugated antibody/ligand.

The chemical binding of the toxin-conjugated antibody/ligand with the biocompatible material can involve covalent bonding to the biocompatible material with reactive agents such as glutaraldehyde or other

suitable crosslinking agents. This is an especially suitable procedure for the strong binding of polypeptide ligands and antibodies with biocompatible material formed from tissue or other polymers with suitable functional groups. A typical procedure for the crosslinking of the toxin conjugated antibody/ligand to the surface of a tissue makes use of glutaraldehyde, which crosslinks proteins by way of two aldehyde groups.

Since glutaraldehyde is typically used for fixation of tissue, the crosslinking to bind the toxin-conjugated antibody/ligand to the tissue can be performed simultaneously with fixation of the tissue. Alternatively, crosslinking to covalently bond the toxin-conjugated antibody/ligand with a tissue or synthetic substrate can be performed as a separate step before or after the completion of a fixation process, assuming a fixation step is performed. Other chemical reagents for covalent bonding of toxin conjugated antibody/ligand to a biocompatible material include, for example, epoxies and other difunctional aldehydes, such as glyoxal.

Other polyfunctional linkers can be designed to react with specific functional groups both in the toxin-conjugated antibody/ligand and in the biocompatible material, such that the linkers generally bond simultaneously with the toxin-conjugated antibodies/ligands and biocompatible material. The linkers incorporate one or more functional groups that covalently bond to corresponding functional groups on the biocompatible material and one or more functional groups that covalently bond to functional groups on the toxin-conjugated antibody/ligand. For example, the approaches described above using either disulfide bridges with a 5-methyl-2-iminothiolane linker or

glutaraldehyde crosslinking are specific examples of using polyfunctional linkers. Thus, the linker covalently links the toxin-conjugated antibody/ligand to the biocompatible material.

Alternatively, chemical binding of the toxin-conjugated antibody/ligand to the biocompatible material can involve specific binding interactions. The specific binding interactions can be used to target specific locations within the biocompatible material. The targeting of specific locations in the biocompatible material can be useful, for example, if specific locations are particularly susceptible to colonization by microorganisms. An example of a possible target location includes sewing cuffs of a prosthesis, such as a mechanical heart valve.

In particular, a method of targeting a particular location involves the use of linkers that target specific cellular or extracellular binding sites within a natural tissue or other biocompatible material. In certain embodiments, the linker is covalently bonded to the toxin-conjugated antibody/ligand, and the linker associates with the biocompatible material by a plurality of non-covalent interactions, such as antibody-antigen interactions. Alternatively, the linker can be covalently bonded to the biocompatible material and the toxin-conjugated antibody/ligand can be associated with the linker by a plurality of non-covalent interactions. A variety of commercially available antibodies and other specific binding reagents may be used as linkers. Alternatively, antibodies and other specific binding reagents can be prepared by conventional techniques.

A toxin-conjugated antibody/ligand having a second attached antibody/ligand or any other comparable

targeting molecule/linker for binding to a biocompatible material is considered a toxin-conjugated antibody/ligand for the purposes of the present application. Similarly, an engineered chimera of the toxin-conjugated antibody/ligand and the targeting molecule is considered a toxin-conjugated antibody/ligand for the purposes of the present application. The chemical binding of compounds to antibodies/ligands as well as the development of chimeras is well established, especially where the compound is a protein.

In an alternative embodiment, photochemical coupling can be used for covalent coupling. Photochemical coupling is based on the use of high energy light, e.g., ultraviolet light, to form reactive intermediates of certain functional groups. These reactive intermediates can form carbon-carbon bonds between two compositions. Aryl ketone functional groups are particularly useful in this respect.

Photochemical coupling can be used for attachment of toxin-conjugated antibodies/ligands to tissue or other biocompatible materials. For a general discussion of photochemical coupling, see, for example, Dunkirk et al., J. Biomaterials Applications 6:131-156 (1991), incorporated herein by reference. The tissue may or may not be separately crosslinked since the photochemical coupling generally also crosslinks the tissue, i.e., photofixation. Photochemical coupling can be used to attach a linker to the biocompatible material either before, after, or during binding of the linker to a toxin-conjugated antibody/ligand.

Empirical adjustments can be made to ensure that the activity of the toxin is not significantly impaired when the toxin-conjugated antibody/ligand is associated with the biocompatible material. In

particular, the conditions used to associate the toxin-conjugated antibody/ligand with the biocompatible material can be altered to ensure appropriate activity of the toxin, for example, by changing the association approach or the particular binders used. The activity of the toxin can be verified in vitro. The use of in vitro assays to establish the toxicity of a toxin-conjugated antibody against human cells is described in U.S. Patent 5,756,699, entitled "Immunotoxins Comprising Ribosome-Inactivating Proteins," incorporated herein by reference. This procedure can be adapted to the evaluation of effectiveness against microorganisms. Other standard procedures can also be used to assess antimicrobial efficacy, such as determination of minimal inhibitory concentration to inhibit 90% of growth (MIC90) and minimum bacteriocidal concentration to kill 90% of bacteria tested (MBC90). Procedures to evaluate the MIC90 and MBC90 values are described in Figura et al., "In vitro activity of lansoprazole and Helicobacter pylori," J. Antimicrobial Chemotherapy, 39:585-590 (1997), incorporated herein by reference.

The activity of the toxin and antibody/ligand generally have a finite lifetime, preferably long enough to accomplish the intended purpose of the conjugate. Loss of activity may be due to proteolytic activity, other biological degradation processes and/or denaturing of the materials due to other chemical processes, whether or not the toxin conjugated antibody/ligand is associated with a medical article. Furthermore, toxin-conjugated antibodies/ligands may become disassociated from the biocompatible material. The effective time frame for association of the toxin-conjugated antibody/ligand with the biocompatible material will vary depending on the nature of the toxin, nature of the

antibody/ligand, and the approach for associating the antibody/ligand with the biocompatible material.

Thus, the toxin-conjugated antibodies/ligands may gradually lose effectiveness with respect to preventing colonization of the biocompatible material by microorganisms. If the lifetime of the toxin-conjugated antibodies/ligands is shorter or longer than desired, the characteristics of the toxin-conjugated antibodies/ligand, such as by the selection of a different toxin, or the association characteristics can be altered based on the description above to yield a different effective lifetime. Alternatively, additional toxin-conjugated antibodies/ligands or other antimicrobial agents can be associated with the medical article to achieve a greater antimicrobial activity and/or longer term antimicrobial activity.

Combination With Other Antimicrobials

When the toxin-conjugated antibodies/ligands are used as antimicrobial agents either by direct delivery or by association with a medical device, additional antimicrobial agents can be used effectively along with the toxin-conjugated antibodies/ligands. In some embodiments, additional antibiotics can be administered directly to the patient, in addition to the toxin-conjugated antibody/ligand that is directly administered to the patient or is associated with a medical article that contacts a patient's bodily fluids or tissues. For direct delivery, the additional antibiotics can be administered vascularly or by introduction into particular tissue. For example, toxin-conjugated antibodies/ligands with lysostaphin bacteriocin toxin can be coadministered along with penicillin, the combination of which may have a

synergistically enhanced effectiveness against staphylococcal bacteria.

Similarly, it may be desirable to associate one or more additional antimicrobial agents with the medical article along with the toxin-conjugated antibodies/ligands. The additional antimicrobial agents can be associated with the same biocompatible materials that are associated with the toxin-conjugated antibodies/ligands. The additional antimicrobial agents can be associated with the entire medical article. Alternatively, the medical article can be constructed such that the additional antimicrobial agents are associated with only a particular region of the medical article, such as at different portions of a biocompatible material or in different biocompatible materials of the medical device. In particular, if a region of the medical article is not associated with toxin-conjugated antibodies/ligands, that region can be associated with an alternative antimicrobial agent to reduce the risk of infection.

If selected appropriately, the supplementary antimicrobial agents can have different time scales over which they are effective. For association with medical devices, toxin-conjugated antibodies/ligands may have a limited time frame of effectiveness, so a longer term of effectiveness may be maintained by the supplemental antimicrobial agents. The additional antimicrobial agents can enhance the effectiveness of the toxin conjugated antibodies/ligands with respect to the prevention of infection over comparable time frames.

A particularly suitable approach to associate alternative antimicrobial agents with a biocompatible material involves the use of exogenous storage structures. Exogenous storage structures are not

inherent to the biocompatible material to which they are attached. In other words, the exogenous storage structures are in addition to or an alternative to any naturally occurring structures that are inherently part of the biocompatible material. Exogenous storage structures are attached to the biocompatible material at a molecular level without necessarily forming a coating that could possibly disrupt the effectiveness of a toxin-conjugated antibody/ligand.

The exogenous storage structures can store significant quantities of antimicrobial agents. In a particularly preferred embodiment, the exogenous storage structure is a metal binding protein, such as ferritin, and the antimicrobial agents are silver ions or other antimicrobial metal ions. The exogenous storage structure can be bound to the substrate, for example, by crosslinking or using appropriate antibodies. A more complete description of the use of exogenous storage structures for the delivery of antimicrobial agents can be found in copending and commonly assigned U.S. Patent Application serial No. 08/787,139 to Tweden et al., entitled "Medical Article with Adhered Antimicrobial Metal Ions and Related Methods," incorporated herein by reference.

Alternatively, a deposit of antimicrobial agents can be placed on and/or within the biocompatible material. The deposition of antimicrobial elemental metal (such as silver metal) and/or antimicrobial metal compounds (such as silver chloride) with a biocompatible material is described further in copending and commonly assigned U.S. Patent Application Serial No. 08/974,992 to Ogle, entitled "Medical Article with Adhered Antimicrobial Metal," incorporated herein by reference, and U.S. Patent Application Serial No. 09/143,989 to

Ogle et al., entitled "Medical Article with Adhered Antimicrobial Metal," incorporated herein by reference. The selection of the additional antimicrobial agent(s) can be based on the availability of an antimicrobial agent with long term effectiveness to supplement effective shorter term protection supplied by the toxin-conjugated antibodies/ligands, as well as any synergistic enhancement of antimicrobial effectiveness.

Packaging, Distribution, And Use

Following formation of the toxin-conjugated antibodies/ligands, the antibodies/ligands can be stored in solution, frozen preferably at low temperatures, such as -80°C, or lyophilized for storage in dried form. Appropriate storage times will depend on the storage approach and on the nature of the toxin-conjugated antibody/ligand. Certain storage approaches, such as lyophilization, may not be suitable for particular toxins or ligands.

For direct administration of the toxin-conjugated antibodies/ligands, the toxin conjugated antibodies/ligands preferably are formulated into a pharmaceutical composition for packaging and distribution. The packages generally include instructions for administration of the composition and other labels including labels required by the Food and Drug Administration. For the preparation of pharmaceutical compositions, the toxin-conjugated antibodies/ligands can be formulated into injectable preparations, preparations for oral administration and/or topical preparations. Preferred injectable formulations include therapeutically effective amounts of toxin-conjugated antibodies/ligands in a sterile liquid solution or a sterile liquid suspension, particularly a glycerol suspension. Lyophilized

versions of the formulations can be prepared, which are reconstituted later with injectable liquid carriers, such as sterile water, saline, or 0.3% glycine.

Generally, the injectable formulations are administered in amounts to deliver from about 0.01 mg of toxin-conjugated antibody/ligand per kilogram of patient body weight to about 10 mg/kg, preferably from about 0.1 mg/kg to about 2 mg/kg. An effective dose can be administered daily over several days to several weeks and can be provided in a sequential dose-escalation regimen. Injectable formulations are suitable for administration through, for example, intramuscular, intravascular, subcutaneous, intrathecal, intra-articular, intrarectal, intrauteral, intravaginal, and/or intraperitoneal injection.

The toxin-conjugated antibodies/ligands can be incorporated into a dermatological vehicle for topical administration. Suitable dermatological vehicles include, for example, hydrogels, oil-in-water emulsions and water-in-oil emulsions, where the oil phase can include mineral oils, petroleum products and the like. The concentration of toxin-conjugated antibody/ligand in the topical formulation generally ranges from about 0.1 mg/ml to about 25 mg/ml, and preferably from about 1 mg/ml to about 20mg/ml. Suitable vehicles for topical administration include transdermal administrative approaches, such as a variety of transdermal patches and the like used for the administration of a variety of drugs. Topical formulations can include components to facilitate compositions to enhance adsorption of the toxin-conjugated antibodies/ligands, such as surfactants. Topical vehicles can include suitable additives, such as emollients, stabilizers and

preservatives, such as antioxidants. Topical formulations can be applied by way of suppositories.

In alternative embodiments, toxin-conjugated antibodies/ligands are administered into a patient's lungs as an aerosol. The aerosols are formed generally from sterile aqueous solutions, aqueous dispersions, liposomal preparations and/or dispersions of solid particles containing toxin-conjugated antibodies/ligands along with pharmaceutically acceptable carriers and stabilizers. Suitable carriers and stabilizers generally include nonionic surfactants, such as Tweens, Pluronics or polyethylene glycol, sorbitan esters, oleic acid, innocuous proteins, such as albumin or lecithin, amino acids, as well as buffers, salts, sugars, and/or sugar alcohols. Formulations for aerosol delivery can further include lung surfactants, mucolytic agents and/or bronchodilators.

Furthermore, the toxin-conjugated antibody/ligand can be delivered orally as a capsule or a liquid. Suitable capsules can be formulated by proteinoid encapsulation or using other approaches including conventional approaches. A therapeutically effective dose for oral delivery per day generally would be in the range of 0.05 mg of toxin-conjugated antibody/ligand per kg of patient body weight to about 50 mg/kg, and preferably from about 0.05 mg/kg to about 5 mg/kg. Liquid preparations for oral delivery preferably have a pH from about 5 to about 9.5 and more preferably from about 6.5 to about 7.5. The liquid preferably includes a physiologically acceptable buffer such as phosphate, tris(hydroxymethyl)aminomethane-HCL, or citrate, at a concentration from about 1 mM to about 100 mM. The liquid can also include a stabilizing agent, such as albumin or gelatin, as well as a salt, such as sodium

chloride or potassium chloride, at a concentration from about 50 mM to about 150 mM.

Generally, the pharmaceutical preparations are prepared for use and placed in an appropriate container. While the preparations may contain preservatives and stabilizers, the containers generally are marked with a date to indicate the expiration past which it is not recommended to use the product. The pharmaceutical preparations are packaged along with any instructions for use and any FDA required documentation.

Following association of the toxin-conjugated antibody/ligand with the biocompatible material, the biocompatible material, possibly formed into a medical device, can be stored. Preferred storage techniques minimize the risk of microbial contamination. For example, the biocompatible material can be stored in a dry sterile container or a sealed container with sterile buffer and/or saline solution, as appropriate.

In a sealed container the biocompatible material is not subjected to a continuous supply of fluids. Nevertheless, consideration should be given to possible loss during storage of toxin-conjugated antibody/ligand from the biocompatible material or loss during storage of activity of the toxin-conjugated antibody/ligand. If excessive loss is a possibility, the storage time can be limited appropriately to keep the loss to an acceptable level.

For distribution, the medical device generally is placed in sealed and sterile containers. The containers can be dated such that the date reflects the maximum advisable storage time accounting for possible loss of the toxin or degradation of activity. The containers generally are packaged with instructions for the use of the medical devices along with desired and/or

required labels. The containers are distributed to health care professionals for surgical implantation/ application of the medical device by a qualified health care professional. If the medical device is a prosthesis, the surgical implantation generally involves the replacement or repair of damaged tissue with the prosthesis.

As an alternative to the above storage and distribution approaches, the modification of the biocompatible material by addition of an toxin-conjugated antibody/ligand can be performed at a hospital or site remote from the manufacturing site, if desired. This is a particularly suitable approach if the storage time for the biocompatible material modified with the toxin-conjugated antibody/ligand is relatively short or if storage following modification is problematic. In such a case, the medical device prepared for modification is distributed with a solution of the toxin-conjugated antibody/ligand and, optionally with a binding agent, adhesive or the like.

Alternatively, a solution of the toxin-conjugated antibody/ligand is packaged separately from the medical article along with instructions for performing modification of a medical article and, optionally, distributed together as a kit. For embodiments based on chemical binding or adhesive attachment, a kit can be distributed comprising a container of the toxin-conjugated antibody/ligand and a separate container of a chemical binding agent or an adhesive, optionally along with instructions for modification of a biocompatible material. In these embodiments, a selected medical device can be modified using the compositions from the kit prior to medical use. Once the medical device is modified with the

toxin-conjugated antibody/ligand, it can be implanted/applied or stored for a reasonable period of time.

Incorporation of a toxin-conjugated antibody/ligand into a medical device can reduce the risk of infection associated with the contact of the medical article with the patient's bodily fluids or tissue. Reduction in the risk of infection thereby reduces associated health risks to the patient from microbial infections associated with implantation of the medical article. By reducing the risk of infection, there is a corresponding reduction in the need for replacement of the medical article. The toxin-conjugated antibodies/ligands are particularly effective for the treatment of microorganisms that are resistant and infections, such as biofilms, which are difficult to treat. Furthermore, the toxin-conjugated antibodies/ligands are more easily able to target specific microorganisms.

The embodiments described above are intended to be illustrative and not limiting. Additional embodiments are within the claims. Although the present invention has been described with reference to preferred embodiments, workers skilled in the art will recognize that changes may be made in form and detail without departing from the spirit and scope of the invention.

CLAIMS:

1. An antimicrobial conjugate comprising an antibody or ligand chemically bound to an antimicrobial agent, the antibody or ligand having a specific affinity for prokaryote adhesins or fungi adhesins the antibody or ligand having an affinity for bacteria or fungi of at least a factor of ten greater than their affinity for mammalian cells.

2. The antimicrobial conjugate of claim 1 wherein the antimicrobial agent has specific toxicity for microorganisms.

3. The antimicrobial conjugate of claim 1 wherein the antimicrobial agent comprises ribosome inhibiting protein.

4. The antimicrobial conjugate of claim 3 wherein the ribosome inhibiting protein comprises a proenzyme having a mutated amino acid sequence such that the proenzyme is not susceptible to cleavage by a eukaryotic protease to form the active form of the ribosome inhibiting protein.

5. The antimicrobial conjugate of claim 1 wherein the antimicrobial agent comprises a proenzyme with a domain susceptible to cleavage by a microbial enzyme, such that cleavage at the domain results in an active microbial toxin.

6. The antimicrobial conjugate of claim 5 wherein the proenzyme comprises a proenzyme of ribosome inhibiting protein.

7. The antimicrobial conjugate of claim 1 wherein the antibody or ligand comprises a humanized antibody.

8. The antimicrobial conjugate of claim 1 wherein the antibody or ligand comprises an antibody fragment.

9. The antimicrobial conjugate of claim 1 wherein the antibody or ligand comprises a ligand that binds to a microbial cell surface receptor.

10. The antimicrobial conjugate of claim 1 wherein the antimicrobial agent comprises a protein and the

antibody or ligand comprises a protein, and wherein the association involves chemical bonding between amino acid side chains of the antibody or ligand and amino acid side chains of the antimicrobial agent.

11. An antimicrobial conjugate comprising an antibody or ligand chemically bound to an antimicrobial agent, the antibody or ligand having a specific affinity for antigens or receptors associated with prokaryote, fungi or protozoa, wherein the antimicrobial agent comprises a protein and the antibody or ligand comprises a protein and wherein the association of the antibody or ligand and the antimicrobial agent involves peptide linkages such that the conjugate comprises a chimera.

12. The antimicrobial conjugate of claim 1 wherein the antibody or ligand has an affinity for a microorganism at least a factor of one hundred greater than the corresponding affinity for mammalian cells.

13. The antimicrobial conjugate of claim 1 wherein the antimicrobial agent is selected from the group consisting of omeprazole, lansoprazole, phytotoxins, syringomycins, pardaxin, actinomycin, bacitracin, circulin, fungisporin, gramicidin S, malformin, mycobacilin, polymyxin, tyrocidine, valinomycin, penicillin, phosphonomycin, vancomycin, streptomycin, tetracycline, chloramphenicol, erythromycin and bacteriocins.

14. Use of an antimicrobial conjugate of claim 1 in the manufacture of a preparation for eliminating viable microorganisms by placing said preparation in contact with a solution from which viable microorganisms are to be eliminated.

15. Use of claim 14 wherein the solution comprises a patient's bodily fluids or tissues and wherein the placement of said preparation comprises direct delivery of the preparation to the patient.

16. Use of claim 14 wherein the solution comprises a patient's bodily fluids or tissues and wherein the placement of the preparation comprises associating the antimicrobial conjugate with a prosthesis and implanting the prosthesis within the patient.

17. A medical device comprising a biocompatible material associated with an antimicrobial conjugate, the antimicrobial conjugate comprising an antibody or ligand associated with an antimicrobial agent, and the antibody or ligand having an affinity for microbial antigens or receptors.

18. The medical device of claim 17 wherein the biocompatible material comprises tissue.

19. The medical device of claim 17 wherein the biocompatible material comprises a synthetic material.

20. The medical device of claim 19 wherein the biocompatible material is associated with the antimicrobial conjugate by incorporating the antimicrobial conjugate within the synthetic material.

21. The medical device of claim 17 wherein the biocompatible material is associated reversibly with the antimicrobial conjugate by covalent bonding or noncovalent bonding.

22. The medical device of claim 17 wherein the biocompatible material is associated with the antimicrobial conjugate with an adhesive.

23. The medical device of claim 17 wherein the antibody or ligand has an affinity for a microorganism at least a factor of one hundred greater than the corresponding affinity for the patient's eukaryotic cells.

24. The medical device of claim 17 further comprising a linker associating the antimicrobial conjugate with the biocompatible material.

25. A method of forming an antimicrobial conjugate, the method comprising associating an antimicrobial agent with an antibody or ligand having an affinity for antimicrobial antigens or receptors.

26. The method of claim 25 wherein the antimicrobial agent comprises ribosome inhibiting protein.

27. The method of claim 25 wherein the bonding involves covalent bonding.

28. The method of claim 25 wherein the bonding involves noncovalent interactions.

29. The antimicrobial conjugate of claim 1 wherein the antibody or ligand has an affinity for a microorganism at least a factor of one thousand greater than the corresponding affinity cells.

30. The antimicrobial conjugate of claim 1 wherein the antibody or ligand comprises antiadhesin antibodies.

31. The antimicrobial conjugate of claim 30 wherein the antiadhesin antibodies have affinity for adhesin filamentous hemagglutinin.

32. The antimicrobial conjugate of claim 1 wherein the antibody or ligand comprises a ligand having an RGD domain.

33. The antimicrobial conjugate of claim 1 wherein the antimicrobial agent comprises a Shiga toxin.

34. The method of claim 25 wherein the association of the antibody or ligand and the antimicrobial agent involves peptide linkages such that the conjugate comprises a chimera.

35. An antimicrobial conjugate comprising an antibody or ligand chemically bound to an antimicrobial toxin, the antibody or ligand having an affinity for microbial antigens or receptors, wherein the antimicrobial toxin comprises a ribosome inhibiting

protein, the antibody or ligand having an affinity for bacteria, fungi or protozoa of at least a factor of ten greater than their affinity for mammalian cells.

36. The antimicrobial conjugate of claim 35 wherein the antimicrobial toxin comprises a proenzyme with a cleavage region that is selectively cleaved by prokaryotic proteases preferentially over eukaryotic proteases.

37. The antimicrobial conjugate of claim 35 wherein the antimicrobial toxin has effectively no toxicity against human cells.

38. The antimicrobial conjugate of claim 35 wherein the antimicrobial toxin has at least a factor of 100 greater IC_{50} value for microbial cells for some type relative to any mammalian cells.

39. The antimicrobial conjugate of claim 35 wherein the antibody or ligand have a greater specific affinity for prokaryotes, fungi or protozoa relative to mammalian cells.

40. An antimicrobial conjugate comprising a ligand chemically bound to an antimicrobial agent, the ligand having a specific affinity for antigens or receptors associated with prokaryote, fungi or protozoa.

41. The conjugate of claim 40 wherein the ligand has an affinity for bacteria, fungi or protozoa at least a factor of ten greater than their affinity for mammalian cells.

42. A substance or composition for use in a method of eliminating viable microorganisms, said substance or composition comprising an antimicrobial conjugate of claim 1, and said method comprising placing said substance or composition in contact with a solution from which viable microorganisms are to be eliminated.

43. A substance or composition for use in a method of treatment of claim 42 wherein the solution comprises a patient's bodily fluids or tissues and wherein the

placement of said substance or composition comprises direct delivery of said substance or composition to the patient.

44. A substance or composition for use in a method of treatment of claim 42 wherein the solution comprises a patient's bodily fluids or tissues and wherein the placement of said substance or composition comprises associating the antimicrobial conjugate with a prosthesis and implanting the prosthesis within the patient.

45. A conjugate as claimed in claim 1, or claim 35, or claim 40, substantially as herein described and illustrated.

46. Use as claimed in claim 14, substantially as herein described and illustrated.

47. A device as claimed in claim 17, substantially as herein described and illustrated.

48. A method as claimed in claim 25, substantially as herein described and illustrated.

49. A substance or composition for use in a method of treatment as claimed in claim 42, substantially as herein described and illustrated.

50. A new conjugate, a new use of a conjugate as defined in claim 1, a new device, a new method of forming a conjugate, or a substance or composition for a new use in a method of treatment, substantially as herein described.

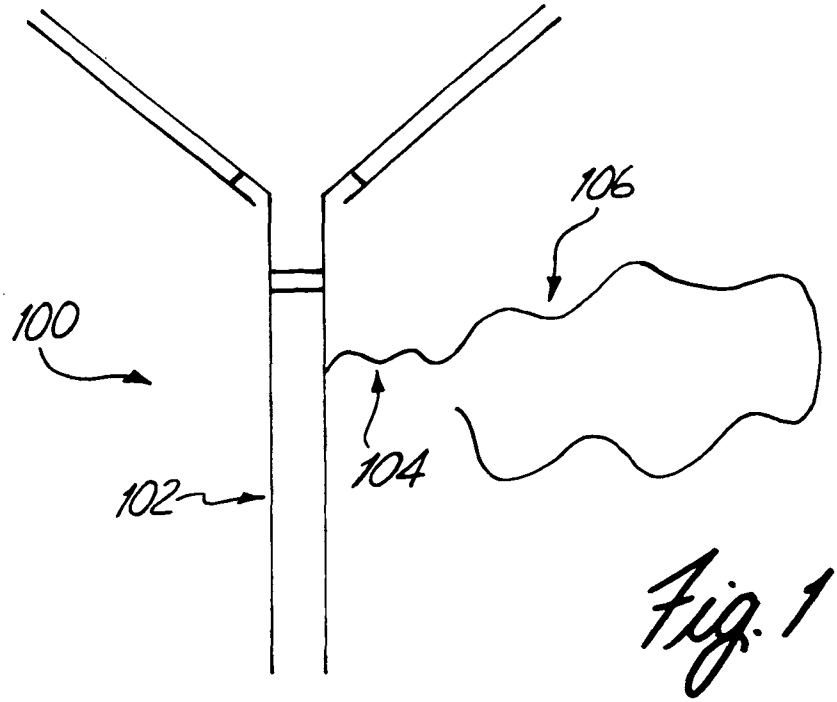

Fig. 1

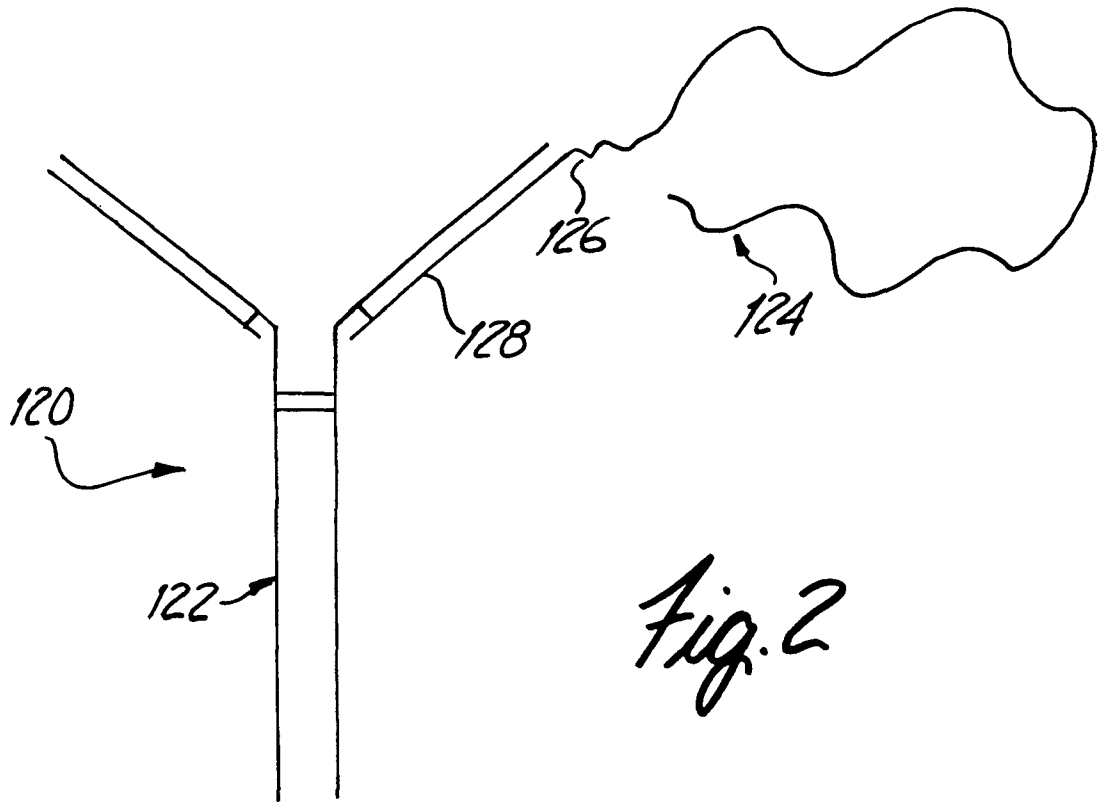

Fig. 2

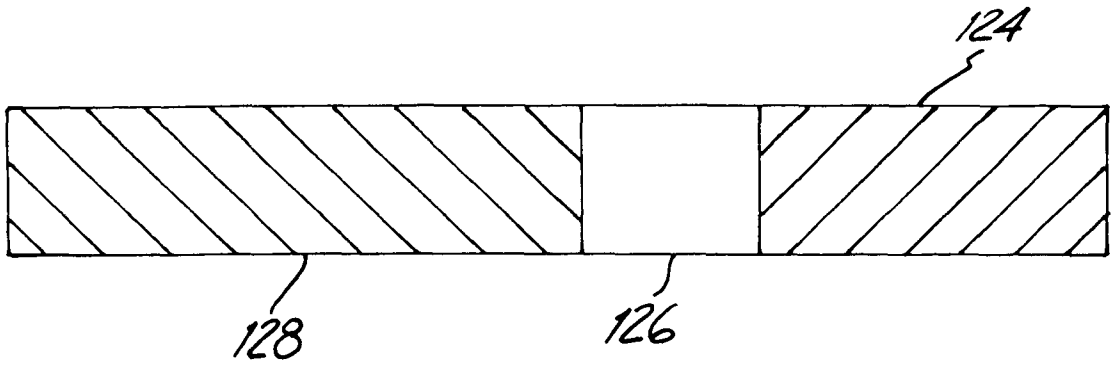

Fig. 3

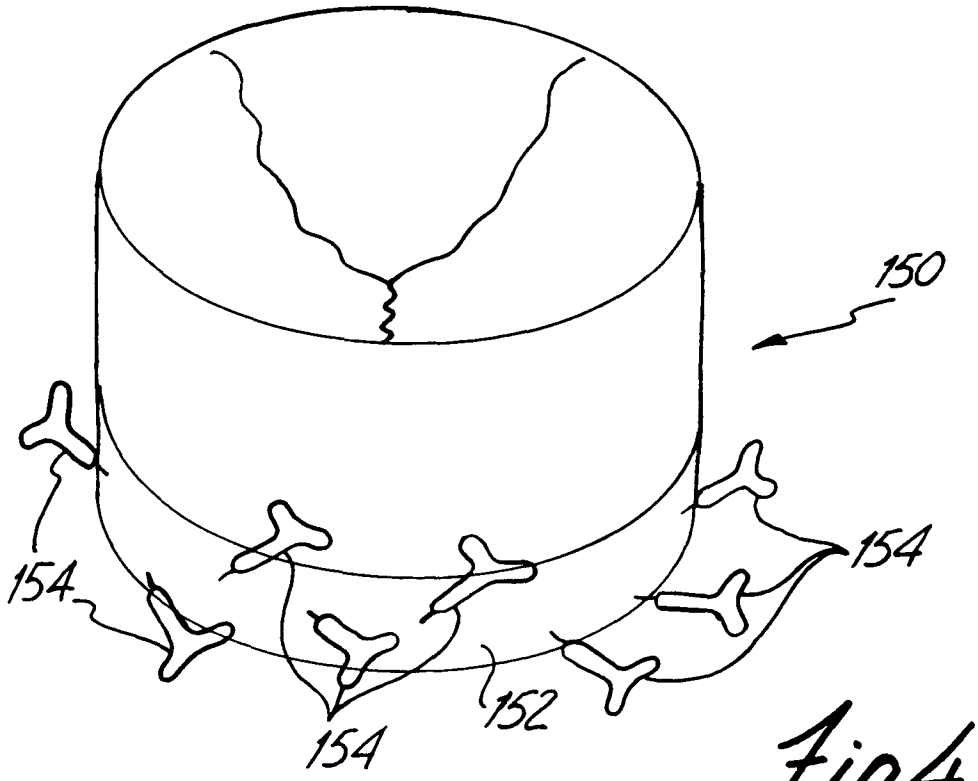

Fig. 4