Abstract: This invention relates to a method of preventing wound infection by administering a therapeutic formulation comprising a peptide to a wound site. More specifically, the invention relates to the use of amphiphilic peptides in preventing and treating infections. The invention also relates to preventing wound infection in a host by administering a biologically active amphiphilic peptide or biologically active protein to a host having a wound wherein the peptide or protein is administered in an amount effective to prevent wound infection. Even more specifically the invention relates to the use of cationic amphiphilic peptides in preventing and treating infections associated with transcutaneous osseointegrated implants.
COMPOSITIONS AND METHODS FOR PREVENTION OF INFECTION IN TRANSCUTANEOUS OSSEOINTEGRATED IMPLANTS

RELATED APPLICATIONS
This application claims the benefit of priority to U.S. Provisional Application No. 60/990,430, filed on November 27, 2007, the entire disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION
This invention relates to the use of peptides in preventing and treating infections. More specifically, the invention relates to the use of amphiphilic peptides in preventing and treating infections. Even more specifically the invention relates to the use of cationic amphiphilic peptides in preventing and treating infections associated with transcutaneous osseointegrated implants.

BACKGROUND OF THE INVENTION

Bone is the major biological tissue designed to carry the biomechanical loads associated with motion in the human body. Therefore, the ideal strategy for rehabilitating the amputee is to develop skeletal implants that are maintained without infection and remain attached to the human skeleton for the lifetime of the amputee. Branemark recently demonstrated that osseointegrated implants affixed to the skeleton can substantially improve the quality of life for amputee patients (Hagberg K. Transfemoral amputation, quality of life and prosthetic function. Goteborg, Sweden: The Sahlgrenska Academy at Goteborg University; 2006) as they avoid the limitations associated with socket technology.

In these types of situation, where there is a constant risk for infection, current treatment regimens are insufficient. It is clear that frequent dressing changes and treatment with antiseptics are not sufficient to prevent the onset of infections. Chronic treatment with common antibiotics, either to treat repeated infection, or as a prophylactic are also insufficient and can contribute to the development of antibiotic resistant strains. In the future, with continued antibiotic resistance, more infections may result in reamputation or revision surgeries unless new infection prevention strategies are developed.


proliferation in wounds. Clinical Research. 1991 Apr(39):566A). Unlike many other peptide-based therapies, with cytotoxicity due to increased local peptide concentrations at the wound site, pexiganan appears to remain safe and effective when used topically in vitro and in vivo.

Although, the use of this class of peptides as anti-infectives is well established and they have been shown to be effective in wound healing, no study to date has explored the ability of these peptides, when administered chronically, to prevent infection in any type of wound let alone prevent infection in transcutaneous implants.

SUMMARY OF THE INVENTION

This invention comprises a method of preventing infections using amphiphilic peptides. More specifically, the invention relates to the use of cationic amphiphilic peptides in preventing infections associated with transcutaneous implants. Preferably, the cationic amphiphilic peptide is a magainin peptide or magainin peptide derivative. Most preferably the magainin peptide is pexiganan or derivative thereof.

In one aspect the invention comprises a method of preventing wound infection by the topical application of a cationic peptide to a wound site. In one embodiment the cationic peptide is a magainin. In a preferred embodiment the magainin peptide is pexiganan or a analog thereof. In a further preferred embodiment the pexiganan is an acid, amid or salt of the peptide shown in SEQ ID NO: 1.

In another aspect, the invention comprises a process for preventing wound infection, comprising topically administering a therapeutic formulation of a magainin peptide having the following peptide structure:

\[(\text{NH}_2)\text{-GIGKFLHSAKKFGKAFVGEIMNS-(NH}_2)\];

In yet another aspect, the invention comprises a process for preventing wound infection, comprising topically administering a therapeutic formulation of a magainin peptide having the following peptide structure:

\[(\text{NH}_2)\text{-GIGKFLHSAKKFGKAFVGEIMNS-(NH}_2)\];
In one aspect, the invention comprises a process for preventing wound infection by administering topically a therapeutic formulation comprising a cationic peptide wherein the peptide has the amino acid sequence of SEQ ID NO: 1.

In a further aspect, the invention comprises a method of preventing wound infection by the topical application of a therapeutic formulation comprising a cream, the cream further comprising a cationic peptide to a wound site. In a preferred embodiment the peptide is a salt, amide, or acid of pexiganan. In a most preferred embodiment the therapeutic formulation comprises a 1% pexiganan acetate cream. In another preferred embodiment, the therapeutic formulation is applied to the wound site once a day.

In a further aspect, the invention comprises a process for preventing wound infection in a host, comprising administering to a host having a wound at least one biologically active amphiphilic peptide and/or biologically active protein, said peptide or protein being an ion channel-forming peptide or protein, said at least one biologically active amphiphilic peptide or protein being administered in an amount effective for preventing wound infection in the host. In one embodiment, the peptide of the process of the invention is a basic polypeptide having at least sixteen amino acids, wherein said basic polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids. In a preferred embodiment, the amphiphilic peptide is cationic.

More preferably the peptide is a magainin peptide.

In a further aspect, the invention comprises a process for preventing wound infection in a host, comprising administering to a host having a wound a magainin peptide having the following basic peptide structure:

\[-R\pi -Rj] -Ri2 -Ri3 -Rn -Ru -Ri2 -Rn -Ri4 -

\[R_{12} -Rn -Rn -Ri^* - (Ris)n -Ri4a -RM -\]

wherein Rn is a hydrophobic amino acid, Ri2 is a basic hydrophilic amino acid, Ri13 is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid, R14 and Ri4a are hydrophobic or basic hydrophilic amino acids, Ri5 is glutamic acid or aspartic acid, a hydrophobic amino acid, or a basic hydrophilic amino acid, and n is 0 or 1.
In a further aspect, the invention comprises a process for preventing wound infection in a host, comprising administering to a host having a wound a magainin peptide having the following basic peptide structure:

\[-R_i^4 -R_n -R_{i4a} -R_{i2} -R_n -R_{i2} -R_n -R_{i2} -R_{i4} -R_n -\]

wherein \(R_n\) is a hydrophobic amino aid, \(R_{i2}\) is a basic hydrophilic amino acid, \(R_{i3}\) is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid, and \(R_{i4}\) and \(R_{i4}\) are hydrophobic or basic hydrophilic amino acids.

In one aspect, the invention comprises a process for preventing wound infection in a host, comprising administering to a host having a wound at least one biologically active amphiphilic peptide and/or biologically active protein, wherein the wound is a transcutaneous wound. In another embodiment, the transcutaneous wound is the site of a medical implant. In a more preferred embodiment, the wound is the site of implantation of an osteointegrated implant.

In another aspect, the invention comprises a process for preventing wound infection in a host, comprising administering to a host having a wound at least one biologically active amphiphilic peptide and/or biologically active protein wherein the peptide has the following peptide structure:

Gly-Ue-Gly-Lys-Phe-Leu-Lys-Ala-Lys-Phe-Gly-Lys-Ala-Phe-Val-Lys-Ile-Leu-Lys-Lys (SEQ ID NO: 1); as well as amides, salts and acids of the foregoing.

**BRIEF DESCRIPTION OF THE FIGURES**

**FIGURE 1** is a depiction of the surgery involved in inserting transcutaneous implants. The implant went through the tibialis anterior muscle and remained transcutaneous on the lateral side. The ends of the cerclage wire were twisted together, trimmed, and pressed flat against the medical side of the tibia underneath the soft tissue.
FIGURE 2 is a scanning electron image of a tantalum implant depicting the area analyzed for bone ingrowth.

FIGURE 3 shows that certain porous tantalum implants used in the procedures of the examples were prone to breaking and to skin healing over the implant, thereby, sealing the implant from the external environment. The rabbits were removed from the study since they no longer had a pathway for pin track infection.

Figure 4 shows a Kaplan-Meier curve showing that Group 2 with pexiganan acetate statistically differed from the Group 1 without an antimicrobial indicating that daily application of pexiganan acetate helped to reduce pin track infection rates by 75% (p=0.019). The porous tantalum pin used in Group 3 did not prevent infection any better than the smooth titanium pin used in Group 1 (p = 0.230).

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

As used herein, "a" or "an" refers to one or more of the term modified. Thus, the compositions of the present invention include one or more polypeptides.

"Amino acid" is used herein to refer to a chemical compound with the general formula: \( \text{NH}_2\text{CRH-COOH} \), where R, the side chain, is H or an organic group. Where R is an organic group, R can vary and is either polar or nonpolar (i.e., hydrophobic). The amino acids of this invention can be naturally occurring or synthetic (often referred to as nonproteinogenic). As used herein, an organic group is a hydrocarbon group that is classified as an aliphatic group, a cyclic group or combination of aliphatic and cyclic groups. The term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" refers to mono- or polycyclic aromatic hydrocarbon groups. As used herein, an organic group can be substituted or unsubstituted.
The terms "polypeptide" and "peptide" as used herein, are used interchangeably and refer to a polymer of amino acids. These terms do not connote a specific length of a polymer of amino acids. Thus, for example, the terms oligopeptide, protein, and enzyme are included within the definition of polypeptide or peptide, whether produced using recombinant techniques, chemical or enzymatic synthesis, or naturally occurring. This term also includes polypeptides that have been modified or derivatized, such as by glycosylation, acetylation, phosphorylation, and the like.

The following abbreviations are used throughout the application:

A = Ala = Alanine
T = Thr = Threonine
V = Val = Valine
C = Cys = Cysteine
L = Leu = Leucine
Y = Tyr = Tyrosine
I = He = Isoleucine
N = Asn = Asparagine
P = Pro = Proline
Q = GIn = Glutamine
F = Phe = Phenylalanine
D = Asp = Aspartic Acid
W = Trp = Tryptophan
E = GIu = Glutamic Acid
M = Met = Methionine
K = Lys = Lysine
G = GIy = Glycine
R = Arg = Arginine
S = Ser = Serine
H = His = Histidine
The ability of a protein or peptide of the invention to prevent infection can be evaluated against a variety of bacteria, preferably Gram-negative bacteria, but the types of bacteria can include Pseudomonas spp including P. aeruginosa and P. cepacia. E. coli strains, including E. coli B, Salmonella, Proteus mirabilis and Staphylococcus strains such as Staphylococcus aureus. A preferred organism is Staphylococcus aureus.

The polypeptides of the invention can be in their free acid form or they can be amidated at the C-terminal carboxylate group. The present invention also includes analogs of the polypeptide of the invention, which typically have structural similarity with the disclosed polypeptides. An "analog" of a polypeptide includes at least a portion of the polypeptide, wherein the portion contains deletions or additions of one or more contiguous or noncontiguous amino acids, or containing one or more amino acid substitutions. Substitutes for an amino acid in the polypeptides of the invention are preferably conservative substitutions, which are selected from other members of the class to which the amino acid belongs. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can generally be substituted for another amino acid without substantially altering the structure of a polypeptide.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr, and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn, and Gln (carboxyl group containing side chains): Class IV: His, Arg, and Lys (representing basic side chains); Class V: He, Val, Leu, Phe, and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr, and His (representing aromatic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in Class II; 2-aminoacidipic acid, 2-aminopimelic acid, .gamma.-carboxyglutamic acid, .beta.-carboxyaspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and hydroxylysine in Class IV; substituted phenylalanines, norleucine, norvaline, 2-
aminooctanoic acid, 2-aminoheptanoic acid, statine and beta-valine in Class V; and
naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid,
and halogenated tyrosines in Class VI.

Polypeptide analogs, as that term is used herein, also include modified
polypeptides. Modifications of polypeptides of the invention include chemical and/or
enzymatic derivatizations at one or more constituent amino acid, including side chain
modifications, backbone modifications, and N- and C-terminal modifications including
acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate
or lipid moieties, cofactors, and the like.

A preferred polypeptide analog is characterized by having at least one of the
biological activities described herein. Such an analog is referred to herein as a
"biologically active analog" or simply "active analog." The biological activity of a
polypeptide can be determined, for example, as described in the Examples Section.

The polypeptides of the invention may be synthesized by the solid phase method
using standard methods based on either t-butyloxy carbonyl (BOC) or 9-
fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described
New York, N.Y., pp. 77 183 (1992). The present peptides may also be synthesized via
recombinant techniques well known to those skilled in the art. For example, U.S. Pat.
No. 5,595,887 describes methods of forming a variety of relatively small peptides
through expression of a recombinant gene construct coding for a fusion protein which
includes a binding protein and one or more copies of the desired target peptide. After
expression, the fusion protein is isolated and cleaved using chemical and/or enzymatic
methods to produce the desired target peptide.

II. Compositions of the Invention

A. Peptides of the Invention

In general, the biologically active peptides or biologically active proteins
employed in the present invention are ion channel-forming peptides or proteins. An ion
channel-forming peptide or protein or ionophore is a peptide or protein which increases
the permeability for ions across a natural or synthetic lipid membrane. B. Christensen
et al. PNAS Vol. 85 P. 5072-76 (July, 1988) describes methodology which indicates
whether or not a peptide or protein has ion channel-forming properties and is therefore an ionophore. As used herein an ion channel-forming peptide or protein is a peptide or protein which has ion channel-forming properties as determined by the method of Christensen et al.

The peptides employed in this invention are preferably amphiphilic peptides and more preferably amphiphilic acationic peptides. An amphiphilic peptide is a peptide which includes both hydrophobic and hydrophilic peptide regions. A cationic peptide is one that carries a positive charge.

The administration of the biologically active amphiphilic peptides or proteins to a host may is preferably by topical administration in a consistent and repeated manner in order to prevent infection of the host. The topical administration is preferably performed at or near a wound site where infection would tend to occur.

In accordance with an embodiment, the peptide employed, or derivatives or analogues thereof, is a basic (positively charged) polypeptide having at least sixteen amino acids wherein the polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids. Still more particularly, the hydrophobic amino acids are in groups of two adjacent amino acids, and each group of two hydrophobic amino acids is spaced from another group of two hydrophobic amino acids by at least one amino acid other than a hydrophobic amino acid (preferably at least two amino acids) and generally by no greater than four amino acids, and the amino acids between pairs of hydrophobic amino acids may or may not be hydrophilic.

The hydrophilic amino acids are generally also in groups of two adjacent amino acids in which at least one of the two amino acids is a basic hydrophilic amino acid, with such groups of two hydrophilic amino acids being spaced from each other by at least one amino acid other than a hydrophilic amino acid (preferably at least two amino acids) and generally no greater than four amino acids, and the amino acids between pairs of hydrophilic amino acids may or may not be hydrophobic.

In accordance with a particularly preferred embodiment, the polypeptide comprises a chain of at least four groups of amino acids, with each group consisting of four amino acids. Two of the four amino acids in each group are hydrophobic amino acids, and two of the four amino acids in each group are hydrophilic, with at least one of the hydrophilic amino acids in each group being a basic hydrophilic amino acid and the
other being a basic or neutral hydrophilic amino acid.

The hydrophobic amino acids may be selected from the class consisting of Ala, Cys, Phe, Gly, He, Leu, Met, Val, Trp, and Tyr. The neutral hydrophilic amino acids may be selected from the class consisting of Asn, Gln, Ser, and Thr. The basic hydrophilic amino acids may be selected from the class consisting of Lys, Arg, His, and ornithine (O).

Each of the groups of four amino acids may be of the sequence ABCD, BCDA, CDAB, or DABC, wherein A and B are each hydrophobic amino acids and may be the same or different, one of C or D is a basic hydrophilic amino acid, and the other of C or D is a basic or neutral hydrophilic amino acid and may be the same or different. In a preferred embodiment, the polypeptide chain may comprise 5 or 6 groups of this sequence. In each group, each of A, B, C and D may be the same in some or all of the groups or may be different in some or all of the groups.

The polypeptide chain preferably has at least 20 amino acids, and no greater than 50 amino acids. It is to be understood, however, that the polypeptide does not have to consist entirely of the groups described above. The polypeptide may have amino acids extending from either or both ends of the noted groups forming the polypeptide chain and/or there may be amino acids between one or more of the at least four groups and still remain within the scope of the invention.

The groups of amino acids may be repeating groups of amino acids, or the amino acids in the various groups may vary provided that in each group of the at least four groups of amino acids there are two hydrophobic and two hydrophilic amino acids as hereinabove noted.

Thus, in a preferred embodiment, the biologically active polypeptide comprises a chain including at least four groups of amino acids, each containing four amino acids. Two of the four amino acids in each group are hydrophobic, at least one amino acid is basic hydrophilic, and the remaining one is basic or neutral hydrophilic, with the polypeptide chain preferably having at least 20 amino acids but no greater than 50 amino acids.

In one embodiment, each of the at least four groups of amino acids which are in the peptide chain is of the sequence A-B-C-D, B-C-D-A, C-D-A-B or D-A-B-C wherein A and B are hydrophobic amino acids, one of C or D is basic hydrophilic amino acid,
and the other of C or D is basic or neutral hydrophilic amino acid. The resulting polypeptide chain, therefore, may have one of the following sequences:

\[(X_0)_a (A-B-C-D)_n (Y_0)_b\]

\[(X_2)_b (B-C-D-A)_n (Y_2)_b\]

\[(X_3)_a (C-D-A-B)_n (Y_3)_b\]

\[(X_4)_a (D-A-B-C)_n (Y_4)_b\]

wherein

\[X_1\] is D, C-D, or B-C-D,

\[Y_1\] is -A or -A-B or -A-B-C

\[X_2\] is A-, D-A, or C-D-A-

\[Y_2\] is -B, -B-C or B-C-D

\[X_3\] is B-, A-B-, D-A-B-

\[Y_3\] is -C, -C-D, -C-D-A

\[X_4\] is C-, B-C, A-B-C-

\[Y_4\] is D, -D-A, -D-A-B

\[a\] is 0 or 1; \[b\] is 0 or 1.

and \[n\] is at least 4.
It is to be understood that the peptide chain may include amino acids between the hereinabove noted groups of four amino acids provided that the spacing between such groups and the charge on the amino acids does not change the characteristics of the peptide chain which provide amphiphilicity and a positive charge and do not adversely affect the folding characteristics of the chain to that which is significantly different from one in which the hereinabove noted group of four amino acids are not spaced from each other.

As representative examples of peptides in accordance with the present invention, there may be mentioned.

I - Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys (SEQ ID NO:2)

II - Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys (SEQ IDNO:3)

III - Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala (SHQ ID Nü:4)

IV - Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala (SEQ ID NO:5)

V - Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser-Lys-Ala-Phe-Ser (SEQ ID NO:6)

The peptide may have amino acids extending from either end of the chain. For example, the chains may have a Ser-Lys sequence before the "Ala" end, and/or an Ala-Phe sequence after the "Lys" end. Other amino acid sequences may also be attached to the "Ala" and/or the "Lys" end.

Similarly, in any polypeptide chain having at least four groups of amino acids of the sequence as described above, the chain may have, for example, a C-D sequence before the first A-B-C-D group. Also other amino acid sequences may be attached to
the "A" and/or the "D" end of one of these polypeptide chains. Also there may be amino acids in the chain which space one or more groups of the hereinabove noted four amino acids from each other.

The peptides may be produced by known techniques and obtained in substantially pure form. For example, the peptides may be synthesized on an automatic synthesizer. Journal of the American Chemical Society, Vol. 85 Pages 2149-54(1963). It is also possible to produce such peptides by genetic engineering techniques.

In accordance with another preferred embodiment, the peptide employed may be a magainin peptide.

A magainin peptide is either a magainin such as Magainin I, II or III or an analogue or derivative thereof. The magainin peptides may include the following basic peptide structure X_{12}:

\[ -R_n -R_\text{n} -R_{i_2} -R_{i_3} -R_n -R_{i_4} -R_{i_2} -R_n -R_{i_4} - \]

\[ R_{i_2} -R_n -R_n -R_{i_4} -R_{i_4} - (R_{i_5})_n -R_{i_4} -R_{14} - \]

wherein \( R_n \) is a hydrophobic amino acid, \( R_{12} \) is a basic hydrophilic amino acid; \( R_{13} \) is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid; \( R_{i_4} \) and \( R_{i_4} \) are hydrophobic or basic hydrophilic amino acids, \( R_{i_5} \) is glutamic acid or aspartic acid, or a hydrophobic or basic hydrophilic amino acid, and \( n \) is 0 or 1. In a preferred embodiment, \( R_n \) is a hydrophobic or neutral hydrophilic amino acid, \( R_{i_4} \) is a hydrophobic amino acid, and \( R_{15} \) is glutamic acid or aspartic acid.

Thus, for example, a magainin peptide may include the following structure:

\[ Y_{12} -X_{i_2} - \]

where \( X_{i_2} \) is the hereinabove described basic peptide structure and \( Y_{i_2} \) is:
A magainin peptide may also have the following structure:

\[-X_{12} - Z_{12} -\]

wherein \( X_{12} \) is as previously defined and \( Z_{12} \) is:

- (i) \( R_{16} \) where \( R_{16} \) is a basic hydrophilic amino acid or asparagine or glutamine; or
- (ii) \( R_{16} - R_{17} \) where \( R_{17} \) is a neutral hydrophilic amino acid, a hydrophobic amino acid, or a basic hydrophilic amino acid. Preferably, \( R_{17} \) is a neutral hydrophilic amino acid.

A magainin peptide may also have the following structure:

\((Y_{12})_a - X_{12} - (Z_{12})_b\)

where \( X_{12}, Y_{12}, \) and \( Z_{12} \) are as previously defined, and \( a \) is 0 or 1 and \( b \) is 0 or 1.

The magainin peptides may also include the following basic peptide structure \( X_{13} \):

\[ R_{14} - R_{14} - R_{12} - R_{11} - R_{11} - R_{12} - R_{14} - \]

\[ R_{12} - R_{11} - R_{11} - R_{12} - . \]
wherein \( R_n, R_{i2}, R_n, R_{i4a} \) are amino acids as hereinabove described.

The magainin peptide may also include the following structure \( X_{i3} - Z_i3 \); wherein \( X_{i3} \) is the herein above described basic peptide structure and \( Z_i3 \) is:

\[
(RlOn -(Rl)n -(RlOn -(Rl4a)n -(Rl5)n -(Rl4a)n -(RlOn -(Rl6)n -(Rn))n)
\]

wherein \( R_n, R_{i3}, R_{i4a}, R_{i5}, R_{i6}, \) and \( R_{i7} \) are amino acids as hereinabove described, and \( n \) is 0 or 1, and each \( n \) may be the same or different.

In a preferred embodiment the peptide of the invention is a magainin peptide or analog thereof. The magainin peptides generally include at least fourteen amino acids and may include up to forty amino acids. A magainin peptide preferably has 22 or 23 amino acids. Accordingly, the hereinabove described basic peptide structures of a magainin peptide may include additional amino acids at the amino end or at the carboxyl end, or at both ends.

Magainin peptides are described in Proc. Natl. Acad. Sci. Vol. 84 pp. 5449-53 (Aug. 1987). The term "magainin peptides" as used herein refers to the basic magainin structure as well as derivatives and analogs thereof, including but not limited to the representatives derivatives derivatives or analogs disclosed herein.

As representative examples of such magainin peptides, there may be mentioned peptides having the following primary sequence (expressed as a single letter code) as well as appropriate analogues and derivatives thereof:

(a) \((\text{NH}_2)\ GIGKFLHSAGKFGICAFVGEIMKS(\text{OH}) \) or \((\text{NH}_2)(\text{Magainin I}) \) (SHQ ID NO:7)

(b) \((\text{NH}_2)\ GIGKFLHSAKKFGKAFVGEIMNS(\text{OH}) \) or \((\text{NH}_2)(\text{Magainin II}) \) (SKQ ID NO:8)
The following are examples of peptide derivatives or analogs of the basic structure:

(d) \((\text{NH}_2)\) IGKFLHASKKFKGVGEIMNS(OH) or \((\text{NH}_2)\) (Magainin III) (SHQ ID NO: 9)

(e) \((\text{NH}_2)\) GKFLHSAKKFKGVGEIMNS(OH) or \((\text{NH}_2)\) (SI-Q ID NO: 11)

(f) \((\text{NH}_2)\) KFLHSAKKFKGVGEIMNS(OH) or \((\text{NH}_2)\) (SEQ ID NO: 12)

(g) Gly-Ile-Gly-Lys-Ph\(\varepsilon\)-Leu-L\(\geq\)s-Lys-Ala-L\(\geq\)s-Lys-Phe-Gly-Lys-Ala-Phe-Val-Lys-Ile-Leu-Lvs-Lys(OH) or \((\text{NH}_2)\) (SBQ ID NO: 1).

B. Formulations of the Invention

The present invention also provides a composition that includes one or more active agents (i.e., polypeptides) of the invention and one or more pharmaceutically acceptable carriers. One or more polypeptides with demonstrated biological activity can be administered to a patient in an amount alone or together with other active agents and with a pharmaceutically acceptable buffer. The polypeptides can be combined with a variety of physiological acceptable carriers for delivery to a patient including a variety of diluents or excipients known to those of ordinary skill in the art. For example, for parenteral administration, isotonic saline is preferred. For topical administration, a cream, including a carrier such as dimethylsulfoxide (DMSO), or other agents typically found in topical creams that do not block or inhibit activity of the peptide, can be used. Other suitable carriers include, but are not limited to, alcohol, phosphate buffered saline, and other balanced salt solutions.

The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Preferably, such methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are
prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations. The methods of the invention include

administering to a patient, preferably a mammal, and more preferably a human, the composition of the invention in an amount effective to produce the desired effect. The peptides can be administered as a single dose or in multiple doses. Useful dosages of the active agents can be determined by comparing their in vitro activity and the in vivo activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art; for example, see U.S. Pat. No. 4,938,949.

The agents of the present invention are preferably formulated in pharmaceutical compositions and then, in accordance with the methods of the invention, administered to a patient, such as a human patient, in a variety of forms adapted to the chosen route of administration. The formulations include, but are not limited to, those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic, or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, and intravenous) administration.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile powders of the active agent, which are preferably isotonic with the blood of the recipient. Isotonic agents that can be included in the liquid preparation include sugars, buffers, and sodium chloride. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage. The necessary fluidity can be achieved, for example, by using liposomes, by employing the appropriate particle size in the case of dispersions, or by using surfactants. Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze drying of the sterile injectible solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption of the active agents over a
prolonged period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

In general, the peptide or protein is employed to provide peptide or protein dosages of from 0.1 mg to 250 mg per kilogram of host weight, when administered systemically. When administered topically, the peptide or protein is administered in an amount of from about 0.1% to about 10%, preferably from about 0.5% to about 2%. The topical composition may be in the form of an ointment, cream, or solution. In a preferred embodiment the composition is a topical cream or ointment comprising about 1% pexiganan or its derivatives, analogs, salts or amid

III. EXAMPLES
A. IMPLANTS
The implants were 36-mm long and 2.7-mm diameter. A hexagonal cross-section was used to prevent rotational motion. The implants were either titanium alloy (Ti-6Al-4V) free of surface finish or tantalum with uniform volume porosity of 75% to 80% (Trabecular Metal™, Zimmer, Inc., Warsaw, IN) (Hacking SA, Bobyn JD, Toh KK, Tanzer M, Krygier JJ. Fibrous tissue ingrowth and attachment to porous tantalum. J Biomed Mater Res. 2000 Dec 15;52(4):631-8). All implants were manufactured by the Zimmer Corporation. The implants were pre-sterilized by gamma radiation and conformed to Zimmer’s human implant sterile lot release procedures. The investigators added a less than 1.0-mm diameter hole to the tip of the implant to allow it to be secured the tibia with a cerclage wire. The implants were then passivated by MedicineLodge, Inc (Logan, UT), re-sterilized in an autoclave, and placed in a sterile pouch.

B. ANIMALS AND EXPERIMENTAL GROUPS
All animal protocols were reviewed and approved by the Salt Lake City, Utah, Veterans Affairs Institutional Animal Care and Use Committee and the USAMRMC Animal Care and Use Review Office. A total of 37 New Zealand white rabbits, approximately 5 months old and weighing between 3.3 kg and 4.2 kg, were used in this study. Eleven rabbits were in the untreated control group (Group 1). Rabbits in Group 1 received the titanium alloy implant and did not receive an antimicrobial. Eight rabbits were in Group 2. Rabbits in Group 2 received the titanium alloy implant and also
received topical pexiganan acetate 1% (LOCILEX™, Macrochem Inc., Wellesley Hills, MA) at the pin site the day after surgery and then daily starting on the fifth day following surgery. The five day gap minimized handling during the post-operative recovery. Group 2 was halted at n=8 while alternative study designs were considered since the study design for this group was not optimal because the rabbits appeared to remove the antimicrobial after application. Group 1 and Group 2 were compared to address the first hypothesis (Table 1).

Eighteen rabbits were in Group 3. Group 3 received porous tantalum implants and did not receive an antimicrobial. More rabbits were needed in the Group 3 with tantalum implants relative to Group 1 and 2 with titanium alloy implants because initial data indicated that the tantalum implants had a higher probability of failure due to the implant breaking or the skin slipping and healing over the implant thereby eliminating a path for pin track infection. Therefore, a total of 18 rabbits were placed into the Group 3 to maintain a statistically reliable sample size. Group 1 and Group 3 were compared to address the second hypothesis (Table 1).

A previous study and our pilot work indicated that rabbits have less than a 20% chance of pin track infection when left to the environment for 12-weeks (Gerritsen M, Lutterman JA, Jansen JA. Wound healing around bone-anchored percutaneous devices in experimental diabetes mellitus. J Biomed Mater Res. 2000;53(6):702-9). Therefore, all rabbits were inoculated with $1 \times 10^8$ CFU/0.2 ml concentration of exogenous *S. aureus* fourteen days post-surgery and weekly thereafter, to increase the infection challenge (An YH, Friedman RJ. Animal models of orthopedic implant infection. Journal of Investigative Surgery. 1998 Mar-Apr; 11(2): 139-46). The solution for suspending the *S. aureus* consisted of phosphate buffered saline with 25% glycerol. The bacteria solution was frozen until just prior to inoculation. Inoculation was performed by dripping the bacteria solution over the implant site with a sterile transfer pipet. The *S. aureus* used in this study came from an American Type Culture Collection (ATCC) 49230, which was from a patient with osteomyelitis.
Table 1 - Description and sample size of each experimental rabbit group. Group 1 was compared to Group 2 for addressing the first hypothesis, and Group 1 was compared to Group 3 for addressing the second hypothesis.

<table>
<thead>
<tr>
<th>Rabbit Group (n = sample size)</th>
<th>Implant Material</th>
<th>Exogenous Bacteria Applied to Implant Exit Site</th>
<th>Topical Antimicrobial Applied to Implant Exit Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Control (n = 11)</td>
<td>Titanium alloy free of surface finish</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2 (n = 8)</td>
<td>Titanium alloy free of surface finish</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3 (n = 18)</td>
<td>75% - 80% Porous Tantalum</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

C. Surgical Technique

Anesthesia was induced by an intramuscular injection of ketamine (35mg/kg) and Xylazine (5mg/kg) into the rabbit’s semi tendonosis or semi membranosis muscle. To determine normal skin flora, swab samples were obtained at the implant site. After endotracheal intubation the rabbit breathed isoflurane (3-5%) in oxygen and respiration was monitored with an apnea detection device. An alpha 2 antagonist, Yohimine or Tolazine HCL, was administered IV (0.5mg/kg) in the lateral ear vein to partially reverse the effect of Xylazine. Approximately two minutes after the Yohimine, buprenorphine (0.02-0.1 mg/kg) was injected subcutaneously into the rabbit’s neck region. For postoperative pain management, a 25 µg transdermal fentanyl patch was sutured to the shaved spot in between the rabbit’s shoulders. Suturing prevented the patch from coming off during the rabbit’s recovery. The hind leg was close-shaved and prepared for surgery by scrubbing with a povidine iodine scrub followed by a 70% alcohol wipe repeated three times. After a brief drying time, the area was prepped with a final spray of povidine solution and again allowed to dry.

During surgery, the rabbit’s heart rate, mucus membrane color, corneal reflex, capillary refill time and respiratory rate were monitored and recorded. After the rabbit’s right leg was draped, a direct anterolateral incision (1.0±0.2cm) was made over the
proximal tibia while the limb was positioned in extension. The skin and fascia were reflected. The fibers of the tibialis anterior muscle were separated so that the implant traversed the muscle belly. In preparation for implantation, a bicortical hole was drilled in the proximal tibia approximately 1-cm distal to the tibia tuberosity. The implants were tapped into the hole with a metal mallet for press-fit fixation.

After the implant crossed the tibia, a 24 gauge cerclage wire was inserted through the hole in the medial end of the implant. One end of the wire was placed along the anterior cortex of the tibia, taken around the shaft of the implant on the lateral side, and then both ends of the wire were twisted together on the medial side. The ends of the wire were trimmed to about 2-mm and pressed flat with needle holders against the tibia (Figure 1).

A 2-mm puncture was made in the skin about 2-cm proximal to the incision. The implant exited the skin at this site. This allowed for less tension on the skin when the rabbit was in its normal resting posture and more closely mimicked the skin and post placement of Branemark's osseointegrated implant for human amputees (Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the trans-femoral amputee with an osseointegrated prosthesis: the United Kingdom experience. Prosthet Orthot Int. 2003 Aug;27(2):1 14-20; Branemark P-I, Chien S, Gröndahl H-G, Robinson K, editors. The Osseointegration Book. From Calvarium to Calcaneus. Berlin, Germany: Quintessenz Verlags-GmbH; 2005). After placement of the implant, the skin was closed with a subcuticular stitch. The rabbit recovered on a heating pad and towels. After the rabbit was awake and standing upright, the rabbit was placed into its cage. The fentanyl patch gave pain relief for 72 hours. All rabbits were monitored daily to determine their general health and infection status.

D. Study Endpoint

The rabbits were euthanized as per protocol when they showed clinical signs greater than a Grade I pin track infection but no greater than a Grade II infection (Checketies RG, MacEachern AG, Otterburn M. Pin track infection and the principles of pin site care. In: Giovanni De Bastiani AGaAG, editor. Orthofix External Fixation in Trauma and Orthopaedics. Great Britain: Springer-Verlag London Limited; 2000. p. 97-103). Grade I infection was defined as "slight redness around the pin together will a
little discharge" whereas a Grade II infection was defined as the presence of swelling, redness, purulent discharge, and tenderness of the implant site (Checketts RG, MacEachern AG, Otterburn M. Pin track infection and the principles of pin site care. In: Giovanni De Bastiani AGAaAG, editor. Orthofix External Fixation in Trauma and Orthopaedics. Great Britain: Springer-Verlag London Limited; 2000. p. 97-103). This was a subjective evaluation made by the same team of investigators. If euthanasia occurred for other reasons, the rabbit was removed from its study group. Rabbits that remained healthy were euthanized at the end of the 24-week study trial. Euthanasia was performed by an intravenous injection of phenytoin sodium and pentobarbital sodium at a dose of 1 mL/4.5 kg.

E. Tissue Processing and Culturing

Using sterile technique, samples were obtained of blood, muscle, and bone. All skin surfaces were disinfected with a povidone-iodine solution and all instruments were sterilized or disinfected prior to tissue collection. Immediately prior to euthanasia, blood was drawn from a vein or artery from the inside of the ear and injected into a blood culture bottle. After euthanasia, a biopsy punch was used to collect the muscle samples. This sample extended from the skin surface to the bone. In order to preserve the soft tissue/implant interface for imaging, the muscle sample was taken 5-mm away from the implant site in the direction towards the knee joint. The muscle sample was then placed into fastidious broth for culturing. To obtain the bone sample, the soft tissue around the tibia was cut with a scalpel circumferentially 4-cm distal from the implant site. The tibia was transected using an autopsy saw. Bone chips were collected by twisting a 1/8 inch drill bit inside the medullary canal near the implant. The entire drill bit was placed into fastidious broth. After using standard laboratory protocols to culture the tissue samples, the presence or absence of bacteria growth was noted as well as the type of organism such as S. aureus or E. coli. The technician performing the cultures and analysis was not aware of the rabbit study groups the samples belonged.

F. Histology

After obtaining culture specimens, the tibia was disarticulated and placed in 70% ethanol. After taking photos and radiographs of the gross specimens, they were fixed in
1:10 buffered formalin. Within days, a transverse 3-mm thick slice of the tibia was taken so that the side of the slice closest to the implant was at a distance of 1 to 3-mm. Histology ink was used to mark the side of the section that was furthest from the implant and the lateral side of the skin, muscle, and bone. Skin and muscle were placed into one cassette and the bone in another cassette. Care was taken to maintain the physiological orientation of the tissue. The bone was decalcified and then both the bone and soft tissue were dehydrated, infiltrated (Tissue Tek VAccum Infiltration Process, Miles Scientific), and embedded in paraffin (Histocentre 2, Shandon). A microtome was used to cut 4-μm slices of the specimens. At least three slices were taken of each soft tissue and bone specimen. These were stained with Hematoxylin & Eosin (H&E) and histologically examined for inflammation, fibrosis, and bone remodeling. Periodic acid-Schiff (PAS) stain was used to detect the presence of fungus, and Brown-Brenn stain was used to detect the presence of bacteria. The H&E and PAS stain was performed using a Microm DS 50 Slide Stainer (Richard-Allan Scientific). The Brown-Brenn stains as well as all histological analyses were performed by the Department of Pathology at Harvard Vanguard Medical Associates (Boston, MA). This analysis performed while being blinded to the specimen’s study group. Positive histology, indicative of infection, was defined as the presence of acute inflammation and microorganisms on the Brown-Brenn and/or PAS stain.

*G. Bone Ingrowth*

The residual tibia was dehydrated, infiltrated, and embedded in methyl methacrylate using standard procedures (Emmanuel J, Hornbeck C, Bloebaum RD. A polymethyl methacrylate method for large specimens of mineralized bone with implants. Stain Technol. 1987;62(6):401-10). An approximately 4-mra thick transverse section, encompassing the entire implant, was made with a custom, water cooled, high-speed, cut-off saw with a diamond edged blade. The section was ground down using a variable-speed grinding wheel (Buehler Incorporated, Lake Bluff, IL) to approximately 2-mm and polished to an optical finish. The implant was centered in the finished section.
Each section was sputter coated with a conductive layer of gold for 1-min using a Hummer-VI-A sputter unit (Anatech, Alexandria, Virginia). The sections were then examined in a scanning electron microscope (JSM T-330A; JEOL, Peabody, Massachusetts) with the backscattered electron detector (Tetra; Oxford Instruments, Cambridge, United Kingdom).

To measure bone ingrowth, images were taken along the implant in both cortical bone regions. The magnification and number of images varied since the goal was to cover the entire cortical bone region without overlapping images. Images also did not go beyond the implant or beyond the periosteal and endosteal cortical bone boundary (Figure 2). When cortical bone was not present at the bone/implant interface, the imaging boundary was defined by the thickness of the cortical bone most adjacent to the implant (Figure 2). Cancellous and woven bone were also imaged and measured if they were present in the imaging field (Figure 2).

Link ISIS software revision 3.35 (Oxford Instruments pic) was used to determine the percent bone present in the images by measuring the amount of grey material representing bone relative to the low mineral tissue in black and the metal implant in white (Figure 2). To determine reproducibility, measurements were repeated on 45 images. The average difference between the measurements was 0.0015% ± 0.0030% for the investigator performing all ingrowth analyses. Error was also found from the greylevel threshold technique with ISIS software since it counted bone around the perimeter of each implant piece whether or not bone was present. To determine the amount of error, seventeen images were taken between 43x to HOx and analyzed for percent bone even though they were from locations on the implant where bone was not present. The outlines around the implant averaged 2.70% ± 1.18% of the image indicating that bone ingrowth measures could be overestimated by this amount.

H. Statistics

A Kaplan-Meier survivorship curve was used to compare infection rates in each rabbit group. The rabbit was classified as infected if it had at least two of the three infection criteria: 1) greater than Grade I clinical signs of infection, 2) positive culture for one or more of the tissue samples, and/or 3) positive histology indicative of infection for one or more of the tissue samples. Statistical differences between the groups were
tested with a log-rank test for equality of survivor functions. A p-value of ≤ 0.05 was considered statistically significant.

1. Results

(i) Animal Health

The main reason rabbits had to be withdrawn from the study was because of rabbit's skin healing over the implant (Table 2). However, up to five of the eight rabbits in Group 3 may have had the skin heal over the implant after the implant broke at the bone/muscle interface (Figure 3). When an implant broke, the implant did not protrude as far from the lateral side of the tibia. Therefore, swelling and loose skin could more easily slide over the implant and heal. Once an implant was sealed from the outside environment, pin track infection could not occur so the rabbit was removed from the study group and euthanized. Other reasons for removal from the study included one rabbit prematurely pulling out stitches at the surgical site, four animals with fractured tibias, and one animal death due to unknown causes.

Table 2 - Number of rabbits that remained in study for the measurement of pin track infection rates. Abbreviations: Titanium implant (Ti) and Tantalum implant (Ta)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Number in Group</th>
<th>Animals that Remained in Study</th>
<th>Animals Removed from Study</th>
<th>Skin Healing Over Implant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Control (Ti, No antimicrobial)</td>
<td>11</td>
<td>9</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2: (Ti, Pexiganan acetate)</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3: (Ta, No antimicrobial)</td>
<td>18</td>
<td>9</td>
<td><strong>8</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

*Other reasons for a rabbit to be removed from the study included one rabbit prematurely pulling out its stitches at surgical site, four animals with fractured tibias, and one animal death due to unknown causes.

** Five of the eight rabbits had broken implants at the bone/muscle interface in the radiographs taken after necropsy. It is unknown whether the implant broke before or after the skin healed over the implant (Figure 3).
(ii) Infection Rates

Of the rabbits euthanatized due to clinical signs of infection, 85% (17 out of 20) had bacteria cultured, and 30% (6 out of 20) had positive histology indicating infection. Inconclusive histological results occurred in 25% (5 out of 20) of the rabbits. Two rabbits euthanatized due to clinical signs of infection were not classified as infected because of negative culture and histology results. Of the 18 rabbits classified as infected, five (28%) were because of clinical signs, positive cultures, and positive histology, 12 (67%) were because of clinical signs and positive cultures, and one (6%) was because of clinical signs and positive histology.

The first hypothesis was supported since Group 2 receiving pexiganan acetate had a lower pin track infection rate than its Group 1 control (p=0.019, Figure 4). After 24-weeks, Group 2 had a 75% chance of not getting an infection whereas Group 1 had a 0% chance of not getting an infection (Figure 4).

The second hypothesis was not supported since the porous tantalum implant (Group 3) did not prevent infection any better than the non-porous titanium (Group 1) (p = 0.248, Figure 4). The failure of the porous tantalum implants, in preventing infection, may have been due to lack of skin ingrowth. Qualitative observations showed that the skin was mobile at the implant exit site. The skin appeared more mobile around the tantalum implants since more rabbits in Group 3 with tantalum implants were removed from the study due to skin slipping over the implant (Table 2).

(Uii) Bone Ingrowth

When examining the amount of bone ingrowth into the tantalum implant, an average of 20 ± 15% of cortical, cancellous, and/or woven bone was found inside the tantalum pores at an average in situ time of 47 ± 21 days (Table 3). The degree of osseointegration did not appear related to implant time in situ (r²=0.024, p=0.692). Other factors such as implant micromotion may have influenced the variation in amount of bone ingrowth since the rabbits were observed manipulating the implant.
Table 3 - Bone ingrowth analysis per rabbit with porous tantalum implant in Group 3.

<table>
<thead>
<tr>
<th>Group 3 Animal Number</th>
<th>Percent Bone Ingrowth</th>
<th>In Situ Time (days)</th>
<th>Location of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5%</td>
<td>16</td>
<td>S. aureus in muscle</td>
</tr>
<tr>
<td>2</td>
<td>2.8%</td>
<td>70</td>
<td>S. aureus in muscle and blood</td>
</tr>
<tr>
<td>3</td>
<td>3.6%</td>
<td>80</td>
<td>S. aureus in bone</td>
</tr>
<tr>
<td>4</td>
<td>10.0%</td>
<td>34</td>
<td>S. aureus in muscle</td>
</tr>
<tr>
<td>5</td>
<td>28.6%</td>
<td>58</td>
<td>S. aureus in muscle and blood</td>
</tr>
<tr>
<td>6</td>
<td>30.3%</td>
<td>31</td>
<td>E. coli in muscle and blood</td>
</tr>
<tr>
<td>7</td>
<td>31.2%</td>
<td>35</td>
<td>E. coli in muscle, blood, and bone</td>
</tr>
<tr>
<td>8</td>
<td>34.4%</td>
<td>41</td>
<td>S. aureus in muscle</td>
</tr>
<tr>
<td>9</td>
<td>36.2%</td>
<td>58</td>
<td>S. aureus and E. coli in muscle</td>
</tr>
</tbody>
</table>


First, the low sample size and the rabbit’s removal of the pexiganacetate in Group 2 could have made it more difficult to detect a statistical decline in infection rates due to this antimicrobial. However, a statistical reduction was found despite the low sample size and possibly lower than ideal exposure to pexiganan acetate. Nevertheless, we attempted address this limitation. Three pilot studies (each n=5) were performed to test ideas for increasing pexiganan acetate’s exposure to the implant exit site. Two of these pilot studies used two different plastic cap designs to prevent the rabbit from orally removing the antimicrobial but resulted in the rabbits using the caps as leverage to bend or break the implants. Another pilot study used Elizabethan collars, but rabbits were able to remove the collars and sometimes able get its mouth to the antimicrobial while still wearing the collar. Therefore, different implantation sites and/or different animals with fewer tendencies to manipulate the implant site should be considered in future studies testing topical antimicrobials.

The second limitation was the high drop out rate of the porous tantalum implants in Group 3 due to breaking and/or having the skin heal over the implant resulting. The low strength of porous tantalum in bending may have contributed to its breaking. Porous tantalum’s ultimate bending strength has been reported as $110 \pm 14 \text{ MPa}$ for a disk $2.5 \times 4.0 \times 35$-mm (Zardiackas LD, Parsell DE, Dillon LD, Mitchell DW, Nunnery LA, Poggie R. Structure, metallurgy, and mechanical properties of a porous tantalum foam. J Biomed Mater Res. 2001;58(2):180-7). The implant was not made stronger by increasing its diameter from $2.7$-mm since a larger implant could have further weakened the tibia and led to more tibia fractures. However, the remaining n=9 in Group 3 (porous tantalum implants) was able to demonstrate that porous tantalum in this animal model could not prevent pin track infection; therefore, a larger sample size in this study was not needed.
The ability of pexiganan acetate to reduce infection was consistent with previous studies. In vitro, pexiganan acetate has been shown to kill gram-positive and gram-negative anaerobic and aerobic bacteria. In vivo, pexiganan incorporated into collagen matrices reduced *P. aeruginosa* and *S. aureus* growth in infected wounds on rats. The current study supported pexiganan acetate's effectiveness against gram-positive and gram-negative bacteria since *S. aureus* and *E. coli* were found to be the infecting organisms in the rabbits with pin track infections. Interestingly, the *S. aureus* infecting the rabbits did not appear to be from the *S. aureus* strain applied exogenously but from *S. aureus* native to the rabbits. Since the implications of this finding exceed the purpose of this study, it was addressed in a separate publication (Williams DL, Bloebaum RD, Petti CA. Characterization of *Staphylococcus* aureus strains in a rabbit model of osseointegrated pin infections. *J Biomed Mater Res Part A*. 2007 Aug 9;In Press).

The bone ingrowth into porous tantalum appeared equivalent to other porous materials (Willie B, Bloebaum R, Bireley W, Bachus K, Hofmann A. Determining relevance of a weight-bearing ovine model for bone ingrowth assessment. *J Biomed Mater Res Part A*. 2004 June;69A(3):567-76; Bloebaum RD, Mihalopoulus NL, Jensen JW, Dorr LD. Postmortem analysis of bone growth into porous-coated acetabular components. *J Bone Joint Surg [Am]*. 1997;79-A(7): 1013-22). Soft tissue ingrowth was not quantified because the soft tissue/implant interface appeared disturbed by the technical process of removing tissues around the implant for culturing and histology and the process of embedment. However, observations of the implant exit site showed that the skin was mobile around the implant. One result of skin mobility, in some cases, was the skin healing over the implant. To reduce skin mobility by increasing dermal attachment to the implant, Pendergrass et al. (Pendergrass CJ, Goodship AE, Blunn GW. Development of a soft tissue seal around bone-anchored transcutaneous amputation prostheses. *Biomaterials*. 2006 Aug;27(23):4183-91; Pendergrass CJ, Goodship AE, Price JS, Blunn GW. Nature's answer to breaching the skin barrier: an innovative development for amputees. *J Anat*. 2006 Jul;209(1):59-67) tested an implant with a porous flange that was placed just under the epithelium. This strategy statistically reduced epithelial downgrowth and enhanced the collagenous tissue-implant interface. While the results indicated that a porous flange may reduce interfacial movement at the skin/implant interface, it is not yet known if the flange technique can reduce pin track
In conclusion, pexiganan acetate is an important antimicrobial for transcutaneous osseointegrated implants since it helped to prevent organisms from traversing the skin barrier into muscle, blood, and bone. Porous tantalum, as a coating on an osseointegrated implant, will not likely prevent pin track infection without additional methods of soft tissue immobilization around the implant site.
CLAIMS

1. A method of preventing wound infection comprising topically administering a therapeutic formulation comprising cationic peptide to a wound site.

2. The method of claim 1 wherein the cationic peptide is a magainin.

3. The method of claim 2 wherein the magainin peptide is pexiganan.

4. The method of claim 3 wherein the pexiganan comprises the peptide of SEQ ID NO:1.

5. The process of claim 2 wherein said magainin peptide is:

\[(\text{NH}_2)\text{GIGKFLHSAKKFGKAFVGEIMNS(OH)}.\]

6. The process of claim 2 wherein the peptide is:

\[(\text{NH}_2)\text{GIGKFLHSAKKFGKAFVGEIMNS(NH}_2).\]

7. The method of claim 1 wherein the peptide has the amino acid sequence of SEQ ID NO:1.

8. The process of claim 1 wherein the peptide is administered topically.

9. The method of claim 1 wherein therapeutic formulation is a cream.

10. The method claim 9 wherein the cream is applied topically to the wound site.

11. The method of claim 9 wherein the therapeutic formulation comprises approximately 1% pexiganan acetate.

12. The method of claim 9 comprising applying the therapeutic formulation to the wound site once a day.
13. A process for preventing wound infection in a host, comprising administering to a host having a wound at least one biologically active amphiphilic peptide and/or biologically active protein, said peptide or protein being an ion channel-forming peptide or protein, said at least one biologically active amphiphilic peptide or protein being administered in an amount effective for preventing wound infection in the host.

14. The process of claim 13 wherein the peptide is a basic polypeptide having at least sixteen amino acids, wherein said basic polypeptide includes at least eight hydrophobic amino acids and at least eight hydrophilic amino acids.

15. The process of claim 13 wherein the amphiphilic peptide is cationic.

16. The process of claim 13 wherein the peptide is a magainin peptide.

17. The process of claim 16 wherein said magainin peptide includes the following basic peptide structure:

\[-R_\pi -R_n -R_{i2} -R_{i3} -R_n -R_{12} -R_n -R_{13} -R_\pi -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} \]

wherein $R_n$ is a hydrophobic amino acid, $R_{i2}$ is a basic hydrophilic amino acid, $R_{13}$ is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid, $R_{i4}$ and $R_{i4a}$ are hydrophobic or basic hydrophilic amino acids, $R_{i5}$ is glutamic acid or aspartic acid, a hydrophobic amino acid, or a basic hydrophilic amino acid, and $n$ is 0 or 1.

18. The process of claim 16 wherein said magainin peptide is of the following basic peptide structure:

\[-R_{14} -R_n -R_{i4a} -R_{12} -R_n -R_{12} -R_\pi -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} -R_n -R_{12} -R_{i4} \]

wherein $R_n$ is a hydrophobic amino acid, $R_{i2}$ is a basic hydrophilic amino acid, $R_{i3}$ is a hydrophobic, neutral hydrophilic, or basic hydrophilic amino acid, and $R_{i4}$ and $R_{j1a}$ are hydrophobic or basic hydrophilic amino acids.

19. The process of claim 13 wherein the wound is a transcutaneous wound.
20. The process of claim 19 wherein the transcutaneous wound is the site of a medical implant.

21. The process of claim 13 wherein the wound is the site of implantation of an osteointegrated implant.

22. The process of claim 13 wherein the peptide is:
   (SEQ ID NO:1).

23. The process of claim 13 wherein the peptide is:
   Gly-Ile-Gly-Lys-Phe-Leu-Lys-Ala-Lys-Lys-Phc-Gly-Lys-Ala-Phe-Val-Lys-