Supplementary Material: Antimicrobial Peptides for Topical Treatment of Osteomyelitis and Prevention of Implant-Related Infections: Study in the Spongy Bone

Figure S1. Effect of peptide V on the eradication of infection inside the bone sample compared to gentamicin

*Staphylococcus epidermidis* (clinical isolate from the University Hospital in Motol)

Peptide V vs. gentamicin loaded in ChronOS calcium phosphate cement

a) ChronOS without peptide

b) ChronOS with peptide

c) ChronOS with gentamicin
Figure S2. Effect of the mixture of peptides I and II on the eradication of infection inside the bone sample compared to vancomycin

*Staphylococcus aureus* MRSA 6271

Peptide I + peptide II (4:1) vs. vancomycin loaded in ChronOS calcium phosphate cement

a) ChronOS without peptides

b) ChronOS with peptides

c) ChronOS with vancomycin
**Figure S3.** Effect of the mixture of peptides I and II on the eradication of infection inside the bone sample compared to vancomycin.

*Staphylococcus aureus* MRSA (clinical isolate from Regional Hospital in Liberec)

Peptide I + peptide II (4 : 1) vs. vancomycin loaded in ChronOS calcium phosphate cement

a) ChronOS without peptide

b) ChronOS with peptide

c) ChronOS with vancomycin
Figure S4. Peptide I prevents *P. aeruginosa* biofilm formation on Palacos®

*P. aeruginosa* penetrated from the hole (arrow) towards implants made from Palacos®

1. Palacos® without peptide

2. Palacos® without peptide

3. Palacos® with peptide I

4. Palacos® with peptide I
Figure S5. Identification of human defensins in bone marrow tissue

The sample of bone marrow tissue taken from femur head was extracted with acetonitrile-water (1:1) mixture containing 1% trifluoroacetic acid (TFA) and then ultrafiltered using Amicon centrifugal filter units 0.5 mL with a 50-kDa molecular weight cut-off membrane. The filtrate was lyophilized and then fractioned by RP-HPLC. The material of the peak eluted at 25.4 min was analyzed by mass spectrometry (Fig. S5b).

a) RP-HPLC profile of bone marrow extract at 220 nm.
An elution gradient of solvents from 5% to 70% acetonitrile-water-0.1% TFA was applied for 60 min at a flow rate 1 mL/min. Arrow indicates the peak containing the mixture of three human defensins (HNP1, HNP2, and HNP3).

b) ESI-MS spectrum of the compounds corresponding to the peak of ts 25.4 min (Fig S-5)

\[ \text{MR} = 3368.4 \quad \text{CYCRIPACIAGERRYGTYQGRLWAFCC} \] (HNP2)
\[ \text{MR} = 3439.5 \quad \text{ACYCRIPACIAGERRYGTYQGRLWAFCC} \] (HNP1)
\[ \text{MR} = 3483.1 \quad \text{DCYCRIPACIAGERRYGTYQGRLWAFCC} \] (HNP3)