INTRODUCTION Polyethyl methacrylate (PMMA) beads containing gentamicin are still an effective treatment for osteomyelitis. However, the emergence of anti-microbial resistance in general and the increasing prevalence of MRSA in nosocomial infections indicate the need for new antibiotics. Anti-microbial peptides (AMP) are a promising class of antibiotics and have a low tendency to induce anti-microbial resistance.

We previously investigated the release of Dhvar-5, a synthetic anti-microbial peptide with in vitro bactericidal activity against MRSA, from PMMA mini beads in vitro. This study showed that the release pattern of Dhvar-5 resembles that of gentamicin in low quantities but increases exponentially with the added amount of peptide. Furthermore, the addition of peptide also causes an increase in the rate of gentamicin. Known factors that influence antibiotic release from PMMA are porosity and connectivity density (CD; number of interconnected voxels per mm³). Consequently, the addition of antibiotics to bone cement influences its mechanical properties.

In this study we investigated the porosity, CD and compressive strength of PMMA cement mixed with Dhvar-5 and or gentamicin.

METHODS The minibeads were prepared as previously reported. In short, different amounts of Dhvar-5 and or gentamicin were mixed with cement powder (Osteopal®, Biomet Merck, Darmstadt, Germany), liquid monomer was added and after applying a slight vacuum in a syringe, the paste was injected into a custom made mould. The beads were loaded with: 50 mg/g cement powder Dhvar-5 (D50), 100 mg/g Dhvar-5 (D100), 25 mg/g gentamicin (Osteopal®; G 25), and both 50 mg/g dhvar-5 and 25 mg/g gentamicin (G 25 + D 50). Further, empty beads and Septopal® beads were tested. The latter is a commercially available bead for the treatment of osteomyelitis containing 1.7 mg gentamicin bead. These beads were examined after 28 days of release with a scanning electron microscope (SEM; XL 20, Philips) and with a micro computed tomography scanner (µ-CT; µCT-20, Scanco Medical, Bassersdorf, Switzerland) at 12 µm voxel resolution with an integration time of 150 ms. Volumes of interest of 1 mm³ were selected in each slice image and thresholded with a gaussian filter. The threshold was selected with the adaptive threshold function of image processing language (IPL). These slice images were compiled, inverted and analysed with IPL to render 3D images and obtain quantitative architectural parameters (porosity and CD). Additionally, the compressive strength of empty, G25, D50 and D100 mixtures was tested in accordance with ISO standard 5833 on an Instron 8872 universal testing machine.

RESULTS The SEM micrographs showed an increase in the porosity of the cement mixtures containing antibiotics compared to the plain cement. The gentamicin containing cement mainly showed an increase in the number of micro-pores (≤ 0.1 mm) and the dhvar-5 containing cement an increase in the number of very small pores (≤ 0.1 mm) (Figure 1). The number of very small pores increased with the addition of more Dhvar-5.

The porosity value of plain PMMA cement was 6.4% (Figure 2a). The porosity of the D50 group was not significantly increased, in contrast with the D100 (16.2%) and the G 25 (21.6%) groups who both had an increased porosity. Adding both gentamicin and Dhvar-5 did not further increase the porosity. The Septopal® bead was the most porous bead tested (27%). The CD values of both empty and D50 beads were very low, however the G 25 and D100 beads had a higher CD value, of 521 and 270 respectively (Figure 2b).

DISCUSSION The results of the compressive strength testing are shown in figure 3. The empty PMMA cement was the strongest tested cement (93 MPa). The G 25 and D50 mixtures both had a significantly decreased yield strength (84 MPa and 87 MPa respectively) and the D100 mixture had the lowest yield strength (77 MPa).

The increased porosity in combination with sufficient compressive strength provides a basis for further development of anti-microbial peptide containing bone cements.

REFERENCES

AFFILIATED INSTITUTIONS
** Department of Oral Cell Biology, ACTA-VU, Amsterdam
*** Department of Clinical Physics and Informatics, VUmc, Amsterdam, The Netherlands.