Novel Silicone Composite for Prolonged Release of Antimicrobial Peptides

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ABSTRACT SUMMARY
A novel approach for constructing a bacteria-resistant polymer surface was developed. The methodology involves formation of a silicone/hydrogel composite starting from an existing silicone network and subsequent loading of the hydrogel part of the matrix with an antimicrobial agent. This is likely to allow for a prolonged and controlled release of the antibiotic at the surface of the material.

INTRODUCTION
Colonization by bacteria is a frequently occurring problem with polymer materials used in medical devices. Several approaches have been utilized as an attempt to inhibit or prevent bacterial growth on such polymer surfaces. Antimicrobial peptides are a promising new class of potential antibiotics that cause no cross-resistance with conventional antibiotics. However, because of the inherent problems with systemic administration of peptide drugs their clinical use has been highly limited. Through topical application, many of the issues associated with labile drugs are circumvented thus presenting an interesting new avenue for the utilization of such compounds.

In this work, the broad spectrum antimicrobial peptide novicidin was employed in a novel silicone/hydrogel composite. A hydrogel network is formed inside an existing silicone network. This hydrogel is subsequently loaded with the peptide that is slowly released during the use of the material.

EXPERIMENTAL METHODS
In a typical experiment, a 16 mL custom made stainless steel high-pressure reactor was loaded with 60 µL EGDMA, 2.0 mL HEMA, 4.0 mL ethanol and 10 silicone discs. The discs (Ø 10 mm) were stamped out from a 2 mm silicone sheet. The reactor was closed and pressurized with CO2 to approximately 250 bar at 75 °C. After 2 h of impregnation, 500 µL 0.2 M DEPDC in hexane and CO2 were added to the reactor to ensure a polymerization pressure of approximately 300 bars. After two hours of polymerization, the reactor was allowed to cool to room temperature before the pressure was slowly decreased to ambient pressure. The samples were cleaned in ethanol and dried at 75 °C.

N-terminal carboxyfluorescein-tagged novicidin (CF-KNLRRIRKGIHIKKYF-NH2) was synthesized by Fmoc solid-phase peptide synthesis and purified by reverse phase HPLC.

High pressure impregnation was performed by mixing the peptide solution, co-solvent and samples in custom made 2.65 mL stainless steel high-pressure reactors. The reactors were pressurized with CO2 to 300 bars at 25 °C. After 24 h, the pressure was slowly decreased to ambient pressure.

The distribution of peptide in the Z-plane of the disc was visualized using a Zeiss LSM 510 CLSM with a 20X/0.75 air objective and an Argon laser at 488 nm. Emission was recorded between 505 and 530 nm. During acquisition, each line was scanned 8 times and averaged to reduce noise. The pixel intensity in the generated images was analyzed using ImageJ.
RESULTS AND DISCUSSION

The defining property of hydrogels is their ability to take up water, which is usually characterized by the equilibrium water content (EWC). This was determined by fitting water uptake data (Figure 1) to one-site saturation kinetics. The calculated EWC is $8.5 \pm 0.1$% of total material weight for samples containing $19.5 \pm 0.5$% hydrogel. When normalized to the HEMA content, this value is similar to what has previously been reported for HEMA hydrogels with similar degrees of crosslinking. The result indicates that the entire hydrogel network is surface-connected as water cannot penetrate the silicone. The water uptake kinetics, however, are markedly slower than previously reported for pure hydrogels.

![Figure 1. Water content in percentage (%WC) plotted against time normalized to the starting mass. Each point is an average of 7 samples ± SD. Insert shows first day.](image)

Typically, drug loading in hydrogels is performed by soaking the material in a solution containing the drug. However, the very slow rate of water uptake raised the need for an alternative way to facilitate uptake of peptide into the material, since the diffusion of peptide is limited by the water uptake, and potentially is significantly slower. Thus, the traditional soaking approach was considered unfeasible.

Instead, to increase the rate of peptide uptake, the influence of high pressure was investigated. The uptake after 24 h at a pressure of 300 bars showed a deep penetration of the labeled peptide into the hydrogel network (Figure 2), whereas similar experiments performed at ambient pressure showed peptide diffusion lengths of less than 50 µm.

![Figure 2. A) Penetration depth of CF-novicidin in silicone composite. B) Intensity of fluorescent dye as a function of penetration depth. Averaged over 20 µm along the arrow shown in slide A.](image)

It is seen that the tagged peptide has penetrated several hundred µm into the material after 24 h. It may be argued that addition of the fluorophore to the peptide changes the physicochemical properties of the peptide, which may influence the uptake in the material. However, as carboxyfluorescein is hydrophobic and bulky compared to the peptide, it is assumed that the addition of the tag will decrease the rate of uptake or cause increased adsorption to the matrix and thus lowering the rate of uptake.

CONCLUSION

In conclusion, an interconnected hydrogel was synthesized inside an existing silicone material. Uptake of the antimicrobial peptide, novicidin, was demonstrated, and it was shown that increased pressure has a positive effect on peptide uptake; however, the mechanism underlying this needs to be investigated further.

REFERENCES


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