SUMMARY

Due to the increased resistance of bacteria against traditional antibiotics, antimicrobial peptides (AMPs) are attracting research interest as alternative therapeutics. However, the delivery of AMPs is challenging due to their local and systemic toxicity at high therapeutic doses, as well as to susceptibility towards both proteolytic and chemical degradation. In addition, AMPs are large molecules, generally carrying a net positive charge and containing a substantial fraction of hydrophobic amino acids. As a result of this, AMPs bind extensively to anionic serum proteins, glycoproteins, and other tissue components, precluding efficient in vivo delivery of AMPs at therapeutically relevant concentrations. In relation to this, various delivery systems have been identified to provide advantages, one of these being microgels. Microgels are cross-linked polymeric networks which may display swelling responsiveness to a broad range of stimuli. Besides providing protectiveness against enzymatic/physical degradation, microgels may also allow the triggered exposure and release of encapsulated drug. The kinetics of drug release depends on the structure of the microgel particles, and the latter on properties of peptide, the microgel, and ambient conditions. For a rational design of AMP-loaded microgels, it is therefore important to understand how such factors affect peptide loading and release, interactions of such particles with lipid membranes, and how the latter translate into antimicrobial effects and cell toxicity. In addition, as commonly employed approaches for microgel preparation may be time-consuming or unsuitable for upscaling, a key aspect for the development of microgels as AMP delivery systems concerns identification of alternative approaches for such aim. Considering this, we here investigated the use of three-dimensional (3D) printed micromixers for continuous generation of peptide-loaded microgels. For this purpose, we initially investigated how the hydrodynamic flow conditions in micromixers of different designs affected the properties of alginate microgels, loaded with the AMP polymyxin B and cross-linked by Ca$^{2+}$, while varying components concentration. The effect of pH and resulting charge contrast between polymer and AMP on particle formation was addressed in a subsequent study, as was the destabilizing effects of the resulting microgels against bacteria-mimicking lipid membranes. The structural evolution of peptide-loaded microgels upon a change in ionic strength was also investigated on a system composed of hyaluronic acid and the AMP polymyxin E. From such studies, microgel structure was found to depend on the flow patterns of the employed micromixer, as well as on composition and charge contrast between the components. Despite the structural differences, however, the peptides were found to localize in the interior of the particles with high encapsulation efficiencies for all investigated micromixers,
charge contrasts, and microgel component concentrations investigated. Increasing ionic strength resulted on microgel deswelling accompanied by peptide release. As a consequence of the latter, similar destabilization effects on lipid membranes were observed, regardless of composition or conditions used on microgel preparation. These findings demonstrate 3D-printed micromixers provide good opportunities for the continuous generation of peptide-loaded microgels with refined control of their properties, and mixing at widely different compositions and conditions results in microgel particles of similar properties from a functional perspective.