Abstract

Droplet-Based Technology for Studying the Phenotypic Effect of Microplastics on Antimicrobial Resistance †

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Keywords: antimicrobial resistance; microplastic pollution; droplet microfluidics; droplet-based technology; biofilm formation; plastisphere; phenotypic analysis; single cell incubation; Fluorescence microscopy

Plastic pollution is a global emergency [1,2]. One key problem is that microplastics (MPs) (1 µm–5 mm) [3] and nanoplastics (NPs) (<1 µm) [4] enhance the already severe threat of antimicrobial resistance (AMR) by providing a micro-environment termed the “plastisphere” for bacteria to form biofilms [5,6]. However, exact knowledge of the severity of the plastisphere and its impact on AMR is currently still scarce [7]. Here, we show how droplet-based technology can be used to study the potential phenotypic effect of MPs on AMR. For this we used (i) polydisperse water-in-oil droplets generated via vortexing, (ii) GFB-labelled Escherichia coli JEK 1036 as our study object, (iii) cefotaxime as the test antibiotic, and (iv) 10 µm carboxylated polystyrene microspheres (PS). In parallel, we encapsulated single cells of E. coli into droplets with different concentrations of cefotaxime and with or without PS. After overnight incubation at 37 °C, we imaged droplets as a monolayer via confocal microscopy and analyzed droplets via Software Ilastik [8], CellProfiler™ [9] and EasyFlow [10]. Our results show that E. coli’s minimal inhibitory concentration (MIC) shifts slightly towards a higher cefotaxime concentration when PS is present in droplets. Image analysis of E. coli growth patterns in individual droplets illustrates that E. coli tends to clump together in droplets with PS, versus exhibiting an evenly distributed growth pattern in droplets without PS. In conclusion, we see that PS in droplets might enhance the MIC of E. coli resistance against cefotaxime. This possible enhanced resistance may be related to the observed tendency for clumping (indication of biofilm formation) of E. coli when PS is present. Droplet-based technology is thus a suitable tool for studying the phenotypic effect of MPs on AMR. Further experiments with different antibiotics and MP types and sizes will shed more light on the interesting and worrying tendency of MPs to potentially enhance AMR that was found in this study.

Author Contributions: Conceptualization, S.B. and O.S.; methodology, S.B., F.L.S. and P.P.; software, S.B. and I.S.; validation, S.B. and F.L.S.; formal analysis, S.B., F.L.S. and O.S.; investigation, S.B. and O.S.; resources, O.S.; data curation, S.B. and I.S.; writing—original draft preparation, S.B.; writing—review and editing, S.B. and O.S.; visualization, S.B., F.L.S. and I.S.; supervision, S.B. and O.S.; project administration, O.S.; funding acquisition, S.B. and O.S. All authors have read and agreed to the published version of the manuscript.

Funding: The project was partially funded by Tallinn University of Technology Development Program 2016–2022, project no. 2014–2020.4.01.16.0032; Tallinn University of Technology, grant no. GFLKSB22; Estonian Research Council, grant no. PRG620. The conference participation was funded by COST Action: CA20101 (PRIORITY).
Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We would like to thank Margit Heinlaan, National Institute of Chemical Physics and Biophysics, Tallinn, for kindly providing us with carboxylated polystyrene microspheres used in this work. We would also like to thank Piotr Garstecki at the Institute of Physical Chemistry, Polish Academy of Sciences, Poland, for the kind provision of surfactant and microfluidic chip mold used in laboratory.

Conflicts of Interest: The authors declare no conflict of interest.

References

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