Reproducible biofilm cultivation of chemostat-grown *Escherichia coli* and investigation of bacterial adhesion on biomaterials using a non-constant-depth film fermenter.

Lüdecke C, Jandt KD, Siegismund D, Kujau MJ, Zang E, Rettenmayr M, Bossert J, Roth M (2014) Reproducible biofilm cultivation of chemostat-grown *Escherichia coli* and investigation of bacterial adhesion on biomaterials using a non-constant-depth film fermenter. *PLOS One* 9(1), e84837.

Abstract

Biomaterials-associated infections are primarily initiated by the adhesion of microorganisms on the biomaterial surfaces and subsequent biofilm formation. Understanding the fundamental microbial adhesion mechanisms and biofilm development is crucial for developing strategies to prevent such infections. Suitable in vitro systems for biofilm cultivation and bacterial adhesion at controllable, constant and reproducible conditions are indispensable. This study aimed (i) to modify the previously described constant-depth film fermenter for the reproducible cultivation of biofilms at

non-depth-restricted, constant and low shear conditions and (ii) to use this system to elucidate bacterial adhesion kinetics on different biomaterials, focusing on biomaterials surface nanoroughness and hydrophobicity. Chemostat-grown Escherichia coli were used for biofilm cultivation on titanium oxide and investigating bacterial adhesion over time on titanium oxide, poly(styrene), poly(tetrafluoroethylene) and glass. Using chemostat-grown microbial cells (single-species continuous culture) minimized variations between the biofilms cultivated during different experimental runs. Bacterial adhesion on biomaterials comprised an initial lag-phase I followed by a fast adhesion phase II and a phase of saturation III. With increasing biomaterials surface nanoroughness and increasing hydrophobicity, adhesion rates increased during phases I and II. The influence of materials surface hydrophobicity seemed to exceed that of nanoroughness during the lag-phase I, whereas it was vice versa during adhesion phase II. This study introduces the non-constant-depth film fermenter in combination with a chemostat culture to allow for a controlled approach to reproducibly cultivate biofilms and to investigate bacterial adhesion kinetics at constant and low shear conditions. The findings will support developing and adequate testing of biomaterials surface modifications eventually preventing biomaterial-associated infections.

Involved units

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