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Direct surface visualization of biofilms with high spin coordination clusters using Magnetic Resonance Imaging

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ABSTRACT

Magnetic Resonance Imaging is a powerful tool for the investigation of a biofilms' physical structure determining mass transport behavior which is of major importance in biofilm research. The entire biofilm is imaged *in situ* non-invasively and non-destructively on a meso-scale. In this study, different contrast agents were applied to study the biofilm's properties with the focus on mass transport, which is achieved by varying the contrast agents with respect to their NMR and interaction properties. The spatio-temporal tracking of these *cluster*, *molecular* and *particulate* contrast agents in biofilms was achieved by T_1-, T_2-weighted and proton density images during short (20 h) and long (14 d) term exposures. The best biofilm surface visualization was observed when applying a new high spin coordination cluster ($Fe_{10}Gd_{10}$) showing a high affinity to the biofilm's surface and a fast immobilization within minutes. Contrarily, the small molecular contrast agents show no immobilization and fully penetrated into the biofilm. A concentration equilibrium was observed which was confirmed in back diffusion experiments. Interactions between larger nanoparticulate contrast agents and the biofilm required hours to achieve immobilization. Thus, the penetration depth into the biofilm is predominantly size-dependent. Here, it is shown that biofilm surface interactions can be observed *in situ* and spatio-temporarily resolved. The reported methodology demonstrates a new means to explore mass transfer of various substances in biofilms.

Statement of significance

In biofilm research, the investigation of the biofilms' physical structure is of high relevance for the understanding of mass transport processes. However, commonly used imaging techniques for biofilm imaging such as CLSM or electron microscopy rarely visualize the *real* biofilm due to their invasiveness and destructiveness. Magnetic Resonance Imaging (MRI) represents the ideal tool to image the biofilm *in situ*, *non-invasively* and *non-destructively* with a spatial resolution of several 10 μm. To gain specific structural and functional information, a variety of MRI contrast agents (molecular and particulate) was applied with different properties for the first time. Results elucidate the interactions between the biofilms' surface and the contrast agents and open a new field for biotechnological applications by functional contrast enhancement.

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1. Introduction

The impact of physical structure, composition and morphology of living biofilms on mass transport in technical, medical and environmental systems is of major importance with respect to local convective and diffusive transport of substrates on and within biofilms [1]. The dynamic interplay between diffusion [2] and advection [3] directly correlates with substrate conversion in biofilm systems. Different imaging techniques, such as confocal laser scanning microscopy (CLSM), optical coherence tomography (OCT) and Magnetic Resonance Imaging (MRI), have significantly contributed to the understanding of biofilm structure and functionality [4–9]. Specifically, CLSM techniques offer the possibility to investigate the biofilm structure as well as mass transfer, however, specific staining agents are necessary and only a selective (probe depended) visualization is possible with limited depth resolution in the opaque biofilms and other geometric restrictions [10,11]. Commonly applied techniques to investigate the mass transport into biofilms, such as micro electrodes (limited to certain substances e.g. pH, O_2, NO_3^-) [12,13] are invasive and destructive, influencing the biofilm's structure and consequently the mass transfer.

^{1}H MRI has proved to be particulary suited for non-destructive and *in situ* biofilm investigations and has a spatial resolution of a few tenth of μm [7,8] with the advantage that various parameters beyond the ^{1}H spin density of the NMR experiment can be exploited to generate contrast in the images. For example, longitudinal (T_1) and transverse relaxation (T_2) times of ^{1}H nuclei can be exploited to tailor image contrast in order to characterize biofilm structure and functionality. Thus, whereas pure water exhibits relatively long T_2 relaxation times in the range of seconds, T_2 of water inside biofilms is reduced to values about 100 ms [14,15]. There are also different characteristics for T_1 and the effective diffusion coefficient. An additional factor pertinent to the contrast of the image is the paramagnetic relaxation enhancement (PRE) provided by relaxation agents [16,17]. PRE results from the hyperfine relaxation phenomena introduced through the presence of paramagnetic centers, such as iron ions (Fe^{3+} and Fe^{2+}) [18] or lanthanide ions [19–23]. To date, it has been demonstrated that MRI contrast agents enhance the contrast between bulk water and biofilm [24–26] as shown in the investigation of the spatial distribution and adsorption of heavy metal ions in alginate [27]. The high spin cluster Fe_{10}-Gd_{10} was shown to exhibit large positive and negative contrasts due to longitudinal and transverse relaxivities, which was proven in a first example on a biofilm [26]. Of particular environmental interest is the study of heavy metals, which can be tracked using level of penetration of contrast agents containing metal ions into biofilms. This is assessed through the study of their transport and immobilization in the biofilm by constructing concentration maps based on T_1 and T_2 values in homogeneous and monocultured biofilms using in 2D and 3D studies [8,28–31]. Most of the mentioned MRI studies focus on these artificial and homogenous mono-culture biofilms to trace the fate of specific paramagnetic compounds. To the author's knowledge data of a set of contrast agents for studies on real biofilms is available with the aim to gain a realistic insight into mass transfer as well as deposition of differently shaped, sized and interacting moieties with real biofilm systems.

Here, we report on the interactions of different MRI contrast agents within heterogeneous, heterotrophic biofilm systems. Comparison of the image contrast reveals the relative distributions of (1) molecular contrast agents, (2) paramagnetic coordination clusters and (3) super paramagnetic iron oxide nanoparticles (SPION), and insights into mass transport processes and adsorption can be gained. The main objective here was firstly the exploration and optimization of the image contrast created by different contrast agents in biofilms. A secondary objective was to use contrast agents to gain insights in terms of the biofilm structure. Finally, investigations of the interactions and reversibility of the attachment of contrast agents in real biofilm systems was assessed.

2. Materials and methods

2.1. Biofilm cultivation

The study focusses on heterotrophic biofilms involved in water treatment systems and their interaction with nanoparticles of different size and interaction properties. Therefore, a laboratory scale moving bed biofilm reactor (MBBR) (volume V = 1 L) was operated over 90 days with a plastic carrier material, type K1 (AnoxKaldnes AB, Sweden). The MBBR was inoculated using activated sludge from a local wastewater treatment plant. This K1 carrier (polyethylene, length: 9.2 mm, height: 7 mm) is cylindrical, has 4 combs and a specific surface area of 500 m^2/m^3 (Fig. 1(I)) and has the advantage of being compact, easy to handle as well as characteristic for water treatment systems. The MBBR was continuously fed with acetate (1200 mg/L) as main substrate to grow heterogeneous, mixed culture biofilms on the carrier material. The reactor was continuously aerated with pressurized air to guarantee mixing and sufficient oxygen supply. The cultivated K1 carriers were stored in tap water at 4 °C before the experiment to minimize biological activity. No substrate was added; consequently no biological growth is expected and was also not observed in visual inspections.

2.2. Contrast agents: preparation and characterization

MRI contrast agents with different size, surface functionalization and paramagnetic centers were applied (Table 1). The molecular contrast agents Hydroxy-Tempo (Sigma–Aldrich, St. Louis, USA) and Gadovist (Gd-DO3A-butrol6, Bayer Healthcare,

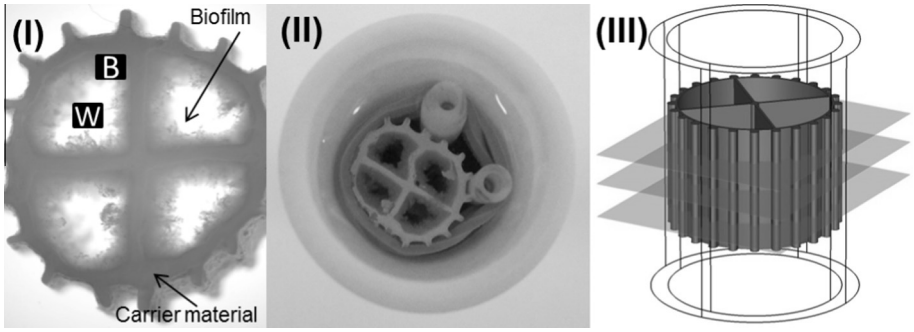

Fig. 1. Biofilm on carrier material type K1 (AnoxKaldness. Sweden). (I) Carrier material with biofilm. Black indications depict regions of interest defined as biofilm (B) and bulk water (W) for image processing. (II) The biofilm-carrier-system (consisting of biofilm, carrier material and surrounding bulk water) is fixed with a rubber band in the MRI sample holder. (III) A model of the system: The horizontal layers indicate the orientation of the measured axial slices (here exemplary for 3 out of 8).