FLUORESCENCE MICROSCOPY AS A TOOL TO STUDY THE TRANSPORT OF MOLECULES AND NANOPARTICLES IN BACTERIAL BIOFILMS AND CYSTIC FIBROSIS SPUTUM

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Today, it is known that the number of bacteria associated in biofilms greatly outnumbers planktonic bacteria in both natural and pathogenic environments. Biofilms are highly structured communities of bacteria embedded in a protective extracellular matrix consisting primarily of polysaccharides, proteins and nucleic acids [1]. Biofilm-based infections are very persistent since biofilms can evade the immune system and show increased antibiotic resistance compared to their planktonic counterparts [2]. Rapid decline in lung function and increased mortality was seen in cystic fibrosis (CF) patients infected with bacteria belonging to the Burkholderia cepacia complex [3]. Antibiotic treatment fails to clear these infections, a possible cause being limited diffusion of the antibiotic into the biofilms. To investigate if this diffusion problem exists and to develop a suitable nanocarrier for improved delivery of antibiotics into the biofilm, the transport of fluorescent molecules and nanoparticles is studied in both Burkholderia multivorans (LMG 18825) biofilms and CF sputum using various microscopy techniques. To probe the diffusion problem, FRAP measurements of fluorescent FITC-dextrans (MW 10000) are conducted into various areas of the biofilm using a newly developed FRAP method that makes use of the full spatio-temporal information [4]. Via single particle tracking, diffusion of fluorescent polystyrene nanospheres of different sizes and surface charges is investigated in CF sputum and biofilms. Confocal microscopy is used to get detailed information on the interaction of the nanospheres with the biofilms.

Figure 1: Biofilm development [1]