Establishment of a flow-chamber system for the investigation of biofilm formation on implant surfaces

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Abstract The aim of this study was the development and evaluation of a flow-chamber system for the investigation of innovative implant materials for their antibacterial properties
Keywords: Flow chamber system, Biofilm formation, Bacterial infection, Implant surface

1 Introduction

Within the last decades, implant systems have become more and more sophisticated, and have thus improved tremendously the patient's quality of life. However, bacterial infections are still one of the main causes for implant failure. These inflammatory processes are caused by bacterial colonization of the implant surfaces and subsequent formation of sessile microbial communities, called biofilms. Biofilm-inhabiting bacteria are embedded in an extracellular polymeric matrix that shields from antimicrobials and greatly reduces the success rate of therapeutic interventions. Consequently, there is a great demand for new implant materials that abolish initial microbial attachment and thus protect from biofilm formation. However, appropriate in vitro biofilm models for the assessment of these implant surfaces are missing. Therefore, the aim of this study was the development and evaluation of a flow-chamber system for the investigation of innovative implant materials for their antibacterial properties

2 Material and Methods

The biofilm formation of Streptococcus oralis ATCC 9811, Porphyromonas gingivalis DSM 20709, Staphylococcus aureus DSM 20231 and Streptococcus gordonii DSM 20568 was assessed in a flow chamber system. Biofilms were grown on 12 mm titanium discs (grade 4) that underwent surface treatment with a 45µm diamond abrasives grinding disc to generate a uniform surface pattern. The flow chamber experiments were conducted either in a closed circuit model (P. gingivalis and S. oralis) or an open system (S. aureus, S. gordonii). In the first model, the fixed test specimens were overflowed by a suspension of the respective bacterial species cultured in Brain Heart Infusion (BHI) supplemented with Vitamin K and 5 % sucrose. The culture medium was pumped in a closed circuit (Fig. 1A) from a temperature-controlled bioreactor (37°C) at a flow rate of 100 µL/min over the test specimen and back into the reactor. In the open circuit system (Fig. 1B), the experimental setting was the same, except that the bacterial suspension was collected in a waste container after passing the specimens and that Tryptic Soy Broth (TSB) supplemented with 50mM glucose was used as growth medium. Optical density of the suspension was continuously recorded with an inline photometer (Elocheck, Biotronix, Berlin). The attached biofilms were stained live/dead (BacLight, Life Technologies) directly in the chambers and analyzed by confocal laser scanning microscopy (CLSM) with a Leica Upright MP microscope connected to a TCS SP2 AOBS scan head. The average biofilm height was calculated from the acquired z-stacks with the Imaris Scientific 3D image processing software (Version 6.2.1, Bitplane, Oxford instruments). Experiments were performed in triplicate and repeated three times.

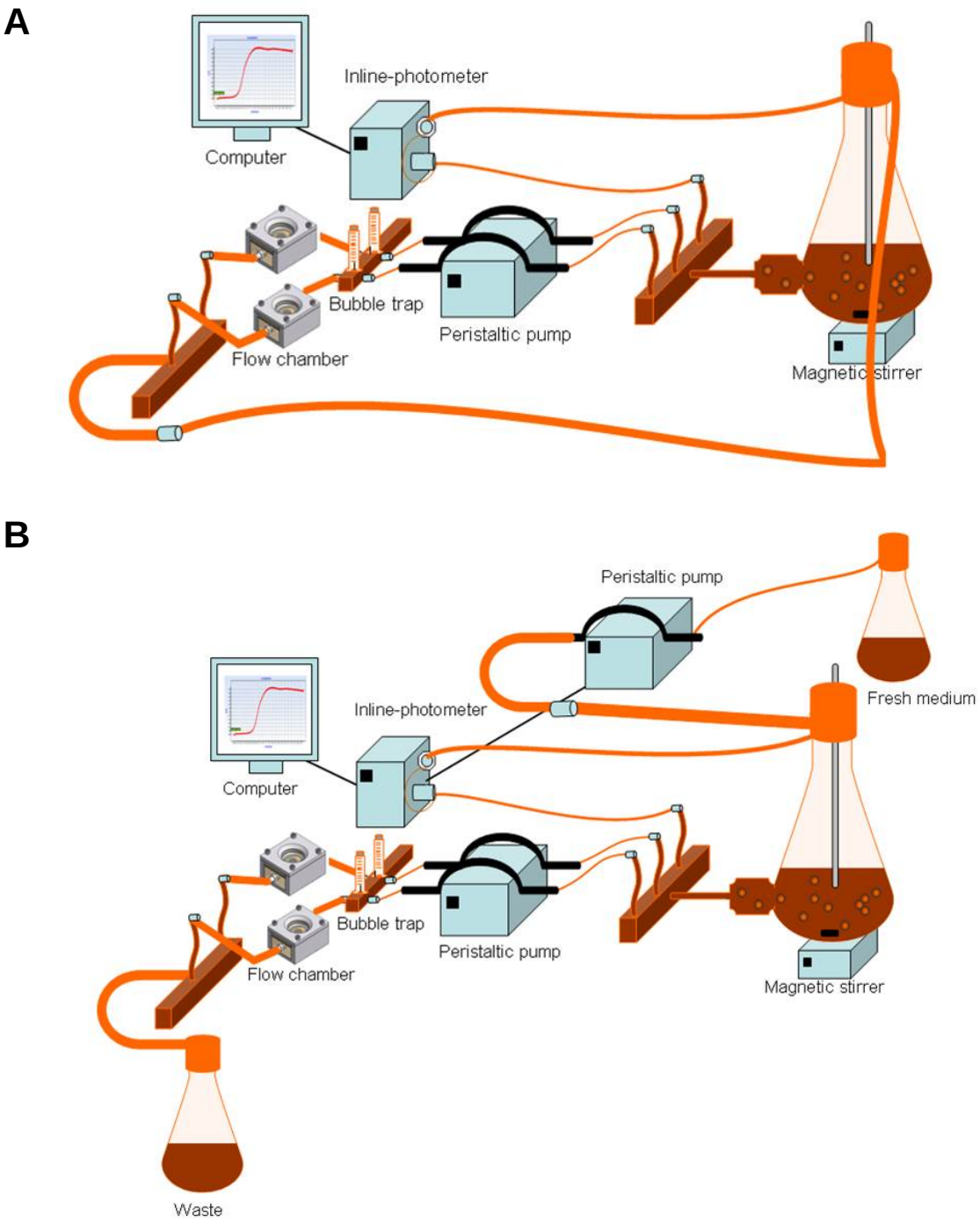
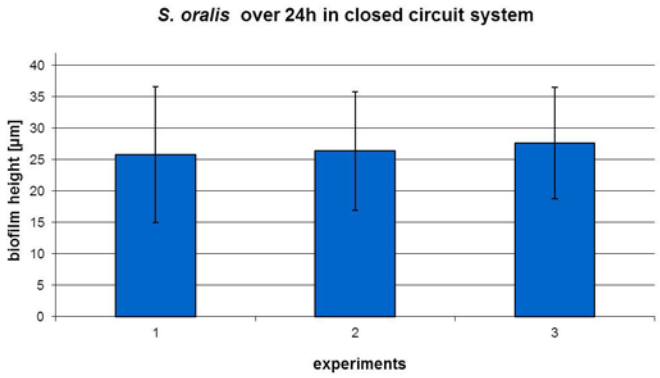


Figure 1: Flow-chamber systems. The experimental setting of both flow chamber models is depicted above: A) Closed circuit model and B) Open system.

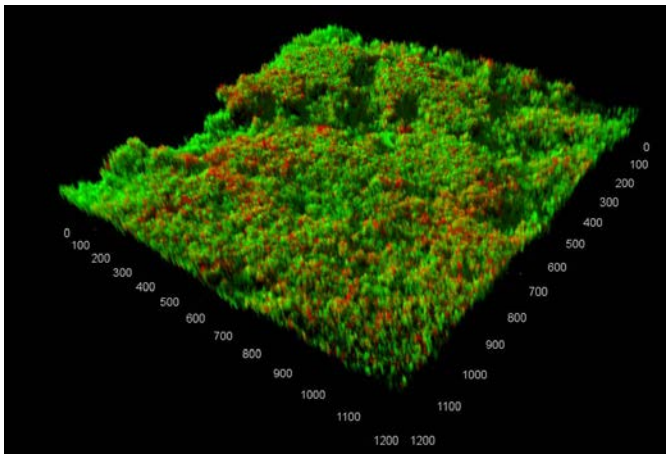
3 Results

In both flow chamber systems a reproducible biofilm formation in terms of biofilm height (Fig. 2, Row A) and biofilm morphology (Fig. 2, Row B) was observed for all tested species. The systems proved to be suitable for the evaluation of biofilm formation on implant-derived surfaces.

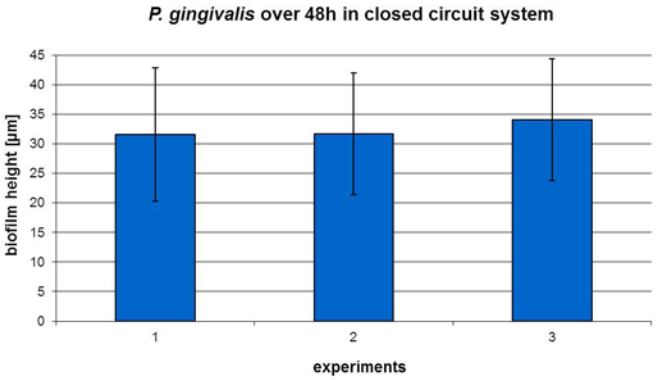
1A



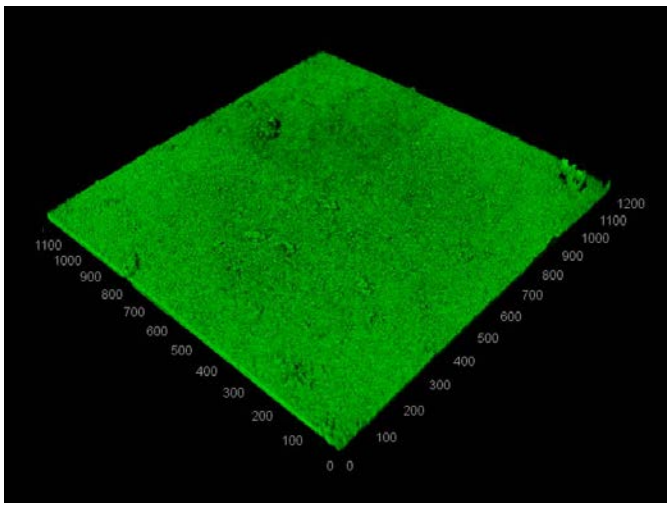
1B



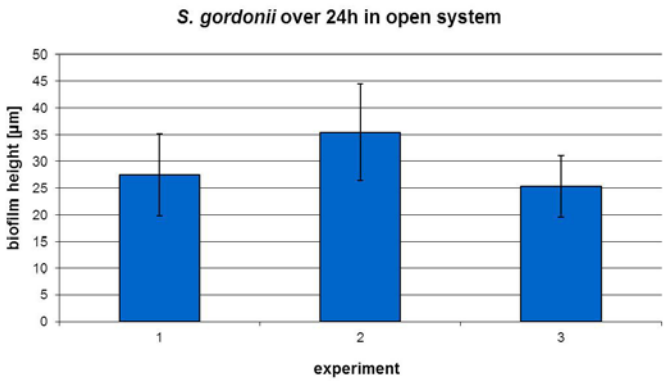
2A



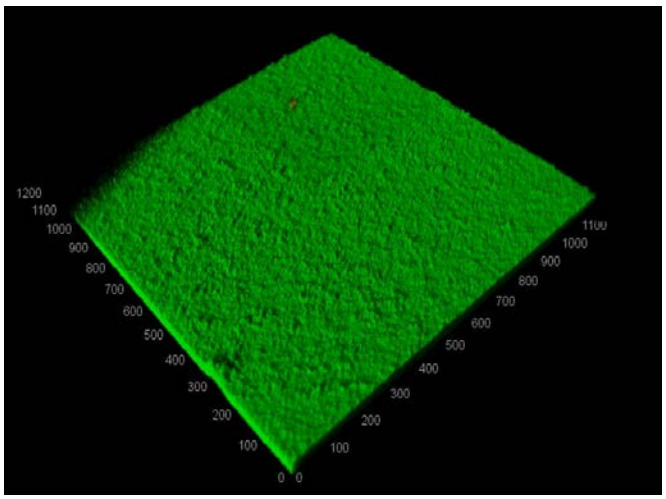
2B



3A



3B



4A **4B**

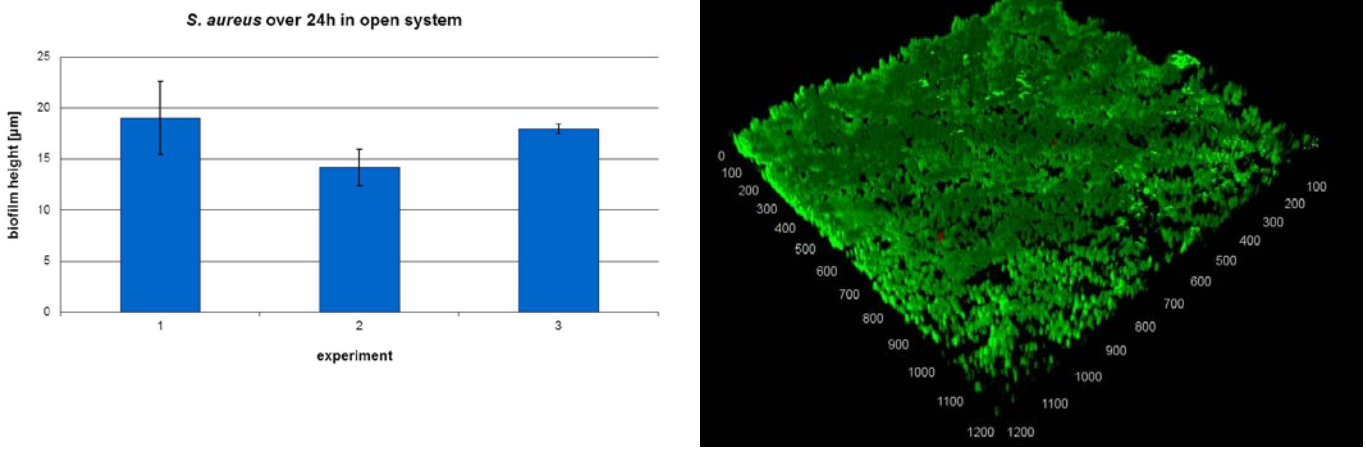


Figure 2: **Biofilm height and 3D reconstruction of biofilms**: *S. oralis* (1), *P. gingivalis* (2), *S. gordonii* (3) and *S. aureus* (4). Right row: 3D reconstructions of live/dead stained biofilms (green = vital, red = membrane impaired/dead); left row: average biofilm height calculated from CLSM acquired z-stacks.

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4 Conclusion

We have successfully established a reliable flow camber system for the evaluation of microbial adhesion to innovative implant surfaces. Future experiments aim at developing the systems further to be used with complex bacterial multi-species biofilms. This is of great medical importance for the in vitro evaluation of implant surfaces exposed to highly diverse microbial environments like in the oral cavity..