Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Material

Bacterial Cultures and Biofilm Staining

1. Methods

Streptococcus salivarius, *S. sanguinis*, and *S. oralis* isolated from clinical specimens and identified by mass spectrometry were routinely maintained on brain-heart infusion (BHI; BD Difco, Milan, Italy) agar plates. To assess biofilm formation, bacteria were grown in BHI broth overnight at 37°C, then plated ($1x10^6$ CFU/mL) on 4 mg of each biomaterial. Bacteria were grown in a total volume of 1 mL of fresh BHI broth for 48 h at 37°C without agitation; these conditions were previously set in our laboratory to ensure a biofilm formation. At the end of incubation, the LB medium was discarded and samples were washed three times with sterile PBS to remove planktonic cells. Adhering bacteria were stained with 150 µl of 0.1% (w/v) Crystal violet (CV) solution for 15 min at room temperature. Samples were washed, air-dried, and CV was solubilized in 125 µl of 30% (v/v) glacial acetic acid per well. The optical density was measured at 570 nm using a microplate reader (MultiPlateReader VictorX2). Bacteria incubated with BHI alone assigned 100% biofilm formation.

2. Results

To evaluate the role of different sizes of biomaterials in bacterial biofilm formation, *Streptococcus salivarius*, *S. sanguinis*, and *S. oralis* were isolated from clinical specimens, characterized, and cultured. Bacterial strains were grown on biomaterials for 2 days to form bacterial biofilms. The staining of biofilms with CV revealed that BO and sphene did not have any antibacterial activity. Indeed, the optical density relative to the biofilms was comparable to the values recorded for the different biomaterials not incubated with bacteria. Among the same type of biomaterials, the different sizes did not affect the adhesion and proliferation of the tested bacterial strains.