Electronic Supplementary Material (ESI) for Biomaterials Science.

Electronic Supplemental Information

S1 Analysis of bacterial adhesion with and without saliva coating

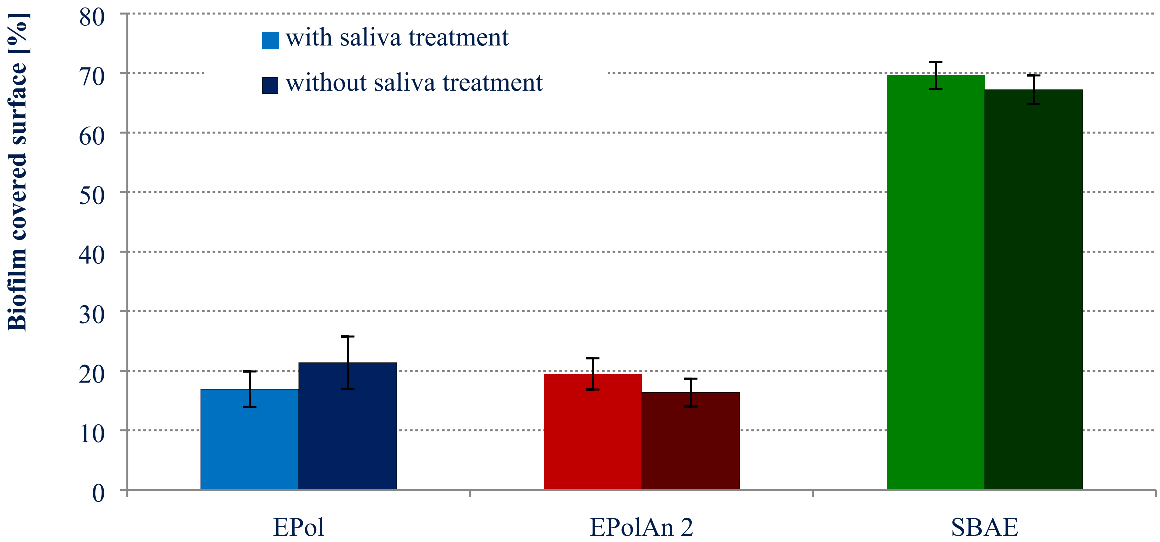

Fig. S1 Analysis of bacterial adhesion and biofilm formation of Streptococcus sanguinis (biofilm covered surface [%]) on electropolished (EPOL), nanotubular (EPOLAN) and sandblasted and acid etched (SBAE) titanium oxide with and without saliva treatment. Fifteen microscopic images of each sample were taken and analysed. Data shown represents the average value of three samples and its mean deviation.

S2 Calculations of the flow conditions

The Reynold's number (Re), often used to describe flow characteristics, can be calculated using Eq. (1), with the hydraulic diameter of the flow cross-section d_h (Eq. 2-4) [1]. Wall shear rate and shear stress are given by Eq. (5) and Eq. (6) respectively, where $\dot{V}$ is the volumetric flow rate (Eq. (7)) [2, 3].

$$Re = \frac{v_m \cdot d_h \cdot \rho}{\mu_{dyn}} \tag{1}$$

$$d_h = \frac{4 \cdot A}{U} \tag{2}$$

$$A = h \cdot b \tag{3}$$

$$U = 2(h + b) \tag{4}$$

$$\gamma = \frac{3 \cdot \dot{V}}{2 \cdot \left(\frac{h}{2}\right)^2 \cdot b} \tag{5}$$

$$\tau = \gamma \cdot \mu_{dyn} \tag{6}$$

$$\dot{V} = v_m \cdot A \tag{7}$$

The Re values (Table 1) estimated for a 10 mm (b) × 0.3 mm (h) channel were 0.1 (unstimulated flow) and 4.9 (stimulated flow) as well as 17.6 (rinsing), assuming that the density (ρ) and dynamic viscosity (μ_{dyn}) of the medium were equal to those of water at 30 °C (995.7 kg·m^3 and 7.977·10^{-4} Pa·s, respectively). This describes a laminar flow, since the transition from laminar to turbulent flow in pipes occurs at Re > 2300 [4]. The calculated shear stress varies in the range of 0.002 – 0,285 Pa (wall shear rate: 2.3 - 418 s^{-1}).

Table 1 flow conditions and calculated shear stress for a 10 mm (b) × 0.3 mm (h) channel

average flow velocity [m/s]		flow rate [m^3·s^{-1}]	Reynolds number [-]	wall shear stress [Pa]	wall shear rate [s^{-1}]
unstimulated flow	$0.1 \cdot 10^{-3}$	$3.5 \cdot 10^{-10}$	0.1	0.002	2.3
stimulated flow	$5.8 \cdot 10^{-3}$	$2.0 \cdot 10^{-8}$	4.9	0.079	116.1
rinsing	$20.9 \cdot 10^{-3}$	$4.3 \cdot 10^{-8}$	17.6	0.285	417.8

It is believed that high shear stress leads to detachment of biomass from surfaces [5, 6]. For biofilms, resistance towards shear stress induced detachment depends on the shear stress conditions the biofilm was cultivated in, since the biofilm density can adapt to shear forces [7]. Paul et al. precultivated biofilms under very low shear stress (0.01 Pa) and then exposed it to high shear stress in the range of 0.3 - 13 Pa. An exponential and asymptotic decrease of the biofilm thickness and mass with increasing τ was observed. At lower shear stress increments, from 0.01 to up to 2 Pa, only detachment is observed and no increase in the compactness is detected. A lower shear stress allows an extension of the biofilm thickness but this is characterized by low cohesion. Therefore, for biofilms developed under low shear stress, detachment would prevail for the superficial layers and compression for the deep layers [7].

Fig. S2A Explanation for calculation of shear stress within the flow chamber

Abbreviation

A_{cs}	cross sectional area [m^2]
b	channel width [m]
d_h	hydraulic diameter of the flow cross-section [m]
h	channel height [m]
Re	Reynold's number [-]
U	circumference [m]
v_m	average flow velocity [m/s]
V	volume [m^3]
$\dot{V}$	volumetric flow rate [m^3·s^{-1}]

Greek symbol

μ_{dyn}	dynamic viscosity [Pa·s]
ρ	density [kg·m^{-3}]
τ	shear stress [Pa]

References

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[6] Rao, K. R., Srinivasan, T., and Venkateswarlu, C., Mathematical and kinetic modeling of biofilm reactor based on ant colony optimization. *Process Biochemistry* 2010,*45*, 961-972.

[7] Paul, E., Ochoa, J. C., Pechaud, Y., Liu, Y., *et al.*, Effect of shear stress and growth conditions on detachment and physical properties of biofilms. *Water Research* 2012,*46*, 5499-5508.

Fig. S2B Abbreviations and references for calculation of shear stress within the flow chamber

S3 Cell proliferation for donor 2

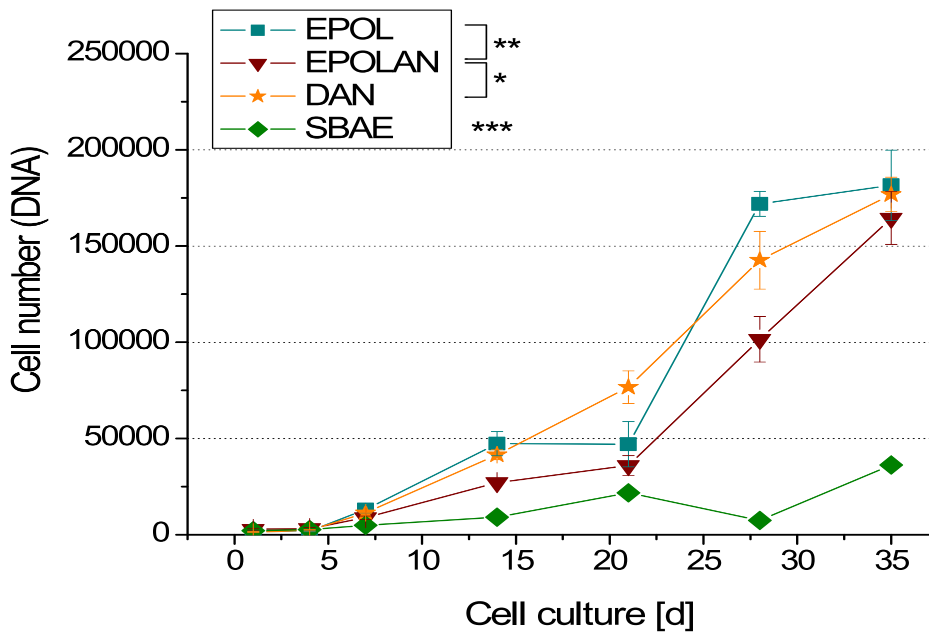

Fig. S3 Cell proliferation of donor 2 determined via DNA content, mean of two experimental runs with n=4 for each. Error bars reperesent standard error. Two-way Anova was performed with factor A being the experiemental run and factor B being the samples types for timepoint 14 – 35 d. Significances marked besides the legend display the obtained significance levels between the surface types in this range.

S4 ALP activity for donor 2

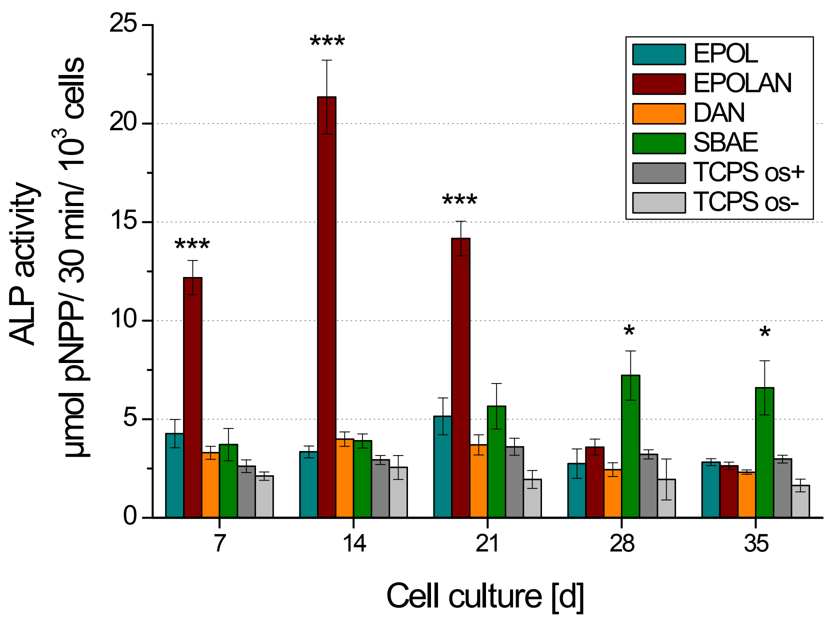

Fig. S4 ALP activity of donor 2, normalized to cell number either via DNA content,
mean of two experimental runs with n=4 for each. Error bars reperesent standard error. Two-way Anova was performed with factor A being the experimental run and factor B being the sample types for each timepoint. Significant differences are labeled above the columns.

S5 Mineral deposition for donor 2

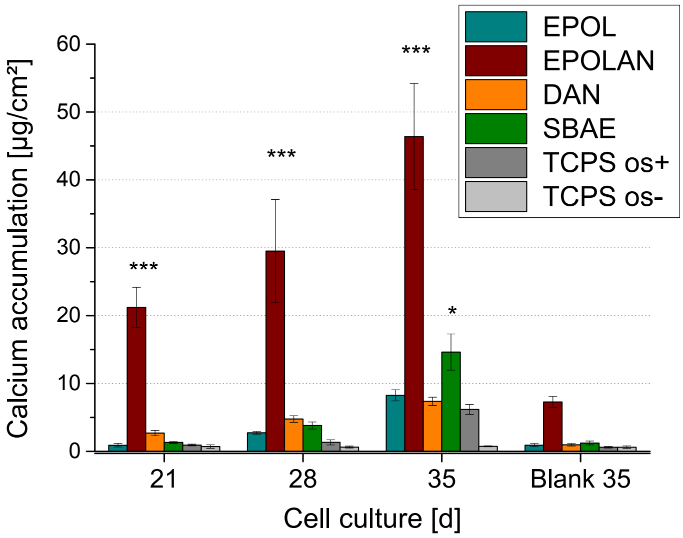

Fig. S5 Mineral deposition from cells on samples into their excreted extracellular matrix for donor 2. Determination of the deposited amount of calcium as mean of 2 experiments, each with n=4 and error bars representing standard error. Asterisks show the significance level of differences to all other samples of the same day