

Lab Report

“Interphase Phenomena – Wettability and Cell Adhesion”

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I hereby declare that my elaboration was made independently and is not a copy of an existing protocol.

Date: July, 21st 2022

Signature:

1 Introduction

Dental implants are widely used among the human population to replace missing teeth. To enable optimal ingrowth and osseointegration of these implants into the human jaw bone, and thus ensuring a long-term integration, the choice of an appropriate material, and especially its surface is crucial. Cell adhesion after implantation is mainly influenced by surface parameters such as surface chemistry, surface topology as e.g. roughness, and surface free energy (SFE) which is related to the wettability. To obtain the desired osseointegration, hydrophilicity of the implant surface plays an essential role, as it promotes fast interaction with the biological surrounding, such as blood, immediately after implantation [1]. With the method of crystal violet staining, living cells adhered on a surface can be quantified. Hereby, the dye binds to proteins and DNA of the cells in a sample [2]. Therefore, the aim of this practical was to investigate different materials regarding their wettability and surface free energy, and thus making conclusions about their hydrophilicity. Furthermore, cell adhesion on these different materials was observed via crystal violet staining.

2 Methods

2.1 Wettability

Different potential implant materials (titanium machined (Machined), titanium modified by anatase (Anatase), titanium grit blasted/acid etched (SLA), and titanium modified by amorphous Teflon (Teflon)) were observed regarding their wettability properties. Therefore, static contact angles of fluids (water, ethylene glycol (EG), and diiodomethane (DM)) on these solid materials were measured via the sessile drop method, and the surface free energy was determined by different approaches. Furthermore, dynamic contact angle measurement was conducted via the Wilhelmy method. Both experiments were performed as described in the instruction manual.

2.2 Cell Adhesion Assay

To evaluate the cell adhesion of SAOS-2 cells on different potential implant materials (Machined, titanium machined hydrophilized by plasma (Machined-P), SLA, Teflon), crystal violet staining was used to quantify the number of living cells attached on the material surface. Therefore, after dying the samples with crystal violet, the samples were washed, leading to an elution of the dye from the cells into the supernatant. By measuring its absorbance, a conclusion about the cells attached on the samples could

be made, as the measured intensity is directly proportionally related to the cell amount. The experiment was performed as described in the instruction manual.

3 Results

3.1 Wettability

Tab. 1 lists the results of the contact angles (CA) from different fluids measured on the different surfaces at room temperature.

Table 1: Measurement results of contact angles

CA	M		Anatase		SLA	Teflon
Water	before O ₂ plasma	89°	before UV-A	94°	92°	118°
	after O ₂ plasma	0°	after UV-A	0°		
EG	26°		-		-	102°
DM	26°		-		-	96°

Fig. 1 depicts the Zisman plots for Machined (left) and Teflon (right). The critical surface tensions (for $\cos(\theta) = 1$) were determined as $\gamma_{\text{krit, Machined}} = 46.59 \text{ mN/m}$, and $\gamma_{\text{krit, Teflon}} = -38.92 \text{ mN/m}$.

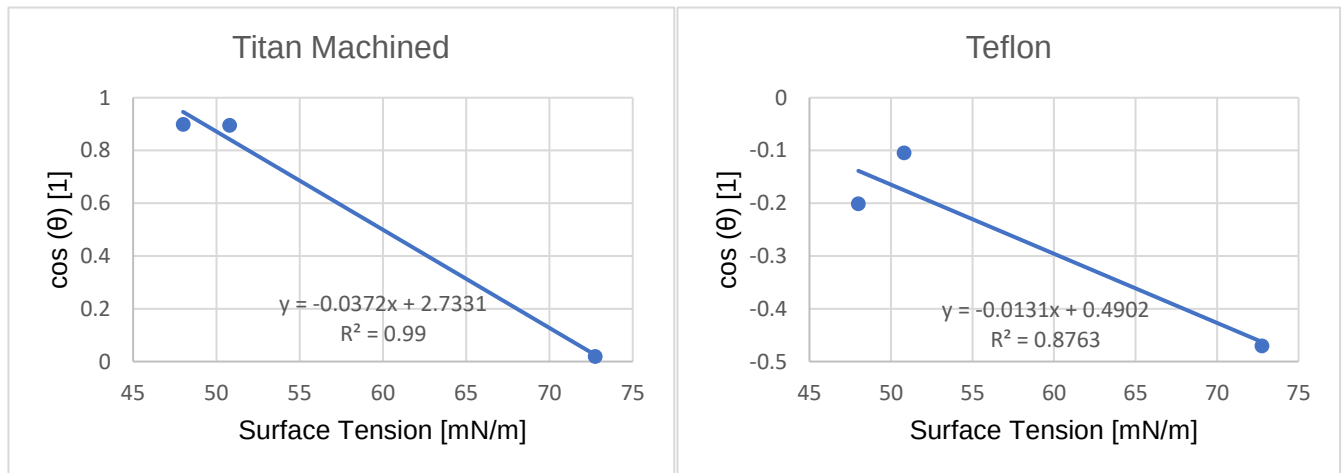


Figure 1: Zisman plots of Titan Machined (left) and Teflon (right)

In Tab. 2 the SFEs obtained by the different approaches and their related parameters are listed.

Table 2: SFE, related parameters and critical surface tension for different approaches

	LvdW/acid-base [mN/m]	Owens-Wendt [mN/m]	Zisman [mN/m]
Titan Machined	SFE = 45.63 γ_{LW} = 45.63 acid = 1.57 base = 0	SFE = 50.25 dispers = 49.69 polar = 0.56	γ_{krit} = 46.59
Teflon	SFE = 10.35 γ_{LW} = 10.13 acid = 0.01 base = 0.58	SFE = 10.03 dispers = 9.64 polar = 0.39	γ_{krit} = -38.92

Fig. 3 shows the measurement curve for dynamic contact angle measurement of a titanium screw with the Wilhelmy method. The advancing θ_a and receding θ_r contact angles were determined from linearizing the first cycle measurements (orange and yellow), and applying the formula $\theta = \arccos \frac{F_W/L}{\gamma_{LV}}$.

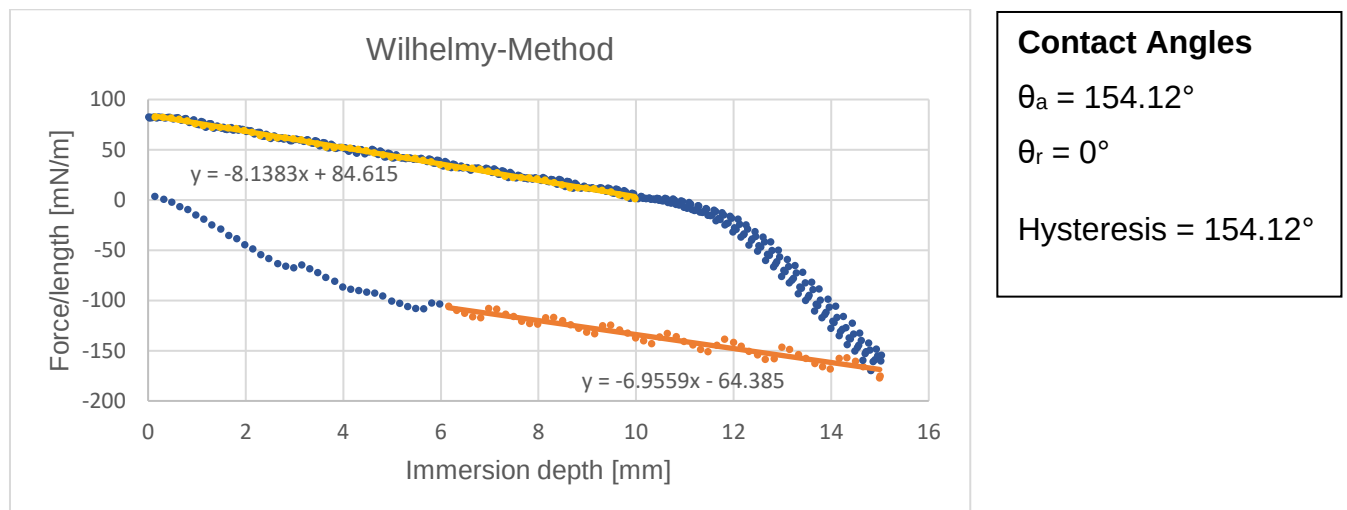


Figure 2: Results of dynamic CA measurement on titanium screw with Wilhelmy method

3.2 Cell Adhesion Assay

In Fig. 3 the different surfaces stained with crystal violet can be seen.

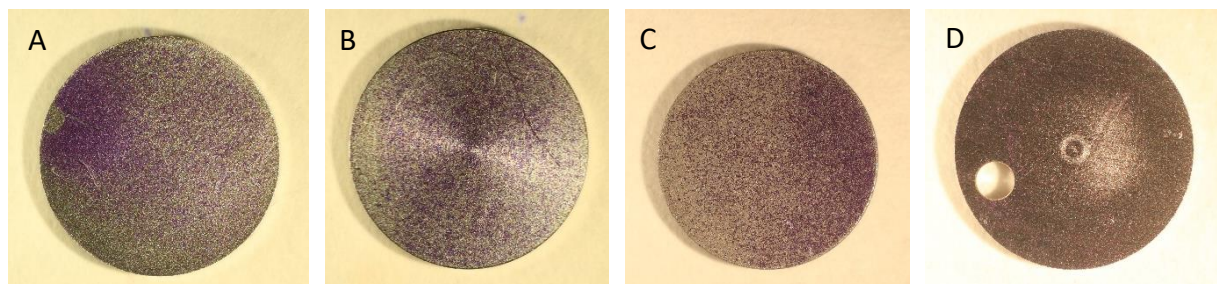


Figure 3: Cells stained with crystal violet adhered on the different implant surfaces, Machined (A), Machined-P (B), SLA (C), Teflon (D)

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The machined titanium shows a high density of cell coverage on the upper left side, whereas the plasma treated titanium is homogeneously stained, but with a lower density. The SLA sample also shows cell attachment, and the Teflon surface has almost no cell attachment. In Fig. 4 the average cell coverage of the different samples and the standard deviations (SD) are depicted in relation to the machined titanium sample that was taken as reference with 100% coverage. The mean values, as well as the SD related to the machined titanium are listed in Tab. 3. t-Tests were conducted between all samples to determine whether the difference in the average cell attachment between the samples is not significant (ns), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Before each t-Test, a F-Test was done to decide whether an independent t-Test assuming different or equal variances should be conducted. Significant differences are marked in the diagram. Comparing the other mean values showed no significant difference between them.

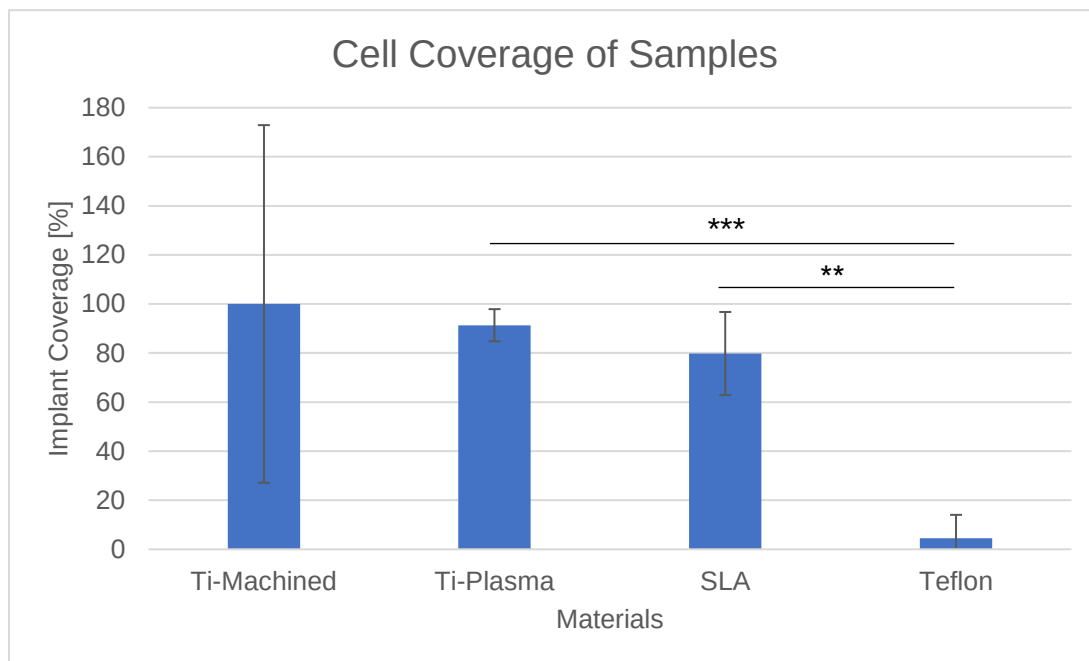


Figure 4: Cell coverage of observed samples in relation to machined titanium, significant differences between the mean cell coverage values of the samples are marked in the diagram

Table 3: Mean values and SD related to machined titanium in %

	Mean Value [%]	SD [%]
Ti-Machined	100	72.89
Ti-Plasma	91.33	6.56
SLA	79.80	16.93
Teflon	4.44	9.60

4 Discussion

During the static CA measurement, it was shown, that the four materials observed are hydrophobic when putting a drop of water on them. However, after treatment with plasma and UV-A, machined titanium and Anatase became hydrophilic, and therefore enable good wettability. Machined titanium is also wetted by EG and DM. Teflon was always hydrophobic. These results were confirmed by doing a Zisman plot. As the critical surface tension of machined titanium is 46.59 mN/m, fluids with lower or the same surface tension would completely wet the surface. Since water has a surface tension of approx. 72 mN/m a drop arises. The calculated critical surface tension of Teflon is very low, thus all fluids will form drops when being in contact with Teflon. In addition to the Zisman plot the acid-base and Owens-Wendt approaches were used to calculate the SFE. Due to the different approaches, the results for the SFE are slightly different, but in the same range. The negative value for $\gamma_{krit,Teflon}$ might be due to a measurement mistake. Dynamic CA measurement with the Wilhelmy method revealed a much higher CA than with the static one. This might be due to the dynamic approach, where the interface was perhaps not in equilibrium during the CA determination.

As expected, the cell adhesion experiment revealed a varying cell attachment density on the different sample surfaces. For clinical application of dental implants it is crucial to know which surfaces allow high cell attachment, and thus enable osseointegration of the implant in the human jaw bone. According to this experiment, machined titanium allows the highest cell attachment, and is therefore the best suitable material. Teflon should not be used due to the low cell attachment because of its high hydrophobicity. With regard to the future, there will be made improvements regarding implant surfaces to enable even better ingrowth into the bone. By using advanced materials and surface pre-treatments the implants will be hydrophilic, what enables good cell attachment, but prevent from additional bacterial attachment, due to e.g. improved surface roughness, what might enhance inflammation and implant rejection.

5 Literature

1. Gittens, R.A., et al., *A review on the wettability of dental implant surfaces II: Biological and clinical aspects*. Acta Biomater, 2014. **10**(7): p. 2907-18.
2. Feoktistova, M., P. Geserick, and M. Leverkus, *Crystal Violet Assay for Determining Viability of Cultured Cells*. Cold Spring Harb Protoc, 2016. **2016**(4)

