

Grade 1,3

Protocol for the experiment

Interface Phenomena

Master Biomedical Technologies
Modul: Implantology

July 21, 2022

1 Introduction

Osseointegration plays a decisive role in the implantation of dental implants, as it leads to the formation of new bone tissue on the implant surface and thus to the firm anchoring of the implant in the jaw bone [1]. In order for bone tissue to form on the implant, good adhesion and proliferation of fibroblasts on the implant surface is important, which is positively influenced by physic-chemical factors such as surface wettability, surface free energy (SFE) and roughness. To optimize these factors, the choice of suitable materials and optimal surface modification are decisive [2]. For this reason, various tests with biocompatible materials are performed in laboratories. These include cell adhesion assays, in which the adhesion of living cells to various modified materials is analysed by staining their nuclei using crystal violet staining, and wettability testing by measuring the contact angle (CA) and calculating the SFE of various liquid droplets on the surface of different materials (see experimental manual "Interface Phenomena").

In this experiment, the above two tests were performed and evaluated using different material samples.

2 Methods

Cell adhesion assay

In the first part of the laboratory work, a cell adhesion assay was performed, in which four titanium samples (see results) incubated with living human osteosarcoma-derived cell-line (SAOS-2) were stained using crystal violet staining. This allowed the attachment of the living cells to be examined under the microscope. In addition, a methanol-cell suspension was prepared by adding methanol to the stained samples, the cell concentration of which could be determined by absorbance measurement at 595 nm. The exact procedure of the assay is described in the experimental manual "Interface Phenomena Part II - Cell Adhesion Assay".

Wettability

In the second part of the laboratory work, a wettability test was performed by placing a drop of various liquids on the surface of different material samples (see results) and measuring the resulting CA. During the experiment, some samples were also subjected to O₂ plasma or UV-A light. In the conclusion of the experiment, the dynamic CA (DCA) of a cylindrical titanium specimen (diameter = 4 mm) in water was investigated using the Wilhelmy plate technique by immersing and emerging the specimen in the liquid three times. The exact steps of the experiment are described in the experimental instructions "Interface Phenomena Part I - Wettability".

3 Results

Cell adhesion assay

The microscope images of the four different material samples machined titanium (Titanium), titanium hydrophilized by plasma (Titanium-Plasma), grid blasted/acid etched titanium (SLA) and Teflon coated titanium (Teflon) are shown in figure 1, where the purple areas represent the adhered cells. Images 1A and 1C show the Titanium sample and the SLA sample, respectively. Both samples show uneven colonization of the material by cells, with one side densely covered and the other half showing medium colonization. A similar accumulation of cells can be seen on the titanium plasma sample as in the more sparsely populated area of the previously described samples, although these are now evenly distributed throughout the material surface (see figure 1B). In figure 1D, hardly any adhered cells can be observed on the Teflon sample.

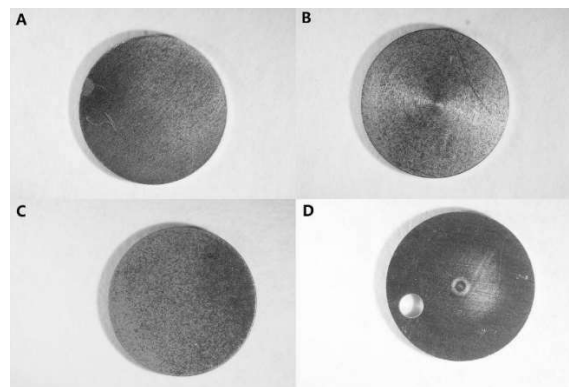


Figure 1: Microscope images of the material samples Titanium (A), Titanium-Plasma (B), SLA (C) and Teflon (D) with attached cells in purple

In addition, the photometric measurement of the cell-methanol suspensions of the triplicates of the four samples was quantitatively evaluated. For this purpose, the duplicates of each sample were averaged, the results of the background measurements were subtracted from these, and all samples were normalized to the Titanium sample. Finally, the mean and standard deviation (SD) of the triplicates were calculated (see figure 2).

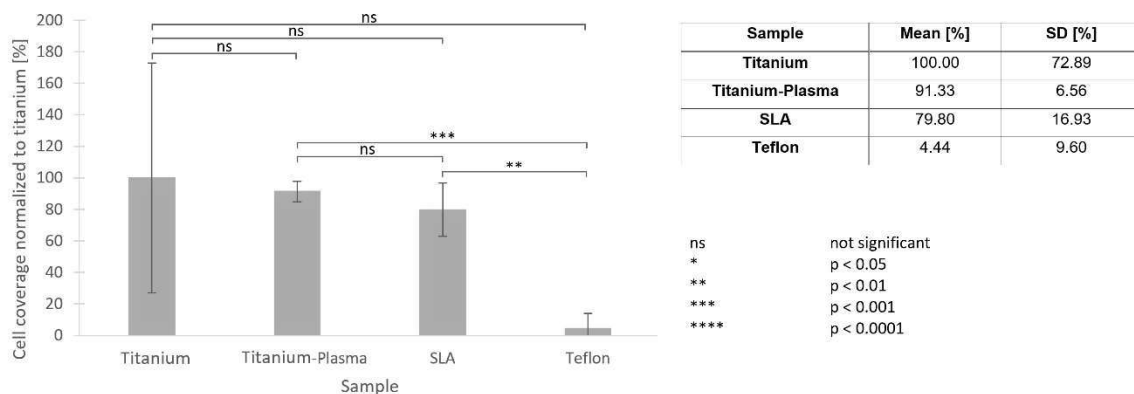


Figure 2: Cell coverage of the four different material samples

Additionally, F-tests and t-tests with the significance level of 0.05 were performed between all samples to determine significant differences between the cell coverage of each sample. It can be seen that Teflon has significantly lower cell adhesion compared to the Titanium-Plasma and SLA samples. The t-tests among the other samples showed no significant difference in cell coverage.

Wettability

The measured contact angles of the droplets of the different liquids on the materials Teflon, anatase covered titan (Anatase), SLA and Teflon are plotted in table 1. Considering only the untreated specimens, it can be seen that all of the different liquid droplets have the lowest CA on the Titanium, whereas the highest occurs on the Teflon sample. The CA of the water droplet on the untreated materials Anatase and SLA is slightly higher than that of Titanium. For the Titanium and Anatase samples treated with O₂ plasma and UV-A, respectively, the CA of the water droplet is reduced to 0° and is thus significantly lower than for the same untreated material samples.

Table 1: Measured contact angles of different liquid droplets on the material samples

	Titanium		Anatase		SLA	Teflon
Water	Before O ₂ plasma	89°	Before UV-A	94°	92°	118°
	After O ₂ plasma	0°	After UV-A	0°		
Ethylene glycol	26°		-		-	102°
Diiodomethane	26°		-		-	96°

Using the literature values of the surface tension of the three liquids and the measured contact angles on Titanium and Teflon, a Zisman plot was created for these two material samples to determine the critical surface tension γ_{crit} . The calculated values, as well as the regression line for the two materials, can be seen in the plot in figure 3.

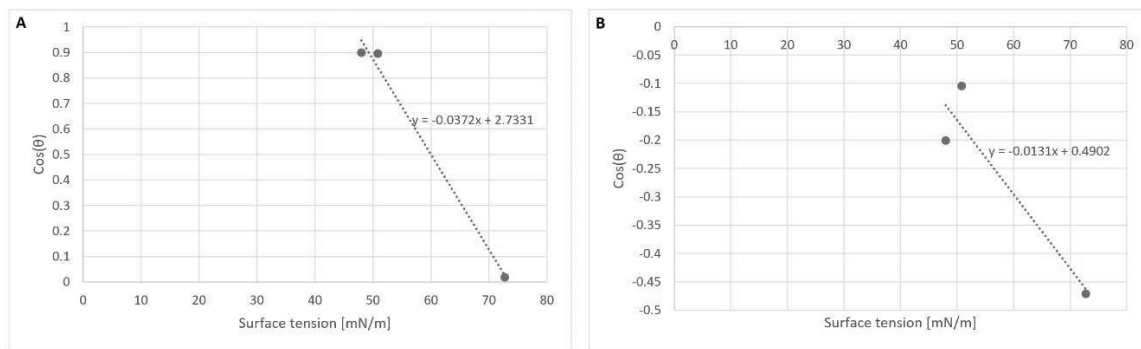


Figure 3: Zisman plots of the material samples Titanium (A) and Teflon (B)

For the determination of the critical surface tension γ_{crit} , the following formula applies according to the Zisman approach (see formula 1):

$$\gamma_{crit} = \frac{1 - b}{m} \quad (1)$$

Where m is the slope and b is the y-axis intercept of the regression line.

After inserting the slope and the y-axis intercept from the Zisman plots, the critical surface tension amounted to 46.59 mN/m for Titanium and -38.92 mN/m for Teflon. The two values are plotted in table 2 together with the calculated values of the SEF of the other approaches performed by the computer during the experiment.

Table 2: SFE and critical surface tension of different approaches

	Zisman [mN/m]	Acid-Base [mN/m]	Owens-Wendt [mN/m]
Titanium	$\gamma_{crit} = 46.59$	SFE = 45.63	SFE = 50.25
		$\gamma_{LW} = 45.63$	Dispers = 49.69
		Acid = 1.57	Polar = 0.56
		Base = 0	
Teflon	$\gamma_{crit} = -38.92$	SFE = 10.35	SFE = 10.93
		$\gamma_{LW} = 10.13$	Dispers = 9.64
		Acid = 0.01	Polar = 0.39
		Base = 0.58	

Based on the data of the DCA measurement, a hysteresis loop was created (see figure 4). Three cycles are shown, whereby only the first cycle was used for the linear regression of the linear subranges of immersion and emersion, since the Titanium specimen was already wet in the other cycles, which falsified their measurement.

The calculation of the dynamic contact angle θ was performed according to formula 2:

$$\theta = \arccos\left(\left(\frac{F_W}{L}\right) \cdot \frac{1}{\gamma_{lv}}\right) \quad (2)$$

Where F_W/L describes the immersion force and γ_{lv} the surface tension of the water. By inserting the y-axis intercept of the regression lines of immersion and emersion, the DCA for immersion amounted to 154.12° and for emersion to 0°.

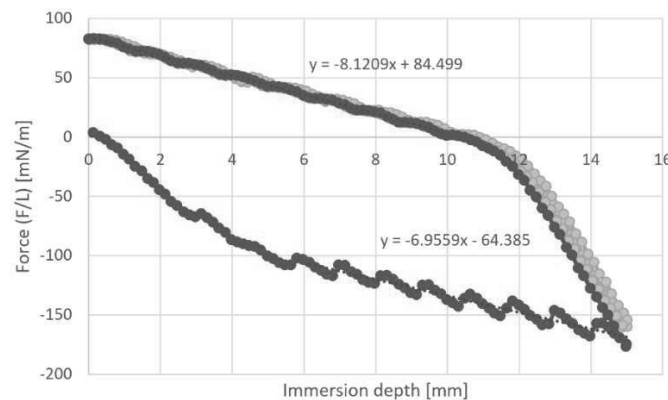


Figure 4: Hysteresis loop of the DCA measurement (blue: first cycle, grey: second and third cycle, red: regression lines of linear immersion and emersion subranges)

4 Discussion

The results of the cell adhesion assay showed that the Teflon sample had a significantly lower cell adhesion than the other samples, which was demonstrated both qualitatively under the mi-

croscope and quantitatively by photometric measurement. In the case of Teflon, no significant difference was found only in comparison with Titanium. The reason for this is assumed to be the high standard deviation of the Titanium triplicates, which probably resulted from errors during the preparation. However, it is suspected from the optical images that Titanium possessed similar cell coverage as the Titanium-Plasma and SLA sample and thus the cell adhesion is also significantly higher for this sample. Thus, the Titanium, Titanium-Plasma and SLA, would be equally suitable for dental implants despite the difference in surface structure, as the described cell attachment would drive osseointegration. However, further tests would have to be used to make more accurate statements, as few replicas were used in this trial and thus statistical fluctuations can be assumed. Furthermore, measurement errors and contamination during the test cannot be ruled out.

In the wettability test, Titanium showed lower contact angles and a higher calculated critical surface tension than Teflon, which indicates a higher wettability. The different approaches also showed that positive factors influencing wettability, such as the SFE, are higher for Titanium than for Teflon. It can be assumed that the wettability of the samples Anatase and SLA are between those of Titanium and Teflon, since their contact angles when using water were between those of Titanium and Teflon. It was found that by reducing contamination using O₂ plasma or UV-A, the contact angle could be reduced and thus the wettability increased. Based on these tests, it can be assumed that machined Titanium is best suited for dental implants due to its high wettability, as water-like fluids such as blood have the best adhesion to the hydrophilic surface, thus favouring osseointegration. In addition, a treatment to reduce contamination prior to implantation is recommended as it can further increase the wettability. The DCA resulting from the Wilhelmy plate techniques amounted to 154.12° for Titanium, which was in the hydrophobic range and thus deviates strongly from the previous measurements of the static contact angle. Reasons for this could be measurement errors and contamination in both tests, as well as the non-uniform diameter of the titanium sample in the dynamic measurement. Further in-vitro and in-vivo tests should be carried out to obtain a more accurate statement on the suitability of the individual materials as dental implants and their influence on osseointegration.

5 References

- [1] H. S. Alghamdi, "Methods to Improve Osseointegration of Dental Implants in Low Quality (Type-IV) Bone: An Overview," *Journal of Functional Biomaterials* 9(1), 2018.
- [2] C.-P. Hao et al., "The osseointegration and stability of dental implants with different surface treatments in animal models: a network meta-analysis," *Scientific Reports* 11, 2021.