Basic Medical Research

Evaluation of surface properties of local machinery metal-based implant and the correlation with the viability of osteoblast and fibroblast

Ronald Iskandar[1]

[1]Department of Orthopaedic and Traumatology, Faculty of Medicine, Gadjah Mada University, Yogyakarta - Indonesia

ABSTRACT

Introduction: Indonesia has started to make local machinery metal-based implant. Evaluation of local machinery processing can be done with the measurement of surface properties of the materials. The surface properties of a medical implant is of great importance since the surface is in direct contact with the host tissue. Surface properties influence osteoblastic proliferation.

Methods: This was an experimental study of 5 local machinery metal-based materials from stainless steel and titanium alloy. Those materials were measured for the surface roughness, contact angle, and viability of osteoblast and fibroblast. These parameters were analyzed and compared with the results from common production method, and the correlation among those parameters was then analyzed.

Results: The highest surface roughness was in Titanium Ti-GR2 material (Ra 3.986 ± 2.043 µm) and the lowest was in Stainless steel AISI 316 L COR (1.640 ± 0.960 µm). The highest contact angle was in Stainless steel AISI LVM material (78.721 ± 2.833^o) and the lowest was in Titanium Ti-V-Al (43.218 ± 4.586^o). The viability of osteoblast was ranging from 68.25% - 84.79% and the viability of fibroblast was ranging from 63.98 % - 90.55 %. The correlation between the surface roughness on these materials and the viability of osteoblast gave *p value* = 0.014, r 0.857 and fibroblast gave *p value* = 0.003, r 0.929.

Conclusion: Surface roughness of our local machinery metal-based materials is consistent with the result of grinding by common production method. The local machinery metal-based material had hydrophilic nature. The higher the surface roughness, the greater the viability of osteoblast and fibroblast.

ABSTRAK

Pendahuluan: *Indonesia telah memulai membuat implan berbahan metal yang diproduksi lokal. Evaluasi dari produksi lokal tersebut dapat dilakukan dengan pengukuran sifat permukaan material, di mana struktur permukaan material mempunyai pengaruh yang besar karena permukaan berkontak langsung dengan jaringan tubuh. Struktur permukaan implan mempengaruhi proliferasi osteoblas.*

Metode: *Penelitian ini merupakan penelitian eksperimental dari 5 jenis material berbahan metal yang diproduksi lokal. Material tersebut diukur untuk kekasaran permukaan, sudut kontak, bio availabilitas osteoblast dan fibroblas. Parameter ini dianalisis dan dibandingkan dengan hasil dari produksi standar, dankorelasi antar parameter tersebut dianalisis.*

Hasil: *Hasil kekasaran permukaan tertinggi ditunjukkan pada Titanium Ti-GR2 (Ra 3.986 ± 2.043 µm) dan yang terendah pada Stainless steel AISI 316 L COR (1.640 ± 0.960 µm). Hasil sudut kontak yang paling besar diberikan oleh Stainless steel AISI LVM (78.721 ± 2.833^o) dan yang terendah pada Titanium Ti-V-Al (43.218 ± 4.586 ^{o}). Bioavailabilitas osteoblas berkisar dari 68.25 – 84.79 % dan bioavailabilitas fibroblas berkisar dari 63.98 – 90.55 %. Korelasi antara kekasaran permukaan dengan bioavailabilitas osteoblas adalah p= 0.014, r 0.857 dan fibroblas p= 0.003, r 0.929.*

Kesimpulan: *Kekasaran permukaan dari material berbahan metal yang diproduksi lokal sesuai dengan hasil proses grinding yang dihasilkan oleh metode produksi pada umumnya. Material berbahan metal yang diproduksi lokal memiliki sifat hidrofilik. Semakin tinggi kekasaran permukaan akan meningkatkan bioavailabilitas osteoblas dan fibroblas.*

Keywords: Stainless steel, Titanium alloy, surface roughness, wettability, viability of osteoblast, viability of fibroblast

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Corresponding author : Ronald Iskandar, MD. dr.ronald.iskandar@gmail.com

INTRODUCTION

Orthopedic implants are medical device used in various orthopedic cases related to joints and bones for reconstructive joint replacement, spinal implants, ortho biology, and trauma implants.[1] Most orthopedic implants are produced overseas and sold at high prices. Indonesia, especially UGM (Universitas Gajah Mada), is faced with the challenge of being able to make its own implants with local raw materials, so the price will reduce and the implants will also be more appropriate with the size of the average Indonesian people.

The technology for orthopedic implants is very dependent on the development and the use of biomaterials. Biomaterials are synthetic materials that are used to replace parts of living systems and interact directly with the living tissue.[2] To develop materials as basic materials for orthopedic implants, a series of tests is needed to assess their physical property, durability and biocompatibility. One of the factors that influence material biocompatibility is the surface properties of the material. The surface structure of the material reacts with the cells of the body and can affect the response of proteins and cells. Surface structure can also be used to assess whether the manufacturing process of the material is good. Some methods for testing the surface structure include contact angle test, surface roughness test, and Scanning Electron Microscopy (SEM).[3]

Cytotoxicity test is an important indicator in evaluating material biocompatibility because the test is simple, fast and has high sensitivity.[4] This test is conducted by using tissue cells, in vitro, to find out whether the material to be used in the body can cause toxic effects on cells and tissues. There are three types of toxicity test in accordance with ISO 10993-5, namely extract test, direct contact test and indirect test.[5] To determine the possible application of stainless steel and titanium alloy as implant base material, investigation on the surface structure of these materials is needed. The aim of this study is to evaluate the surface roughness and wettability of our local machinery metal-based materials and to find correlation between the surface properties and the viability of human osteoblast and fibroblast.

METHODS

This study is an experimental study conducted from April – October 2018. This study used stainless steel and titanium alloy-based materials consisting of 5 types: AISI 316 L/ROD, AISI 316 L/COR, AISI LVM, Ti-V-AL, Ti-GR2. The materials had passed the process of cutting, machining, and grinding. The materials were made with cylindrical shape with diameter of 3.7-3.8 mm and height of 4.7-4.8 mm.

Figure 1. Sample material

The study was conducted in the machinery engineering laboratorium and LPPT UGM. We collected the data consisting of surface roughness, water contact angle (wettability), and viability of osteoblast and fibroblast. We used Surfocom 120 A and Scanning Electron Microscope to measure the surface roughness. Water contact angle is an angle between the edge of the water and the material surface, if the angle is less than 90^{o} the material has hydrophilic surface, if the angle is more than 90^{o} the material has hydrophobic surface.

Figure 2. Measurement of surface roughness and water contact angle

Viability test was done *in vitro*. This study used osteoblast and fibroblast cells with MTT assay and IC50 technique according to the toxicity test procedure. If the percentage of cell viability is smaller than 60%, the material exposed to the cells is said to be toxic.

RESULTS

There were 5 types of material tested with 3 cylindrical samples for each material. Table 1 shows the average height and diameter of each material. It can be seen from these results that the samples of each material have rela tively the same average of height and diameter indicated by the results of normality of the data ($p > 0.05$), which means that the sample sizes do not statistically different.

The results from the surface roughness test were obtained in the form of mean surface roughness value (Ra) in micrometer yield. The results of Ra for each material were not statistically different as indicated by the results of normality test of the data with the Shapiro Wilk test, where $p > 0.05$ was obtained.

Surface roughness test using SEM was carried out on 4 materials, namely AISI 316L COR, AISI LVM, Ti-V-Al, and Ti-Gr2. The test results can be seen in the (Figure 3).

The results of the contact angle test are obtained as shown in Table 3. The contact angle results are the average contact angle from the left and the right sides.

Table 1. Average data on the height and diameter of each material

Material	Average Height	*P*	Average Diameter	*p*
AISI 316 L ROD	5.283 ± 0.057 mm	0.637	4.083 ± 0.057 mm	1.000
AISI 316 L COR	5.033 ± 0.378 mm	0.253	4.100 ± 0.100mm	0.637
AISI LVM	5.125 ± 0.170 mm	0.850	4.062 ± 0.050 mm	0.224
Ti-V-Al	5.550 ± 0.173mm	0.195	4.10 ± 0.050 mm	1.000
Ti-GR2	5.733 ± 0.305mm	0.637	4.016 ± 0.115 mm	0.463

Table 2. Results of the average surface roughness (Ra) and the normality test for each material

Material	n	Average Surface Roughness (Ra)	*P*
AISI 316 L ROD	3	1.973 ± 1.308 µm	0.780
AISI 316 L COR	3	1.640 ± 0.960 µm	0.931
AISI LVM	4	2.760 ± 1.275 µm	0.948
Ti-V-Al	4	3.285 ± 0.757 µm	0.523
Ti-GR2	3	3.986 ± 2.043 µm	0.778

Table 3. Average contact angle and normality test for each material

Material	n	Contact angle	*P*
AISI 316 L ROD	3	64.845 ± 19.016 ^{o}	0.805
AISI 316 L COR	3	81.713 ± 0.706 ^{o}	0.816
AISI LVM	4	78.721 ± 2.833 ^{o}	0.513
Ti-V-Al	4	43.218 ± 4.586 ^{o}	0.648
Ti-GR2	3	47.735 ± 2.108 ^{o}	0.532

Table 4. Viability of osteoblast and fibroblasts and normality test for each material

Material	n	Osteoblast	*p*	Fibroblast	*p*
AISI 316 L ROD	3	68.25 ± 6.66 %	0.184	63.98 ± 6.76 %	0.682
AISI 316 L COR	3	76.23 ± 10.29 %	0.743	72.41 ± 7.64 %	0.980
AISI LVM	3	70.99 ± 3.54 %	0.991	77.87 ± 13.18 %	0.385
Ti-V-Al	3	84.79 ± 7.82 %	0.910	90.55 ± 1.27 %	0.634
Ti-GR2	3	78.57 ± 6.26 %	0.517	83.79 ± 10.28 %	0.939

Table 5. Correlation between surface roughness and contact angle and proliferation of osteoblast and fibroblast

	Viability	*P*	r
Surface Roughness	Osteoblast proliferation	0.014	0.857
	Fibroblast proliferation	0.003	0.929
Contact angle	Osteoblast proliferation	0.702	0.179
	Fibroblast proliferation	0.760	0.143

The result of viability of osteoblast and fibroblast are shown in Table 4.

Statistical analysis was conducted to determine whether there was a correlation between the results of surface roughness and contact angle and the results of viability of osteoblast cells and fibroblasts. The correlation analysis used Spearman's analysis. In Table 5, it is shown that there is a strong correlation between the average material surface roughness and the average viability of osteoblast ($p = 0.014$, $r = 0.857$) and there is a strong correlation between the average material surface roughness and the average viability of fibroblast ($p = 0.003$, $r = 0.929$). However, there is no correlation between the average material contact angle and the average viability of both osteoblast and fibroblast, with p value of > 0.05.

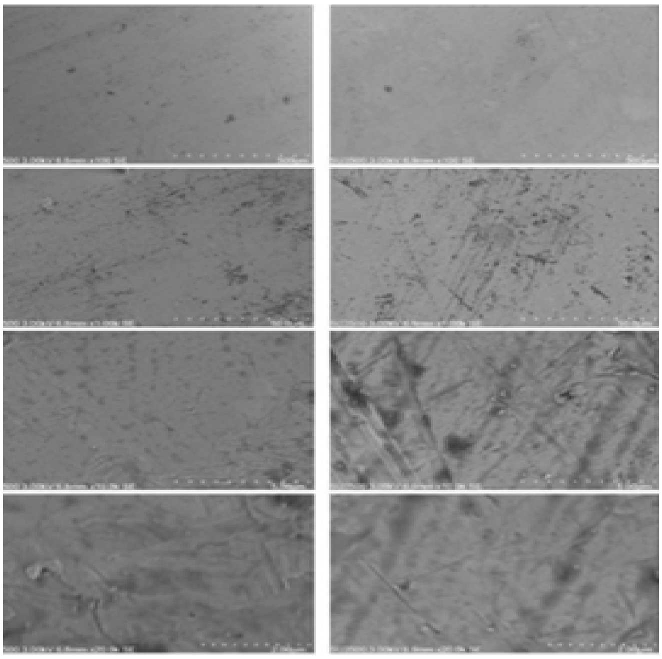

Figure 3. Comparison of SEM imaging at 100x, 1000x, 10000x and 20000x magnification of AISI 316L COR (Left) and AISI LVM (Right**)**

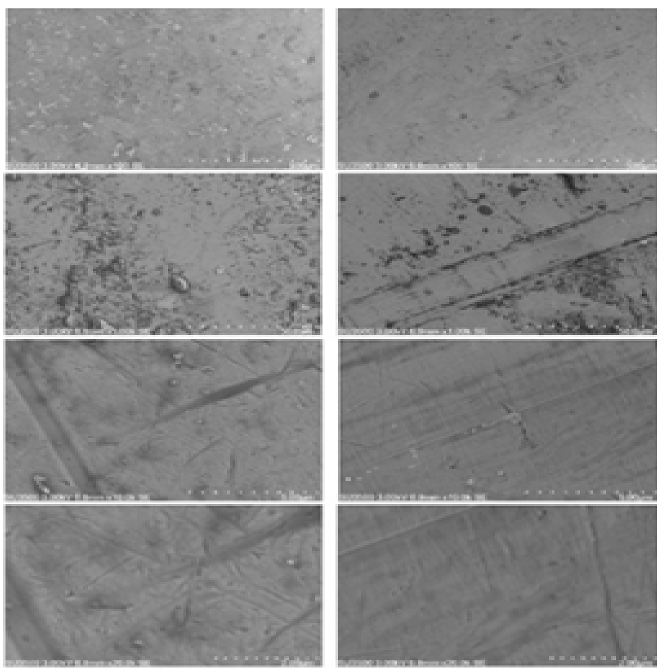

Figure 4. Comparison of SEM imaging at 100x, 1000x, 10000x and 20000x magnification of Ti-V-Al (Left) and Ti-Gr2 (Right)

DISCUSSION

According to the Table of surface roughness of several production methods in Oberg E. et al., 2012 the production of several materials in the present study is in accordance with the results of surface roughness that is common in the grinding process. The results of surface roughness for each type of material statistically showed a significant difference ($p = 0.01$) even though undergoing the same production process. This indicates that the base material of the material can also influence the results of surface roughness.

From the results of contact angle test, it was found contact angle that was less than 90^o, or hydrophilic, in all materials. This is in accordance with the basic nature of stainless steel which is hydrophilic.[7] In the study of Wang et al. It was also shown that the titanium alloy was hydrophilic, only the contact angle in the study was slightly larger, 70^o.[8] Similar results were also reported by Panjwani et al. with contact angle of 55.1^o, but the material in this study had reached the polishing stage.[9] Many things can affect the results of the contact angle, such as the measuring instruments used, surface roughness, surface heterogeneity, and the liquid used.[3] Hydrophobicity of the material surface affects the adhesion of bactericides, eukaryotic cells and proteins.[10]

The viability of osteoblast and fibroblast showed almost similar results where the highest viability was in the Ti-V-Al material and the lowest was in the AISI 316L ROD material. Similar result was also shown by a study of Sumarta et al. where the result of viability of fibroblast in stainless steel was 62.33% and titanium was 82.4%.[11] Gupta et al. showed the viability of fibroblast in Ti-6Al-4V that was ranging from 60-90% and in 316L Stainless Steel that was ranging from 70-90% after 48 hours in various surface modifications.[12] Fibroblast and osteoblast come from the same origin, namely mesenchyma, and knowing the viability of fibroblast and osteoblast may be used as a guide to the viability estimation of other cells from the same origin although in vitro condition cannot be equated with in vivo condition.[12]

In this study, the higher the surface roughness, the higher the viability of osteoblasts and fibroblasts. ($p = 0.014$, $r = 0.857$ for osteoblast viability and $p = 0.003$, $r = 0.929$ for fibroblast viability). This is similar to a study of Zareidoost et al. where titanium material that was given a rougher surface modification would produce higher osteoblast viability.[13]

CONCLUSION

The surface roughness of our local machinery metal-based materials is consistent with the standard of the common production method. The wettability of the local machinery metal-based materials is hydrophilic in nature. There is correlation between the surface roughness and the viability of human osteoblast and fibroblast. For further study, it is suggested to compare with the material from standard implant or performing in vivo instead of in vitro study.

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