Topographic characteristics of implants surface and osteoblast adhesion: a literature review

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Objective: This study aimed at correlating the topographical features of implants surface and osteoblast adhesion. **Methods:** a search for publications using the BIREME and PubMed databases was performed. The search was limited to in vitro studies published in English, in the last 5 years (January, 2010 to November, 2014). **Results:** A total of 145 abstracts were recovered and only 23 articles were selected after reading the title and abstract; 61% of the articles found a positive correlation between the roughness and the adhesion of osteoblasts and 39% of the studies found no correlation. Among the surfaces most cited by the selected articles, the machined surface was in the first place, with 56.5% of citations; followed by the microstructured surface, with 52.1%; biomimetics, with 30.4%; macrotexturized and nanotexturized, with 26% each. It was also observed different cell types used for osteoblast adhesion analysis, and different methods for adhesion analysis. **Conclusion:** Most of the selected studies showed that osteoblasts exhibit greater initial adhesion on roughened titanium surfaces and there was no standardization of the cell type used for osteoblast adhesion analysis and of the method used for adhesion analysis. **Keywords:** Dental implants, subperiosteal. Surface properties. Cell adhesion. Osteoblasts.

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» Patients displayed in this article previously approved the use of their facial and intraoral photographs.

INTRODUCTION

Titanium and titanium-related alloys have been the material of choice for dental implants in the last three decades due to having excellent physical properties, satisfactory corrosion resistance and favorable biocompatibility. However, titanium is not bioactive and requires a minimal 3-month period without being subjected to any type of load in order to achieve success in osseointegration. In order to decrease waiting time, implant surface treatment methods have been studied with a view to enhancing bioactivity and speed up osseointegration.[1]

Those methods encompass from different products/chemical substances to physical changes to the implant surface. Changes made to biomaterial surface composition have led to significant improvements in cell response, especially regarding osteoblast adhesion to implant surface, both in vitro and in vivo.[2]

Implant surfaces are classified into machined surface,[2-14] macrostructured,[6-17] microstructured,[5,6,16,17-22] nanostructured,[3,4,6,8] and biomimetic surface.[1,2,7,11,22]

Smooth or machined surfaces are characterized by smoothness due to being subjected to machining processes. However, once the process is over, grooves can be detected, thus resulting in surfaces not 100% smooth.[2-14]

Macrostructured surfaces, on the other hand, are associated with roughness values greater than 10 micrometers (μm). In order to achieve such roughness, surface treatment methods, such as plasma spray (titanium, hydroxyapatite or fluorhydroxyapatite) or particle blasting (aluminum oxide, titanium oxide or calcium phosphate), are carried out. The particle blasting procedure involves surface modification as a result of bombarding specific particles at high speed. Roughness pattern is directly related to the size of the macrostructured particle.[6,9,14-17]

At present, one of the most widely employed surface treatment methods is hydroxyapatite (HA) plasma spray. A new surface with a triple-layer coating consisted of hydroxyapatite (HA), fluorhydroxyapatite (FHA) and TiO_2, manufactured by means of the bio-gel method, was developed by He et al.[1] Those three different layers provide optimal balance between biocompatibility and stability of coating surfaces.

Microstructured surfaces have surface roughness values ranging from 1 to 10μm. In order to achieve that type of surface, acid etching or a combination of acid etching and particle blasting is carried out, with the latter providing macrostructuring while the former provides microstructuring. Additionally, Selective Laser Sintering might also be carried out to produce this type of surface.[5-14]

Nanostructured surfaces (1 to 2μm) have subtler surface roughness interfering in adsorption of proteins and cells involved in the process of osseointegration with osteoblasts. In order to produce that type of surface, implant surface treatment is carried out by means of anodic oxidation, an electrochemical process resulting in thicker oxide layer (TiO_2) and increased roughness. The oxidation process modifies the oxide layer, thus providing better cell adhesion and guidance, in addition to speeding up osseointegration.[3,4,6]

Lastly, biomimetic surfaces are produced by means of heterogeneous precipitation of calcium phosphates, such as hydroxyapatite, onto metallic substrates immersed in blood plasma-like ion solutions, so as to enhance implant osseointegration.[1,2,7,11]

The connection between implant surface properties and in vitro osteoblast response remains unclear. Therefore, the objective of the present study is to carry out a critical analysis on

the topographical characteristics of implant surface and osteoblast adhesion, with a view to identifying different implant surfaces and the most appropriate one relative to osteoblast adhesion.

METHODS

An electronic search was conducted in BIREME and PubMed databases in June, 2015. The following keywords were used: "Dental Implants;" "Surface Properties;" "Cell Adhesion;" and "Osteoblasts." Research was limited to studies published in English from January/2010 to November/2014, as well as in vitro studies.

Articles found in BIREME and PubMed databases were compared and studies selected according to previously determined inclusion as well as exclusion criteria. Titles and abstracts were read for initial selection. The article was fully read only if both title and abstract were seen as inconclusive.

RESULTS

A total of 145 abstracts were retrieved as a result of search conducted in BIREME and PubMed databases. Out of this total, only 23 articles were selected after title and abstract reading. The the main reasons for exclusion were studies not focusing on osteoblastic cell adhesion, studies carried out by means of other methods but in vitro, studies published in both databases.

Table 1 shows a compilation of all 23 selected articles. It comprises the following: author/year, types of surface assessed, types of osteoblastic cells used for adhesion analysis, methods of osteoblast adhesion analysis, and conclusion.

Out of 23 articles (100%), 61% (14 articles) found a connection between surface roughness and osteoblast adhesion, whereas nine articles (39%) found no such connection.

Tables 2, 3 and 4 represent the abstracts of all selected articles, and disclose the following: types of surfaces assessed by each author, osteoblastic cell lineages used for osteoblast adhesion analysis, and methods of adhesion analysis employed by each author.

Table 3 shows the different types of cells of osteoblastic lineage used by the studies. Out of the 23 selected studies, 21.7% (5 articles) used hMSCs cells; 21.7% (5 articles) used MC3T3-E1 cells; 17.4% (4 articles) used SAOS-2 cells; 13% (3 articles) used MSCs; 4.3% (1 article) used osteoblast-like cells (from mouse skull); 4.3% (1 article) used rat osteosarcoma cell line (UMR-106); 4.3% (1 article) used human osteoblast-like sarcoma cells (HOS); 4.3% (1 article) used human osteoblasts (hFOB); 4.3% used human osteoblasts (HOB); and 4.3% (1 article) used MG-63 (human osteosarcoma cells).

Table 4 shows all methods of osteoblast adhesion analysis used in the selected articles.

Table 1: Compilation of the 23 articles selected in this literature review.

AUTHOR/YEAR	TYPE OF SURFACE	TYPE OF CELL
Xing et al.[3], 2014	Nanostructured surface (treatment with aqueous NaOH 2.5, 5.0, 7.5, 10.0 and 12.5 M solution) x CP Titanium Degree 2 (machined surface)	MSCs (mesenchymal stem cells or mesenchymal stromal cells)
He et al.[1], 2014	Surface treated with hydroxyapatite (HA), fluorhydroxyapatite (FHA), and TiO2 (Biomimetic surface) x HA-treated surface (Biomimetic surface)	Osteoblast-like cells (from mouse skull)
Kim et al.[18], 2013	SLA surface + Mg ion (Mg-SLA Ti) (Microstructured surface) x SLA surface (SLA Ti) (Microstructured surface)	hMSCs (human mesenchymal stem cells)
Zuo et al.[4], 2013	Machined surface (Ti-m) RA: 345.73 x Surface treated by polishing with abrasive paper (P1200 grit) and alumina (Ti-p) RA: 67.22 x Surface treated by dielectric barrier discharge (Ti-tr) (Nanostructured surface) RA: 187.44	MC3T3-E1 (mouse osteoblastic cell)
Chen e Ko[5], 2013	Machined surface (G) x Machined surface + silane coupling agents (S) x Machined surface + silane coupling agents + RGD peptide (P) x SLA surface + silane coupling agents + RGD peptide (SLA-P) (Microstructured surface)	hMSCs (human mesenchymal stem cells)
Santander et al.[2], 2012	Machined surface x Biomimetic surface (Al oxide blasting + Anodization with electrolyte solution rich in Ca and P)	hMSCs (human mesenchymal stem cells)
Conserva et al.[6], 2013	Machined surface x Blasted surface (Macrostructured) x Acid etching-treated surface (Microstructured) x SLA surface (Microstructured) x Anodic oxidation-treated surface (Nanostructured)	SAOS-2 (human osteoblastic osteosarcoma cell)
Zhang et al.[19], 2012	Blasted surface + double acid etching with H2O2/HCl (Microstructured) x Blasted surface + thermally treated with double acid etching with H2O2/HCl (Microstructured)	MC3T3-E1 (mouse osteoblastic cells)
Lavenus et al.[23], 2011	30-nm diameter nanopore surface x 150-nm diameter nanopore surface x 300-nm diameter nanopore surface	hMSCs (human mesenchymal stem cells)
Annunziata et al.[15], 2011	Titanium plasma spray (TPS)-treated surface (Macrostructured) x TiN-coated titanium plasma spray (TiN-TPS)-treated surface (Macrostructured)	MSCs (mesenchymal stem cells or mesenchymal stromal cells)
Chung et al.[7], 2011	Machined surface x Hydroxyapatite (HAp)-coated surface (Biomimetic) Hydroxyapatite + Sr (Sr-HAp)-coated surface (Biomimetic)	MC3T3-E1 (mouse osteoblastic cells)
Palaiologou et al.[8], 2012	Machined surface x Double acid etching-treated surface (Microstructured) x Nanostructured surface (Calcium phosphate-coated)	UMR-106 (rat osteosarcoma cell line)
Silva et al.[9], 2011	Machined surface x Plasma nitriding-treated surface (Macrostructured)	MC3T3-E1 (mouse osteoblastic cels)
Ramaglia et al.[10], 2011	Machined surface x SLA surface (Microstructured)	SAOS-2 (human osteoblastic osteosarcoma cell)
Mamalis e Silvestros[20], 2011	SLA surface (Blasting with specific particles + acid etching) (Microstructured) x SLActive surface (Blasting with specific particles + acid etching processed with nitrogen and stored in NaCl isotonic solution) (Microstructured)	hMSCs (human mesenchymal stem cells)
Kim et al.[11], 2011	Machined surface x Body-fluid-modified machined surface x Biomimetic surface	HOS (human osteoblast-like sarcoma cells)
Bello et al.[13], 2010	Machined surface x Surface subjected to thermal oxidation (Nanostructured surface)	hFOB (human osteoblasts)
Park et al.[12], 2010	Machined surface x HA-coating by aerosol deposition (Biomimetic)	HOB (human osteoblasts)
Guida et al.[16], 2010	Surface subjected to blasting with titanium oxide (Macrostructured) x SLA surface (Microstructured)	MSCs (mesenchymal stem cells or mesenchymal stromal cells)
Zhang et al.[21], 2010	Blasted surface + double acid etching (Microstructured) x Blasted surface + acid etching (Microstructured)	MC3T3-E1 (mouse osteoblastic cells)
Bucci-Sabattini et al.[22], 2010	SLA surface (Microstructured) x Biomimetic surface	SAOS-2 (human osteoblastic osteosarcoma cells)
Rosales-Leal et al.[14], 2010	Machined titanium (pTi) x Acid etching-treated surface (eTi) (Microstructured) x Surface subjected to blasting with particles (bTi) (Macrostructured) x Surface subjected to blasting combined with acid etching (beTi) (Microstructured)	MG-63 (human osteosarcoma cells)
Conserva et al.[17], 2010	Surface subjected to blasting with particles (Macrostructured) x Surface subjected to blasting combined with acid etching (GBAE) (Microstructured)	SAOS-2 (human osteoblastic osteosarcoma cells)

	METHOD OF ANALYSIS	CONCLUSIONS
	Fluorescence microscopy	Osteoblast adhesion was greater in NaOH-treated samples. The amount of osteoblasts increased with a higher NaOH concentration.
	SEM and optical microscopy	Greater osteoblast adhesion to HA/FHA/TiO2-treated surface due to HA outer layer. HA is a good osteoconductive substance.
	SEM	The amount of osteoblasts adhered to the Mg-SLA Ti surface was 2.15 times greater in comparison to the SLA Ti surface. This suggests that initial adhesion is affected by Mg ions.
	Fluorescence microscopy	Initial osteoblast adhesion within the first four hours was significantly greater to Ti-tr surface than Ti-p and Ti-m surfaces. There was no statistically significant difference in osteoblast adhesion when Ti-p and Ti-m surfaces were compared.
	SEM	Comparison between P and SLA-P reveal roughness is key to osteoblast adhesion.
	SEM	Better osteoblast adhesion to biomimetic surface than machined surface.
	SEM	All surfaces had SAOS-2 osteoblasts adhered.
	SEM	There was no statistically significant difference in adhesion between groups.
	SEM	There was greater osteoblast adhesion to 30-nm diameter nanopore surface.
	SEM	Satisfactory cell adhesion to both surfaces.
	SEM	Sr-HAp surface allowed greater osteoblast adhesion at early stages (four hours) than machined and HAp-coated surfaces.
	Fluorescence microscopy	Initial osteoblast adhesion was similar when machined and double acid etching-treated surfaces were compared. Decreased osteoblast adhesion was found in the nanostructured surface.
	SEM	Greater osteoblast adhesion to plasma nitriding-treated surface.
	SEM	There was statistically significant difference in osteoblast adhesion between surfaces. SLA surface allowed greater osteoblast adhesion.
	Spectrophotometry	There was no statistically significant difference in initial osteoblast adhesion between SLA and SLActive surfaces.
	SEM	Greater osteoblast adhesion to biomimetic surface.
	SEM	There was no statistically significant difference in osteoblast adhesion between machined surface and surface subjected to thermal oxidation.
	SEM	Osteoblast adhesion to both surfaces. Osteoblasts were more evenly distributed and found in a greater amount in the machined surface.
	SEM	There was no statistically significant difference in osteoblast adhesion between surfaces.
	SEM	There was greater osteoblast adhesion to the Blasted surface + double acid etching.
	SEM	Both surfaces proved to be satisfactory substrates for osteoblast adhesion.
	Atomic-force microscopy	Increased cell adhesion rates in all treated surfaces. 180 minutes and 24 hours later, contact and cell adhesion improved in both bTi and beTi surfaces.
	SEM	GBAE surface treatment and chemical composition allowed greater osteoblast adhesion.

Table 2: Types of surface, as cited by the authors.

TYPE OF SURFACE	AUTHORS
Machined surface	Xing et al.[3]; Zuo et al.[4]; Chen e Ko[5]; Santander et al.[2]; Conserva et al.[6]; Chung et al.[7]; Palaiologou et al.[8]; Silva et al.[9]; Ramaglia et al.[10]; Park et al.[12]; Kim et al.[11]; Bello et al.[13]; Rosales-Leal et al.[14]
Macrostructured surface	Conserva et al.[6]; Annunziata et al.[15]; Silva et al.[9]; Guida et al.[16]; Rosales-Leal et al.[14]; Conserva et al.[17]
Microstructured surface	Kim et al.[18]; Chen e Ko[5]; Zhang et al.[19]; Conserva et al.[6]; Palaiologou et al.[8]; Ramaglia et al.[10]; Mamalis e Silvestros[20]; Guida et al.[16]; Zhang et al.[21]; Bucci-Sabattini et al.[22]; Rosales-Leal et al.[14]; Conserva et al.[17]
Nanostructured surface	Xing et al.[3]; Zuo et al.[4]; Conserva et al.[6]; Lavenus et al.[23]; Palaiologou et al.[8]; Bello et al.[13]
Biomimetic surface	He et al.[1]; Santander et al.[2]; Kim et al.[18]; Chung et al.[7]; Park et al.[12]; Kim et al.[11]; Bucci-Sabattini et al.[22]

Table 3: Types of cells used to assess adhesion, as cited by the authors.

TYPE OF CELL	AUTHORS
hMSCs (human mesenchymal stem cells)	Kim et al.[18]; Chen e Ko[5]; Santander et al.[2]; Lavenus et al.[23]; Mamalis e Silvestros[20]
MC3T3-E1 (mouse osteoblastic cells)	Zuo et al.[4]; Zhang et al.[19]; Chung e Long[7]; Silva et al.[9]; Zhang et al.[21]
SAOS-2 (human osteoblastic osteosarcoma cells)	Conserva et al.[6]; Ramaglia et al.[10]; Bucci-Sabattini et al.[22]; Conserva et al.[17]
MSCs (mesenchymal stem cells or mesenchymal stromal cells)	Xing et al.[3]; Annunziata et al.[15]; Guida et al.[16]
Osteoblast-like cells (from mouse skull)	He et al[1]
UMR-106 (rat osteosarcoma cell line)	Palaiologou et al.[8]
HOS (human osteoblast-like sarcoma cells)	Kim et al.[11]
hFOB (human osteoblasts)	Bello et al.[13]
HOB (human osteoblasts)	Park et al.[12]
MG-63 (human osteosarcoma cells)	Rosales-Leal et al.[14]

Table 4: Methods of osteoblast adhesion analysis.

METHOD OF ADHESION ANALYSIS	AUTHORS
SEM	He et al.[1]; Kim et al.[18]; Chen e Ko [5]; Santander et al.[2]; Zhang et al.[19]; Conserva et al.[6]; Kim et al.[11]; Lavenus et al.[23]; Annunziata et al.[15]; Chung et al.[7]; da Silva et al.[9]; Ramaglia et al.[10]; Park et al.[12]; Bello et al.[13]; Guida et al.[16]; Zhang et al.[19]; Bucci-Sabattini et al.[22]; Conserva et al.[17]
Optical Microscopy	He et al.[1]
Fluorescence Microscopy	Xing et al.[3]; Zuo et al.[4]; Palaiologou et al.[8]
Spectrophotometry	Mamalis e Silvestros[20]
Atomic-force Microscopy	Rosales-Leal et al.[14]

DISCUSSION

With a view to enhancing osseointegration, a number of implant surface titanium treatments have been proposed. As a result, studies aimed at assessing the best surface treatment have been carried out.[2]

Surface roughness seems to have an influence on osteoblastic cell behavior. In the present study, the majority of articles (61%) revealed osteoblasts have greater initial adhesion to rough titanium surfaces.[5,21,23]

However, 39% of them evinced osteoblast adhesion did not improve in rougher surfaces[8] or there was no statistically significant difference in adhesion when surfaces were compared.[6,13,22]

Palaiologou et al[8] assessed machined, microstructured and nanostructured surfaces in terms of osteoblast adhesion and found decreased adhesion to the nanostructured surface. This finding corroborates the outcomes achieved by Park et al.[12] The authors also concluded that rougher surfaces provide less osteoblast adhesion.

Topography, especially implant surface nanotopography, can play a relatively more important role in cell adhesion than surface chemical treatment. A possible explanation for this phenomenon is discussed by Zuo et al.[4] In their study, the authors propose comparing osteoblast adhesion to three different surfaces.

Surface treatment by means of thermal oxidation increases corrosion resistance and decreases ion release as a result of providing a thicker oxide layer.[13] Bello et al[13] assessed osteoblast adhesion on machined surfaces as well as surfaces subjected to thermal oxidation by means of SEM. The authors found no statistically significant difference in osteoblast adhesion.

Kim et al[11] compared machined surfaces with body-fluid-modified and biomimetic surfaces, particularly regarding osteoblast adhesion. The authors found greater adhesion to the biomimetic surface. On the other hand, Bucci-Sabattini et al[22] carried out a study comparing surface treated with a combination of blasting and acid etching and biomimetic surface treated with calcium phosphate precipitation. The authors concluded that cell behavior was acceptable in both cases.

The use of different types of cells, the comparison among different types of surfaces, and different methods of adhesion analysis can lead to distinct research outcomes. The articles selected for the present study focused on five surface treatment methods, as well as different methods of adhesion analysis. Additionally, ten different types of osteoblast precursor cells were identified. As a result of assessing this number of variables, we are able to notice the reason why no consensus has been reached in the literature on this matter.

Out of all studies using hMSCs cells, only Mamalis and Silvestros[20] found similar adhesion among surfaces subjected to comparison. There was no correlation between adhesion and roughness. As for studies using MC3T3-E1 cells, only Zhang et al[19] found no statistically significant difference in osteoblast adhesion among all assessed surfaces. In studies using SAOS-2 cells, both Conserva et al[6] and Bucci-Sabattini et al[22] found no difference in adhesion among treated surfaces. As for studies using MSCs cells, those conducted by Annunziata et al[15] and Guida et al[16] found no differences in adhesion among assessed surfaces. Both the study by Palaiologou et al[18] assessing osteoblast adhesion with rat osteosarcoma cell line (UMR-106) and the study by Park et al[12] using human osteoblasts (HOB) found no connection between osteoblast adhesion and rougher surfaces.

For osteoblast adhesion analysis, the following methods were used: scanning electron microscopy (SEM), optical microscopy, fluorescence microscopy, spectrophotometry, and

atomic-force microscopy. Lack of standardization of methods of analysis might have led to discordant results on osteoblast adhesion to rougher surfaces.

FINAL CONSIDERATIONS

Out of 23 selected articles, 61% revealed osteoblasts have greater initial adhesion to rough titanium surfaces. Although most articles suggest that osteoblast adhesion is greater with increased roughness, 39% of them disagree with this finding. This is mainly due to lack of standardization of not only the types of cells subjected to osteoblast adhesion analysis, but also the methods of adhesion analysis and the variety of surface treatment modalities.

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