Titanium Surfaces with Nanostructures Influence on Osteoblasts Proliferation: a Systematic Review

Maxim Goldman¹, Gintaras Juodzbalys¹, Valdas Vilkinis²

¹Department of Maxillofacial Surgery, Lithuanian University of Health Sciences, Kaunas, Lithuania.
²Private practice, Kaunas, Lithuania.

Corresponding Author:
Maxim Goldman
Vilniaus 70-2, LT-44291, Kaunas
Lithuania
Phone: +370 643 76375
Email: maxik1984@gmail.com

ABSTRACT

Objectives: Nanothechnology found to be increasingly implemented in implantology sphere over the recent years and it shows encouraging effect in this field. The aim of present review is to compare, based on the recent evidence, the influence of various nanostructure surface modifications of titanium for implants, on osteoblasts proliferation.

Material and Methods: A literature review of English articles was conducted by using MEDLINE database restricted to 2009 - 2014 and constructed according PRISMA guidelines. Search terms included “Titanium implant”, “Titanium surface with nanostructure”, “Osteoblast”. Additional studies were identified in bibliographies. Only in vitro and/or in vivo studies on nano structured implant surfaces plus control sample, with specific evaluation method for osteoblasts proliferation and at least one Ti sample with nanostructure, were included in the review.

Results: 32 studies with 122 groups of examined samples were selected for present review. Each study conducted in vitro experiment, two studies conducted additional in vivo experiments. All studies were dispensed by type of surface modification into two major groups; “Direct ablative titanium implant surface nano-modifications” with 19 studies and ”Nanocomposite additive implant surface modifications” with 13 studies. Overall 24 studies reporting on positive effect of nanostructured surface, 2 studies found no significant advantage and 6 studies reported on negative effect compared to other structure scales.

Conclusions: From examination of selected articles we can notice marked advantage in implementation of various nanostructures onto implant surface. Yet for discovering the ultimate implant surface nanostructure, further comparable investigations of Ti surface nanostructures need to be done.

Keywords: dental implants; nanotechnology; nanostructured materials; osteoblasts; cell proliferation.
INTRODUCTION

Dental implant treatment is very widely spread and reliable treatment that provides good clinical results with high success rates over 90% [1-2]. Titanium is commonly used as an implant material as it has high biocompatibility and bonding ability with the bone. These characteristics were found in 1952 by the Swedish scientist Per-Ingvar Brånemark [3]. Since then, the results of many studies have demonstrated that titanium has high biocompatibility. Titanium has no adverse effect on the human body and bonds readily with the new bone, which penetrates into the titanium surface [4,5].

Implant survival rate and prognosis depends on quality of osseointegration as more direct bone-to-metal interface take place without interposition of non-bone tissue [6]. However, differences in bonding force between the implant body and bone occur depending on the differences in surface structures of the implant. Titanium surfaces play an important role in affecting osseointegration of dental implants. Many studies have concluded that certain characteristics of the implant surface play an important role in altering the quality of osseointegration [7-9]. It is commonly thought that the slightly roughened implant surface allows better osseointegration compared with the smooth implant surface [10,11]. Moreover nanostructured materials have shown increased cell attachment over microstructured or smooth surfaces [12,13].

An essential role of osseointegration processes is played by osteoblast progenitor stem cells during recruitment, adhesion, proliferation, differentiation, and mineralized matrix deposition during bone regeneration phases [14,15]. Nanoporous topography tend to help the proliferation processes, acting directly on the selective adhesion of osteoblastic cells on the surface, which can accelerate the healing process around implants [16,17]. Low osteoblasts cell number and proliferation have been closely associated with negative results when considering it to osseointegration [18,19].

Many studies were conducted to investigate various implant surface nanostructures and their influence on cell behaviour as proliferation, in contrast to other scopes of surface structures dimensions [20]. Many studies were conducted to compare different nanostructured morphologies as well. However, the optimal implant surface nanostructure covering for osteoblasts proliferation is yet to be established. The aim of the present review is to compare, based on the recent available evidence, the influence of various nanostructure surface modifications of titanium for implants, on osteoblasts proliferation.

MATERIAL AND METHODS

Protocol and registration

The review is registered in international prospective register of systematic reviews ‘PROSPERO’ [21]. The protocol can be accessed at: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42014009436. Registration number: CRD42014009436.

Eligibility criteria

Types of studies

The review included laboratory research studies, in vitro studies that using cells from human or animals and in vivo studies on animals. Studies published on English language between January 2009 and June 2014 with various evaluation methods for osteoblasts proliferation and various evaluation intervals between hours and days. Letters, reviews and abstracts were excluded.

Information sources

The information source was MEDLINE (PubMed) database.

Search

Pubmed resource database was explored through advanced search. The search terms those were used: “Titanium implant”, “Titanium surface with nanostructure”, “Osteoblast”. For more recent and updated information, search included only articles that were published from January 2009 to June 2014 to ensure sensitivity of the review. Additional simultaneous manual screening for related articles was performed. (Figure 1) illustrates the flow diagram of present articles selection according to PRISMA guidelines [22].

Study selection

Inclusion and exclusion criteria

Inclusion criteria for the selection were:
- In vitro and/or in vivo studies.
- Nano structured implant surface + control sample.
- Studies with specific evaluation method for osteoblasts proliferation.
At least one Ti sample with nanostructure must be included in the study.

Exclusion criteria for the selection were:
- No osteoblasts proliferation described.
- Investigation of microstructured surfaces.
- Nanostructure and morphology has not been described.
- Organic coating has been used.

The search displayed 97 results from which 72 abstracts were screened (Figure 1). A total of 55 articles were reviewed in full. Preliminary exclusion was made by the title and its relevancy, later by abstract and its relevancy. Finally, articles that did not meet the inclusion and exclusion criteria, where filtered as followed: No proliferation described (n = 12), Microstructured surface analyzed (n = 4), No titanium sample included (n = 3), Nanostructure and morphology has not been described (n = 1), Organic coating has been used (n = 5). Additional manual selection from references according eligibility was performed (n = 2).
Data collection process

Data was independently extracted from reports in form of variables according the aim and themes of present review as listed onwards.

Data items

Variables on which data were sought are as follow: “TYPE OF STUDY”, indicates whether it was in vivo or in vitro or both and materials respectively. “SAMPLE”, describes the number of particular investigated samples in the study and its singularity (e.g., A-machined, B-polished, C-acid etched). “TOPOGRAPHY”, describes the nanoscale topography of the nanostructures on the surface of the sample, can be interrelated with SAMPLE description of nanostructure (e.g., nanograins 100nm). “EVALUATION”, describes evaluation methods and duration of osteoblasts proliferation cultured on the sample (e.g., Histology, 24, 48 and 72 hours). “RESULT”, describes the impact of surface structure on osteoblast proliferation. Description of nanostructure peculiarities can vary from study to study and may be located under SAMPLE column or TOPOGRAPHY or both.

Risk of bias assessment

Risk of bias (e.g., lack of information or selective reports on variables of interest) was assessed on study level. The risks were indicated as lack of precise information of interest in each individual study that can blind the reader from particular information about examined samples.

The Cochrane Collaboration’s tool for assessing risk of bias [23] was used to assess bias across the studies that can affect cumulative evidence. Particularly “Blinding of outcome Assessment” and “Selective reporting”.

Synthesis of results

Relevant data of interest according stated previously variables, was collected and organized in two tables that divided according type of implant surface modification. The tables include results according individual evaluation of osteoblast proliferation.

Additional analyses

Separation of articles by their samples fabrication methods of nanostructured surfaces into two groups can provide possibility for simple comparison. Numbers of samples that provide positive or negative effect on osteoblasts proliferation are assessed in each article for each modification method. In addition samples of three topography types (e.g., control, microstructured and nanostructured) are included in each modification methods groups for better understanding of nanostructured surface superiority. Quantitative and relative comparison between examined groups of samples and illustration of their relative efficiency in cells proliferation by means of diagrams.

RESULTS

Study selection

The search displayed 97 results from which 72 abstracts were screened. A total of 55 articles were reviewed in full. Preliminary exclusion during screening stage was made by the titles and abstracts relevancy (n = 13) and (n = 4) respectively. During eligibility stage articles that did not meet the inclusion and exclusion criteria, where filtered as followed: No proliferation described (n = 12), Microstructured surface analyzed (n = 4), No titanium sample included (n = 3), Nanostructure and morphology has not been described (n = 1), Organic coating has been used (n = 5). Additional relevant articles were added after manual selection from references according eligibility (n = 2). Finely 32 studies with 122 groups of examined samples were included in present review (Figure 1).

Study characteristics

All 32 studies finally selected for the review were in vivo and in vitro studies published in English with description of osteoblast proliferation. Each study conducted in vitro experiment by culturing osteoblasts on several investigated samples and controls when three studies conducted additional in vivo experiments. All authors except four, reported on conducting the experiments at least twice to ensure statistical validity.

The duration of osteoblasts proliferation evaluation varied from 2 hours to 14 days across all the studies except three articles of Gittens et al. [24-26] where evaluation was performed after culture confluence. The main evaluation methods for osteoblasts proliferation across the studies were: histology, MTT assay [27], alamarBlue™ assay [28], DNA assay and WST-1 or BrdU marker evaluations [29,30], additional visual evaluation by scanning electron microscope (SEM) was described by six authors,
when Tetè et al. [31] uses SEM as main evaluation method.

All examined studies were assessed for specific variables described previously and were further divided into two groups characterized by type of implant surface modification. The division provided better understanding of nanosurface structure characteristics and contributed to sensitivity of the review.

First group “Direct ablative titanium implant surface nano-modifications” deals with titanium implant samples that were treated by various methods directly without addition of other materials to the implant surface, include 19 studies with 70 samples (Table 1).

Second group ”Nanocomposite additive implant surface modifications” deals with samples that were treated by addition of variable non organic particles, includes 13 studies with 50 samples, showed in Table 2 respectively.

Risk of bias within studies

Only 22 from 32 studies fulfilled the expected markers of validity. The risk of bias that indicated within other 10 studies presented as lack of information values that grouped as followed: “Nano scale topography was not indicated”, “Evaluation methods with timing description” and “Significance of experiments indicated in the study result (P < 0.05) (Table 3).

Synthesis of results

Present review focused on describing the studies, their results and qualitative synthesis rather than meta-analysis because the analyzed studies were not presented with clear quantitative results of osteoblasts proliferation, furthermore examined samples, evaluation methods and duration varied markedly.

Overall 24 studies with 89 examined samples from which 63 are various nanostructured patterns, reporting positive effect of 33 nanostructure modified Ti samples, thus enhance osteoblasts proliferation on nanostructured features with significant differences (P < 0.05) between nanostructured surface, and microstructured or smooth control surfaces in each study.

No significant difference in positive effect on proliferation of cells cultured on nanostructured samples compared to microstructured or smooth control samples, was described in 2 studies with 6 examined samples; one study reports no significant differences between all three examined samples, another study result reveals equal proliferation on smooth sample and sample with micronanohybrid surface, whereas microstructured sample showed impaired proliferative activity.

Negative effect was described in 6 studies with 27 samples from which 15 are nanostructured samples, when results vary from, microstructured surface that promote osteoblasts proliferation to smooth or control samples showed better proliferation results.

Risk of bias across studies

All studies examined didn’t show numerical data on the osteoblast proliferation results, what did not allow us to estimate precisely the advantage of one nanostructured sample on another.

All reviewed studies except two indicated significance of their results by (P < 0.05) what can be interpreted as study’s quality guaranty, but as mentioned previously, absence of quantitative results and calculations prevent conformation of significance and comparison across the studies by the reviewer.

In addition there are 10 studies with 41 examined samples were we could not find exact information as, nanoscale topography of the specific surface structure and/or evaluation timing. Review with and without inclusion of these studies found no differences in the patterns of our review results but only leave the reader blinded by lack of specific features of investigated sample. One study selectively reports on specific result from complete outcome [32].

Additional analysis

The division of articles into two groups by surface modification type contributed to better understanding of nanosurface structure characteristics and provide possibility for comparison between two main nanostructure modification methods (Table 4).
Table 1. Direct ablative titanium implant surface nano-modifications

<table>
<thead>
<tr>
<th>Author</th>
<th>Structure (sample)</th>
<th>Type</th>
<th>Topography</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gittens et al. 2012 [14]</td>
<td>PT-smooth</td>
<td>In vivo</td>
<td>MG63</td>
<td>Cell number, which decreases as cells transition from a proliferative to a more mature state, was lower for MG63s on the micro-rough surfaces compared to the micro-smooth control, with the lowest levels on the combined micro-rough SLA and nanostructured NMsAIav surfaces. P &lt; 0.05</td>
</tr>
<tr>
<td>Gittens et al. 2013 [5]</td>
<td>SLA — NMIT (45 μm) proteoblast — NMIT (90 μm) and NMIT (180 μm) corner str. - NMsAIAV</td>
<td>In vivo</td>
<td>MG63 cell</td>
<td>The number of MG63 osteoblast from data measurements for the NMsAIAV, SLA and NMsAIAV samples were lower than for the PT. This reduction in cells paralleled an increase in mean nanoscale roughness (NMsAIAV vs. PT) and the microscale roughness (SLA and NMsAIAV vs. PT). P &lt; 0.05</td>
</tr>
<tr>
<td>Zhao et al. 2013</td>
<td>- Acetich-Ti surface - Acid-etched-TiO2 deposits-UV treated</td>
<td>In vitro</td>
<td>Rat BMs</td>
<td>Increase in the proliferative activity of cells on the nanoscale features both before and after UV treatment, with that on the 300 nm nanoscale being the greatest. P &lt; 0.05</td>
</tr>
<tr>
<td>Han et al. 2011</td>
<td>- Ti6Al4V Smooth - Ti6Al4V porous</td>
<td>In vivo</td>
<td>MC3T3-E1</td>
<td>The growth curves showed that the osteoblasts on nanoporous Ti6Al4V substrate appeared to have a not only higher but also longer growth phase compared to those cultured on any other surface. P &lt; 0.01</td>
</tr>
<tr>
<td>Yu et al. 2010 [37]</td>
<td>- Smooth</td>
<td>In vitro</td>
<td>MC3T3-E1 Mouse pre OB</td>
<td>The proliferation of osteoblast cultured on anatase or rutile/titane nanotube layered showed significantly higher than smooth layer and anatase nanotube layers, which means the crystal structure of nanotube layers can over-rule the chemistry effect and plays a major role in cell proliferation and mineralization. P &lt; 0.01</td>
</tr>
<tr>
<td>Zhu et al. 2011 [40]</td>
<td>- Nanotubes - Microstructure - Flat (control)</td>
<td>In vitro</td>
<td>Rat OB</td>
<td>The number of adhered cells on the smallest 30 nm diameter nanotubes was notably higher than all the other sizes of nanotubes, but the cells started to further elongated on nanotubes diameters above 70 nm. P &lt; 0.05</td>
</tr>
<tr>
<td>Ranis et al. 2012 [44]</td>
<td>- Nanotubes</td>
<td>In vitro</td>
<td>MC3T3-E1</td>
<td>The large nano-sawtooth structure approximately 30 nm produced the largest cell responses, including adhesion, proliferation, and differentiation performances. P &lt; 0.05</td>
</tr>
<tr>
<td>Ross et al. 2013 [57]</td>
<td>- Smooth</td>
<td>In vitro</td>
<td>MC3T3-E1</td>
<td>The proliferation rate of preosteoblasts was statistically similar at 24h and statistically lower on nano-foveolae structures at 72h. P &lt; 0.05</td>
</tr>
<tr>
<td>Hori et al. 2010 [62]</td>
<td>- Smooth (machined) - Micros</td>
<td>In vitro</td>
<td>MC3T3-E1 Mouse pre OB</td>
<td>The proliferation rate of preosteoblasts was statistically similar at 24h and statistically lower on nano-foveolae structures at 72h. P &lt; 0.05</td>
</tr>
</tbody>
</table>

Note: The table above summarizes various studies on the effects of different surface modifications on osteoblast activity. Each row corresponds to a different study, with the columns indicating the type of surface modification, cell type used, and the results observed. The studies range from in vitro to in vivo models, with various cell types used, such as MG63, Rat BMs, and MC3T3-E1. The results include changes in cell proliferation, adhesion, and differentiation, with statistical significance indicated by P-values. The table highlights the importance of surface roughness and nanostructure in influencing osteoblast behavior on titanium implants.
### Table 2. Nanocomposite additive implant surface modifications

<table>
<thead>
<tr>
<th>Autor</th>
<th>Structure (sample)</th>
<th>Topography</th>
<th>Type</th>
<th>Evaluation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocchini et al. 2013 [32]</td>
<td>- A-Not porous - B-Mesoporous - C-Mesoporous + Mg</td>
<td>- Not indicated - 6 mm pore - 6 mm pore</td>
<td>In vitro</td>
<td>hFOB (human fetal OB)</td>
<td>- MTT assay at 24 h - SEM 1 to 24 h X 3 times</td>
</tr>
<tr>
<td>Zhao et al. 2011 [39]</td>
<td>- TiO2-N TiO2-Al0.5M - NT-Ag 1.0 M - NT-Ag 1.5 M - NT-Ag 2.0 M - Flat-Ti</td>
<td>- NT-130 nm - NT-130 nm - NT-130 nm - NT-130 nm - Not indicated (All Ag particles 10 to 20 nm amount increase with concentration)</td>
<td>In vitro</td>
<td>Primary rat OB</td>
<td>- Histology - DNA analysis 1 and 4 days</td>
</tr>
<tr>
<td>Zhao et al. 2013 [46]</td>
<td>- TiO2 coating - Nb2O5 doped TiO2 - SiO2 doped TiO2 coating</td>
<td>- grains &lt; 50 nm - nanoplates - hairy protrusions</td>
<td>In vitro</td>
<td>Primary HOBs</td>
<td>- alamarBlue™ assay 2 to 24 h, 3 d, 7 d, 14 d</td>
</tr>
<tr>
<td>Roy et al. 2011 [47]</td>
<td>- HA coating Ti - Sr-HA coating Ti</td>
<td>- 23 ± 3.9 nm grains - 21 ± 3.7 nm grain - 24 ± 3.5 nm grain</td>
<td>In vitro</td>
<td>hFOB1.19 cells</td>
<td>- Histology - MTT assay 3, 7, 11 days X 3 times</td>
</tr>
<tr>
<td>Zhou et al. 2013 [50]</td>
<td>- Nanoporous TiO2 - S67 interspace - S96 interspace - S137 interspace</td>
<td>- Not indicated - diam 71.4 nm - diam 68.9 nm - diam 67.6 nm</td>
<td>In vitro</td>
<td>hFOB1.19 (human fetal OB)</td>
<td>- MTT assay 3, 7, 14 days X 3 times</td>
</tr>
<tr>
<td>Bayram et al. 2012 [49]</td>
<td>- Ti - TiN (nanotubes) - TiN-SBF(1 h) HA - TiN-SBF(2 h) HA - TiN-SBF(3 h) HA - TiN-SBF(5 h) HA - TiN-SBF0.1J/HA</td>
<td>- Not indicated - 45 ± 50 nm diameter 10 nm wall - 1 to 2 μm HA particles</td>
<td>In vitro</td>
<td>Sae-2/An1 (OB like human bone osteogenic sarcoma cell)</td>
<td>- MTT assay 3, 5, 7 days X 3 times</td>
</tr>
<tr>
<td>Portan et al. 2012 [60]</td>
<td>- A-Ti - Bi-TiO2 nanotube - C-TiO2; nanoHA</td>
<td>- Not indicated - 80 to 200 nm - according SEM image</td>
<td>In vitro</td>
<td>Human Bone marrow cells</td>
<td>- Histology - SEM 1 week incubation</td>
</tr>
<tr>
<td>Gu et al. 2012 [51]</td>
<td>- A-Bare Ti (control) - B-nanotub - C-nanotub-HA</td>
<td>- Not indicated - 90 nm</td>
<td>In vitro</td>
<td>MC3T3-E1</td>
<td>- MTT proliferation assay 1, 4, 7 days</td>
</tr>
<tr>
<td>Dimitrijevska et al. 2011 [52]</td>
<td>- UncorTei64 (ertl.) - HA coating - TiO2 coating - TiO2-HA coating</td>
<td>- only roughness indicated</td>
<td>In vitro</td>
<td>hMSC-derived OB</td>
<td>- Histology - SEM</td>
</tr>
<tr>
<td>Wang et al. 2012 [53]</td>
<td>- Ti controls - nHA coated Ti - BS-WCNT Ti - nHA-N-SWCNT Ti</td>
<td>- Not indicated - 20 to 30 nm Rod HA</td>
<td>In vitro</td>
<td>hFOB (human fetal OB)</td>
<td>- Histology (fluorescence microscopy) 1, 3 and 5 days X 3 times</td>
</tr>
<tr>
<td>Tran et al. 2010 [54]</td>
<td>- Uncoated Ti - Low-SrSe-Ti - Medium-SrSe-Ti - High-SrSe-Ti</td>
<td>- low density - medium density - high density</td>
<td>In vitro</td>
<td>PHCO</td>
<td>- Histology fluorescence microscopy 4, 17, 24, 40, 53 and 65 h X 3 times</td>
</tr>
<tr>
<td>Mazzaola et al. 2011 [55]</td>
<td>- UncorTiO2 (IPPla ion plating plasma assisted deposition)</td>
<td>- Not indicated</td>
<td>In vitro</td>
<td>hFOB1.19</td>
<td>- DNA assay 24 h</td>
</tr>
<tr>
<td>Hu et al. 2013 [56]</td>
<td>- A-Ti pure - B-TiO2 - C-TiO2-CaSiO3</td>
<td>- Not indicated - nanograins 20 to 100 nm - CaSiO3 nanocrystals</td>
<td>In vitro</td>
<td>MG63</td>
<td>- SEM 1, 3, 5, and 7 days</td>
</tr>
</tbody>
</table>
### Table 3. Assessment of the risk of bias

<table>
<thead>
<tr>
<th>Reference</th>
<th>Nano scale topography description</th>
<th>Evaluation methods and timing description</th>
<th>Significance of result indicated in the study (P &lt; 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gittens et al. 2012 [24]</td>
<td>Not complete</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gittens et al. 2012 [25]</td>
<td>No</td>
<td>Not complete</td>
<td>Yes</td>
</tr>
<tr>
<td>Gittens et al. 2011 [26]</td>
<td>Yes</td>
<td>Not complete</td>
<td>Yes</td>
</tr>
<tr>
<td>Tetè et al. 2010 [31]</td>
<td>Not complete</td>
<td>Not complete</td>
<td>Yes</td>
</tr>
<tr>
<td>Rani et al. 2012 [44]</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Zhuang et al. 2014 [45]</td>
<td>Not complete</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Portan et al. 2012 [50]</td>
<td>Not complete</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Dimitrievska et al. 2011 [52]</td>
<td>Not complete</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Mazzola et al. 2011 [55]</td>
<td>Not complete</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ross et al. 2013 [57]</td>
<td>Not complete</td>
<td>Not complete</td>
<td>No</td>
</tr>
</tbody>
</table>

### Table 4. Division of samples by modification and topography

<table>
<thead>
<tr>
<th>Topography division</th>
<th>Direct ablative nanomodifications</th>
<th>Nanocomposite additive modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control/smooth</td>
<td>Microstructured</td>
</tr>
<tr>
<td>Sample amount</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Sample promote proliferation</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Percent</td>
<td>25%</td>
<td>6.7%</td>
</tr>
</tbody>
</table>

Additionally the examined groups of samples can be compared for their relative influence on cell proliferation by surface topography. Percentage of groups of samples that promote proliferation in each surface topography, within nanostructures been the greatest illustrated in (Figure 2).

### DISCUSSION

#### Summary of evidence

Overall, in most of reviewed articles 24 in number, nanostructured surfaces enhanced osteoblast proliferation compared to microstructured or smooth surfaces.

Dielectric barrier discharge (DBD) modification of Ti with spherical nanoparticles of 50 - 125 nm, significantly enhance cell proliferation, adhesion and spread without negative effect on differentiation [33]. Strong evidence delivered by two different studies that acid etched microfeature and TiO deposited samples with nanonodules of 100 nm, 300 nm and 500 nm increase in proliferation, with that on 300 nm being the greatest [34,35].

Porous Ti6Al4V substrate with 10 - 20 nm grains appeared to have a not only higher but also longer growth phase for cell proliferation [36].

Full contact coverage coating that was obtained by galvanotactic anodizing process in a phosphate-sulfate bath, (FCC) characterized by unique nanotopography of regular volcanoes with circular pores of 10 µm and 700 nm shows greater amount of cell proliferation than micro and nanopore of 2 µm and 150 nm [31].

Yu et al. [37] stated that crystal structure of nanotube layer can override the chemistry effect and plays a main role in cell proliferation, when anatase/rutile ~80 nm nanotube layers showed significantly higher proliferation than smooth layer and amorphous nanotube layers. This statement confirmed with evidence found in another study where ~80 nm nanotube surface increase cell number [38].

![Figure 2. Percentage of groups of samples that promote proliferation in each surface topography.](http://www.ejomr.org/JOMR/archives/2014/3/e1/v5n3e1ht.htm)
when the same author reports on reduced cell number cultured on 130 nm nanotubes [39] and in other study of same author, no difference was found between ~80 nm nanotubular pattern and polished sample [40]. Final conformation for nanotubes benefit in such dispute was found in in vivo and in vitro experiment [41]. One of the most advantageous nanotube diameters for better cell proliferation appears to be ~30 nm [42]. Additional nanofeature with ~30 nm was reported to have largest cell response, including proliferation adhesion and differentiation, as ~30 nm saw tooth nanonetwork surface with 200 - 300 nm inter tooth distance was examined [43].

Interesting findings were observed in additional combined in vivo and in vitro studies when one reports on higher cell proliferation rate on nanoleaf feature with roughness of 228 nm than on 60 - 80 nm nanotubs and significant reduction in proliferation on nanoneedle feature with 940 nm roughness [44], whereas another latest in vivo and in vitro study controversially reports on enhanced proliferation on nanoneedle structure [45].

Concerning nanocomposite materials incorporated into the implants surface we saw niobium (NbO$_3$) doped TiO$_2$ producing nanoplantate structure that promote cell adhesion and proliferation [46]. Strong evidence brought to us in two studies that reported on strontium doped hydroxapatite with 21.6 ± 3.7 nm grain morphology, accelerates cell proliferation [47,48]. Furthermore, cell proliferation can be directly regulated by Sr1-HA interrod spacing, with 71.4 nm interrod space three-dimensional patterns being the greatest. Controversial results were noticed about incorporation of HA within nanotubes as two authors [49,50] claim that ‘there is an obvious positive change in the spreading and viability of osteoblasts on HA coated ~45 - 50 nm titania nanotubes layer comparing to cells on pure titanium or TiO$_2$ nanotubes’ and in contrast Gu et al. [51] reports on reduced proliferation on 90 nm nanotubules with HA compared to same nanotubes without HA.

Another report reveals higher cell numbers on HA - TiO$_2$ nanocomposite coated sample with 300 nm spherical TiO$_2$, 20 - 30 nm in diameter and 50 - 100 nm in length HA nanorods [52].

Significantly improved bone cell proliferation on the biomimetic nano coatings compared to uncoated Ti and nano-HA coated Ti was reported [53], as nano-HA combined with both magnetically and non magnetically treated ‘single walled carbon nanotubuls’ can achieve the highest osteoblasts proliferation density when diameter of SWCNT is 1.19 and 1.52 nm respectively. Structures with 80 nm selenium clusters incorporated onto Ti implant surface, significantly increase healthy cell density compared to untreated Ti, on which cancerous osteoblasts found to be prevailed [54]. TiC layer deposited on Titanium sample by ion plating plasma assisted deposition, increase osteoblast growth rate as was claimed in an in vitro and in vivo studies [55]. Another nanocomposite material that succeeds to increase proliferation rate and vitality was TiO$_2$/CaSiO$_3$ which exists on the surface as CaSiO$_3$ nanocrystals on 20 - 10 nm TiO$_3$ grains pattern [56]. Magnesium contained nanocomposite coatings found to be without any benefits for osteoblasts proliferation [30,47].

In contrast to 23 articles that describe positive effect of nanostructured surfaces, we are dealing with negative reports as follow. Ross et al. [57] reporting superiority of microstructure surface with pore size of 1 - 2 μm over nanostructured samples. Yu et al. [58] described lower proliferation rate on 80 nm nano-foveolae structure compared to smooth control sample. Gittens et al. [24-26] in three different studies describes negative results for micro and nano-modified samples compared to smooth and control surfaces in regard to osteoblasts proliferation, possibly due to transcriptionally-restricted transition. Transcriptionally-restricted transition between proliferation and differentiation is a process that forces osteoblasts to stop dividing once they start maturing [59-61]. Zhao et al. [39] from three articles included in this work, first reporting on significant smaller osteoblasts numbers cultured on nanotubules of 130 nm than on flat Ti sample, the number become even smaller when 10 - 20 nm Ag particles added to the surface. Second article finds no significant difference in cell numbers between polished sample, 25 nm nanonet texture and 80 nm nanotubular texture [40]. Third article reports on slightly enhanced cell number on the ~80 nm NT acid-etched/20 V anodized surface [38].

Hori et al. [62] describe relatively equivalent proliferation level on TiO$_2$ smooth and 198.5 ± 22.3 nm TiO$_2$ micronanohybrid.

After all, the most mentioned advantageous pattern is nanotubular structure with nanotube diameter of ~30 nm. Another superior morphological pattern across the studies was nanonoduls of 300 nm. Other nanostructures mentioned in our review need to be further investigated and compared for most advantageous nanoscale within each particular nanostructure. Furthermore this review analysed articles that present synergetic effect of ablative and additive nanocomposite surface coating to
osteoblasts proliferation. Most articles that were including hydroxyapatite incorporation into Ti implant nanostructures describe obvious positive effect on cells proliferation especially when doped with strontium. Strontium doped HA nanorods appear to be beneficial with nanoscale of ~20 - 30 nm diameter and with interrod spacing of less than 96 nm. Beside strontium, nanostructures doped with niobium, selenium and CaSiO$_2$ nanoparticles showed promising results, when selenium substrates suggesting a more favourable environment for healthy than cancerous osteoblasts. In contrast magnesium presence in the nanostructure poses some cytotoxicity.

**Limitations**

The main limitation of this overview is that the samples group types, the culture techniques and evaluation methods are not the same across studies and cannot be compared. All studies examined didn’t show numerical data on the osteoblast proliferation results, what did not allow us to estimate precisely the advantage of one nanostructured sample on another. All reviewed studies indicated significance of their results by ($P < 0.05$) what can be interpreted as study’s quality guaranty, but as mentioned previously, absence of quantitative results and calculations prevent conformation of significance and comparison across the studies by the reviewer. In addition there are 10 studies with 41 examined samples were we could not find exact information as, nanoscale topography of the specific surface structure and/or evaluation timing. Review with and without inclusion of these studies found no differences in the patterns of our review results but only leave the reader blinded by lack of specific features of investigated sample.

**CONCLUSIONS**

From examination of selected articles we can notice marked advantage in implementation of various nanostructures onto implant surface. In our review 24 articles reporting on positive effect of nanostructured surfaces on osteoblasts proliferation, when 33 samples with particular nanostructures markedly enhance cell proliferation. Most of the examined nanostructures showed obvious positive impact on osteoblasts proliferation compared to other topography scales. Yet for discovering the ultimate implant surface nanostructure, further investigations of Ti nanopatterns with various nanoscales need to be done, moreover for reaching the most sensitive outcome, the experiments should be statistically compared, what can be achieved only when different studies will use the same concerted evaluation method for osteoblasts proliferation.

**ACKNOWLEDGMENTS AND DISCLOSURE STATEMENTS**

The authors report no conflicts of interest related to this study.

**REFERENCES**


http://www.ejomr.org/JOMR/archives/2014/3/e1/v5n3e1ht.htm J Oral Maxillofac Res 2014 (Jul-Sep) vol. 5 | No 3 | e1 | p.12 (page number not for citation purposes)


