Dental, Oral and Craniofacial Research

Research Article ISSN: 2058-5314

# The influence of microgrooved surfaces on the behavior and celluar function of osteoblasts

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## Abstract

Since 1945 Weiss Paul described the phenomenon 'contact guidance' which means the cell elongates along the direction of the groove and migrates guided by the grooves. Cell could sense the surface topography where it lies and react to these surface cues. Many researches have devoted themselves to reveal the potential mechanisms. The interaction is mainly mediated by the cytoskeleton, the focal adhesions and the extracellular matrix (ECM). But how would the groove dimensions affect the cellular behavior is still obscure. Nowadays, micro fabrication techniques such as electron beam lithography have been applied to the production of micro-textured surfaces. They are relatively fast and cheap, and could fabricate microgrooves of reasonable size. Thus, they have been widely utilized to generate (micro-) nano-topographical surfaces or scaffolds for in vitro cell research. According to the report of P. CLARK, the response of cells to micro-grooved surfaces is cell type-dependent, so the focus of this review is on the osteoblast(s) reaction to micro-grooved surfaces.

## Introduction

A century ago, in 1911 Harrison depicted that cells cultured on spider's webs grew along the fibers [1]. Later on, in 1945 Weiss P initially named the phenomenon 'contact guidance': a tendency of cells to align, grow, or migrate along the grooves [2]. Cell can 'sense' the surface topography and then take reaction to these surface cues. The interaction between substrates and cells is achieved through the effort of the cytoskeleton, the extracellular matrix (ECM) [3-5] and the focal adhesions [6].

In terms of the defined (micro-) nano-topographical surface, they are usually produced by the micromachining technology: lithographic patterning (photolithography, electron beam lithography, colloidal lithography), galvanoformung abformung process LIGA, focused ion beam-chemical vapor deposition FIB-CVD and so on. Some of these techniques such as electron beam lithography (EBL) have been developed for creating well-defined patterns with feature sizes <10 nm [7]. Recent years, femtosecond laser patterning has obtained a position in the microgrooves ` machining [8,9]. These techniques promoted the development of biomaterials and tissue engineering greatly.

As the response of cells to micro-grooved surfaces is cell type-dependent [10], the react of osteoblasts to the micro-machined surface might be different from other cell types. This review is based on the gathered information about the defined microgrooves, ranging from nanometers to microns, role on the osteoblasts, aiming at finding out the interaction between these ultrafine arrays and the bone-forming cells.

## Micro/Nanofabrication technologies

A variety of methods such as Femtosecond laser microtexturing can be applied to the fabrication of microtextured surface. These nano/micro patterning techniques were early used in the semiconductor and microelectronics industries [11], later they were increasingly applied in biology, medicine, and biomedical engineering fields [12]. Researchers [13,14] use these techniques to manufacture materials, attempting to get a value that is optimal for the growth of cells. These technologies both have their adaptations as well as limitations, also they have got developments. Hence it is hard to define the best tech in this field [15].

## Microgrooved surface influences cellular behavior

Cell adhesive to the grafting materials, more importantly, they are in reciprocity with them. Different surface materials and topographies may induce distinct cell morphology, proliferation, and gene expression [16]. Cells can "sense" substrate elasticity [17,18] as long as its surface patterns in the scope of 10 nm to 100 mm [19,20].

Different dimensions are thought to play varied roles in cellular behavior [10,21]. The average size of the osteoblasts is 20-30μm. When the dimensions of grooves/ridges are reduced to the sizes of the cells and less, topographic effects on cell orientation become more prominent [22]. As will be discussed below, a majority of results focused on groove width of the micro-or-nanoscaled surface, some reports show that ridge width is more important in conducting the cellular behavior, while maybe the groove depth is the leading factor inducing cellular activities.

Groove/ridge topographies are important modulators of both cellular adhesion and osteospecific function and that groove width is vital in determining cellular response [23]. Certain groove width guides the cell to align along the direction [8,9,24,25]. The change of the

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***Key words:*** *osteoblast(s), nanotechnology, surface chemistry /properties, cell biology, cell differentiation, cell-matrix interactions*

**Received:** August 29, 2016; **Accepted:** September 19, 2016; **Published:** September 22, 2016

width affects celluar shape [26], attachment [27], cellular proliferation [28] as well as bone forming ability [25,26]. Form these opinions and Table 1 and 2, we can infer that substrates with the microgroove width of 1-5μm, particularly 2μm seems to be optical for the biological behavior of osteoblasts. On 2μm-wide-grooves the cellular adhesion [29], proliferation [28], osteogenic differentiation [28,30] as well as calcification [28]. Also，these nanophase material increased adhesions of osteoblasts compared with the conventional materials [31]. Depicted in table 2, almost all of these dimensions guide the cells to align along the long axis of micropatterns. Some nano-dimensions display an osteogenic influencing function [25,30,32].

Those who focused on the effect of ridge part had some limited findings. Alexey Klymov *et al.* designed the substrates with ridge to groove ratios of 1:1, 1:3 and 3:1. He demonstrated that nano-grooved patterns with the ridge to groove ratio of 1:3 showed cell repelling, meanwhile grooves with the ridge to groove ratio of 3:1 partially showed cell attraction during cellular selective migration [33]. Apart from that the ridge width clearly enhanced differentiation of MSCs towards specific lineages [30]. Tests on other kinds of cells, say fibroblasts, found that ridge width is the main parameter affecting cell alignment (alignment being inversely proportional to ridge width) [34].

Actually there is no defined item about the influence of groove depth on the osteoblast. From the information Azeem A reported, 306nm and 2046nm promoted osteoblasts alignment parallel to underlined topography. Besides this size showed its osteogenesis ability [32]. Kenichi Matsuzaka observed that on a 0.5μm deep and 10μm wide grooved surface, the cell descends into the groove, on a 1.5μm deep and 1μm wide grooved surface, cells attach to the ridges only. Nowhere, differences were observed between specimens with different groove depths. Instead Kenichi Matsuzaka attributed this phenomenon to the width of the ridge merely [27].

*In vivo* studies on effect of the surface micromachining to the osseointegration also take for the positive side. The laser micromachining technology enhances bone [24] and soft-tissue integration and controls the local microstructural geometry of attached bone [35]. The organized pattern of the microgrooved surfaces is capable of resulting in transverse collagen fiber microenvironment reaction to the load, being positive to promote and to maintain the bone remodeling; in addition, blood vessels and bone cells are able to penetrate microgrooved surfaces [36]. What's more, micromachined implants enhances primary and secondary implant stability, preserves crestal bone levels [36,37].

## Conclusions and outlook

With the acceptance of 'contact guidance' theory, many defined patterns were made by various micro/nano technoloies, prompting the study of different dimensions to the cellular behavior. The limited collected data in the table 1 and 2 showed that the groove width is the most influencing factor affecting the osteoblasts. On the micropatterned substrates, osteoblasts adhere and elongate along the long axis of the microgrooves. Improper width of microgrooves may lead to adhesion down growth. On certain groove width cell density, proliferation and osteogenic ability show an improvement. The differentiation also can be affected by the nanotopography. However, the reports based on the virtues of the ridge width and the depth of the array still needs further exploration. Moreover, we can do a further step research on the effect

**Table 1**. The influence of microscale microgrooves on osteoblasts′ function.

| References | Cell and Substrate type | Groov width(μm) | Ridge width (μm) | Groove Depth (μm) | Results |
|---|---|---|---|---|---|
| Delgado-Ruíz *et al.* 2015) [9] | hFOB, zirconia | 30 | 70 | — | LSA, density and cellular activity increase |
| (Matsuzaka *et al.* 2003) [27] | RBM cells, polystyrene | 1, 2, 5, 10 | 1, 2, 5, 10 | 0.5, 1,1.5 | smooth and grooves >5 μm cells extensions close to substrates grooves 2 μm were bridged |
| (Puckett *et al.* 2008) [26] | human osteobalsts, titanium | 80,48,22 | 45, 35 30 | — | Attachment gradually decrease, cellular function increase, cellular shape change |
| (Biggs *et al.* 2009) [39] | HOB, PMMA | 10,100 | 10,100 | 300 nm | 10μm focal adhesions and osteospecific lineage decrease adipospecific genes increased 100μm cellular adhesion increase |
| (Ismail *et al.* 2007) [29] | MG63, silicon | 2, 4, 8,10 | 1.5-2 | cell viability 8, 10 μm grooves increase | smaller groove sizes smooth one's better cell adhesion |
| (Abagnale *et al.* 2015) [30] | MSCs, Polyimide | 2,3,5,10,15 | 2,3,5,10,15 | 15 down to sub-micrometer | 1 5μm ridges increased, adipogenic differentiation,2μm enhanced osteogenic differentiation |
| (Biggs *et al.* 2008) [23] | HOBs, Silicon | 10, 25, 100 | 10, 25,100 | 330nm | planar adhesion more,100μm increased osteospecific function, 25μm reduction SMA increase FX formation,10μm reduced, adhesion and induced an interplay of up- a and downregulation of gene expression |
| (Taniguchi *et al.* 2015) [28] | MC3T3-E1, zirconia, polycrystal | 2μm | — | — | proliferation was significantly greater, The Runx2 mRNA level increased time dependently, calcification and ALP activity and osteocalcin mRNA levels were higher |
| (Lu and Leng 2009) [21] | osteoblast, myoblast, silicon | 8, 24 | — | 2, 4, 10 | 8μm width strongly affect both osteoblasts and myoblasts 24μm strongly affect myoblasts only |
| (Lu and Leng 2003) [40] | osteoblast, myoblast, silicon | 8, 24 | — | 2, 4, 10 | 8μm width strongly affect both osteoblasts and myoblasts, 24μm strongly affect myoblasts only |
| (Lu and Leng 2003) [40] | SaOS-2, Ti and HA | 4, 8, 16, 24, 30, 38 | — | 2, 4, 10 | No difference in orientation angle between HA and Ti microgrooves |
| (Koo *et al.* 2014) [41] | human primary cells, titanium | 15-, 30-, 60- | — | 3.5- ,10- | lower levels of type I collagen α1 gene expression at day 14, extremely increase in osteopontin gene expression at days 21 and 28 |
| (Hamilton and Brunette 2007) [42] | Osteoblast cell, epoxy-resin | Pitch: 30-175 | 5-175 | | Total tyrosine phosphorylation increased Src levels decrease, but FAK and ERK1/2 phosphorylation were highest, Inhibition of Src phosphorylation with PP2 inhibited FAK and ERK 1/2 phosphorylation |
| (Fransiska *et al.* 2013) [43] | ROS, silicon | from 1 to 20 | — | — | width less than 10 μm induced the alignment of osteoblasts, increase osteogenic proteins |

**Table 2**. The influence of nanoscale microgrooves on osteoblasts′ function.

| References | Cell and Substrate type | Groov width(nm) | Ridge width (nm) | Groove Depth (nm) | Results |
|---|---|---|---|---|---|
| (Lamers *et al.* 2010) [25] | rats bone marrow stromal cells, silicon | down to 75 | — | down to 33 | minimal align dimensions' width:75nm nanogroove-to-ridge ratios of 1:1, 1:3 and 3:1 depth:33nm. minimal mineralization width: 50nm, depth:17nm, osteoblast- specific gene expression increased |
| (Yim *et al.* 2007) [44] | hMSCs, Poly(dimethylsiloxane) | 350 ,700 | — | 350 | Upregulation of neuronal markers— MAP2 and GFAP |
| (Yang *et al.* 2009) [45] | MG-63, Silicon | 90,150,250,340,500 | 90,150,250,340,500 | — | elongated and aligned along the direction, so does cell nucleiei |
| (Abagnale *et al.* 2015) [30] | MSCs, Polyimide | pitch of 650 | 200 | — | increased differentiation towards both osteogenic and adipogenic lineages |
| (Azeem *et al.* 2015) [32] | Primary human osteoblasts polystyrene | ~1860 | ~2220 | ~35, 306,2046 | ~306 and 2046 nm promote osteoblast alignment parallel to underlined topography in vitro In vivo showed osteogenic ability |
| (Lenhert *et al.* 2005) [46] | Primary osteoblasts, polystyrene | periodicity of 500 | 50, 150 | — | align, elongate and migrate parallel to the grooves |
| (Lamers *et al.* 2012) [47] | old male Wistar WU rats Osteoblast-like cells, silicon | periods 1000, 300,150 | 32-150 | — | Cells aligned down to 300 nm pitch In vivo 150 nm pitch grooves has the lowest density of multinucleated cells |
| (Prodanov *et al.* 2013) [48] | MC3T3-E1, silicon | 200 | — | 50 | PFF (pulsatile fluid flow) did affect cellular morphology. Cells aligned on nanotexture substrate in a direction parallel to the groove orientation |
| (Prodanov *et al.* 2010) [49] | Rat MSCs, Silicone rubber | 300 (600 pitch) | 1 µm(pitch 2 µm) | ~150, 500 | perpendicular the substrates when parallel stretch to the nanotexture greater than 3% applied |
| (Klymov *et al.* 2015) [33] | Rat MSCs, Silicon, polystyrene | 10 – 1000 ridge to groove ratios of 1:1, 1:3 and 3:1 | — | — | All sizes of squares showed strong cell-repelling capacity. 3:1 partially showed cell attraction |

of the wettability and inclination of ridge. Soluble biochemical cues, dynamic control and regulation of topographical features, as well as cell co-culture systems, have all been declared to act in synergy with physical cues in regulating stem cell fate [38]. When we design a test, multi-factors should be taken into consideration. In conclusion, critical dimensions do play a part in regulating celluar behavior. However, it is a pity that we have not completely revealed the mystery of micro-nanotopographical on the osteoblasts. In addition, which dimension of microarrays is optimal for the adhesion, proliferation, differentiation, and osteogenisis is still under research. We can use the obtained data as a guide and reference for the study in the future. Besides, these results could be helpful in the design and fabrication of implants and biomaterials.

## Acknowledgement


- The authors acknowledge funding from the Chengdu science and technology huimin engineering projects. China. 0040305301462. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.


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