The influence of microgrooved surfaces on the behavior and cellular function of osteoblasts

Qrong Li, Yuchen Guo and Yongyue Wang*
West China School of Stomatology Sichuan University, oral implantology, Chengdu, Sichuan 610041, China

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 microtextured 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 of the 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 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.

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width affects cellular 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 optimal 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 1.5μm deep and 10μm width showed cell attraction during cellular selective migration [33]. Apart from that the ridge width clearly enhanced differentiation of MSCs towards osteogenic influencing function [25,30,32]. 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].

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 groove, the cell descends into the groove, on a 1.5μm deep and 1μm wide groove. Besides, groove 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]. 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 groove, the cell descends into the groove, on a 1.5μm deep and 1μm wide groove, cells attract during cellular selective migration [33]. 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 groove, the cell descends into the groove, on a 1.5μm deep and 1μm wide groove, cells attract during cellular selective migration [33]. 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 groove, the cell descends into the groove, on a 1.5μm deep and 1μm wide groove, cells attract during cellular selective migration [33].

Table 1. The influence of microscale microgrooves on osteoblasts’ function.

| References                  | Cell and Substrate type          | Groove width(μm) | Ridge width (μm) | Groove Depth (μm) | Results                                                                 |
|-----------------------------|----------------------------------|------------------|------------------|-------------------|-----------------------------------------------------------------------|
| Delgado-Ruiz et al. 2015    | hFOB, zirconia                   | 30               | 0                | —                 | LSA, density and cellular activity increase                         |
| (Matsuzaka et al. 2003)     | 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. 2009)       | human osteobalts, titanium       | 80,48,22         | 45, 35 30        | —                 | Attachment gradually decrease, cellular function increase, cellular shape change |
| (Biggs et al. 2009)         | HOB, PMMA                        | 10,100           | 10,100           | 300 nm            | 10μm focal adhesions and osteogenic lineage decrease adipspecific genes increased 100μm cellular adhesion increase |
| (Ismail et al. 2007)        | MG63, silicon                    | 2, 4, 8,10       | 1.5-2            | —                 | smaller groove sizes smooth one’s better cell adhesion               |
| (Abagnale et al. 2015)      | MSCs, Polyimide                  | 2,3,5,10,15      | 2,3,5,10,15      | 15 down to sub-micrometer | 5 μm ridges increased, adipsogenic differentiation,2μm enhanced osteogenic differentiation |
| (Biggs et al. 2008)         | HOBs, Silicon                    | 10, 25, 100      | 10, 25, 100      | 330nm             | planar adhesion more,100μm increased osteospecific function, 25μm reduction SFA increase FX formation, 10μm reduced, adhesion and induced an interplay of up- a and downregulation of gene expression |
| (Taniguchi et al. 2015)     | 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)          | osteoblast, myoblast, silicon    | 8, 24            | —                | 2, 4, 10          | 8μm width strongly affect both osteoblasts and myoblasts 24μm strongly affect myoblasts only |
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| (Lu and Leng 2003)          | 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)           | 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) | Osteoblast cell, epoxy-resin     | Pitch: 30-175    | 5-175            | —                 | Total tyrosine phosphorylation increased Sre levels decrease, but FAK and ERK1/2 phosphorylation were highest, Inhibition of Src phosphorylation with PP2 inhibited FAK and ERK 1/2 phosphorylation |
| (Franssiska et al. 2013)    | ROS, silicon                     | from 1 to 20     | —                | —                 | width less than 10 μm induced the alignment of osteoblasts, increase osteogenic proteins |

Conclusions and outlook

With the acceptance of ‘contact guidance’ theory, many defined patterns were made by various micro/nano technologies, 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 of microneedle micropatterning to examine the influence of groove width and depth. The results show that the groove width and depth significantly affect the cell behavior and function.
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 microgrooves.

Acknowledgement

• The authors acknowledge funding from the Chengdu science and technology humanities and social sciences projects. China. 040305301462.

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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