Review

Dental pulp stem cells response on the nanotopography of scaffold to regenerate dentin-pulp complex tissue

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ABSTRACT

The study of regenerative dentistry receives a fast growing interest. The potential ability of the dentin-pulp complex to regenerate is both promising and perplexing. To answer the challenging nature of the dental environment, scientists have developed various combinations of biomaterial scaffolds, stem cells, and incorporation of several growth factors. One of the crucial elements of this tissue engineering plan is the selection and fabrication of scaffolds. However, further findings suggest that cell behavior hugely depends on mechanical signaling. Nanotopography modifies scaffolds to alter cell migration and differentiation. However, to the best of the author's knowledge, there are very few studies addressing the correlation between nanotopography and dentin-pulp complex regeneration. Therefore, this article presents a comprehensive review of these studies and suggests a direction for future developments, particularly in the incorporation of nanotopography design for dentin-pulp complex regeneration.

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Contents

1. Introduction

The bioactivity of the dentin-pulp complex has been discussed extensively, especially regarding its role in regenerative dentistry. The study of dentin-pulp tissue demonstrates its potential ability to stimulate dentin growth in response to harmful stimuli such as caries by triggering the immune defense mechanism. Dentin-pulp complex, which mainly consists of odontoblast [1], could repair not only the hard tissues, but also the soft tissues in the pulp itself, including angiogenic and neurogenic repair [2]. Growth factors play a significant role in this regeneration mechanism. Several growth factors that are responsible in dentin formation after injury are: platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), bone morphogenetic protein, nerve growth factor (NGF), vascular fibroblast growth factor-2, vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), stromal cell-derived factor-1 (SDF-1), glial cell line-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF). Additionally, some of the pro-inflammatory cytokines display multifunctionality [2,3].

Some of the bioactive molecules found in dentin and pulp induce the proliferation of various stem cells related to growth factors present within the dentin-pulp complex [4]. Specifically, each growth factor stimulates cells' activity in different ways, depending on the type of cells and tissue. For example on dental pulp stem cell (DPSC), PDGF family increases odontoblastic differentiation, cell proliferation, and dentin—pulp complex regeneration [5]. TGF- β family involves in the DPSC mineralization [6]. BMP-2 promotes differentiation of DPSC into early-preosteoblasts [7] and affects its odontoblastic differentiation [8]. BMP-2 and BMP-4 are also the major ligands needed for bone development [9]. However, it has been shown that there is time limit on the efficacy of growth factors in tooth development and regeneration [10].

Sharpe and Young [11] made the first finding about a fully engineered tooth. They proposed a newly developed tooth tube that has positive results, marked by the growth of tiny tooth-like structure. This method utilizes a synthetic biodegradable scaffold. Furthermore, Yang, Yuan, and Chen [12] proposed a scheme of regenerating dental—pulp complex using a scaffold. Since then, the development of dentin-pulp regeneration has improved significantly. Scientists found several ways to conduct the regeneration of dentin-pulp complex using stem cells like DPSC, stem cells from apical papilla (SACP), stem cells from human exfoliated deciduous teeth (SHED), and a sorted subpopulation of dental pulp cells. However, DPSC, dental mesenchymal stem cells, and induced pluripotent stem cells are the most popular for dentin-pulp regeneration [13]. Besides, the incorporation of growth factors and scaffold designs could also significantly enhance cell viability and differentiation.

The next challenge is to provide guidance for cell differentiation as expected. Lately, it has been found that cells are more sensitive to micro and nanoscale topography [14,15]. Nanotopography is a surface characterization of nano-sized pattern (1—100 nm) [16—18]. Within the dentin-pulp complex itself, there are some nanotopography features such as intercellular spaces between odontoblasts (30—40 nm), fibril diameter at the base of odontoblast (approximately 15 nm), and fibril diameter on the calcification area (about 50 nm) [1]. Nanotopography has a more effective modulator than the micron one [19]. This is because nanotopography could promote adhesion to cells, distribute cells, affect the arrangement of the cells, and stimulate morphological changes and gene expression [15].

Recent studies implied that nanoscale modification upon implant surface could modulate the osseointegration because it alters both cellular and tissue responses [16]. Nanotopography increases the secretion of some of the main growth factors in dentin-pulp regeneration such as BMP-2 [9]. Nanotopography is also better compared to chemical surface modification in signaling cells because it is more durable and more comfortable to be tailored to meet the need of the cell environment [20]. Wherefore, authors believe that the incorporation of nanotopography on a scaffold to enhanced dentin-pulp complex regeneration is crucial compared to the usage of growth factors alone in order to get an optimal result.

However, there has not been any discussion on the potential and influence of nanotopography, particularly on scaffold for dentin-pulp complex regeneration. Therefore, this article mainly evaluates the role of nanotopography in a scaffold and emphasize dental stem cells in improving dentin-pulp complex regeneration.

2. Research method

An in-depth literature review approach was used for completing its objectives. All studies related to dentin-pulp complex regeneration and the usage of scaffold and nanotopography design were included. These included all academic journals but excluded reviews, basic research journals, empirical researches, case reports, books, and theses from Google Scholar, PubMed, and Science direct published between 2010 and 2019. There were several keywords entered in the search engines and library directory, such as "Dentin pulp complex" AND "Nanotopography" OR "Dentin pulp complex" AND "Regeneration" OR "Dental pulp stem cells" AND "Nanotopography". These keywords were used due to their close correlation to the topic. Secondly, the keywords "Dentin pulp complex Regeneration" AND "Dental pulp stem cells" AND "Nanotopography" were inputted to test whether any publications on-trend and development had been done. It is concluded that there has not been any article which specifically discusses the effect of nanotopography scaffold on dentin-pulp complex regeneration.

A total of 235 articles were initially selected, with the following inclusion criteria: full text articles, written in English, with topics on dentin pulp complex regeneration, dental pulp stem cells and nanotopography. Abstracts, review papers, and manuscripts in any languages other than English were excluded. However, after careful selection, there were only 22 remaining articles eligible. The flowchart of the article selection can be seen in Fig. 1..

3. Discussion

3.1. Nanotopography on scaffold

Scaffold works by providing suitable environment to regenerate extracellular matrix (ECM). Once cells are attached to a scaffold, a series of physico—chemical reaction will occur between both cells and the scaffold [21]. Since scaffold plays a key role to the regeneration of tooth, several factors should be carefully considered. Porosity, the mechanical reliability and the surface morphology of the scaffold are few of the most important factors [22]. Using X-ray nano-tomography, Forien et al. [23] showed that dentin is composed of complex nanoparticles and shapes. Carbonated hydroxyapatite (cHAP) in dentinal root spans 35 nm in the outer root regions. On the verge of dental pulp, the size reduces around 25 nm. Therefore, the incorporation of nanotopographical cues on a scaffold is crucial to mimic natural environment of dentin-pulp complex.

3.2. Stem cells

Dental stem cells have been used as one of stem cell sources. Since 2000, there have been more than 1000 publications about this interesting stem cell source. In fact these cells are mostly
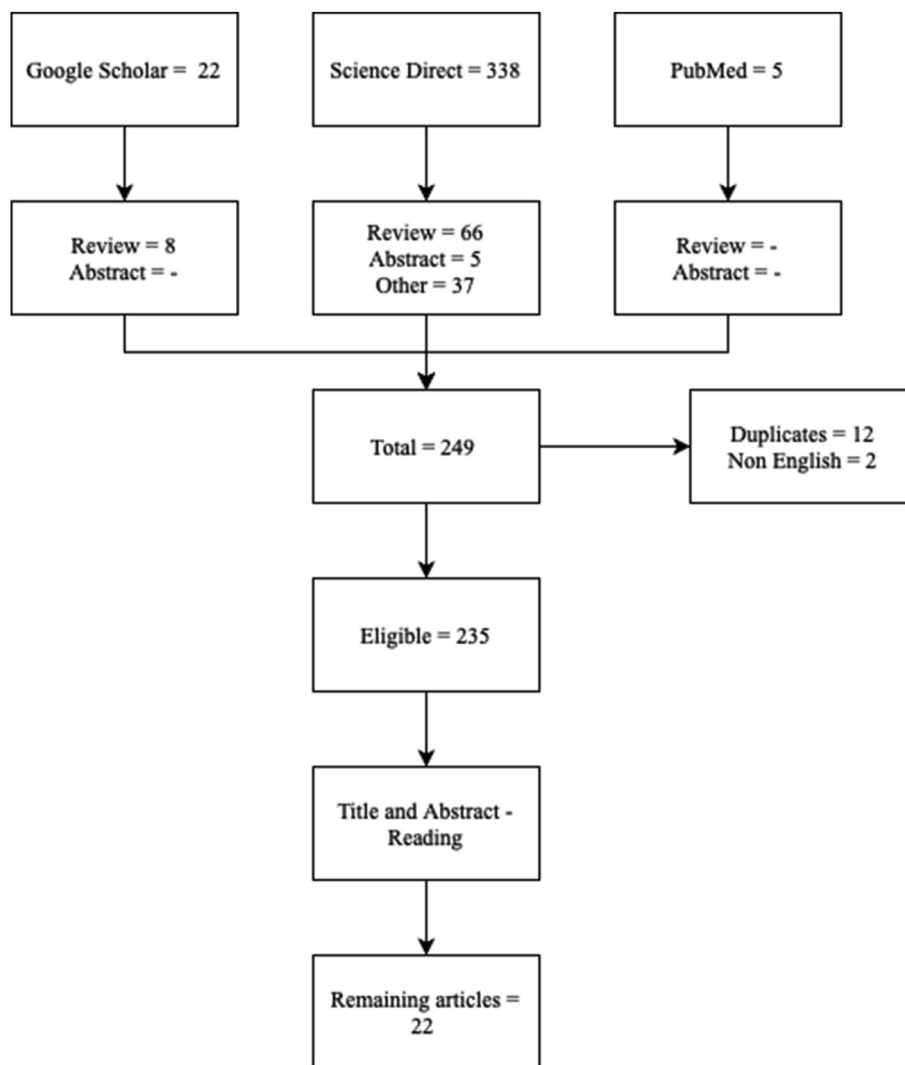
Fig. 1. The process of articles selection.

typical cells that could proliferate to increase their colony. Dental stem cells are also able to provide a long term multilineage and self-renewal capacity. According to The International Society for Cellular Therapy, dental tissues are categorized as mesenchymal stem cells (MSCs). Many investigations have shown that dental pulp tissues can be formed from dental stem cells [24—26].

DPSC and stem cell from human deciduous teeth have a stem-cell-like property such as self-regeneration capability and multi-lineage differentiation. These characteristics enable an *ex vivo* expansion and improve the translational potential of these cells [27]. They show odontogenic, neurogenic, osteogenic, adipogenic, and chondrogenic differentiation [13,28,29].

The DPSC is accessible, non-invasive, can be cryopreserved and restored to be whenever needed [27,30]. The DPSC has been revealed to have a high potential of proliferation, self-renewal, and multilineage capacity *in vitro* under specific conditions [28]. Nakashima and Iohara [31] used a cell injection method to regenerate dental pulp using DPSC. The result shows that the odontoblast-like cells could attach to the dentinal wall and form dentin-like tissue. One consideration in the usage of stem cells in dental pulp regeneration is vascularization. The morphology of the dental pulp complex does not supply enough vascularization for damaged tissue. The source of vascularization in dental morphology is only from apical foramen [32]. The lack of blood and oxygen could harm tissue development. Furthermore, stem cell isolation and storage have to be managed well. Perfect storage of stem cells allows transfer cell from site to site, without losing its capability to differentiate [33]. Therefore, before seeding the cells, the preparation of the cells has to be done carefully to achieve a maximum result.

3.3. Extracellular matrix

In a natural environment, cells behavior is determined by biochemical and mechanical signaling. Mechanical signaling is defined as the strain produced by a cell according to linear elasticity equation (l/r) [34]. Mechanical signaling differs from chemical signaling in several manners as seen in Table 1 in which delivered to cell through an ecosystem provided by ECM.

ECM is formed by nanoscale protein, glycosaminoglycan, and glycoprotein that are position to specific coordination to promote tissue-specific functions [37]. These proteins also provided physical and tensile strength and serve as the surface receptor of attachment site [38]. Additionally, ECM provides physical signaling through architectural, mechanical, and topographical arrangement [36,39,40]. ECM architecture induces cell exposure to the three-

Table 1
Differences of chemical and mechanical signaling.

	Chemical signaling	Mechanical signaling
Signal characteristic	Radial type [34].	Pole or linear type [34].
Mode of transmission	Diffusion or carried by fluid flow [34].	Transmitted through ECM, fast, resulted in farther distance [34,35].
Directionality	Control through chemotaxis [34].	More specific direction [36].
Rate	Generally slower [34].	Faster [34].

dimensional environment and dictates the cell geometry. Turner and Dalby [40] found that flattened cells differentiate into osteogenic lineages, and rounded cells differentiate into adipogenic lineages. This three-dimensional structure is one of the most important features of ECM. It lies on the basement membrane, which comprises of fibers and pores varying from 30 to 400 nm in dimension [41]. This nanotopographical signaling stimulates diverse functions towards cells, including adhesion, migration, proliferation, and differentiation [41,42]. Moreover, to maintain homeostasis and regulate morphogenesis, ECM undergoes constant remodeling. In contrast, disruption of ECM composition, structure, stiffness, and availability creates few pathological disorders, such as fibrosis and cancer [42].

Cells respond to mechanical stimuli such as shear flow, compression, or substrate stiffness from the ECM via integrins and actomyosin cytoskeleton, followed by a mechanotransduction. This process causes deposition, rearrangement, or removal of the ECM to preserve overall form and function [35,36,40]. Considering the importance of three-dimensional features of ECM, scientists are trying to mimic these architectural structures and transform them into an engineered scaffold. There have been several studies reporting that nanotopography alone can produce a similar effect to chemical induction, for example, growth factors. Furthermore, the rapid development of nanofabrication techniques such as soft lithography, photolithography, and electrospinning has made it possible to investigate the effect of nanotopography on an extensive range of materials [43,44].

3.4. Mechanisms of how dental pulp stem cells may interpret nanotopographical signals

At the cellular level, physical forces, such as tension, gravity, shear force, and compression, greatly influence the growth and remodeling of all living tissues [45—48]. Mechanotransduction is a way by which mechanical forces in the extracellular environment are converted into genomic and proteomic changes [49]. The ECM's architectures or nanotopography orientation features are able to regulate the morphology and function of stem cells via specific cell—surface interactions such as mechanotransduction [37]. Moreover, stem cells respond to nanotopography through several behaviors such as adhesion, alteration in gene expression, migration, differentiation, and proliferation, those specifically for DPSC will be described below [15,25,50—52].

3.4.1. Odontogenic, osteogenic, and chondrogenic
A number of studies have found that synthetic polymer materials such as polystyrene, poly-L-lactic acid (PLLA), polyglycolic acid (PGA), and poly-dl-lactic-co-glycolic acid (PLGA) are suitable for scaffold production. In addition to being versatile, these materials also allow scientists to control their degradation characteristic [53]. Wang et al. [54], investigated that nanofibrous poly (L-lactic acid) (NF-PLLA) scaffolds with a nanofiber diameter ranging from 50 to 500 nm showed better cell attachment, proliferation, osteogenic differentiation, and mineralization for DPSC compared to solid-walled (SW) PLLA scaffolds both *in vitro* and *in vivo*. Nanofibrous pattern provides better vitronectin and fibronectin adsorption

which are essential to the attachment of pre-osteoblasts [54]. Nanofibrous pattern increases surface area for extrinsic interchange thereby spreading an effective amount of molecular presentation to substrates [55]. Filopodia also possibly plays a role to enhance cell attachment. Nanofibrous pattern provides better anchorage to filopodia. Furthermore, nanofibrous design allows more nutrient/oxygen supply to cells as well as metabolic waste discharge [56—58]. Similarly, research on another synthetic polymer, poly-hydroxymethylsiloxane (PHMS), showed that 341 nm nano wells exhibit better cell clusters through the formation of scattered actin stress fibers, adhesion, and an increased number of fringe style protrusions compared to 109 nm nano wells [59].

Despite prior evidence, Sung [60] showed that PLLA scaffolds with the size of ridge/groove 250 nm exhibit insignificant effect on the expression of osteogenic marker genes. When osteocalcin and Runx 2 increase, they only show a normal osteogenic differentiation. In addition, a 350-nm nano-pattern on polyurethane acrylate inhibits osteogenic differentiation [61,62]. This contradictory finding might occur due to difference in the scaffold fabrication, which affects mechanical integrity, three-dimensionality, distribution including arrayed surface pattern, and uniformity of a nanotopography scaffold [61—63]. As reflected by the findings of Dolatshahi-Pirouz et al. [50], which showed that from three different architectures of nanotopography (low-nanosurface roughness, hut-nanostructured surface, and dome structures), DPSC exhibits the highest fibronectin mass uptake and adhesion towards the hut-nanostructured surface, while the highest proliferation is shown by dome structures. Additional mechanical forces could also interfere with DPSC behavior towards nanotopography. Uniaxial force (120 rpm) in silk fibroin scaffolds (10 nm diameter) promotes differentiation into a mineral-producing cells, creates more homogenous mineralization, however, decreases differentiation into osteoblast compared to static (0 rpm) scaffold environment [64].

Furthermore, a larger dimension of nanopattern on synthetic polymer scaffold, exhibits chondrogenic differentiation of DPSC. A combination of poly (ethylene glycol) dimethacrylate (PEGDMA), methacrylatedgelatin (GelMA), and hyaluronic acid (PEG-GelMA-hyaluronic acid hydrogels) of which the dimension of ridge × groove × height is 800 × 800 × 500 nm, displays a significant increase in the chondrogenic gene markers (Sox 9, alkaline phosphatase, aggrecan, procollagen type II, and procollagen type X) and the production of collagen type II [65]. Another synthetic polymer like nanofibrous peptide-amphiphile (PA) hydrogel scaffolds, which is cultured using DPSC, has shown a decreasing trend in cell proliferation. However, it generates an osteoblast-like phenotype and mineral deposition, and expresses osteoblast marker genes [66].

Another study conducted by Jang et al. [67] showed that the parallel nanogrooves polycaprolactone (NG-PCL) is equally capable to induce osteogenesis compared to equine bone powders alone as a chemical signalling. Moreover, compared to the flat polycaprolactone (F-PCL), the NG-PCL shows larger cell adhesion area where the cells are elongated in the direction of the nanogrooves. In addition to polymer, bioceramic material is also used as scaffold for DPSC. The usage of bioceramic scaffolds in conjugation

with DPSC has also been intensively studied. A combination of iron oxide nanoparticle-incorporating calcium phosphate cement scaffolds (IONP-CPC) with a spherical γIONPs (7–8 nm) and a spindle αIONPs (10 × 90 nm) has increased the synthesis of bone matrix mineral, the attachment of DPSC and osteogenic differentiation. The incorporation of IONP-CPC decreases crystal size and surface topography, increasing surface area and cell adhesion. Moreover, the nanotopography expands the availability of epitopes which impacts their recognition by specific cell surface receptors [68].

Another study also found that a 20 nm spherical nano-bioglass induces the polarization of odontoblast-like cells from DPSC, which forms dentin-like tissue within six weeks [69]. Graphene oxide with the incorporation of organic nanofibrous scaffolds shows an increased expression of coll I, osteonectin, osteocalcin, and osteopontin I [70]. Another study using monodispersed gold nanoparticle (AuNP) propounded that 68 nm nanotopography is the strongest inducer for cell proliferation. It has to be considered that although a 16 nm nanotopography is powerful enough to promote cell attachment for proliferation, there might be no spaces left available to the cells. Moreover, the density of nanotopography also plays role on DPSC response. The higher the density, the faster the cell proliferation, the better the cell adhesion [71].

3.4.2. Neurogenic

A similar trend is found in terms of the differentiation of DPSC into a neurogenic cell. The usage of a graphene scaffold is popular in combination with DPSC in order to promote neurogenic differentiation due to its biocompatibility, superior physicochemical and mechanical properties, and versatility [70]. It is shown that graphene polycaprolactone (PCL) hybrid nanofibers (TCPS) increase the expression of Tuj-1, a primary marker of neurogenesis, and NeuN. Meanwhile, reduced graphene oxide (RGO) PCL nanofibers enable the alignment of differentiated cells along the direction of nanofibers [72]. Similar research using graphene oxide and PCL

nanofibers used four types of organic nanofibers: randomly (R) oriented PCL with a fiber diameter of 450 ± 160 nm, uniaxially aligned (P) PCL with an average diameter of 580 ± 160 nm, randomly (RG) oriented graphene oxide (GO) coated with an average diameter of 400 ± 130 nm, and uniaxially aligned (PG) GO coated with an average diameter of 430 + 140 nm. It is shown that the incorporation of nanotopography and GO coating affect wettability. Furthermore, osteoblastic, glial, fibroblastic, and neuronal-related gene expression is found on R, RG, P, and PG samples, consecutively [70]. In addition, a study using polystyrene and polybromostyrene indicated that island height affects fibroblast spreading and proliferation in both ways (increase or decrease). A 13-nm island produces the utmost cell area, while 95 nm islands produce the least cell area [57].

3.4.3. Angiogenic

Notwithstanding the fact that DPSC has potential angiogenic properties [73–75], the study of the effect of nanotopography on angiogenic differentiation of DPSC is exceptionally limited. One of which is a study using GO nanosheets with an architecture of irregular nano-flakes with diameters under 500 nm, promoting the secretion of oncostatin M, tumour necrosis factor-alpha (TNF-α), and other factors via nuclear factor-κB pathway. The GO conditioned medium induces the osteogenic differentiation, promotes their tube formation *in vitro*, and stimulates upregulation of the HUVECs of vascular-related receptors [76].

Surface modification on a scaffold orchestrates DPSC into a wide array of lineage emphasizes the cell's multipotent characteristic. Researchers introduced nanohut, nanodome [50], nanofibrous pore wall [54], nanowell[59], ridge/groove nano patterned surface [60–62,67], or nanoparticle [69,71] and received interesting response from human DPSCs (Fig. 2 and Table 2). Fibronectin coating promotes better attachment and proliferation of human DPSC [50,54]. However, this treatment alters the architecture of a
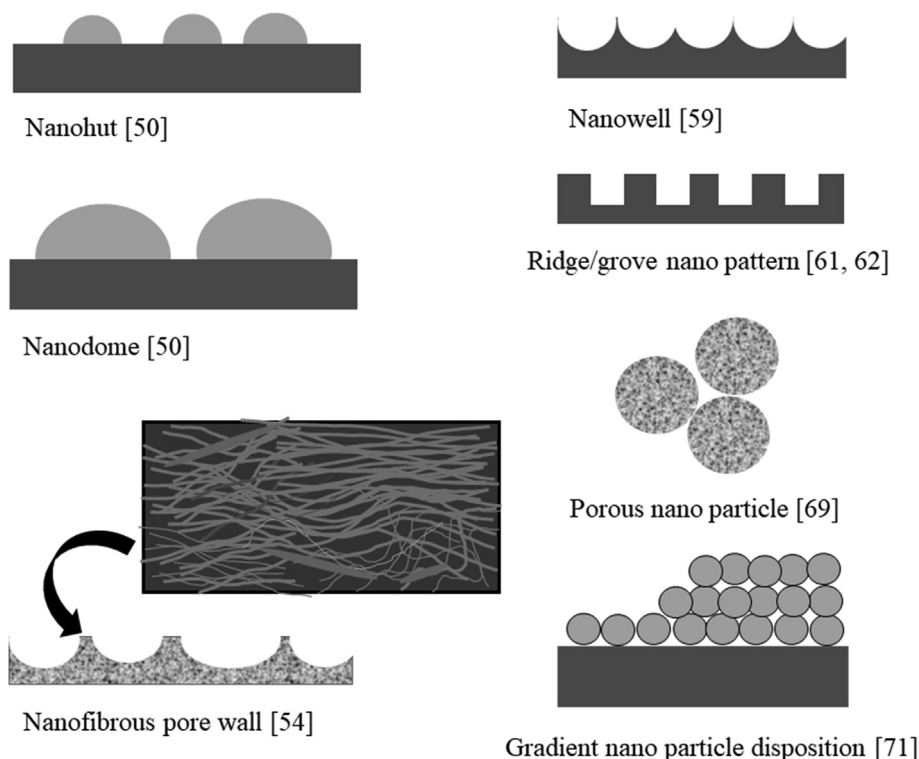

Fig. 2. The illustration of proposed nanotopography in the studies of human DPSC.

Table 2
Size does matter. Nanotopography effects on the behaviour of human DSPC.

No	Author/s	Compared Nanotopography	Effect on human DPSC
1.	Dolatshahi-Pirouz et al. [50]	Sputter-coated tantalum surface <0.2 nm surface roughness versus nanohut (height 2.9 ± 0.6 nm, width 35 ± 8) versus nanodome (height 13 ± 2 nm, width 52 ± 14 nm)	Nanodome is the most powerful signal for proliferation.
2.	Dolatshahi-Pirouz et al. [50]	Sputter-coated tantalum surface with fibronectin <0.2 nm surface roughness versus nanohut (height 2.9 ± 0.6 nm, width 35 ± 8) versus nanodome (height 13 ± 2 nm, width 52 ± 14 nm)	Nanohut promotes the highest number of filopodia and vinculin positive spot in cytoplasm. Nanodome is the most powerful signal for proliferation.
3.	Wang et al. [54]	Poly-ʟ-lactic acid (PLLA) 50—500 nm nanofibrous pore wall versus solid-walled	Nanofibrous pore wall PLLA enhances attachment, proliferation, alkaline phosphatase activity, osteocalcin and dentin sialophosphoprotein expression, and mineral deposition.
4.	Karakeçili et al. [59]	Polyhydroxymethylsiloxane (PHMS) Nanopores diameter 66.9 ± 4.7 nm and height 15.7 ± 1.3 nm versus diameter 274.3 ± 11.3 nm and height 111.7 ± 7.3 nm	PHMS with diameter 274.3 ± 11.3 nm and height 111.7 ± 7.3 nm promotes more cell attachment with well- developed and organized actin skeleton.
5.	Karakeçili et al. [59]	PHMS with Fibronectin coating Nanopores diameter 50.0 ± 4.6 nm and height 20.9 ± 3.9 nm versus diameter 127.3 ± 24.5 nm and height 33.9 ± 13.2 nm	PHMS-Fibronectin with diameter 127.3 ± 24.5 nm and height 33.9 ± 13.2 nm encourages the formation of more mature and higher number of adhesion site.
6.	Roh et al. [61] Kim et al. [62]	Poly-urethane acrylic (PUA) 250 nm ridge/grove pattern arrayed [61] or 350 nm ridge/grove pattern arrayed [62] versus conventional/smooth surface	250 nm ridge/grove patterned scaffold increases the expression of peroxisome proliferator activated receptor-γ, but reduces Runx 2 and osteocalcin expression. 350 nm ridge/grove patterned scaffold promotes higher expression of lipoprotein lipase, but lower Runx-2 expression.
7.	Wang et al. [69]	Bioactive glass (SiO_2, CaO, P_2O_5) 20 nm (nano bioactive glass) versus 2—20 μm (micro bioactive glass) porous particle	Nano bioactive glass induces stronger mineralization capacity and upregulates dentin sialophosphoprotein and dentin matrix protein-1.
8.	Bachhuka et al. [71]	Gradient disposition of gold nanoparticle onto a plasma polymerized allylamine-coated coverslip Gold nano particle size 16 nm versus 38 nm versus 68 nm	16 nm gold nanoparticle gives strongest cue for cell to attach and induces highest alkaline phosphatase intensity. 68 nm gold nanoparticle promotes highest proliferation rate.

scaffold. A PHMS polymer scaffold with nanopores diameter 274.3 ± 11.3 nm and height 111.7 ± 7.3 nm reduces in diameter and height after the fibronectin coating.

Interestingly, fibronectin treatment decreases the diameter but increases the height of a PHMS polymer with nanopores diameter 66.9 ± 4.7 nm and height 15.7 ± 1.3 nm, shows a rim-like conformation. The human DPSC exhibits more adhesion sites and a well-developed cytoskeleton on the more abundant nanopores PHMS scaffold with a fibronectin treatment [59]. Designing the nanotopography of a scaffold by patterning with grooves, pores, or wells, orienting fibers, or incorporating functional molecules directs DPSC differentiation into a chondrogenic, odontogenic, adipogenic, osteogenic, neurogenic, fibroblastic, or glial-like cell [61,62,65,70,71]. These findings encourage nanotopography engineering to drive the differentiation of DPSC for regenerating the dentin-pulp complex in the future.

4. Conclusion

This study has found that generally, regardless of the scaffold materials, nanotopography itself played a significant role in cell response and behavior. This research has thrown up many questions in need of further investigation. Further work needs to be done to determine the reproducible size and architecture needed to tailor DPSC into desired differentiation to mimic the dentin-pulp complex.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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