Preservation of alveolar ridge height through mechanical memory: A novel dental implant design

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\textbf{ABSTRACT}

Irreversible marginal bone loss can hinder recovery around dental implants. Insufficient alveolar osteogenesis and stress concentration during chewing contribute to marginal bone resorption and can result in implant failure. A biomaterial with a micropore-channel structure was developed using 3D printing technology. This design facilitated bony ingrowth and provided similar mechanical stimulation at the implant neck during mastication to a natural tooth. The micropore channels provided a guiding structure for bone mesenchymal stem cell proliferation and differentiation without the need for growth factors. Specifically, this was achieved through mechanical transduction by F-actin remodeling and the activation of Yes-associated protein (YAP). The implants were verified in a canine dental implant surgery model, which demonstrated the promising use of biomaterial-based dental implants in future clinical applications.

1. Introduction

Dental implants have become a popular alternative treatment for edentulism but are often associated with marginal bone loss over time [1]. The marginal bone reaction is of great importance not only because of the cosmetic requirements in some esthetic areas, but also because it is the most recognized standard for the success of the implant [2,3]. The design of an implant directly influences its clinical effect, especially its appearance and surface treatment. Previous studies have demonstrated that an appropriate thread pitch and taper, moderate roughness, nano-modification and hydrophilic treatments, and bioactive molecular modification can be optimized and applied to improve the initial osseointegration for faster bone binding [4–7]. Although the stability of the implant is enhanced by such treatments, the maintenance of crestal bone levels still requires continuous and appropriate medical stimulation around the neck, and other strategies must be systematically investigated. Natural teeth have periodontal ligament fibers between the root and the alveolar bone that continuously produce appropriate mechanical stimulation when chewing to help maintain the inherent height of the alveolar bone [8]. However, dental implants do not offer the same function due to their direct osseointegration between the implant and bone tissue. This study investigated micropore-channel dental implants produced using three-dimensional (3D) printing technology. This design promoted bony ingrowth, while ensuring compatible mechanical stimulation of the osteoblasts surrounding the implant neck. This stimulation during mastication was more similar to the horizontal traction of natural teeth than other implants, which demonstrated the promise of this strategy for achieving better marginal height (Fig. 1).

Biomaterials can be employed to fine-tune the fate and function of...
bone mesenchymal stem cells (BMSCs) around implants [9] and show promise for the innovative modification of clinical dental implants. The physical characteristics of implants with no chemical modification regulate cell behavior via a process called mechanical transduction [10,11]. This reaction between the cells and implant material is affected by the surface topography, where differing biochemical signals caused by this reaction can influence the cellular response to the surface [12,13].

Pore channels are a vital component with a large influence [14]. Cells tend to grow along pore channels, and Zhang et al. reported that the 3D printing of a hollow-pipe structure can induce rapid endothelial cell growth [15]. Further, de Godoy et al. found that increased porosity had a positive effect on osteoblast growth [16]. However, the underlying mechanisms by which pore-channel characteristics affect stem cell differentiation and tissue morphogenesis remain poorly understood.

The main effector of Hippo signaling is the YAP, which has recently been identified as a major regulator of mechanical transduction. YAP plays a vital role in transducing extracellular signals to intracellular effectors, thereby initiating downstream biochemical signals [17,18]. The tension and conformation of the F-actin cytoskeleton is the predominant determinant of YAP activation [19,20]. Previous studies have also shown that stem cells cultured on specific architectural substrates exhibited reorganization of actin bundles to produce cytoskeletal tension, which promoted YAP and the downstream activation of osteogenic genes [21–23]. However, the manner in which pore channels regulate the actin cytoskeleton and cause changes in the downstream YAP signal pathway remains unknown.

Selective laser melting (SLM) is a 3D printing process involving metallic materials, where a 3D implant is produced by layering metallic powder on a computer-aided design (CAD) model and selectively melting the layered powder using a high-energy laser [24,25]. This process involves a small number of production phases and has been used to produce excellent products with almost no waste. Further, SLM may be used to produce precise micro- and nano-designs for medical applications [26,27].

This study aimed to investigate a biomaterial with a micropore-channel architecture as a novel cell-free and growth factor-free approach to maintain the height of alveolar marginal bone around a dental implant. SLM 3D printing was used to prepare metal implants with a specialized micropore-channel structure. The structure physically guided F-actin remodeling and mediated YAP to generate tension, which subsequently initiated downstream signaling molecules for enhanced stem cell recruitment, proliferation, and differentiation. These effects facilitated the inherent healing ability of the alveolar crest. The findings of this study emphasized the importance of mechanically controlling cell phenotype and fate, where this simple and effective approach led to delayed and reduced bone height reduction after implantation. This study provides a theoretical basis for improving the success rate of dental implants.

2. Materials and methods

2.1. Sample fabrication by 3D printing

Gas-atomized Ti-6Al-4V powders (EOS Corp.) with an average particle size of 15–45 μm were used as a printing material. CAD modeling was performed and a direct metal laser melting (DMLM) machine (Concept Laser M2, GE Additive, Germany) was used to produce titanium in vitro templates and in vivo implants with cross-shaped micropore-channel diameters of 0, 500, and 750 μm, referred to as the control (con), 500c, and 750c samples, respectively. A laser power of 150 W and scanning speed of 500 mm/s in continuous wavelength mode were used. The samples were ultrasonically cleaned in formaldehyde, acid, and ethanol before use.

2.2. Characterization

The structure and distribution of the channels in the titanium templates and implants were observed using a stereo zoom microscope (Olympus, Japan). Scanning electron microscopy (SEM, JEOL JSM-
and permeabilized with phosphate buffer containing 4% paraformaldehyde (PFA) at room temperature (RT) for 15 min. The cytoskeleton was stained with tetrafluorinated, where the cells were washed with cold 1X PBS for 3 min, resuspended, and inoculated into a petri dish. The cells were cultured in high-dose Dulbecco’s modified Eagle’s medium for 1, 3, and 7 days. The cytoskeleton morphology was evaluated, while X-ray diffraction (XRD) was used to determine the chemical composition and phase purity of the samples.

2.3. Cells and animal subjects

All animal subjects were provided by the Animal Center of the Ninth People’s Hospital (Shanghai, China). All procedures were conducted in accordance with international ethical guidelines and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, as approved by the Laboratory Animal Management Committee of Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine. BMSC samples were obtained from four-year-old Sprague Dawley rats. Bone marrow was collected from the tibia and femur of each rat under sterile conditions. The sample was washed repeatedly with culture medium, combined with aspirate, centrifuged at 1000 rpm for 15 min, resuspended, and inoculated into a petri dish. The cells were cultured in high-dose Dulbecco’s modified Eagle’s medium (H-DMEM) containing 10% of fetal bovine serum (FBS; Gibco, USA) and 0.1 mg/ml of streptomycin (Gibco, USA). The cell culture was incubated at 37 °C in 5% CO₂. The second and third passages were used.

Six adult Beagle dogs (approximately 18 months old) were used for in vivo mandibular implantation. Dental implantation was performed under general anesthesia administered via intramuscular injection.

2.4. Effect of substrate mechanics on in vitro cytoskeleton and cell proliferation

The BMSCs were implanted on plates and held in full culture medium for 1, 3, and 7 days. The cytoskeleton morphology was evaluated, where the cells were washed with cold 1X PBS for 2–3 min, fixed in 4% paraformaldehyde (PFA) at room temperature (RT) for 15 min and permeabilized with phosphate buffered saline containing 0.1% Triton X (PBS-Tx-0.1%). The cytoskeleton was stained with tetramethylrhodamine isothiocyanate (TRITC)-phalloidin (Yeasen, China), where the nuclei were stained with 4′,6-Diamidino-2-phenylindol (DAPI, ThermoFisher Scientific, USA). The samples were imaged using a fluorescence microscope (Olympus, Japan). The BMSC proliferation induced by different pore channels was evaluated, where cells were cultured in EdU-containing medium (diluted to 1: 1000, 1 mg L-1; Ribobio, China). The samples were collected, and the plates were stained using a 5-ethynyl-2′-deoxyuridine (EdU) Apollo kit (Ribobio, China) according to the manufacturer’s instructions, while the cell nuclei were counterstained with Hoechst 33342. The samples were observed using a fluorescence microscope (Olympus, Japan), where statistical analysis of the images was used to calculate the percentage of EdU positive cells.

2.5. Evaluating the crucial role of YAP

Actin and YAP co-localization analysis was conducted in cells cultured on the substrate for 7 days. The cells were fixed with 4% PFA in PBS, infiltrated with 0.1% Triton X-100, blocked in 5% BSA, and stained with mouse monoclonal antibodies with anti-YAP (Santa Cruz Biotechnology, USA) at 4 °C overnight. The BMSCs were incubated with donkey anti-mouse immunoglobulin G (IgG) (Yeasen, China). Actin was stained with fluorescein isothiocyanate (FITC) -phalloidin (Yeasen, China), while nuclei were counterstained with DAPI. Confocal laser scanning microscopy (CLSM) was used to detect the spatial distribution of YAP and actin.

EdU and YAP co-localization analyses were also conducted in cells cultured on the substrate for 7 days. Proliferation was assessed using an EdU Apollo kit (Ribobio, China) using the same procedure as the actin and YAP co-localization analysis. Immunofluorescence staining of YAP was also performed according to the actin and YAP co-localization analysis procedure, but observation was performed using a fluorescence microscope (Olympus, Japan).

The total RNA from BMSCs cultured for 7 days was harvested using Trizol (TaKaRa, Japan), where the PrimeScript 1st Strand cDNA Synthesis kit (TaKaRa, Japan) were used for reverse transcription of the samples to cDNA. YAP, osteocalcin (OCN), osteopontin (OPN), runt-related transcription factor 2 (Runx2), bone sialoprotein ( BSP), and osterix (OSX) were assayed using quantitative real-time polymerase chain reaction (RT-PCR; LightCycler® 480II; Roche, Switzerland), where glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene. Primer sequences are given in Table 1. The relative gene expression levels were studied using the 2−ΔΔ Ct method. All assays were performed in triplicate.

BMSCs were cultured in a medium containing YAP-TEAD inhibitor 1 (Peptide 17), where a control group without Peptide 17 was used. Osteogenic differentiation was evaluated based on OCN and OPN expression. The primary antibodies targeting YAP were used for co-immunofluorescence. The BMSCs were inoculated on plates for 7 days and the cells were fixed with 4% paraformaldehyde for 15 min, washed with PBS, permeabilized with phosphate buffered saline containing 0.1% Triton X (PBS-Tx-0.1%), and incubated with 5% of bovine serum albumin block in PBS for 1 h. The cells were incubated with primary antibodies, namely mouse anti-YAP (Santa Cruz Biotechnology, USA), rabbit anti-OPN, and rabbit anti-OCN (Abcam, UK) at 4 °C overnight. The BMSCs were incubated with donkey anti-mouse IgG (Yeasen, China) and donkey anti-rabbit IgG secondary antibodies (Yeasen, China) and their nuclei were stained with DAPI. The substrate was washed with PBS and stored at 4 °C before imaging, where follow-up observations were performed using CLSM (Leica, Germany).

2.6. Maintaining alveolar height around in vivo implants

All premolars and first molars of the left mandible of the Beagle dog subjects were extracted under general anesthesia, and the wounds were left to heal for 3 months. 3 months after the tooth extraction, when the alveolar bone reconstruction was completed, the alveoloplasty was performed to make sure the height of alveolar bone consistent. And also during the implantation process, the height of the implant and the alveolar bone were strictly controlled to ensure that the initial alveolar bone height is flush with the top of the implant. The wound was left to heal for 3 months, after which all subjects were euthanized. The mandibles with implants were removed and fixed for 24 h.

The samples were examined and imaged using Micro-CT (μCT50, Scanco Medical, Switzerland), and the system software was used for 3D reconstruction and quantitative analysis. The alveolar height around the implant was analyzed using ImageJ software.

Polychrome sequential fluorescent labeling (SFL) was used to evaluate the formation of new bone and bony ingrowth around the implant. Fluorescent dyes, namely 30 mg/kg of Alizarin Red S (Sigma,
USA) and 20 mg/kg of Calcein (Sigma, USA), were administered intraperitoneally at 4 and 8 weeks after the operation, respectively. The samples were fixed, gradient dehydrated in ethanol, and embedded in polymethylmethacrylate (PMMA). The samples were cut into sections with a thickness of 150 μm using a Leica SP1600 saw microtome (Leica, Germany), and the sections were polished to a final thickness of about 40 μm. The fluorescent labeling was observed using CLSM (Leica, Germany). Van Gieson’s picrofuchsin was also performed for new bone formation observation, where the new bone areas were analyzed using ImageJ software.

2.7. Statistical analysis

The data were presented as a mean ± standard deviation. All statistical analyses were performed using the SAS 8.2 statistical software package (Cary, USA). Statistical comparisons were conducted via one-way ANOVA and Tukey’s post hoc test, where values of *p < 0.05 and **p < 0.01 were considered statistically significant.

3. Results and discussions

3.1. Fabrication and characterization of implants and templates with a pore-channel pattern

The templates and implants were produced using 3D printing, as illustrated in Fig. 2a. The square templates (1 cm*1 cm*1 mm) were used in vitro, while the implants were used in vivo and were designed by CAD. The micropore-channel portion of the implant was an array superposition of pore units. The dental implants had a uniform cross-shaped porous-pipe structure, where channels of different diameters (0, 500, and 750 μm) were formed (Fig. 2b). The channels were regularly arranged on the surface in a circular pore array.

The surface topographies of the 3D printed titanium templates were evaluated using optical microscopy and SEM. Stereo microscopy revealed that all samples consisted of pore-channels, and the diameters of 0, 500, and 750 μm were confirmed in the con, 500c, and 750c samples, respectively. Further, the samples exhibited a uniform texture with obvious pore characteristics. The mechanical properties of the samples were dependent on the pore-channel diameter, which affected cell behavior [9]. The SEM images illustrated the microstructure of the pore-channels, which were tightly bound, dense and complete among all the groups (Fig. 3a). Furthermore, the chemical composition and phase purity were evaluated using XRD analysis, where all of the printed samples exhibited diffraction peaks assigned to the Ti–6Al–4V phase (Fig. 3b).

3.2. Leading cell cytoskeleton alignment depends on pore-channel architecture

Previous research indicates that the mechanical and physicochemical properties of pore-channel architecture have a large effect on cell behavior [28]. The effects of the pore-channel microstructure of the templates on the basic bone regeneration processes were assessed in vitro, where the proliferation of BMSCs and the structure of the cytoskeleton were evaluated. BMSCs were cultured on the templates for 1, 3, and 7 days at the same density, after which the results were visualized using immunofluorescence staining and CLSM. F-actin and the nucleus were stained with phalloidin and DAPI (Fig. 4a). In the early stages of cell culture (Day 1), the cells were scattered uniformly on all of the templates. Further, the cytoskeleton morphology was relatively consistent, and cells showed random arrangements of nuclei and F-actin. The cytoskeleton morphology changed around the micropores compared to the control group during incubation. The cytoskeleton morphology gradually began to correspond with the shape of the micropore channels, where actin bundles began to aggregate and become distributed around the channels after 3 days. This progressed until 7 days incubation.

Pore-edge aggregation of the cytoskeleton was observed more closely using CLSM. The 750-μm micropore-channels exhibited a significant increase in the fluorescence intensity of intracellular F-actin. Further, the cytoskeleton morphology was highly consistent with the shape of the channel after aggregation (Fig. 4b). This was attributed to the consistent expression of F-actin in the single cells, where local fluorescence intensity increased due to morphological aggregation. These results clearly demonstrated that the reassembly of the cytoskeleton was directed by the micropore-channel architecture, which is a new mechanism for regulating pressure applied to actin.

3.3. Cytoskeleton mechanical force induces the expression of YAP

YAP is a transcriptional effector downstream of the Hippo signaling pathway and is related to important mechanical response signaling for tissue development and metabolism [29]. Studies have shown that F-actin can control the biomechanical activation of YAP [30]. The structure of actin and the expression of YAP proteins were evaluated in BMSCs cultured on the various templates. Immunofluorescence co-staining was conducted after 7 days of culturing and indicated that the YAP protein expression was higher in the micropore-channel templates than the flat template (Fig. 5a). The enhanced YAP expression was related to the cell’s position on the template, where actin is significantly deformed and aggregated around the micropore channels.

To verify whether YAP can sense the aggregation and rearrangement of actin bundles due to mechanical forces, the mRNA expression...
level of YAP was quantified using RT-PCR. The mRNA expression levels of the YAP genes on the two templates with micropore-channels were significantly up-regulated compared with the flat template (Fig. 5b), which further confirmed the actin aggregation activation observed by immunofluorescence staining. The differences in the expression of YAP in the templates with different micropore-channel diameters were highly related to the conformation of the F-actin cytoskeleton. These findings were consistent with previous reports, which found that mechanical forces regulate the hippo pathway by remodeling the actin cytoskeleton [31]. Considering this relationship, the morphological conditions must be optimized based on the function of BMSCs.

3.4. YAP activation for BMSC proliferation and differentiation during mechanical memory

YAP plays an important part in cell proliferation, bone differentiation, and cell fate via direct binding interactions with the transcriptional enhanced associate domain (TEAD) protein family. The ability of the micropore-channel structure to stimulate cell proliferation and its links to YAP were evaluated based on BMSC proliferation after culturing in medium for 3 days. EdU labeling revealed that the micropore-channel and flat templates both contained EdU positive cells (stained pink), but the groups exhibited significant differences in the number and location of these cells (Fig. 5c). Immunofluorescence staining
revealed that the EDU-positive cells on the 750-μm micropore-channel templates were concentrated around the micropores. The percentage of EdU-positive cells on the micropore-channel and flat templates are given in Fig. 5d.

The correlation between the kind of proliferation and YAP activation was evaluated based on the co-localization of EdU and YAP. Immunofluorescence analysis revealed that there was a higher number of EdU-positive and YAP-positive cells on the micropore-channel templates. Further, the EdU-positive and YAP-positive cells were found in the same positions, indicating that the expression of YAP in the proliferating cells increased (Fig. 5e). The link between this unique proliferation behavior and locations on the template was related to the activation of upstream YAP proteins.

YAP regulates the balance of adipogenic and osteogenic differentiation in BMSCs and acts as a cofactor of RUNX2, which is essential for homeostasis [32]. The osteogenic regulators (Runx2, BSP, OPN, and OSX) produced by BMSCs were examined to evaluate the effect of YAP activated by cytoskeleton orientation on the regulation of osteogenic differentiation. RT-PCR analysis involved the extraction of total mRNAs from the samples, and revealed that the osteogenic regulator expression levels were slightly increased in BMSCs induced on the 500c template and were significantly increased on the 750c template compared to flat template (Fig. 6a and d).

OCN and OPN were identified as particularly important differentiation regulators and are mainly involved in the regulation of bone matrix mineralization. These regulators are, thus, important markers for bone activity and bone turnover. The correlation between YAP expression and bone development was evaluated using double immuno-fluorescence staining of EDU and YAP.

Fig. 5. Improved cell proliferation and YAP expression, namely (a) YAP levels based on immuno-fluorescence staining, (b) YAP expression during early incubation (n = 4, **p < 0.01), (c–d) percentage of proliferative cells and statistical analysis based on the EdU staining results (n = 3, **p < 0.01, *p < 0.05), and (e) double immuno-fluorescence staining of EDU and YAP.

3.5. Maintaining alveolar bone height after implantation in canines

The micropore-channel templates promoted improved cell viability, proliferation, and osteogenic differentiation. A decrease in alveolar bone height is related to poor osteogenesis at the top of the alveolar socket and a lack of osteoinduction at the apical position [33,34]. This growth-factor free approach was applied to bone remodeling around a standard canine dental implantation (Fig. 7a). Micro-CT revealed a significant marginal bone loss in the control group, while subjects with the micropore-channel templates exhibited superior retention of OCN and OPN levels on the micropore-channel templates were significantly higher than the control group. Selective high YAP expression in the OCN/OPN-positive region was observed around the pore-channel structure (Fig. 6e and g). Further, the high expression of OCN and OPN at the edge of the micropore-channels reflected the same trend observed for actin distribution, which was characterized as an arc-shaped distribution along the edge of the channel. This phenomenon was more pronounced on the 750-μm micropore-channel template. The relationship between YAP and osteogenic differentiation was explored further by adding YAP inhibitors to the culture medium. Immunofluorescence staining after 7 days revealed that the BMSCs were affected by the YAP inhibitors, where the expression of OCN and OPN decreased (Fig. 6f and h). However, the expression was not inhibited completely, indicating that YAP did not promote the osteogenic differentiation of BMSCs alone. Thus, cell differentiation based on mechanical memory was possible using YAP activation. Overall, YAP played a critical role: it achieved the mechanical transduction by F-actin remodeling and thereby activated downstream osteogenic related proteins, which facilitated osteoblastic differentiation without osteogenic supplements.
alveolar bone height on the lingual and buccal sides (Fig. 7b). The buccal alveolar bone height loss was measured (Fig. 7c and d). The 750c group better retained marginal alveolar bone height than the con and 500c groups; the differences were statistically significant. These results prove that the hollow implant design slowed bone resorption of the marginal alveolar bone to some extent. This might be due to the fact that when the cells gathered around the pores, they could undergo the appropriate mechanical stimulation, reshape F-actin, and activate the osteogenic signal, which was similar to the role of periodontal fibrous tissue in natural teeth in stimulating the differentiation of pre-osteoblasts into osteoblasts.

Successful bone tissue regeneration in the side openings and internal channels was required to maintain bone height and evaluated using Van Gieson’s picro fuchsin staining (Fig. 7e). All samples exhibited close adhesion between the bone and implant, while the micropore-channel templates led to new growth within the internal channels. The newly formed bone tissue was qualitatively assessed using fluorescent bone markers at 4 and 8 weeks after implantation. The newly formed bone area was analyzed using confocal microscopy (Fig. 7e). The total area of alizarin red fluorescent bone marker around the implant after 8 weeks was much higher in the micropore-channel templates than in the flat template, where a trend in calcein was observed (Fig. 7f). The newly formed bone area and alveolar bone height around the bone implants in the 500c and 750c micropore-channel templates were significantly higher than in the flat templates, as observed using Micro-CT.
Previous studies have shown that the long-term survival rate of dental implant systems with hollow structures is 85% higher than conventional systems. However, hollow implant systems are associated with large side holes and a cylindrical hollow bucket at the base of the implant. The findings of this study suggest that the micropore-channel implant could be used as a potential treatment with limited alveolar bone loss. Bone regeneration was significantly improved, which promoted the stability and overall success of the dental implantations. This study clearly demonstrated the clinical application prospects of this biomaterial implant.

Fig. 7. Application of novel implant in vivo, illustrated as (a) the surgical procedure, (b) Micro-CT images of the bone repair 3 months after implantation, (c–d) the height of buccal alveolar bone (*n* = 3, **p** < 0.01), (e–f) Van Gieson’s picrofuchsin and immunofluorescence staining 3 months after implantation, and (g) quantitative analysis of the new bone area (*n* = 3, **p** < 0.01).

4. Conclusions
Implants containing cross-shaped micropore-channels were produced using 3D printing technology. The open microstructure promoted bone ingrowth and preserved the height of the alveolar bone without the need for growth factors and bovine-derived porous xenograft. The implant provided a guiding structure for BMSC proliferation and differentiation via mechanical transduction by promoting F-actin remodeling and YAP activation. These findings provide further insights into the relationship between osteogenic factors and altered surface topology, and is expected to aid the future development of new high-quality dental implants for clinical applications.
Disclosure of competing interest

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

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References

[1] R. Tabrizi, H. Mohajerani, B. Ardalan, K. Khiahani, Does preservation of the socket decrease marginal bone loss in the mandible after extraction of first molars? Br. J. Oral Maxillofac. Surg. 57 (5) (2019) 886–890.
[2] H. Tercanli Alkis, N. Turker, Retrospective evaluation of marginal bone loss around implants in a mandibular locator-retained denture using panoramic radiographic images and finite element analysis: a pilot study, Clin. Implant Dent. Relat. Res. 21 (6) (2019) 1199–1205.
[3] E.C. Rosa, T.M. Deliberador, T.C.D.L.D. Nascimento, C.C.D.A. Kintopp, J.S.R. Orsi, L.M. Wambier, C.L.M. Storrer, Does the implant-abutment interface interfere on marginal bone loss? A systematic review and meta-analysis, Braz. Oral Res. 33 (2019).
[4] E. Velasco-Ortega, A. Jimenez-Guerra, L. Monsole-Guil, I. Ortiz-Garcia, A.I. Nicolas-Silvente, J.J. Segura-Egea, J. Lopez-Lopez, Long-term clinical outcomes of treatment with dental implants with acid etched surface, Materials 13 (7) (2020) 1553.
[5] E. Anitza, L. Piñas, M.H. Alkhraisat, Early marginal bone stability of dental implants placed in a transalveolarly augmented maxillary sinus: a controlled retrospective study of surface modification with calcium ions, International Journal of Implant Dentistry 3 (1) (2017) 1–5.
[6] D.M.D. Ehrenfest, P.G. Coelho, B.S. Kang, Y.T. Schul, T. Albrektsson, Characterization of osseointegrated implant surfaces: materials, chemistry and topography, Trends Biotechnol. 28 (4) (2010) 198–206.
[7] E. Velasco-Ortega, A. Jimenez-Guerra, L. Monsole-Guil, I. Ortiz-Garcia, A.I. Nicolas-Silvente, J.J. Segura-Egea, J. Lopez-Lopez, Long-term clinical outcomes of treatment with dental implants with acid etched surface, Materials 13 (7) (2020) 1553.
[8] M. Ikar, T. Grobecker-Karl, M. Karl, C. Steiner, Mechanical stress during implant surgery and its effects on marginal bone: a literature review, Quintessence Int. 51 (2) (2020).
[9] A. Petersen, A. Priem, G. Korus, A. Ellinghaus, H. Leemhuis, A. Herrera, S. Geissler, A biomaterial with a channel-like pore architecture induces endochondral healing of bone defects, Nat. Commun. 9 (1) (2018) 1–16.
[10] S. Zhan, B. Ma, F. Liu, J. Duan, S. Wang, J. Qiu, D. Li, Y. Sang, C. Liu, D. Liu, H. Liu, Polyactic acid nanoparticle array-driven osteogenic differentiation of human adipose-derived stem cells determined by pillar diameter, Nano Lett. 18 (4) (2018) 2245–2253.
[11] S.S. Kim, J.H. Kim, B. Kim, Y.S. Park, H.K. Kim, H.T. Tran, S.H. Kim, J. Heon, S. Kim, J.H. Sim, H.M. Shin, A specific groove pattern can effectively induce osteoblast differentiation, Adv. Funct. Mater. 27 (44) (2017) 1703569.
[12] S. Park, H.H. Park, K. Sun, Y. Gwon, M. Seong, S. Kim, T.E. Park, H. Huyun, Y.H. Choung, J. Kim, H.E. Jeong, Hydrogel nanoparticles patch as a flexible anti-panothigenic scaffold for regulating stem cell behavior, ACS Nano 13 (10) (2019) 11181–11193.
[13] Z. Chen, A. Bachhuka, S. Han, F. Wei, S. Lu, R.M. Visalakshah, K. Vasilev, Y. Xiao, Tuning chemistry and topography of nanogengineered surfaces to manipulate immune response for bone regeneration applications, ACS Nano 11 (5) (2017) 4492–4506.
[14] L.S. Wray, J. Rnjak-Kovacina, B.B. Mandal, D.F. Schmidt, E.S. Gil, D.L. Kaplan, A silk-based scaffold platform with tunable architecture for engineering critically-sized tissue constructs, Biomaterials 33 (36) (2012) 9214–9224.
[15] W. Zhang, C. Feng, G. Yang, G. Li, X. Ding, S. Wang, Y. Dou, Z. Zhang, J. Chang, C. Wu, X. Jiang, 3D-printed scaffolds with synergistic effect of hollow-pipe structure and bioactive ions for vascularized bone regeneration, Biomaterials 135 (2017) 85–95.
[16] R.P. De Godoy, S. Hutchens, C. Campion, G. Blunn, Silicate-substituted calcium phosphate with enhanced strut porosity stimulates osteogenic differentiation of human mesenchymal stem cells, J. Mater. Sci. Mater. Med. 26 (1) (2015) 54.
[17] V.A. Godina, K.D. Irvine, Hippo signaling goes long range, Cell 150 (5) (2012) 1075–1079.
[18] J. Gao, L. He, L. Zhou, Y. Jia, F. Wang, Y. Shi, M. Cai, J. Sun, H. Xiu, J. Jiang, L. Zhang, Mechanical force regulation of YAP by F-actin and GPCR revealed by super-resolution imaging, Nanoscale 12 (4) (2020) 2703–2714.
[19] C. Shi, Y. Cai, Y. Li, Y. Li, N. Hu, S. Ma, H. Zhou, Yap promotes hepatocellular carcinoma metastasis and mobilization via governing coflin/F-actin/lamellipodium axis by regulation of JNK/Itk3/3/SECA/CaMKII pathways, Redox Biology 14 (2018) 59–71.
[20] S. Nasrollahi, C. Walter, A.J. Loza, G.V. Schimizzi, G.D. Longmore, A. Pathak, Past matrix stiffness primes epithelial cells and regulates their future collective migration through a mechanical memory, Biomaterials 146 (2017) 146–155.
[21] V. Le, J. Lee, S. Chaterey, A. Spencer, Y.L. Liu, P. Kim, H.C. Yeh, H.K. Kim, A.B. Baker, Syndecan-1 in mechanosensing of nanotopographical cues in engineered materials, Biomaterials 155 (2018) 13–24.
[22] L.Y. Chen, J.C. Huang, C.H. Lin, C.T. Pan, S.Y. Chen, T.L. Yang, D.Y. Lin, H.K. Lin, J.S.C. Jiang, Anisotropic response of Ti-6Al-4V alloy fabricated by 3D printing selective laser melting, Mater. Sci. Eng., A 682 (2017) 389–395.
[23] A. Basalah, Y. Shanjani, S. Esmaeili, E. Torskovsky, Characterizations of additive manufactured porous titanium implants, J. Biomed. Mater. Res. B Appl. Biomater. 100 (7) (2012) 1970–1979.
[24] Z.X. Khou, Y. Liu, J. An, C.K. Chua, Y.F. Shen, C.N. Kuo, A review of selective laser melted Ti6Al7N titanium alloy, Materials 11 (4) (2018) 519.
[25] X. Pei, B. Zhang, Y. Fan, X. Zhu, Y. Sun, Q. Wang, X. Zhang, C. Zhou, Bionic mechanical design of titanium bone tissue implants and 3D printing manufacturing, Mater. Lett. 208 (2017) 133–137.
[26] S. Shi, T. Fujie, A. Saito, S. Takeoka, Y. Hou, Y. Shu, M. Chen, H. Wu, A. Khademhosseini, Periosteum-mimetic structures made from freestanding microfabricated nanoshapes, Adv. Mater. 26 (20) (2014) 3296–3296.
[27] S. Lee, A.E. Stantont, X. Tong, F. Yang, Hydrogels with enhanced protein conjugation efficiency reveal stiffness-induced YAP localization in stem cells depends on biochemical cues, Biomaterials 202 (2019) 26–34.
[28] J. Oliver-De La Cruz, G. Nardono, J. Vrbsky, A. Pompeiano, A.R. Perestrello, F. Capradosso, K. Melakov, P. Filipensky, G. Forte, Substrate mechanics controls adipsogenesis through YAP phosphorylation by dictating cell spreading, Biomaterials 205 (2019) 64–80.
[29] H.P. Lee, R. Stowers, O. Chaudhuri, Volume expansion and TRPV4 activation regulate stem cell fate in three-dimensional microenvironments, Nat. Commun. 10 (1) (2019) 1–13.
[30] S. Wan, F. Xu, Y. Ji, M. Li, X. Shi, Y. Wang, PAK and YAP/TAZ dependent mechanotransduction pathways are required for enhanching immunomodulatory properties of adipose-derived mesenchymal stem cells induced by aligned fibrinous scaffolds, Biomaterials 171 (2018) 106–111.
[31] V.V. Kumar, K. Sagheb, P.W. Kämmerer, B. Al-Nawas, W. Wagner, Retrospective clinical study of marginal bone level changes with two different screw-implant types: comparison between tissue level (TE) and bone level (BL) implant, Journal of Maxillofacial and Oral Surgery 13 (3) (2014) 259–266.
[32] A.L. Lima, D.D. Bosshardt, L. Chambrone, M.G. Araújo, N.P. Lang, Excessive occlusal load on chemical modifications and moderately rough titanium implants stored with cantilever reconstructions. An experimental study in dogs, Clin. Oral Implants Res. 30 (11) (2019) 1142–1154.