Lasers Med Sci (2017) 32:1197–1206
DOI 10.1007/s10103-017-2217-7

REVIEW ARTICLE

In vitro biological outcome of laser application for modification or processing of titanium dental implants

Ahmed Hindy[1] · **Farzam Farahmand**[2] · **Fahimeh sadat Tabatabaei**[1,3]

Received: 29 October 2016 / Accepted: 19 April 2017 / Published online: 27 April 2017

Abstract There are numerous functions for laser in modern implant dentistry including surface treatment, surface coating, and implant manufacturing. As laser application may potentially improve osseointegration of dental implants, we systematically reviewed the literature for in vitro biological responses to laser-modified or processed titanium dental implants. The literature was searched in PubMed, ISI Web, and Scopus, using keywords "titanium dental implants," "laser," "biocompatibility," and their synonyms. After screening the 136 references obtained, 28 articles met the inclusion criteria. We found that Nd:YAG laser was the most commonly used lasers in the treatment or processing of titanium dental implants. Most of the experiments used cell attachment and cell proliferation to investigate bioresponses of the implants. The most commonly used cells in these assays were osteoblast-like cells. Only one study was conducted in stem cells. These in vitro studies reported higher biocompatibility in laser-modified titanium implants. It seems that laser radiation plays a vital role in cell response to dental implants; however, it is necessary to accomplish more studies using different laser types and parameters on various cells to offer a more conclusive result.

✉ Fahimeh sadat Tabatabaei
f.s.tabatabaei@gmail.com; f.tabatabaei@sbmu.ac.ir

[1] Department of Dental Biomaterials, School of Dentistry, Shahid Beheshti University of Medical Sciences, Evin, Tehran 1983963113, Iran

[2] Department of Mechanical Engineering, Sharif University of Technology, Tehran, Iran

[3] Department of Tissue Engineering, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Keywords Biocompatibility · Biological response · Differentiation · Laser · Surface modification · Surface coating · Titanium dental implants

Introduction

Developing materials that are physically and biologically compatible with alveolar bone remains a challenge in dental implant design. Titanium is commonly used in dental implant manufacturing due to their proper physical properties. Titanium oxide forms a dense, protective, and strongly adherent layer over dental implants, which is called the passive film, and is excellent in resisting corrosion [1–4]. Under optimal conditions, bone differentiation occurs directly adjacent to the implanted material. This process is called osseointegration and is the direct structural and functional connection between living bone and load-carrying dental implant surfaces and is the main requirement for long-term success of dental implants [5, 6]. Surface topography, chemistry, degree and scale of roughness, and wettability can modify cellular behaviors such as adhesion, proliferation, differentiation, and migration during the osseous healing period and can promote osseointegration [7, 8].

Although titanium dental implants have high clinical success rates [9], multiple approaches have been developed during the last few decades to enhance physical and chemical aspects of osseointegration and to reduce the duration of its formation. Surface-roughened implants and ceramic coatings are well-established practices while three-dimensional (3D) printing remains an experimental technique [6, 7, 10–15]. Several studies have suggested that the roughness of titanium dental implants can promote cytocompatibility, enhance surface area of implants adjacent to bone cells, and increase biochemical interaction of implants with bone osteoblasts [14,

16–18]. Compared to 2D surface roughness, porous implants decrease stress shielding and increase bone-implant interlocking such that a high porosity implant that mimics natural tissue is capable of stimulating osteoblast differentiation [19, 20]. Gradient porosity is another important issue in producing dental implants. It confers reactive properties to the implant surface and decreases its modulus of elasticity to match that of bone [21]. Three-dimensional printing gives the chance to directly produce dental implants with different shapes, textures, and gradient of porosity and also minimize post-processing requirements [22].

Nowadays, investigators use laser irradiation for surface or structural modification of dental implants. Applications of laser are versatile and laser can be used to heat, melt, or vaporize materials based on the type of laser used [1]. Laser application for various purposes depends on properties such as direction, divergence, wavelength, and frequency of laser beam, which can be adjusted by the laser components [13]. Considering the importance of cell-implant interaction on the success rate of dental implants, the effect of laser on biological properties of dental implants has gained interest. The aim of this review was to analyze the influence of laser treatments of uncontaminated titanium implant surfaces on in vitro behavior of cells (cellular morphology, cell adhesion, viability, proliferation, and differentiation). Furthermore, the in vitro biological responses as the outcome of the laser application in surface coating were evaluated. Lastly, special attention was focused onto the laser application in additive manufacturing of titanium dental implants to determine whether this new technology is as good as or better than other tools for supporting cell growth.

Materials and methods

Search strategy

We searched PubMed, ISI Web, and Scopus databases to find relevant articles published between 2000 and 2016 using the following keywords: laser, AND modification (OR processing, melting, coating), AND titanium dental implants (OR titanium surface), AND biocompatibility (OR in vitro bioresponse OR cell activity). Subsequently, each article's references were reviewed to identify other relevant articles.

This systematic review assessed whether laser enhanced the in vitro biological response of titanium dental implants. Table 1 outlines the questions that were addressed with reference to participants or population (P); intervention (I); comparison, control, or comparator (C); outcome (O); and study design (S) (PICOS elements).

Inclusion criteria

Titanium dental implants which had been treated/coated by use of laser or processed using laser were included. There was no distinction made with regard to the grade of titanium, type of laser, or parameters of laser. In vitro studies which reported some measures of biological responses as an outcome were included. Interventions based on combinations of laser and other modalities such as ceramic coating or growth factors were also considered for review.

Exclusion criteria

Studies carried out on animal models and studies other than in vitro's were excluded. Publications in languages other than English were excluded. In vitro studies on contaminated implants in which disinfection of the implant surface was stimulated by laser were excluded. Similarly, studies on zirconia implants, orthopedic implants, and those on abutments were excluded.

Selection of studies and quality assessment

Two trained reviewers (A.H. and F.T.) performed independent searches, assessed publication validity, and extracted the data in duplicate. Disagreements were resolved by discussion, rereading, and consultation with the third member of the research team (F.F.) when necessary. All citations were imported into an electronic database (EndNote). The quality rating of studies was based on comprehensiveness and reproducibility of the methodology, the use of standard methods to appraise the biological response, and the absence of apparent bias in results.

Table 1 PICOS format of the question used in this systematic review

Component	Description
Population	Studies of titanium dental implants and the use of laser
Intervention	Laser application for surface treatment, surface coating, or manufacturing of dental implants
Comparison	Different types of laser that were used (pulsed vs continuous and types of laser device)
Outcome	Cell behavior on the surface of dental implants
Study design	In vitro studies

Table 2 Studies on surface modification of dental implants with laser

	Author	Ti type	Laser type	Cell type	Assay	Result
1	Groessner-Schreiber et al. [23]	Cp Ti grade II	Nd:YAG, 50 J	Mouse fibroblasts (balb/3T3; ATCC)	Cell spreading (SEM and fluorescence microscopy) MTT assay BCA protein assay	SEM showed cells cultured on laser-treated titanium surfaces and on discs coated with TiN appeared to be stronger and well spread and showed a polygonal shape, but differences in mean values between cells cultured on polished, oxidized, or laser-treated titanium discs did not reach statistical significance.
2	Schwarz et al. [24]	Cp Ti	Er: YAG laser at an energy level of 100 mJ/pulse and 10 Hz	SAOS-2 cells	Cell count	Discs treated with laser demonstrated nearly the same cell count as the untreated surfaces
2	Hao et al. [25]	Ti6Al4V	1.5-kW high-power diode laser	Human osteoblastic cell line hFOB 1.19	Cell attachment, MTT assay	Favorable cell response was observed on the HPDL laser-treated Ti6Al4V alloy than on either un-treated samples or a mechanically roughened samples
3	Lawrence et al. [26]	Ti6Al4V	Nd:YAG, 200 W	Human osteoblastic cell line (hFOB 1.19)	LDH, cell adhesion, MTT assay	Cell adhesion and proliferation on laser-treated samples was considerably better than those on untreated samples.
4	Biswas et al. [13]	Ti6Al4V	1.5-kW continuous wave (CW) diode laser	L-929 (mouse fibroblast cell line)	MTT assay	Cell counts on laser-treated surfaces exceeded those of the positive control. Highest numbers were observed on laser-nitrided surfaces, and lowest numbers were observed on laser-melted surfaces.
5	Ulerich et al. [12]	Ti6Al4V	Nd:YVO4 laser (355 nm), (0–300 μJ) [27]	Human osteosarcoma cells	Cell growth (fluorescence imaging microscope)	Cell growth exhibited a greater density and a greater degree of cell alignment, which led to contact guidance, but higher energy and greater roughness resulted in a much larger number of cells covering multiple grooves.
6	Heinrich et al. [28]	Plasma-sprayed Ti implants	KrF excimer laser, (248 nm), energy density 15–17 J/cm^2	Fibroblast	Cell attachment ESEM	Improved cell attachment to the laser hole boundary.
7	Erdogan et al. [29]	Ti6Al4V	Nd laser, 1060 and 1035 nm, 1 W	Saos-2	Cell attachment, and cell count	Cell attachment and proliferation on picosecond-laser-textured surfaces are as good as commercially available surfaces (sandblasting, acid etching, and the SLA method)
8	Dolores Paz et al. [30]	Ti6Al4V	Nd:YAG (1064 nm), 6.19 J/cm^2 ArF excimer lasers (193 nm) 240 mJ/cm^2	Human fetal osteoblastic cell line (Hfob 1.19)	Cell proliferation, ALP activity	Laser macrostructuration alone did not promote cell response. However, UV laser oxidation enhanced cell proliferation.
9	Ayobian-Markazi et al. [31]	SLA titanium	Er:YAG, 2940 nm, pulse energy 60–100 mJ	Sarcoma osteogenic (SaOs-2)	Cell attachment, MTT assay	Significant differences were noted in proliferation and viability between the experimental and control groups, but the difference in the mean MTT score did not reach statistical significance.
10	Györgyey et al. [32]	Cp Ti grade IV	Doubled Q-switched Nd:YAG 532 nm, 1–1.5 J/cm^2, KrF excimer laser (248 nm, 0.4–0.6 J/cm^2)	Osteoblast-like MG-63	Cell attachment (AB), MTT, ALP	MTT, AB, and ALP methods did not reveal any significant differences between laser-ablated surfaces and the controls, although cells were more homogenously spread in laser-treated disks.
11	AyobianMarkazi et al. [27]	SLA titanium	Er:YAG, 2940 nm 12.8 J/cm^2, pulse energy up to 500 mJ	Sarcoma osteogenic (SaOs-2)	MTT assay	Significantly higher cell viability was observed in the test group.
12	Chikarakara et al. [33]	Ti6Al4V	1.5-kW CO_2 laser	BALB 3T3 cells (mouse embryonic fibroblast cell line)	Cell viability assay, MTT assay, Hoechst 33258 DNA assay, AB assay	Laser-treated surfaces promoted cell attachment and proliferation and enhanced bioactivity compared to untreated samples.
13	Vignesh et al. [34]	Cp Ti grade II	Nd:YAG, Q-switched laser; 1.5–4.5 J/cm^2	L929 murine fibroblasts	Cell attachment	Laser-treated surfaces showed strong cell adhesion and spread widely and had denser cell growth compared to machined or acid-etched surfaces but did not have any particular orientation to particular surfaces.
14	Mariscal-Muñoz et al. [15]	Cp Ti grade IV	Yb:YAG 1064 nm; average pulse power of 10 kW	Mouse calvarial osteoblasts (primary cell)	MTT assay, alizarin red assay, ALPase activity, real-time PCR	Laser treatment of surfaces induced calcified nodules, stimulated ALPase, decreased cell proliferation, and upregulated osteoblastic gene expression

Table 2 (continued)

	Author	Ti type	Laser type	Cell type	Assay	Result
15	Mukherjee et al. [35]	Ti6Al4V	Yb continuous wave fiber laser, different laser frequency, and duty cycle	MG63 cells	MTT, ALP activity, cell spreading	The sample with the lowest wavelength and highest duty cycle proved to be the best.
16	Hsiao et al. [36]	Ti6Al4V, HA-coated Ti implant,	ArF excimer 355 nm, pulse energy 150 μJ	MC3T3 osteoblast precursor cells, fibroblasts	Cell growth (immunofluorescence)	Potential growth of both fibroblasts and bone cells

Cp Ti commercially pure titanium, *Nd:YAG* neodymium-doped yttrium aluminum garnet, CO_2 carbon dioxide, *CW* continuous-wave, *Er:YAG* erbium-doped yttrium aluminum garnet, *Nd:YVO4* neodymium-doped yttrium orthovanadate, *ATCC* American Type Culture Collection, *BCA* bicinchoninic acid, *TiN* titanium nitride, *HFOB* human fetal osteoblastic cells, *HPDL* high-power diode laser, *LDH* lactate dehydrogenase, *KrF* krypton fluoride laser, *MG-63* osteosarcoma cell line, *PCR* polymerase chain reaction, *Yb* ytterbium-doped, *HA* hydroxyapatite, *Saos-2* sarcoma osteogenic cell line, *MTT* dimethylthiazol-diphenyl tetrazolium bromide, *SEM* scanning electron microscopy, *ESEM* environmental scanning electron microscope, *FL* fluorescence microscopy, *VM* video microscope, *AB* Alamar Blue, *ALP* alkaline phosphatase activity, *SLA* sandblasted large-grit acid etch, *PCR* polymerase chain reaction

Data extraction

We extracted data from different scenarios: (1) studies reporting surface modification or treatment of dental implants with laser, (2) studies reporting laser-assisted titanium coating, and (3) studies reporting laser-based manufacturing of titanium dental implants. Given the heterogeneity of the dose, type of laser, and type of cells, no statistical analysis was used to synthesize the data.

Results

The initial search identified 332 articles, 136 of which were chosen after screening their titles and abstracts. After retrieving the articles' full texts, 28 were included in this systematic review. Sixteen articles studied surface modification or treatment using laser, while six articles studied laser-assisted coating, and another six were different articles that studied using laser for manufacturing titanium dental implants. All 29 articles were in vitro studies published between 2000 and 2016. Tables 2, 3, and 4 summarize the results according to the technique that was utilized.

Discussion

Today, biocompatibility is a grand area of concern in dental biomaterial properties. Most dental implants support cell attachment by conferring suitable areas for cell adhesion [48]. Laser offers a high energy that can be applied to modify surfaces made of different materials and to produce three-dimensional nano- and microstructures. It is used in different surface modification techniques because of its ability to rapidly and effectively induce physical and/or chemical changes such as surface roughness and deformation and assist coating of biomaterial surfaces [49]. Some advantages of laser include generation of complex features with high resolution, high degree of purity [50], suitability for selective changes in implant surfaces, and its precision [51, 52]. Regulation agencies such as the Food and Drug Administration in the USA require biocompatibility testing per ISO 10993 (International Standard Organization: Standard for Biological Evaluation of Medical Devices) or ASTM F748 (American Society for Testing of Materials: Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices) prior to device approval. Consequently, there is a need to carry out biocompatibility testing for any new material or processing method [53].

In vitro experiments are the first step in biocompatibility testing of new materials by the observation of viability and biofunctionality of cells on a material surface; therefore, in this review, we focused on the in vitro biological responses of dental implants processed with laser as a new processing method. Based on our review, the most common in vitro assays were MTT assays, cell attachment, proliferation, and cell counting.

This review was limited to the study of titanium and its alloys since titanium is used commonly in dental implants, and there are many studies of the long-term outcomes of titanium dental implants. Most of the studies reviewed in this article used Ti6Al4V alloy, and only nine studies used Cp Ti. It has been demonstrated that bone cell interactions are mainly determined by the chemistry of the substrate, the structure of the implanted material, and the production method. However, the topography of the surface is more important in cell behavior than the chemistry of implant material or the processing method [54], although it should be noted that these effects are difficult to separate as they are interrelated [48].

Surface modification of titanium with laser can promote micron-level surface texturing, increase the surface area, and

Table 3 Studies on laser-assisted coating of dental implants

No.	Author	Ti type	Laser type	Coat material	Cell type	Test	Result
1	Lusquinos et al. [37]	Ti6Al4V	Nd:YAG 1064 nm, 2 kW/cm^2	TCP	MG-63 osteoblast-like cells	AB MTT assay	Cells on TCP coat exhibited a significantly higher proliferation rate, but in longer periods of time, no statistically significant differences were observed.
2	Seydlova et al. [38]	Ti6Al4V	KrF excimer 248 nm 450 mJ, ArF excimer 193 nm 330 mJ	ZrO2 interlayers, HA coat	3T3 murine line fibroblasts, human dermal fibroblasts	Monoclonal antibody (fluorescence microscope) MTT assay	Laser-coated surfaces were not cytotoxic. Fibroblast cell morphology did not change, and rapid cell proliferation resulted in almost confluent growth.
3	Teuberova et al. [39]	Ti6Al4V	KrF excimer laser 248 nm, 4 J cm^{-2}	ZrO_2 buffer layer, HA coat	Human embryonal lung fibroblasts (LEP19)	Cell count MTT assay	PLD zirconia/HA coating can promote the growth of fibroblasts as a biomimetic coating.
4	Bose et al. [40]	Cp 99.8%	Nd:YAG 400–500 W	TCP	Osteoblastic precursor cell line (OPC1)	Cell attachment ALP (CSLM) MTT assay	Laser TCP coat and titania nanotube surfaces showed good cell attachment, high cell proliferation, and early differentiation.
5	Gao et al. [41]	Cp Ti	5 kW CW CO_2 laser	CaP	Osteoblasts	RT-PCR MTT assay Cell morphology (SEM)	Cell growth showed significantly higher optical density and TGβ1 mRNA expression, and BMP2 was significantly upregulated, resulting in better surface cytocompatibility
6	Oyane et al. [42]	Cp Ti	Excimer 355 nm, 4 W/cm^2	CaP	Chinese hamster ovary-K1 cells	Cell adhesion assay	Cell adhesion assays indicated that laser Ti surface CaP biofunctionalization enhanced cell adhesion.

CaP calcium phosphate, *TGβ1* transforming growth factor β1, *mRNA* messenger RNA, *BMP2* bone morphogenetic protein 2, *TCP* tricalcium phosphate, *ZrO2/HA* zircon oxide/hydroxyapatite, *CaP* calcium phosphate, *PLD* pulsed laser deposition, *CSLM* confocal scanning laser microscopy

significantly enhance micromechanical properties of titanium dental implants [13, 55]. Laser can also modify the surface roughness as well as the physical and chemical properties and the biocompatibility of the titanium surfaces compared with a smooth surface [34, 55–57], depending on the type of laser and the parameters used [58, 59], as well as the contamination

Table 4 Studies on manufacturing dental implants using laser

	Author/year	Ti type	Laser	Cell type	Test	Result
1	Hollander et al./2006 [43]	Ti6Al4V	Nd: YAG laser	Human primary osteoblasts (HOB)	XTT assay, enzymatic photo-metric assay(alkaline phosphatase)	High growth of human osteoblasts on laser prepared construct. DLF Ti6Al4V guides osteoblast-specific differentiation.
2	Xue et al./2007 [44]	Cp Ti	Nd:YAG 1064 nm, 250–300 W	Osteoblast precursor cell line 1 (OPC1)	MTT assay, cell morphology (SEM)	Evidence of cell proliferation, adhesion, and differentiation. Proliferation was improved, with obvious ALP production
3	Mangano et al./2009 [45]	Ti6Al4V	Ytterbium fiber laser 1054 nm, 200 W	Primary osteoblasts	Cell culture SEM	Conducive to cell attachment and proliferation
4	Mangano et al./2010 [22]	Ti6Al4V	Ytterbium fiber laser system 1054 nm, 200 W	DPSCs	Adhesion assays, PCR analysis ELISA PCR	Better and quicker osteoblast differentiation of DPSCs, and bone morphogenetic protein production was obtained in laser-sintered titanium.
5	Shishkovskii et al./2012 [46]	Cp Ti/HA GA-P85d, nitinol	Nd:YAG 1064-nm laser	Human dermal fibroblasts	Cell adhesion assay; cell count	Not cytotoxic, pronounced cell adhesion with a high density of cells. The cells retained their structural and proliferative activity. HAP did not significantly affect the behavior of fibroblasts.
6	Cheng et al./2014 [47]	Ti6Al4V	Ytterbium fiber laser 1054 nm, 200 W	MG63 human osteoblast--like cell	DNA content and total protein content, ALP, cell adhesion	3D constructs with the highest porosity and surface modification supported the greatest osteoblast differentiation

DLF direct laser forming, *DPSCs* dental pulp stem cells, *GAP85d* a grade of hydroxyapatite, *HAP* hydroxyapatite, *DNA* deoxyribonucleic acid, *SEM* scanning electron microscopy, *XTT* assays (colorimetric assay based on the oxidation of the tetrazolium derivate XTT by vital cells), *SBF* simulated body fluid, *OM* optical microscopy, *ELISA* enzyme-linked immunosorbent assay, *MMSC* multipotent mesenchymal stromal cells, *OPC1* osteoblast precursor cell line 1

control [60]. Laser adjusts the titanium oxide layer and improves biocompatibility [12, 13, 33, 36, 61]. Assessment of surfaces for roughness, microhardness, and phase development after melting with laser showed titanium oxide formation, which has a sterilizing effect and provides a contaminant-free surface that can effectively enhance biocompatibility [33, 56].

The laser parameters play an important role in determining bioresponses [62]. The main parameters related to processing include laser power and peak power for continuous wave (CW) and pulsed lasers, respectively, as well as laser spot diameter [63]. The main advantage of pulsed lasers compared with CW lasers is the ability to deliver high peak power in a short pulse length, resulting in effective melting with a small heat-affected zone [64]. In contrast, evaluation of the processing window for pulsed lasers is more troublesome because peak power, pulse width, and frequency need to be optimized. One study showed that the best parameters for using selective laser melting with pulsed laser were a scan speed of 6 mm/s, laser peak power of 1 kW, and hatching pitch of 0.4 mm, yielding a tensile strength of 300 MPa and torsional fatigue strength of 100 MPa [63]. As we summarized in tables, different laser devices were used in different studies. It seems that the power or energy used depends on the desired effect (melting vs surface texturing or coating).

The growth and differentiation of osteoblasts are essential for the regeneration of bone around dental implants. This may explain the greater use of osteoblast-like cells in biocompatibility investigation. However, it appears that cell type does not play a great role in determining the biological response of laser-processed dental implants as most of the articles showed that biofunctionalization with laser led to higher levels of cell bioactivity, proliferation, and attachment to titanium surfaces [33, 65]. Observation of Alamar Blue proliferation assay measurements showed positive cellular metabolic activity [37] while MTT assays showed an increase in the number and viability of cells [27, 31–33]. Also, creation of hybrid nano- and microscale titanium surface roughness by laser treatment allows stimulation of osteoblast differentiation and bioactivity to form mineralized zones [15] and improves the bone response to the laser-modified titanium surfaces [66, 67]. The main mechanism governing the cell adhesion on the laser-treated groups could be change of the wettability characteristics [25]. However, Györgyey et al. did not find any significant differences in cell bioactivity or attachment between laser-treated and controlled groups. In their study, they used osteogenic sarcoma cells (SaOs-2) treated with Er:YAG laser (60–100-mJ pulse energy) [32]. Ayobian-Markazi et al., also found that the difference between laser-treated and controlled groups in the mean MTT score did not reach statistical significance [31]. Variations in the irradiation protocols could be the cause of such discrepancies. It seems that surface features dimensionally closer to the cell dimensions are able to positively affect the viability and spreading of cells [35]. Alkaline phosphatase activity and gene expression were assays used in six studies to investigate osteogenic differentiation of cells.

In order to enhance the integration of titanium into living tissues, researchers have used laser to coat implant with materials such as bioactive ceramics that imitate bone [41, 42, 68]. Surface modification with laser, in association with biomimetic coating, shortens implant healing period by increasing bone implant interaction [39]. Cell adhesion assays indicate that laser Ti surface CaP biofunctionalization enhances cell adhesion to the surface and provides osteoconductivity [42]. Also, Nd:YAG laser-assisted nitride titanium surface (TiN) treatment appears to support tissue growth on the surface of dental implants [23]. The Q-switched Nd:YAG laser titanium surface microscale patterning plays a significant role in enhancing metal-ceramic bond strength and is a promising method for manufacturing dental implant biomaterial with high osteoconductivity, cell growth, and differentiation and better adherence to bone surfaces compared to oxidized titanium surfaces [69]. Commercially available dental implants coated by pulsed laser deposition demonstrate uniform coating thickness around the corners and sidewalls of implants [42, 70, 71].

Surface treatment and coating of dental implants could be further customized with additive manufacturing. Some additive manufacturing techniques use laser as energy source for printing with high accuracy and maneuverability [21, 43, 72–74]. Laser additive manufacturing is a scalable manufacturing method that can create complex structures with high dimensional accuracy and controllable density and reduce material waste. It also provides the ability to produce costume made dental implants with enhanced osseointegration [73]. Titanium dental implants are made by laser-forming techniques such as laser sintering and laser melting. Laser sintering is an efficient method, which meets the required micromechanical and surface criteria for dental implant biomaterials. It is better adapted to the elastic properties of bone and minimizes stress-shielding effects while improving long-term performance [21, 43, 75]. One advantage of laser melting is the ability to fabricate parts with controlled porosity. Implants manufactured by this technique have a porous surface structure that increases bone osseointegration and a compact core that enhances mechanical strength [76]. Three-dimensional laser synthesized porous titanium constructs have improved cell bioactivity and stimulate osteoblast differentiation and maturation. Osteoblasts retain their structural proliferative activity activated by high-porosity laser additive manufacturing [22, 46, 77]. Bone shows active growth into the intricate porous structure of

titanium implant surface with no signs of inflammation, indicating high compatibility of the titanium implants [63, 77]. Enhanced bone growth and osseointegration into the surface with adequate micro- to nanoroughness support osteoblastic differentiation and increase the production of local factors important for creating an osteogenic environment [78].

The main lasers used in metal forming or manufacturing of titanium dental implants include 1054 nm Yb-doped fiber laser system and 1064 nm Nd:YAG, with an average power of 200–300 W [44, 45]. Nd:YAG laser creates efficient constructs with functionally graded complex structures and costume-made dental implants with high chemotaxis for cells that stimulate osteoblast differentiation and maturation and are activated by high porosity laser additive manufacturing [21, 22, 43, 44, 46, 63, 73, 74, 78]. Hollander and colleagues in their study on porous blasted *direct metal laser-sintered* (DMLS) specimens demonstrated that DMLS-fabricated Ti6Al4V allowed structure-oriented growth of human osteoblasts on its surface. In their study, the biocompatibility of *selective laser melting* (*SLM*) Ti-64 material was also studied. Comparisons were made between SLM surfaces and commercially available Thermanox® (Nalge Nunc Int., New York, NY, USA) control and conventional bulk titanium. The authors concluded that the increased metabolic activity of osteoblasts on SLM discs compared to the controls may have been due to the greater surface area of the SLM material, which took longer to be covered by the cells [43].

Mangano et al. seeded human dental pulp stem cells (DPSCs) on direct laser-sintered titanium scaffolds and acid-etched surfaces. They observed that gene expression and protein secretion were faster on laser-sintered scaffolds [79]. These results were confirmed by another study on cell cultures, where rat calvarial osteoblasts were seeded and cultured on disc specimens produced by DMLS. Cell density was similar to that of commercially available rough microtextured surfaces but lower than that of machined and smooth-textured grit-blasted, acid-etched surfaces [45]. Finally, in another in vitro study, human osteoblasts and human DPSCs were cultured either on acid-etched or DMLS titanium surfaces, in order to investigate their osseointegration and clinical applicability of the derived implants. When stem cells were exposed to DMLS titanium surface, osteoblastic differentiation of DPSCs and bone morphogenetic protein production occurred more quickly. These successful results suggest that DMLS titanium surfaces may represent a promising alternative for clinical use in implants [22].

Witek et al. measured bone implant contact and removal torque of dental implants that had a porous layer, which were produced by laser sintering and compared them with sandblasted acid-etched implants (i.e., those with a rough, but not porous, surface) and concluded that porous dental implants produced by laser sintering showed better biocompatibility [80]. It seems that laser engineered net shaping to construct porous structures from Ti6Al4V alloy across the range of 23–32% porosity with low modulus (7–60 GPa), which can be tailored to match human cortical bone [81].

The studies reviewed in this article may have been inconsistent with regard to laser parameters disclosed. This can make the studies heterogeneous and difficult to compare, even if lasers with the same wavelength were used.

In addition, studies of in vitro biocompatibility of laser-processed dental implants are limited in number. More research is needed to investigate the effect of cell type, laser type, and laser power on biocompatibility and functionality of titanium dental implants that are manufactured by a range of additive and advanced manufacturing technologies that are available.

Conclusions

In the present review, an attempt was made to summarize in vitro biological outcome of different applications of laser technology in titanium dental implants (surface treatment, assisted coating, and 3D construction). Based on the obtained results, the following conclusions can be drawn:

- Almost, all examined surface modifications by laser were as good as or better than other treatments for supporting cell attachment and growth. However, the property of laser (type, wavelength, and time of radiation) might affect the cell proliferation or at least the cell spreading.
- Laser-assisted coating of Ti dental implants might produce uniform coating thickness around the corners and sidewalls of implants and shorten the healing period.
- Three-dimensional laser forming of titanium implants is a reasonable and effective technology to produce titanium constructs with controlled porosity, which can be further modified to enhance their biocompatibility.
- The environment or atmosphere that the titanium surface is modified in or coated in can affect the surface characteristics.
- Laser type and parameters used in all three applications examined may affect the dental implant's biocompatibility outcome.
- Diverse assays and cells have been used by various researchers as biological assessment of laser application in implantology.

Acknowledgments This study was supported by a grant from Iran National Science Foundation (Project no. 95819948).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Sakaguchi RL, Powers JM (2013) Craig's restorative dental materials, 13th edn. Mosby: Elsevier, USA
2. Anusavice KJ, Shen C, Rawls HR (2012) Phillip's science of dental materials, 12th edn. Saunders, St. Louis
3. Kasemo B (1983) Biocompatibility of titanium implants: surface science aspects. J Prosthet Dent 49(6):832–837
4. Saini M, Singh Y, Arora P, Arora V, Jain K (2015) Implant biomaterials: a comprehensive review. World J Clin Cases 3(1):52–57
5. Branemark P, Adell R, Breine U, Hansson B, Lindström J, Ohlsson A (1969) Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg 3(2):81–100
6. Anil S, Alghamdi H, Jansen J, Anand P (2011) Dental implant surface enhancement and osseointegration. In: Turkyilmaz I (ed) Implant dentistry—a rapidly evolving practice. InTech, Croatia, pp 83–108
7. Wall I, Donos N, Carlqvist K, Jones F, Brett P (2009) Modified titanium surfaces promote accelerated osteogenic differentiation of mesenchymal stromal cells in vitro. Bone 45(1):17–26
8. Raines AL, Olivares-Navarrete R, Wieland M, Cochran DL, Schwartz Z, Boyan BD (2010) Regulation of angiogenesis during osseo integration by titanium surface microstructure and energy. Biomaterials 31(18):4909–4917
9. Buser D, Janner SF, Wittneben JG, Bragger U, Ramseier CA, Salvi GE (2012) 10-year survival and success rates of 511 titanium implants with a sandblasted and acid-etched surface: a retrospective study in 303 partially edentulous patients. Clin Implant Dent Relat Res 14(6):839–851
10. Dohan Ehrenfest DM, Coelho PG, Kang BS, Sul YT, Albrektsson T (2010) Classification of osseointegrated implant surfaces: materials, chemistry and topography. Trends Biotechnol 28(4):198–206
11. Novaes AB Jr, de Souza SL, de Barros RR, Pereira KK, Iezzi G, Piattelli A (2010) Influence of implant surfaces on osseointegration. Braz Dent J 21(6):471–481
12. Ulerich JP, Ionescu LC, Chen J, Soboyejo WO, Arnold CB, editors. Modifications of Ti-6Al-4V surfaces by direct-write laser machining of linear grooves. Lasers and Applications in Science and Engineering; 2007: International Society for Optics and Photonics.
13. Deppe H, Horch HH (2007) Laser applications in oral surgery and implant dentistry. Lasers Med Sci 22:217–221
14. Brunette DM (1988) The effects of implant surface topography on the behavior of cells. Int J Oral Maxillofac Implants 3(4):231–246
15. Mariscal-Munoz E, Costa CA, Tavares HS et al (2016) Osteoblast differentiation is enhanced by a nano-to-micro hybrid titanium surface created by Yb:YAG laser irradiation. Clin Oral Investig 20(3): 503–511
16. Hara T, Matsuoka K, Matsuzaka K, Yoshinari M, Inoue T (2012) Effect of surface roughness of titanium dental implant placed under periosteum on gene expression of bone morphogenic markers in rat. Bull Tokyo Dent Coll 53(2):45–50
17. Velasco-Ortega E, Jos A, Camean AM, Pato-Mourelo J, Segura-Egea JJ (2010) In vitro evaluation of cytotoxicity and genotoxicity of a commercial titanium alloy for dental implantology. Mutat Res 702(1):17–23
18. Le Guehennec L, Lopez-Heredia MA, Enkel B, Weiss P, Amouriq Y, Layrolle P (2008) Osteoblastic cell behaviour on different titanium implant surfaces. Acta Biomater 4(3):535–543
19. Parthasarathy J, Starly B, Raman S, Christensen A (2010) Mechanical evaluation of porous titanium (Ti6Al4V) structures with electron beam melting (EBM). J Mech Behav Biomed Mater 3(3):249–259
20. Sumner DR, Galante JO (1992) Determinants of stress shielding: design versus materials versus interface. Clin Orthop Relat Res 274:202–212
21. Traini T, Mangano C, Sammons RL, Mangano F, Macchi A, Piattelli A (2008) Direct laser metal sintering as a new approach to fabrication of an isoelastic functionally graded material for manufacture of porous titanium dental implants. Dent Mater 24(11): 1525–1533
22. Mangano C, De Rosa A, Desiderio V et al (2010) The osteoblastic differentiation of dental pulp stem cells and bone formation on different titanium surface textures. Biomaterials 31(13):3543–3551
23. Groessner-Schreiber B, Neubert A, Muller WD, Hopp M, Griepentrog M, Lange KP. Fibroblast growth on surface-modified dental implants: an in vitro study. J Biomed Mater Res A 15:64(4):591–9.
24. Schwarz F, Rothamel D, Sculean A, Georg T, Scherbaum W, Becker J (2003) Effects of an Er:YAG laser and the vector ultrasonic system on the biocompatibility of titanium implants in cultures of human osteoblast-like cells. Clin Oral Implants Res 14: 784–792
25. Hao L, Lawrence J, Chian KS (2005) Osteoblast cell adhesion on a laser modified zirconia based bioceramic. J Mater Sci Mater Med 16(8):719–726
26. Lawrence J, Hao L, Chew HR (2006) On the correlation between Nd:YAG laser-induced wettability characteristics modification and osteoblast cell bioactivity on a titanium alloy. Surf Coat Tech 200(18–19):5581–5589
27. Ayobian-Markazi N, Karimi M, Safar-Hajhosseini A (2013) Effects of Er: YAG laser irradiation on wettability, surface roughness, and biocompatibility of SLA titanium surfaces: an in vitro study. Lasers Med Sci 30(2):561–566
28. Heinrich A, Dengler K, Koerner T, Haczek C, Deppe H, Stritzker B (2008) Laser-modified titanium implants for improved cell adhesion. Lasers Med Sci 23(1):55–58
29. Erdogan M, Oktem B, Kalaycioglu H et al (2011) Texturing of titanium (Ti6Al4V) medical implant surfaces with MHz-repetition-rate femtosecond and picosecond Yb-doped fiber lasers. Opt Express 19(11):10986–10996
30. Paz MD, Alava JI, Goikoetxea L et al (2011) Biological response of laser macrostructured and oxidized titanium alloy: an in vitro and in vivo study. J Appl Biomater Biomech 9(3):214–222
31. Ayobian-Markazi N, Fourootan T, Zahmatkesh A (2012) An in vitro evaluation of the responses of human osteoblast-like SaOs-2 cells to SLA titanium surfaces irradiated by erbium:yttrium-aluminum-garnet (Er:YAG) lasers. Lasers Med Sci 29(1):47–53
32. Györgyey Á, Ungvári K, Kecskeméti G et al (2013) Attachment and proliferation of human osteoblast-like cells (MG-63) on laser-ablated titanium implant material. Mater Sci Eng C Mater Biol Appl 33(7):4251–4259
33. Chikarakara E, Fitzpatrick P, Moore E et al (2015) In vitro fibroblast and pre-osteoblastic cellular responses on laser surface modified Ti-6Al-4V. Biomed Mater 10(1):015007
34. Vignesh, Nayar S, Bhuminathan, Mahadevan, Santhosh S (2015) Comparative evaluation of the three different surface treatments—conventional, laser and Nano technology methods in enhancing the surface characteristics of commercially pure titanium discs and their effects on cell adhesion: an in vitro study. J Pharm Bioallied Sci 7(Suppl 1):587–591
35. Mukherjee S, Dhara S, Saha P (2015) Enhancing the biocompatibility of Ti6Al4V implants by laser surface microtexturing: an in vitro study. Int J Adv Manufac Tech 76(1):5–15
36. Hsiao W-T, Chang H-C, Nanci A, Durand R (2016) Surface microtexturing of Ti–6Al–4V using an ultraviolet laser system. Mater Des 90:891–895

37. Lusquinos F, De Carlos A, Pou J et al (2003) Calcium phosphate coatings obtained by Nd:YAG laser cladding: physicochemical and biologic properties. J Biomed Mater Res A 64(4):630–637
38. Seydlova M, Teuberova Z, Dostalova T et al (2006) Biological properties of titanium implants covered with hydroxyapatite and zirconia layers by pulsed laser: in vitro study. J Appl Phys 99(1): 014905
39. Teuberova Z, Seydlova M, Dostalova T et al (2007) Biological and physical properties of pulsed-laser-deposited zirconia/ hydroxyapatite on titanium: in vitro study. Laser Phys 17(1):45–49
40. Bose S, Roy M, Das K, Bandyopadhyay A (2009) Surface modification of titanium for load-bearing applications. J Mater Sci Mater Med 20(Suppl 1):S19–S24
41. Gao Y, Hu J, Guan TH, Wu J, Zhang CB, Gao B (2014) Physical properties and cellular responses to calcium phosphate coating produced by laser rapid forming on titanium. Lasers Med Sci 29(1):9–17
42. Oyane A, Matsuoka N, Koga K et al (2015) Laser-assisted biomimetic process for surface functionalization of titanium metal. Colloids Interface Sci Comm 4:5–9
43. Hollander DA, von Walter M, Wirtz T et al (2006) Structural, mechanical and in vitro characterization of individually structured Ti–6Al–4V produced by direct laser forming. Biomaterials 27(7):955–963
44. Xue W, Krishna BV, Bandyopadhyay A, Bose S (2007) Processing and biocompatibility evaluation of laser processed porous titanium. Acta Biomater 3(6):1007–1018
45. Mangano C, Raspanti M, Traini T, Piattelli A, Sammons R (2009) Stereo imaging and cytocompatibility of a model dental implant surface formed by direct laser fabrication. J Biomed Mater Res A 88(3):823–831
46. Shishkovskii IV, Morozov YG, Fokeev SV, Volova LT (2012) Laser synthesis and comparative testing of a three dimensional porous matrix of titanium and titanium nickelide as a repository for stem cells. Powder Metall Met Ceram 50(9):606–618
47. Cheng A, Humayun A, Cohen DJ, Boyan BD, Schwartz Z (2014) Additively manufactured 3D porous Ti-6Al-4V constructs mimic trabecular bone structure and regulate osteoblast proliferation, differentiation and local factor production in a porosity and surface roughness dependent manner. Biofabrication 6(4):045007
48. Bidan CM, Kommareddy KP, Rumpler M, Kollmannsberger P, Fratzl P, Dunlop JW (2013) Geometry as a factor for tissue growth: towards shape optimization of tissue engineering scaffolds. Adv Healthc Mater 2(1):186–194
49. Gaković B, Desai T, Trtica M, et al. Laser ablation of multi-layered targets with short laser pulses. 3rd international conference on the frontiers of Plasma Physics and Technology 2007.
50. Gaggl A, Schultes G, Muller WD, Karcher H (2000) Scanning electron microscopical analysis of laser-treated titanium implant surfaces—a comparative study. Biomaterials 21(10):1067–1073
51. Pachauri P, Bathala LR, Sangur R (2014) Techniques for dental implant nanosurface modifications. J Adv Prosthodont 6(6):498–504
52. Sjostrom T, Brydone AS, Meek RM, Dalby MJ, Su B, McNamara LE (2013) Titanium nanofeaturing for enhanced bioactivity of implanted orthopedic and dental devices. Nanomedicine (Lond) 8(1):89–104
53. Chen H, Sago A, West S, Farina J, Eckert J, Broadley M (2011) Biocompatibility of metal injection molded versus wrought ASTM F562 (MP35N) and ASTM F1537 (CCM) cobalt alloys. Biomed Mater Eng 21(1):1–7
54. Kumar G, Tison CK, Chatterjee K et al (2011) The determination of stem cell fate by 3D scaffold structures through the control of cell shape. Biomaterials 32(35):9188–9196
55. Poulon-Quintin A, Watanabe I, Watanabe E, Bertrand C (2012) Microstructure and mechanical properties of surface treated cast titanium with Nd:YAG laser. Dental Mater 28(9):945–951
56. Ciganovic J, Stasic J, Gakovic B et al (2012) Surface modification of the titanium implant using TEA CO2 laser pulses in controllable gas atmospheres—comparative study. Appl Surf Sci 258(7):2741–2748
57. Trtica M, Gakovic B, Batani D, Desai T, Panjan P, Radak B (2006) Surface modifications of a titanium implant by a picosecond Nd: YAG laser operating at 1064 and 532 nm. Appl Surf Sci 253(5): 2551–2556
58. Ricci J, Charvet J, Frenkel S, et al. Bone response to laser microtextured surfaces. In: JE D, editor. Bone engineering. Toronto: Em2 Inc 2000. p. 1–11.
59. Vorobyev AY, Guo C, editors. Femtosecond laser surface structuring of biocompatible metals. Commercial and Biomedical Applications of Ultrafast Lasers IX; 2009: Proc. of SPIE.
60. Karacs A, Joob Fancsaly A, Divinyi T, Pető G, Kovách G (2003) Morphological and animal study of titanium dental implant surface induced by blasting and high intensity pulsed Nd-glass laser. Mater Sci Eng C Mater Biol Appl 23(3):431–435
61. Braga FJC, Marques RFC, Filho EA, Guastaldi AC (2007) Surface modification of Ti dental implants by Nd:YVO4 laser irradiation. Appl Surf Sci 253(23):9203–9208
62. Tabatabaei F, Torshabi M, Mojahedi Nasab M, Khosraviani K, Khojasteh A (2015) Effect of low-level diode laser on proliferation and osteogenic differentiation of dental pulp stem cells. Laser Phys 25(9):095602
63. Laoui T, Santos E, Osakada K et al (2006) Properties of titanium dental implant models made by laser processing. J Mech Eng Sci 220(c):857–863
64. Ready JF (1997) Industrial applications of lasers. Academic Press, San Diego
65. Fuchs E, Mandel K, Bouazza S, Rosin A, Weiss E, Willert-Porada M. Surface modification of porous titanium composites obtained by different processing methods. 17th Plansee Seminars 2009. p. 1–7.
66. Guo Z, Zhou L, Rong M, Zhu A, Geng H (2010) Bone response to a pure titanium implant surface modified by laser etching and microarc oxidation. Int J Oral Maxillofac Implants 25(1):130–136
67. Tavangar A, Tan B, Venkatakrishnan K (2011) Synthesis of biofunctionalized three-dimensional titania nanofibrous structures using femtosecond laser ablation. Acta Biomater 7(6):2726–2732
68. Bruschi M, Steinmüller-Nethl D, Goriwoda W, Rasse M (2015) Composition and modifications of dental implant surfaces. J Oral Implants 2015:14
69. Rajesh P, Muraleedharan CV, Komath M, Varma H (2011) Laser surface modification of titanium substrate for pulsed laser deposition of highly adherent hydroxyapatite. J Mater Sci Mater Med 22(7):1671–1679
70. Filho EA, Fraga AF, Bini RA, Guastaldi AC (2011) Bioactive coating on titanium implants modified by Nd:YVO4 laser. Appl Surf Sci 257(10):4575–4580
71. Symietz C, Lehmann E, Gildenhaar R, Koter R, Berger G, Krüger J (2011) Fixation of bioactive calcium alkali phosphate on Ti6Al4V implant material with femtosecond laser pulses. Appl Surf Sci 257(12):5208–5212
72. Murr LE, Quinones SA, Gaytan SM et al (2009) Microstructure and mechanical behavior of Ti-6Al-4V produced by rapid-layer manufacturing, for biomedical applications. J Mech Behav Biomed Mater 2(1):20–32
73. Chen J, Zhang Z, Chen X, Zhang C, Zhang G, Xu Z (2014) Design and manufacture of customized dental implants by using reverse engineering and selective laser melting technology. J Prosthet Dent 112(5):1088–95.e1
74. Liu J, Han G, Pan S, Ge Y, Feng H, Shen Z (2015) Biomineralization stimulated peri-titanium implants prepared by selective laser melting. J Mater 1(3):253–261
75. Lin WS, Starr TL, Harris BT, Zandinejad A, Morton D (2013) Additive manufacturing technology (direct metal laser sintering) as a novel approach to fabricate functionally graded titanium implants: preliminary investigation of fabrication parameters. Int J Oral Maxillofac Implants 28(6):1490–1495

76. Osakada K, Shiomi M (2006) Flexible manufacturing of metallic products by selective laser melting of powder. Int J Mach Tool Manu 46(11):1188–1193
77. Mizutani M, Honda R, Yuda A, Komotori J, Ohmori H (2013) Effects of nanosecond laser fabrication on bioactivity of pure titanium. Procedia CIRP 5:242–246
78. Han G, Shen Z (2015) Microscopic view of osseointegration and functional mechanisms of implant surfaces. Mater Sci Eng C Mater Biol Appl 56:380–385
79. Mangano C, Piattelli A, d'Avila S et al (2010) Early human bone response to laser metal sintering surface topography: a histologic report. J oral implantol 36(2):91–96
80. Witek L, Marin C, Granato R et al (2012) Characterization and in vivo evaluation of laser sintered dental endosseous implants in dogs. Biomed Mater Res Part B Appl Biomater 100(6):1566–1573
81. Bandyopadhyay A, Espana F, Balla VK, Bose S, Ohgami Y, Davies NM (2010) Influence of porosity on mechanical properties and in vivo response of Ti6Al4V implants. Acta Biomater 6(4):1640–1648