# *In vitro* biocompatibility and bioactivity of calcium silicate-based bioceramics in endodontics (Review)

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**Abstract.** Calcium silicate-based bioceramics have been applied in endodontics as advantageous materials for years. In addition to excellent physical and chemical properties, the biocompatibility and bioactivity of calcium silicate-based bioceramics also serve an important role in endodontics according to previous research reports. Firstly, bioceramics affect cellular behavior of cells such as stem cells, osteoblasts, osteoclasts, fibroblasts and immune cells. On the other hand, cell reaction to bioceramics determines the effect of wound healing and tissue repair following bioceramics implantation. The aim of the present review was to provide an overview of calcium silicate-based bioceramics currently applied in endodontics, including mineral trioxide aggregate, Bioaggregate, Biodentine and iRoot, focusing on their *in vitro* biocompatibility and bioactivity. Understanding their underlying mechanism may help to ensure these materials are applied appropriately in endodontics.

**Contents**



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## 1. Introduction

The stem cell population in dental pulp possesses multilineage differentiation potential and the pulp vascular system participates in the reaction of dental pulp tissue to external stimulus, the initiation of dental pulp inflammation and pulp tissue repair (1). When sound dentin suffers damage, such as tooth wear, fracture, or caries, bacterial infection and the subsequent inflammatory response lead to damage of pulp tissue and impaired periapical tissue through blood circulation (2). Therefore, endodontic therapy has become a necessary option to preserve teeth by removing microorganisms, their by-products and residual necrotic tissue (3). However, the success rate of traditional endodontic therapy was only 70-80% over the past decade globally (4-6). The apical seal is important to improve the success rate in endodontic therapy; an excellent apical seal by root-end filling prevents the spread of dental pulp inflammation to the periapical tissue (7). In order to achieve a higher success rate in endodontic therapy, an ideal root-end filling material is required that possesses excellent root-end sealing capacity, good biocompatibility with surrounding cells/tissue, superior antibacterial properties and ability to promote tissue regeneration.

Mineral trioxide aggregate (MTA) has been used in root-end filling as calcium silicate-based bioceramic and displays better root apical sealing ability and higher success rates compared with conventional root-end filling materials, such as amalgam and intermediate restorative material (8-10). Furthermore, given its clinical effect in root-end filling in endodontic therapy, MTA has also been used in other endodontic application, such as pulp capping and regeneration and root perforation repair, and is currently considered as the gold standard in endodontics. As ProRoot MTA (Dentsply Sirona) has shown good clinical performance in endodontics, other calcium silicate-based bioceramics have been developed, including Bioaggregate (Innovative Bioceramix, Inc.), Biodentin (Septodont Holding) and iRoot BP/FS/SP (Innovative Bioceramix, Inc.) (11,12).

The chemical constituents of these novel calcium silicate-based bioceramics are similar to that of MTA, but Bioaggregate, Biodentine and iRoot BP/BP Plus display better color stability than MTA because bismuth oxide is replaced by

tantalum or zirconium oxide as a radiopacifier. Bioaggregate exhibits superior stable bond strength but inferior mechanical properties and bond strength in comparison with MTA. Biodentine shows increased mechanical strength and longer setting time as it does not contain calcium aluminate and calcium sulfate, which are present in MTA (13). iRoot BP/BP Plus, novel calcium silicate-based bioceramics applied in permanent root canal repair and filling, exhibit easier manipulation and faster setting time compared with MTA (14).

It is essential to clarify the effect and mechanism how these bioceramics influence the surrounding cells/tissue when used in endodontics. Numerous studies have investigated the biocompatibility and bioactivity of bioceramics in endodontics. Materials with good biocompatibility should not induce notable and continuous toxic effects on surrounding cells and tissue (15,16). Biocompatibility can be defined as the interaction between implanted biomaterials and the associated tissue (17). Bioactive materials also induce apatite layer formation and biomineralization (18). Increased deposition of hydroxyapatite over time is observed when calcium silicate-based bioceramics are exposed to PBS, which suggests that these materials are bioactive (19-22). Bioceramics have been demonstrated to have excellent biocompatibility and lasting bioactivity during and after setting by the secretion of molecules. When calcium silicate-based bioceramics are applied in endodontics, the interaction between the materials and cells affect the biological behavior, such as proliferation, differentiation, migration and apoptosis (23,24). Various types of cell are involved in changes of biological behavior when bioceramics are used in endodontics (Fig. 1). For example, calcium silicate-based bioceramics affect the biological behavior of dental pulp stem cells (DPSCs) in dental pulp capping, whereas osteoblasts/osteoclasts are influenced when bioceramics are applied as root-end filling material (25,26). Despite the weakness of *in vitro* studies in mimicking the human body reaction to environmental stimuli and providing accurate results compared with animal or human studies, it is essential to investigate biocompatibility and bioactivity *in vitro* to clarify the mechanism underlying how calcium silicate-based bioceramics influence cell behavior. The present review focuses on *in vitro* biocompatibility and bioactivity when calcium silicate-based bioceramics are applied in endodontics. All information is summarized in Table I.

## 2. Stem cells

Mesenchymal stem cells (MSCs) derived from dental tissue originate from teeth and surrounding support tissue, possess similar biological characteristics to bone marrow-derived MSCs and differentiate into osteoblasts, adipocytes, chondrocytes and neural cells (27,28). MSCs derived from dental tissue are capable of dentinogenesis or angiogenesis and secretion of growth factors, which influence behavior, such as proliferation, differentiation and mineralization (29). MSCs derived from dental tissue include DPSCs, periodontal ligament stem cells (PDLSCs), stem cells from human exfoliated deciduous teeth (SHED) and stem cells from apical papilla (SCAPs) that are involved in renewal and regeneration of dental tissue via the repair of injured dentin, root structure and the pulp-dentin complex (26,30,31) (Fig. 2). Calcium silicate-based bioceramics significantly promote attachment and survival of stem cells derived from dental tissue but their effects on biological behavior appear to be cell type-dependent (25). Several common markers are used to test the osteo/odontogenic and angiogenic potential of stem cells in the presence of calcium silicate-based bioceramics. For example, alkaline phosphatase (Alp) is a marker protein of mineralization and is associated with early osteo/odontogenic differentiation (32,33). Collagen type I (COL1), osteocalcin (Ocn) and osteopontin (OPN) are expressed in the extracellular matrix and serve an important role in osteoblastic mineralization (34). Runt-related transcription factor 2 (Runx2) acts as a marker of osteogenesis in the early stage (35,36), while Ocn functions in the late stage of osteogenic differentiation (37,38). Regarding dentinogenesis, Runx2 and its downstream molecule osterix (Osx), dentin sialoprotein and dentin sialophosphoprotein (DSPP) and its downstream molecule dentin matrix protein 1 (DMP1) (39,40) are considered as protein markers closely associated with the formation and mineralization of odontoblasts.

*DPSCs*. MTA promotes the proliferation and survival of human DPSCs, bone marrow stromal/stem cells (BMSCs) and PDLSCs via the ERK signaling pathway (25), and also exhibit a positive effect on viability of human DPSCs (41). MTA at high concentrations (20 and 10 mg/ml) is toxic to human DPSCs but MTA at low concentrations (2.0, 1.0, 0.2 and 0.1 mg/ml) enhances viability of human DPSCs but has no effect lower concentrations (0.020 and 0.002 mg/ml) (42). Similarly, undiluted MTA extract slightly increase survival of human DPSCs, while 1/2 and 1/4 dilutions of MTA extract have no effect on cell viability (43). In addition, MTA at high concentrations (20 mg/ml) decreases proliferation of DPSCs under inflammatory conditions but has no effect at low concentrations (0.020 and 0.002 mg/ml) (44). Moreover, various commercial MTA extracts, such as Angelus MTA (Angelus Dental Products Industry) and Root MTA (University of Tabriz, Iran), show similar effects on human DPSCs but were more biocompatible when applied at lower concentration (1:2) and longer exposure times compared with MTA. These results suggested that the biocompatibility of MTA is dependent on not only dosage but also exposure time (45). In terms of time-dependent biocompatibility of MTA, the cytotoxicity of MTA decreased over time and the viability and proliferation of human DPSCs increased following two aging cycles, which further supported the aforementioned time- and concentration-dependent effects of MTA (46). Moreover, the proliferation and viability of DPSCs decreased significantly when in direct contact with MTA for the first day but subsequently raised after three days (47). The initial cytotoxicity of MTA to growth and viability of DPSCs (47-49) may be partly ascribed to the relatively rough surface of biomaterials (50) or leakage of components such as bismuth oxide (51) and Al (52) and Si ions (53). Higher levels of Si ion concentrations from the $SiO_2$ phase of materials may lead to hyperosmoticity and subsequently stimulates production of inflammatory cytokines (54). Furthermore, production of MTA during the hydration reaction and its exposure concentration may result in early slight cytotoxicity of MTA. Calcium hydroxide is produced when calcium silicate contained in MTA reacts with water and increases pH of the culture media (55,56).

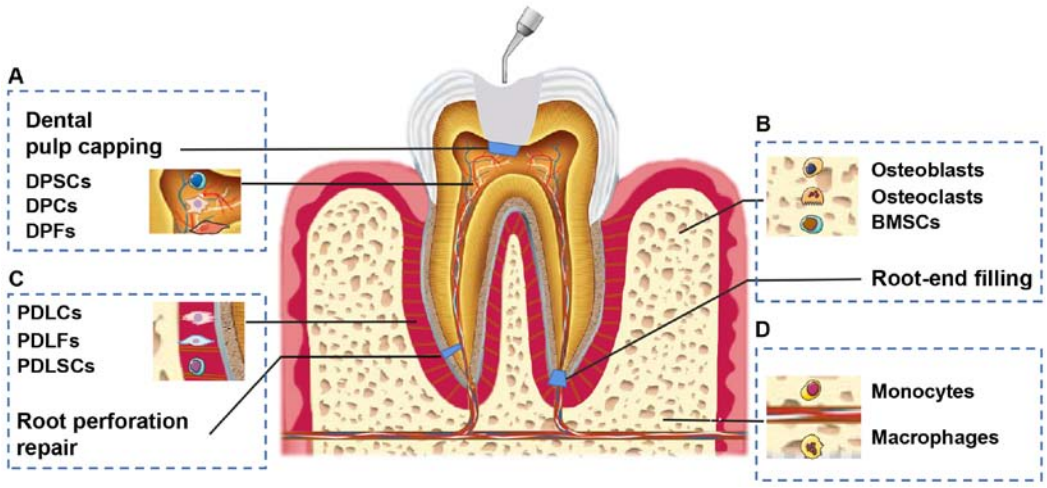


Figure 1. Application of calcium silicate-based bioceramics in endodontics and associated cells. Calcium silicate-based bioceramics affect the biological behavior of (A) DPSCs, DPCs and DPFs when used in dental pulp capping, (B) PDLSCs, PDLCs and PDLFs when applied in root perforation repair and (C) BMSCs, osteoblasts and osteoclasts when used as root-end filling material. (D) Immune cells, such as monocytes and macrophages, respond to implantation of calcium silicate-based biomaterials into tissue. DPSCs, dental pulp stem cells; DPCs, dental pulp cells; DPFs, dental pulp fibroblasts; PDLSCs, periodontal ligament stem cells; PDLCs, periodontal ligament cells; PDLFs, periodontal ligament fibroblasts; BMSCs, bone marrow stromal/stem cells.

Basic pH environment and release of inorganic salts induced by high concentrations of MTA significantly decreases cell proliferation (57). Furthermore, 24-h set MTA promotes viability of human DPSCs, whereas 1-h set MTA exhibits an inhibitory effect after 7 days, which suggests that incompletely solidified MTA is cytotoxic (58). The subsequent rise in cell viability may be caused by hydroxyapatite layer formation on the hydrated bioceramic surface (59) controlled continuous production of calcium, silica and phosphate ions from bioceramics along with alkaline pH in the later stage (60,61). Regarding differentiation of human DPSCs, in the first week following induction with MTA in odontogenic differentiation medium, DPSCs began to change from spindle to rounded shape upon reaching confluency and then migrated to form clusters (62). DPSCs began to differentiate at day 7 (63) and mineralization was observed by Alizarin Red staining until day 21. Combination of MTA and odontogenic differentiation medium enhanced odontogenic differentiation of DPSCs but MTA extract-alone did not induce this (62). Treatment with MTA for 1 day affected more genes in uninduced DPSCs than in DPSCs induced by odontogenic differentiation medium, which suggested that MTA exhibits a greater stimulative effect on odontogenic differentiation of uninduced DPSCs compared with induced DPSCs (64). In addition, MTA at a concentration of 0.2 mg/ml displayed the strongest capacity to induce odontoblastic differentiation of human DPSCs via the p42/44 ERK signaling pathway (42). MTA-conditioned medium at the same concentration enhances the odonto/osteogenic capacity of DSPCs from inflammatory sites by activating the NF-κB pathway, as shown by significantly upregulated odonto/osteoblastic gene expression levels, such as *ALP, RUNX2, OSX, OCN* and *DSPP* (44). Consistent with the changes in genes associated with osteo/dentinogenic differentiation, MTA promotes mineralized nodule formation of human DPSCs (65,66). Increased secretion of angiogenic factor VEGF has been detected in human DPSCs induced by MTA (63), which in turn affects viability and function of DPSCs (67). MTA contributes to dentin bridge formation in endodontics (68,69). In addition, MTA is applied in pulp capping because of its excellent bioactivity, which has been confirmed by the elongated shape of DPSCs, formation of collagen fibers and calcified deposition in the presence of MTA in a model simulating indirect pulp capping (70). In previous studies, accelerants, including 5% $CaCl_2$ and 2.5% $Na_2HPO_4$, have been mixed with MTA to shorten setting time. Compared with MTA in the presence of distilled water, MTA in the presence of 5% $CaCl_2$ and 2.5% $Na_2HPO_4$ is more biocompatible and exhibits greater ability to promote odontoblastic differentiation of DPSC niches (48,71). Propolis, a natural alternative endodontic material produced by honeybees from tree resin, also enhances the ability of MTA to promote odontogenic differentiation and mineralization of DPSCs via the ERK pathway (72).

Compared with human PDLSCs and tooth germ stem cells (TGSCs), human DPSCs exhibit better viability in the presence of both Biodentine and MTA (73). Moreover, Biodentine displays a superior ability to promote viability, adhesion and migration of human DPSCs compared with MTA. Human DPSCs spread on the surface of Biodentine show a spindle, polygonal and flattened morphology (74). Similar to MTA, high concentrations of Biodentine extract (20 mg/ml) exhibit slight cytotoxicity, whereas 0.2 mg/ml Biodentine enhances biological behaviors of human DPSCs, including cell proliferation, viability, migration, adhesion and mineralization formation. In addition, low concentrations of Biodentine (0.2 mg/ml) promote odontoblast differentiation and biomineralization of human DPSCs by activating ERK1/2 and JNK and attenuating the NF-κB pathway (75,76). Increased Alp activity and dentin matrix protein expression levels have been observed in human DPSCs stimulated with Biodentine (75-78). Furthermore, Biodentine significantly increases calcium deposition (79) and enhances the production of Ocn and Runx2 in human DPSCs stimulated with lipopolysaccharide (LPS) although there is no change in *ALP* expression levels (53). In terms of the inflammatory response, Biodentine does not affect high expression of IL-6 and IL-8 in DPSCs induced by LPS stimulation but decreases levels of the anti-inflammatory cytokine TGF-β1 (53).

Table I. *In vitro* studies of biocompatibility and bioactivity of calcium silicate-based bioceramics in endodontics.

| Bioceramic | Cell type | Subtype | Biocompatibility and bioactivity | References |
|---|---|---|---|---|
| MTA | Stem cells | DPSCs | Promotes proliferation and survival of human DPSCs, BMSCs and PDLSCs via ERK signaling pathway | (25) |
| | | | Concentration- and time-dependent biocompatibility | (42-45,48,49,99) |
| | | | Set MTA shows better biocompatibility than incompletely set MTA | (58) |
| | | | Cells change shape and migrate to form clusters during the first week, and apparent mineralization at day 21 following induction with MTA | (62) |
| | | | Begin to differentiate at day 7 following induction with MTA | (63) |
| | | | Affect more genes in uninduced DPSCs than in DPSCs induced by odontogenic differentiation medium | (64) |
| | | | Promotes odonto/osteogenic differentiation via p42/44 ERK and NF-κB pathways | (42,44) |
| | | | Enhances formation of collagen fibers and mineralized nodules | (65,66,70) |
| | | | Increases secretion of angiogenic factors, such as VEGF | (63) |
| | | PDLSCs | Bioroot BC Sealer is more biocompatible than Endoseal MTA and MTA Fillapex | (100-102) |
| | | | Induces odonto/osteogenic differentiation by activating NF-κB and MAPK pathways | (103) |
| | | BMSCs | Respond more rapidly to MTA than human PDLSCs and DPSCs | (25) |
| | | | Rat BMSCs respond more rapidly to MTA than human BMSCs | (107-109) |
| | | | Induces proliferation and odonto/osteoblastic differentiation in a dose-dependent manner via ERK and JNK signalling pathways | (25,110,111) |
| | | SHED | Enhances attachment, proliferation, migration and odontogenic differentiation | (125,126) |
| | | | Fresh mixed MTA and direct incubation with MTA induce cytotoxicity | (130) |
| | | SCAPs | Concentration-/time-dependent biocompatibility | (25,133-135,138) |
| | | | Induces odonto/osteogenic differentiation via NF-κB, p38 and ERK signaling | (138-140,142) |
| | | | Increases expression of pro-inflammatory cytokines IL-1α, IL-1β and IL-6 | (139) |
| | | | Increases expression of angiogenic genes VEGFA and FIGF/VEGFD | (134) |
| | | TGSCs | Biocompatible and increases release of PDGF, FGF-2 and VEGF | (73) |
| | | | Direct incubation with MTA inhibits viability and odontogenic differentiation | (151) |
| | Osteoblasts | Primary osteoblasts | Inhibit cell proliferation and differentiation | (156-158) |
| | | | Biocompatible with primary osteoblasts cultured in 3D culture system and promotes differentiation | (166) |
| | | MC3T3-E1 | Promotes viability, osteoblastic differentiation and by activating transcription factor 6 and endoplasmic reticulum stress response | (168-171) |
| | | MG-63 | Cytotoxicity and inflammation decrease as material sets | (167,174) |
| | | Saos-2 | Promote the adhesion, spreading, proliferation and secretion of collagen | (176,177) |
| | | | Enhances osteogenic differentiation | (178) |

Table I. Continued.

| Bioceramic | Cell type | Subtype | Biocompatibility and bioactivity | References |
|---|---|---|---|---|
| | Osteoclasts | Osteoclasts | Inhibits bone resorption and osteoclast differentiation by preventing migration and fusion of osteoclast precursors via attenuation of the autophagic pathway | (191,193,194) |
| | | | Inhibit osteoclastogenesis dose-dependently | (195,196) |
| | Fibroblasts | DPCs | More biocompatible with rat pulp cells (RPC-C2A) than SuperEBA and Vitrebond | (218) |
| | | | Promotes proliferation and odontogenic differentiation, and decreases secretion of IL-1β and IL-6 | (219,220) |
| | | | Enhances expression of VEGF and angiogenin | (222) |
| | | DPFs | Less cytotoxic than $Ca(OH)_2$ | (232) |
| | | | No cytotoxicity or genotoxicity | (233) |
| | | PDLCs | Inhibits proliferation, viability and differentiation | (111,244) |
| | | | Enhances calcification and BMP-2 expression | (245) |
| | | PDLFs | Fresh MTA inhibits proliferation, attachment and differentiation of PDLFs | (250-253) |
| | | | More biocompatible than commonly used endodontic materials | (105,254-257) |
| | | | Induces differentiation | (105) |
| | | | Concentration-/time-dependent biocompatibility | (251,258-261) |
| | Immune cells | Monocytic cells | Biocompatible with THP1 cells and increases secretion of cytokines by THP1 cells | (272) |
| | | | Induces THP-1 polarization toward M2 phenotype by activating Axl/Akt/NF-κB signaling pathway | (286) |
| | | Neutrophils | Increases expression of IL-1β and IL-8 | (278) |
| | | | Enhances chemotaxis and chemokinesis by activation of calcium-sensing receptors and downstream pathways | (279) |
| | | Macrophages | Induces release and upregulates expression of neutrophil chemotactic factor substances from macrophages and mast cells | (275-277) |
| | | | Biocompatible | (282-284) |
| | | | Increases expression of inflammatory cytokines and induces M2 polarization in RAW 264.7 macrophages | (282,285) |
| | | Lymphocytes | No DNA breakage to human peripheral lymphocytes | (287) |
| Bioaggregate | Stem cells | BMSCs | Biocompatible | (115) |
| | Osteoblasts | MC3T3-E1 | Shows no cytotoxicity and increases expression of COL1, OCN and OPN | (180) |
| | Osteoclasts | Osteoclasts | Similar ability to MTA to prevent migration and fusion to inhibit bone resorption and differentiation via NF-κB signaling pathway | (191-193) |
| | Fibroblasts | DPCs | Similar compatibility and ability to enhance odontogenic differentiation by activation of MAPK signaling pathway to MTA | (223,224,226) |
| | | | Superior to MTA in promoting cell adhesion and migration | (225) |
| | | | Stronger potential to induce osteogenic differentiation than MTA | (223) |
| | | PDLCs | Better biocompatibility than MTA | (246) |
| | | | Promotes mineralization and osteogenic differentiation in a concentration-/time-dependent manner via miR-146a | (247) |
| | | PDLFs | Comparable biocompatibility with MTA | (262) |
| Biodentine | Stem cells | DPSCs | Superior to MTA in biocompatibility and mineralized nodule formation | (74,82,86) |

Table I. Continued.

| Bioceramic | Cell type | Subtype | Biocompatibility and bioactivity | References |
|---|---|---|---|---|
| | | | Promotes osteo/odontogenic differentiation and mineralization by activating ERK1/2 and JNK, and attenuating NF-κB pathways | (53,75-78) |
| | | | No effect on expression of IL-6 and IL-8 but decreases expression of TGF-β | (53) |
| | | | More compatibility when stored in acid rather than saline | (58) |
| | | | Slight toxicity and more odontogenic differentiation when directly contacting DPSCs | (49,77,80) |
| | | | Similar ability to improve expression of VEGF compared with MTA | (49) |
| | | PDLSCs | Similar dose-dependent biocompatibility to MTA | (104) |
| | | BMSCs | Promotes proliferation | (116-118) |
| | | | Inferior to MTA in promoting proliferation and osteoblastic differentiation | (119) |
| | | SHED | Similar dose-dependent biocompatibility to MTA | (126,126,128,129) |
| | | | Superior to MTA in promoting proliferation and calcified matrix deposition | (125,127) |
| | | SCAPs | Similar biocompatibility to MTA | (133,134,136) |
| | | | Induce odonto/osteogenic differentiation in dose-dependent manner | (138,139) |
| | | | Superior to MTA in inducing odontoblastic differentiation | (133,134,137) |
| | | | Similar capacity to enhance expression of pro-inflammatory cytokines, such as IL-1α, IL-1β, IL-6 and TNF-α, compared with MTA | (139) |
| | | | Similar ability to promote expression of VEGFA and FGIF | (134) |
| | | TGSCs | Similar to MTA in biocompatibility and release of angiogenic | (73) |
| | Osteoblasts | Primary osteoblasts | Similar cytocompatibility to MTA | (164,181) |
| | | MG-63 | Similar cytocompatibility to MTA | (182) |
| | | MC3T3-E1 | Similar ability to promote calcification compared with MTA | (183) |
| | | Saos-2 | Similar dose-dependent biocompatibility to MTA and induces expression of ALP and mineralization | (184) |
| | Osteoclasts | Osteoclasts | Lower inhibitory effect on differentiation and activity via ERK1/2 and NF-κB signaling pathways | (197) |
| | Fibroblasts | DPCs | Biocompatible and promotes odontoblastic differentiation and biomineralization | (224,229) |
| | | | Similar ability to MTA in increasing TGF-β1 secretion | (228) |
| | | DPFs | Similar biocompatibility to MTA in concentration-/time-dependent manner | (233,234) |
| | | | Affects differentiation in a concentration-dependent manner by modulating TGF-β1 secretion | (228,234) |
| | | | More biocompatibility and less inflammation compared with TheraCal | (78,240,243) |
| | | PDLCs | Similar biocompatibility to MTA | (55,181) |
| | | | Superior to MTA in terms of attachment and proliferation | (181) |
| | | PDLFs | Viability increases with time | (261) |
| | | | More cell aggregates on surface compared with MTA | (267) |
| | | | Higher expression of Integrin β1 and Vinculin compared with MTA | (268) |
| | Immune cells | Monocytes | Decreases adhesion of THP-1 cells to endothelial cells, migration and activation to macrophages | (240) |

Table I. Continued.

| Bioceramic | Cell type | Subtype | Biocompatibility and bioactivity | References |
|---|---|---|---|---|
| | | | Similar biocompatibility compared with MTA | (289) |
| | | Macrophages | No effect on activation and inflammatory response of THP-1 macrophages | (288) |
| | | | Greater inhibitory effect on expression of inflammatory cytokines compared with MTA | (183) |
| iRoot BP Plus | Stem cells | DPSCs | Greater ability to promote adhesion, migration and mineralization than MTA | (93) |
| | | | Similar ability to enhance formation of focal adhesions and reorganization of the actin cytoskeleton compared with MTA | (94) |
| | | BMSCs | Enhances osteo/odontogenic differentiation via MAPK pathway and autophagy | (114) |
| | | SHED | Similar in promoting proliferation but superior in enhancing migration, adhesion and osteogenetic differentiation | (93) |
| | Osteoblasts | Primary osteoblasts | More cytotoxic than MTA | (185) |
| | | MC3T3-E1 | Improved viability under inflammatory acidic environment compared with MTA | (186) |
| | Fibroblasts | DPCs | Superior to MTA in proliferation, mineralization and odontoblastic differentiation | (226,230) |
| | | | Promotes migration and upregulates expression of focal adhesion molecules via FGFR-mediated ERK 1/2, JNK and Akt pathways | (231) |
| iRoot FS | Stem cells | DPSCs | Promotes proliferation, migration and osteogenic differentiation | (97) |
| | | | Superior to Biodentine in terms of proliferation and migration | (98) |
| | | SCAPs | Similar biocompatibility but stronger capacity to enhance migration and osteo/odontogenesis via the Wnt/β-catenin pathway in comparison with MTA | (147) |
| | Osteoblasts | MC3T3-E1 | More biocompatible than iRoot BP Plus or MTA | (187) |
| | | MG63 | More biocompatible than iRoot BP Plus or MTA | (188) |
| | Fibroblasts | PDLCs | Superior to Biodentine in cell viability, proliferation and osteogenic differentiation | (248) |
| iRoot FM | Stem cells | SCAPs | Induces proliferation and osteo/odontogenic differentiation dose-dependently without affecting cell morphology | (148) |
| iRoot SP | Stem cells | TGSCs | Biocompatible | (152) |
| | | | Inferior to MTA in inducing odontogenic differentiation and hard tissue deposition | (153) |
| | Osteoblasts | MG63 | Biocompatible and enhances expression of COL1, OCN and BSP | (190) |
| | Osteoclasts | Osteoclasts | More cytotoxicity than MTA, but similar potential to inhibit osteoclastogenesis | (200) |
| | Fibroblasts | PDLCs | Displays biocompatibility and enhances osteoblastic differentiation via the integrin-mediated signaling pathway | (249) |
| | Immune cells | Macrophages | More cytotoxic and primarily induces M1 macrophage polarization compared with MTA | (200) |
| | | | Similar ability to MTA in enhancing expression of inflammatory cytokines in RAW 264.7 macrophages | (200,282,285) |
| | | | Similar ability to shift balance of M1/M2 polarization to M2 polarization compared with MTA | (282,285) |

MTA, mineral trioxide aggregate; DPSCs, dental pulp stem cells; DPCs, dental pulp cells; DPFs, dental pulp fibroblasts; PDLSCs, periodontal ligament stem cells; PDLCs, periodontal ligament cells; PDLFs, periodontal ligament fibroblasts; BMSCs, bone marrow stromal/stem cells.

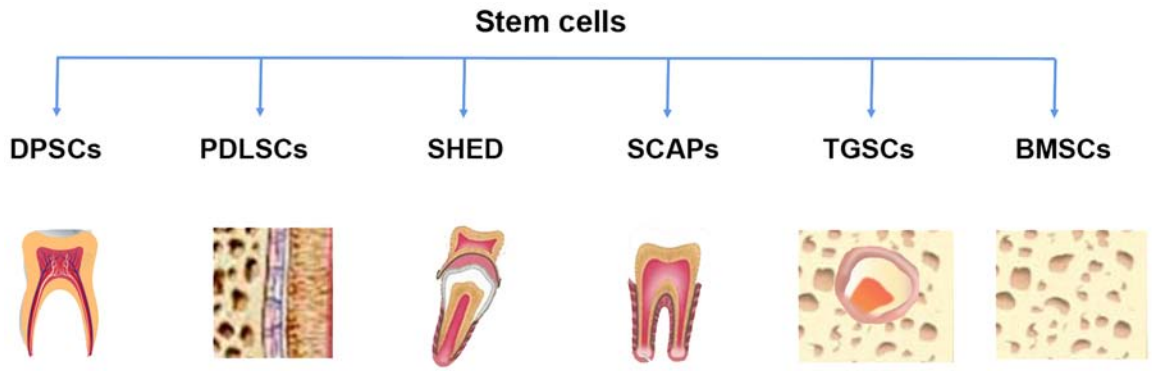


Figure 2. Stem cells involved in the interaction with calcium silicate-based bioceramics in endodontic application. (A) DPSCs. (B) PDLSCs. (C) SHEDs. (D) SCAPs. (E) TGSCs. (F) BMSCs. DPSCs, dental pulp stem cells; PDLSCs, periodontal ligament stem cells; SHED, stem cells from human exfoliated deciduous teeth; SCAPs, stem cells from apical papilla; TGSCs, tooth germ stem cells; BMSCs, bone marrow stromal/stem cells.

Although Biodentine medium is biocompatible with DPSCs, when in direct contact with DPSCs, Biodentine exhibits a slight toxic effect and delays closure of wound edges, which implies that direct contact between Biodentine and DPSCs leads to cell death or decreased proliferation (80,81). However, unaltered expression levels of actin, tubulin and vimentin indicate that Biodentine neither induces apoptosis, inflammation and genotoxicity nor impairs cellular architecture. The inhibitory effect of Biodentine on cell proliferation and migration may result from the decreased space for cell growth in a direct culture model and prolonged cell doubling time (80). Similarly, other studies have observed that the viability of human DPSCs in direct contact with Biodentine is initially decreased (49,77), which may be due to substantial calcium ion released from Biodentine in the first 3 h (82). Relatively large amounts of Ca and Si ions and the absence of Sr, Al and S in Biodentine extract contribute to the biocompatibility of Biodentine (74). Considering the increased release of Ca and Si ions (83) and microstructural (84,85) changes induced by acidic conditions, human DPSCs cultured in Biodentine stored in acidic environment display more spindle-shaped formation and higher adherent cell density compared with that in Biodentine stored in saline (58) Similar to MTA, Biodentine also increases gene expression levels of osteogenic and odontogenic markers, such as *OPN* and *DSPP* in human DPSCs when in direct contact with DPSCs (49,77). However, Biodentine promotes odontogenic differentiation of DPSCs more significantly than osteogenic differentiation, as indicated by detection of expression levels of odontoblastic marker *DSPP* and osteogenic gene markers *ALP, COL1A1* and *OPN* (80). Moreover, Biodentine promotes biomineralization and secretion of extracellular mineral matrix in human DPSCs cultured with osteogenic medium for 21 days (77) and induces more mineralized nodules in the osteogenic medium compared with MTA, suggesting that increased calcium ion release, along with a neutral pH, promotes differentiation and mineralization of DPSCs and subsequently generates a greater number of structured dentin bridges (82). Similarly, when DPSCs are cultured with three-dimensional models, Biodentine induces higher viability compared with MTA. Furthermore, expression levels of *COL1A1, ALP* and *DSPP* in DPSCs on MTA and Biodentine are initially upregulated significantly and then decrease gradually until day 21; however, expression of *RUNX2* in three-dimensional cultures remains lower than that in control group (86). The reason for this may be that *COL1A1, ALP* and *DSPP* are associated with initiation of dentinogenesis and mineralization. Low expression levels of *RUNX2* contribute to odontoblast differentiation and cell maturation, whereas increased expression is observed during terminal odontoblast differentiation (87,88). MTA and Biodentine stimulated angiogenesis by improving the expression levels of *VEGF* in human DPSCs on day 14 (49). *VEGF* enhances the proliferation, migration, and tubulogenesis of endothelial cells close to microvessels, which regulates both vascularization and angiogenesis. Angiogenesis is a key factor for wound healing and tissue regeneration of damaged dental pulp (89-91). The pro-angiogenic capacity of bioceramics depends on dissolution products, such as Si, Mg and Ca ions, which induce secretion of angiogenic factors from cells. Dissolution of calcium ions and subsequent precipitation reactions on the surface of bioceramics leads to vascular penetration and osteoblastic differentiation. Interconnections and pore size of the scaffold also influence the size and amount of the blood vessels, which are necessary for the vascularization of bioceramic material (92).

iRoot BP Plus promotes proliferation, attachment, migration and mineralization of DPSCs compared with MTA (93). Furthermore, iRoot BP Plus releases more Si ions than MTA, which may explain why iRoot BP Plus induces greater apatite formation. iRoot BP Plus and MTA promote stretched and highly organized stress fibre assembly of DPSCs, which is indicative of reorganization of the actin cytoskeleton. Moreover, iRoot BP Plus and MTA enhance phosphorylation of both Paxillin and focal adhesion kinase (FAK) and increase protein expression levels of Vinculin, FAK and Paxillin in human DPSCs (94); this is associated with the formation of focal adhesions (95). Cytoskeleton reorganization and focal adhesion formation is essential for cell adhesion and migration (96). These results confirmed that both iRoot BP Plus and MTA promote attachment and migration of human DPSCs. Likewise, iRoot FS enhances proliferation, migration, and osteoblastic differentiation of human DPSCs (97). Additionally, iRoot FS displays superior ability than Biodentine to promote proliferation and migration of human DPSCs on day 7. However, iRoot FS showed no significant effect on osteogenic differentiation on day 7 (98), which implied that iRoot FS affects proliferation and migration of human DPSCs and later influenced osteoblastic differentiation. A longer experimental

observation period should be used to investigate the bioactivity of calcium silicate-based bioceramics on human DPSCs.

*PDLSCs*. MTA exhibits concentration-dependent cellular compatibility with human PDLSCs; MTA at higher dilution exhibits better biocompatibility with human PDLSCs (99). Due to the biological characteristics of MTA, other MTA-based endodontic materials have been developed, including Endoseal MTA (ES; Maruchi Co., Ltd.), Nanoceramic Sealer (NCS; B&L Biotech USA, Inc.), Bioroot BC Sealer (BR; Septodont Holding) and MTA Fillapex (Angelus Dental Products Industry). Although human PDLSCs in the presence of these MTA-based bioceramics maintain high expression levels of MSC markers, including CD105, CD73 and CD90, the capacity of human PDLSCs to migrate, adhere and grow is higher when treated with BR at different concentrations compared with ES and NCS. ES and MTA Fillapex show cytotoxicity to human PDLSCs at 24, 48 and 72 h and give rise to worse cell attachment and spread, which may be caused by tungsten contained in MTA Fillapex (100-102). By contrast, 2 mg/ml MTA extract is the optimal concentration to markedly increase calcified nodule formation, Alp activity and odonto/osteogenic differentiation in human PDLSCs; these effects are mediated by activating NF-κB and MAPK signaling pathways (103). Likewise, 2 mg/ml Biodentine is the most biocompatible concentration to promote migration, attachment, and mineralization of human PDLSCs. Biodentine at low concentrations (2.00, 0.20 and 0.02 mg/ml) significantly enhances viability of human PDLSCs, while Biodentine at higher concentrations (20 mg/ml) exhibits cytotoxicity, which may be associated with high pH (104). High pH of Biodentine results in an increased concentration of iron and calcium ions in the extracellular environment (105). The inhibitory effect of Biodentine at high concentration (20 mg/ml) on the viability of human PDLSCs may be explained by increased or unbalanced ions levels, which generate a toxic effect on cells (106).

*BMSCs*. BMSCs are used as an *in vitro* model of MSCs associated with wound healing and tissue regeneration of alveolar bone. MTA promotes the adhesion and proliferation of human DPSCs, PDLSCs and BMSCs, which display an elongated morphology and are well-spread. Moreover, positive effects of MTA on proliferation are cell type-dependent (25). Human PDLSCs and DPSCs rapidly respond to MTA with significantly increased cell number by day 3, whereas human BMSCs stimulated with MTA show slow increase by day 5 (25). Furthermore, MTA promotes migration of human BMSCs significantly after 18 h and cells spread well on the surface of MTA after 24 h. MTA increases proliferation of human BMSCs when incubated in normal medium, whilst the same phenomenon was delayed by 7 days when cultured in differentiation medium (107). When human BMSCs are cultured in osteogenic differentiation medium, MTA does not stimulate osteogenic differentiation by day 10, as indicated by decreased mRNA and protein expression levels of osteogenic markers, such as *RUNX2, OSX, MSX2* and *OCN* (108). A positive effect of MTA on viability of rat BMSCs is observed up to day 7, which is sooner than in human BMSCs. Simultaneously, MTA stimulates rat BMSC differentiation into osteoblast-like cells over 7 days, which is confirmed by enhanced ALP staining and upregulation of gene expression levels of bone morphogenetic protein 2 (*BMP-2), ALP,* bone sialoprotein (*BSP*) and osteocalcin *(OC*) (109). Low concentrations of MTA-conditioned media (2.000, 0.200, 0.020 and 0.002 mg/ml) are not toxic to BMSCs and 0.02 mg/ml MTA is the optimal concentration to upregulate odonto/osteogenic capacity of BMSCs originating from rat mandible; this finding was supported by enhanced Alp activity, calcified nodule formation and expression levels of odonto/osteoblastic genes in BMSCs, such as *ALP, RUNX2, OSX, OCN* and *DSPP*. However, MTA at lower dilution inhibits the proliferation of BMSCs (110). Likewise, 1:5 MTA medium decreases viability and osteogenic differentiation of primary BMSCs from the hind limb of mice, as indicated by decreased von Kossa staining and low expression levels of *OCN* and *BSP* (111). Furthermore, tricalcium aluminate, a primary composite of MTA, contains aluminum and exhibits significant toxicity to rat BMSCs from femur and tibia bone marrow *in vitro*, whereas good tissue compatibility has been observed with tricalcium or dicalcium silicate (112). In addition, bismuth oxide in MTA is cytotoxic to human BMSCs (113). Therefore, the negative effect of MTA on the viability of BMSCs at high concentrations may partly be caused by tricalcium aluminate and bismuth oxide. Investigation of the mechanism underlying how MTA affects behavior of BMSCs has demonstrated that the ERK signaling pathway is associated with the positive effect of MTA on proliferation (25) and odonto/osteogenic differentiation of BMSCs. JNK signaling pathway is also involved in the odonto/osteogenic capacity of BMSCs induced by MTA (110). In addition, iRoot BP Plus promotes osteogenic/odontogenic differentiation of BMSCs via the MAPK signaling pathway and autophagy (114).

Bioaggregate is non-toxic to human BMSCs throughout a culture period of 24 h (115). Similarly, Biodentine promotes proliferation of rat (116) and human BMSCs (117,118). Both Biodentine and MTA at high concentrations (1:2) show cytotoxicity to human BMSCs, while Biodentine at low concentrations (1:10 and 1:20) and long incubation periods exhibits an inferior ability to enhance proliferation and osteoblastic differentiation of human BMSCs compared with MTA (119). Moreover, Biodentine and MTA display an inhibitory effect on survival of human BMSCs in a concentration-dependent manner over 7 days but do not affect cell morphology (120). The cytotoxicity of Biodentine to human BMSCs may be ascribed to the calcium chloride contained in its liquid (121), which has been reported to exhibit less biocompatibility when added to MTA compared with MTA mixed with water (122). Therefore, Biodentine prepared following complete setting is more compatible with human BMSCs than MTA (120). High Alp activity with mineral deposits in rat BMSCs have been found in the presence of Biodentine after 12 days (116) but the capacity of Biodentine to induce osteogenic differentiation in human BMSCs is inferior to that of MTA (117,118). MTA exhibits earlier and more pronounced calcium deposits than Biodentine (120), which may be explained by the fact that MTA produces high pH and Alp activity and promotes production of high concentration of Ca ions (123).

*SHEDs*. SHEDs originate from deciduous teeth and regenerate bone and dentin, but not dentin/pulp-like complexes as human DPSCs do (124). Due to the good porous microstructures in

MTA or Biodentine, SHEDs attach and spread well on the surface of MTA and Biodentine, which helps SHEDs maintain mesenchymal properties in the presence of MTA or Biodentine with positive expression of CD105, CD90 and CD73 (125). Moreover, the capacity of SHEDs to adhere and proliferate is enhanced by MTA or Biodentine after 48 h (125). MTA and Biodentine exhibit a comparable ability to promote migration of SHEDs. In addition, the viability and proliferation of SHEDs cultured with 1 mg/ml MTA- or Biodentine-conditioned medium is similar to that of negative control during the whole incubation period, which implies that both MTA and Biodentine at 1 mg/ml are non-toxic to SHEDs (126). However, MTA shows greater potential to promote odontogenic differentiation compared with Biodentine (126), whereas Biodentine has better capability to promote proliferation and calcified matrix deposition in SHEDs than MTA (125,127). Furthermore, Biodentine affects behavior of SHEDs in a concentration-dependent manner. Biodentine at low concentrations (2.00, 0.20 and 0.02 mg/ml) stimulates proliferation, viability and migration of SHEDs, whereas high concentrations of Biodentine (20 mg/ml) exhibit slight cytotoxicity to SHEDs. Changes in the concentration of Biodentine have no impact on the adhesion ability of SHEDs (128). Similarly, Biodentine at higher dilutions (1:16 and 1:32) is more effective in promoting proliferation, odontogenic differentiation and biomineralization of SHEDs, which may be because Sr and Si are gradually released from Biodentine as the concentration of Biodentine decreases over time (129). By contrast, fresh mixed MTA impairs the viability and migration of SHEDs and enhance apoptosis over 7 days. Furthermore, the cytotoxicity of MTA to SHEDs is more apparent when SHEDs directly contact MTA (130). The different results may be associated with the preparation of MTA. For example, freshly mixed MTA is frequently used in endodontics whereas bioceramic eluate or aged bioceramics were used in the aforementioned *in vitro* studies (86). However, the cytotoxicity of these bioceramics generally decreased as the bioceramic set and pH changed of medium over time. Freshly mixed MTA caused severe damage to cells due to the initially high concentration of calcium hydroxide and subsequent raise in pH to 12.5 after mixing for 3 h (131,132). Although iRoot BP Plus possesses a similar capacity to MTA in terms of SHEDs proliferation, it displays more prominent capacity to enhance adhesion, migration and osteogenesis of SHEDs compared with MTA (93).

*SCAPs*. Both MTA and Biodentine have been shown to promote the proliferation, odontoblastic differentiation and biomineralization of SCAPs over 14 days (133,134). However, Schneider *et al* (135) found MTA induces early short-term proliferation of SCAPs over 5 days and promotes the migration of SCAPs after 6 h. Saberi *et al* (136) discovered that the cytotoxicity of both complete set MTA and Biodentine to SCAPs decreased over time. By contrast, Miller *et al* (137) revealed that incompletely set MTA inhibits proliferation of SCAPs, whereas Biodentine promotes proliferation of SCAPs. The difference in results may be due to the method of cytotoxicity assessment, contact between cells and material, concentration of material and assessment time points. MTA affects survival of SCAPs in concentration-dependent manner. MTA at lower concentrations (0.02, 0.20 and 2.00 mg/ml) exhibits excellent biocompatibility with SCAPs; however, proliferation of SCAPs is inhibited and normal morphological cells disappeared when treated with MTA at higher concentrations (10 and 20 mg/ml) (138). Low concentrations of MTA or Biodentine (2.00, 0.20 and 0.02 mg/ml) enhance Alp activity and osteoblastic/odontoblastic differentiation in SCAPs, while high concentrations of MTA or Biodentine (20 mg/ml) exhibit a negative effect (138,139). MTA extract enhances the ability of osteogenic medium to induce mineralization and increase expression of mineralization-associated genes, such as Ocn (140). In comparison with MTA, SCAPs treated with Biodentine display greater odontoblastic differentiation, as demonstrated by positive alizarin red staining and expression of genes encoding DMP-1, DSPP, OCN and matrix extracellular phosphoglycoprotein (133,134). Biodentine enhances expression of odontoblast specific marker *DSPP*, while MTA promotes osteoblastic differentiation of SCAPs by increasing expression of the osteoblastic marker integrin-binding sialoprotein (137). Both MTA and Biodentine enhance the secretion of pro-inflammatory cytokines, such as IL-1α, IL-1β and IL-6 (139), and MTA activates NF-κB signaling pathway, which affects the odonto/osteogenic differentiation of SCAPs (141,142). In addition, p38 and ERK signaling pathways serve an essential role in odontoblastic/osteoblastic differentiation of SCAPs stimulated by MTA (138). MTA and Biodentine enhance the angiogenic potential of SCAPs; these bioceramics promote the expression of angiogenic genes in human SCAPs, such as *VEGFA* and c-fos induced growth factor (*FIGF)* (134), which induce endothelial cell proliferation, migration and differentiation, and promote formation of endothelial tubules (143-146).

Compared with MTA, iRoot FS exhibits similar biocompatibility with human SCAPs but possesses markedly stronger capacity to enhance migration and osteo/odontogenesis differentiation of human SCAPs, and mineralized nodule formation via the Wnt/β-catenin signaling pathway (147). iRoot FM at low concentrations (0.5 mg/ml) increases proliferation and osteo/odontoblastic differentiation of SCAPs, whereas there is no marked effect on SCAPs stimulated with iRoot FM at high concentrations (1.0 and 2.5 mg/ml). Moreover, mineralized nodule formation and expression of *DMP-1* and *ALP* are enhanced by iRoot FM compared with $Ca(OH)_2$. However, iRoot FM at different concentrations has no impact on morphology of SCAPs (148).

*Human TGSCs*. TGSCs, a popularized stem cell source derived from wisdom teeth, display MSC properties and can differentiate into endothelial or epithelial cells in dental tissue regeneration (149,150). Consistent with human DPSCs and PLSCs, MTA and Biodentine exhibit no cytotoxicity to TGSCs (73). Nonetheless, viability and odontogenic differentiation of TGSCs are inhibited slightly when in direct contact with MTA, which has been confirmed by decreased numbers of attached cells and Alp activity (151). MTA and Biodentine induce angiogenesis of TGSCs by promoting the release of angiogenic growth factors (platelet-derived growth factor, fibroblast growth factor-2 and VEGF) and enhancing tube formation of human umbilical vein endothelial cells (73). Furthermore, iRoot SP exhibits good biocompatibility with human TGSCs and promote their attachment (152). iRoot SP

possesses an inferior capacity to MTA in terms of inducing odontogenic differentiation of human TGSCs and hard tissue deposition; human TGSCs in the presence of MTA exhibit higher Alp activity and enhanced odontoblastic differentiation compared with those in the presence of iRoot SP (153).

### 3. Osteoblasts/osteoclasts

Restoration of bone tissue around teeth with lesions relies on the amount of, and balance between, osteoblasts and osteoclasts (154). When calcium silicate-based bioceramics are used in perforation repair, apical plugs in necrotic teeth or root-end filling in endodontics, the interaction between the bioceramics and osteoblasts in periapical tissue is key to inflammation control and wound repair of (155). The biological influence of calcium silicate-based bioceramics on osteoblasts/osteoclasts must be characterized.

*Osteoblasts*. Proksch *et al* (156) found that MTA impairs proliferation, osteogenic differentiation and extracellular matrix mineralization of primary human osteoblasts derived from the alveolar bone; this could be restored by addition of fluoride to MTA. Similarly, MTA inhibits proliferation and *COL1* gene expression in bone marrow osteoblasts from rat femur (157). MTA inhibits proliferation and differentiation of rat primary calvarial osteoblasts, as demonstrated by decreased calcified nodule formation and osteoblastic differentiation (158). When primary osteoblasts are cultured with MTA, the highest ionic concentration in areas around MTA results in an inhibitory effect on primary osteoblasts in the central area (158). High dissolution rate of MTA and large release of calcium hydroxide from MTA contribute to highest ionic concentration in areas around MTA (159). Excess calcium concentration induces cell death by apoptosis or necrosis (160), whereas slight increases in extracellular $Ca^{2+}$ concentration (161) and continuous low levels of calcium ion release, along with slower change in pH, promote proliferation and differentiation of osteoblasts (162,163). The decreased viability of primary human osteoblasts induced by MTA may be due to the cumulative effect of the release of toxic components, such as Bi and Al ions, following contact with bioceramic extract (164). These different results may be due to different methods and concentrations of bioceramics used in the experiments. MTA exhibit higher biocompatibility with osteoblasts cultured in three-dimensional culture systems and are attracted toward the material compared with cells cultured directly on materials or in extract. Increased percentage of mature osteoblasts or osteocytes with high expression levels of green fluorescent protein and osteogenic genes, including *ALP, BSP* and *OSX*, in primary mouse mandibular osteoblasts demonstrates that MTA promotes differentiation of primary mouse osteoblasts in a three-dimensional cell culture model without differentiation medium, which is required for osteoblastic differentiation in a two-dimensional cell culture model (165,166).

MTA exhibits minimal cytotoxicity to the human osteoblast MG-63 cell line (167). MTA promotes viability and increases mRNA expression levels of *COL1* and *OCN* in MC3T3-E1 cells induced by MTA, accompanied by enhancement of Alizarin Red-S staining and Alp activity, which suggests that MTA promotes bone matrix formation and mineralization of MC3T3-E1 cells (168-171). Moreover, when incubated on the surface of set MTA, a thick mineralized matrix layer covered with multilayered flat cells is observed in MC3T3-E1 cells after three weeks (169). The mechanism underlying MTA-induced osteoblastic differentiation, mineralization and bone matrix formation in MC3T3-E1 cells *in vitro* may be associated with the activation of activating transcription factor 6 by MTA and the subsequent endoplasmic reticulum stress response (170). Moreover, Ca, Si and P ions released from MTA (157,172) and alkaline pH value of MTA extract (171) contribute to the bioactivity of MTA. Alkaline pH neutralizes lactic acid, which dissolves mineralization of dentin, and promotes formation of hard tissue by activating alkaline phosphatase (173). MTA with high surface pH exhibits cytotoxicity to MG-63 cells initially (174); the inhibitory effect of MTA on viability of MG-63 cells weakens with the setting of MTA (174), which may be due to the fact that the expression of pro-inflammatory cytokines in MG-63 cells are increased by MTA during the first 24 h but decreased over time (167). Growth factor BMPs promote osteoblastic differentiation and biomineralization (175); the mixture of BMPs and MTA attenuates the cytotoxic effect of MTA to MG-63 cells and inflammation response of tissue to MTA (174). MTA promotes the adhesion, spreading, proliferation and collagen secretion in Saos-2 cells (176,177). Moreover, MTA enhances osteogenic differentiation, Alp activity and calcified nodule formation in Saos-2 cell line (178). By contrast, Modareszadeh *et al* (179) reported that MTA does not affect survival and Alp activity in Saos-2 cell line. The inconsistency between these studies may due to differences in the preparation of MTA, experimental methods and assessment of Alp activity. Bioaggregate shows no cytotoxicity to MC3T3-E1 and markedly increases expression levels of mineral-associated genes, including *COL1, OCN* and *OPN,* in MC3T3-E1 cells compared with MTA (180).

Compared with MTA, Biodentine displays good cytocompatibility with primary human osteoblasts, indicated by enhancement of cell viability, attachment and proliferation (164,181). Similar biocompatibility of MTA and Biodentine has been observed with human osteoblast-like cell line MG63; both enhance viability, adhesion and proliferation of MG63 cells, which may be because Biodentine and MTA have similar surface roughness, heterogeneous morphology and particle size (182). Biodentine and MTA both exhibit positive effects on viability and calcification of MC3T3-E1 cells (183). Biodentine and MTA show dose-dependent effects on viability of Saos-2 cells. Biodentine or MTA at lower concentrations (1:4 and 1:8) result in higher viability of Saos-2 cells. Furthermore, Biodentine stimulates proliferation and migration of Saos-2 cells and induces expression of *ALP* and mineralization (184).

Compared with MTA, iRoot BP Plus induces greater cytotoxicity to primary human osteoblasts but is still considered as biocompatible because cell viability in the presence of iRoot BP Plus remained >70% compared with that in the control group (185). When MTA or iRoot BP Plus is applied in acidic pH conditions, the secretion of Ca and Si ions is enhanced and apatite formation is decreased. However, cell attachment of MC3T3-E1 on these bioceramics is not affected significantly in an acidic environment. Furthermore, MTA decreases viability, whereas iRoot BP Plus increases survival of MC3T3-E1 cells,

which suggests that, compared with MTA, iRoot BP Plus may be more suitable as root-end filling material under inflammatory acidic conditions when used in endodontics (186). By contrast, iRoot FS exhibits better biocompatibility with human osteoblast-like MC3T3-E1 and MG63 cells by promoting their attachment and proliferation (187,186). iRoot FS shows better biocompatibility than MTA or iRoot BP Plus because certain toxic metal substances, such as bismuth (189), aluminium and manganese, are excluded in iRoot FS to enhance its compatibility. On the other hand, smaller particle size on the surface of iRoot FS results in higher cell attachment and subsequent proliferation (188). iRoot SP is non-cytotoxic to MG63 cells and enhances osteoblastic differentiation, which is beneficial to healing inflammatory periapical tissue (190).

*Osteoclasts*. The migration and fusion of osteoclast precursors is key to osteoclast formation. MTA and Bioaggregate inhibit bone resorption and osteoclast differentiation via preventing the migration and fusion of osteoclast precursors, including mouse bone marrow macrophages (191) and RAW264.7 macrophages (192-194). In addition, MTA inhibits osteoclastogenesis in a dose-dependent manner in the co-culture of mouse bone marrow cells with primary osteoblast cells. Furthermore, MTA suppresses expression of osteoprotegerin in primary osteoblast cells without affecting receptor activator of NF-κB ligand (RANKL) expression levels (195,196). Moreover, MTA solution at low concentration (20%) impairs phosphorylation of c-Src, decreases expression levels of genes encoding MMP-9 and cathepsin K and disrupt formation of actin rings. MTA solution at high concentration (50%) upregulate expression levels of Bim to increase apoptosis of osteoclasts (196). The mechanism underlying MTA-induced inhibition of osteoclastogenesis is associated with attenuation of the autophagic pathway, as demonstrated by decrease in autophagic vacuole and expression levels of autophagic genes and proteins (194). Bioaggregate inhibits osteoclastogenesis via the NF-κB/RANK signaling pathway by decreasing expression levels of Rank, TNF receptor-associated factor 6, NF-κB and nuclear factor of activated T cells 1 (192,193). Bioaggregate and MTA possess comparable ability to decrease osteoclast numbers and attenuate bone resorption (191). Bioaggregate or MTA inhibit osteoclast differentiation and bone resorption due to activation of autophagy in osteoclast differentiation; MTA inhibits osteoclast differentiation via inhibition of the autophagic pathway (194). Compared with MTA, Biodentine exhibits a lower inhibitory effect on osteoclast differentiation and activity of murine bone marrow macrophages by inhibiting ERK1/2 and NF-κB signaling pathways (197). Moreover, the inhibitory effect on osteoclast differentiation and activity of both MTA and Biodentine is similar to that of alendronate, which has been reported to prevent root resorption by inhibiting macrophages (197), which suggests the application of calcium silicate-based bioceramics as treatment to prevent root resorption in endodontics (198,199). iRoot SP shows more cytotoxicity to RAW264.7 cells than MTA but possesses a similar ability to inhibit osteoclastogenesis (200). The aforementioned studies suggested that calcium silicate-based bioceramics attenuate osteoclast differentiation and the primary mechanism is associated with their bioactive elements. Bioactive elements contained in these bioceramics, such as Ca, Mg, Si and Sr, enhance osteoblastic differentiation and suppress RANKL-induced osteoclastogenesis (201-204). A significant increase in Si and Sr ions has been observed in extracts of calcium silicate-based bioceramics (192,205). Si ions enhance the viability, adhesion, differentiation, mineralization and angiogenesis of osteoblasts via the Wnt/β-catenin and MAPK signaling pathways (206-210). Meanwhile, the effect of Si ion on surface roughness is characterized by increased adhesion and proliferation of human osteoblast cell lines (211). Furthermore, Si and Sr ions suppress RANKL-mediated osteoclastic differentiation and bone resorption by inhibiting expression levels of cathepsin K, tartrate-resistant acid phosphatase and c-Fos (205,212,213). In addition, Si and Sr ions create alkaline conditions, which neutralize lactic acid from osteoclasts and promotes accumulation of mineralized components of teeth (214,215). Therefore, the bioactive elements exhibit synergistic effects on osteogenesis, osteoclastogenesis and angiogenesis of associated cells in endodontics (216).

## 4. Dental pulp or periodontal ligament cells/fibroblasts (PDLC/Fs)

Dental pulp or PDLC/Fs are associated with wound healing and tissue regeneration of dental or periapical tissue, respectively (217). When calcium silicate-based bioceramics are used in pulp capping, cells/fibroblasts from dental pulp are involved in interactions between cells and bioceramics. Cells/fibroblasts from periodontal ligament are affected by bioceramics applied in perforation repair or root-end filling. Numerous studies have investigated the effect on biocompatibility and bioactivity of cells/fibroblasts from dental pulp or periodontal ligament.

*Dental pulp cells (DPCs)*. Compared with SuperEBA and Vitrebond, MTA exhibits decreased suppression of mitochondrial activity in the rat DPC RPC-C2A cell line (218). Furthermore, MTA significantly promotes proliferation, odontogenic differentiation and mineralization of human DPCs but inhibits secretion of lL-1β and IL-6 (219,220). In order to improve the bioactivity potential of MTA, the growth factor FGF-2 has been added to MTA to enhance its effect on proliferation and osteogenic differentiation of human DPCs (221). Set and fresh MTA display similar biocompatibility with human DPCs. In addition, MTA increases expression of the angiogenic factors VEGF and angiogenin (222). In comparison with MTA, Bioaggregate and Biodentine possess equal biocompatibility with human DPCs. Moreover, Bioaggregate, Biodentine and MTA enhance mRNA expression levels of ostogenic/odontogenic genes, such as ALP, OPN, OCN, DSPP and DMP-1, increase Alp activity and promote mineralization nodule formation due to activation of the MAPK signaling pathway induced by these calcium silicate-based bioceramics (223,224). In addition, Bioaggregate exhibits superior capacity to MTA in terms of adhesion, attachment and migration of human DPCs (225). Moreover, compared with MTA, Bioaggregate induces enhanced mineralization and odontoblastic differentiation in human DPCs (226). In terms of osteogenic differentiation of human DPCs, Bioaggregate displays stronger potential than MTA (223). Both Biodentine and MTA promote mineralization by increasing secretion of TGF-β1 from human DPCs, which mediates mineralization-associated cellular

activity and subsequent dentin bridge formation (227,228). Biodentine enhances cell proliferation, viability, migration, adhesion, odontoblastic differentiation and biomineralization of the immortalized murine DPC OD-21 cell line (229). iRoot BP Plus exhibits a higher proliferation rate of human DPCs compared with MTA during the whole culture period (230). Moreover, iRoot BP Plus promotes migration and upregulates the expression of focal adhesion molecules in human DPCs via the ERK 1/2, JNK and Akt signaling pathways (231). In addition, iRoot BP Plus possesses stronger potential than MTA to enhance the mineralization and odontoblastic differentiation of human DPCs (226).

*Dental pulp fibroblasts (DPFs).* Given that $Ca(OH)_2$ and MTA are used in pulp capping, Kierat *et al* (232) compared cytotoxicity to human DPFs; MTA was less cytotoxic to human DPFs compared with $Ca(OH)_2$. Likewise, Zakerzadeh *et al* (233) compared the biocompatibility of MTA and Biodentine with human DPFs; MTA and Biodentine did not display cytotoxicity or genotoxicity at 0-1,000 $\mu$g/ml concentration. Similarly, Biodentine does not influence the morphology, proliferation or cell integrity of human DPFs, but affects cell survival in a concentration- and time-dependent manner (234). Moreover, higher concentration of Biodentine suppress differentiation of DPFs by decreasing production of COL1 and TGF-β1 (234), whereas Biodentine at lower concentrations stimulate TGF-β1 secretion (228). Both TGF-β1 and COL1 exhibit notable effects on collagen synthesis, mineralization and hard dental tissue generation (228,235-237). TGF-β1 and FGF-2 are also involved in proliferation, differentiation and migration of DPFs (228,238,239). Giraud *et al* investigated the effect of Biodentine on injured human DPFs; Biodentine increased cellular survival and migration, as well as the secretion of FGF-2 and TGF-β1 in lipoteichoic acid-stimulated and physically injured human DPFs. Moreover, injured DPFs exhibit decreased levels of pro-inflammatory cytokines, such as IL-6, and inflammatory cell recruitment in the presence of Biodentine compared with that in the presence of resin-containing TheraCal (240). TheraCal inhibits proliferation of human DPFs while Biodentine has no effect on proliferation of human DPFs. In addition, decreased release of pro-inflammatory cytokine IL-8 and higher mineralization have been observed in human DPFs induced by Biodentine compared with TheraCal (78). In addition, complement, particularly the C5a fragment, is involved in initiation of inflammation (241) and recruitment of DPSCs in regeneration (242). Due to the fact that C5a is produced by local DPFs when dental pulp is injured, Giraud *et al* compared several pulp capping materials and their effect on release of C5a in human DPFs. The results revealed that C5a secretion of injured human DPFs was not affected by Biodentine, whereas TheraCal increased C5a secretion. Furthermore, compared with TheraCal, Biodentine significantly decreased recruitment of THP-1 cells involved in inflammation by affecting C5a/C5a receptor interactions (243). These results suggested that calcium silicate-based Biodentine affects the balance between initial pulp inflammatory reaction and subsequent pulp healing by affecting complement activation following pulp injury. Calcium silicate-based bioceramics shift the balance toward pulp healing, whereas resin-containing materials shift the balance toward pulp inflammation (78,240).

*PDLCs.* Vidovic Zdrilic *et al* (111) found that MTA-conditioned medium promotes healing of injured periapical tissue *in vivo*, whereas MTA inhibits viability and biomineralization in mouse PDLCs *in vitro*. Eluate extract from MTA results in decreased viability and increased death of human PDLCs compared with those in a control group (medium-only) (244). By contrast, when human PDLCs are incubated with set MTA, MTA enhances mineralization and *BMP-2* expression levels, which is caused by gradual production of $Ca^{2+}$ from MTA and the interaction of BMP2/BMP-2 receptors in human PDLCs (245). These contradictory results may be due to different preparation methods of MTA. Set MTA releases calcium gradually and calcium at low concentrations stimulates cells mildly, whereas MTA-conditioned medium leads to immediate release of calcium at high concentrations; this may exert a negative effect on bioactivity of MTA to PDLCs. Compared with MTA, Bioaggregate displays better biocompatibility with human PDLCs, which may be due to the absence of aluminium in Bioaggregate. Moreover, the primary components of Bioaggregate, calcium silicate, calcium hydroxide and hydroxyapatite, stimulate proliferation of human PDLCs (246). In addition, Bioaggregate significantly increases mineralization in PDLCs in a concentration- and time-dependent manner. Furthermore, Bioaggregate enhances differentiation of PDLCs, particularly osteogenic differentiation, via microRNA-146a upregulation, as demonstrated by increased expression levels of *ALP*, *DMP1* and *BMP* and decreased cementum protein 1 expression levels in PDLCs (247). In comparison with MTA, Biodentine exhibits equally good biocompatibility with human PDLCs (55,181). Moreover, Biodentine displays greater potential in terms of attachment and proliferation of human PDLCs compared with MTA, which may be due to its hydroxyl apatite-like surface characteristics and greater release of calcium and silicon ions compared with MTA (181). Luo *et al* compared bioactivity of Biodentine and iRoot FS to human PDLCs and showed that both Biodentine and iRoot FS increased the adhesion of human PDLCs. iRoot FS possesses superior ability to Biodentine to promote viability, proliferation and osteoblastic differentiation of human PDLCs (248). In order to clarify the bioactivity of iRoot SP to human PDLCs, Chang *et al* investigated the biocompatibility and bioactivity of four root canal sealers: iRoot SP, Sealapex (Kerr Corporation), ARS (Dentsply-Sankin KK) and MTA Fillapex. The results revealed that all sealers were non-toxic to human PDLCs and increased Alp activity and formation of mineralization nodules. However, compared with Sealapex, an epoxy resin-based material, the other three sealers induced lower inflammatory response and enhanced osteogenic differentiation in PDLCs via the integrin-mediated signaling pathway (249).

*PDLFs.* Human PDLFs exhibit decreased proliferation rate when cultured on the surface of MTA compared with culturing on the surface of a coverslip (250); PDLFs on the surface of MTA have a rounded morphology with blunted extensions, while PDLFs on the surface of glass coverslips show good attachment and spreading (251). Balto investigated the effect of MTA surface characteristics on attachment of human PDLFs; human PDLFs did not attach to fresh MTA and the surface of cells appeared less smooth and exhibited more vacuoles. By contrast, human PDLFs on the surface of set MTA were

round and flattened with smooth surfaces and attached well to MTA (252). Similarly, Bonson *et al* reported that human PDLFs exposed to washed MTA possess greater proliferation capacity those exposed to fresh MTA. Moreover, compared with fresh MTA, washed MTA exhibits stronger potential to induce osteogenic differentiation of human PDLFs (253). Compared with other endodontic materials, such as Diaket (ESPE; 3M), Super-EBA (Harry J Bosworth Company) and amalgam, MTA also displays better biocompatibility with human PDLFs and does not induce apoptosis and necrosis of human PDLFs (254-257). Compared with these other materials in root perforation models *in vitro*, MTA also results in higher viability in human PDLFs and induces mRNA expression levels of *COL1* and *RUNX2* in human PDLFs, which suggests that MTA has potential to induce osteogenic differentiation of PDLFs, which is key for periodontal regeneration (105). Likewise, MTA-conditioned medium at low concentrations (0.5, 5.0 and 50.0 $\mu$g/ml) possesses superior capacity to formocresol and ferric sulphate in maintaining the viability of human PDLFs, whereas MTA at higher concentrations (5,000 $\mu$g/ml) shows slight cytotoxicity to human PDLFs (258,259). Bioaggregate displays biocompatibility with human PDLFs comparable to that of MTA, whereas viability of human PDLFs in the presence of Biodentine is slightly decreased compared with MTA (260-262). By contrast, Akbulut *et al* (263) reported that Biodentine possesses better biocompatibility with human PDLFs. This discrepancy may be associated with chemical composition of the material, assessment time point and surface characteristics. In terms of chemical composition, calcium chloride used in the liquid of Biodentine as an accelerator decreases the setting time (264) and results in early production of calcium hydroxide, which contributes to relatively decreased cell survival after 24 h in the presence of Biodentine (265). Moreover, zirconium oxide in Biodentine is non-toxic to murine PDLFs, but bismuth oxide in MTA has no impact on cell growth (266). MTA supports higher cell viability during the first 24 h but decreases cell viability to 80% later (251,260). Biodentine maintains lower cell viability during the first 24 h but viability increases gradually over time (261). Human PDLFs attach well to the surfaces of both MTA and Biodentine and maintain their original morphology. Nevertheless, more cell aggregates have been observed on the surface of Biodentine, whereas human PDLFs tend to show greater spread and elongation on MTA (267). Moreover, expression levels of Integrin β1 and Vinculin, which are associated with focal contacts between human PDLFs and bioceramics, are higher in human PDLFs treated with Biodentine than in those treated with MTA, which suggests that the surface characteristics of Biodentine promote the adhesion and survival of human PDLFs more strongly compared with MTA (268).

## 5. Immune cells

Immune cells, such as monocytes and macrophages, respond immediately when biomaterials are placed into tissue, which causes the initial inflammatory response and tissue healing. Macrophages release pro-inflammatory cytokines in the beginning of an acute inflammatory response, such as TNF-α, IL-1 and IL-12, but release anti-inflammatory cytokines during regeneration and healing of tissue, such as IL-4, which contributes to the production of fibronectin (269-271). MTA displays detectable, but not statistically significant, cytotoxicity to human monocytic cell line THP1 and alters secretion of inflammatory cytokines (272). In addition, macrophages and mast cells participate in leukocyte recruitment and extravasation via secretion of inflammatory cytokines that regulate inflammation control and tissue healing in pulpitis and apical periodontitis (273,274). Moreover, neutrophil chemotactic factor is induced from macrophages and mast cells by MTA; the upregulation of these neutrophil chemotactic factor substances participates in migration and accumulation of neutrophils, monocytes and lymphocytes (275-277). Similarly, Cavalcanti *et al* (278) found that MTA increases secretion of IL-8 and IL-1β, which supports the migration of human neutrophils. Chang *et al* (279) discovered that MTA enhances migration of immune cells, which is regulated by calcium-sensing receptors and the PI3K pathway for chemotaxis, as well as the $Ca^{2+}$-calmodulin-dependent MLCK pathway for chemokinesis.

M1/M2 macrophage polarization is associated with the inflammatory response and subsequent tissue regeneration following biomaterial implantation (270,280,281). Tu *et al* found that iRoot SP induces greater cytotoxicity to RAW264.7 macrophages than MTA. Furthermore, both MTA and iRoot SP induce expression of pro-inflammatory cytokines without inducing osteoclastogenesis in RAW264.7 macrophages. In addition, MTA primarily induces M2 macrophage polarization, whereas iRoot SP induces M1 macrophage polarization (200). Both MTA and iRoot SP are non-toxic to RAW264.7 cells (282). Moreover, MTA does not affect the viability and adherence of M1 and M2 macrophages isolated from mice (283,284). MTA and iRoot SP reinforce expression of inflammatory cytokines in RAW264.7 cells. Furthermore, MTA and iRoot SP possess equal capacity to stimulate M1/M2 macrophage polarization but greater M2 macrophage polarization is induced, which implies that calcium silicate-based bioceramics shift M1/M2 polarization balance to M2 macrophage polarization under inflammatory conditions (282,285). Yeh *et al* reported that MTA induces THP-1 cells toward M2 polarization by activating the Axl/Akt/NF-κB signaling pathway. MTA promotes tissue regeneration and wound healing via M2 macrophage polarization (286). MTA does not induce DNA breakage of human peripheral lymphocytes, which is the first step in carcinogenesis. This suggests that MTA exhibits no potential carcinogenic risk when used in endodontics (287).

Compared with other pulp capping materials, such as TheraCal and Xeno III, Biodentine decreases migration and adhesion of THP-1 cells to endothelial cells and inhibits their activation to macrophages *in vitro* (240). In addition, Biodentine does not stimulate expression of inflammation-associated enzymes *in vitro*, such as prostaglandin E2 and thromboxane (288). Compared with MTA, Biodentine displays a more notable inhibitory effect on mRNA and protein expression levels of inflammatory cytokines in RAW264.7 macrophages (183). Biodentine is biocompatible with immune cells, which is consistent with another study that demonstrated that Biodentine exhibits similar biocompatibility with human monocytes compared with MTA (289).

## 6. Conclusion

There have been numerous studies on the *in vitro* biocompatibility and bioactivity of calcium silicate-based bioceramics in endodontics (16,79,147,192). MTA has been investigated most thoroughly and is considered the gold standard. However, compared with MTA, there are not enough studies to assess the biocompatibility and bioactivity of other calcium silicate-based bioceramics, such as Bioaggregate, Biodentine and iRoot BP/FS/SP, in endodontics. Therefore, further studies are required. Results from various *in vitro* models are inconsistent due to several reasons. Considering the discrepancy in cell types and methods to prepare the sample and evaluate the effect of MTA, it is difficult to compare the results and conclusions from different studies. Firstly, (patho-) physiological and anatomical differences between cells may lead to discrepancies in results. Secondly, experimental procedures and associated assessment criteria are distinctive so it is difficult to compare results directly and apply them to use in humans. It is essential to establish a well-defined gold standard model, experimental procedures and evaluation criteria to overcome this. Although calcium silicate-based bioceramics display excellent biocompatibility and bioactivity, the combined use of calcium silicate-based bioceramics with other materials/procedures improves efficiency of the calcium silicate-based bioceramics in endodontics. For example, the addition of TGF-β1 and VEGF to bioceramics displays better biocompatibility with human DPSCs and promotes formation of dentin bridge in rat pulp capping compared with MTA (290). Further studies are required to determine the effects of combined bioceramics.

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## Authors' contributions

WS conceived and wrote the manuscript. WS and SL contributed to data acquisition, analysis and interpretation. QT made substantial contributions to conception and design. LC and ZY critically revised the manuscript. Data sharing is not applicable. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

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## Competing interests

The authors declare they have no competing interests.

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