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Biodentine™ induces TGF-β1 release from human pulp cells and early dental pulp mineralization

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Abstract

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Aim To assess the ability of a recently developed tricalcium silicate-based cement (Biodentine™) to induce reparative dentine synthesis and to investigate its capacity to modulate pulp cells TGF-β1 secretion.

Methodology Biodentine™ was directly applied onto the dental pulp in an entire human tooth culture model. After various culture periods, the interaction of the material with dental pulp tissue was analysed on tissue sections. The effect of increasing surface area of this material on TGF-β1 secretion was investigated on pulp cell cultures and compared with that of MTA, calcium hydroxide and Xeno®III adhesive resin. After performing artificial injuries on pulp cell cultures, the materials eluates were added for 24 h and then TGF-β1 secretion was quantified by ELISA. Controls were performed by incubating intact cells with the culture medium, while injured cells TGF-β1 level was used as the baseline value.

Results Biodentine™ induced mineralized foci formation early after its application. The mineralization appeared under the form of osteodentine and expressed markers of odontoblasts. Biodentine™ significantly increased TGF-β1 secretion from pulp cells ($P < 0.03$) independently of the contact surface increase. This increase was also observed with calcium hydroxide and MTA, but not with the resinous Xeno®III. The statistical analysis showed statistically significant differences between capping materials and the resinous Xeno®III ($P < 0.001$).

Conclusions When Biodentine™ was applied directly onto the pulp, it induced an early form of reparative dentine synthesis, probably due to a modulation of pulp cell TGF-β1 secretion.

Keywords: Biodentine, odontoblast, pulp, reparative dentin, TGF-β1.

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Introduction

Maintaining pulp health following carious, traumatic or iatrogenic injuries remains a challenge and is of prime importance particularly in immature permanent teeth where pulp vitality allows completion of root formation.

Several biomaterials are used in vital pulp therapies, with the prognosis depending on several factors such as its biocompatibility and its ability to prevent bacterial microleakage; the outcome also depends on the pulp's ability to respond to injury (Camps *et al.* 2000).

Portland cements such as ProRoot®MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) have been developed and used for pulp capping. Recent data suggest that this biomaterial stimulates reparative dentine formation faster than calcium hydroxide cements (Accorinte *et al.* 2008a) and provokes less pulpal inflammation (Sawicki *et al.* 2008). The mineralized barrier is thicker with MTA than with Dycal® and shows fewer tunnel defects (Nair *et al.* 2008). The recent direct application onto the pulp of both calcium hydroxide and ProRoot®MTA in entire human tooth culture model confirmed their potential in

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inducing reparative dentine secretion (Tecles *et al.* 2008).

Based on the outstanding biological properties of Portland cements, a new calcium silicate-based cement called Biodentine™ (Septodont, Saint-Maur-des-Fosses, France) has been developed recently. The powder is mainly composed of tricalcium silicate, calcium carbonate and zirconium oxide. The liquid contains water, calcium chloride (used as a setting accelerator) and a modified polycarboxylate (a superplasticising agent). A single dose of liquid is dropped in a disposable cap containing the powder and then mixed with an amalgamator for 30 s. The cement can be applied directly in the restorative cavity with a spatula and a plugger as a bulk dentine substitute without any conditioning pre-treatment. With improved physical properties (Goldberg *et al.* 2009, Villat *et al.* 2010) and reduced setting time to 12 min (Goldberg *et al.* 2009) as compared to Portland cements, the new biomaterial can be used as dentine substitute in several clinical indications. Investigating its interactions with the pulp cells demonstrated its biocompatibility and its ability to induce odontoblast differentiation and mineralization in cultured pulp cells (Laurent *et al.* 2008). However, its capacity to promote reparative dentine remains to be demonstrated in direct pulp capping situations.

Interactions between pulp capping materials and injured pulp tissue in the initiation and the development of wound healing and regenerative processes remain unclear. Many hypotheses have been evoked, but recent studies highlighted the role of growth factors in angiogenesis, recruitment of progenitor cells, cell differentiation and finally mineralization in the pulp area beneath the biomaterial. Amongst these bioactive molecules, TGF-β1 is known to be involved as a key factor. Indeed, calcium hydroxide has been shown to solubilize bioactive molecules such as TGF-β1 from the dentine to signal reparative dentinogenesis (Graham *et al.* 2006). Similarly, TGF-β1 release was obtained from powdered human dentine with MTA (Tomson *et al.* 2007). However, these materials are often applied directly onto the pulp, and it is still unclear whether they affect the secretion of this growth factor from pulp cells.

In a previous study, an increase of growth factors secretion after physical injury to pulp cells was demonstrated (Tran-Hung *et al.* 2008). The presence of a toxic adhesive resin component (HEMA) decreased the factors secreted by injured pulp cells in culture, and this could explain their inhibitory role in the early steps of pulp wound healing and dentine regeneration (Tran-Hung *et al.* 2008).

It has been hypothesized that bioactive materials such as calcium hydroxide, MTA and Biodentine™ might locally increase TGF-β1 secretion from the injured pulp tissue; this might partially explain their stimulating effect on dentine–pulp complex regeneration.

Thus, the objectives of this study were (i) to determine whether Biodentine™ induces the reparative dentine synthesis in pulp capping situation on entire human teeth culture as reported earlier with calcium hydroxide and MTA (Tecles *et al.* 2008) and (ii) to investigate the capacity of several commonly used biomaterials including Biodentine™ to modulate TGF-β1 secretion by pulp cells.

Materials and methods

Direct pulp capping with Biodentine™ using a human entire tooth culture model

Tooth culture

The detailed procedure of this protocol has been described previously (Tecles *et al.* 2008). Briefly, 15 human immature third molars extracted for orthodontic reasons were collected in agreement with French legislation (informed patients and parents' consent and Institutional Review Board approval of the protocol used) and stored for 2 h at 4 °C in a Dulbecco's Modified Eagle medium (DMEM) (Lonza, Vervier, Belgium) supplemented with 300 UI mL^{-1} penicillin, 300 μg mL^{-1} streptomycin and 0.75 μg mL^{-1} amphotericin B (Lonza). A cavity with pulp exposure was performed on each tooth (Fig. 1a,b). Biodentine™ (Lot no. 48770) was prepared by squeezing out the liquid of a single-dose container into the powder-containing capsule. The capsule was then placed in a mixing device and mixed for 30 s at 4000 rpm. The cavity was dried with a sterile cotton pellet, and Biodentine™ was applied as a direct pulp capping material without any conditioning treatment of enamel/dentine (Fig. 1c). A sterile metallic wire was sealed on the crown with a little drop of Embrace™ photopolymerized resin (Pulpdent Company; Watertown, MA, USA). The roots of the treated teeth were suspended into DMEM supplemented with 10% foetal bovine serum (Lonza), 200 UI mL^{-1} penicillin, 200 μg mL^{-1} streptomycin and 0.5 μg mL^{-1} amphotericin B in 12-well cell culture plates (Falcon®; Becton Dickinson, Franklin Lakes, NJ, USA) (Fig. 1d). The culture medium was changed every day. The cultured teeth were incubated for 2 days (n = 5), 14 days (n = 5) or 28 days (n = 5).

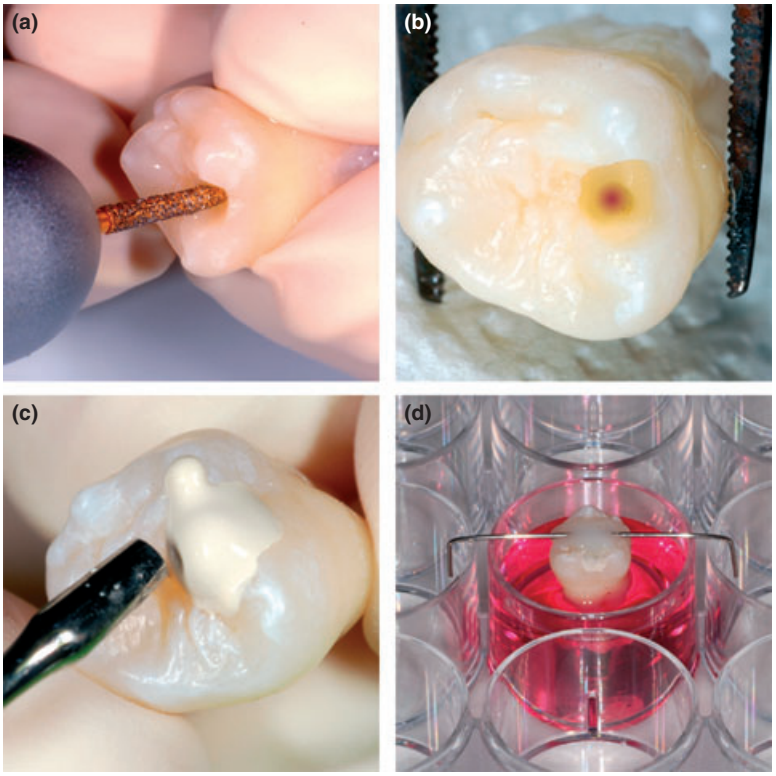

Figure 1 Direct Pulp capping with Biodentine™ on entire tooth culture. A cavity was performed *ex vivo* with a truncated diamond bur mounted on a high-speed hand-piece and under sterile saline cooling (a) until the pulp exposure was obtained (b). The cavity was gently dried and immediately restored with Biodentine™ (c). A metallic wire was used to suspend the teeth in the culture dish with their roots dipped into the culture medium (d).

Histology

At the end of each culture period, the teeth were fixed in 4% formalin solution, demineralised, paraffin embedded and routinely processed as described previously (Tecles *et al.* 2005). Five slides per tooth were stained with haematoxylin and eosin.

Immunohistochemistry

To assess pulp cell differentiation and newly formed reparative dentine, odontoblast and matrix dentine molecular markers were investigated. This was performed on rehydrated paraffin-embedded sections with antibodies against collagen I (Southern Biotechnology Associates Inc., Birmingham, AL, USA), osteonectin (Takara Shuzo Co. Ltd, Shigo, Japan), dentine sialoprotein (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and nestin (Chemicon International, Temecula, CA, USA). Primary antibodies were diluted in Dulbecco's phosphate-buffered saline (Lonza) containing 0.1% bovine serum albumin. The incubation with primary antibodies was performed overnight at 4 °C at the following concentrations: anti-collagen I at 40 μg mL^{-1}, anti-osteonectin at 10 μg mL^{-1}, anti-dentine sialoprotein at 1/200 and anti-nestin antibodies at 5 μg mL^{-1}. The staining was revealed using the labelled streptavidin–biotin kit (DakoCytomation, Carpinteria, CA, USA) according to the manufacturer's instructions. Controls were performed by omitting primary antibodies or incubations with unrelated primary antibodies (Cytokeratin 19). All controls gave negative results.

TGF-β1 secretion by pulp cells in contact with Biodentine™ eluates

Pulp cell culture

Human dental pulp cells were cultured as described previously (Tran-Hung *et al.* 2008). Briefly, immature third molars were obtained from 16-year-old adolescents in compliance with French legislation (informed patients' and parents' consent, and Institutional Review Board approval of the protocol used). After extraction, the teeth were washed and the apical

portions removed. The extirpated dental pulp was minced, and explants were cultured in 100-mm-diameter culture dishes (Falcon®; Becton Dickinson) containing DMEM supplemented with 10% foetal bovine serum, 100 UI mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 0.25 μg mL^{-1} amphotericin B.

Confluent cells from 3rd passage were collected by trypsination and subcultured in 35-mm-diameter culture dishes (Falcon®; Becton Dickinson, Plymouth, UK) for 24 h. The cell density was 30 000/cm^2.

Conditioned media

All the materials were prepared according manufacturer's instructions. White ProRoot®MTA powder was mixed for 30 s with sterile water. Biodentine™ (Lot no 48770) was prepared as described in the tooth culture section. Calcium Hydroxide XR (Dentsply, Montigny-le-Bretonneux, France) was a ready-to-use paste. All materials were then put directly in sterile calibrated silicone moulds. Four drops of each components of the self-etching adhesive Xeno®III (Dentsply De Trey GmbH, Konstanz, Germany) were mixed before placement in silicone moulds and photo-polymerized in the absence of oxygen for 1 min. Both materials were then stored in an incubator at 37 °C for 6 h to achieve complete setting. Each sample was finally removed from the moulds and sterilized using UV rays for 15 min.

Samples of set Biodentine™ were then incubated in the culture medium (DMEM supplemented with 200 UI mL^{-1} penicillin, 200 μg mL^{-1} streptomycin and 0.5 μg mL^{-1} amphotericin B) for 24 h. Increasing surface areas were used so that the contact surface between the material and the culture media volume was 0.05, 0.5, 5 and 50 mm^2 mL^{-1}. Similarly, samples of ProRoot®MTA, Calcium Hydroxyde XR and Xeno®III were also incubated in culture media under similar conditions at 0.05 mm^2 mL^{-1}.

Contact between pulp cells and conditioned medium

Injuries to pulp cells were performed with a sterile scalpel to disrupt the cell monolayer in 35-mm culture dishes. Ten straight lines per dishes were performed immediately after the culture medium removal.

- Injured cells were then incubated for 24 h with 2 mL of each conditioned medium (n = 3).
- Injured cells incubated with the unconditioned medium were used as baseline values (n = 3).
- Intact cells (without lesions) were also incubated with unconditioned medium and used as the negative (C-) control (n = 3).
- Each experiment was carried out on cultures from three different subjects.

Sandwich enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to quantify transforming growth factor-beta1 (TGF-β1) in the cell culture supernatants (Quantikine Cytokine ELISA kit; R&D system, Lille, France).

Prior to running the assays, samples were treated to activate latent TGF-β1 to the immunoreactive form. This was achieved by acid activation with 1N HCl and neutralization with 1.2N NaOH/0.5 mol L^{-1} HEPES.

A 96-well polystyrene microplate coated with a monoclonal antibody specific for TGF-β1 is provided with this kit. Standards, controls and activated samples were added to the wells and incubated for 2 h at room temperature. After washing away any unbound material, a horseradish peroxidase–conjugate polyclonal antibody to TGF-β1 was added to each well and then incubated for 2 h at room. After washing, a freshly prepared substrate solution was added to each well and then incubated for 20 min in the dark at room temperature. The colour development was stopped with a diluted hydrochloric acid solution, and the optical density of each well was determined within 30 min using a microplate reader (Metertech Inc., Taipei, Taiwan) at 450 nm.

A standard curve was generated for each experiment and was used to calculate the TGF-β1 concentration (pg mL^{-1}).

Statistical analysis

A Kruskall and Wallis test was performed to compare the effect of the presence of Biodentine™ and increasing its surface area on TGF-β1 secretion. Mann and Whitney U test was performed to compare the effect of the materials with the negative control and with each other. The confidence level was set at 95%.

Results

Direct pulp capping with Biodentine™

Haematoxylin and eosin staining revealed early and dense mineralized foci formation in the pulp connective tissue after culture for 2 days just beneath the capping material (Fig. 2a,b). These foci were small, dense and amorphous and were observed in 2/5 teeth. After 14 and 28 days, these spherical foci appeared in all teeth, increased in size and were more numerous. Small

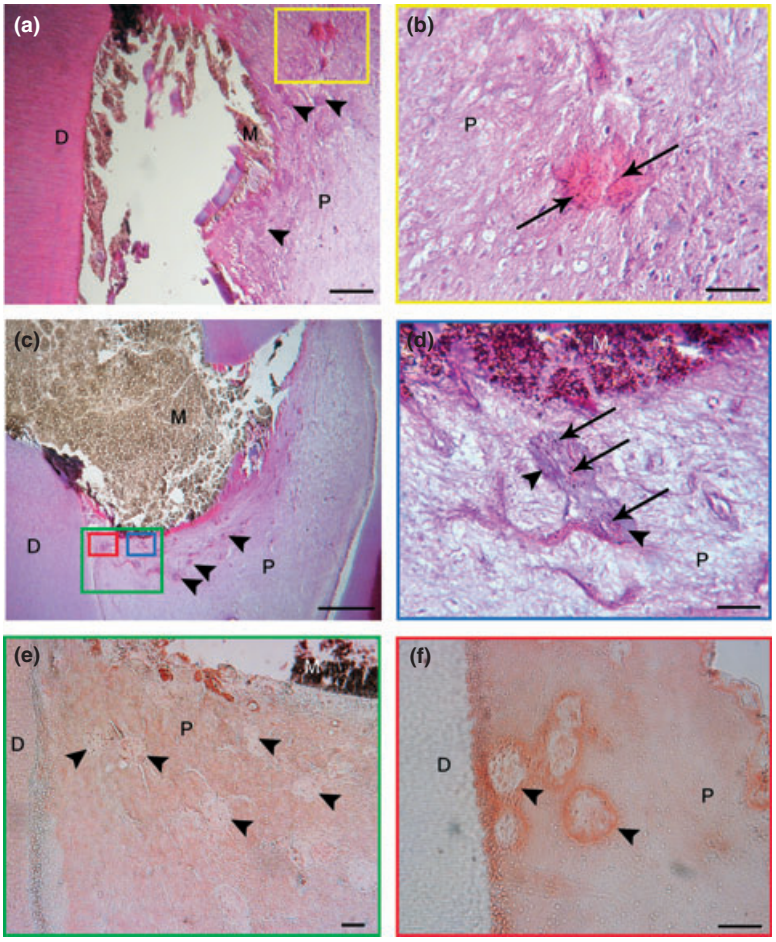

Figure 2 Pulp mineralization after capping with Biodentine™. Mineralized foci (arrowheads) appeared just beneath the material in the pulp wound area after 2 days (a,b). Their number increased after 14 days and took the appearance of osteodentine (c,d). Some particles of the material (arrows) appeared entrapped into the matrix of the foci (b,d). Collagen I (e) and osteonectin (f) are expressed in the mineralized matrix and the sequestered cells. a–d: H&E staining. D, dentine; P, pulp; M, biomaterial. Scale bars: a,e = 100 μm; b,d,f = 50 μm; c = 500 μm.

irregular cavities were observed in the foci matrix; some of them contained sequestered cells showing an osteodentine aspect (Fig. 2c,d). Interestingly, particles of cement were visible inside the foci, but not in the surrounding connective tissue (Fig. 2b,d).

Immunohistochemistry revealed expression of type I collagen (Fig. 2e) and osteonectin (Fig. 2f). Dentine sialoprotein was strongly expressed in the mineralized matrix and sequestered cells (Fig. 3a,b). An intense expression of nestin within the sequestered cells in the foci was also observed (Fig. 3c). No labelling was observed with the negative control (Fig. 3d).

TGF-β1 secretion by pulp cells

Intact pulp cells were found to secrete TGF-β1 (374 pg mL^{-1}, mean value). This secretion level was used as the negative control (C-). Artificial injuries did not modify this TGF-β1 secretion level (400 pg mL^{-1}, mean value). When compared with the negative control, Biodentine™ significantly increased TGF-β1 secretion by pulp cells ($P < 0.03$). The differences in TGF-β1 secretion level were not statistically significant (ns) amongst the surface areas used (Fig. 4a). It can be concluded that Biodentine™ increases TGF-β1 secretion by pulp cells and that the increase is not dependent of the exposed contact surface area between Biodentine™ and the injured pulp.

To compare the effect of Biodentine™ with other capping materials, the effect of conditioned media prepared under identical conditions with injured pulp cells were investigated (Fig. 4b). When compared to the negative control, both Biodentine™ and MTA significantly increased TGF-β1 secretion ($P < 0.001$) and when Biodentine™ was compared with MTA, the difference was not significant. Calcium hydroxide slightly increased TGF-β1 secretion, whilst XENO®III decreased its secretion, but these modifications were not statistically significant (ns). When TGF-β1 level with Biodentine®, MTA and calcium hydroxide was

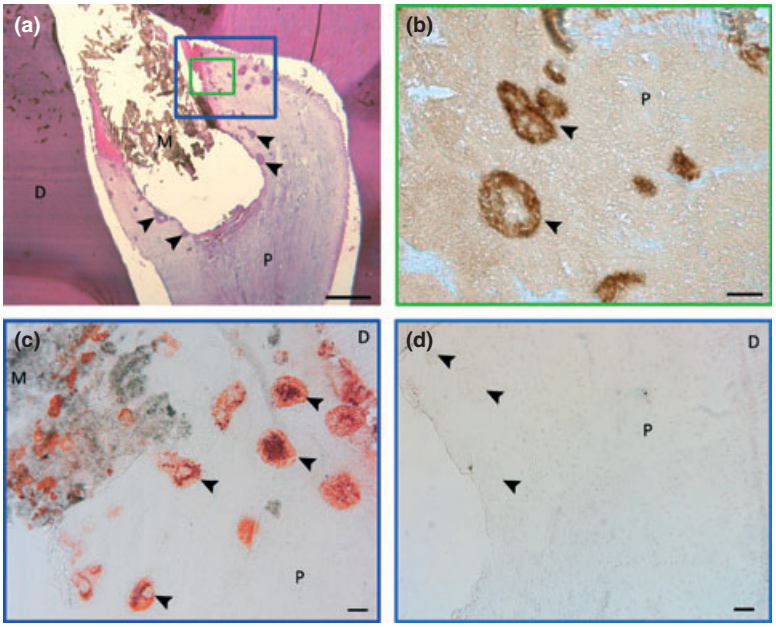

Figure 3 Expression of odontoblast markers. Numerous dense foci (arrowheads) can be viewed beneath the biomaterial after H&E staining (a). Dentine sialoprotein (b) and nestin (c) are expressed in the mineralized foci. No labelling is observed when primary antibodies were replaced with unrelated (Cytokeratin 19) ones (d). D, dentine; P, pulp; M, biomaterial. Scale bars: a = 500 μm; b,c,d = 100 μm.

compared with that with Xeno®III, the difference was statistically significant ($P < 0.001$).

Discussion

The results demonstrate that direct pulp capping with Biodentine™ using an *ex vivo* human tooth culture model gives a similar response to that observed with MTA and calcium hydroxide. All three materials induced odontoblast-like cell differentiation and mineralization, and this effect might be because of an increase of TGF-β1 secretion by pulp cells.

The mineralization obtained was observed after 2 days of culture and was numerous and of various sizes at 14 and 28 days. They appeared under the form of mineralized foci with a morphological appearance of osteodentine.

This early form of mineralization has already been observed after MTA and calcium hydroxide application using the same entire tooth culture model (Tecles *et al.* 2008). Similar mineralized foci organization were observed beneath an incomplete dentine bridge in human teeth capped with MTA after 1 month (Nair *et al.* 2008) and during the early phase of dentine bridge formation with calcium hydroxide (Schröder 1985). Mineralization foci were also observed *in vivo* after pulp capping with MTA for 2 weeks in dogs where irregular osteotypic matrix depositions were observed (Tziafas *et al.* 2002), whilst the reparative tubular dentine bridge formation with this material was obtained after 2 months in human teeth (Aeinehchi *et al.* 2003). Thus, the mineralization obtained in this work corresponds to an early step of reparative dentine synthesis reported *in vivo*. It appears after an equivalent delay and precedes tubular dentine bridge formation.

Immunohistochemistry revealed that the molecular markers of dentine were expressed in the mineralized foci matrix. Moreover, molecular markers of the odontoblasts such as type I collagen, osteonectin, dentine sialoprotein and nestin were expressed in the sequestered cells. Dentine sialoprotein and nestin expression in the sequestered cells is very significant. Dentine sialoprotein is widely expressed at 14 days. This noncollagenous protein is considered as a specific marker of dentine even if it is expressed at a lower level in bone tissue (Qin *et al.* 2002). Its role in dentine formation is essential as it initiates and regulates the dentine mineralization (Suzuki *et al.* 2009). Nestin is an intermediate filament protein that has been shown to be specific and characteristic of the secretory human odontoblast (About *et al.* 2000). Thus, the expression of these proteins in the sequestered cells strongly suggests that these cells are odontoblastic and brings a confirmation that the mineralized foci seem to correspond to a form of early reparative dentine production (Tecles *et al.* 2008). This reparative dentine synthesis is directly related to a disruption of the odontoblastic layer, and the subsequent pulp healing requires the recruitment and differentiation of pulp progenitor cells to protect the underlying pulp tissue

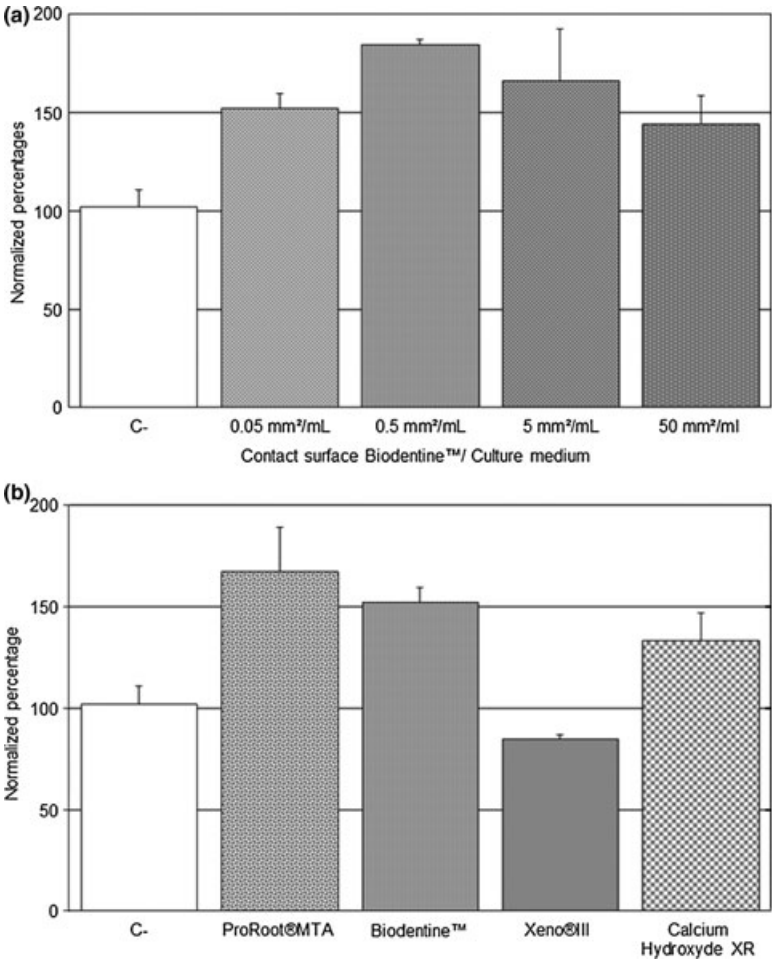

Figure 4 Effect of Biodentine™ and other biomaterials on TGF-β1 release from pulp cells. (a) Injuries to pulp cells were performed with sterile scalpels. The injured cells were cultured with conditioned media obtained after incubation with increasing Biodentine™ surfaces. After 24 h, ELISA was performed to quantify the secreted TGF-β1. A statistically significant difference in TGF-β1 level was obtained when pulp cells were incubated with all Biodentine™ eluates ($P < 0.03$). No statistical difference was observed upon increasing the Biodentine™ contact surface. (b) Comparison of Biodentine™ effect on TGF-β1 secretion with that of other biomaterials (0.05 mm^2 mL^{-1}). Biodentine™ and ProRoot®MTA significantly increased TGF-β1 secretion level as compared to the negative control ($P < 0.001$). There was a statistically significant difference between Biodentine™, MTA and calcium hydroxide when compared to Xeno®III ($P < 0.001$). All the results were normalized and expressed as percentage of TGF-β1 concentration in injured cells, but without any contact with the conditioned media.

(Fitzgerald 1979). Previous work has shown that perivascular progenitor pulp cells can be activated and migrate to the injury site after pulpal injury in human teeth (Tecles *et al.* 2005). This work provides further evidence that, in entire human tooth cultures, these cells can differentiate into odontoblast-like cells and secrete a form of reparative dentine after capping with Biodentine™. This form of mineralization was not the typical tubular one usually observed after longer delays.

This is because of the fact that the *ex vivo* model used here has some drawbacks such as a culture period limited to 1 month, absence of noxious components clearance, absence of circulation and a limited inflammatory reaction. However, it allows investigating the early steps of dentine regeneration in a whole-tooth environment. It also allows prediction of dental pulp cells behaviour after application of restorative materials, thus reducing the use of animal experiments before studies on human beings.

Surprisingly, particles of Biodentine™ were entrapped in the newly formed foci, but not in the surrounding pulp tissue. Similar particles were observed after pulp capping with calcium hydroxide and self-etching adhesive resin, but these particles were entrapped into macrophages in the vicinity of the capping materials (Kitasako *et al.* 2006). It is important to note that the particles of Biodentine™ seemed to be completely integrated in the newly formed mineralized structures, suggesting that their physicochemical properties might promote the mineralization process as shown with MTA-based cements (Gandolfi *et al.* 2010).

To further investigate the interactions between Biodentine™ and injured pulp tissue, it was hypothesized that TGF-β1 concentration could increase locally, and thus contribute to odontoblast-like cells differentiation and mineralization. The role of this growth factor has been widely studied in pathological simulation implicating reactionary dentine (Smith *et al.* 2001), but its functions and mechanism of action in direct pulp capping situation, when native odontoblast layer is destroyed, remain unclear.

The hypothesis of this study was based on the fact that it has been well demonstrated that TGF-β1 acts as a modulator of many reparative processes in various tissues. In the dental pulp, TGF-β1 promotes progenitor cell migration (Howard *et al.* 2010) and odontoblast differentiation (Begue-Kirn *et al.* 1992). Additionally, previous work has shown that dentine matrix contains sequestered TGF-β1 (Finkelman *et al.* 1990) and other bioactive molecules including angiogenic growth factors (Roberts-Clark & Smith 2000). During dentine demineralization through carious lesions or acidic etchant application, these growth factors are solubilized and can be released to the pulp tissue where they could mediate dentine regeneration processes. Additionally, pulp capping materials such as MTA (Tomson *et al.* 2007) and calcium hydroxide (Graham *et al.* 2006) have been shown to solubilize TGF-β1 from dentine. However, after surgical pulp amputation and application of capping materials directly onto the pulp, TGF-β1 release from dentine dissolution cannot explain the observed odontoblastic differentiation and mineralization in the injured pulp tissue just beneath the applied material.

With this regard, the study revealed that cultured human pulp cells secrete TGF-β1. After artificial injury, these cells continued to secrete TGF-β1 almost at the same level, indicating that the mechanical stress did not seem to affect this growth factor secretion by the surrounding cells. However, TGF-β1 secretion significantly increased after incubating the cells with Biodentine™ eluates ($P < 0.03$) whatever the surface contact between the material and the culture medium. The clinical relevance of this result is that whatever the size of the pulp exposure, its direct capping with Biodentine™ induces a significant increase of TGF-β1 secretion.

To better understand the modulatory effect of pulp capping materials on this growth factor, the effect of Biodentine™ on TGF-β1 secretion was compared with that of other direct pulp capping materials and to an adhesive resin. Interestingly, all direct pulp capping materials induced an increase in TGF-β1 secretion as compared to the baseline values. This increase was statistically significant only with Biodentine™ and ProRoot®MTA ($P < 0.01$). When the results obtained with Biodentine™, ProRoot®MTA or calcium hydroxide were compared with those obtained with Xeno®III, there was a significant difference between the three mineral-based materials and Xeno®III ($P < 0.001$). This might explain, at least in part, the successful therapeutic use of pulp capping materials such as Biodentine™, ProRoot®MTA or calcium hydroxide, and the fact that adhesive resins such as Xeno®III are not recommended for this purpose.

The decrease in TGF-β1 secretion obtained with Xeno®III is in agreement with previous long-term human clinical pulp capping studies with adhesive resins where no reparative dentine formation has been observed (Gwinnett & Tay 1998, Hebling *et al.* 1999, Pereira *et al.* 2000, de Souza Costa *et al.* 2001, Accorinte *et al.* 2008b, Fernandes *et al.* 2008). Several reasons were used to explain this failure: difficulties to obtain blood clot formation, no antibacterial activity, incomplete polymerization and cell toxicity with adhesive resin. The present study revealed another putative reason: the inhibition of TGF-β1 secretion by pulp cells subjected to these resinous materials.

The effect of TGF-β1 on pulp cells has been studied *in vitro*. The results obtained using an organ culture where dental pulp cells treated with TGF-β1 differentiated into odontoblast-like cells expressing dentine relative proteins such as dentine sialophophoprotein and dentine matrix protein-1 and formed a pulp–dentine complex whilst when the dental pulp cells were incubated with TGF-β1, they formed mineralization nodules (Nie *et al.* 2006). These observations might explain the indirect effects of ProRoot®MTA and Biodentine™ on the increase in TGF-β1 secretion and consequently odontoblast differentiation and mineralization.

Whilst the signalling processes involved in this odontoblast-like cell differentiation under these materials are still not completely elucidated, a recently developed

calcium silicate-based bone cement with similar composition to that of Biodentine™ has been investigated in simulated body fluid conditions. Scanning electron microscopy and X-ray diffraction showed that calcium silicate-based cement stimulated cell growth and induced hydroxyapatite formation on the surface of the material (Zhao *et al.* 2005). It has been suggested that alterations in calcium levels in the cellular environment might invoke odontoblast-like cells differentiation (Kardos *et al.* 1998). Accordingly, calcium is one of the principal components of Biodentine™ and may be involved in pulp cells differentiation. A recent study highlighted the effect of calcium ions released by calcium hydroxide on fibronectin production. Indeed, increasing extracellular matrix calcium levels induced pulp cells fibronectin synthesis and up-regulated mineralized tissue forming cell-specific gene expression (Mizuno & Banzai 2008). More recently, extracts of tricalcium silicate were able to induce human dental pulp stem cells proliferation, differentiation and mineralization. When compared with calcium hydroxide extracts, all these processes were enhanced by the calcium silicate-based cement. This might be because of the silicon ions released from silicate-containing cements (Peng *et al.* 2011).

Thus, whilst one may speculate that the presence of both calcium and silicon ions released by Biodentine™ and ProRoot®MTA may be involved in the early odontoblastic differentiation and the initiation of mineralization, the stimulation of TGF-β1 secretion reported in this work provides an additional argument to explain these effects.

Conclusion

This study provides a better understanding of the molecular events resulting from interactions between calcium silicate-based cements and the dental pulp tissue. When they are applied directly onto the pulp, they induce an early form of reparative dentine, which seems to be because of a modulation in TGF-β1 secretion from dental pulp cells. This modulation is also obtained with other pulp capping materials and may affect reparative dentine formation. This is of prime importance particularly in young immature teeth with deep carious lesions.

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