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Effect of Biodentine™ on the proliferation, migration and adhesion of human dental pulp stem cells

Zhirong Luo [a,1], Dongmei Li [b,1], Meetu R. Kohli [c], Qing Yu [a], Syngcuk Kim [c], Wen-xi He [a,*]

[a] Department of Operative Dentistry & Endodontics, Fourth Military Medical University, Xian, China
[b] Department of VIP Dental Care, Fourth Military Medical University, Xian, China
[c] Department of Endodontics, University of Pennsylvania, Philadelphia, USA

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ABSTRACT

Objectives: To investigate the proliferative, migratory and adhesion effect of Biodentine™, a new tricalcium silicate cement formulation, on the human dental pulp stem cells (hDPSCs).
Methods: The cell cultures of hDPSCs obtained from impacted third molars were treated with Biodentine™ extract at four different concentrations: Biodentine™ 0.02 mg/ml (BD 0.02), Biodentine™ 0.2 mg/ml (BD 0.2), Biodentine™ 2 mg/ml (BD 2) and Biodentine™ 20 mg/ml (BD 20). Human dental pulp stem cells proliferation was evaluated by MTT (3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) and BrdU (5-bromo-2′-deoxyuridine) viability analysis at different times. Migration was investigated by microphotographs of wound healing and transwell migration assays. Adhesion assay was performed as well in presence of BD 0.2, BD 2 and blank control, while qRT-PCR (quantitative real-time reverse-transcriptase polymerase chain) was used for further analysis of the mRNA expression of chemokine and adhesion molecules in hDPSCs.
Results: Biodentine™ significantly increased proliferation of stem cells at BD 0.2 and BD 2 concentrations while decreased significantly at higher concentration of BD 20. BD 0.2 concentration had a statistically significant increased migration and adhesion abilities. In addition, qRT-PCR results showed that BD 0.2 could have effect on the mRNA expression of chemokines and adhesion molecules in human dental pulp stem cells.
Conclusions: The data imply that Biodentine™ is a bioactive and biocompatible material capable of enhancing hDPSCs proliferation, migration and adhesion abilities.
Clinical significance: Biodentine™ when placed in direct contact with the pulp during pulp exposure can positively influence healing by enhancing the proliferation, migration and adhesion of human dental pulp stem cells.

1. Introduction

Pulpal healing after direct pulp exposure is a complex molecular and cellular process. The sequence of events in pulpal healing is similar to a connective tissue injury.[1] Schroder describes the first stage to involve vascular and inflammatory cell migration, proliferation and adhesion, to control and eliminate the irritant. In the second stage, repair

* *Corresponding author at*: 145 Chang-le Xi Road, Xian 710032, China. Tel.: +86 29 84776476; fax: +86 29 84776476.
E-mail address: hewenxi@fmmu.edu.cn (W.-x. He).

[1] These authors contributed equally to this work.

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occurs with migration, proliferation and adhesion of mesenchymal and dental pulp stem cells. Regeneration of dentine–pulp complex after severe injury involves dental pulp stem cell differentiation to secondary or replacement odontoblast and subsequent dentinogenesis.[1] An ideal pulp capping material should have the ability to stimulate and modulate the healing process for a complete dentine–pulp complex recovery.

Mineral trioxide aggregate (MTA) has become the material of choice for direct pulp cap in the last couple decades.[2] In-vivo and in-vitro studies show formation of dentine-bridge under exposed sites, when sealed with MTA.[2,3] MTA however has its drawbacks: long setting time, handling properties and discoloration of the remaining tooth structure. In order to overcome some of these limitations other bioactive tricalcium silicate cements have been recently introduced in the market. One such material is Biodentine™, introduced by Septodont, St. Maur des Fosses, France. It is being marketed as a bioactive dentine substitute with active biosilicate technology. The material is dispensed in powder liquid form in a single dose capsule to be triturated in an amalgamator for 30 s. The powder is mainly composed of tricalcium and dicalcium silicate, calcium carbonate and zirconium oxide. The liquid contains water, calcium chloride (used as a setting accelerator) and a modified polycarboxylate (a super-plasticizing agent).[4] Like MTA, Biodentine™ belongs to the larger group of bioactive materials called bioceramics which are essentially tricalcium silicate cement. These cements have been shown to induce dental pulp stem cell proliferation and differentiation.[5]

Dental pulp stem cells (DPSCs) play an important role in the healing process through odontoblast-like cell differentiation. DPSCs are clonogenic and are capable of self-renewal and multi-lineage differentiation.[6] The ability of the material to aid or induce this differentiation and maturation affects its bioactivity and biocompatibility. Tricalcium silicate-based cements have shown to induce DPSCs proliferation and differentiation.[5,7] Previous studies have demonstrated that many chemokines and adhesion molecules can regulate stem cells adhesion, migration, and growth.[8–15] Understanding the mechanisms that regulate adhesion and migration in hDPSCs will have important implications for the development of new therapeutic strategies in dental pulp injury. As effect of Biodentine™ has never been examined on human dental pulp stem cells, the purpose of this study was to investigate the effect of Biodentine™ on the response of hDPSCs in terms of proliferation, migration, adhesion and the involvement of different chemokines and adhesion molecules in cultured hDPSCs.

2. Materials and methods

2.1. Reagents

Biodentine™ was obtained from Septodont (St. Maur des Fosses, France), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, dimethyl sulphoxide (DMSO) were obtained from Sigma. Transwell chambers were purchased from Corning (Corning, NY, USA).

2.2. Preparation of Biodentine™ materials

The method for preparation of Biodentine™ in this study was according to Zhao X et al.[7] and Zanini M et al.[16] with minor modification. Briefly, single dose capsule of Biodentine™ was mixed as per manufacturer's instructions in an amalgamator. The set mix was ground and sterilized by dry heat. The powder was added to alpha-minimum essential medium (a-MEM; Invitrogen) to prepare four concentrations of solutions: Biodentine™ 0.02 mg/ml (BD 0.02), Biodentine™ 0.2 mg/ml (BD 0.2), Biodentine™ 2 mg/ml (BD 2) and Biodentine™ 20 mg/ml (BD 20). The solutions were vortexed until completely suspended, and then incubated for 3 days (5% CO_2 at 37 °C). Supernatant from these preparations was filtered and used. Cells were treated every other day with freshly prepared Biodentine™ solutions.

2.3. Cell cultures

All the experiments were performed with the approval of Ethics Committee of the Fourth Military Medical University (FMMU). Freshly extracted teeth were collected from human adult (18 to 25-year-old) patients after obtaining informed consent. The isolated dental pulps were cut into small pieces and digested in a solution of 3 mg/ml type I collagenase with 4 mg/ml dispase (Sigma) for 45–60 min at 37 °C. Subsequently, the solution was filtered through a 70 mm cell strainer (Becton/Dickinson, Franklin Lakes, NJ, USA). The single cell suspensions were seeded in 35 mm culture dishes and maintained in a culture media consisting of a-MEM supplemented with 15% fetal bovine serum (FBS; Hyclone), 2 mmol/L glutamine (Invitrogen), 100 units/ml penicillin G, 100 mg/mL streptomycin and 50 mg/ml ascorbic acid (Sigma) and incubated in a humidified atmosphere containing 5% CO_2 at 37 °C. The medium was changed every 3 days. The characterization of DPSCs were identified by flow cytometric analysis and multiple lineage differentiation potential as described in previous studies.[17,18] The cells between 2 and 5 passages were used in the following experiments.

2.4. Cell proliferation assay

For cell proliferation assay, hDPSCs were plated at a density of 3×10^3 cells per well and a volume of 100 μl in a 96-well plates. The cells were grown in α-MEM medium containing 10% FBS and allowed to adhere for 24 h. The samples were incubated for 24 h in media without FBS before being treated with 10% α-MEM medium containing different concentrations of Biodentine™ (BD 0.02, BD 0.2, BD 2 and BD 20). Cells cultured in 10% α-MEM medium without Biodentine™ served as a blank control. Each group contained 5 wells, and the medium was changed every 3 days. The rate of proliferation was estimated by performing MTT analysis on day 1, 3, 5 and 7, respectively. Briefly, on the day of harvest, MTT (5 mg/ml in PBS) of 20 μl was added to each well and incubated at 37 °C for 4 h. The culture medium was then replaced with DMSO of 150 μl per well and plates were shaken at room temperature for 10 min to dissolve the purple crystals. Cell number and viability were determined by measuring the absorbance of the converted dye at a wavelength of 570 nm on a multi-plate reader (BIO-TEK, Winooski, VT, USA).