Dentin Conditioning with Bioactive Molecule Releasing Nanoparticle System Enhances Adherence, Viability, and Differentiation of Stem Cells from Apical Papilla

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Abstract

Introduction: Temporal-controlled bioactive molecule (BM) releasing systems allow the delivery of appropriate concentration of BM to enhance the interaction of stem cells to dentin matrix and subsequent odontogenic differentiation in regenerative endodontics. **Objectives:** The goal of this study was to evaluate the effect of dentin conditioning with 2 variants of dexamethasone (Dex) releasing chitosan nanoparticles (CSnp), (1) Dex-CSnpI (slow releasing) and (2) Dex-CSnpII (rapid releasing), on adherence, viability, and differentiation of stem cells from apical papilla (SCAP) on root dentin exposed to endodontic irrigants. **Methods:** Slab-shaped dentin specimens were prepared parallel to the root canal and treated with 5.25% sodium hypochlorite (NaOCl) for 10 minutes and/or 17% EDTA for 2 minutes. Dentin was then conditioned accordingly by (1) no nanoparticle treatment, (2) CSnp, (3) Dex-CSnpI, and (4) Dex-CSnpII. The effect of nanoparticle conditioning on SCAP viability was determined by cell count and a circularity index. SCAP adherence and viability on dentin were assessed by fluorescence and scanning electron microscopy and odontogenic differentiation by immunofluorescence. **Results:** SCAP on dentin treated with NaOCl alone or NaOCl as the last irrigant showed the least adherence, minimal cytoplasmic extensions, and higher circularity. SCAP adherence and viability on Dex-CSnpI and Dex-CSnpII conditioned dentin were increased and had a well-developed cytoplasmic matrix and significantly lower circularity ($P < .05$). SCAP cultured in Dex-CSnpII group expressed higher levels for DSPP and DMP-1 than in CSnp or Dex-CSnpI groups. **Conclusions:** Dex-CSnpI and Dex-CSnpII conditioning of dentin enhanced SCAP adherence and viability. Temporal-controlled release of Dex from Dex-CSnpII enhanced odontogenic differentiation of SCAP. This study highlighted the ability of dentin conditioning with temporal-controlled BM releasing nanoparticles to improve the local environment in regenerative endodontics. (*J Endod 2016;42:717–723*)

Key Words

Cell adherence, chitosan nanoparticles, dentin, dexamethasone, odontogenic differentiation, regenerative endodontic procedures, stem cells from apical papilla

Regenerative endodontic procedures are biologically based procedures designed to replace damaged, diseased, or missing portions of the pulp-dentin complex (1). Although disinfection of root canal is an essential prerequisite for successful regeneration of tissue (2), many of the agents used for this purpose are cytotoxic and potentially damaging to the dentin. This, in turn, can alter the bioactivity of dentin matrix and compromise the survival, adherence, proliferation, and odontogenic differentiation of the stem cells (3). Conversely, treatment of the dentin with ethylenediaminetetraacetic acid (EDTA) appears to improve stem cell adherence and survival, but when the root canal is irrigated with sodium hypochlorite (NaOCl) after irrigation with EDTA, its positive effect is negated and dentin erosion accelerated (4). This further compromises the interaction between the dentin and stem cells and impedes neotissue integration.

Chitosan is a cationic natural biocompatible polymer with broad-spectrum antibacterial properties and excellent biodegradable characteristics when used in regenerative endodontic procedures (5). Its molecular structure is similar to extracellular matrix component and contains numerous free hydroxyl and amino groups that allow it to be easily modified when necessary (5). The incorporation of chitosan nanoparticles (CSnp) with dentin matrix appears to enhance the mechanical properties and its resistance to bacterial enzymatic degradation (6). CSnp possesses favorable physicochemical characteristics such as a nanoscale size, a large surface area/mass ratio, and an increased chemical reactivity, which makes it useful in the local delivery of bioactive molecules (BMs) in regenerative procedures (7). Temporal-controlled release of bovine serum albumin from CSnp, for example, has been shown to increase the viability and the alkaline phosphatase activity of stem cells from apical papilla (SCAP) (8). More recently, temporal-controlled release of dexamethasone (Dex) from CSnp enhanced the odontogenic differentiation of SCAP *in vitro*. Furthermore, the rapid releasing variant of CSnp, Dex-CSnpII, has been shown to be more effective in increasing biomineralization and expression of odontogenic markers in cultured SCAP than a slow-releasing variant Dex-CSnpI (9).

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.01.026

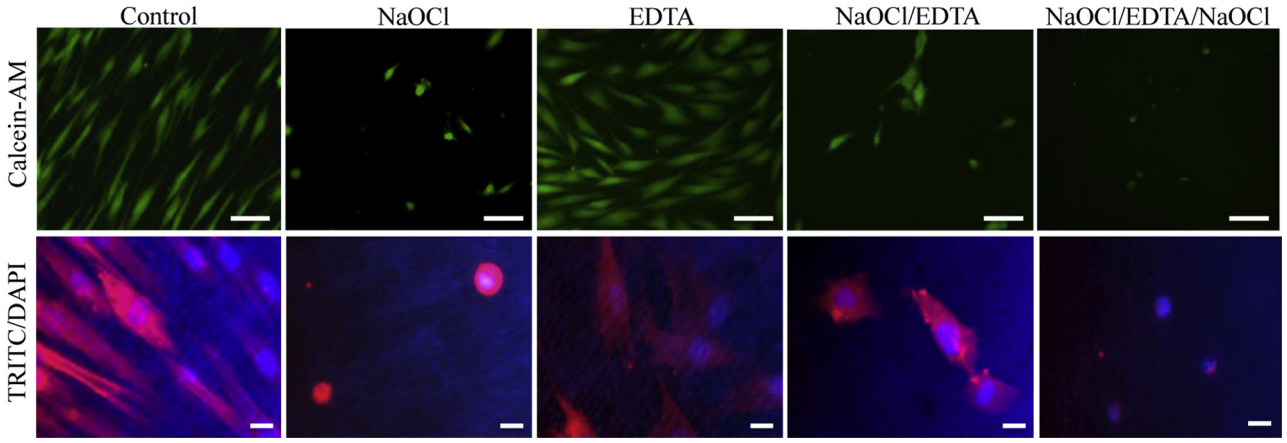

Figure 1. Representative images of SCAP adherence on chemically untreated/treated dentin specimens. SCAP were cultured on dentin specimens for 24 hours and stained with calcein-AM (bar: 100 μm) and phalloidin-TRITC/DAPI (bar: 20 μm). Few cells with cytoplasmic extensions were present in NaOCl/EDTA group. Significantly less numbers of cells with rounded morphology and devoid of cytoplasmic extensions were observed in NaOCl and NaOCl/EDTA/NaOCl groups.

Strategies to improve the success of tissue engineering should allow for (1) favorable interaction between stem cells and dentin matrix and (2) availability of BMs at an optimal time and concentration. It has been hypothesized that dentin conditioned with a temporal-controlled Dex releasing system can neutralize the detrimental effect of some root canal irrigants on dentin and provide a bioactive extracellular matrix that promotes SCAP adherence, viability, and differentiation. The purpose of this study was to evaluate the effects of a slow Dex releasing nanoparticle system (Dex-CSnpI) and a rapid Dex releasing nanoparticle system (Dex-CSnpII) in promoting SCAP adherence, viability, and odontogenic potential of SCAP seeded on dentin exposed to endodontic irrigants.

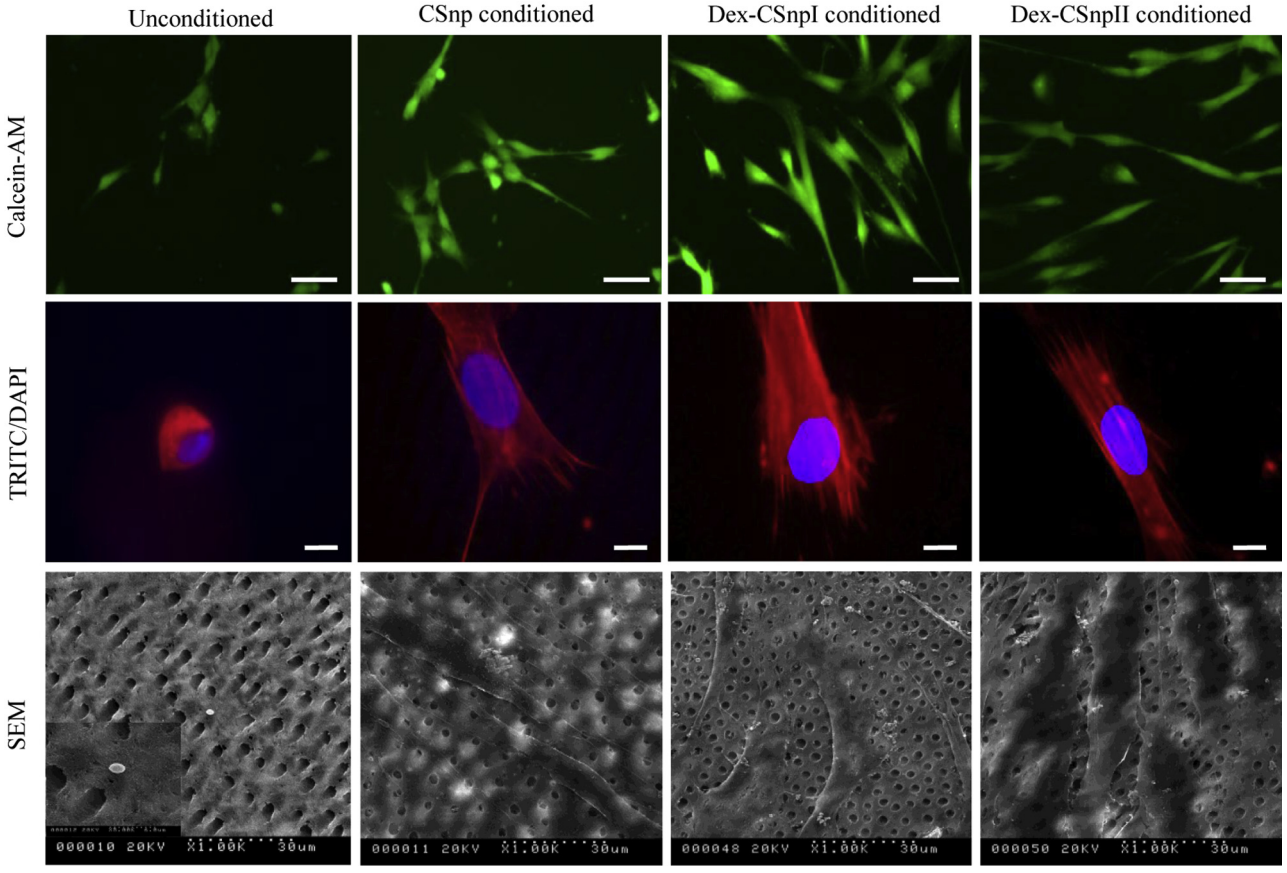

Figure 2. Representative images of SCAP adherence on NaOCl/EDTA-treated dentin specimens without/with nanoparticle conditioning. SCAP were cultured on dentin specimens for 24 hours and stained with calcein-AM (bar: 100 μm) and phalloidin-TRITC/DAPI (bar: 10 μm). SCAP ultrastructure was also observed under scanning electron microscope (SEM). Cell adherence and morphology were improved by nanoparticle conditioning on dentin specimens. Unconditioned: NaOCl/EDTA-treated specimens.