



University of Pennsylvania
ScholarlyCommons

Departmental Papers (Dental)

Penn Dental Medicine

9-4-2009

Dynamic Hydrostatic Pressure Promotes Differentiation of Human Dental Pulp Stem Cells

V. Yu

University of Pennsylvania

M. Damek-Poprawa

University of Pennsylvania

S.B. Nicoll

University of Pennsylvania

Sunday O. Akintoye

University of Pennsylvania

Follow this and additional works at: https://repository.upenn.edu/dental_papers

 Part of the [Dentistry Commons](#)

Recommended Citation

Yu, V., Damek-Poprawa, M., Nicoll, S., & Akintoye, S. O. (2009). Dynamic Hydrostatic Pressure Promotes Differentiation of Human Dental Pulp Stem Cells. *Biochemical and Biophysical Research Communications*, 386 (4), 645-649. <http://dx.doi.org/10.1016/j.bbrc.2009.06.106>

This paper is posted at ScholarlyCommons. https://repository.upenn.edu/dental_papers/85
For more information, please contact repository@pobox.upenn.edu.

Dynamic Hydrostatic Pressure Promotes Differentiation of Human Dental Pulp Stem Cells

Abstract

The masticatory apparatus absorbs high occlusal forces, but uncontrolled parafunctional or orthodontic forces damage periodontal ligament (PDL), cause pulpal calcification, pulp necrosis and tooth loss. Morphology and functional differentiation of connective tissue cells can be controlled by mechanical stimuli but effects of uncontrolled forces on intra-pulpal homeostasis and ability of dental pulp stem cells (DPSCs) to withstand direct external forces are unclear. Using dynamic hydrostatic pressure (HSP), we tested the hypothesis that direct HSP disrupts DPSC survival and odontogenic differentiation. DPSCs from four teenage patients were subjected to HSP followed by assessment of cell adhesion, survival and recovery capacity based on odontogenic differentiation, mineralization and responsiveness to bone morphogenetic protein-2 (BMP-2). HSP down-regulated DPSC adhesion and survival but promoted differentiation by increasing mineralization, *in vivo* hard tissue regeneration and BMP-2 responsiveness despite reduced cell numbers. HSP-treated DPSCs displayed enhanced odontogenic differentiation, an indication of favorable recovery from HSP-induced cellular stress.

Keywords

Dental pulp stem cells; hydrostatic pressure; pulp calcification; tooth; cellular stress

Disciplines

Dentistry

Published in final edited form as:

Biochem Biophys Res Commun. 2009 September 4; 386(4): 661–665. doi:10.1016/j.bbrc.2009.06.106.

Dynamic Hydrostatic Pressure Promotes Differentiation of Human Dental Pulp Stem Cells

V Yu^{1,§}, M. Damek-Poprawa², S. B. Nicoll³, and S.O. Akintoye^{2,*}

¹Department of Orthodontics, School of Dental Medicine, University of Pennsylvania, Philadelphia PA

²Department of Oral Medicine, School of Dental Medicine, University of Pennsylvania, Philadelphia PA

³Department of Bioengineering, School of Engineering, University of Pennsylvania, Philadelphia PA

Abstract

The masticatory apparatus absorbs high occlusal forces, but uncontrolled parafunctional or orthodontic forces damage periodontal ligament (PDL), cause pulpal calcification, pulp necrosis and tooth loss. Morphology and functional differentiation of connective tissue cells can be controlled by mechanical stimuli but effects of uncontrolled forces on intra-pulpal homeostasis and ability of dental pulp stem cells (DPSCs) to withstand direct external forces are unclear. Using dynamic hydrostatic pressure (HSP), we tested the hypothesis that direct HSP disrupts DPSC survival and odontogenic differentiation. DPSCs from four teenage patients were subjected to HSP followed by assessment of cell adhesion, survival and recovery capacity based on odontogenic differentiation, mineralization and responsiveness to bone morphogenetic protein-2 (BMP-2). HSP down-regulated DPSC adhesion and survival but promoted differentiation by increasing mineralization, *in vivo* hard tissue regeneration and BMP-2 responsiveness despite reduced cell numbers. HSP-treated DPSCs displayed enhanced odontogenic differentiation, an indication of favorable recovery from HSP-induced cellular stress.

Keywords

Dental pulp stem cells; hydrostatic pressure; pulp calcification; tooth; cellular stress

Introduction

The human masticatory apparatus absorbs high occlusal forces under physiological conditions. However, chronic parafunctional forces from clenching and bruxism cause pulpal inflammation, elevated intra-pulpal pressure and deposition of calcified nodules [1;2;3]. Clinically, uncontrolled orthodontic forces cause pain, root resorption, tooth mobility and pulp

© 2009 Elsevier Inc. All rights reserved.

*Corresponding Author, Please address all correspondence to: Sunday O. Akintoye BDS,DDS,MS, University of Pennsylvania, School of Dental Medicine, Department of Oral Medicine, The Robert Schattner Center Room 209, 240 South 40th Street, Philadelphia PA 19104, Phone: 215-898-9932, Fax: 215-573-7853, E-mail: akintoye@dental.upenn.edu

§This work was based on a thesis presented in partial fulfillment of the Masters of Science in Oral Biology, School of Dental Medicine, University of Pennsylvania.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

devitalization [4;5;6;7]. While orthodontic forces cause mechanical stimulation of periodontal ligament (PDL) cells, the impact on intra-pulpal cellular homeostasis is often overlooked [6; 8]. Mechanical stimulation alters morphology and differentiation of connective tissue cells. Specifically, hydrostatic pressure (HSP) alters cellular cytoskeleton and metabolic activity in osteoblast-like cells and chondrocytes [9;10;11;12;13]. Likewise, trauma-induced dentin formation and pulp calcification occur in response to chronic rise in intra-pulpal pressure [14]. It is conceivable that increased intra-pulpal pressure disrupts activity of dental pulp stem cells (DPSCs), a unique population of post-natal stem cells within the pulp [15;16]. DPSCs maintain pulp homeostasis by forming new dentin and other tooth components to protect the pulp [17;18;19], but their ability to withstand external forces are still unclear. Also, cellular mechanisms underlying trauma-associated intra-pulpal calcification, specifically the role of DPSC differentiation and their response to sustained intra-pulpal pressure have not been fully elucidated. This study tested the hypothesis that direct HSP disrupts DPSC survival and odontogenic differentiation and evaluated responsiveness of HSP-treated DPSCs to stimulatory effects of BMP-2, a growth factor associated with development of functional odontoblasts and deposition of mineralized hard tissue matrix [20]. We showed that HSP down-regulated DPSC survival, and promoted odontogenic differentiation, early *in vitro* mineralization, *in vivo* hard tissue regeneration and BMP-2 responsiveness. These effects may be associated with pathogenesis of heterotopic pulp calcification in response to sustained intra-pulpal pressure.

Materials and Methods

Isolation of Dental Pulp Stem Cells

After written informed consent, four patients were enrolled in a protocol approved by University of Pennsylvania Office of Regulatory Affairs. Healthy premolars, without history of trauma, caries or restorations were collected after routine dental extractions performed prior to orthodontic treatment. DPSCs were isolated and expanded in culture as previously described [16;21]. Briefly, premolars were cleaned with dental scaler to remove attached soft tissue before splitting the teeth at the cemento-enamel junction with a sterile dental drill to remove pulp tissues. The pulp tissues were digested with 3 mg/ml collagenase type I (Worthington Biochemical, Lakewood, NJ) and 4 mg/ml dispase II (Roche Applied Science, Indianapolis IN) for 1 hour at 37 °C. Single cell suspensions obtained by passing the cells through a 70- μ m strainer were expanded in culture using growth medium consisting of α -modified minimum essential medium α -MEM, GIBCO® Invitrogen, Carlsbad, CA), supplemented with 20% fetal bovine serum (FBS) (Equitech Bio, Kerville, TX), 100 U/ml penicillin, 100 mg/ml streptomycin sulfate and 2 mM glutamine maintained at 37°C in the presence of 5% CO₂). At 70% confluence, primary DPSCs were either sub-cultured or stored in liquid nitrogen. Passage 1 or 2 cells were used for all experiments.

Application of Hydrostatic Pressure

A dynamic hydrostatic pressure (HSP) device was used to simulate increased intra-pulpal pressure [22]. The peak pressure and frequency of pressurization was regulated by feedback control using a custom software program (Lab View 6.1). In all experiments, a frequency of 0.5 Hz (5 cycles /10 sec) was used while the duration and magnitude were varied based on experimental design. Circular 22 mm-diameter glass coverslips (Fisher Scientific, Pittsburgh PA) were coated with poly-L-lysine and seeded with 8.3×10^4 DPSCs for 24 hours before testing with HSP. Seeded glass coverslips were placed in custom-made sterile stainless steel holders and heat-sealed in sterile plastic pouches containing 10 ml α -MEM growth medium devoid of air bubbles. The sealed plastic bags were placed in water-filled pressure chamber prior to application of HSP. Controls consisted of cells seeded on glass coverslips that were

sealed in plastic bags and maintained under identical conditions as experimental groups, but were not subjected to HSP. All experiments were performed at 37°C.

Refinement of Hydrostatic Pressure Parameters

To determine effective HSP (P_{eff}) and exposure time (t_{eff}) necessary to induce morphological changes and cell viability, we seeded early passage (passage 1 or 2) DPSCs on separate glass coverslips and subjected them to different magnitudes of HSP from 0 (control) to 1.5, 2.5 and 3.0 MPa at 0.5 Hz of HSP for 1, 2, 3 or 4 hours based on an earlier study on osteocytes [23]. Cell attachment and morphological changes were evaluated microscopically under each experimental parameter to determine optimum time and pressure for subsequent experiments.

In separate experiments, DPSCs were recovered immediately after HSP application by trypsinization and sub-cultured at 5×10^3 cells/well in a 96-well plate. Control untreated DPSCs were similarly sub-cultured in parallel experiments. Proliferating cells were assessed 12 and 24 hours post-HSP using WST-1 cell viability assay (Roche Applied Science, Indianapolis IN) following manufacturer's protocol and the absorbance measured at 450 nm with a Multiskan MCC microplate reader (Thermo Fisher Scientific, Waltham, MA). Cell viability was used to determine magnitude-dependent cell survival and selection of an effective HSP (P_{eff}) for differentiation experiments.

Hydrostatic Pressure Modulation of Dental Pulp Stem Cell Differentiation

DPSCs (8.3×10^4) seeded on triplicate coverslips were subjected to 2.5 MPa of HSP for 2 hours at 0.5 Hz. Growth medium was changed 24 hours after HSP application to differentiation medium of α -MEM, 20% fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin sulfate and 2 mM glutamine, and 10^{-4} M L-ascorbic acid-2-phosphate (GIBCO® Invitrogen, Carlsbad, CA) for additional 48 hours. Protein lysates of HSP-treated and control cells were collected using M-PER® protein extraction reagent (Pierce Biotechnology, Rockford, IL). Protein concentration was quantified using the BCA protein assay (Pierce Biotechnology, Rockford, IL) with bovine serum albumin as a standard. 15 μ g protein per sample was separated on 4-12% Bis-Tris gel (Invitrogen, Carlsbad, CA) under reducing conditions, transferred onto nitrocellulose membrane for Western blot immunoreactivity. Blots were probed with 1:1000 dilution of mouse monoclonal antibody to dentin matrix protein 1 (LFMb-31; anti-DMP-1; a gift from Dr. Larry Fisher, National Institute of Health, Bethesda MD) [24] followed by incubation with horseradish peroxidase-conjugated sheep anti-mouse secondary antibody (GE Healthcare, UK). Blots were stripped and re-probed with 1:1000 dilution of mouse monoclonal antibody to α -tubulin (12G10, Abcam, Cambridge, MA) serving as loading control. Blots were analyzed digitally with Kodak Image Station 4000MM (Molecular Imaging Systems, Carestream Health, Rochester, NY) and results were normalized to α -tubulin. In similar experiments, total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) following manufacturer's recommendations. Primer express software (Applied Biosystems, Foster City, CA) and GenBank RNA sequences were used to design primer sets for dentin sialophosphoprotein (DSPP) - forward, 5'-TCACCAAGTGAAGGAAGTGG-3', reverse, 5'-AAAGCCCAAGGTGGATTTTT-3'; intercellular adhesion marker-1 (ICAM-1) - forward, 5'-GGAGGTCACCCGCAAGGT-3', reverse, 5'-CACAGTGATGATGACAATCTCATAC-3'; vascular cell adhesion marker-1 (VCAM-1) - forward, 5'-GAAGGAATTAACCAGGCTGGAA-3', reverse, 5'-AGTTTTATGTCTTTTGGAGTAACTTGGAT-3' and TATA binding protein (TBP) - forward, 5'-GGAGCTGTGATGTGAAGTTTCCTA-3', reverse, 5'-CCAGGAAATAACTCTGGCTCATAAC-3'. cDNA was prepared from 2 μ g mRNA using oligo(dT) and the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Real-time PCR was performed with an ABI 7300 Real-Time PCR System using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) with TATA-

binding protein (TBP) as endogenous control. A negative PCR control without template was included in each assay. Gene expression of assessed markers normalized to TBP was presented as relative fold change using the $\Delta\Delta C_t$ method (Applied Biosystems, Foster City, CA) and was used to quantify changes in mRNA levels in response to HSP.

Calcium Accumulation by Hydrostatic Pressure-Treated Dental Pulp Stem Cells

DPSCs subjected to 2.5 MPa of HSP for 2 hours at 0.5 Hz were kept in culture until confluent before changing to mineralization medium consisting of α -MEM supplemented with 2.5% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin sulfate, 2 mM glutamine, 10^{-4} M L-acid-2-phosphate, 50 mM β -glycerol phosphate. The medium was replenished at 3-day intervals for 2 weeks. Cells were fixed with 70% ethanol, and stained with 1% Alizarin Red-S monohydrate (Sigma Aldrich, St. Louis, MO) dissolved in 2% ethanol to assess calcium deposition [25].

Bone Morphogenetic Protein-2 -Induced Differentiation of Hydrostatic Pressure-Treated Dental Pulp Stem Cells

DPSCs seeded in triplicates (0.5×10^6 cells/well) on poly-L-lysine -coated circular 22 mm-diameter glass coverslips (Fisher Scientific, Pittsburgh PA) were kept overnight before application of 2.5 MPa of HSP for 2 hours at 0.5 Hz. Control cells were not pressurized. Twenty four hours post-HSP, growth medium was changed to serum-free medium of α -MEM, 1 % ITS Universal Culture Supplement Premix (BD Biosciences, San Jose, CA), 10^{-4} M L-ascorbic acid-2-phosphate (Wako Pure Chemical Industries, Ltd., Japan) and either 1) no BMP-2, 2) 100 ng/ml rhBMP-2 (recombinant human bone morphogenetic protein, BioVision, Mountain View, CA) or 3) 100 ng/ml rhBMP-2 + 500 ng/ml noggin (BioVision, Mountain View, CA) for 48 hours. Odontogenic differentiation and BMP-2 responsiveness of HSP-treated DPSCs were assessed by real time PCR analysis of DSPP and DMP-1 as above.

In vivo Hard Tissue Regeneration by Hydrostatic Pressure-Treated Dental Pulp Stem Cells

DPSCs subjected to 2.5 MPa of HSP for 2 hours at 0.5 Hz were allowed to recover for 24 hours before further *ex vivo* expansion. *In vivo* differentiation of HSP-treated DPSCs was performed under a University of Pennsylvania-approved animal protocol following as previously described [25]. Briefly, 2×10^6 HSP-treated and control untreated DPSCs were seeded on 40 mg of spheroidal hydroxyapatite/tricalcium phosphate (HA/TCP, particle size 0.5-1.0 mm, Zimmer, Warsaw, IN) before transplantation into separate subcutaneous pockets of 4-week-old immunocompromised nude female mice (NIH-III-nu, Charles River Laboratories, Wilmington, MA). Transplants were harvested at 6 weeks, fixed in 4% paraformaldehyde in PBS (pH 7.4), decalcified in 10% EDTA/PBS (pH 8.0) and embedded in paraffin. 5- μ m sections were stained with hematoxylin/eosin for histological evaluation.

Statistical Analysis

Results from triplicate experiments from four patients were expressed as mean \pm standard deviation. Data analysis was by two-way analysis of variance (ANOVA). A Mann-Whitney Rank Sum Test was used to compare HSP-treated and control DPSCs. Donor variability was assessed using Kruskal-Wallis ANOVA on Ranks and statistical significance was set at $p < 0.05$.

Results

DPSCs from premolars of four 12-18 year-old patients were tested to minimize age variability (Table 1). HSP-treated DPSCs changed from spindle to round morphology and progressively detached from glass slips following dose and time-dependent patterns (Figures 1A-D). Representative images 2 hours after HSP application showed marked morphological changes

and decreased DPSCs attachment when HSP was greater than 1.5 MPa (Figures 1A-D), and exposure times longer than 2 hours (data not shown). Therefore, effective experimental parameters were set at 2.5 MPa HSP applied for 2 hours (P_{eff} and t_{eff} respectively). Although not statistically significant, HSP decreased DPSC viability within 12 hours post-HSP, but viability moderately improved within 24 hours of re-introduction to regular culture conditions (Figure 1E). This time-dependent recovery was observable at 2.5 and 3.0 MPa but absent at 1.5 MPa, the HSP at which the cells exhibited least observable changes in morphology and attachment (Figure 1B). Apparently, DPSCs recovered within 24 hours from HSP-induced stress and resumed proliferation (Figures 1E and F). However, when DPSCs expanded to the periphery of glass coverslips, control cells maintained proliferation (Figure 1F) while HSP-treated cells partially detached (Figure 1G) as demonstrated by reduction in the number of cells able to cross and reattach beyond the glass-plastic interface. Real time PCR analyses supported HSP-induced decrease in DPSC attachment based on moderate reduction of gene transcripts of one of two adhesion markers, ICAM-1 and VCAM-1 observed in 3 of 4 patients (Figure 1H). While DPSC viability and attachment were diminished by HSP, their moderate recovery was sufficient to promote rapid *in vitro* calcium accumulation as early as 2 weeks post-HSP while untreated DPSCs were still unresponsive (Figure 2A-C). However, compared with control cells, western blot assessment of odontogenic differentiation did not show any changes in the expression levels of two odontogenic markers, DSPP and DMP-1 (Figure 2D and E). Interestingly, HSP-treated DPSCs were responsive to the stimulatory effects of rhBMP-2 based on statistically significant increase in the expression of DSPP, a more specific odontoblast marker ($p = 0.009$) (Figure 3F), while DMP-1 was unaffected (data not shown). This synergistic HSP-rhBMP-2 effect on DPSCs was attenuated to baseline levels by noggin, a known BMP antagonist (Figure 3F). Within 6 weeks of transplantation into mice, HSP-treated DPSCs regenerated appreciable *in vivo* hard tissues within the bed of HA/TCP than control cells (Figure 3 A-D). Unlike controls, newly regenerated hard tissues displayed variable cellularity similar to cementoid or osteoid tissues (Figure 3B-D).

Discussion

DPSCs were isolated from a semi-homogeneous group within the age range of many orthodontic patients [26;27]. They represented an appropriate study population to minimize confounding effects of physiological age changes. Testing DPSCs from four patients was in accordance with earlier studies that showed sample size of 4 was adequate to demonstrate statistically significant cellular response in mesenchymal stem cells from a semi-homogeneous group [25;28].

In orthodontics, the piezoelectric theory of tooth movement indicates that force is transferred from PDL to bone [6;29], while the alternative pressure-tension theory suggests tooth movement is related to altered osmotic pressure, and that release of chemical messengers activates different cells within the pulp and periodontal ligament [6;29;30]. Based on pressure-tension theory, we simulated increased intra-pulpal pressure with HSP after establishing effective pressure. 2.5 MPa HSP caused cellular stress that presented as altered cell morphology, loss of attachment and moderately reduced gene expression of one or two adhesion markers, ICAM-1 and VCAM-1 in 3 of 4 patients. It is possible that similar physiological increases in intra-pulpal pressure induce DPSCs within the pulp to trigger cellular and molecular survival mechanisms that could protect the pulp and overall tooth. Interestingly, increased mineralization may be a protective mechanism initiated by DPSCs since there was early *in vitro* calcium accumulation in HSP-treated cells relative to control DPSCs that usually display mineralization after 6 weeks [21].

Ability to promote hard tissue mineralization was further supported by *in vivo* experiments because HSP-treated DPSCs formed histologically variable hard tissues that resembled

cementoid or osteoid tissues. These DPSC responses may relate to higher occurrence of pulp calcifications in patients with chronic parafunctional habits such as teeth grinding and clenching. Disrupted pulp homeostasis can progress to root resorption detectable early by altered levels of DMP-1, DSP (dentin sialoprotein) and DPP (dentin phosphoprotein) in gingival crevicular fluids [17]. Since BMPs are associated with hard tissue formation, higher odontogenic differentiation and DSPP level by BMP-2 responsive HSP-treated DPSCs may favor pulpal deposition of calcified materials that normally occur in response to external forces, dental caries and pulpal inflammation [31;32]. This BMP-mediated response suggests enhanced differentiation capacity of surviving HSP-treated DPSCs and possible role of BMP signaling in sustaining pulp vitality despite increased mineral deposition. Also, the increased DSPP expression by pressurized DPSCs treated with BMP-2 support recent evidence of synergistic effects of mechanical stimulation and growth factor supplementation on cell differentiation and matrix production [33;34].

The limited number of DPSC samples may account for the individual variability in the expression levels of adhesion markers tested. However, testing DPSCs from more patients in future studies will illuminate the role of intra- and inter-cellular adhesion and other cellular pathways associated with DPSC survival of HSP-induced cellular stress. Taken together, our data demonstrate that while HSP disrupted DPSC survival, it promoted odontogenic differentiation, early *in vitro* mineralization, enhanced *in vivo* hard tissue regeneration and responsiveness to BMP-2 stimulation. The potential of DPSCs to promote mineralization and hard tissue formation in response to HSP supports formation of heterotopic pulp calcification in response to parafunctional masticatory forces.

References

- [1]. Burke FM, Samarawickrama DY. Progressive changes in the pulpo-dentinal complex and their clinical consequences. *Gerodontology* 1995;12:57–66. [PubMed: 9084291]
- [2]. Neville, BW.; Damm, DD.; Allen, CM.; Bouquot, JE. Pulpal and Periapical Disease, Oral and maxillofacial pathology. WB Saunders; Philadelphia, Pennsylvania: 2002. p. 107-136.107
- [3]. White, SC.; Pharoah, MJ. Oral radiology: principles and interpretation. Mosby; St. Louis, Mo.: 2000.
- [4]. Brezniak N, Wasserstein A. Orthodontically induced inflammatory root resorption. Part I: The basic science aspects. *The Angle Orthodontist* 2002;72:175–179. [PubMed: 11999941]
- [5]. Brezniak N, Wasserstein A. Orthodontically induced inflammatory root resorption. Part II: The clinical aspects. *The Angle Orthodontist* 2002;72:180–184. [PubMed: 11999942]
- [6]. Proffit, WR.; Fields, HW. Contemporary orthodontics. Mosby; St. Louis: 2000.
- [7]. Quinn RS, Yoshikawa DK. A reassessment of force magnitude in orthodontics. *American Journal of Orthodontics* 1985;88:252–260. [PubMed: 3862348]
- [8]. Khouw FE, Goldhaber P. Changes in vasculature of the periodontium associated with tooth movement in the rhesus monkey and dog. *Archives of Oral Biology* 1970;15:1125–1132. [PubMed: 4995399]
- [9]. Wang QG, Magnay JL, Nguyen B, Thomas CR, Zhang Z, El Haj AJ, Kuiper NJ. Gene expression profiles of dynamically compressed single chondrocytes and chondrons. *Biochem Biophys Res Commun* 2009;379:738–42. [PubMed: 19118531]
- [10]. Liu J, Zou L, Wang J, Schuler C, Zhao Z, Li X, Zhang J, Liu Y. Hydrostatic pressure promotes Wnt10b and Wnt4 expression dependent and independent on ERK signaling in early-osteoinduced MSCs. *Biochem Biophys Res Commun* 2009;379:505–9. [PubMed: 19118527]
- [11]. Thorpe SD, Buckley CT, Vinardell T, O'Brien FJ, Campbell VA, Kelly DJ. Dynamic compression can inhibit chondrogenesis of mesenchymal stem cells. *Biochem Biophys Res Commun* 2008;377:458–62. [PubMed: 18851955]
- [12]. Parkkinen JJ, Lammi MJ, Inkinen R, Jortikka M, Tammi M, Virtanen I, Helminen HJ. Influence of short-term hydrostatic pressure on organization of stress fibers in cultured chondrocytes. *J Orthop Res* 1995;13:495–502. [PubMed: 7545746]

- [13]. Smith RL, Lin J, Trindade MC, Shida J, Kajiyama G, Vu T, Hoffman AR, van der Meulen MC, Goodman SB, Schurman DJ, Carter DR. Time-dependent effects of intermittent hydrostatic pressure on articular chondrocyte type II collagen and aggrecan mRNA expression. *J Rehabil Res Dev* 2000;37:153–61. [PubMed: 10850821]
- [14]. Spahr A, Lyngstadaas SP, Slaby I, Haller B, Boeckh C, Tsoulfidou F, Hammarstrom L. Expression of amelin and trauma-induced dentin formation. *Clin Oral Investig* 2002;6:51–7.
- [15]. Gronthos S, Akintoye SO, Wang CY, Shi S. Bone marrow stromal stem cells for tissue engineering. *Periodontology* 2006;41:188–195.2000
- [16]. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. Stem cell properties of human dental pulp stem cells. *Journal of dental research* 2002;81:531–535. [PubMed: 12147742]
- [17]. Balducci L, Ramachandran A, Hao J, Narayanan K, Evans C, George A. Biological markers for evaluation of root resorption. *Archives of Oral Biology* 2007;52:203–208. [PubMed: 17097600]
- [18]. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthod.Craniofac.Res* 2005;8:191–199. [PubMed: 16022721]
- [19]. Teclas O, Laurent P, Zygouritsas S, Burger AS, Camps J, Dejou J, About I. Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. *Archives of Oral Biology* 2005;50:103–108. [PubMed: 15721135]
- [20]. Heikinheimo K, Begue-Kirn C, Ritvos O, Tuuri T, Ruch JV. Activin and bone morphogenetic protein (BMP) signalling during tooth development. *Eur J Oral Sci* 1998;106(Suppl 1):167–73. [PubMed: 9541221]
- [21]. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97:13625–13630. [PubMed: 11087820]
- [22]. Reza AT, Nicoll SB. Hydrostatic pressure differentially regulates outer and inner annulus fibrosus cell matrix production in 3D scaffolds. *Annals of Biomedical Engineering* 2008;36:204–213. [PubMed: 18026839]
- [23]. Takai E, Mauck RL, Hung CT, Guo XE. Osteocyte viability and regulation of osteoblast function in a 3D trabecular bone explant under dynamic hydrostatic pressure. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research* 2004;19:1403–1410. [PubMed: 15312240]
- [24]. Ogbureke KU, Fisher LW. Expression of SIBLINGs and their partner MMPs in salivary glands. *Journal of dental research* 2004;83:664–670. [PubMed: 15329369]
- [25]. Akintoye SO, Lam T, Shi S, Brahim J, Collins MT, Robey PG. Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. *Bone* 2006;38:758–68. [PubMed: 16403496]
- [26]. Locker D, Jokovic A, Tompson B, Prakash P. Is the Child Perceptions Questionnaire for 11-14 year olds sensitive to clinical and self-perceived variations in orthodontic status? *Community Dent Oral Epidemiol* 2007;35:179–85. [PubMed: 17518964]
- [27]. Dugoni SA, Aubert MM. Orthodontic treatment in the early mixed dentition: is this the optimum time to start care? *J Calif Dent Assoc* 2006;34:807–12. [PubMed: 17087395]
- [28]. Stefanik D, Sarin J, Lam T, Levin L, Leboy PS, Akintoye SO. Disparate osteogenic response of mandible and iliac crest bone marrow stromal cells to pamidronate. *Oral Dis* 2008;14:465–471. [PubMed: 18938273]
- [29]. Davidovitch Z. Tooth movement. *Crit Rev Oral Biol Med* 1991;2:411–50. [PubMed: 1742417]
- [30]. Davidovitch Z, Nicolay OF, Ngan PW, Shanfeld JL. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. *Dent Clin North Am* 1988;32:411–35. [PubMed: 2900159]
- [31]. Neuhaus KW. Teeth: malignant neoplasms in the dental pulp? *Lancet Oncol* 2007;8:75–8. [PubMed: 17196513]
- [32]. Yu C, Abbott PV. An overview of the dental pulp: its functions and responses to injury. *Aust Dent J* 2007;52:S4–16. [PubMed: 17546858]

- [33]. Elder BD, Athanasiou KA. Synergistic and additive effects of hydrostatic pressure and growth factors on tissue formation. PLoS ONE 2008;3:e2341. [PubMed: 18523560]
- [34]. Mauck RL, Nicoll SB, Seyhan SL, Ateshian GA, Hung CT. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. Tissue Eng 2003;9:597–611. [PubMed: 13678439]

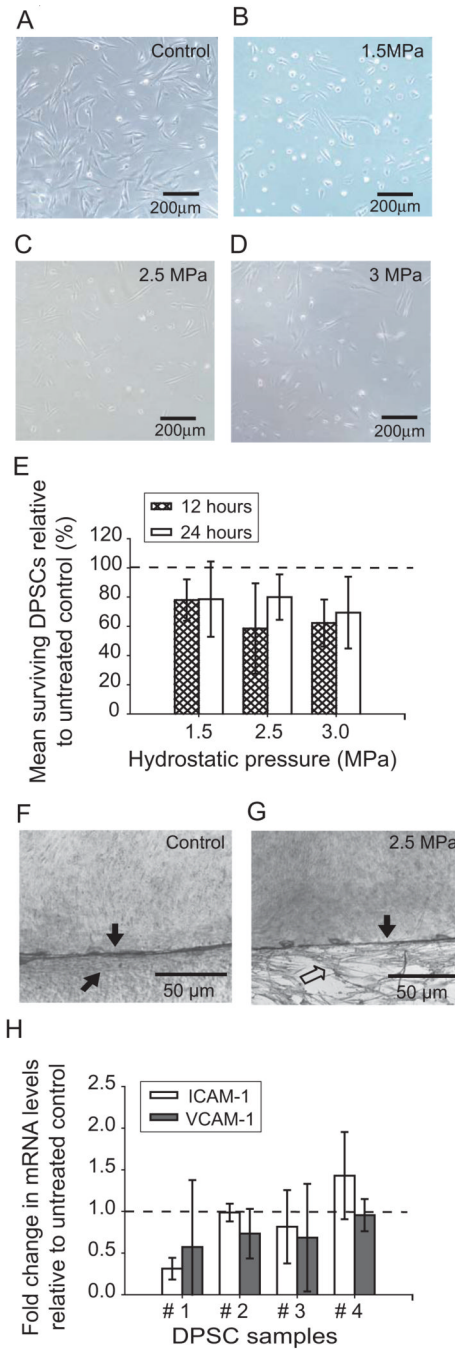


Figure 1.

Recovery capacity of HSP-treated DPSCs. HSP-treated DPSCs acquired round morphology, detached from glass discs and displayed fewer cells per unit area in a magnitude-dependent pattern as shown in representative images 2 hours after HSP application (A-D). There were fewer surviving HSP-treated DPSCs (n= 4 patients) within the first 12 to 24 hours after HSP treatment (E). HSP-treated cells continued to proliferate until confluence similar to control untreated cells (F and G, solid arrows) but HSP-treated cells partially lost adherence to the culture dish at the glass-plastic interface resulting in fewer surviving cells transitioning from the glass coverslip to tissue culture plastic (G, clear arrow). Real time PCR further showed a decrease in mRNA of one or both adhesion markers, ICAM-1 and VCAM-1, in HSP-treated

DPSCs from 3 of 4 patients (H). [A to G are representative patterns displayed by all patients; mRNA levels were normalized to TATA-binding protein; differences in cell survival and mRNA levels between HSP-treated and control cells were not statistically significant].

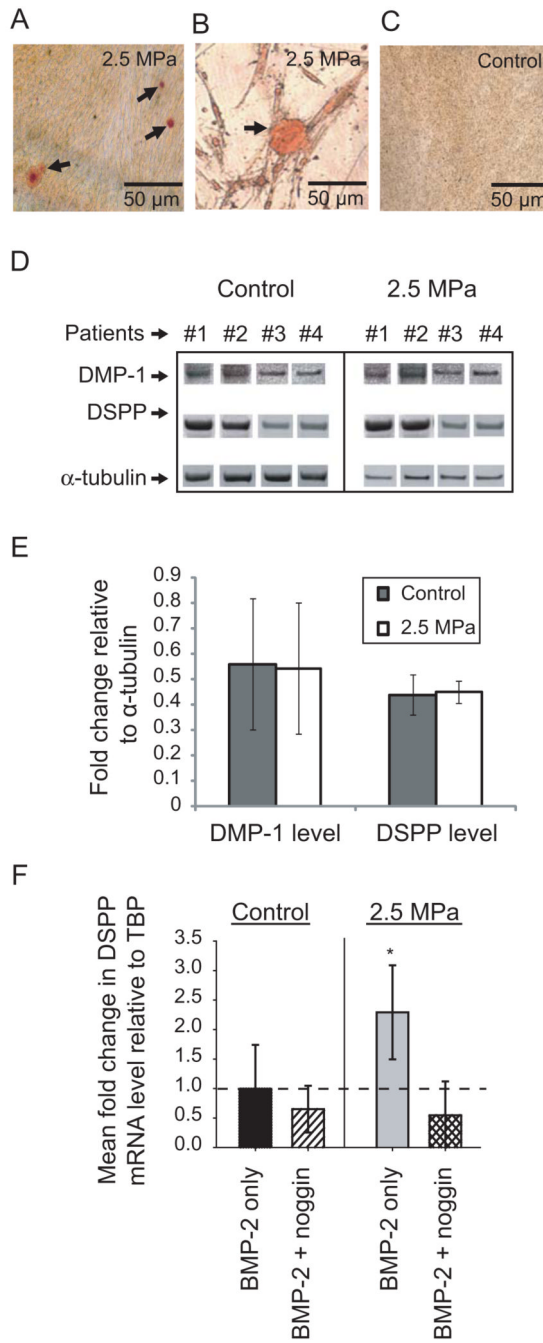


Figure 2.

HSP modulates DPSC *in vitro* differentiation and BMP-2 responsiveness. Representative Alizarin Red staining showed calcium accumulation occurred earlier at 2 weeks in HSP-treated DPSCs (A and B, arrows) while control DPSCs were yet to display similar calcium accumulation (C). Western blotting with anti-human DMP-1 or DSPP demonstrated individual variability in the expression of odontogenic markers in both control and HSP-treated DPSCs from four patients (D). But densitometric analysis of immunoreactive bands normalized to α -tubulin did not show any appreciable differences between control and 2.5 MPa HSP (E). Real time PCR analysis showed that pre-treatment of DPSCs with HSP significantly enhanced DSPP levels in response to bone morphogenetic protein-2 (BMP-2) stimulation (mean of n =

4 patients) while DMP-1 was unchanged (data not provided). Enhanced BMP-2 response was attenuated to baseline level by noggin, a BMP antagonist (G). [*= statistically significant at $p = 0.009$].

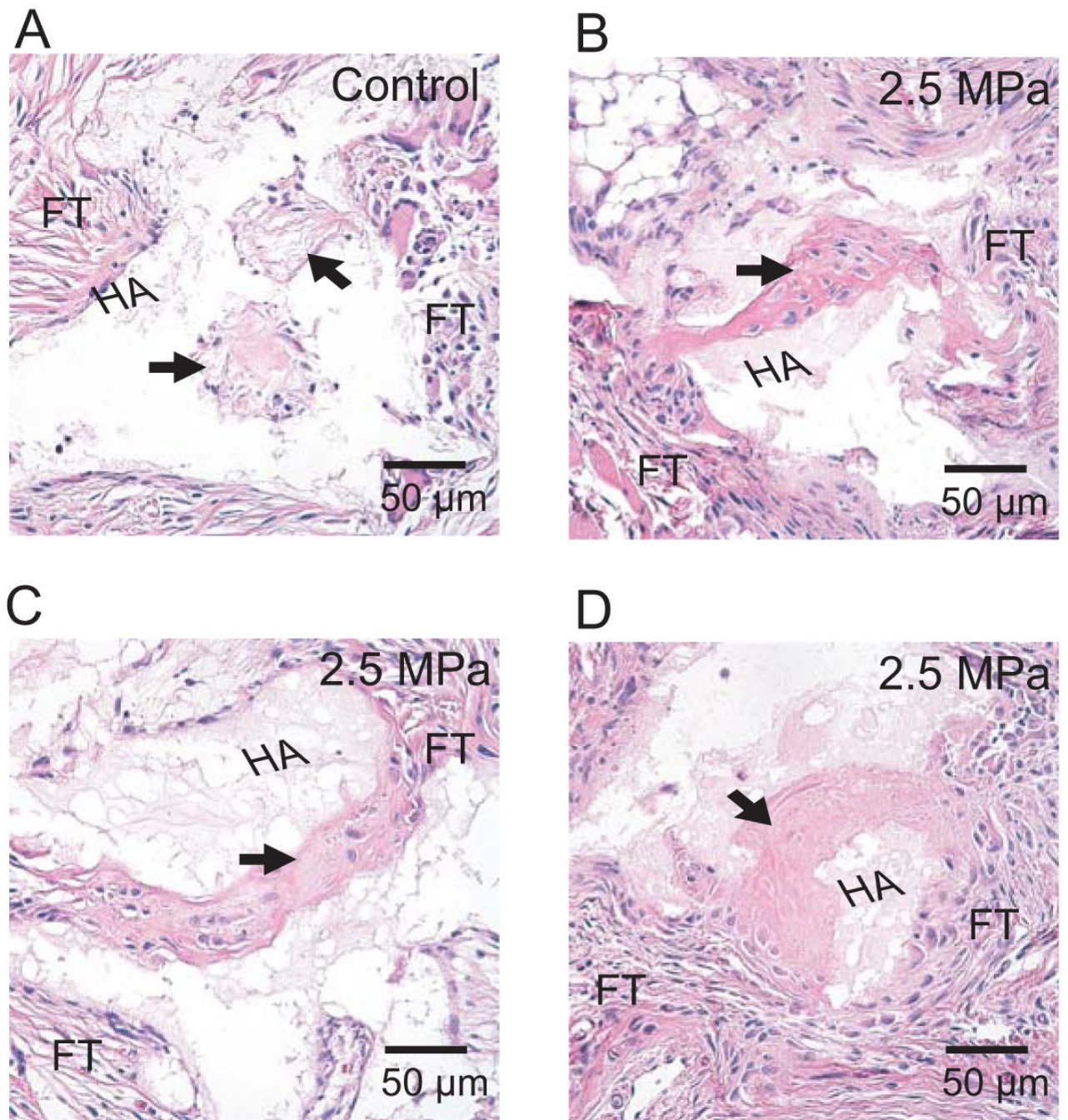


Figure 3.

HSP modulates *in vivo* differentiation and BMP-2 response of DPSCs.

HSP-treated DPSCs retained ability to regenerate hard tissues within a bed of hydroxyapatite/tricalcium phosphate (HA) six weeks after transplantation. Relative to control DPSC (A), transplanted HSP-treated DPSCs regenerated appreciable hard tissue (arrows) and displayed highly cellular (B), partially cellular (C) and acellular (D) histological features surrounded by host fibrous tissue (FT).

Table 1

Subject characteristics

Patient #	Age	Sex	Ethnicity
1	18	Male	African-American
2	14	Female	African-American
3	13	Female	Caucasian
4	12	Male	Caucasian