

S.A. Feitosa<sup>1,2†</sup>, J. Palasuk<sup>1,3†</sup>, K. Kamocki<sup>1</sup>,  
S. Geraldeli<sup>4</sup>, R.L. Gregory<sup>5</sup>, J.A. Platt<sup>1</sup>,  
L.J. Windsor<sup>5</sup>, and M.C. Bottino<sup>1\*</sup>

<sup>1</sup>Department of Restorative Dentistry, Division of Dental Biomaterials, Indiana University School of Dentistry, Indianapolis, IN, USA; <sup>2</sup>Department of Dental Materials and Prosthodontics, São Paulo State University–UNESP, São José dos Campos, SP, Brazil; <sup>3</sup>Department of Restorative Dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand; <sup>4</sup>Restorative Dental Sciences, Operative Division, College of Dentistry, University of Florida, Gainesville, FL, USA; and <sup>5</sup>Department of Oral Biology, Indiana University School of Dentistry, Indianapolis, IN, USA; <sup>†</sup>authors contributing equally to this work; <sup>\*</sup>corresponding author, mbottino@iu.edu

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## APPENDIX

### MATERIALS AND METHODS

#### Cytotoxicity of Adhesive Eluates on Human Dental Pulp Stem Cells

Adhesive disks (5 × 2 mm; *n* = 4 per group) were fabricated as mentioned. Eluates from the distinct specimens were prepared as previously described and following the guidelines of the International Standards Organization (1993). In brief, we used a 1:2 ratio between the elution solution (Dulbecco's Modified Eagle's Medium [DMEM], Gibco, Grand Island, NY, USA) and the adhesive disk volume (*i.e.*, 1 mL of DMEM to 2 mm<sup>3</sup>), respectively (Zhang *et al.*, 2013a, 2013b). The disks were ultraviolet sterilized (30 min/side) and then individually immersed in 19.625 mL of DMEM supplemented with 10% FBS (Hyclone, Logan, UT, USA) and 1% penicillin-streptomycin (Sigma), placed in a shaker and mixed at 37°C at 60 rpm for 24 hr. To mimic clinically relevant conditions, the eluate solutions were serially diluted as follows: 32-fold (1 mL of original eluate + 31 mL of full DMEM), 64-fold, and 128-fold (Zhang *et al.*, 2013a, 2013b).

Human dental pulp stem cells cultured in supplemented DMEM were seeded at the density of 3,000 cells per well in 96-well plates in 100 μL of medium (Bottino *et al.*, 2013). After 4 hr of incubation (37°C and 5% CO<sub>2</sub>), the medium was removed and replaced with 100 μL of previously prepared

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experimental eluates (Bottino *et al.*, 2013). Following culture for another 48 hr, 15 μL of dye solution (CellTiter 96 Non-Radioactive Cell Proliferation Assay/MTT; Promega, Madison, WI, USA) was added to each well. After 4 hr of incubation, 100 μL of Solubilization/Stop Mix was added into each sample, and after overnight incubation the optical density was measured at 570 nm and subtracted by background optical density, measured at 680 nm. Human dental pulp stem cells grown without any eluate served as a negative control. Cell viability percentages were presented as determined from the ratio of experimental value to value from untreated cells.

### APPENDIX REFERENCES

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**Appendix Table.** Mean Microtensile Bond Strength and Failure Mode

Group	μTBS (MPa)	Failure Mode, %			
		Adhesive	Mixed	Cohesive Dentin	Cohesive Resin
SBMP	51.7 ± 23.3	25.00	52.78	2.78	19.44
HNT	44.6 ± 15.2	31.71	60.98	4.88	2.44
HNT+DOX	54.3 ± 19.1	23.26	53.49	11.63	11.63

Microtensile bond strength (μTBS; mean ± SD) showed no statistically significant differences among the groups (*p* = .07). HNT, SBMP with halloysite nanotubes; HNT+DOX, SBMP with doxycycline-encapsulated halloysite nanotubes; SBMP, Scotchbond Multi-Purpose.