Advances in Computer Science and Information Technology (ACSIT)
p-ISSN: 2393-9907; e-ISSN: 2393-9915; Volume 2, Issue 14; October-December, 2015 pp. 43-46

http://www.krishisanskriti.org/Publication.html

Imaging of Smart Dental Composites Using Mesoscopic Fluorescence Molecular Tomography: An *ex Vivo* Feasibility Study

Feixiao Long[1], Aditya Srinivasan[2], Xavier Intes[3] and Shiva P. Kotha[4*]

[1,2,3,4]*Department of Biomedical Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA*
E-mail: [1]longf@rpi.edu, [3]intesx@rpi.edu, [4]kothas2@rpi.edu

Abstract—*One of the many reasons for failure of dental restorations is secondary caries caused by bacteria invading the margins of the restoration. Dental composites containing upconversion nano-particulate can be used to induce bactericidal activity upon irradiation with near-infrared light, while providing contrast that can enable imaging of the location of the restoration. In this study, we present data from our preliminary studies indicating the feasibility of reducing bacterial burden by irradiating composites with nanoparticulate with NIR light as well as imaging nano-particulate in a simulated tooth cavity using optical tomography.*

Keywords*: NIR imaging; mesoscopic molecular fluorescence tomography; optical tomography; bactericidal activity.*

1. INTRODUCTION

Dental caries or tooth decay has a very high prevalence, occurring in 92% of adults between 20 and 64 years [1]. Caries is generally treated by mechanical removal of the lesion followed by placement of a particulate reinforced photo-polymerizing composites. However, direct or indirect bacterial activity at the tooth-dental restoration interface has been implicated in loosening of the restoration and its resulting failure [2], [3]. To overcome this, novel dental composites are being developed to exhibit intrinsic anti-bacterial efficacy or lack of susceptibility to degradation by bacteria [4], [5]. A limitation of these strategies is that they are insufficient to prevent degradation of the tooth structure at the interface because these materials cannot promote regeneration at the tooth-composite interface. To overcome this limitation, we propose to use dental composites that contain upconversion particulate as smart materials that can not only be used to generate bactericidal activity [6], [7], but simultaneously promote regeneration at the tissue-dentin interface upon exposure to NIR light [8]. Upconversion is a process where light of lower wavelength (e.g. 470nm) is emitted upon irradiation with light at higher wavelength (e.g. 975nm). Note that light in the UV-blue range (e.g. 470nm) is bactericidal against bacteria that are known to be present in carious lesions (e.g. S. Mutans)[9], [10] whereas light in the NIR range (e.g. 975nm) is known to stimulate tissue regeneration [11]. An additional benefit of using these composites with light-activated bactericidal and regeneration activity is the possibility of enabling 3-D reconstruction of the composite [12], in order to better target desired therapeutic effects (anti-bactericidal or regeneration). Herein, we present preliminary results indicating the bactericidal activity of the composites containing upconversion nano-particulate upon irradiation with NIR light as well as 3-D reconstructions of a simulated composite containing upconversion nano-particulate.

2. MATERIALS AND METHODS

2.1. Effects on NIR irradiation on bacterial and mammalian cells plated on composites with and without upconversion particulate

Bis-GMA/TEGDMA based resins were photopolymerized in 96-well tissue culture plates in the presence and absence of upconversion nanoparticles (5% w/v of ytterbium and thulium doped sodium yttrium fluoride). S. mutans (bacterial) or NIH3T3 fibroblasts (mammalian) were inoculated onto the surface of the plasma treated Bis-GMA as well in control wells (no Bis-GMA) following plasma treatment and media deposition. Plates with bacteria were irradiated with 975nm wavelength light at 820 mW/cm^2 (which generated 70 mW/cm^2 blue light) at 14-16 hrs after plating, while plates with mammalian cells were irradiated at 24hrs after plating. Live/ dead analysis was performed using appropriate kits at appropriate times after irradiation.

2.2. 3-D reconstruction of nanoparticulate placed in a simulated tooth cavity

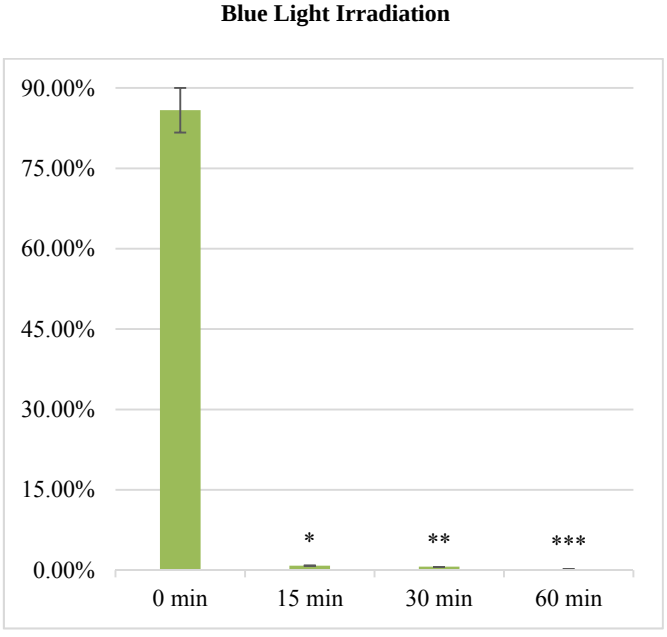
A hole was drilled in human molar teeth obtained from local dental clinics with appropriate IRB and IBC approvals. A capillary containing upconversion nanoparticulate was placed in the simulated cavity and imaging was performed by scanning a laser diode light source in a grid-like pattern over the lingual surface while using an EMCCD camera to record

an image of light emitted from the buccal surface (transmission mode). Once the data was collected, an inverse optical problem was solved to obtain 3-D images of a fluorescent inclusion. Micro-CT of the molar tooth was performed to enable registering of the reconstructed image in order to provide metrics about the photon-based reconstruction.

3. RESULTS

Bacteria (*S. mutans*) plated in a 96-well cell culture plate (without composite) were irradiated with blue light for 0, 15, 30, and 60 minutes. The green/red fluorescence ratio was determined for each sample and subsequently converted into percentage of surviving bacterial cells by the equation G/R Ratio = .0331(% Cells Alive) - .002 (Fig. 1). ANOVA analysis shows that the mean survival rates differ from one another ($p < .0001$). Further post-hoc analysis using the Turkey HSD test shows that each mean survival rate is different from the others ($p < .01$).

S. mutans were also grown on Bis-GMA composite or Bis-GMA composite with particles. Each well was irradiated with near infrared light for 0, 15, 30, or 60 minutes. Using the equation G/R Ratio = .0331(% Cells Alive) - .002, the ratio between green/red fluorescence for each group was converted into percentage of cells alive. ANOVA analysis at each stimulation time (15 minutes, 30 minutes, and 60 minutes) shows that mean survival rates of S. mutans are not similar for each irradiation time ($p < .0001$). Post-hoc analysis using the Turkey HSD test shows that the S. mutans mean survival rate on irradiated Bis-GMA plates with the upconversion particle differs from the survival rate of all other groups for each treatment time ($p < .01$ for all treatment times).



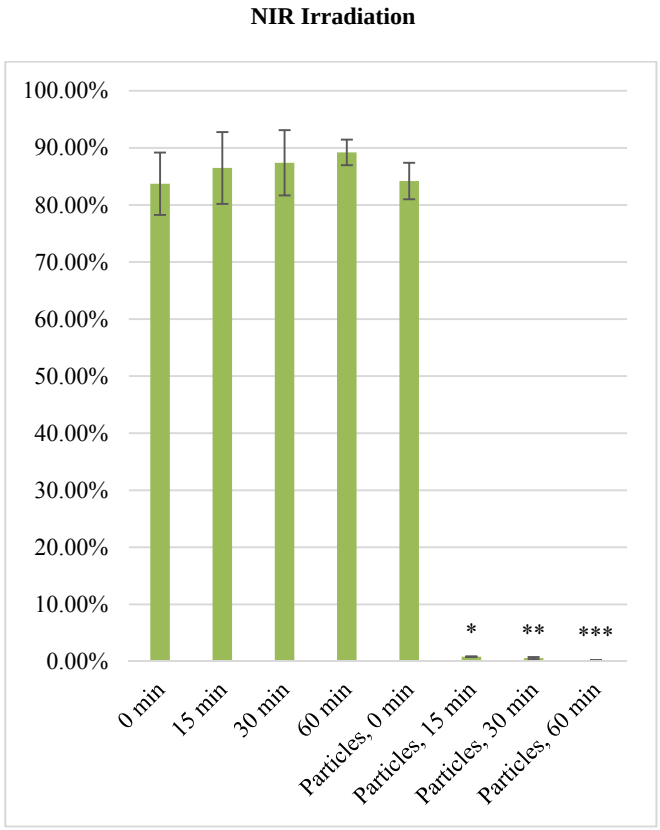
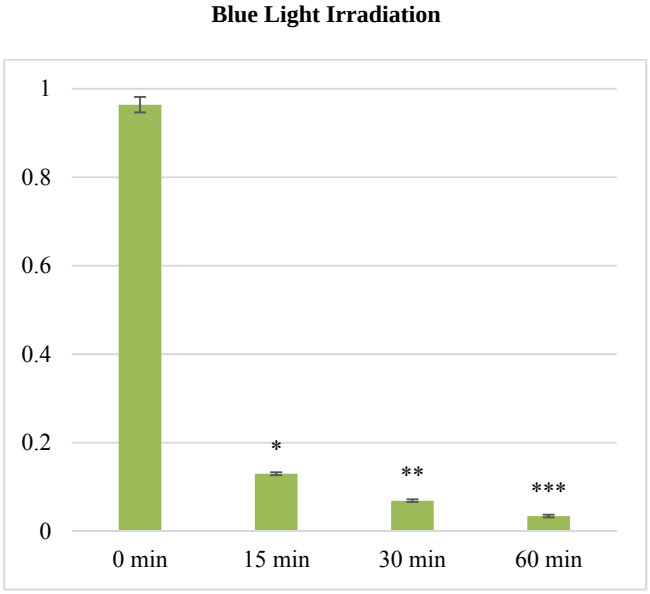


Fig. 1: Percentage of S. mutans alive following light irradiation, indicating irradiation with blue light or NIR irradiation of upconversion particles reduces percentage of alive S. mutans. Asterisks denote mean survival percentages that differ between groups ($p < 0.01$).



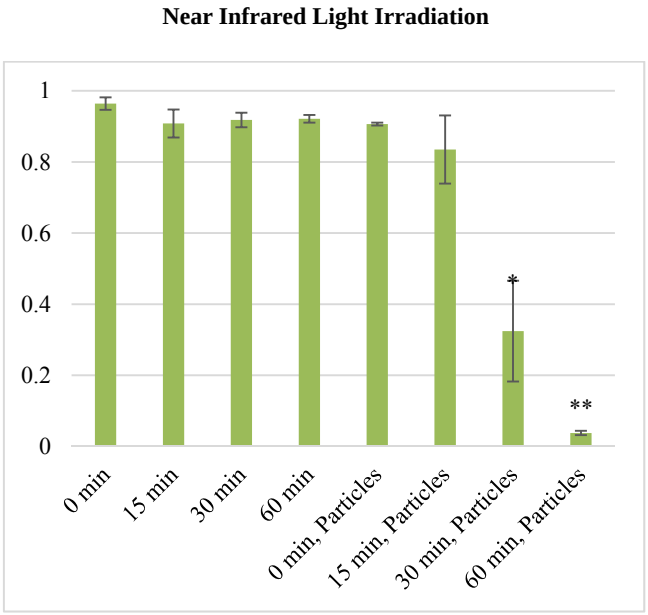


Fig. 2: Live/Dead Ratios of NIH3T3 Fibroblasts, indicating irradiation with blue light or NIR irradiation of upconversion particles for longer than 15 minute reduces the proportion of live NIH3T3 fibroblasts, determined by the B/G+R fluorescence ratio. Asterisks denote mean fluorescence B/G+R ratios that differ between groups ($p < 0.01$).

NIH3T3 fibroblasts plated in a 96-well plate as well as on top of Bis-GMA photopolymerized in the 96-well plate were irradiated by blue light for 0, 15, 30, and 60 minutes. The blue/(green +red) – B/G+R – fluorescence ratio was found on each plate to determine relative ratios of cells alive (blue) against apoptotic cells (green) and necrotic cells (red). ANOVA shows that the mean fluorescence ratios differ across treatment groups ($p < .0001$, Fig. 2). Further post-hoc analysis shows that mean fluorescence ratio differs from the others ($p < .01$).

NIH3T3 fibroblasts were also plated on Bis-GMA composite and Bis-GMA composite with the upconversion particle. Each well was irradiated with near infrared light (NIR) for 0, 15, 30, or 60 minutes following which live/dead staining was performed to evaluate the effects of irradiation with NIR light in the presence and absence of composite with and without upconversion nanoparticulate. ANOVA analysis for the 15 minute treatment time shows that the mean B/G+R fluorescence ratios do not differ significantly. For the 30 minute and 60 minute treatment times, ANOVA analysis does a show a significant difference in B/G+R ratios ($p < .0001$ for both treatment times). Post-hoc analysis using the Turkey HSD test shows that the B/G+R mean fluorescence ratio on the 30 minute irradiated Bis-GMA and particle well differs from the B/G+R mean fluorescence ratio of the Bis-GMA control, Bis-GMA and particle control, and 30 minute NIR irradiated Bis-GMA wells ($p < .01$). The Tukey HSD test also shows that the B/G+R mean fluorescence ratio on the 60 minute irradiated Bis-GMA and particle well differs from the B/G+R mean fluorescence ratio of the Bis-GMA control, Bis-GMA and particle control, and 60 minute NIR irradiated Bis-GMA wells ($p < .01$).

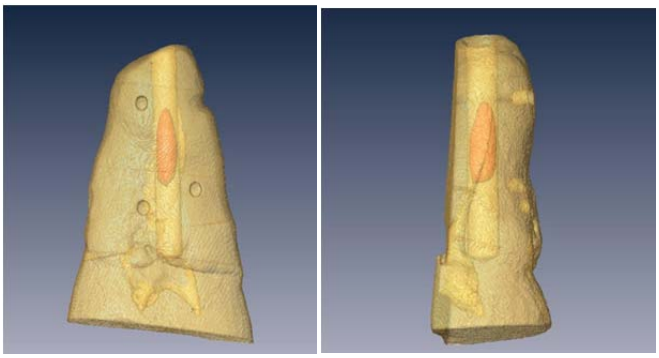


Fig. 3: Two different views of micro-CT and photon based 3-D image reconstruction superimposed on one other demonstrating that nanoparticles are localized to a cavity (red) drilled in tooth (identified from microCT). This suggests that photon based 3-D image reconstruction can be used to localize the presence of upconversion nanoparticles.

Registration of X-ray micro-CT and photon based 3-D images with one other indicated that the photon based reconstruction, which detected the presence of nanoparticles, was localized to the region in the cavity.

4. DISCUSSION AND CONCLUSION

In this study, we have demonstrated that irradiation of NIR light on composites reinforced with upconversion nanoparticles can be used to induce bactericidal activity, at doses and durations that do not significant photon toxicity to mammalian cells. The benefit of using NIR light is that it penetrates deeper into tissues (even ones that have high scatter and absorption coefficients, like teeth), and it has been known to induce tissue regeneration. Another benefit of using NIR light in conjunction with upconversion particles is that the blue light that causes bactericidal activity is only generated where it is required (i.e. close to where the composite is present). Furthermore, we have demonstrated the utility of diffuse imaging to localize the nanoparticles within teeth. These results indicate that it may be possible to localize the position of the composite within teeth surfaces and calibrate photon intensities to engender site-specific therapy.

5. ACKNOWLEDGMENTS

This work was partly funded by the National Science Foundation NSF CMMI 1200270.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] "Dental Caries (Tooth Decay)." [Online]. Available: http://www.nidcr.nih.gov/datastatistics/finddatabytopic/dentalcaries/. [Accessed: 19-Nov-2015].

[2] J. L. Drummond, "Degradation, fatigue, and failure of resin dental composite materials," *J. Dent. Res.*, vol. 87, no. 8, pp. 710–719, Aug. 2008.

[3] "untitled - compositefailure.pdf." [Online]. Available: http://www.dwdav.com/dds/compositefailure.pdf. [Accessed: 19-Nov-2015].

[4] N. Beyth, S. Farah, A. J. Domb, and E. I. Weiss, "Antibacterial dental resin composites," *React. Funct. Polym.*, vol. 75, pp. 81–88, Feb. 2014.

[5] X. Xu, Y. Wang, S. Liao, Z. T. Wen, and Y. Fan, "Synthesis and characterization of antibacterial dental monomers and composites," *J. Biomed. Mater. Res. B Appl. Biomater.*, vol. 100, no. 4, pp. 1151–62, May 2012.

[6] Y. Ye, Y. Li, and F. Fang, "Upconversion nanoparticles conjugated with curcumin as a photosensitizer to inhibit methicillin-resistant Staphylococcus aureus in lung under near infrared light," *Int. J. Nanomedicine*, p. 5157, Nov. 2014.

[7] S. L. Cates, E. L. Cates, M. Cho, and J.-H. Kim, "Synthesis and Characterization of Visible-to-UVC Upconversion Antimicrobial Ceramics," *Environ. Sci. Technol.*, vol. 48, no. 4, pp. 2290–2297, Feb. 2014.

[8] P. R. Arany, A. Cho, T. D. Hunt, G. Sidhu, K. Shin, E. Hahm, G. X. Huang, J. Weaver, A. C.-H. Chen, B. L. Padwa, M. R. Hamblin, M. H. Barcellos-Hoff, A. B. Kulkarni, and D. J Mooney, "Photoactivation of endogenous latent transforming growth factor-β1 directs dental stem cell differentiation for regeneration," *Sci. Transl. Med.*, vol. 6, no. 238, p. 238ra69, May 2014.

[9] Y.-H. Lee, H.-W. Park, J.-H. Lee, H.-W. Seo, and S.-Y. Lee, "The photodynamic therapy on Streptococcus mutans biofilms using erythrosine and dental halogen curing unit," *Int. J. Oral Sci.*, vol. 4, no. 4, pp. 196–201, Jan. 2013.

[10] R. Lubart, A. Lipovski, Y. Nitzan, and H. Friedmann, "A possible mechanism for the bactericidal effect of visible light," *Laser Ther.*, vol. 20, no. 1, pp. 17–22, 2011.

[11] P. Avci, A. Gupta, M. Sadasivam, D. Vecchio, Z. Pam, N. Pam, and M. R. Hamblin, "Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring," *Semin. Cutan. Med. Surg.*, vol. 32, no. 1, pp. 41–52, Mar. 2013.

[12] F. Long, M. S. Ozturk, M. S. Wolff, X. Intes, and S. P. Kotha, "Dental Imaging Using Mesoscopic Fluorescence Molecular Tomography: An ex Vivo Feasibility Study," *Photonics*, vol. 1, no. 4, pp. 488–502, Dec. 2014.