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Abstract. In this work we demonstrate the potential use of gold nanoparticles as contrast agents for the optical coherence tomography (OCT) imaging technique in dentistry. Here, a new *in situ* photothermal reduction procedure was developed, producing spherical gold nanoparticles inside dentinal layers and tubules. Gold ions were dispersed in the primer of commercially available dental bonding systems. After the application and permeation in dentin by the modified adhesive systems, the dental bonding materials were photopolymerized concurrently with the formation of gold nanoparticles. The gold nanoparticles were visualized by scanning electron microscopy (SEM). The SEM images show the presence of gold nanospheres in the hybrid layer and dentinal tubules. The diameter of the gold nanoparticles was determined to be in the range of 40 to 120 nm. Optical coherence tomography images were obtained in two- and three-dimensions. The distribution of nanoparticles was analyzed and the extended depth of nanosphere production was determined. The results show that the OCT technique, using *in situ* formed gold nanoparticles as contrast enhancers, can be used to visualize dentin structures in a non-invasive and non-destructive way. [DOI: 10.1117/1.JBO.17.6.066003]

Keywords: optical coherence tomography; gold nanoparticles; dental bonding agents.

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1 Introduction

Contemporary restorative procedures are based on the adhesive properties of tooth colored resin-based materials.[1] Even though several studies revealed excellent immediate and short-term bonding effectiveness of dental adhesives,[2] bond stability for a long time period still remains an important concern.[3] Stable bonding is created by full impregnation of the dentin substrate by blends of resin monomers, thus avoiding different degrees of incomplete impregnation[4,5] and producing a compact and homogenous hybrid layer.[1] This Hybrid layer is created by a mixture of the dentin organic matrix, residual hydroxyapatite crystallites, resin monomers, and the solvent. Each of these individual components may be affected by aging and/or degradation phenomena within the hybrid layer.[1] There are two known degradation patterns: degradation of resin (hydrolysis) and degradation of exposed collagen fibrils (disorganization).[6] Hybrid layer degradation may increase the water content of the bonded interface which has been claimed as one of the major causes for collagen breakdown.[1]

A variety of photo-induced synthetic methods for producing metal nanoparticles and nanostructures have been developed to obtain monometallic and bimetallic nanoparticles, and composite materials. In particular, it is known that direct photoreduction of $AuCl_4^-$ results in formation of gold nanoparticles in aqueous solution[7] and polymer matrices.[8] Gold nanoparticles are key materials in nanoscience and nanotechnology due to their strong absorption and scattering derived from an intense localized plasmonic resonance.[9–11] This plasmonic resonance can be tuned over a broad spectral range in various gold nanostructures (such as nanorods and nanoshells) Gold is biocompatible and nanoparticles can be fabricated with excellent colloidal stability. Because of these features, gold nanoparticles have extensively been used for bioimaging. In particular, multiplexed dark-field imaging as well as near-infrared-enabled deep tissue imaging can be achieved. Additionally, they can be surface-functionalized with biomolecules and used for targeted delivery to specific cells.[12–14] Gold nanoparticles have also been explored in the biomedical field to reduce the pore size of collagen, to reduce collagenase biodegradation formation of multiple cross-links with the collagen structure.[15]

Optical coherence tomography (OCT) is a relatively new imaging modality that uses near-infrared broad band light sources to scan tissues. These systems offer cross-sectional and 3D images with resolution on the order of few microns and a depth of penetration of one to two millimeters. It generates a signal based on the presence of refractive index mismatches and scattering events within the imaged volume. In particular, an agent that modifies the scattering properties of a sample can improve the image contrast. Contrast agents could lead to

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improvement of *in situ* OCT image quality to a degree comparable to biopsy, reducing the need to remove, section, and stain tissues.[10]

Here, we demonstrate the potential use of gold nanoparticles as contrast agents for OCT in dentistry. Spherical gold nanoparticles were produced inside the hybrid layer and tubules by a photothermal reduction *in situ*. Gold ions were dispersed in the primer of several dental bonding systems. A new procedure was developed to simultaneously reduce the gold ions and photopolymerize the adhesive system. Furthermore, OCT was used to visualize dentin structures in a non-invasive and non-destructive way, and to identify the gold nanoparticle distribution within the tissue.

2 Methodology

2.1 *Dental Bonding Agent Preparation*

Two commercially available dental bonding agents (DBAs) were modified in this study. Adper Scotchbond Multi-Purpose from 3M ESPE Corporation is a three-step etch and rinse adhesive which removes the smear layer completely and demineralizes the subsurface of dentin with phosphoric acid. Adhese from Ivoclair Vivadent is a two-step self-etch adhesive, which incorporates the smear layer into the hybrid layer via the use of an acidic primer, and does not require further rinsing. In spite of this difference, for both agents, the bond and the primer are in different vials. Therefore, the gold precursor can be added only to the primer and the bond is applied as a final solvent-free coating. Figure 1 shows the steps for the preparation of the modified adhesive systems. The gold precursor ($HAuCl_4 \cdot 3H_2O$) was purchased from Sigma-Aldrich. Due to the hygroscopic nature of the gold precursor, it was kept in a glove box with a dry argon atmosphere. The gold precursor was dissolved in a small amount of acetone. The DBA primer/gold precursor solution was mixed in a glove box and stirred for 20 min, then ultrasonicated for 5 min.

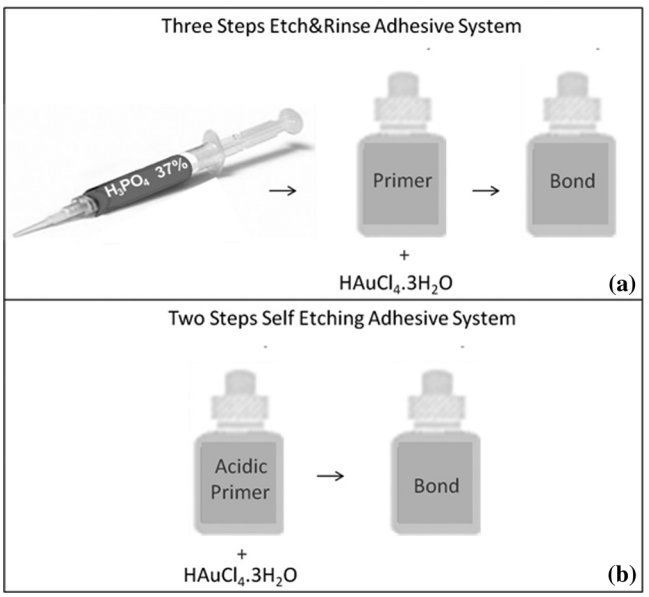

Fig. 1 DBA preparation with (a) a three-step etch and rinse adhesive system, Adper Scotchbond Multi-Purpose, and (b) a two-steps self etching adhesive system, Adhese.

2.2 *Tooth Preparation*

Human third molars were collected from the Teeth Bank of the Universidade Federal de Pernambuco. The teeth were stored in distilled water until the start of the experiment. The occlusal one-third of each tooth was removed by means of a diamond saw (Isomet 1000) under water lubrication. The teeth were sectioned perpendicularly to the longitudinal axis, 1 to 1.5 mm into the sample, removing the enamel to obtain a superficial dentin. The dentin surfaces were polished with 600-grit SiC paper (DP 10 from Panambra) for one minute under running water to create a standardized smear layer, as described in Ref. 16, and stored in water for one week. The sectioned teeth were randomly distributed into four groups corresponding to the adhesive materials used: Adper Scotch Bond Multi-Purpose and Adhese were each tested with and without the gold nanoparticle precursor. Both adhesives were applied following the manufacturers' instructions. In our procedure, warm air (~50°C) was used to improve the evaporation of the solvent as suggested previously,[17] as well as to accelerate the reduction of gold nanoparticles in the primer of the experimental adhesive systems. Light curing for 60 s from a commercially available light source (Ultrablue LED/DMC) was used to photopolymerize the DBA concurrently with formation of the gold nanoparticles.

2.3 *Scanning Electron Microscopy Imaging*

Scanning electron microscopy (SEM) was used to characterize the gold nanoparticle formation. A Hitachi S-4000 field-emission scanning electron microscope in the back scattered mode and secondary electron emission mode was used.

2.4 *OCT Imaging*

Samples were imaged by the OCT technique. A commercial spectral optical coherence tomography instrument, the Ganymede system from Thorlabs, was used to produce cross-sectional and volumetric optical imaging of the samples. The system is based on a Michelson interferometer configuration. The main unit contains a super luminescent diode (SLD) light source. A fiber-optic coupler was used to direct the light from the broadband SLD source to the scanner probe. The central wavelength of the SLD was 930 nm, with a spectral width of 100 nm, providing an axial resolution of 5 μm. With an A-scan rate of 29 kHz, the system can produce 29 frames per second (fps) with 512 lines per frame. At this speed, it can rapidly generate 2D and 3D images of the samples. The images were taken by scanning the oclusal surface in a bucco-lingual direction. The long wavelength light penetrated deep into the dentin, and a tomographic image (OCT slice) parallel to the long axis of teeth was obtained.

3 Results and Discussion

In dental restorative techniques, stable bonds are obtained by full impregnation of the dentin substrates with the adhesive. The stability of the bonded interface is based on the formation of a homogeneous hybrid layer. Preparation of tooth with dental burs creates a smear layer of debris into the dentin. An adhesive system removes or treats the smear layer, creating a hybrid layer of resin bond, collagen fibers, dentin surface structure and intertubular structure. Over the hybrid layer, an adhesive layer forms. Under the hybrid layer, resin tags are established into the tubules.[1,4,5]

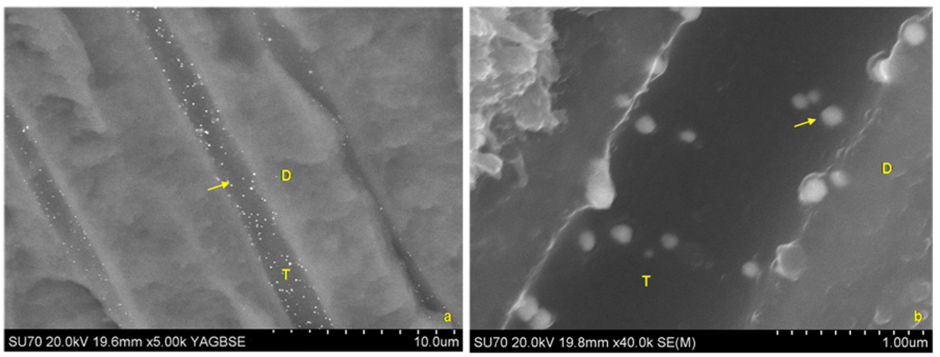

Fig. 2 SEM images of gold nanoparticles (arrows) showing penetration into dentinal tubules. Dentin (*D*) and tubules (*T*) are identified.

To confirm the formation and distribution of gold nanoparticles in the bonded dentin structure, SEM and OCT were performed. The SEM images in Fig. 2 show the presence of gold nanoparticles within the dentinal tubules. Nine randomly selected teeth were prepared and sectioned, then subjected to SEM evaluation. The fracture of the specimens avoided the formation of a new smear layer. The dentin contains many microscopic tubules of 0.5 to 4.0 μm diameter which extend parallel to each other from the pulp chamber to the amelo- or cement-dentinal junctions.[18,19] Gold nanoparticles were observed in the dentin with diameters of 40 to 120 nm. No difference was observed in the production of gold nanoparticles with the two adhesive systems used. In the photoreduction procedure, gold was not expected to interfere with the penetration of the modified DBA into dentinal tubules since the nanoparticles are formed after DBA application and light irradiation.

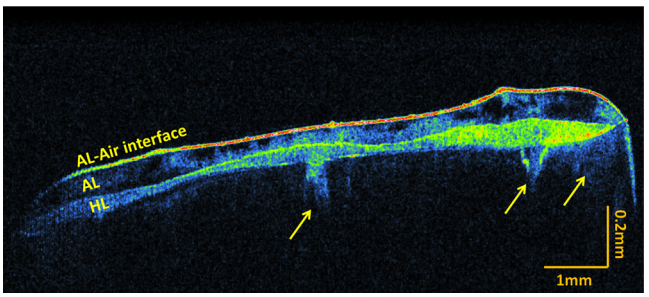

Fig. 3 OCT images of the adhesion process on dentin using DBAs prepared with gold nanoparticles. The adhesion process regions are identified: adhesive layer (AL), hybrid layer (HL), and resin tags (arrows).

OCT images of the samples with and without gold nanoparticles were obtained. Figure 3 shows a typical OCT cross-sectional image of the dental bonding structure. Hyperintense regions of OCT images represent highly scattering portions and high levels of direct backscatter of light due to the presence of gold nanoparticles within the sampled volume. In Fig. 3, this information is displayed in false colors, using warm and cool colors for higher and lower scattering intensities, respectively. The data displayed in false colors allows better distinguishability of different image sections, since on a colored image, 256^3

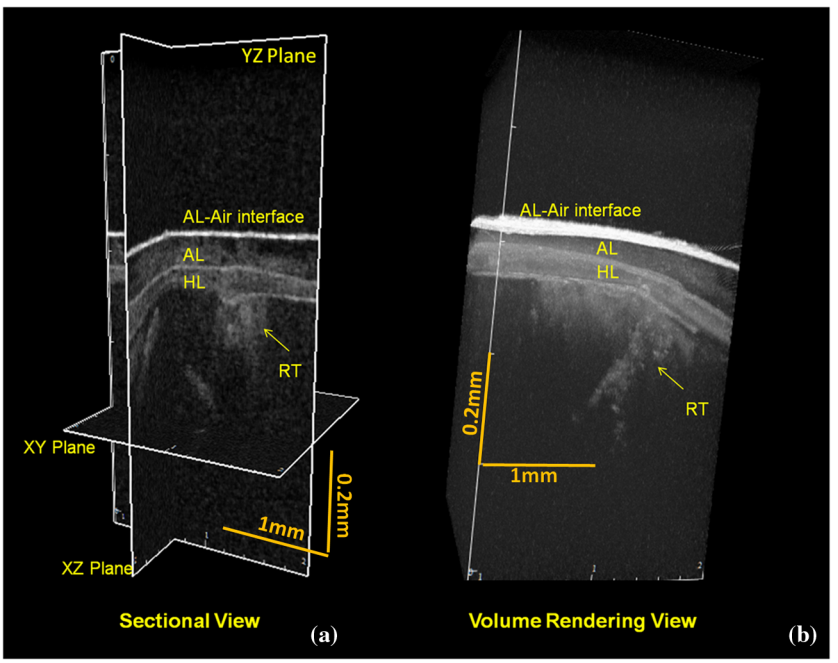

Fig. 4 Three-dimensional reconstruction with (a) sectional and (b) volume rendering views. The adhesive layer (AL), hybrid layer (HL), and resin tags (RT) are identified.

different shades of color and intensity can be identified.[20] In Fig. 3, all the regions of the adhesion process are identifiable: adhesive layer, hybrid layer, and resin tags. OCT measurements revealed a hybrid layer thickness of 56 to 89 μm. On the average, Resin Tags extended 150 μm deep into the dentin tissue, with an extreme depth value of 350 μm observed in Fig. 4. As consequence of the localized variation of the dentin structure,[21] the resin penetration is not uniformly distributed.

In the OCT images of the dental adhesive systems without nanoparticles, the interface between the adhesive layer and the hybrid layer was not observable. Moreover, we were not able to identify the resin tags and the hybrid layer extension in the samples without gold particles.

OCT was also performed in 3D imaging mode. In this mode, the OCT software acquires multiple adjacent (*Y* direction) 2D OCT frames (slices) in the *XZ* plane, forming a stack of images as a 3D data set. The 3D reconstruction can be examined in a sectional or volume rendering view, as shown in Fig. 4. The sectional view projects optical sections to one plane, or section level, in the *XY*, *XZ* or *YZ* planes as shown in Fig. 4(a). The volume rendering view is the full 3D volume representation shown in Fig. 4(b). The image background often limits the visibility of structures, but one can get a better understanding of the sample volume configuration by the use of various rendering methods.

Selected section planes of the OCT 3D images are shown in Fig. 5. With the *YZ* and *XZ* section planes, one can identify the limits of the three bonding regions, while the *XY* section exhibits the distribution and characterization of gold nanoparticles in each adhesion layer. The *XY* image plane of the adhesive layer shows the formation of gold nanoparticles, but not in a homogenous way. The *XY* image plane of the hybrid layer shows a homogenous distribution of the nanoparticles. The *XY* section plane of the resin tags shows that the nanoparticles were formed only in some limited regions.

The OCT imaging technique also allowed the identification of cavities surrounded by the demineralized area in the dentin structure, shown as a white spot lesion, that were not observed by the naked eye during tooth preparation. The white spot lesion is the manifestation of earlier local destruction of hard tissue due to a non-continuous demineralization processes. Nanoparticles can have the same dimensions as those of the missing hydroxyapatite and therefore have the potential to infiltrate deep into demineralized area. Figure 6 shows that by OCT characterization, one can identify a high concentration of gold nanoparticles not only at the surface, but also into all demineralized areas surrounding a small cavity in dentin. A 3D image in the attenuation mode exhibits a global view of the dentin sample and is presented in Fig. 6(a). One can identify a cavity in the dentin structure. The extension of the cavity is characterized by a cross-section image in the *XZ* plane shown in Fig. 6(b). The high-intensity areas surrounding the small cavity are associated with a higher concentration of the nanoparticles. The gold nanoparticle distribution is identified with clip-plane views in the *XZ* plane from the surface to the internal region, as shown in Fig. 6(c).

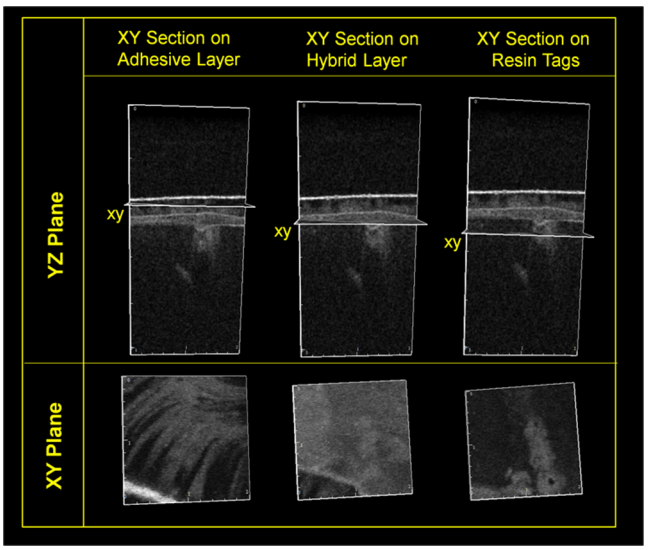

Fig. 5 OCT sectional reconstruction of each bonding region: adhesive layer, hybrid layer and resin tags (left to right). The *XY* plane is 2 × 2 mm and the *YZ* Plane is 2 × 1 mm.

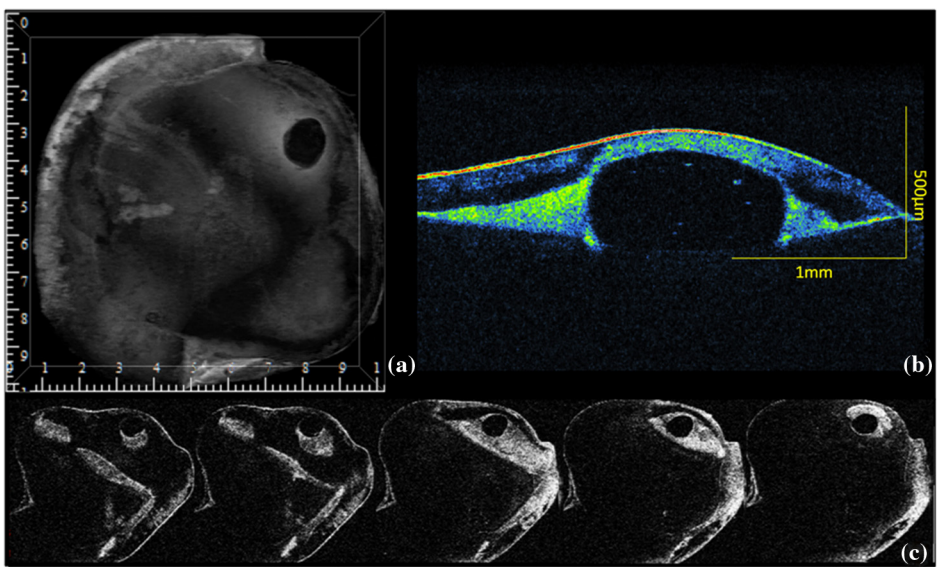

Fig. 6 An OCT image of the dentin structure: (a) 3D attenuation mode, 10 × 10 mm, (b) cross-section in the *XZ* plane, and (c) clip-plane views in the *XZ* plane from the surface (left) to the internal region (right).

We developed a gold nanoparticle/polymer hybrid material by photo-induced gold reduction in conjunction with current dental bonding agents. The metallic nanoparticles were evaluated as a contrast agent for OCT, and allowed the structural characterization of adhesive and hybrid layers which were not distinguishable in samples without nanoparticles. In the future, contrast agents could be explored to identify dental bonding materials under restorative material layers. Besides serving as contrast agents for optical imaging techniques, gold nanoparticles may be functionalized to provide sites in the surface with specialized functionality. Many works have already presented positive results about crosslinking between type I collagen and gold nanoparticles in biomedicine.[15,21] In particular, type I collagen is one of the main components and represents the backbone of the dentin organic matrix,[22] thus different approaches to slow or prevent the disintegration of the collagen network is pivotal to increase bonding stability.

4 Conclusion

In summary, we have demonstrated photo-induced gold reduction in dental bonding agents *in situ* to obtain a gold nanoparticle/polymer hybrid dental bonding material. The formation of the gold nanoparticles was characterized by scanning electron microscopy (SEM) and OCT. With OCT sectional reconstruction, we observed distinct distributions of nanoparticles in each bonding region. The bonding structures were identified and measured, finding a maximum penetration of 350 μm into the dentin tissue. We demonstrated the use of gold nanoparticles as contrast enhancing agent for OCT imaging in dentistry. Contrast agents for OCT produces a better image quality, allowing an improvement in the identification of tooth structures, and therefore extending the application of this technique to numerous diagnostic procedures.

Acknowledgments

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