Facile dental resin composites with tunable fluorescence by tailoring Cd-free quantum dots

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Non-toxic indium phosphide (InP) quantum dots (QDs) were synthesized by the solvothermal method and then embedded into dental resin to tune the emission color of the resin. Cell viability was investigated with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and a reactive oxygen species (ROS) assay to evaluate biological applications. The results clearly indicate that employing InP QDs in creating dental resin composites allows for the fabrication of restorative materials with biocompatible fluorescence properties.

1. Introduction

With the development of modern aesthetic dentistry in recent years, dental ceramics and composite resins have been widely used as filling materials for artificial crowns and restoration. The clinical resin restorative materials are mainly composed of a resin matrix and inorganic filler particles; thus, these materials are called composite resins. 1–4 Other additives in composite resins are used to cause polymerization, which eases the clinical operation and strengthens the durability of the materials. The restorative material can match the shade of the natural teeth to achieve aesthetic requirements by adding pigments and stains. 5 Luminophores with similar fluorescence properties are group III, IV, and V elements such as europium, cerium, ytterbium, and other rare earth oxides or organic molecules. Luminophores may be blended with the filler and then dispersed in the resin matrix. However, the fluorescence exhibited by these luminophores becomes more complex when mixed and is affected by the matrix. Different brands of commercial dental composite resins exhibit various excitation and emission spectra as well as fluorescence intensities when exposed to ultraviolet light. Restorative materials must show good stability to endure the damp environment and fluctuating temperature in the oral cavity.

Semiconductor quantum dots (QDs) have several advantages over conventional luminophores because their optical properties can be tuned by their size and shape due to quantum-confinement effects. 6–8 It is easier to perform various colours with minimal spectral overlap. Due to their broad excitation spectra, it is possible to excite all colours of QDs simultaneously with a single light source. 9 In past decade many reports have demonstrated their high potential in applications such as light emitting diodes (LEDs), 10,11 solar cells, 12 and biomedical labeling 13 because they display an excellent photostability compared with molecular luminophores. 14

In 2010, a composite dental resin (A2 shade) containing CdSe–ZnS core–shell quantum dots (QDs) was studied and the dependence of the fluorescence intensity of the dental resin composite on the QD concentration was measured. 15 However, their biological use is very limited due to the presence of cadmium as a major component in most commercially available QDs, despite being passivated by a ZnS shell. 16 Some reports have discussed the toxic effects of cadmium-based QDs, as cadmium ions may diffuse into the biological environment with time. 17–19 Moreover, as well as the possible effect on health, there are significant environmental concerns regarding the use and disposal of cadmium-based nanomaterials.

In the last two decades, research has generally shifted towards the synthesis of non-toxic group III–V QDs, particularly, indium phosphide (InP). The covalent bonds in III–V semiconductors, are robust compared with the ionic bonds in II–VI semiconductors. 20 InP has a smaller band gap (1.35 eV at room temperature) 21–23 and exhibits a wider emission range from blue to near-infrared than most of the II–VI semiconductors.

In the past, a hot injection technique was usually used in synthetic strategies because it allowed the nucleation step to be segregated from the growth step. 24 Thus, InP nanocrystals (NCs) of various sizes could be acquired using different heating processes. However, this synthetic method involves complicated reaction conditions, such as a relatively high temperature, long reaction time and vacuum atmosphere. The
solvolothermal method involves a short reaction time at a low temperature. Moreover, extremely expensive, unstable, and hazardous precursors such as tris(trimethylsilyl)phosphine and tris(trimethylsilyl)arsine are used to obtain these NCs. Recently, an effort has been made to use alternative safe P sources such as tris(dimethylamino)phosphine (P(N(CH$_3$)$_2$)$_3$) through their synthetic reactions.

This study presents the use of biocompatible InP QDs obtained by a solvolothermal reaction, with a safe P source tris(dimethylamino)phosphine (P(N(CH$_3$)$_2$)$_3$), as a fluorescence source for dental composite resins. In vitro cytotoxicities of InP QD-embedded resins were investigated by employing human gingival fibroblast cells through a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and a reactive oxygen species (ROS) assay, which were compared with those of the free QDs.

2. Experimental section

Materials
Indium(in) chloride (InCl$_3$, 99.99%), dodecylamine (98%), and tris(dimethylamino)phosphine (P(N(CH$_3$)$_2$)$_3$) (98%) were purchased from Alfa Aesar. Tri-n-octylphosphine oxide (TOPO) and chloroform were acquired from Acros Organics. Toluene, methyl alcohol, acetonitrile, and hydrofluoric acid (HF) were purchased from J. T. Baker, Mallinckrodt Chemicals, Fluka, and Riedel-de Haen, respectively.

Synthesis of InP QDs
InP core QDs were synthesized by a solvolothermal route, which used InCl$_3$, (1.81 mmol, 0.40 g) and dodecylamine (27 mmol, 6.21 mL) as the starting materials, which were mixed with 5 mL of toluene in a Teflon-lined autoclave. Tris(dimethylamino)phosphine (P(N(CH$_3$)$_2$)$_3$) (2.75 mmol, 0.50 mL) was added to the mixture in a glovebox. The autoclave was sealed and heated at 200 °C for 24 h. After cooling to room temperature, a dark-brownish InP QD crude solution was obtained. To sort the size of the InP QDs in solution, the solution was mixed with 10 mL of chloroform and 5 mL of methanol and then centrifuged to remove byproducts from the first stage. The InP QD solution was suspended in 1 mL of methanol and then centrifuged up to 7000 rpm for 5 min to generate size-selective precipitation. The isolated InP QD precipitates were dispersed in 2 mL of chloroform. This prepared InP QD solution was added into a butanol solution (25 mL) with TOPO (0.25 g) and then stirred for 30 min. An HF solution (0.2 mL) composed of distilled water (0.065 mL), butanol (5 mL), and HF (0.527 mL, 49 wt% solution), was added into the above solution and then it was stirred continually under 365 nm UV irradiation for 12 h to achieve photo-etching. These photo-etched InP QDs were isolated by adding acetonitrile (25 mL) and then collected by centrifugation (7000 rpm, 10 min). The precipitate was treated by photo-annealing, in which the precipitate was re-dispersed in chloroform and then exposed under UV irradiation for 10 h.

Characterization
The UV-visible spectroscopy absorption spectra of the colloidal InP semiconductor NCs were obtained at room temperature using a SHIMADZU UV-700 spectrophotometer with a 1 cm-wide quartz cell. Photoluminescence (PL) spectra were collected using a FluoroMax-3 spectrophotometer equipped with a 150 W Xe lamp and a Hamamatsu R928 photomultiplier tube. The nanoparticles were analyzed by X-ray diffraction (XRD) using a Bruker D2 PHASER diffractometer operated in transmission mode with Cu-Kz radiation ($\lambda = 1.5418 $ Å), Data were collected over a 2θ range from 20° to 70° at intervals of 0.02° with a counting time of 30 s per step. The photoluminescence (PL) quantum yield (PLQY) of InP core QDs dispersed in chloroform was calculated by comparing their integrated emissions with that of rhodamine 6G ethanol solution (PLQY of 96%).

Preparation of the resin
The homogenized resin was inserted into the cavity of a Teflon matrix in a single step and interposed between glass sheets to prevent bubbles and obtain a smooth surface. The resin was polymerized with a LED source (MR. LIGHT, cordless type, Dent Zar, LA, USA). LED light with its emission centered at 470 nm (B type, 500 mW cm$^{-2}$ to 700 mW cm$^{-2}$) was applied directly to the resin composites for 40 s on both sides.

Preparation of InP QD-embedded resin
InP QDs were blended with an A2 enamel shade composite dental resin (Filtek™ Z350 XT, 3M ESPE, St. Paul, MN, USA) to simulate the emission spectrum of natural teeth. Different powder forms of InP QDs were obtained after the solvent was evaporated and then they were washed with 95% ethanol thrice and then embedded in the composite resins. The Teflon matrix containing a hole (10 mm diameter × 2 mm deep) was used to standardize the QD-doped resin samples. After weighing the desired amount of resin and QD powder, the powder and the uncured resin were mixed and homogenized in a dim room with the aid of a spatula. The homogenized resin was then inserted into the cavity of the Teflon matrix in a single step and interposed between glass sheets to prevent bubbles and then embedded in the composite resins. The Teflon matrix was polymerized with the same LED source. The LED light was applied directly to the resin composite for 40 s on both sides.

Cytotoxicity assay
The cells used in the cytotoxicity test were human gingival fibroblasts (GFs). The GF tissue was removed from the surface of an extracted premolar, rinsed twice in a phosphate buffer saline (PBS) supplement, and placed in tissue-culture dishes. The GFs were incubated in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS), 100 U mL$^{-1}$ penicillin, and 100 mg mL$^{-1}$ streptomycin (Biological Industries Israel Beit Haemek LTD.) at 37 °C under 5% CO$_2$ and 100% humidity. The sixth-passage cells were used for this experiment. After weighing the desired amount of A2 shade composite resins and 1 mg of QD powder, the powder and the uncured resin were mixed as previously described to obtain a sample, 8 mm wide and 1.2 mm thick. The ratio of the disc...
surface area to the medium volume was 1.386 cm² mL⁻¹, which was within the range (0.5 cm² mL⁻¹ to 6.0 cm² mL⁻¹) recommended by the International Organization for Standards and other researchers. After incubation and subculture, the GF cells were plated in 96-well cell culture dishes with 1 × 10⁴ cells per well (100 μL per well). The old medium in the 96-well dishes was removed after 24 h and rinsed with PBS. The 24 h-aged medium was then placed into the wells such that the cells were incubated with the aged medium (n = 6 for each group). The negative control group was placed in the fresh medium only (n = 6). After incubation for 24, 72 and 120 h, the succinate dehydrogenase (SDH) activity of the cells was obtained using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cell viability of the experimental groups was expressed as the absorbance ratio compared with the negative control group.

Reactive oxygen species (ROS) assay

The production of intracellular ROS was measured using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to form the non-fluorescent compound DCFH-DA. DCFH-DA is rapidly oxidized to form the highly fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of ROS. The DCF fluorescence intensity is believed to be proportional to the amount of intracellularly formed ROS. The human gingival fibroblast (GF) cell incubation and subculture as well as the sample treatment were the same as for the MTT assay. After incubation for 24 h, the cells were stained with 10 μmol of DCFH-DA for 30, 60 and 90 min at 37 °C, detached using trypsin–EDTA, washed with PBS, and then immediately subjected to a flow cytometric analysis. The ROS production of the experimental groups was expressed as the absorbance ratio compared with the negative control group.

3. Results and discussion

Characterization of InP

InP core QDs were solvothermally synthesized at 200 °C for 24 h and exhibited a broad size distribution due to the simultaneous nucleation and growth during the solvothermal reaction. Size-sorting of the as-prepared InP QDs was conducted by incremental addition of methanol into the InP QD crude solution dispersed in a chloroform–methanol-mixed solvent and then centrifuged. InP QD fractions with the largest to the smallest sizes were collected by repeated size sorting, and denoted as InP1, InP2 and InP3. Their respective absorption spectra are shown in Fig. 1(a). Due to their unusual optical and electronic properties that are tunable with size, the excitonic absorption peaks were at 567, 530 and 460 nm, indicating that size sorting was satisfactorily performed. A series of InP QD fractions were photo-etched and consecutively photo-radiated under identical conditions. Normalized photo-luminescence (PL) emission spectra of the QD samples at 614 nm (denoted as InP1), 553 nm (denoted as InP2) and 520 nm (denoted as InP3), and as well as the corresponding photo-

<table>
<thead>
<tr>
<th>InP</th>
<th>PL wavelength (λ, nm)</th>
<th>Quantum yield (η, %)</th>
<th>Particle size (nm)</th>
</tr>
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<tbody>
<tr>
<td>InP1</td>
<td>614</td>
<td>6.2</td>
<td>2.9</td>
</tr>
<tr>
<td>InP2</td>
<td>553</td>
<td>8.0</td>
<td>2.6</td>
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<tr>
<td>InP3</td>
<td>520</td>
<td>4.7</td>
<td>2.1</td>
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graphs of red to green-emitting QDs under UV irradiation, are shown in Fig. 1(b). Table 1 shows that the PLQYs of the QD samples varied, between 4.7% to 9.8%. All of the size-sorted InP QDs exhibited low PL emission intensities. Through HF photochemical etching using a 365 nm multiband hand-held UV lamp, the QDs began to brighten and their emission was almost saturated after an etching period 10 h. As is well explained in the literature, upon the dramatic PL emission enhancement of InP QDs after HF photo-etching treatment, the phosphorus (P) dangling states on the surface serve as luminescent centres.
The average sizes of the InP QDs were estimated by Zunger et al., they proposed the relation of \( E_g = 1.45 + \frac{37.295}{D^{1.16}} \) between the InP QD diameter \( (D, \text{in units of Å}) \) and energy gap \( (E_g \text{in units of eV}) \) through a local-density approximation calculation instead of the well-known effective mass theory and found that the calculated band gaps of InP QDs were in agreement with the experimentally determined ones. By plugging the excitonic absorption peak energies of QD InP1, InP2 and InP3 (which are 2.18, 2.33, and 2.69 eV, respectively) into the equation, the QD diameters of each InP were calculated to be 2.94, 2.62 and 2.10 nm, respectively. The high-resolution transmission electron microscopy (HRTEM) image of the InP QDs in Fig. 2(a) shows that the InP QDs have spherical shapes with clear fringes. The average size of the QDs is around 2.5 nm \( ± \) 0.7 nm. These TEM observations are in fairly good accord with the above calculated diameter values. The lattice fringes with a \( d \)-spacing of 3.38 Å, as shown in Fig. 2(a), match the interspacing of the (111) planes of the InP bulk crystal. The XRD pattern further confirmed that the InP exhibits a zincblende structure, which is in accordance with the JCPDS card No.10-0216 shown in Fig. 2(b).

**Characterization of InP-embedded resin**

The InP QDs were encapsulated with dodecylamine the hydrophobic carbon chains of which are completely compatible with the resin. The InP QD powders were obtained after the solvent was evaporated and they were washed thrice with 95% ethanol. After weighing the desired amount of Filtek™ Z350 XT resin (denoted as Z350) and QD powder, the powder and the uncured resin were mixed and homogenized in a dim room with the aid of a spatula. The homogenized resin was then inserted into the Teflon matrix cavity in a single step and interposed between glass sheets to prevent bubbles and obtain a smooth surface. The resin was polymerized with a LED source. This process is illustrated in Scheme 1.

The photoluminescence (PL) properties of the InP-embedded resin were also investigated by comparing the QD-incorporated resin with the Z350 resin, as shown in Fig. 3(a). The shape of the PL spectrum was changed by embedding InP2 or InP3. The PL of the InP3 composite shows a small shoulder around 520 nm, indicating that the contribution from InP3 is green. The InP-embedded resin evidently changed the shape of the PL spectrum of the resin. The PL spectrum of the composite shows a hump around 553 nm, indicating that the contribution from InP2 is yellow in color, as shown in Fig. 3(b). The PL intensity of the resin decreased. This decrease may be due to the reabsorption from the resin by the InP QDs. The Commission Internationale de l’Eclairage (CIE) chromaticity coordinates of the Z350 resin, InP3 QD-embedded Z350 resin (denoted as InP3–Z350), and InP2 QD-embedded Z350 resin (denoted as InP2–Z350) calculated from the emission spectra are shown in Fig. 4(a). The CIE chromaticity coordinates of InP3–Z350 and InP2–Z350 are located in the purplish-blue region at (0.2406, 0.2456) and (0.2773, 0.3133), respectively. The emission color of the Z350 resin is blue, with color coordinates of (0.2007, 0.2043). Along with corresponding photographs under room light, photographs of the Z350 resin, InP3–Z350 and InP2–Z350 under UV irradiation are shown in Fig. 4(b). Therefore, we can successfully tune different emissions of the resin by embedding various InP QDs.

**Cell viability assay of the resin containing QDs**

The in vitro cell viability presented in Fig. 5(a) was obtained to investigate the biosafety of the resin containing QDs to evaluate potential for biological applications. The human gingival fibroblast (GF) cells were incubated with Z350, InP2 QDs and Z350 containing InP2 QDs (denoted as InP2–Z350) for 24 h. The cytotoxic effect of the resin-based InP QD composite was evaluated by the widely established 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay performed with GF cells. This cell line was selected because the resin containing InP2 QDs is to be placed in the oral cavity. The reported irreversible binding of formazan to nanofilaments was found to be negligible under our assay conditions, that is, dissolving formazan crystals in a solution containing dimethyl
sulfoxide. Consequently, the method exhibits high sensitivity compared with direct counting of the cells in the dishes by microscopic methods. However, GF cell cultures treated for 24, 72 and 120 h in parallel with the resins and InP QDs were compared by determining the intensity of the MTT signals to validate the results presented in Fig. 5(a).
The cell viabilities of Z350 (92.96 ± 2.17%), InP2 QDs (90.96 ± 6.97%), and InP2–Z350 (99.59 ± 6.12%) were observed after 24 h. Moreover, after 120 h treatment, the cell viabilities of Z350 (94.71 ± 9.89%), InP2 QDs (95.57 ± 6.40%), and InP2–Z350 (98.95 ± 1.85%) were obtained. The MTT signals decreased in all treated samples compared with the untreated cells because the MTT assay measures the combined effects of cell proliferation and metabolic activity of the cells and has been reported to be prone to artifacts under certain experimental conditions.30 The cell viability of the resin containing QDs remained above 90% and showed no significant difference (ANOVA; \( p > 0.05 \)). This result indicated that QD-incorporated resin was non-toxic toward the GF cells. Some studies have also identified InP QDs as highly biocompatible and biodegradable.21,31

In addition, the results of Z350, InP2 QDs and InP2–Z350 on the reactive oxygen species (ROS) production levels in GF cells were analyzed using the fluorescent probe DCFH-DA. The DCFH oxidation level was measured by intracellular ROS every 30 min for 90 min. As shown in Fig. 5(b), DCFH-DA is esterified into DCFH inside the cytosol, and intracellular ROS oxidize DCFH intoDCF. The lower fluorescence intensity indicates less DCFH-DA oxidation, which corresponds to less intracellular ROS production. Compared with the control group, Z350 and InP2 showed slightly lower fluorescence after 30 (91.77 ± 2.32% and 93.33 ± 5.45%), 60 (98.09 ± 5.21% and 95.05 ± 7.04%) and 90 min (101.30 ± 5.84% and 96.37 ± 8.39%), indicating lower ROS production. The InP2-Z350 composite led to more ROS production (96.33 ± 5.45%, 101.94 ± 6.47% and 105.19 ± 5.51%) than the control group, but this was not a significant difference (ANOVA; \( p > 0.05 \)). Thus, this result clearly indicates that the Z350 XT resins blended with InP2 QDs are biocompatible toward GF cells.

4. Conclusions

We characterized the fluorescence of dental resins containing InP QDs as luminophores. The fluorescence peaks of dental resin composites can be tailored by incorporating InP QDs. Additionally, in vitro cytotoxicity of the InP QD-embedded resin was investigated by employing human gingival fibroblast cells through a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and a reactive oxygen species (ROS) assay, which was compared with that of the free QDs. The results of the cell viability studies indicate that the InP QD-embedded resin didn’t show cytotoxicity. Therefore, employing InP QDs in the creation of dental resin composites allows for the fabrication of restorative materials with biocompatible fluorescence properties.

This work introduces a new bio-friendly material that and can be wildly utilized as a restoration material in aesthetic dentistry. The material was made via a facile approach, and is an applicable restoration material. Therefore, these results will have broad appeal to those interested in dental materials and biological chemistry.

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