The Effects of CdSe/ZnS Quantum Dots on the Photosynthesis Rate of the Chlorella Vulgaris Beads

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Abstract

Photosynthesizing microalgae produce more than 50% of oxygen in the atmosphere and are crucial for the survival of many living systems such as coral reefs. To address the declining of coral reefs, artificial reefs have been introduced to encapsulate the algae cells in a polymer matrix but the effects of nanoscale pollutants on these engineered systems have not been fully understood. In this work, quantum dots with a size smaller than 10 nm are being used to elucidate the photosynthesis performance of the sodium alginate beads encapsulated with Chlorella vulgaris (C. vulgaris). The fluorescent quantum dots can move into the alginate matrix and the fluorescence intensity in the algae beads is correlated with the quantum dot concentration. We further show that the photosynthesis of the algae beads are sensitive to the quantum dot concentration and are also time sensitive. In the first 48 min of quantum dot exposure, both carbon dioxide absorption and oxygen production are low, suggesting limited photosynthesis. After the initial incubation, the photosynthesis rate quickly increases even though more inhibition is still observed with higher concentration of the quantum dots.
**Introduction**

Green algae, such as *Chlorella vulgaris* (*C. vulgaris*), has shown great potential to be used in pollution control\(^1\), biofuel\(^2\), and dietary supplement\(^3\). Containing chloroplasts, algae can photosynthesize and produce half of the atmospheric oxygen\(^4\). They are also crucial to the marine systems via the formation of symbiotic relationships with other organisms, particularly coral reefs. Coral reefs play an essential role in shoreline protection by reducing the wave energy by 97%\(^5\). However, the reefs have decreased by ~50% since 1950 due to rapid global warming, thus increasing the risks of global natural hazards\(^6\). A recent study has shown that heat-evolved algae can endure elevated temperatures and enhance coral bleaching tolerance to marine heat waves\(^7\). To further enhance the photosynthetic efficiency of coral reefs and potentially create artificial living reefs to combat climate change, 3-D printed structures containing algae cells have been introduced to mimic the morphological features of coral reefs\(^8\)^9. Alginate hydrogel shows great promise as the scaffold material, in which the cells are viable for several days without nutrition\(^10\).

Another emerging challenge for the coral reefs and future engineering living reefs is the continuous exposure to chemical waste, especially at nanoscale\(^11\)^12. Early results reported decreases in cell growth rate and chlorophyll content when the cell cultures were exposed to nanomaterials such as quantum dots\(^13\), nanoplastics\(^14\), and oxide nanoparticles\(^15\). On the other hand, gold nanoparticles can enhance photosynthesis in microalgae by transferring light into photogenerated electrons. A 42.7% increase in the carotenoids has been reported with this method\(^16\). To date, most of the research on nanomaterials/microalgae interactions has focused on cell cultures in solution, and little has been investigated with the immobilized microalgae cells that can be constructed as engineering living systems.

Here, we use quantum dots as a model to study the interaction of nanostructures with engineering living systems by encapsulating *C. vulgaris* in sodium alginate beads (Figure 1a). We show that more quantum dots are presented in the algae beads as the quantum dot concentration in the solution increases. We apply the bicarbonate indicator and gas chromatography-mass spectrometry to study the effect of quantum dots on algae photosynthesis. In the first 48 min after incubation, the photosynthesis is significantly inhibited by
the presence of quantum dots. However, the photosynthesis rate increases after the initial incubation, suggesting that some of the quantum dots might be released or digested by the algae cells. At 120 min, algae beads incubated with quantum dots show less oxygen production than the samples without quantum dots, and the oxygen production is inversely correlated with the quantum dot concentration. The results indicate that the microalgal-based living systems are sensitive to the environment with nanoscale pollutants.

Materials and Methods

Materials: C. vulgaris beads were obtained from Algal Research Supply and were manufactured using Sodium Alginate and Calcium Chloride\(^\text{17}\). The bright field and TEM image of a dissected bead are shown in Figure 1b and 1c, respectively. Algal cultures were purchased from Algal Research Supply and grown using Bold’s Basal Medium. Streptavidin - ZnS/CdSe quantum dots (5 nm size), with 525 nm emission maxima, were purchased from Thermofisher Scientific (Figure 1d). Philips T12, 40-watt, cool white fluorescent lights were used to incubate and grow the microalgal cells on a 12hr/12hr light-dark cycle.

Incubation of microalgal beads with quantum dots: Ten microalgal beads were placed into a 200 µL PCR tube with 100 µL of 0 µM, 0.01 µM, 0.05 µM, and 0.1 M of quantum dot solution. The solution was first mixed for 2 min, then centrifuged at 1500 rpm for 15 min, followed by 90 min of room temperature incubation under a T12, cool white, fluorescent light.

Bright-field and fluorescence imaging: The microalgal bead was dissected into hemispheres and prepared on a wet glass slide with a cover. The slide was then viewed using the Bright Field mode of an Amscope XD-RFL microscope. Bright-field imaging was used to identify clusters of the C. vulgaris cells and to quantify the relative concentration of the cells. The Amscope XD-RFL was also used to image the slides fluorescently.

TEM imaging: Intact microalgal beads were first fixed with 2% glutaraldehyde in 0.2 M cacodylate buffer for 2 hr. They were then rinsed three times (15 min each) in 0.2 M cacodylate buffer. They were then postfixed in 1% OsO\(_4\) in 0.2 M cacodylate buffer for two hours, followed by three rinses (15 min each) in
0.2 M cacodylate buffer. The beads were then dehydrated in a series of increasing concentrations (25%, 50%, 75%, and 95%) of ethanol for 10 min at each concentration. Finally, the beads were immersed in 100% acetone for 10 min. The dehydrated beads were immersed in 3/1 acetone/Embed 812 (Electron Microscopy Sciences) for 12 hr, followed by 1/1 acetone/Embed 812 for four hr, followed by 3/1 acetone/Embed 812 for four hr. The beads were then embedded in Embed 812 for 12 hr, followed by polymerization at 60 °C for 12 hr.

Thin sections (70 nm) for imaging were made with a Leica Artos 3D ultramicrotome. The thin sections were floated on water and transferred to 150 mesh copper grids. Staining was performed with UranyLess EM stain (Electron Microscopy Sciences) for two min, followed by two 15 s rinses in distilled water. Imaging was performed with a JEOL 2010 transmission electron microscope operating at 200 kV. Images were captured with an AMT digital camera.

**Quantification of fluorescence:** The fluorescence images were quantified using Fiji ImageJ. Several areas of cells containing quantum dot fluorescence were analyzed to find integrated density. Several areas of background were analyzed to find mean background fluorescence. Thereby the ImageJ program could calculate a baseline for fluorescence which could be used to correct the average fluorescence found from the selected areas of note, using the equation:

\[
CTCF = \text{Integrated Density} - (\text{Area of selected cell} \times \text{Mean fluorescence of background})
\]  

(1)

The area of fluorescence was identified by using threshold values of 83 and 87 HSG, the values most closely matched the emission of the quantum dots, to isolate the fluorescence of the quantum dots. After isolation, the area of these regions was integrated to obtain a total area of fluorescence.

**Production of CO₂:** Stored CO₂ was generated by the chemical reaction:

\[
\text{CaCO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 + \text{CO}_2 + \text{H}_2\text{O}
\]  

(2)
Two 4-liter sealable glass containers were connected by Teflon tubing. We added 22 mL of HCl to one of the containers and followed by another 47 g of CaCO₃. The glass was then promptly sealed, only allowing gas to pass through the Teflon tubing into the second container, separating CO₂ from other products. Using a thin 100 µL Hamilton syringe, 3.6 L of CO₂ gas was extracted from the second container for later use.

**Results and Discussions**

The microalgae beads are large in size (diameter: 1.5 mm) and tend to sediment to the bottom of the microtube. We exploited a short and low-speed centrifuge process to facilitate the interaction between quantum dots and algae beads. As shown in Figure 2, CTCF values of 28,331 and 37,856 are achieved by two different centrifuge processes of 950 rpm for 3 min and 1,500 rpm for 5 min, respectively. While previous studies showed absorption of quantum dots in free-floating *Chlamydomonas* with only 960 rpm for 3 min¹³, fluorescence imaging of the dissected *Chlorella* beads showed minimal absorption into the cells with this protocol. Compared to *Chlamydomonas* species, *Chlorella* species have a much more resilient cell wall due to the “algaenan” outer coating of their cell walls¹⁸. Other studies reported that free-floating *Chlorella* absorbed high concentrations of quantum dots when centrifuged at 13,400 rpm for 15 min¹⁹. However, this high speed would damage the alginate substrate holding *Chlorella* cells in this study. Therefore, we selected the 1,500 rpm/5 min process for all the following experiments.

After establishing the incubation protocol, we then evaluated the fluorescence intensity of the microalgae beads versus quantum dot concentration (0, 0.01, 0.05, and 0.1 µM) by using both bright-field and fluorescence imaging. As shown in Figure 3, increasing the quantum dot concentration from 0 to 0.1 µM increases the CTCF value of the microalgae beads. The calculated CTCF value and the total fluorescence area shown in Figures 4a and 4b both present a logarithmic relationship with the quantum dot concentration. This dependence indicates that the uptake capacity for the cells decreases with the increasing amount of the quantum dots.
Next, we investigate the changes in the rate of photosynthesis at different concentrations of the quantum dots through a bicarbonate indicator solution. This solution comprises a pH indicator and HCO$_3^-$ ions. At higher levels of CO$_2$, the number of bicarbonate ions in the solution increases, resulting in a decrease in pH and a corresponding color change from orange to yellow hue. In contrast, lower levels of CO$_2$ reduce the number of bicarbonate ions and thus increase pH. The solution color changes to a purple or blue hue. The characterization starts with the preparation of PCR tubes with 100 µL of bicarbonate indicator and 25 µL of CO$_2$. The tubes were then mixed for 30 s, causing the red-hued indicator to turn yellow. Afterward, five beads were added to the PCR tubes, and the tubes were subsequently sealed and placed under a cool white fluorescent light for 2 hr. Every 30 min, the vials were mixed at low speed for 30 s and then photographed. As shown in Figure 5, the rate of pH change in the solution is lowered with increasing quantum dot concentration. Although qualitative, this visual observation confirms a negative correlation between quantum dot concentration and the rate of algae photosynthesis.

A Shimadzu gas chromatography-mass spectrometry was used to quantify the concentration of CO$_2$ and O$_2$ in the microalgae beads solution. Thirty-six chromatography vials were used to measure the concentrations every 24 min for 2 hr. Five beads with different concentrations of the quantum dots (0 µM, 0.01 µM, 0.05 µM, 0.1 µM) were added to the chromatography vials, and each vial was filled with 200 µL of water. Two hundred microliters of CO$_2$ were added to the first set of vials at time 0, after which the vials were sealed and placed under a fluorescent lamp. At 24 min, 200 µL of CO$_2$ were added to the second set of vials, and then they were placed under a fluorescent lamp. This process was repeated for all sets of vials until the last set produced at 120 min. The vials of the last set were filled with 200 µL of water but not placed under the fluorescent light. After 120 min, all vials were moved away from light and then taken to measure in the chromatography. The measurement of the time 0 set represents the final concentrations of gases, while the set produced at 120 min gives the initial concentrations of gases. As shown in Figure 6a, the CO$_2$ concentration present in the sample over time is positively correlated with the concentration of quantum dots. On the other hand, the percent oxygen concentration present in the samples over time is
negatively correlated with the concentration of quantum dots (**Figure 6b**). These quantitative data confirm a decrease in the rate of photosynthesis as the concentration of quantum dots increases.

The scale-up of microalgae cultivation with photosynthesis requires living systems based on microalgae cells and polymer matrix to be created with high spatial cell densities\(^8\). However, the influence of engineering nanomaterials and debris on these living systems has not been explored\(^{15,20}\). Despite that alginate and other polymer building blocks can immobilize the cells and impede the quantum dots uptake\(^{21}\), we have found that the photosynthesis of the algae beads remains significantly affected, especially within the first 48 min after the incubation. Previous studies have found a correlation between the introduction of nanostructures to aquaponics plants and the inhibition of the cell wall functions\(^{22,23}\). The quantum dots could have similar adverse effects by inhibiting the vital movement of resources needed for photosynthetic activity in the algae beads, thus indirectly causing the plants’ rate of photosynthesis to decrease. Another possible cause is that the quantum dots directly interfere with the cells’ light absorption in the matrix\(^{24}\). This mechanism would decrease photosynthesis, but not cellular respiration, unlike the inhibition of the cell walls.

We found that the photosynthetic rate increases over time for the algae beads incubated with the quantum dots, regardless of the quantum dot concentration. For example, at 72 min, the \(O_2\) level is only 0.3% for the 0.1 \(\mu\)L sample, compared to 0.8% without quantum dots. On the other hand, at 120 min, the difference between the two samples decreases to \(~0.3\)%.

As the pore size on the cell wall is 5-20 nm, the quantum dots can enter the cell wall through endocytosis to damage the cell structure, thus lowering the photosynthetic efficiency. However, the ubiquitous rate increase for all the samples indicates that the cells can adapt to the hazardous environment and still perform photosynthesis by either exocytosis or quantum dot digestion\(^{25,26}\).
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Figure 1. (a) The schematic showing the endocytosis and photosynthesis process of microalgae beads with quantum dots. (b) Bright-field image of the algae beads (scale bar: 20 µm). TEM image of (c) Microalgae cell (scale bar: 400 nm) and (d) Quantum dots (scale bar: 10 nm).
Figure 2. (a) Bright-field and (b) fluorescence image of *Chlorella vulgaris* cells encapsulated alginate beads centrifuged at 950 rpm for 3 min. The CTCF shown in (b) is 28,331. (c) Bright-field and (d) fluorescence image of the beads centrifuged at 1,500 rpm for 5 min. The CTCF shown in (d) is 37,856. The scale bar is 50 µm.
Figure 3. Bright-field and Fluorescent images of alginate beads incubated with different concentrations of quantum dots. (a) and (b): Control (0 µM). (c) and (d): 0.01 µM. (e) and (f): 0.05 µM concentration. (g) and (h): 0.1 µM. Scale bar is 50 µm.
Figure 4. (a) CTCF graphed alginate beads versus quantum dot concentration, showing a logarithmic relationship. (b) Fluorescence area graphed versus concentration, as well showing a logarithmic relationship.
Figure 5. Qualitative analysis of the rate of photosynthesis using bicarbonate indicator, for quantum dots with a concentration ranging from 0 to 0.1 µM and an incubation time of (a) 0 min. (b) 30 min. (c) 60 min. (d) 120 min. The color of the solution indicates a change in CO$_2$ concentration. The decrease in the CO$_2$ level changes the solution from yellow to purple.
Figure 6. (a) CO\textsubscript{2} and (b) O\textsubscript{2} level versus time of the alginate beads solution with different concentrations of the quantum dots.
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