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Monitoring of Swelling and Degrading Behavior of Alginate Beads using Optical Tweezers

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Abstract

Calcium alginate beads are widely used in drug delivery studies due to their high biocompatibility and the simple gelatinization process. It is well known that the alginate bead size changes in solutions with time, increasing initially and decreasing at a later stage. Therefore, it is essential to monitor or predict the size change of the beads since it affects the drug delivery efficiency significantly. We used the optical tweezers, a non-contact method, to investigate the temporal changes of the alginate beads in solutions instead of using the traditional drying and weighing technique. Responses to alginate concentration or external stimuli such as pH were also studied. The power spectrum method was utilized to estimate the trapping forces on the beads, which is related to the particle size changes. The results of our experiment indicate that the optical tweezers technique can continuously monitor the swelling and degrading of an alginate bead in an aqueous medium over hours which poses a high potential for drug encapsulation and release efficiency studies in the future.

Keywords: Swelling, Degradation, Alginate bead, Optical tweezers, Power spectral density

Introduction

Light carries linear and angular momentum. Hence, it can exert forces and torques on matters, which enables the optical tweezers to trap and manipulate a micro-sized dielectric objects at the focal point of a tightly focused laser beam[1-3]. Up to the present, optical tweezers technique has been applied to a variety of scientific areas such as single level molecular biology[4-6], cell sorting[7,8], microfabrications[9], microrhelogy[10], metallic particles[11,12].

Alginates are widely used as encapsulation carrier for cells or tissue in the development of artificial organs due to their high biocompatibility and stability[13]. Also, they pose much potential in the treatment of a variety of diseases[14,15]. Alginates are naturally derived linear copolymers with blocks of (1-4)-linked β-D-mannuronate (M) and α-L-guluronate residues (G). There is no regular repeat unit in alginate polymers and the chains can be described as a different sequence of regions termed M blocks, G blocks, and MG blocks. The ratio and sequential distribution of blocks along the alginate molecules depends on different origins (brown seaweeds). The season when the algae are harvested also affects the block composition and sequence. Water solutions of polysaccharides form hydrogels in the presence of multivalent cations, for example, Ca^{2+}, Ba^{2+}, or Al^{3+} ions, via ionic interactions between acid groups on G blocks and the chelating ions, generally Ca^{2+}. As a result, calcium alginate gels are physically cross-linked systems with mechanical properties dependent on the proportion and length of the G blocks in a given alginate chain[15-17]. The probable chelation of ions by G blocks has been described by the "egg-box" model in which each divalent ion interacts with two adjacent G residues as well as with two G residues in an opposing chain[18,19].

Alginate beads are commonly used in drug delivery studies such as drug release efficiency test from the alginate matrix during transportation to a target position. Therefore, it is important to investigate the property changes of the alginate beads as a drug or cell carrier. Many researchers have studied the degradation characteristics of the alginate beads in solution using the conventional drying and weighing technique[20]. To the best of our knowledge, however, there has been no report on direct monitoring of calcium alginate hydro-

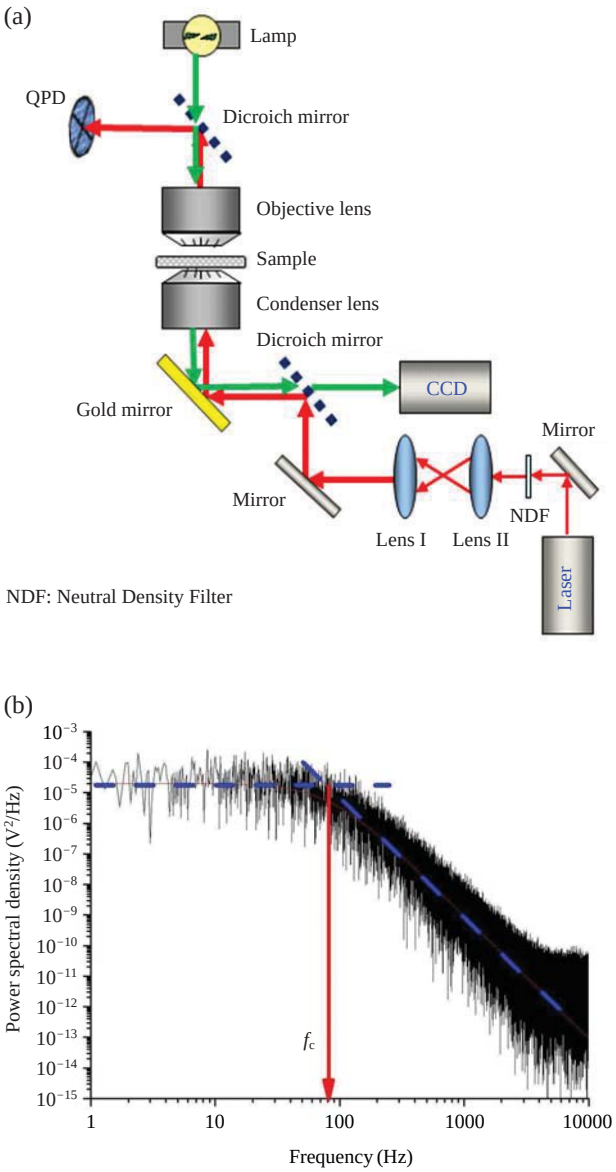

Figure 1. Optical tweezers setup (a) and power spectral density curve (b).

gel beads in solution as yet.

In the present study, we utilized the power spectrum method to characterize swelling and degrading behaviors of calcium alginate beads. The alginate beads were trapped by optical tweezers over many hours and its swelling and degrading under various conditions were continuously monitored through corner frequency measurement. It provides convenient and fast method for studying response to polymer concentration and external stimuli such as pH and so on over the conventional method of drying and weighing alginate beads.

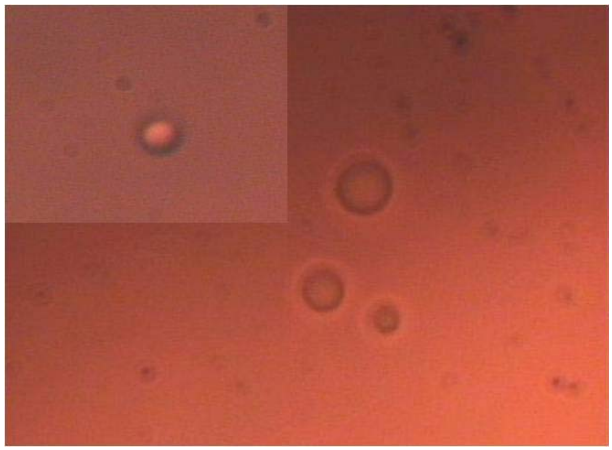

Figure 2. Images of calcium alginate beads. Inside: trapped bead using optical tweezers.

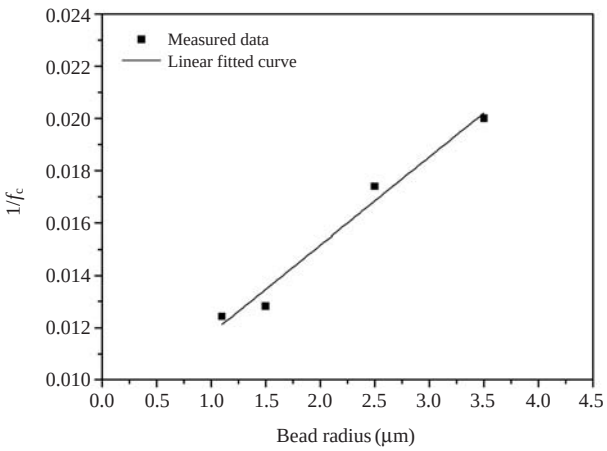

Figure 3. Dependence of polystyrene bead radius on corner frequency (f_c).

Results and Discussion

To evaluate the performance of our tweezers system and the algorithm, polystyrene beads (Bangs Laboratories, Inc., and Spherotech, Inc.) with known sizes were trapped using the optical tweezers and the results are shown in Figure 3. As expected, the reciprocal of corner frequency increases with increasing bead radius showing proportional relationship. All the beads were trapped at the same laser power of 40 mW.

It is important to trap the beads at the right distances from the boundaries such as cover glasses. When the bead is too close to the cover glass (bottom surface in this case), high corner frequency value is obtained due

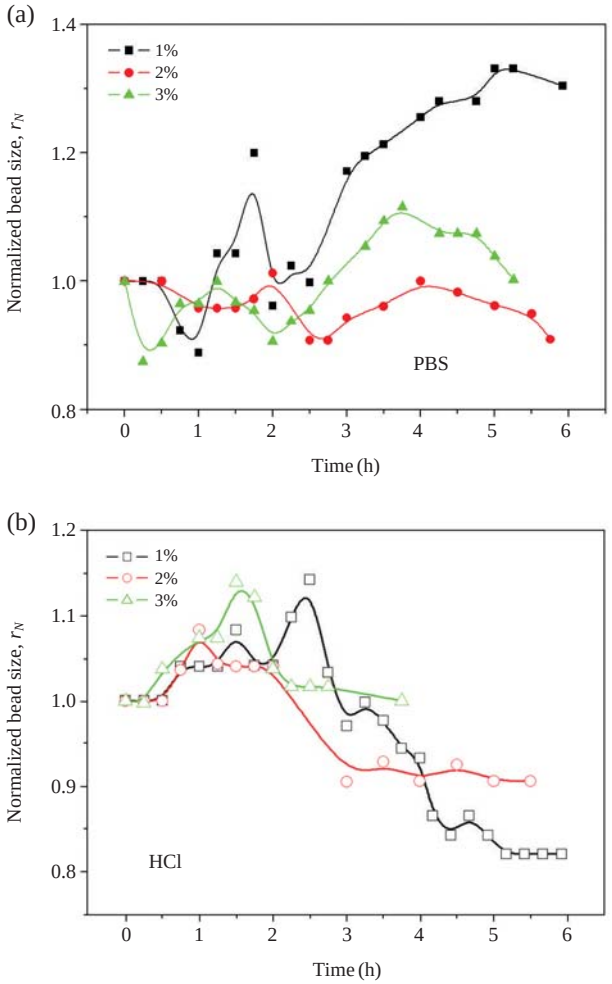

Figure 4. Swelling and degrading of calcium alginate beads during 6 hours of observation in (a) PBS (pH 7.2) and (b) HCl (pH 1.2).

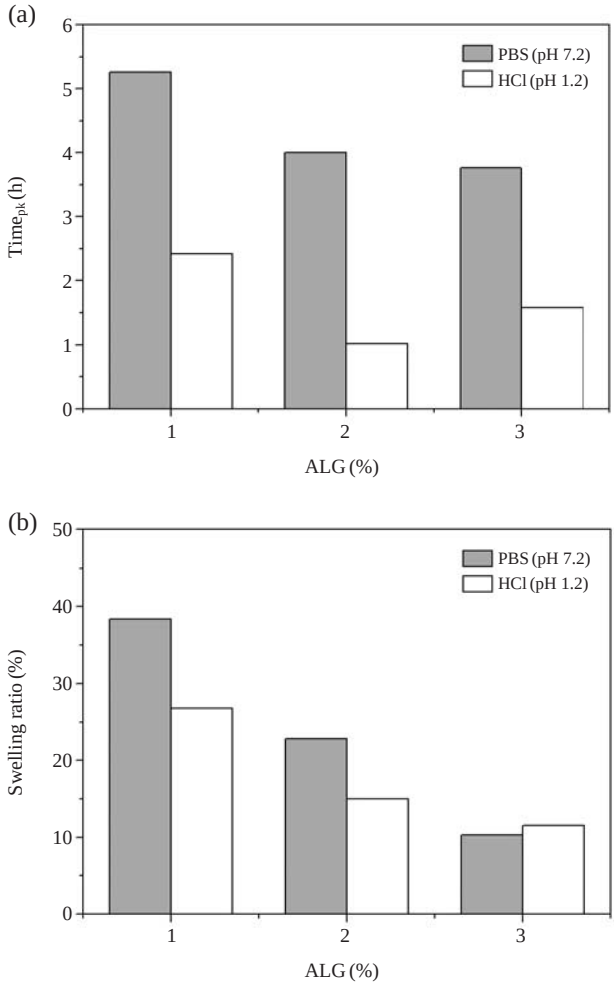

Figure 5. Dependence of beads swelling on alginate (ALG) concentration at different pH solutions, (a) peak time ($Time_{pk}$) and (b) Swelling ratio, respectively.

to friction. On the other hand, if the beads are trapped far from the surface, spherical aberration occurs because of the refractive index mismatch between oil, cover glass and water (not shown in the paper). Therefore, we trapped the beads at an optimal trap depth (approximately ten times to bead radius[22] of 1-3.5 μm) from the cover glass. We verified our hypothesis by evaluating the viscosity of the solution using the calculated corner frequency and stiffness values. Thus, the influence of trap depth on trap stiffness was ignored. The change of bead size leads to a change both in trap stiffness and corner frequency as shown in Eq. 2. The trap stiffness change mainly depends on the relative refractive index of the trapped matter and laser power, whereas less depends on the corner frequency changes[21]. Therefore, it seems convenient to describe the relationship as:

$$\frac{k}{f_c} \propto r\eta \tag{3}$$

In this way, we can relate the bead size changes with measured data assuming that the viscosity of surrounding medium is constant.

Physical and chemical structure changes of hydrogels are accompanied by a volume change as they absorb or desorb water. It is well known that the volume of the calcium alginate beads initially increases due to absorbed water and then decreases in the later stage due to degradation. Figure 4(a) shows the temporal bead size changes for alginate beads of concentrations 1, 2, and 3% in phosphate buffer saline (PBS, pH 7.2) solution. The total monitoring time was 6 hours. To evaluate the effects of the external stimulation such as pH changes, we performed the same experiment using

the same beads in HCl (pH 1.2) solutions for 6 hours and the results are shown in Figure 4(b). For all data set, the bead size change curves agree with the known data showing the initially increasing and later decreasing trends[20]. We tried to trap the beads with similar size which was measured separately via the CCD images. Small variations in bead size were normalized in our graph.

The normalized bead size (r_N) increased to a maximum value and then decreased. Here, the time for reaching the maximum swelling state is designated as a peak time ($Time_{pk}$) and the fractional size change ($[(r_{N\max} - r_{N\min})/r_{N\min}] \times 100\%$) is represented as swelling ratio. Figure 5 shows that beads swelling behavior is dependent on alginate (ALG) concentration at different pH solutions. First of all, the higher alginate concentration beads display a shorter peak time and lower swelling ratio in both kinds of solutions. Moreover, the peak time shortened over 50% comparing the beads in HCl (pH 1.2) with PBS (pH 7.2) as shown in Figure 5(a). It is indicated that faster disintegration of egg-box occurs in acid solutions. Furthermore, the swelling ratios of the beads at pH 1.2 decreased by 20% compared with those at pH 7.2 due to enhanced ionization of carboxylic acid groups in higher pH solutions as illustrated in Figure 5(b). The decrement of swelling ratio is much smaller than that reported in previous literature[20]. It is mainly because the size change measurements were performed in aqueous medium other than the traditional drying and weighing method.

All these results can be explained by taking into account the gel formation process. The Ca^{2+} in the solution would first cross-link the bead surface extensively, which results in calcium alginate beads with hard surface due to cross-linkage between G blocks and the chelating ions (Ca^{2+})[23]. When calcium alginate beads are placed in the PBS, the Na^{+} ions present in the solution undergo the ion-exchange process with Ca^{2+} ions which are binding with carboxyl groups mainly in the polymannuronate sequences. As a result the electrostatic repulsion among the ionized carboxyl groups increases, thus causes the chain relaxation and enhances gel swelling. Finally, the alginate beads begin to disintegrate when Ca^{2+} ions in the egg-box buckled structure diffuse out to the medium[20,24-26]. Therefore, the beads start to degrade. On the other hand, the number of binding sites for Ca^{2+} ions increases when increasing the sodium alginate concentration of the solution, which results in tighter gel network and produce greater cross-linkage[27,28] and consequently lower degree of swelling ratio. An appropriate alginate concentration would enhance drug encapsulation and drug release efficiency.

Conclusions

An alginate bead was successfully trapped and monitored under optimum laser power and trap depth condition using optical tweezers. The relation between polystyrene bead size and corner frequency was demonstrated, which was consistent with previous reports. This method can be used in continuous tracking of calcium alginate beads swelling and degrading in an aqueous medium as a function of time. Increasing the sodium alginate concentration of the solution leads to a tighter gel network and provides many binding sites for Ca^{2+} ions and consequently results in lower degree of swelling ratio and peak time. The swelling ratios of test beads at pH 7.2 increased significantly compared with those at pH 1.2 due to enhanced ionization of carboxylic acid groups at increased pH values. It provides convenient and fast method for analyzing response to polymer concentration and external stimuli such as pH and so on compared with the conventional method of drying and weighing beads. Therefore, optical tweezers technique shows high potential for drug encapsulation and drug release efficiency study.

Materials and Methods

Optical Tweezers Setup

Figure 1(a) illustrates the main components of our optical tweezers setup. The trapping beam (1064 nm DPSS Infrared Lasers, SDL-1064-XXXT, Shanghai Dream Lasers Technology Ltd.) is tightly focused into the sample through an oil-immersion infinity corrected objective lens (100×, 1.23 N.A., Zeiss). We employed non-fluorescent immersion oil (Cargille, Type DF) and cover glass to reduce optical noise. The spot size of laser measured at the focal plane was approximately 1 μm and the laser power was adjusted to 40 mW using a Neutral Density Filter (NDF) to avoid unnecessary heating of the sample. The beads in solution were loaded into home-made sample holder, which consists of two sandwiched 150 μm thick cover glasses separated by an acrylic chamber. The size of the holder was 65 mm long, 48 mm wide and 500 μm thick. The chamber was completely sealed with high vacuum grease to avoid evaporation and leakage. The forward scattered light from the trapped object is collected by a condenser lens positioned over the sample and projected onto a quadrant photodiode (QPD), a position sensor, to track the Brownian motion and displacement of the trapped particle. The resulting signals are then transferred to a computer for analysis through a GPIB

interface. An alginate bead is trapped and the sum and the differential signals from the QPD are tracked and analyzed by means of power spectral density curve as shown in Figure 1. A halogen lamp was used to illuminate the samples and an image of the trapped particle was obtained by using a CCD camera. The measurements were performed at intervals of 15 minutes.

Power Spectrum Method

Power spectrum method has been used to determine trap stiffness of an optical tweezers which is related to the size of the trapped particle. It is well known that Langevin equation governs Brownian motion of the trapped bead. According to the Langevin equation for a solution, the theoretical Power Spectral Density (PSD) of a trapped particle is given by the function[21].

$$S_x(f)=\frac{k_BT}{\gamma\pi^2(f_c^2+f^2)}, \quad (1)$$

where T is the absolute temperature, k_B the Boltzmann constant and f the frequency. The corner frequency f_c of the Lorentzian is related to the trap stiffness, k, as follows:

$$f_c=\frac{k}{2\pi\gamma}. \quad (2)$$

The drag coefficient γ for a sphere is known from Stokes law $\gamma=6\pi\eta r$, η is the viscosity of the surrounding medium (water), and r the radius of optically trapped bead. By fitting the experimental PSD with Eqs. 1 and 2, we could find the corner frequency.

Preparation of Hydrogel Alginate Beads

Sodium alginate (61% L-guluronic acid), calcium chloride, and other reagents were purchased from Sigma-Aldrich Ltd. and used without additional purification. Alginate beads were prepared as follows. Sodium alginate powder was dissolved into three kinds of concentration (1%, 2%, and 3% (w/v)), respectively. An amount of 1.2 g calcium chloride was melted in 50 mL isobutanol at 60˚C for 6 hours. Then, 50 mL oleic-acid was added to $CaCl_2$ solution and stirred at 120˚C for 12 hours in order to enhance roundness of beads. The oleic acid-calcium chloride solution (0.1 M) was prepared in Petri dish. The alginate solution was loaded into syringe and sprayed to the $CaCl_2$ solution under gentle stirring at room temperature. Finally, we rinsed the alginate beads with ethanol twice to eliminate impurities and washed them with distilled water for three times. Finally the beads were diluted to 1%. The alginate beads were suspended in different pH solutions in this study. Figure 2 shows the CCD images of the beads. The bead size ranges from a few hundred nanometers to several hundred micrometers. Here, we used 2-3 micrometer diameter beads selectively for monitoring swelling and degrading behavior.

Acknowledgements

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