Throughput application of quantum dots for the lifespan study of *Caenorhabditis elegans*

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ABSTRACT

The progress in identifying drugs that extend the lifespan of *Caenorhabditis elegans* has been slow partly due to the tedious and labor-intensive nature of drug screening. We describe a method that will facilitate drug screening for the longevity of *C. elegans*. We labeled *C. elegans* by mixing quantum dots, FITC, or rhodamine with *Escherichia coli* as food source. The staining was localized at the intestine of nematode and was visible under a dissection microscope equipped with a fluorescence filter. The parental nematode was distinguished from its progeny by fluorescence. The intensity of emitted fluorescence by quantum dots remained 90 % of the initial intensity for more than 6 weeks, while fluorescence emitted by FITC and rhodamine decayed quickly and dropped below 60 % during the second week. The application of all fluorescence dye did not affect the lifespan of *C. elegans*. We applied this quantum dots labeling system to survey 9 different compounds for their effect on the extension of lifespan. In particular, quercetin prolonged lifespan 5 days, while cappilarisin shortened lifespan 8 days compared to normal control. The fast scoring for the potency of compounds in affecting the lifespan of nematode demonstrated the utility of this protocol.

Keywords: *Caenorhabditis elegans*, quantum dots

1 INTRODUCTION

C. elegans is a useful model system applied both in mechanistic studies and drug screening for the aging process. Genetic studies have elucidated mechanisms that govern the aging process, for example, the insulin signaling pathway which includes the genes age-1, daf-2, and daf-16. Compounds that are effective in slowing aging are likely to fight against age-related diseases. However, the progress identifying drugs that extend lifespan has been slow. High throughput screening for compounds affecting longevity will be of great interest and utility.

The normal lifespan for *C. elegans* is approximately 25 days at 20 °C. Populations of *C. elegans* exist primarily as hermaphrodites. Hundreds of progeny are generated per individual during its short lifetime. The newly born progeny mature within 24 hours and soon are indistinguishable from their parents. To keep track of the lifespan of the original nematode, daily transfer of parental nematodes is mandatory. However, constant picking and plucking of worms might create stress that may affect their normal lifespan.

To avoid this daily stress, 5'flurodeoxyuridine (FUdr) was applied at a concentration of 5mg/mL to inhibit growth of progeny. FUdr directly inhibits thymidylate synthase enzyme, leading to a block in the *de novo* biosynthesis of thymidine monophosphate from the precursor dUMP. Inevitably, application of FUdr introduces complications: FUdr induces apoptosis in mouse cells; FUdr shows clear inhibition during the early phase of the multiplication of adeno type-4 or type-2 viruses [1]. Application of FUdr is likely to retard the growth of *C. elegans* and interact with drugs to be tested.

The short lifespan of *C. elegans* makes it attractive for the development of whole-organism compound screening. There has been progress in developing high throughput screen; i.e. introduction of flow cytometry methods for the sorting and dispensing of worms onto microplates [2], or the application of fluorescent proteins and fluorescent dyes [3, 4] that monitor cellular and morphological features over time. The fluorescent pigment accumulates in the aging nematode [5]. This accumulation correlates with the lifespan of mutants [6, 7].

From genetic studies, mutants that show a prolonged lifespan also show resistance to heat shock [8], oxidative stress [9, 10], UV radiation [11], and heavy metals [12]. Screening candidate genes resistant to stress has been successfully leading to novel aging genes [13, 14].

Measuring stress resistance provides an alternative for throughput drug screening.

Alternatively, somatic cells of normal adult worms can be pulse-labeled by fluorescence dyes. The fluorescence will not transfer to progeny. Counting of surviving nematodes may proceed under a fluorescence microscope without frequent transfer of worms. The current study applied quantum dots, FITC (fluorescein) and rhodamine for in vitro labeling of *C. elegans*. Lifespan analysis was performed with fluorescence-labeled nematodes without daily picking. Quantum dot-labeled *C. elegans* provided a throughput approach for regular laboratories to facilitate the lifespan study.

2 MATERIALS AND METHODS

2.1 Strains and chemicals

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Cultures of *C. elegans* used in this research were obtained from the *Caenorhabditis* Genetics Center (CGC), University of Minnesota, St Paul. Populations of *C. elegans* exist primarily as hermaphrodites. Strains were propagated at 16 °C on nematode growth-medium (NGM) plates (Brenner, 1974) with *Escherichia coli* strain OP50 as a food source (13). Quantum dots (Qdot nanocrystals, 3 nm) were purchased from Invitrogen, USA. FITC and rhodamine were purchased from Sigma. All culture media and related chemicals including Bacto agar, Bacto tryptone, and yeast extract were purchased from Gibco Co. Other chemicals of analytical grade or higher were purchased from Sigma or Merck

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2.2 Labeling procedure and lifespan experiment

Lifespan assays were performed at 16 °C. *E. coli* were grown overnight at 37 °C in LB medium. This culture (2 ml) was used to inoculate a 100-ml culture and allowed to grow to OD600 at 0.6. The bacteria were precipitated and re-suspended in 10 ml of M9 buffer. The suspension was used to seed NGM plates. Seeded plates were dried overnight at 22–25 °C and then stored at 4 °C for subsequent use.

Adult worms were used to start synchronous aging populations by transfer of appropriate parents to fresh NGM plates preseeded with *E. coli* OP50. After 4-12 hr of egg laying, the adults were removed, and the synchronized F2 progeny were allowed to mature. This procedure was designed to eliminate possible effects of differences in parental age on progeny lifespan. The fourth larval stage or young adults were then transferred to fresh NGM plates. Animals were tapped every 2–3 days and scored as dead when they did not respond to the platinum wire pick. Lifespan was defined as the number of days that worms were alive after they were transferred to the first plate (day 1).

Quantum dots and fluorescence dye were diluted to the designated concentration in PBS and added into the *E coli* culture. Adult *C. elegans* were picked and transferred onto the growth medium containing the fluorescence label and incubated at 16 °C overnight.

2.3 Fluorescence microscopy

The observation and acquisition of fluorescent images were performed with a Leica DM2500M fluorescent microscope equipped with a Leica DFC 490 digital camera. A Leica MZ FLIII fluorescence stereomicroscope equipped with a FLUOIIITM filter system was used for the routine picking of fluorescent nematodes.

3 RESULTS AND DISCUSSION

3.1 Fluorescence-labeled C. elegans

C. elegans were fed with 2 nM quantum dots/E. coli mixture overnight and examined under a fluorescence microscope (Fig 1). Fluorescence staining was visible from anterior to posterior and localized at area corresponding to intestine. Detailed examination confirmed the intestine-specific pattern. At the anterior, the pharynx lacked fluorescence. Lumen outside of intestine also lacked fluorescence. No staining was observed with proximal gonad, uterus, or distal gonad. In fact, the gonads were well-outlined by the absence of fluorescence.

C. elegans was also stained with 2 nM FITC and 2 nM rhodamine. The fluorescent images revealed an anterior-to-posterior staining pattern but in a more diffuse manner. For FITC, the stained area was patchy and non-continuous. Area outside of intestine was also lighted up. However, the staining pattern seemed randomly diffused; thus, no specific organs were outlined by FITC labeling. The fluorescence pattern of rhodamine revealed an intermediate staining between quantum dots and FITC. Major staining was clearly defined in the intestinal area. Organs surrounding intestine were also stained but with much less intensity as distinguished from the major staining.

In general, feeding C. elegans with fluorescence probes resulted in intestinal staining. Quantum dots were confined to the intestinal area, while FITC and rhodamine were diffused into the surrounding tissues.

To examine the utility of fluorescence labeling, we performed daily transfers of the labeled C. elegans to normal growth medium and examined the nematodes under a fluorescence microscope every day (Fig 2). Quantum dot-

labeled worms remained bright at the end of week 3 while the fluorescence of FITC- or rhodamine-labeled nematodes decayed quickly in the first week. The average fluorescence intensity of quantum dots remained above 90% throughout the 3-week period while the intensities of FITC and rhodamine dropped below 60% at the end of the third week (Fig 3).

3.2 Fluorescence labeling did not affect the normal lifespan of C. elegans

C. elegans was synchronized and labeled with quantum dots, FITC, and rhodamine at 0.25 nM, 0.5 nM, and 2.5 nM respectively. Daily picking and plucking was performed to obtain viability (Fig 5). Application of fluorescence dyes did not affect mean lifespan of nematode.

3.3 Drug screening

Capillarisin, catalpol, glycyrrhizin, geniposide, isovitexin, Japanese privet, tea melanin, psoralen, and quercetin were incorporated into the fluorescence-labeled C. elegans culture to assay for their effects on lifespan. Among them, capillarisin, catalpol, glycyrrhizin, geniposide, isovitexin, psoralen, and quercetin were pure compound, while Japanese privet and tea melanin were plant extracts. Oxidative stress has been known to accelerate the aging process. These compounds/extracts are known for their antioxidant activity, thus were expected to slow the aging process. The transfer of worms to fresh plates was performed every three days to avoid a shortage of nutrients while counting surviving worms was performed daily using a dissection microscope equipped with a fluorescence filter (Fig 4). Quercetin at 100 μM extended the medium lifespan of C. elegans from 17.9 ± 0.4 days to 22.7 ± 0.7 days (Figure 6). Capillarisin applied at 100 μM shortened the medium lifespan from 13.2 ± 0.8 days to 6.1 ± 0.4 days. Isovitexin at 100 μM extended the medium lifespan from 16.8 ± 0.6 days to 19.1 ± 0.7 days, but reduced it to 15.1 ± 0.5 days at 400 μM. The highest dosage seemed to shorten medium lifespan. Other compounds did not show dosage-dependent interference on the mean lifespan of C. elegans. However, no treatment altered the maximum lifespan.

The current study provides an alternative throughput platform for drug screening. Combined with flow cytometry methods for the sorting and dispensing of worms, the current methodology is capable of development into a high throughput for general drug screening.

4 ACKNOWLEDGMENTS

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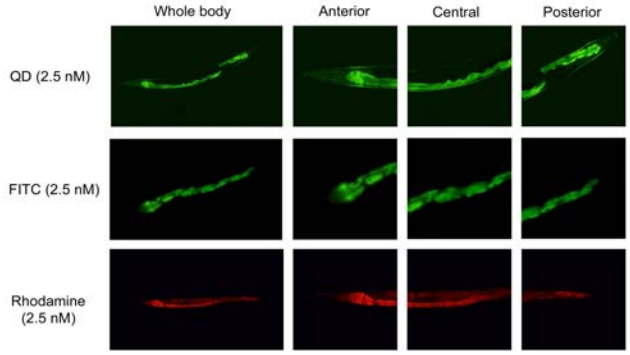

Fig 1: Fluorescence images of *C. elegans* labeled by quantum dots (QD), FITC, and rhodamine.

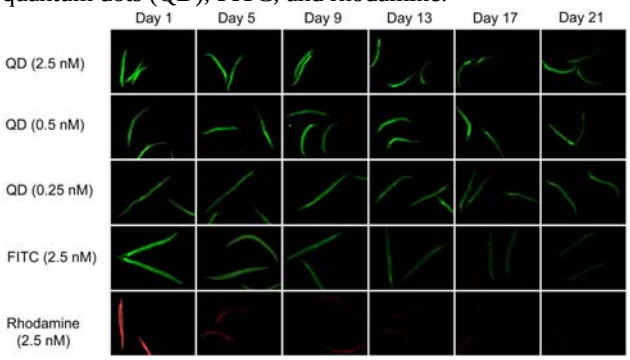

Fig 2: Fluorescence images of labeled *C. elegans* during 3-week period. The labeled nematodes were transferred to new plates daily to mimic regular lifespan operation.

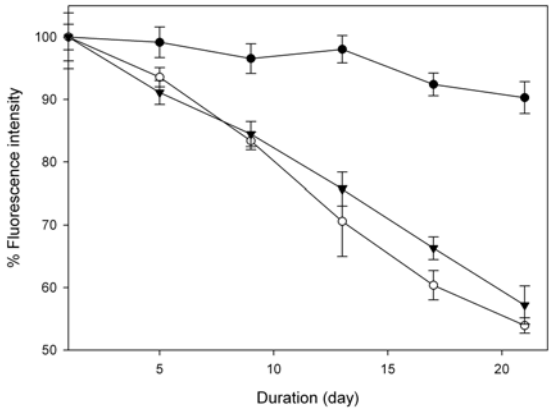

Fig 3: Intensity decay of labeled fluorescence after daily exposure to incident light. Quantum dot- (●), FITC- (▼), and rhodamine- (○) labeled nematodes were transferred daily. The data depict mean value of fluorescence intensity from 30 to 60 individuals. Standard deviation is shown as error bars.

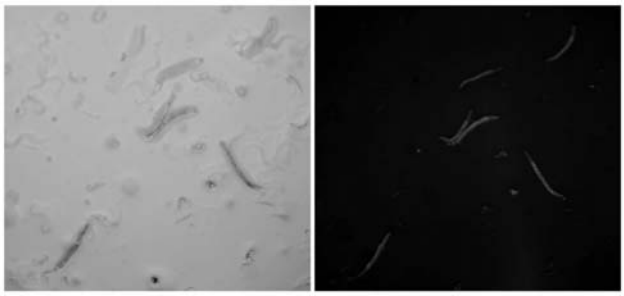

Fig 4: Images of quantum dot-labeled *C. elegans* under light microscope (lift) and fluorescence microscope (right)

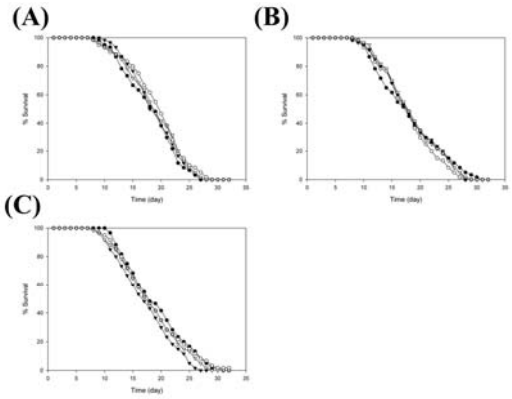

Fig 5: Lifespan study for *C. elegans* labeled by quantum dots (A), FITC (B), and rhodamine (C). Each experiment was performed at 0.25 nM (●), 0.5 nM (▼), and 2.5 nM (▽) and compared to unlabeled control (○).

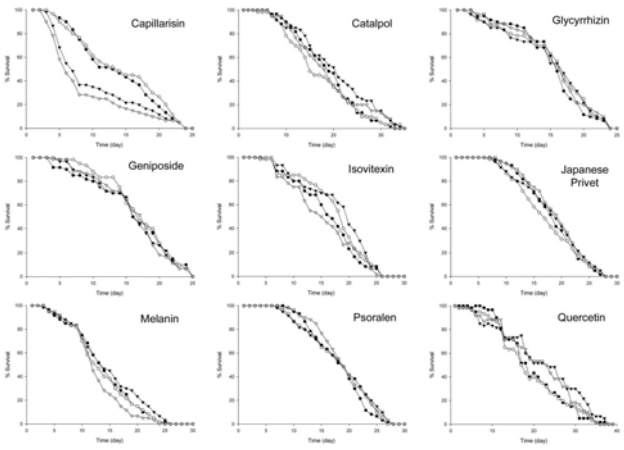

Fig 6: Lifespan study using the quantum dot-labeled *C. elegans*. Catapol, geniposide, isovitexin, quecetin, psolalin, tea melanin, glyrrhzin, capillarisin, and Japanese privet extract were incorporated into the fluorescence-labeled *C. elegans* culture to assay their effects on lifespan. For each drug, 25 μM (●), 100 μM (▼), and 400 μM (▽) were added into the *E. coli* mixture as daily nutrient for *C. elegans*. The results are compared to control group (no drug added, ○).

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