Biomaterials 42 (2015) 78–86

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# Evaluation of the influence of fullerenol on aging and stress resistance using *Caenorhabditis elegans*

Wenshu Cong [a, b, 1], Peng Wang [b, 1], Ying Qu [b], Jinglong Tang [b], Ru Bai [b], Yuliang Zhao [a, b], Chunying Chen [b, **], Xiaolin Bi [a, c, *]

[a] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[b] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China
[c] Institute of Cancer Stem Cell, Cancer Center, Dalian Medical University, Dalian 116044, China

ARTICLE INFO

*Article history:*
Received 20 September 2014
Accepted 25 November 2014
Available online 13 December 2014

*Keywords:*
Fullerenol
Anti-aging
*Caenorhabditis elegans*
Lifespan
DAF-16
Insulin/IGF-1 pathway

ABSTRACT

Fullerene derivatives have attracted extensive attention in biomedical fields and polyhydroxyl fullerene (fullerenol), a water-soluble fullerene derivative, is demonstrated as a powerful antioxidant. To further assess their anti-aging and anti-stress potential, we employed *Caenorhabditis elegans* (*C. elegans*) as a model organism to evaluate the effects of fullerenol on the growth, development, behavior and anti-stress ability in vivo. The data show that fullerenol has no obviously toxic effect on nematodes and can delay *C. elegans* aging progress under normal condition. Further studies demonstrate that fullerenol attenuates endogenous levels of reactive oxygen species and provides protection to *C. elegans* under stress conditions by up-regulating stress-related genes in a DAF-16 depend manner and improving lifespan. In summary, our data suggest that fullerenol might be a safe and reasonable anti-aging candidate with great potential in vivo.



## 1. Introduction

Oxidative stress is induced by endogenous or exogenous reactive oxygen species (ROS) which can cause damage to a series of cell components, such as nucleic acid, protein, lipid etc. Mitochondrial respiration transforms 0.4–4% consumed oxygen into a variety of reactive byproducts, especially $O_2^-$ and $^{\cdot}OH$ free radicals, and results in random deleterious oxidative damage to a variety of tissues [1]. Previous studies have shown that both ROS generation and the corresponding response to oxidative stress including expression of stress-responsive genes are key factors in determining the longevity of an organism [2,3]. The dauer mutants displaying long-lived phenomena possess increased resistance to oxidative stress and up-regulation of antioxidant genes [4]. The insulin/IGF-1 pathway is an essential pathway which controls longevity and stress resistance in nematode *Caenorhabditis elegans* [5,6]. *C. elegans* mutant of *daf-2*, the homolog of an insulin/IGF receptor, lives twice longer as wild type [7]. DAF-16, the master transcription factor in the regulation of longevity and an important target of insulin/IGF-1 pathway, is required for the lifespan extension of insulin/IGF-1 pathway mutants of *C. elegans* [8,9]. DAF-16 can translocate into nucleus and regulate the transcription of a plethora of genes functioning at stress resistance and lifespan, such as superoxide dismutase, heat-shock protein, metallothionein [8,9].

Free-living *C. elegans* is a versatile model system widely used in biomedical and toxicological research [10,11]. Its short lifespan, similar aging process as in human, conserved genetic information and signaling pathways make *C. elegans* an ideal model for aging assay [12]. Since aging is characterized by progressive degenerative changes in tissue organization and functions, feasibility of measuring age-related changes in neuromuscular behaviors such as pharyngeal pumping and biochemicals such as lipofuscin accumulation adds advantage to *C. elegans* [13]. Additionally, high-

* Corresponding author. Institute of Cancer Stem Cell, Cancer Center, Dalian Medical University, China. Tel./fax: +86 411 86110150.
** Corresponding author. CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, China. Tel.: +86 10 82545560; fax: +86 10 62656765.
*E-mail addresses:* chenchy@nanoctr.cn (Chunying Chen), bixl@dlmedu.edu.cn (X. Bi).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2014.11.048
0142-9612/

throughput assays are convenient to be performed because large population of *C. elegans* is easy to get. Loss-of-function or gene-knockout mutants can be achieved by genetic manipulation [14].

Novel nanomaterials such as semiconductor nanocrystals, carbon-based materials (fullerenes, graphenes, carbon nanotubes) and so on have attracted extensive attention in biomedical applications over the past decades due to their unique properties [15,16]. Fullerene and its derivatives, known as spheric carbon molecule with a unique cage structure, can function as the strong radical sponge [16,17] The antioxidant activity of fullerene is several hundred-fold higher than that of other antioxidants [18] and can induce dendritic cell maturation and activate Th1 immune responses [19]. Moreover, fullerene and its derivatives have low toxicity to cells, rodents and fish [17–22]. In the biodistribution and excretion study, about 56% of total injected $^{125}I-C_{60}(OH)_x$ excretes within 72 h post dosing, in which about 92% via urine, and 8% via feces [22]. Recently, fullerene was reported to elongate lifespan of rats due to the attenuation of age-associated oxidative stress [21]. Therefore, in this study, the potential effect of fullerenol on lifespan elongation and stress resistance was investigated in *C. elegans*. Moreover, we further explored whether DAF-16, a key player in the regulation of longevity in insulin/IGF-1 pathway, was responsible for the intrinsic mechanism.

## 2. Materials and methods

### 2.1. Regents and worm strains

Fullerenol, referred to as polyhydroxylated fullerene, was synthesized as described [23] and characterized as shown in Supplementary information, S1. The existence and number of hydroxyl groups attached on fullerenol molecule are determined by IR spectrometer and XPS examination, respectively [22]. It was stored in water solution at 4 °C. Membrane-permeable non-fluorescent dye 2,7-dichlorodi-hydrofluorescein-diacetate (H2-DCF-DA) was used to measure level of intracellular ROS [24]. Juglone (5-hydroxy-1,4-naphthoquinone), a reactive oxygen species-generating compound, was used as an oxidative stress inducer [24]. Ethanol (100%) was used as a co-solvent of Juglone. Sonication treatment for 10 min was applied to the mixture to ensure solubility and validity of Juglone. Levamisole (1%) was used to anesthetize the worms during the fluorescence observation.

Wild-type Bristol N2 and all mutant strains were provided by the Caenorhabditis Genetics Center (University of Minnesota, St. Paul, MN, USA), which was

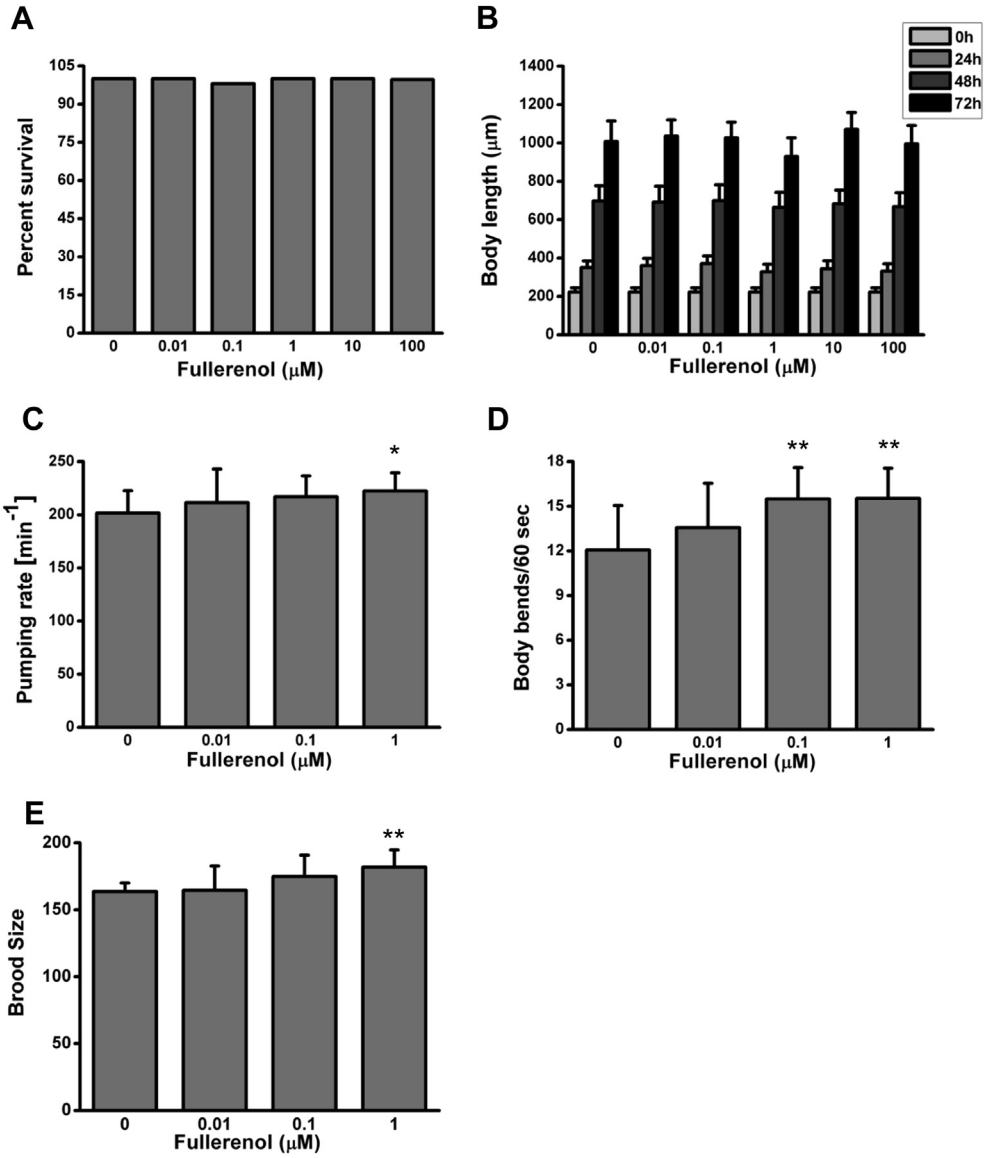


**Fig. 1.** Toxicity assessment of fullerenol on *Caenorhabditis elegans*. Effects of fullerenol on worms were shown in (A) Lethality. (B) Growth. (C) Pumping rate. (D) Body bends. (E) Brood size. N2 at L1 larva stage was treated with fullerenol for three days. Bars represent means ± SD.