



# Caenorhabditis elegans: A Model Organism to Decipher Biological Activities of Nanoparticles

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## Abstract

In recent decades, the range of nanoparticles that have been authorized by many biomedical agencies globally has increased exponentially. In their therapeutic and treatment potentials, nanoparticles have gained greater importance in drug delivery because of their carrying capacity, stability, and specificity. Thus, understanding the broad spectrum of nano-bio interactions and the challenges of the interface may provide new opportunities for novel designing of nano-based materials for different biological applications. *Caenorhabditis elegans* (*C. elegans*) as a model organism has gained popularity, and its feasibility as an in vivo model has also been extended to nano-biotechnology. In the process, understanding the basic biology of *C. elegans* as a model system before the elucidation process of various biological activities of nanoparticles is critical for productivity and translational capability. The applicability of different high-end chemical-based and material science techniques in studying modes of nanoparticle exposure and their routes of absorption, bio-distribution, and excretion has made the process of nano-bio interaction studies in *C. elegans* model more accurate and informative. Furthermore, major factors affecting nano-bio interactions which might hinder the interactions of the model system such as the alterations in media, growth conditions, chemical composition, etc. are highlighted. The final discussion pertains to commonly reported biological activities of different nanoparticles using this model organism such as antioxidants, antimicrobial, toxicity studies, etc. The advantages and disadvantages of different reported activities of nanoparticles as elucidated from this model organism are also elaborated.

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D. B. Siddhardha et al. (eds.), *Model Organisms to Study Biological Activities and Toxicity of Nanoparticles*, [https://doi.org/10.1007/978-981-15-1702-0\\_8](https://doi.org/10.1007/978-981-15-1702-0_8)

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**Keywords**

Nanoparticle · *C. elegans* · Nano-bio interactions · Factors affecting the interactions · Techniques · Biological activities

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## 8.1 Introduction

Modern advances in nanoscience and nanotechnology fields have seen faster development of various novel applications of nanoparticles ranging from electronic appliances to pharmaceutical industry and biomedical sciences. Nanotechnology represents new ways to perturb cells and treat patients, through novel designing of specific nano-based therapeutics that can target diseased cells directly, bypassing unwanted biological barriers. In this process, elucidation of these effects requires functional characterization of the different nano-bio interactions in a complete in vivo model organism. This will allow for an in-depth understanding of the broad spectrum of interactions in an active living organism. Thus, knowledge obtained after its safety validations in vivo would pave a way for the productivity of new nano-based materials in the market for various useful applications.

In the field of biomedical sciences, researchers are currently employing the unique design of these entities in a size range of 1–100 nm for their potential in improving novel targeted therapeutics and for accurate diagnostics. Thus, priorities should be made to identify routes of exposure and absorption and the consequences from their biological effects either after short- or long-term exposure. The basic framework for understanding nano-bio interfaces represents many challenges. An approach in measurement and detection of the interactions in nanoscale range with single-cell requires exquisite sensitivity both in vitro and in vivo. Similarly, it is equally important to have an in-depth understanding of the pharmacokinetics and pharmacodynamics of the whole process right from its uptake, bio-distribution, and translocation to excretion. Thus, translating these proofs of concept in nanotechnology from a controlled laboratory environment to widespread usage will require extensive testing and validations.

In terms of practicality, model organisms have always played a vital role in the field of discovery and research. The popularity and fitness of *Caenorhabditis elegans* (*C. elegans*) as an in vivo model in screening and evaluation of nanoparticles have increased exponentially in recent years (Zhao et al. 2013). The ability of worms to self-fertilize and generate large numbers of progeny aided with the presence of complex tissue systems is ideal for different biological studies in terms of both mechanistic and high-throughput screening approaches.

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## 8.2 *Caenorhabditis elegans*: A Model Organism

*Caenorhabditis elegans* (*C. elegans*) was introduced in 1965 by Sydney Brenner as the whole model organism for studying developmental genetics. Since then, the utility of this model animal has been expanded to explore diverse areas of modern

biology and allied sciences. It is a free-living, soil-loving nematode (roundworm) measuring 1 mm in length. It is non-parasitic and likes to live and thrive in temperate/warm soil environments. In the laboratory, it is maintained and cultured on nematode growth media (NGM) plates and fed with *E. coli* OP50 as diet source (Fig. 8.2). The body of these animals is transparent that enables tracking of an individual cell and cell lineages. Due to its shorter life span, it's well suited for aging studies and is also widely used in neurological and host-pathogen interaction studies as a model organism (Horvitz and Sulston 1980). As a host model, it has been employed in studying microbial and parasitic infections. Similarly, *C. elegans* is at the forefront of high-throughput screening (HTS) for evaluations of biological activities of various chemicals, drugs, and nanoparticles (Brenner 1974; Brinke et al. 2011).

### 8.2.1 Origin

*Rhabditis elegans* was the initial name of *Caenorhabditis elegans*, and this name was coined by Maupas in 1900. In 1952, Osche later placed it in the subgenus *Caenorhabditis* which was later promoted to the generic status in 1955 by Dougherty. N2 wild type was an isolate reference that was first obtained from mushroom compost of England by L. N. Staniland in 1965. A laboratory strain was obtained from Bristol culture by Sydney Brenner in 1964 from Dougherty. It is assumed to share a common ancestor with humans, 500–600 million years ago during the pre-Cambrian era (Fig. 8.1).

### 8.2.2 *C. elegans* Tissues and Body Plan

Despite its simple body, *C. elegans* has some well-characterized and well-defined tissues. The worm body is a tubular structure, with a cuticle forming the external surface and skeleton necessary for its movement. Its basic body system which includes the nervous, alimentary, reproductive, and excretory systems is protected by a collagenous cuticle. The alimentary system of *C. elegans* consists of organs like the mouth, pharynx, intestine, rectum, and anus. The digestive system is organized in the form of a tube covering the whole length of the animal and is made up of a pairwise arrangement of 20 long polyploidy epithelial cells. The excretory system is considered to regulate osmolality and waste elimination from the body. It has muscles, a hypodermis, a cuticle (body)-protective covering, connective tissues, and basement membranes. Gaseous exchange and nutrient supply occur through the body cavity by passive diffusion especially through the cuticle layer of the gut surface, since they lack circulatory system (Altun et al. 2009; Kerr 2006).

In a mixed population of *C. elegans*, both male (XO) and the self-fertilizing hermaphrodite (XX) sexes are seen. After the L4 stage, the worm becomes an adult egg-laying organism of 1 mm long with ~959 cell nuclei, of which 302 are neurons. On the other hand, males have 1031 cell nuclei and can be produced rarely at about 0.1% spontaneously from nondisjunction of the X chromosome and/or up to 50% of the outcross progeny from a mating between a hermaphrodite and a male worm.

**Fig. 8.1** Taxonomy of *C. elegans*

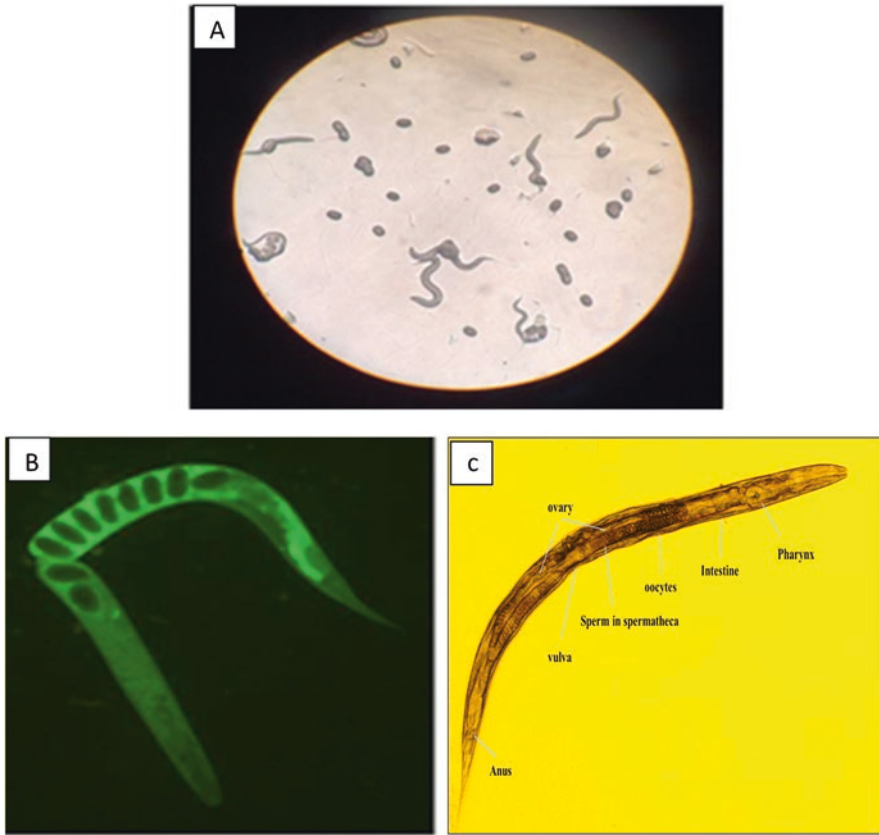
<b>KINGDOM</b>	<b>Animalia</b>
<b>PHYLUM</b>	<b>Nemata</b>
<b>CLASS</b>	<b>Secernentea</b>
<b>ORDER</b>	<b>Rhabditida</b>
<b>FAMILY</b>	<b>Rhabditidae</b>
<b>GENUS</b>	<i>Caenorhabditis</i>
<b>SPECIES</b>	<i>elegans</i>

Slight modifications in reproductive systems of hermaphrodite and male are observed with hermaphrodite having a uterus, gonad, spermatheca, and vulva (Sternberg 2005), whereas the male has gonad, cloaca, vas deferens, and seminal vesicle (Emmons 2005). Although both adult male and hermaphrodite have the same body length, phenotypically they can be differentiated with the presence of male worm with unique features such as blunt tail with rays, the spicules, the hook, a proctodeum, and a thinner body (Fig. 8.2).

Inside the alimentary tract lies a pseudocoelomic fluid called coelomocytes which houses the main organ systems besides its roles in endocytosis and fluid balance (Corsi et al. 2015). Furthermore, due to the lack of circulatory system, this pseudocoelomic fluid performs additional homeostatic roles ranging from lubrication of different tissues, trace metal enrichment of fluid, and maintenance of hydrostatic pressure balance in the worm body. It also serves as a medium for cell-to-cell communication, signal networking, and the passive diffusion and transport of O<sub>2</sub>, CO<sub>2</sub>, and nutrients (Wood 1988). Well-defined tissues with similar architectures of organs like intestines, muscles, pharynx, and hypodermis and gonads of higher organisms are also present in *C. elegans*. Thus, their presence allows for understanding the specific molecular interactions of different nanoparticles that can perturb/affect cellular physiology. Subcellular changes can also be used as parameters for an in-depth understanding of the process involved (Garigan et al. 2002; Herndon et al. 2002; Golden et al. 2007; Haithcock et al. 2005).

### 8.2.3 Reproductive System

The existence of two sexes, which include the most common hermaphrodite (XX) and the rare occurrence male (XO), has given rise to the phenomenon of sexual dimorphism. A highly sexually dimorphic tissue in the worm is the reproductive system which differs between hermaphrodites and males. The space for fertilization and laying eggs is provided by the hermaphrodite, and this is divided into three distinct major parts consisting of the somatic gonad, germline, and egg-laying



**Fig. 8.2** Images of *C. elegans* depicting (a) mixed worm population, (b) transgenic green fluorescent-tagged adult hermaphrodite worm, and (c) bright-field image of adult worm showing organs and tissue systems

apparatus. The germline of an adult is well organized into a distal space corresponding to the distal end and the proximal space which lies near the embryo exit point from the worm. As they move to the proximal part, they can enter into different stages of meiosis. The somatic gonad comprises the spermatheca, gonadal sheath, distal tip cell, and uterus. The egg-laying apparatus is made of the vulva and the muscles of the uterine. Hermaphrodites are considered to be the self-fertilized female because the soma is female. However, the germline produces male gametes in a fixed number before the production of female gametes (L'Hernault 1997; Schedl 1997). They produce ~300 embryos through self-fertilization process. During copulation, male-derived fertilization takes place, and maturation occurs in the spermatheca (Singson 2001). A fertilized egg then passes onto the uterus, and the egg-laying apparatus makes the egg to move out through an opening ventrally called the vulva.

### 8.2.4 Alimentary System

The complex system in the worm's anatomy is the alimentary system with various tissues and cells (White 1988). The connections between the body and the alimentary system network are less but direct. The worm has a cylindrical body wall with an epithelial tube divided by a pseudocoelomic space which is parallel to the gonads. The system has anterior and posterior regions. The pharyngeal epithelium connecting the arcade cells of the lips is the anterior region, and the posterior region ends up in the rectal epithelium and anus. This system which comprises 127 cells is also divided into 3 major parts such as the foregut, midgut, and hindgut. During molts, the stomodeum and proctodeum (parts of the foregut and midgut) line along with the cuticle (Bird and Bird 1991). The stomodeum has the openings for the pharyngeal glands, while the proctodeum has the openings for the rectal glands. *C. elegans* intestine shares similar cellular architecture with higher animals in respect to cell polarity of the intestinal cells (enterocytes), apical and basolateral domains, cell junctions, and microvilli forming the brush border. Ingested food materials reach the intestine through anterior pumping and peristalsis of the pharynx, and the excretory materials are eliminated out through the anal opening. The muscles of the body also have a role in controlling the internal pressure and concentration of contents in the guts before excretion of waste from the body (Bird and Bird 1991). The excretory system is a unicellular tubular system comprising three different individual unicellular tubes such as the canal, duct, and pore connected to form a continuous lumen in the body (Nelson et al. 1983).

### 8.2.5 Nervous System

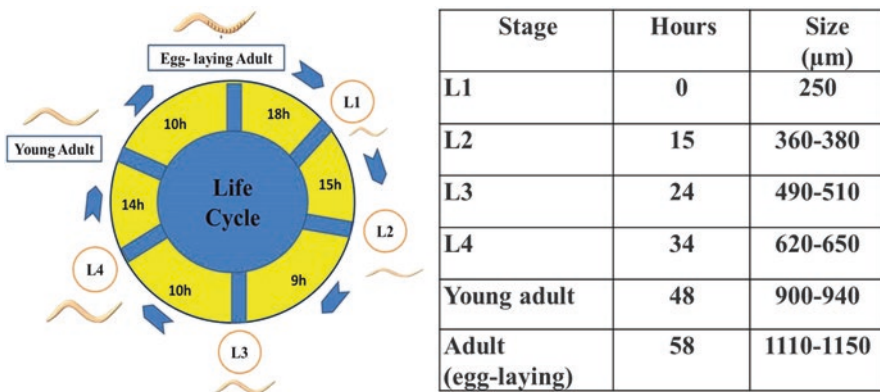
The hermaphroditic adult nervous system is comprised of 302 neurons that can be subdivided into 3 unique and independent systems, with a larger somatic system of 282 neurons and a smaller pharyngeal nervous system (Ward et al. 1975; Sulston and Horvitz 1977; Sulston et al. 1983; White et al. 1986). Majority of neurons in the somatic nervous system lie between the hypodermis and body muscles separated by the basal lamina. On the other hand, pharyngeal neurons are directly located in the pharyngeal muscles and are not divided by any basal lamina. Worm neuronal system has 1500 neuromuscular junctions, 900 gap junctions, and 6400 chemical synapses (Durbin 1987). The male population has 473 additional cells, 79 neurons, and 36 supporting cells than the hermaphrodites. These additional cells have roles in enhancing the male mating behavior (Sulston et al. 1980; Emmons and Lipton 2003; Emmons 2005). This nervous system can manage complex behaviors along with basic behaviors such as feeding, defecation, locomotion, etc. (Bono and Villu Maricq 2005). It also allows the animal to find the presence of nearby diffusible sex pheromone signals or simply to sense changes in the O<sub>2</sub> levels (Jeong et al. 2005). Both sexes have some sex-specific behaviors such as egg-laying and mating behaviors (Schafer 2005).

### 8.2.6 *C. elegans* Life Cycle

Perhaps, the best utility of this organism is due to its ease of growing and maintenance in the laboratory besides its simple and short life cycle. The reproductive life cycle of *C. elegans* is about approximately 72–120 h, and its life span is about 2–3 weeks under proper living conditions at 20 °C. During its life cycle, it has to undergo through four larval stages of development designated as L1–L4, and its reproductive phase is temperature-dependent (García-Sancho 2012). Embryonic development proceeds with the generation of an L1 larva which is about 0.25 mm long and is made up of 550 cells; later 131 cells die during the developmental process through apoptosis (Elmore 2007). On maturity, adult worms become fertile for 4 days, and each adult hermaphrodite can lay ~200–300 eggs. Alternatively, when the prevailing environmental condition is not favorable (i.e., insufficient food, temperature changes, and overpopulation), the late L1 stage nematodes can enter into dauer stage. In this stage, worms require lesser nutrients and are resistant to environmental stresses. When favorable condition returns, it reenters the normal life cycle directly to the L4 stage from the dauer stage (Klass 1977; Kenyon et al. 1993; Williams et al. 2017) (Fig. 8.3).

### 8.2.7 Homologous Genes and Genetic Manipulation

The first organism to be sequenced at the multicellular level (1998) was *C. elegans*. With approximately more than 20,000 genes and with the genome size of more than 100 million base pairs, there are substantial numbers of overlaps and conservative regions among *C. elegans* and human genes (Hodgkin 2005). It also exhibits greater levels of conservatives with other vertebrates in terms of gene functions and metabolic pathways. Algorithm-based studies have reported that greater than 60% of these genes are homologous to humans (Kaletta and Hengartner 2006). These characteristics make it a suitable model organism to study functional genomics and to



**Fig. 8.3** Life cycle of *C. elegans* at 20 °C with different body growth and development parameters as a function of time

get knowledge of the genotypic and phenotypic relationships at a genomic level globally. Furthermore, these insights on multiple biological processes and roles can be correlated to human disease genes which are homologous of *C. elegans* (Bird et al. 1999; Corsi 2006; Walhout 2006).

In a multicellular organism, an important penetration into functions of genes comes from the prevailing environmental conditions during which a gene gets expressed. At the forefront, gene expression studies and analysis in *C. elegans* have several advantages over other species. The availability of its entire genome sequence has provided a simplified functional genomic approach, where manipulations like forward and reverse genetics can be carried out with ease. Strategies that can generate valuable information on expression patterns of various genes in correspondence to different manipulations and amelioration studies have been facilitated. Additionally, by tagging a gene of interest with a reporter gene of green fluorescent protein (GFP), real-time monitoring of expression patterns can be carried out both qualitatively and quantitatively. These approaches of in vivo GFP labeling methods can also give accurate information on the localization of the translated product of a particular gene which can be visualized in live worms. As a result of the Promoterome project, GFP fusions with promoters are being created on a genome-wide scale that led to the development of promoter fusions up to 2 kb from the nematode genes (Dupuy et al. 2004). Another commonly used approach to find the unknown functions of a gene comes from the usage of available *C. elegans* mutant strains. The RNA interference (RNAi) and sequence-specific degradation of homologous messenger RNAs produced by double-stranded RNA were also regularly used to inhibit a particular gene function in *C. elegans*. Understanding of worm's biology through gene manipulation techniques like RNAi knockdowns, reporter gene assays, and protein-protein interaction networks has contributed to the proper understanding of basic and translational biology immensely (Timmons and Fire 1998).

## 8.2.8 High-Throughput Screening

Employing model organisms in high-throughput screening (HTS) is a useful strategy to facilitate the screening of genes or molecules related to basic biology or disease pathogenesis in humans. *C. elegans* is ideal for HTS screens because a large number of worms can go in a single well, of its transparent body, and it is easy to grow and manipulate. This allows for the design and in-depth dissections of fundamental and conserved biological processes like apoptosis (Ellis et al. 1986) and gene regulation by small RNAs (Fire et al. 1998; Wightman et al. 1993). A recent study using HTS suggests that the intensity of the fluorescent dye Nile red (lipid stain) corresponds with the rate of aging in the animals (O'Rourke et al. 2009). Worms with lesser Nile red signal age more slowly, and animals with increased Nile red signal live shorter than the wild type. Analyses of these HTS experiments were done using two types of imaging techniques including bright-field and fluorescence (FL) imaging. Bright-field images notify about developmental and gross anatomical



defects, and FL images display the distribution of Nile red dye in the worm's body (O'Rourke et al. 2009).

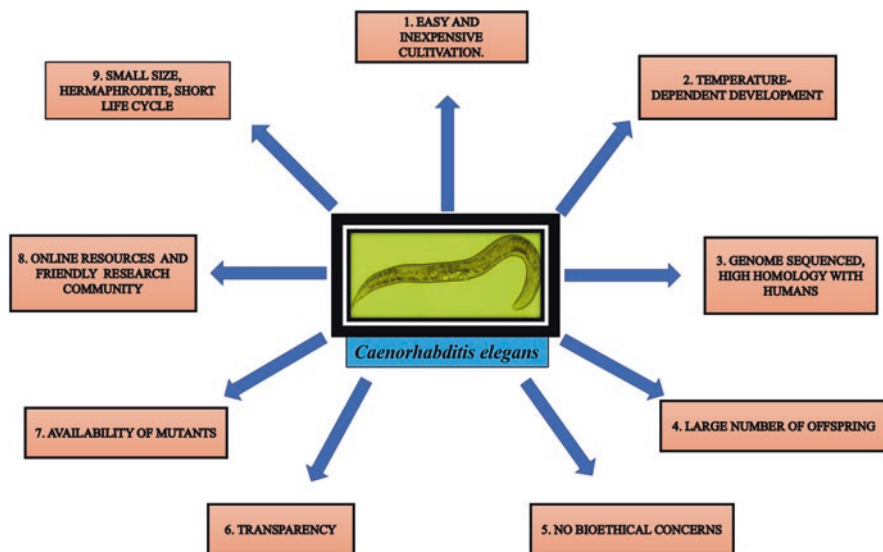
An all-liquid workflow to promote HTS in *C. elegans* in a 96-well form was also developed (Lehner et al. 2006). This method was originally intended to promote genome-wide RNAi screens in a high-throughput mode but now has wider applications. For example, the first HTS approach using *C. elegans* was done in 2006. Kwok and co-workers used worm transfer units of a Complex Object Parametric Analyzer and Sorter (COPAS™ BIOSORT, Union Biometrica) and semi-automated image recovery to screen approximately 14,100 smaller molecules. Molecules were evaluated for different bioactivities by assessing morphological deformities, including slowness of growth, lethality, improper movement, and other phenotypes in wild-type animals. Using this technique, ~308 bioactive molecules were identified. Since this was the first example of HTS screen using *C. elegans*, the screening was done on agar plates (24-well), and phenotypes were marked visually. Furthermore, to enhance the pace and development in *C. elegans* genetic research, libraries covering almost 94% of the 20,000 genes have been established and are also available for researchers (Kamath and Ahringer 2003; Rual 2004; Lamitina et al. 2006).

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### 8.3 Practical Considerations for *C. elegans* as In Vivo Model

Significant delays in our understanding of the different biological activities of different nanoparticles exist. When tested in higher mammalian in vivo models, serious considerations that hinder the process include a shortage in the number of experimental animals, time factor, cost-effectiveness, and ethical procedures. Importantly, with no ethical constraints and ease of cultivation, *C. elegans* counteracts the limitations of many other in vivo models favorably. In the laboratory setting, an invertebrate model such as *C. elegans* has the advantage of getting numbers of animals assigned for the experiment because each adult hermaphrodite can reproduce about ~300 progeny with a life span of only 2–3 weeks. Thus, end numbers of different animals with different stages can be rapidly generated at the minimum cost and time involved. The ease of culturing the worms in either liquid or solid NGM and the minimum requirement of the non-pathogenic bacterium *E. coli* OP50 strain as a standardized food source have further reduced the cost involved. Additionally, for long-term usage, cultures can be easily stored and preserved at –80 °C in 96- or 384-well plates.

The blend of small size (1 mm in adult hermaphrodite) and transparent body of *C. elegans* is also ideal for the normal optical microscopic study even to the single-cell level. Their transparency permits visualization of their various anatomical structures even without dissection and permits colored materials to pass all over their body even without staining (Contag 2002; Pomper and Lee 2005). Due to their smaller size, worms in large numbers can be put in a single petri dish or 96- or 384-well plates which fits high-throughput experiment needs (Stiernagle 2006). Recently, based on their micrometric size from embryo to adulthood, microfluidic platforms have been developed where microfluidic device enables for real-time monitoring of the different parameters of the worms' population. This is advantageous from different aspects



**Fig. 8.4** Diagram depicting common features of *Caenorhabditis elegans* that are ideal for investigating different biological activities of nanoparticles

of time and precision. For example, toxicological profiling of a number of test materials can be done in a high-throughput and computerized manner (Altun et al. 2009; Hulme and Whitesides 2011; San-Miguel and Lu 2013) (Fig. 8.4).

Most importantly, because of its multicellular nature and the availability of complex multiple organ systems, the chances of identifying a particular nanoparticle target and its interactions with biological molecules can be correlated. Further confirmation studies can be carried out in the complex multicellular organisms like humans. For example, with its functions and composition similar to that of human skin, the external part of worms epithelial and cuticle layer can be used as a simple epidermal model to assess nanoparticle routes of entry and absorption and for an in-depth analysis of its positive or negative effects (Chisholm and Xu 2012). Similarly, the *C. elegans* intestinal cellular architecture, patterning, and growth allow for the detailed examination of delivery or entry of nanoparticles orally (Bossinger and Hoffm 2012). Therefore, *C. elegans* is a sensitive and feasible whole animal model system to study the interactions of nanoparticles with various biological barriers like cuticle and intestine and through various levels of organizations ranging from a single cell to the whole organism (Fig. 8.4).

## 8.4 Nano-Bio Interactions: Features of *C. elegans*

Understanding the different interactions of various nanoparticles with multicellular organisms is still in progress. Thus, assessment of interactions between different nanoparticles and *C. elegans* often serves as information-rich resources for an

in vivo behavior and biocompatibility of different nanoparticles. Processes such as uptake, distribution, aggregation, surface adherence, and agglomeration on the exposure of *C. elegans* to nanoparticles have been regularly monitored. Similarly, the roles of different physicochemical properties of nanoparticles on its interactions with *C. elegans* have been reported.

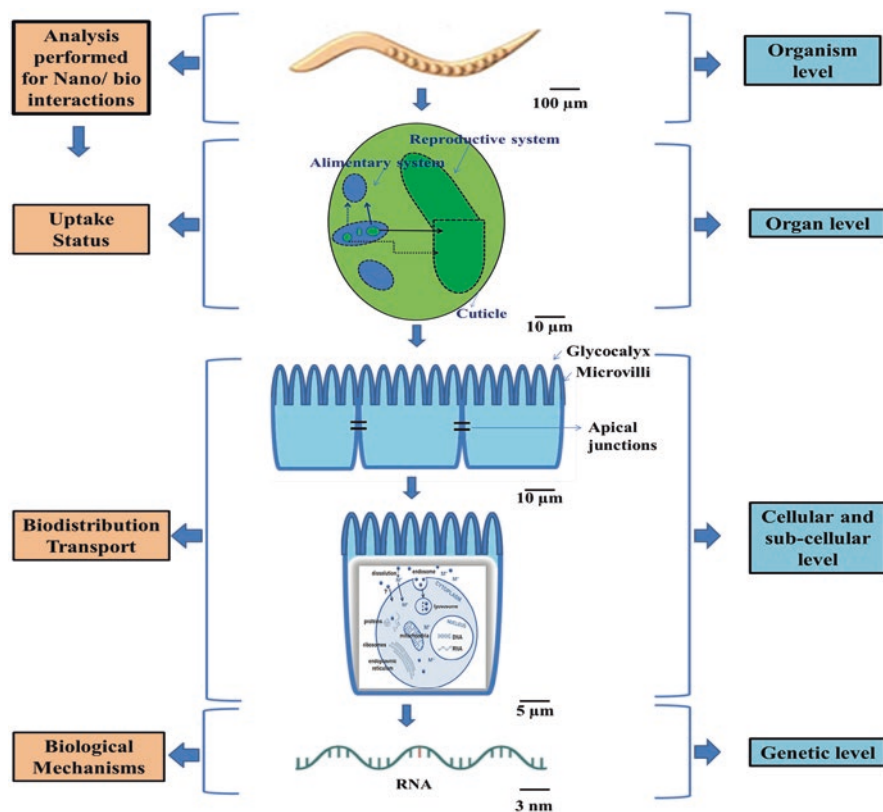
#### 8.4.1 Exposure of Worms

Exposure of worms to nanoparticle is an essential step in studying nano-bio interactions of various nanoparticles starting from the time of its exposure till excretion. Different exposure routes for *C. elegans* can be either in the form of a direct mode of oral feeding, through topical applications, or in some organ-specific cases through microinjection. This exposure can also be in the form of acute and chronic exposure and high-dose and low-dose regimen. Thus, choosing the correct exposure route, concentration, and time is a critical step in determining successful nanoparticle bio-interactions when it is carried out for the first time. Practically, the number of worms required for the study can be obtained by rinsing the worms from NGM plates with Milli-Q water or any buffer of choice. Worm pellets can be concentrated with low-speed centrifugations to the desired number of the worm population before nanoparticle exposure. For larger-population exposure, 24- or 96-well plates with equal volumes in each well can be used to check for the general uptake and bio-distribution of particles (Moragas 2016) (Fig. 8.5).

#### 8.4.2 Uptake, Absorption, Translocation, and Bio-distribution

The uptake of the nanoparticles mainly depends on their physicochemical properties like surface chemistry, size, and shape. In *C. elegans*, nanoparticles are commonly taken through the alimentary system during feeding. Additionally, some studies have shown that nanoparticles can diffuse in worms directly through the cuticle, vulva, anus, and excretory pores (Scharf et al. 2013). In *C. elegans*, the outermost layer of the cuticle contains a negative charge due to the presence of glycoproteins. These negative charges can electrostatically interact with a nanoparticle, which influences its effect on entry into *C. elegans* (Fig. 8.5). For example, superparamagnetic iron oxide nanoparticles (SPIONs) can be absorbed through the worm's epithelial surface (Gonzalez-Moragas et al. 2015).

In the alimentary system, nanoparticle interacts with the pharynx. The pharynx is an encapsulated organ with two muscles, and their movements are necessary for food ingestion and pharyngeal pumping (Song et al. 2013). It was reported that nanoparticle (30 nm–3  $\mu$ m) can pass through the grinder to interact with pharyngeal epithelium where it will get either attached to it or taken up directly by epithelial cells (e.g., quantum dots) (Fang-Yen et al. 2009; Wu et al. 2018). Thus, the pharyngeal pumping rate can be used as an indicator for evaluating nanoparticle interactions with *C. elegans*. For example, silica (Si), silver (Ag), and titanium (Ti)



**Fig. 8.5** Summary of nano-bio interactions of nanoparticles in *C. elegans*

nanoparticles have been reported to interact with the pharynx that resulted in dysfunction or pre-mature reduction of pharyngeal pumping (Scharf et al. 2013; Iannarelli et al. 2016). In the reproductive system, nanoparticles generally get localized after their direct translocation from the alimentary system and rarely get ejected from the vulva. Nanoparticles were also reported to have the capability of translocation, and this, in turn, affects the progeny and reproductive system of the worms (Laromaine 2015). In the nervous system, the interactions of Si nanoparticles with the neuronal cells have led to the segregation of proteins predominantly involved in homeostasis as reported. This observed widespread protein aggregation in axons of serotonergic HSN neurons resulted in a decline of egg-laying capacity and induces internal hatch of worms (Scharf et al. 2015).

A clear comprehension of the route of absorption and modes of bio-distribution of nanoparticles in a living organism is also crucial. *C. elegans* offers an advantage through its transparency and the oral route of entry for nanoparticles. On entry, these nanoparticles pass through the alimentary path in either the presence or absence ( $\pm$ ) of *E. coli* OP50. Irrespective of solid or liquid media, there were speculations

suggesting that *E. coli* OP50 can absorb and decrease the nanoparticle concentration in the suspension. On the contrary, other experiments based on the discharge of metal ions from nanoparticles in both  $\pm$  of *E. coli* OP50 highlighted that the live bacterial metabolism has a lesser influence on nanoparticles (Gonzalez-Moragas et al. 2017).

Notably, Ag nanoparticles and quantum dots have been reported to be diffused in *C. elegans* intestine, subcutaneous tissue, gut lumen, and gonad along with the huge amount of nanoparticles observed to be deposited in the tail (Wu et al. 2014; Luo et al. 2016; Zhi et al. 2016). For those nanoparticles that can enter the membrane layers of cells, delocalization and accumulation in the lysosomes of *C. elegans* were also reported (Wang et al. 2016; Chatterjee et al. 2017; Yu et al. 2016). Some ingested nanoparticles could be seen in the digestive system, starting from the pharynx, intestinal lumen, and rectum besides other organs, like the neurons, muscles, spermatheca, and gonad by crossing their barriers (Qu et al. 2018). In some other reports applying two-photon luminescence microscopy and absorbance microspectroscopy, it was observed that Au nanoparticles ingested by *C. elegans* get accumulated in the intestine but were not internalized by the intestinal cells (Moragas 2016).

### 8.4.3 Recovery and Excretion of Nanoparticles from *C. elegans*

The role of feeding the worms with *E. coli* OP50 plays a vital role in the excretion of nanoparticles. Most of the nanoparticles remain in the body even after defecation, inferring that the worms do not have a preference for the excretion of nanoparticles. In the absence of *E. coli* OP50, the defecation process ceases which leads to the accumulation of the nanoparticles in the gut. Once the animal starts to feed on *E. coli* OP50, the nanoparticles get excreted out (Mohan et al. 2010; Le Trequesser et al. 2014). Previous studies have shown that the excretion of nanoparticles such as nanodiamonds, Fe<sub>2</sub>O<sub>3</sub> nanoparticles, and Au nanoparticles also depends on food availability and even some of the nanoparticles can increase the defecation cycle duration by 80 s. All these experimental results corroborate that caution must be taken in the evaluation of nanoparticles with or without the presence of food in the experimental setup (Fig. 8.5).

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## 8.5 Major Factors Affecting Nano-Bio Interactions in *C. elegans*

### 8.5.1 Effect of Life Span and Period of the Exposure

With worms having four different larval stages, 4 days of active egg-laying period, and a life span of around ~2–3 weeks, exposure of nanoparticles to different worm's population would yield different results. Studies have shown that L3 worms were more resistant than L1 upon exposure with cerium oxide (CeO<sub>2</sub>) nanoparticle (4 nm) (Collin et al. 2016). Similarly, the L4 or adult worms were more resistant than L1 upon exposure to titanium oxide (TiO<sub>2</sub>) nanoparticles (10 nm), and the outcome also relies on time (Zhao et al. 2015). The effects of iron oxide (Fe<sub>2</sub>O<sub>3</sub>) nanoparticles

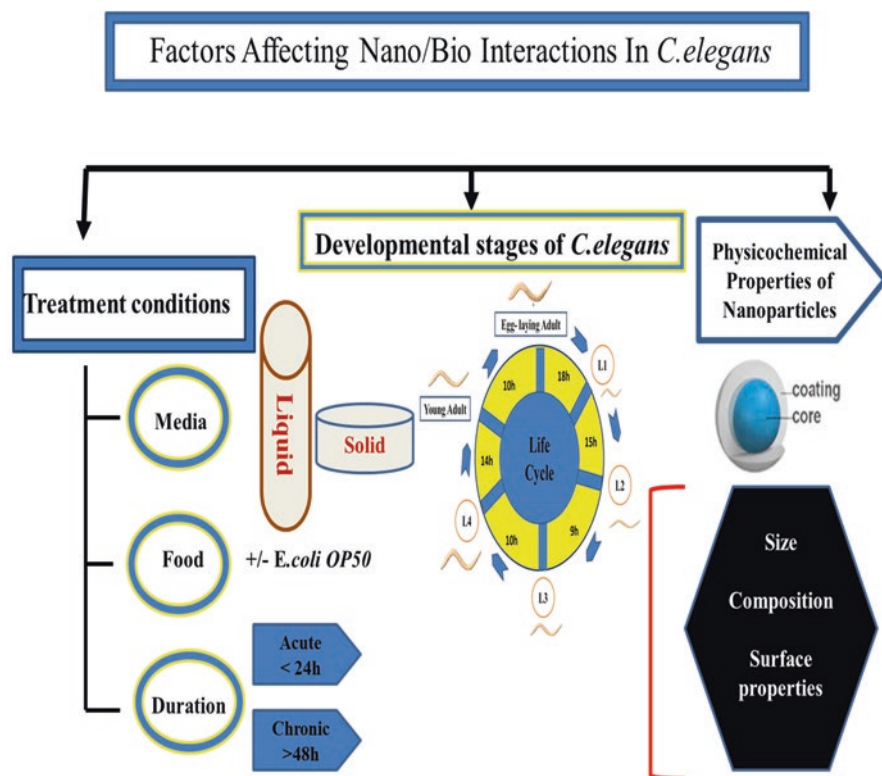
coated with dimercaptosuccinic acid (DMSA) in K-medium were shown in three assay systems such as in 24 h treatment with L4 worms, 3-day treatment (L1 to adult), and treatment from L1 to 8-day-old adult. Higher toxicity was observed with increasing time and at a concentration greater than 50 mg/ml (Wu et al. 2012). TiO<sub>2</sub> nanoparticles show variations in recovery response after acute and chronic treatment in *C. elegans* wherein chronically exposed worms ingested more particles and presented themselves with a reduction in movement, excretion, length (body), and pharyngeal pumping when compared to acutely exposed worms (Yang et al. 2014).

### 8.5.2 Effect of Exposure in Solid Media

During NGM preparation if the nanoparticles are added directly to the medium, it is difficult to confirm the uniformity of particle distribution in the medium fed with the *E. coli* OP50. The solid nature and the ionic strength of the medium can also enhance the colloidal nature of the nanoparticles. Further aggregations can prevent nanoparticles' homogenous distribution from the worms. Thus, care should be taken when the exposure medium is in the form of a solid NGM plate for non-homogenous exposure and that the desired concentration is calculated with precision. Furthermore, the role of live bacteria added as food can perturb the medium biological surface. Bacteria with active metabolism can produce variable outcomes that can change the variability of biological surfaces before and after treatment in *C. elegans*. To overcome these challenges, applications of alternative media with lesser ionic strength were suggested, and these were found to reduce nanoparticle aggregations or precipitation. Some researchers suggested for the application of K-agar over NGM agar. Its high phosphate content allows for interactions with positive charges, and this reduces its availability for unwanted interactions (Maurer et al. 2015). Similarly, acute exposures ( $\leq 24$  h) in liquid media without food or chronic exposures ( $\geq 48$  h) in liquid media (K-medium or S basal) along with food supplementation (Kim et al. 2008; Meyer et al. 2010) have also been reported (Tsyusko et al. 2012; Lim et al. 2012) (Fig. 8.6).

### 8.5.3 Effect of Exposure in Liquid Media

Liquid media have the advantages of uniform nanoparticle exposure and are ideal for different high-throughput studies. However, it can have its disadvantages because of varied ionic strengths that can be used in the maintenance of *C. elegans* under laboratory conditions (Stiernagle 2006). Generally, media with lesser ionic strength are preferred as these can help in maintaining the stability of nanoparticles (M9 buffer, S basal, or K-medium). Interestingly, some studies have shown that the Ag nanoparticle aggregation can take place in K-medium which immediately settles from suspension (Meyer et al. 2010; Ellegaard et al. 2012). The effect of ionic strength on the exposure media in Ag nanoparticle toxicity was also reported, in which lethal doses of 1.5–12-fold higher in low salt containing moderately hard reconstituted water (MHRW) were obtained when compared to K-medium. This



**Fig. 8.6** Major factors that can affect nano-bio interactions in *C. elegans* with different parameters that are commonly employed for elucidation of nanoparticle activity in *C. elegans*

decrease in toxicity due to aggregation was followed by the decrease in surface area for their distribution (Yang et al. 2012) (Fig. 8.6).

Another study has shown lesser toxicity even in the presence of salts (ultrapure water and K-medium) when the worms were treated with ZnO nanoparticles (Wang et al. 2009) conflicting to the previous studies reported. Thus, reducing the time of exposure or the ionic strength has also a major role in preventing aggregation of nanoparticles. Precipitation and aggregation of CeO<sub>2</sub> and TiO<sub>2</sub> nanoparticles in K-medium were also reported at higher concentrations used. Exposure to a lower dose without *E. coli* OP50 for 24 h is ideal for the uniformity and stability of the particle suspensions during this experiment (Roh et al. 2010).

#### 8.5.4 Effect of Organic Components/Food

There were conflicting reports on the effect of *E. coli* OP50 on the stability and activities of different nanoparticles during experimentation. The addition of *E. coli* in the medium, especially in the K-medium, was reported to have enhanced the

toxicity of Ag nanoparticles in the worms due to the increase in their bioavailability (Ellegaard-Jensen et al. 2012). Toxic effects of polyvinylpyrrolidone (PVP)-coated Ag nanoparticles in MHRW with organic matter (natural) along with the *E. coli* were also reported (Yang et al. 2014). On the contrary, some studies have shown that the addition of food can lead to a decreased toxicity of nanoparticles (Fig. 8.6).

### 8.5.5 Effect of Exposure in Standard Conditions

Exposure of nanoparticles even in standardized conditions can have different effects on different nanoparticles, and this indicates the uniqueness of a particular nanoparticle. Pluskota et al. (2009) showed that there was a monodispersion of SiO<sub>2</sub> nanoparticles and polystyrene (PS) nanoparticles in a suspended solution using fluorescence correlation spectroscopy (FCS). The effect was observed after the addition of nanoparticles as a solution along with the bacterial lawn (Pluskota et al. 2009). Polak et al. (2014) showed the different physicochemical properties of ZnO nanoparticle suspension in bacterial culture before the addition into NGM plates for nematode exposure. In LB broth, ZnO nanoparticles formed clusters (agglomerate) with time-dependent variations. Further, TEM investigations revealed that cluster formation resulted in decreased surface charge and weaker electrostatic repulsive force of nanoparticles. Additionally, these clusters did not affect the entry or morphology of bacteria but instead enhance the secretion of polymeric substances which coats the particles within 24 h to influence on their bioavailability (Polak et al. 2014) (Fig. 8.6).

### 8.5.6 Physicochemical Properties of the Nanoparticles

Smaller Ag nanoparticles (10–21 nm) were reported to have been ingested more when compared to larger-sized particle (>75 nm) in K-medium (Meyer et al. 2010). The uptake of polyethylene glycol (PEG)-coated Ag nanoparticles was shown by Contreras et al. (2014), wherein a lesser amount of Ag was internalized in worms when the worms were exposed to smaller-sized nanoparticles than the larger-sized particles. This may be due to the excretion of the smaller-sized nanoparticles. Further studies have shown that there were variations in life span and fertility of worms after frequent exposures to nanoparticles and the same treatment does not have an effect on their body length and movement (Contreras et al. 2014). Similarly, PVP Ag nanoparticles (28 nm) cause higher worm mortality than smaller-sized (1 nm) Ag nanoparticles, and this was associated with a combining effect of higher intake rates, coating, and solubility (Ellegaard-Jensen et al. 2012). On the contrary, in an assessment of Ag nanoparticles of 1–75 nm, no inhibition on growth and size was observed suggesting that there are correlations that exist between dissolved silver and its toxicity. Altogether, these investigations gave us a rough idea of



size-dependent intake, reproductive toxicity, and life span effects of nanoparticles besides its effects on motility and body size.

### 8.5.7 Chemical Composition

The translocation rate of nanoparticles also depends on their composition. Some comparative studies conducted on fluorescently labeled SiO<sub>2</sub> (50 nm), Si, and polystyrene (PS) nanoparticles have reported for the major distribution of Si-based nanoparticles in the lumen of the intestine (primary organ), while other nanoparticles like PS nanoparticles were found in secondary organs and in the cytoplasm of the immature embryos (Pluskota et al. 2009). The toxic levels of TiO<sub>2</sub>, ZnO, and SiO<sub>2</sub> nanoparticles were also reported to be fully dependent on their composition (Nouara et al. 2013) (Fig. 8.6).

### 8.5.8 Effect of Surface Coating and Manipulations

The coating of the surface can significantly alter the toxic levels of nanoparticles by hampering the nanoparticle uptake, bioavailability, and reactivity. To access control over the nano-bio interactions, modifications have to be done properly after synthesis in the animal itself or the environment of nanoparticle engineering. Studies by Collin et al. (2014) have shown the effects of surface charge on nanoparticle toxicity and its accumulation in organs and tissues using 4 nm dextran-coated CeO<sub>2</sub> nanoparticles. The neutral and negative charge carrying nanoparticles exhibit lesser toxicity when compared to positively charged CeO<sub>2</sub> nanoparticles which can be found throughout the animal's body. This finding demonstrated that the higher toxicity of positively charged nanoparticles was due to their direct interaction and disruption of the cell membrane besides an increase in their cellular uptake (Collin et al. 2014).

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## 8.6 *C. elegans* Assays for the Biological Activity of Nanoparticles

The contributions of different researchers with years and years of research in *C. elegans* have led to the development of various assays and standardized protocols that were time tested. They are ideal for the screening of different nanoparticles. Most of these assays are designed to evaluate on *C. elegans* organ systems, including the neural system, digestive system, immune system, and reproductive system (Handy et al. 2012; Marsh and May 2012; Hunt 2017). The information collected from these findings can be extrapolated and further confirmed in higher vertebrate systems. Thus, *C. elegans* is a reliable and valuable experimental biological

platform to evaluate the efficiency of a novel nanoparticle. With the availability of different organs and tissue systems, the targeted biological activity of a particular nanoparticle can also be evaluated specifically. For example, *C. elegans* intestinal enterocytes, cell junctions, and microvilli can be evaluated for the biological effect of nanoparticles on intestinal uptake and integrity. Similarly, to evaluate the effects of nanoparticles on the worm's physiology and metabolism, a simple experiment like monitoring of its pharyngeal pumping rate can be performed.

Generally, upon nanoparticle exposure, survival and mortality rate measurements are performed most frequently for determining the overall fitness and life span of *C. elegans*. This can generate a concentration-response and survival analysis curve upon comparison with the untreated control group of worms (Tejeda-Benitez and Olivero-Verbel 2016). Subsequently, emphasizing on the relevance of oxidative stress in regulating important signaling pathways and worm's biochemistry, different sensitive and effective methods are available for measuring different parameters like pro-oxidants, oxidized biomolecules, and its resistance. The effects of nanoparticles on the nervous system of the worms can also be monitored by different assays with the availability of specific neuron types tagged with GFP. Green fluorescent protein analysis protocols can directly or indirectly allow for the quantification, localization, and specific activity measurements of a particular neuron and/or set of neurons upon nanoparticle treatment. *C. elegans* also provide an ideal platform for whole-organism pathway and network analysis, genetic screening, and analysis through single and double mutant strains available. Recently, transcriptomics and metabolomics approaches have also been reported in *C. elegans* (Kim et al. 2017a). The details of these different assays are described in Table 8.1 and Fig. 8.7.

To examine nanoparticle uptake and bio-distribution, the most commonly used techniques are fluorescent microscopy and hyperspectral dark-field microscopy. Additionally, transmission electron microscopy (TEM), synchrotron-based techniques, and other analytical techniques were also employed (Table 8.2). Recently, the levels of metal exposed on *C. elegans* and their uptake and bio-distribution can also be quantified using inductively coupled plasma mass spectrometry (ICP-MS). The presence of nanoparticles can also be visualized directly in various organ systems of *C. elegans*. For example, TEM and optical microscopy can detect the presence of nanoparticles in the alimentary and reproductive systems. Scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDX) is often used for the visualization of nanoparticles in the cuticular system (Moragas 2016) (Table 8.2) (Fig. 8.7).

Modern imaging techniques like magnetic resonance imaging (MRI) and synchrotron radiation X-ray fluorescence ( $\mu$ -SRXRF) can be used to identify, locate, and characterize individual nanoparticle in the specific area of worm's body. X-ray spectroscopy and synchrotron X-ray absorption near-edge spectroscopy ( $\mu$ -XANES) are used in confirmation of the bio-distribution and status of the nanoparticles along the body of the worm (Moragas 2016). Quantification of the nano-bio interactions between mono-dispersed small and large coated or non-coated nanoparticles in *C. elegans* using two-photon luminescence microscopy (TPLM) has also been reported. Other commonly

**Table 8.1** Different *C. elegans* parameters and techniques involved in elucidation of nanoparticles' biological activity

Parameters	Assays	Biological effects	Techniques involved	Reference(s)
Survival	Lethality assay; classical life span assay; abiotic stress resistance assay; pathogen resistance assay; etc.	Life span decreased; sensitive to stress; increased death rate; sensitivity to pathogens; etc.	Microscopy; counting; staining techniques; statistical analysis; etc.	Kim et al. (2008); Van Voorhies and Ward (1999)
Development growth	Larval development assays; phenotypic and morphological assays; length, width, and density measurements; etc.	Changes in body length and width; dauer formation; abnormal morphology; altered survivability; etc.	Larval counting; body measurement; microscopy; image analyzer; statistical analysis; etc.	Wang and Xing (2009); Boyd et al. (2010); Cha et al. (2012); Rudel et al. (2013)
Behavioral assay	Locomotive assay; feeding and behavioral assay; growth endpoint density assay; etc.	Changes in measurements of head thrash; body bending frequency; and basic movements Food intake frequency, were test nematodes try to avoid contaminated food	Microscopy; counting; statistical analysis; image analyzer tracking; etc.	Matsuura et al. (2013); Jones and Candido (1999)
Reproduction	Fertility assay; egg-laying capacity; gonad size; progeny production; viability; etc.	Decreased or increased brood size; egg-laying capacity changes; changes in gonad morphology; progeny production; and germline alterations	Microscopy; counting; statistical analysis; image analyzer; etc.	Roh and Choi (2011); Cha et al. (2012)
Aging	Life span assays; lipofuscin content; physiological parameters; etc.	Changes in the intestinal lysosomal lipofuscin deposits with age; decreased or increased life span; changes in other physiological parameters; etc.	Microscopy; counting; statistical analysis; image analyzer; etc.	Pluskota et al. (2009); Cha et al. (2012); Wu et al. (2013)

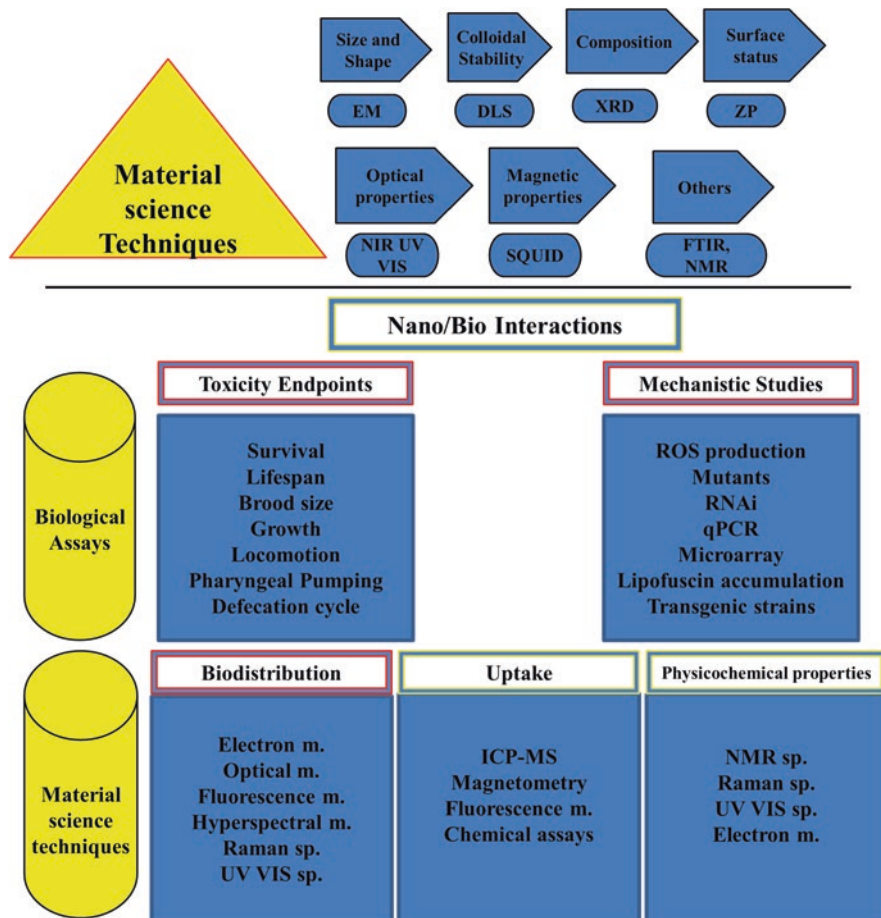
(continued)

**Table 8.1** (continued)

Parameters	Assays	Biological effects	Techniques involved	Reference(s)
Immunity	Assays to check the levels of cytokines; pro-inflammatory markers; innate immune response components; resistance to pathogens Survival assay, etc.	Changes in expression levels of different innate immune response components; susceptible to pathogen infection; etc.	ELISA; Western blots; qPCR; transcriptome and spectrophotometric analysis; etc.	Singh and Aballay (2006); Wang and Ezemaduka (2014)
Nervous	Assessment using fluorescently tagged neurons; measurement of neurons length; locomotive and behavioral assays; etc.	Locomotive and behavior changes; neuron size changes; enhanced or repressed GFP-tagged neurons' expressions; etc.	Counting; fluorescence microscopy; staining; tracking; spectrophotometric analysis; etc.	Yu et al. (2015)
Biochemical	To assess various levels of enzymes; proteins; organelles; metabolic rate assessment; etc.	Broad-spectrum changes in activity and expression levels of different biomolecules (both qualitatively and quantitatively)	Enzyme assays; Western blot; staining techniques; microscopy; spectrophotometric analysis; etc.	Jadhav and Rajini (2009); Kim et al. (2012); Lim et al. (2012)
Genetics	Assays using <i>C. elegans</i> deletion mutants Gene encoding GFP RNAi Gene expression studies, etc.	Expression studies reveal about upregulation and downregulation of genes; changes in the sensitivity and resistance patterns; etc.	Fluorescence microscopy; image analyzer microarray technology; qPCR; etc.	Hofmann et al. (2002); de Pomerai et al. (2009); Roh et al. (2009)

applied techniques to study nano-bio interactions include hyperspectral dark-field microscopy (Meyer et al. 2010; Ahn et al. 2014), fluorescent microscopy (Pluskota et al. 2009), synchrotron-based techniques, transmission electron microscopy (TEM) (Kim et al. 2012), and other chemical techniques (Table 8.2) (Fig. 8.7).

## 8.7 Work Flow to Study Nano-Bio Interaction of Nanoparticles in *C. elegans*



**Fig. 8.7** Common methods and assays to evaluate nano-bio interactions of nanoparticles in *C. elegans*. (Legends: *m.* refers to microscopy, *sp.* spectroscopy, *EM* electron microscope, *DLS* dynamic light scattering, *XRD* X-ray diffraction, *ZP* zeta potential, *SQUID* superconducting quantum interference device, *FTIR* Fourier-transform infrared spectroscopy, *NMR* nuclear magnetic resonance spectroscopy)

**Table 8.2** Common techniques to study the nano-bio interactions in *C. elegans*

Techniques	Use	Nanoparticles	Findings	Reference(s)
Absorbance microspectroscopy	For studying the plasmonic properties of Au NPs in correlation with their confinement inside the intestinal lumen	11 nm Au NPs	No effects of Au NPs on endocytosis and intestinal barrier integrity	Moragas (2016)
Hyperspectral microscopy	To find bio-distribution and localization	Citrate/PVP-coated Ag NPs (10–75 nm) Ag NPs (8–38 nm)	Internalization of several nanoparticles/citrate-coated Ag NPs internalized to germline	Meyer et al. (2010)
Transmission electron microscopy (TEM)	To find the integrity of the intestinal barrier and NP translocation routes	TiO <sub>2</sub> NP/SPIONs	Detection of NPs on the anterior and posterior parts of worm's intestine	Yang et al. (2014)
Scanning electron microscopy (SEM)	To investigate on the morphology, external surface, the cuticle, etc.	Citrate-coated silver nanoparticles (cAg NPs)	Interaction of citrate-coated silver nanoparticles (cAg NPs) with the biological surfaces of <i>C. elegans</i>	Kim et al. (2012)
Synchrotron radiation X-ray fluorescence ( $\mu$ -SRXRF)	To examine metal distribution in <i>C. elegans</i>	24 nm Cu NPs 30 nm TiO <sub>2</sub> NPs	Alterations in metal homeostasis	Gao et al. (2008); Le Trequesser et al. (2014)
Synchrotron X-ray absorption near-edge spectroscopy ( $\mu$ -XANES)	To provide information regarding the oxidation state and coordination environment of metals	30 nm TiO <sub>2</sub> NPs	Alterations in metal homeostasis	Le Trequesser et al. (2014)
ICP-MS	To check for the Au-NP uptake by <i>C. elegans</i>	11 and 150 nm Au NPs	Ion release from 4 nm Au NPs in vitro at pH 4.5 and after 24 h of treatment in <i>C. elegans</i>	Sabella et al. (2014)

(continued)

**Table 8.2** (continued)

Techniques	Use	Nanoparticles	Findings	Reference(s)
Light microscopy	To check for the NPs' bio-distribution (organ level)	Most of the nanoparticles	Confirmation of uptake of nanoparticles by <i>C. elegans</i>	Moragas (2016)
Scanning electron microscopy (SEM-EDX)	To study the interactions between the external part of <i>C. elegans</i> and Au NPs	11 and 150 nm Au NPs	Au NPs did not attach to the external surface of the animal	Moragas (2016)
Fluorescence microscopy	To study the uptake during feeding and translocation to several organs	50 nm polystyrene and SiO <sub>2</sub> NPs	Cytoplasmic uptake of 50 nm polystyrene NPs was observed in early embryos/two entry portals of silica and PS NPs	Scharf et al. (2013); Pluskota et al. (2009)
Hyperspectral dark-field microscopy (HDFM)	To study Au NP distribution inside treated <i>C. elegans</i> at both larval and adult stages	11 and 150 nm Au NPs	Au NPs were constrained to the alimentary system, located inside the intestinal lumen but apparently not internalized by the enterocytes. Translocation to secondary organs such as the reproductive system	Moragas (2016)
Two-photon luminescence microscopy (TPLM)	To find the localization of Au NPs/with better contrast than bright-field or dark-field microscopy	11 and 150 nm Au NPs	Confirming the absence of Au NPs in the cuticle and exclusion of topical entrance of Au NPs	Moragas (2016)

## 8.8 Examples of Common Biological Activities of Nanoparticles Elucidated Using *C. elegans*

### 8.8.1 Nanoparticles in Drug Delivery, Control Release, and Targeting

Delivery systems have been used to enhance the effectiveness of drugs and to decrease the dosage required. Polymeric nanoparticles were often used as particle carriers in various fields because of their subcellular size and sustained release properties that are compatible with tissues and cells. This has led to the applications of

nanoparticles as promising vehicles for drug delivery for various diseases with site specificity in the host system. Nanoparticles provide significant advantages over traditional drug delivery in terms of specificity, stability, drug-carrying capacity, and sustained release. Their ability to deliver both hydrophilic and hydrophobic drug molecules is also feasible for use in various routes of administrations.

Recently, a comparative study on the efficacy of cinnamaldehyde (CNMA) as broad-spectrum antimicrobial agents was tested via its conjugation to the surface of Au nanoparticles (CNMA-GNPs). Different parameters were evaluated which include delivery, compatibility, biofilm formation, and *C. elegans* survival rate. Interestingly, results showed that this antimicrobial nanodrug delivery system (CNMA-GNPs) successfully reduced pathogenic biofilms and antibiotic-resistant strains and significantly ameliorates pathogenic infections. Similarly, it reduced the *C. elegans* mortality rate, whereby a twofold increase in worm survivability was reported on *S. aureus* infection (Ramasamy et al. 2017). A similar study reported that pre-treatment of irradiated *C. elegans* with resveratrol-loaded nanoparticles (RESNPs) enhances the overall life span of the worms by reducing its injury from  $\gamma$ -ray radiation and toxicity from amyloid-peptide overexpression. Additionally, there were enhanced radical scavenging and enhanced expression of SOD-3 observed in the worms that confirmed the successful development of antioxidant nanoparticles (Yin et al. 2014). The significance of peptide multifunctionalized gold nanorods in reducing the toxicity of  $\beta$ -amyloid peptide in the *C. elegans* model of Alzheimer's disease has also been reported (Morales-Zavala et al. 2017).

In another interesting study that highlighted the significance of this model as an effective delivery system is in the form of nanoemulsion-based delivery systems. The incorporation of linoleic acid with conjugation (CLA) or hydrophobic molecules into nanoemulsion based-delivery systems was monitored using the body fat parameter of worms. Overall, their findings have shown that there were significant reductions in whole-body fat of the worms that are exposed to nanoemulsions containing CLA in comparison to the worms that were exposed to linoleic acid only. Thus, it provides a clue that this model is more appropriate in understanding methods or applications of food or drug that are lipophilic in nature (Colmenares et al. 2016). In another development that demonstrated the importance of an in vivo controlled and constant release of molecules in *C. elegans*, the application and design of laser-sensitive Ag nanoparticles from functionalized novel hydrogel shells were also reported using this model system. Thus, consistency in releasing was obtained on radiation with near-infrared light (Lengert et al. 2017).

### 8.8.2 Nanoparticles in Molecular Imaging/Bioprobes/Diagnostics

Transparent body and small size of *C. elegans* are ideal for in vivo optical microscopy. Kim et al. (2013) have reported on a simple immobilization technique that can protect the worms from different toxicants' exposure, besides aiding in its recovery



for long-term imaging using nanoparticle-mediated immobilization (Kim et al. 2013). Recently, a new diagnostic method has also been developed for the accurate measurement of intracellular pH (pHi) using *C. elegans*. This is based on the pH-sensitive Si nanoparticles that can be monitored and visualized by the application of confocal microscopy. The fluorescence intensity patterns that are generated can be used for the quantitative ratio metric analysis of pHi and the overall functions of the organism's metabolic rate. The translational implication is that this economically feasible technique that was formulated in *C. elegans* can have huge applications in the higher model organism for understanding the normal metabolism and its related diseases (Mathew et al. 2014).

In another study that highlighted the significance of *C. elegans* in understanding nanoparticles, bioactivity is in the intercellular transport of lipoproteins whereby fluorescent nanodiamonds were utilized to serve as a marker for observation (Chang et al. 2008). Similarly, Arnhold et al. (2015) have reported that by enhancing Si nanoparticles through fluorescently tagged functionalization, it can be utilized as high-end probes. This has been employed to capture specific images of live worms under an active physiological state. Perturbations of the different cellular components of *C. elegans* can also be monitored in a real-time manner for different useful applications. The feasibility of *C. elegans* as an in vivo model in monitoring the progression of amyloid toxicity through enhanced fibrillation images and its accurate quantification has also been reported (Arnhold et al. 2015). A simple, sensitive, and highly specific lipid targeting Raman probe (Nile red-coated silver nanoparticles) has also been developed to image live *C. elegans* (Charan et al. 2011). This can have wider applications to a higher mammalian system and in understanding lipid droplets, dyslipidemia, or diseases related to lipid metabolism.

### 8.8.3 Nanoparticles as Antioxidants

The central idea of the oxidative damage (or oxidative stress) theory is that “accumulation of molecular damage caused by reactive oxygen species (ROS) which contributes significantly to aging, i.e., to the functional decline and increase in mortality that happens later in life” (Harman 1956; Beckman and Ames, 1998). Recent studies on the biological importance of metal nanoparticles have increased in the field of nano-biotechnology. Kim et al. (2008) investigated whether platinum (Pt) nanoparticles can influence the gathering of the autofluorescent lipofuscin pigment. Lipofuscin is the product of enhanced oxidative damage to cellular components. Its accumulation is most commonly observed in the intestinal cells, and this can serve as a marker for deterioration of overall physiological state with age (Brunk and Terman 2002; Garigan et al. 2002) (Table 8.3).

A study on the biological activity of Pt nanoparticle has shown that it can act as a mimetic of superoxide dismutase (SOD) and catalase in *C. elegans*. Comparative effects of Pt nanoparticle and EUK-8, a synthetic, low-molecular-weight

salen-manganese complex, were also studied based on the life span of wild-type N2 worms and the short-lived *mev-1(kn1)* mutant (Kim et al. 2008). The Pt nanoparticles were more efficient than EUK-8 in prolonging the life span of *C. elegans* (Kim et al. 2008) suggesting that Pt nanoparticles can have a positive effect by reducing enhanced levels of ROS that is mostly seen in diseased conditions. However, it is important to know and monitor the dosage of nanoparticles used because the excess amounts can have harmful effects (Kim et al. 2008).

#### 8.8.4 Nanoparticles as Antimicrobial/Anti-Virulence Activity

The basic principle in assaying and underpinning the use of *C. elegans* in anti-infective/antimicrobial drug discovery is that some pathogens that cause infections in humans infect *C. elegans* too. Indeed, it is estimated that more than 40 human pathogenic strains reported so far can infect *C. elegans* (Sifri et al. 2005). *C. elegans* has become an excellent model for studying the antimicrobial effect of nanoparticles. For example, Au, Ag, and Pt nanoparticles have been extensively used as antimicrobial, antiviral, and anti-inflammatory agents (Hu et al. 2006; Jain et al. 2008). Ramasamy and his colleagues (2017) have shown the antimicrobial effect of Au nanoparticles in the *C. elegans*, where they have conjugated the cinnamaldehyde with Au nanoparticles, which eradicated the biofilm formation of Gram-positive bacteria and Gram-negative bacteria. Besides, these Au nanoparticles attenuate various virulence factors of *S. aureus* and protected *C. elegans* from infection (Ramasamy et al. 2017). Silver nanoparticles have also been reported to have both bactericidal and bacteriostatic properties (Hunt et al. 2013) (Table 8.3).

#### 8.8.5 Nanoparticles' Roles in the Regulation of Metabolism

Feasibility of the worms allows them to be an ideal model for studying energy metabolism, wherein starvation and excess energy can affect the overall physiology of the worms. Recent reports suggest that exposure of worms to nanopolystyrene particles can change various metabolites related to energy metabolisms, such as tricarboxylic acid cycle intermediates, glucose, and lactic acid. Similarly, studies have shown that upon exposure, it reduces the levels of important amino acids that can serve as metabolites for energy metabolism such as glutamic acid, isoleucine, valine, and lysine (Kim et al. 2018; De Lorenzo et al. 2002).

#### 8.8.6 Nanoparticles in Aging and Developmental Studies

During the reproductive cycle, the worm's vulva is the major organ involved in progeny production. The lacking of normal vulva and bag-of-worm (BOW) phenotypes with egg-laying deficiency was seen in reproductive-age worms when treated with plain or

**Table 8.3** Various biological activities of different nanoparticles as reported from *C. elegans* model

Nanoparticle	Metal stress	Oxidative stress	Average size range	Mechanisms	Reference(s)
Pt	-	+	2.4–7 nm	Prevents cellular damage by scavenging endogenous ROS	Kim et al. (2008); Kim et al. (2010)
Ag	+	+	10–21 nm	Impairments in metabolic processes Stress-mediated mitochondrial and DNA damage Dermal effects Cellular damage Early-endosome formation	Scharf et al. (2016); Stames et al. (2015); Ellegaard-Jensen et al. (2012); Ahn et al. (2014); Kim et al. (2012); Maurer et al. (2016); Ahn et al. (2014)
SiO <sub>2</sub>	-	-	50 nm	Aging phenotypic changes	Scharf et al. (2013); Pluskota et al. (2009)
TiO <sub>2</sub>	+	+	7–21 nm	Neuronal control over defecation	Zhao et al. (2014)
CeO <sub>2</sub>	±	±	15–45 nm	Growth defects by inhibition on feeding	Arnold et al. (2013)
GO	-	+	~6 nm	Inhibition of spermatogenesis Modulation in fat metabolism	Kim et al. (2018); Chatterjee et al. (2014)
Al <sub>2</sub> O <sub>3</sub>	±	+	60 nm	Neurotoxic effects and behavioral changes	Li et al. (2012)
Au	±	±	~0.5–6.45 nm	Locomotion and axonal neuron growth	Hu et al. (2018)
CuO	±	-	28 nm	Heat response and unfolding protein function	Mashock et al. (2016)
ZnO	-	-	1.5 nm	Protection against metal toxicity	Ma et al. (2009)
MWCNT	±	±	10–20 nm	Involves in molting cycle Regulation of metabolic pathways	Zhao et al. (2017)
Nanodiamond	-	-	~120 nm	Role in resistance to arsenite and oxidative stress	Mohan et al. (2010)

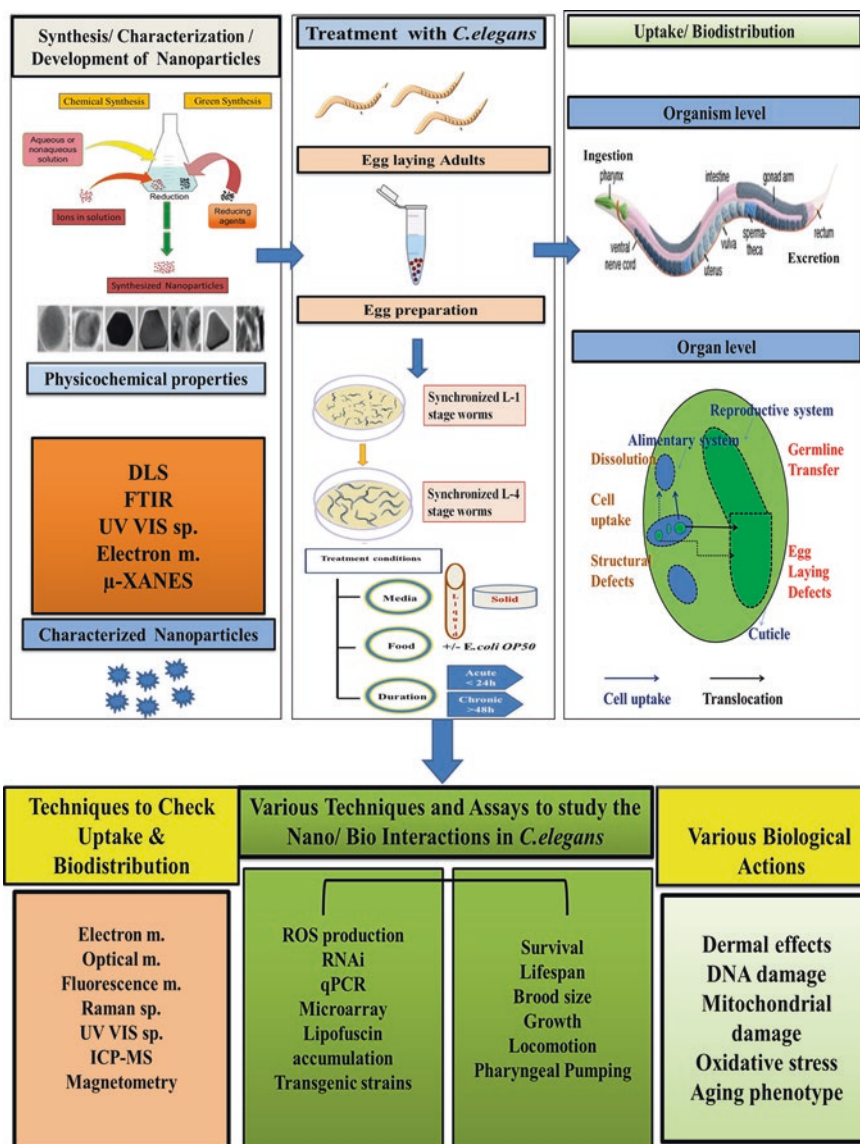
labeled Si nanoparticles. BOW phenotype is characterized by fertilized embryos that come from their shells within the body of hermaphrodite and feeds on the body of their parent worm itself. Further, they ruled out the post-larval stage applications and organ-specific interactions of nanoparticles that affect the reproductive organs. These findings prove that Si nanoparticles can mediate the degeneration of neural and reproductive systems concerning their age (Scharf et al. 2013; Pluskota et al. 2009).

The various effects of nanoparticles on the growth and development of the worms were also linked to defective food intake. Arnold et al. (2013) have seen a decline in *C. elegans* growth on CeO<sub>2</sub> nanoparticle treatment due to a reduced dietary intake mediated by the interactions between CeO<sub>2</sub> and *E. coli* (Arnold et al. 2013). CeO<sub>2</sub> has a greater affinity to bind to *E. coli* (Thill et al. 2006). The decrease in growth and development of the worms may be due to the treatment with CeO<sub>2</sub> nanoparticles. Also, the developmental delay of *C. elegans* is a common physiological response to stress and has been observed after exposure to copper sulfate (CuSO<sub>4</sub>), TiO<sub>2</sub>, and ZnO nanoparticles (Wu et al. 2013).

The effect of SiO<sub>2</sub> nanoparticles on enhancing the aging of cells at the molecular and organism level was also reported (Scharf et al. 2013). Treated animals exhibit enhanced accumulation of ubiquitinated proteins when compared to controls, which resembles the accumulation of endogenous insoluble proteins in older worms. Additionally, fine structures in the intestinal cell nuclei (amyloid-like) were observed in SiO<sub>2</sub> nanoparticle-treated worms. Also, SiO<sub>2</sub> nanoparticle is reported to have a direct influence with pharyngeal use in worms by premature induction of an age-related reduction in pharyngeal motor activity through pharyngeal pumping (Scharf et al. 2013).

### 8.8.7 Nanotoxicity Assessment

*C. elegans* has become a favorable model organism for toxicity assessment. Nanoparticles can have surprisingly both beneficial and toxic effects on macromolecules of cells. Nanoparticle-mediated toxicity in *C. elegans* can be assessed using different standard methods and protocols. For example, assays that determine the worm's growth, mortality rate, reproductive capability, and locomotive changes can provide accurate measurements and predictability when applied to higher mammalian systems. In comparison to different in vitro assays, toxicity assays in *C. elegans* are reproducible. Scientific data can be generated easily from the different lethal and sublethal endpoints of an intact and metabolically active animal with different tissues and organ systems (Boyd et al. 2010; Corsi et al. 2015) (Fig. 8.8).



**Fig. 8.8** Summarized and simplified diagram highlighting the overall process involved in the elucidation of nanoparticle biological activity

## 8.9 Conclusion

Underrating the importance of nanoparticles in research for the usage of mankind has steadily increased the scope of nanotechnology. To serve this purpose, the synthesis and formulations of various nanomaterials should focus on the elimination of as

much of the toxic effects on the biological systems. Thus, a feasible and proper *in vivo* model system such as *C. elegans* represents an ideal platform for fast and reproducible results for the elucidation of nanoparticles biological effects. At the same time, in learning the effects of nanoparticles, it is necessary to examine their interactions since accurate outcome depends on various factors such as media, exposure, physicochemical properties, growth conditions, etc. Different mechanistic studies of nanoparticles for uptake, bio-distribution, and excretion were simplified with the availability of different high-end and sensitive techniques. Biochemical assays and genetic and molecular analyses such as RNAi, qPCR, and microarrays have also enriched our understanding of the whole process of nanoparticle biological activities using *C. elegans*. Lastly, realizing the significant contributions of *C. elegans* in the elucidation of various biological activities of nanoparticles, their safety designs and development can be further improved for various translational applications and human therapeutic values.

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