Review Article

Caenorhabditis elegans as a Model in Studying Physiological Changes Following Heart Failure

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Submission Date: 13-09-2021; Revision Date: 20-10-2021; Accepted Date: 11-11-2021

ABSTRACT

For decades, *Caenorhabditis elegans* (*C. elegans*) has been at the forefront of various advances in aging, cancer, and neurodegeneration research. Despite the complete sequencing of this nematode's genome, there are only a few attempts of using *C. elegans* in studying the circulatory system and other related physiological activities. The absence of the circulatory system poses a significant challenge for researchers to conduct experiments in this organism. In this paper, the association of the heart and pharyngeal muscle in *C. elegans* was reviewed to illuminate new understanding and propose potential methods in investigating the physiological changes following pathogen-induced heart failure.

Key words: *Caenorhabditis elegans*, Heart failure, Infective endocarditis, Pharyngeal muscle, Arrhythmia.

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INTRODUCTION

Caenorhabditis elegans (*C. elegans*) is a non-parasitic nematode widely used as a model organism for various human diseases, such as cancer, neurodegeneration, and infection.[1-3] Various physiological activities like aging, motor behavior, learning, memory, and addiction were elucidated in *C. elegans*.[4,5] Despite these recent advances, heart-associated studies in *C. elegans* remain elusive. Possibly, one reason for this is the absence of the heart in the nematode.

However, research suggests that the *C. elegans*' pharynx and the vertebrate heart are orthologous. The pharynx and the heart are tubes that use binucleate muscles to pump contents through their lumens.[6] Additionally, the pumping of muscle and heart is controlled by similar electrical circuitry.[7] The gap junctions that connect nearby muscle tissues synchronize these contractions without neural input.[8] Neurotransmitters, such as acetylcholine and serotonin, couple to G-protein coupled receptors that modulate the contraction of these organs.[9] LQT potassium channels and L-type voltage-gated calcium channels are used in both the pharynx and the heart.[9]

Conversely, the pharynx of *C. elegans* differs significantly from that of a vertebrate heart. The nematode's pharynx originates from the ectoderm, whereas the heart from the mesoderm.[6] The pharyngeal muscle is divided into four segments, as shown in Figure 1. Posterior to the buccal cavity lies an elongated tube, the procorpus, which marks the start of the pharyngeal muscle. The other segments posterior to the procorpus are the metacorpus, isthmus, and terminal bulb. The pharyngeal pumping is typically observed through the contraction of the terminal bulb, where bacterial cells are ground.[4] A pharyngeal-intestinal valve divides the pharyngeal muscle and intestinal lumen, which regulates the influx of bacteria. Similarly, the electrical conduction

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DOI: 10.5530/ajbls.2021.10.69

in the pharynx and the heart differs. The voltage-gated sodium channel, which usually generates the rapid sodium spike in the heart, is absent in *C. elegans*.[8] The pharynx and skeletal muscles appear to be stimulated by nicotinic receptors in the motor neurons.[10] These distinctions suggest that the organs' similarities may result from convergent evolution between two muscle pumps. However, these contrasts do not invalidate that *C. elegans*' pharynx and vertebrate heart have a similar biological function.

Heart failure results from the ventricle's impaired ability to fill or eject blood due to structural or functional cardiac issues.[11] There are various causes of heart failure, such as coronary artery disease, valvular heart disease, heart muscle disease (dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, amyloidosis/sarcoidosis), hypertension, pericardial disease, congenital heart disease, systemic and infective endocarditis, myocarditis rheumatic fever, tachyarrhythmia, myxoma, and amphetamine abuse.[12] Heart failure is typically asymptomatic until the ejection fraction drops to the point where the heart cannot supply enough oxygen to the brain.[13] Hence, symptoms appear, and the risk of death increases. Some of the significant signs of heart failure are paroxysmal nocturnal dyspnea, neck vein distension, rales, cardiomegaly, acute pulmonary edema, S3 gallop, increased venous pressure, and hepatojugular reflux.[14] Other minor signs and symptoms are ankle edema, night cough, exertional dyspnea, hepatomegaly, pleural effusion, and tachycardia.[14]

This paper hypothesizes that some physiological changes following heart failure, namely remodeling, arrhythmia, edema, and fatigue, can be demonstrated in *C. elegans*. To test this hypothesis, the author proposes experiments to determine whether these consequences are empirical in *C. elegans*. One limitation of these experiments is the compensatory mechanism of the heart following heart failure. It would also be interesting to explore whether the pharyngeal muscle can influence a particular compensatory mechanism in *C. elegans*.

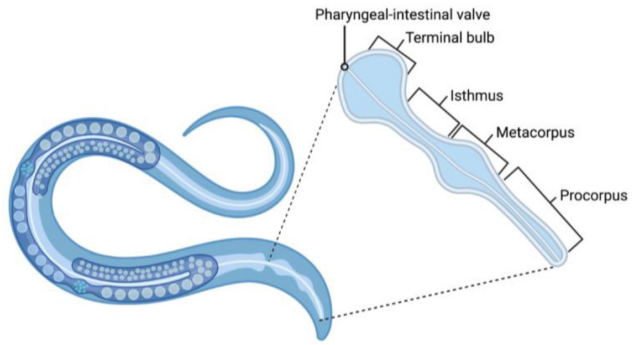

Figure 1: Schematic representation of the *C. elegans* pharyngeal muscle.

Mimicking heart failure in *C. elegans*

Since the vertebrate heart and the pharynx in *C. elegans* appear to have a similar biological function, damaging the nematodes pharynx may affect its basic functionality. One way to damage the nematodes pharynx, similar to the heart, is through bacterial infection, as shown in Figure 2. Feeding the nematodes with pathogenic bacteria, such as *Burkholderia pseudomallei*, *Staphylococci*, and *Streptococci*, may damage the nematodes' pharynx comparable to the vertebrate heart.[1,3,4,15]

Studies have shown that bacteria can cause endocarditis.[16] The inflammation on the inner lining of the heart during the infection leads to scarring. The scarred tissues undergo remodeling, progressing to heart failure.[17] During this remodeling, fibrillar collagen is recruited around the damaged tissue, which accounts for the thickening in the area. This damaged tissue's loss of membrane potential may not conduct regular depolarizations.[18] This hypertrophic cardiac muscle may affect cardiac contraction in the affected area resulting in arrhythmia.[17]

Observing the pharyngeal damage and remodeling in *C. elegans*

C. elegans being transparent is one of the advantages of using this organism as a model in various studies. This feature of the nematode is advantageous in the proposed experiment as it makes the visualization of the pharyngeal muscle easier. There are various ways to visualize the body of *C. elegans*. The conventional way is through stereomicroscope, which can record the pharyngeal pumping rate.

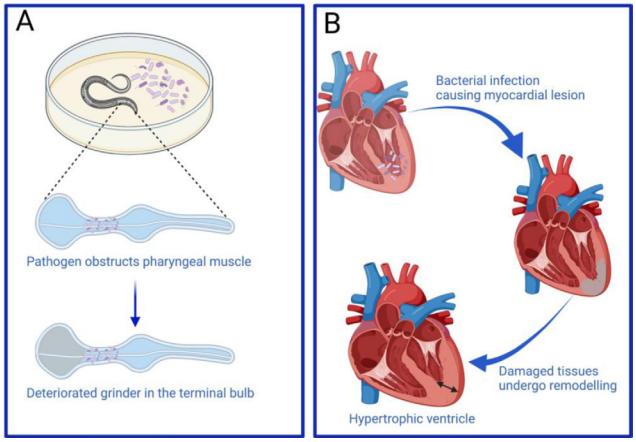

Figure 2: The bacterial infection leads to muscle degeneration. A. Pharyngeal muscle degeneration in *C. elegans*. B. Pathogen-associated myocardial dysfunction in humans.

A fluorescent microscope can also track the pharyngeal muscle in *C. elegans* using various strains. One *C. elegans* strain pCFJ90 myo-2p::m-Cherry highlights the pharyngeal muscle, as shown in Figure 3.[19,20] Using the intensity of the protein may give insight into the number of active muscle cells. Meaning, the lower intensity may depict a high number of dead tissues. Another way to determine the amount of damage in the pharynx is by measuring the length of the pharyngeal muscle and the diameter of the posterior bulb.

Electron microscopy can also visualize the damages in the different pharyngeal muscle layers in *C. elegans*.[21] In this way, one can quickly evaluate pharyngeal muscle remodeling. Usually, the amount of newly formed tissues can be measured by the thickness of the specific muscle layer or by the area of the damaged tissues through Image J.

Measuring arrhythmia in *C. elegans*

In heart muscle, the efficiency of pumping enough blood to the body is an essential indicator of a well-functioning heart. In *C. elegans*, the pharyngeal pumping activity indicates the nematode's feeding behavior.[4] A damaged pharyngeal muscle may have a slower contraction rate, reducing the amount of food transported from the mouth to the gut. This mechanism is like bradycardia, when the heart muscle thickens after injury, which results in difficulty in pumping.[22] One can measure the pharyngeal pumping activity by counting the number of contractions of the posterior bulb of the pharynx for one minute.[4]

Measuring edema in *C. elegans*

The excretory system of *C. elegans* is homologous to the renal system in vertebrates, as shown in Figure 4.[23] Fluids and nutrients passing through the mouth to the gut are excreted out from the anus. This system

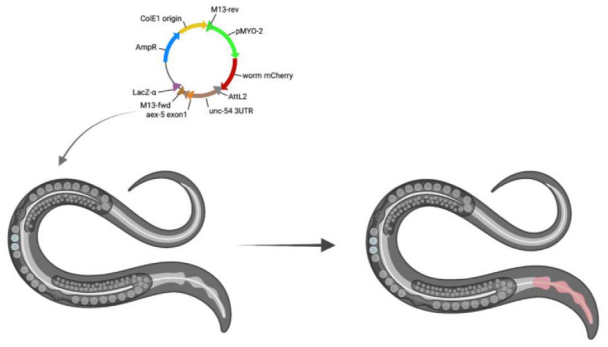

Figure 3: Schematic representation of the visualization of pharyngeal muscle using a fluorescent microscope.

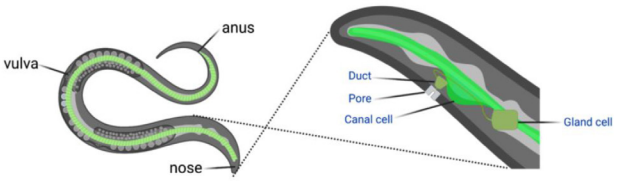

Figure 4: A schematic representation of GFP highlighting the excretory system of *C. elegans*.

is essential for osmoregulation in the nematode, and several genes associated with osmotic stress response influence different sections of this system. Studies have shown that the influx of water expands the lumen of the canal in *C. elegans*.[24] Hence, the author proposes two ways to measure edema in *C. elegans* following pharyngeal muscle damage. The first one is to measure the canal's diameter in at least three sections (anterior, middle, and posterior). Similarly, the other way to determine edema in *C. elegans* is to measure the body width in the previously mentioned body sections.

Measuring fatigue in *C. elegans*

Fatigue is present in both left-sided and right-sided heart failure.[25] In *C. elegans*, the author proposes that the locomotor behavior of the nematode can measure fatigue. Swimming exercises are commonly used to evaluate the locomotor behavior in *C. elegans*.[26] However, one can do more straightforward experiments to evaluate the locomotor behavior, such as response to fine poking and plate tapping.

To measure the nematode's response to fine touch, one should record the distance traveled by the nematode from one point to the next coordinate in a particular period. This experiment can be done by placing a graphing paper underneath the Petri plate and record the event using a camera installed in the stereomicroscope, as shown in Figure 5.

Plate tapping may induce several motor behaviors in the nematode, such as body turns and bends, as shown in Figure 6. The body bend of the nematode can either be a forward behavior (Figure 6A) or a reversal (Figure 6B and 6C). Usually, most researchers consider Figure 6B as the reversal, and only a few consider Figure 6C. Also, the body bends of the nematode can either be a W-shape, Ω-shape, or C-shape, as shown in Figure 6D-F, respectively. The body bending and turning of the nematode depicts its activity to do a particular task, such as feeding.[27] The fewer observed number of these activities may demonstrate fatigue in *C. elegans*.

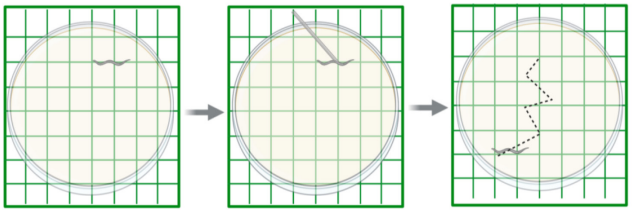

Figure 5: A sketch of the field view representing the experiment to evaluate the motor behavior of the nematode. The green grid lines indicate the graphing paper placed under the plate, while the black circle is the view under the stereoscope. The black line in the second eye field represents the worm picker poking the nematode. The gray curved shape represents the *C. elegans*, whereas the dashed line represents the movement tracked left by the nematode on the agar plate.

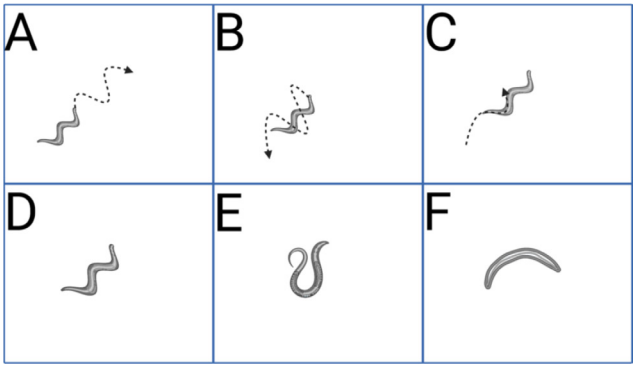

Figure 6: Sketch of the expected body bends, reversal, and turns of *C. elegans* in measuring locomotor behavior. A. Forward turn, B. Reversal, C. Less common reversal, D. W-shape, E. Ω-shape, and F. *C-shape*. The arrowhead represents the head portion of *C. elegans*, whereas the dashed line is the predicted movement of the nematode.

Limitations and Disadvantages

One of the key features of *C. elegans* is its simple structure. However, many limitations entail this feature, such as the classification of heart failure and the availability of assays. From the different classifications of heart failure, namely acute, chronic, systolic, and diastolic, it remains unclear whether the *C. elegans* as a model can further expand in terms of assays that can further be developed. Besides, some empirical observations regarding specific pathological presentations, such as cardiogenic shock and cardiogenic pulmonary edema, may not be possible in this organism due to the absence of circulatory and respiratory organs. Similarly, serological assays appear to be not an option due to the unavailability of serum in this organism. It is not uncommon to perform biochemical assays in *C. elegans*; however, this poses a significant challenge for researchers due to its small size. Although its sizeable reproductive capacity can overcome this limitation, the disparity in the severity of heart-failure-associated physiological changes in *C. elegans* individually may be misrepresented. Specifically, splicing the nematode into sections is uneconomical, thus, rendering tissue-specific examination improbable. Hence, confirmatory tests and stringent statistical design are warranted.

Future Perspective

Whether the advantages of using *C. elegans* as a model for various diseases outweigh its limitations, its application for heart diseases remains elusive. Although it is unlikely to fully elucidate the physiological consequences of heart diseases in *C. elegans*, it does not preponderate the opportunity of developing assays for this model organism. While these physiological changes may depict other phenomena, such as aging and innate response, the possibility of these processes associated with the circulatory system in humans should also be investigated. The use of forward or reverse genetic screening in *C. elegans* to determine genes responsible for these physiological events warrants further examination. Identifying these genes and analyzing certain biochemical pathways may pave the way to understanding the regulation and interaction of these unknown genes in *C. elegans* that may also be comparable with the genes associated with the physiological and molecular changes following heart failure in humans. On this note, enzymes activated after pharyngeal muscle deterioration may also be contrasted with the enzymes associated with compensatory response in humans. Altogether, the absence of a circulatory system in *C. elegans* remains a challenge to develop this model for heart diseases; however, it does not vindicate the opportunity to investigate physiological changes following heart failure assuming that they are comparable.

ACKNOWLEDGEMENT

All figures were created using Biorender.com.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Using *C. elegans* to study heart diseases has its fair share of advantages and disadvantages, primarily because of the absence of this organism's heart and circulatory system. However, the pharyngeal muscle of *C. elegans* resembles a human's heart. Apparently, this resemblance stroke interest, whether damaging the pharyngeal muscle,

would also be comparable with heart failure. Although, there is still a significant knowledge gap in this area due to the lack of evidence. The author hypothesizes that some physiological changes would be evident after pharyngeal muscle damage mimicking infective endocarditis. Hence, empirical yet straightforward methods were reviewed to test this hypothesis.

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Cite this article: Nas JSB. *Caenorhabditis elegans* as a Model in Studying Physiological Changes Following Heart Failure. Asian J Biol Life Sci. 2021;10(3):522-6.