Compact Disc (CD)-Based Automated Animal Assay

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ABSTRACT

Space biology research requires a compact and fully automated system. The unique environment of space flight experiments has limitations of power, size, weight, and crew intervention. In order to address these needs, a compact disc (CD)-based automatic microfluidic system was developed. The long-term goal of this research is to develop an automated microfluidic rotating CD cultivation system for to determine if the space environment is influencing particular genes. *Caenorhabditis elegans* was exploited for this research because it is a well-studied model organism for biological and biomedical research in genetics, aging and disease. Compared to other microfluidic technologies for moving small amounts of fluidic or suspended particles from site to site, the centrifuge-based system is well suited for various microfluidic functions such as flow sequencing, mixing, capillary metering, and flow switching. Those functions can be implemented by exploiting centrifugal, coriolis and capillary forces combined with specific microfluidic network. This automated microfluidic CD culture system contains a cultivation chamber, nutrient chamber, waste chamber, channels, and venting holes. The feeding and waste removal processes were achieved automatically using centrifugal force driven fluidics. The cultivation of *C. elegans* was successfully carried out on the automatic microfluidic CD system.

Keyword : animal assay, automatation, cultivation, *Caenorhabditis elegans*

1 INTRODUCTION

Microfabricated platforms for automated cultivation and behavioral observation of animals would be a valuable tool in physiological and genetic studies. We report an automated microfluidic compact disc (CD) system specialized for cultivating and monitoring *C. elegans*, proficient in automated feeding, waste removal and live-animal microscopy.

The automated CD cultivation platform contains cultivation, nutrient, and waste chamber, channels connecting the chambers, and venting holes. The feeding and waste removal processes are achieved automatically using centrifugal force driven fluidics. Compared to other microfluidic technologies for moving small amounts of fluidic or suspended particles from site to site, this centrifuge-based system is well suited for functions such as flow sequencing, mixing, capillary metering, and switching. Those functions can be implemented by exploiting centrifugal, coriolis and capillary forces combined with specific microfluidic networks. Thus, the CD cultivating system has the capacity to incorporate diverse molecular, biochemical, and pharmacologic assays for real-time physiological and behavioral tests of live animals. This compact and fully automated microfabricated platform is designed to address the unique requirements of space biology due to the stringent limitations on power, size, weight, and crew intervention.

C. elegans is a suitable organism in which to investigate the fundamental genetic basis of stress responses. It exhibits complex developmental and behavioral changes in response to the environment[1]. Its small size (1mm in length of an adult worm) and short life cycle of 3.5 days allow observation of a large and multi-generational population in a short period of time. Its transparent body, completely defined genome and anatomy make possible to monitor gene expression and analyze multicellular processes in living animals at the resolution of individual cells. *C. elegans* has been a prime genetic system for dissecting genes and molecular pathways involved in development, metabolism, stress responses, and ageing, and it has been shown that these molecular mechanisms are conserved in organisms as diverse as worms, fly, mouse, and human[2,3].

Using this microfluidic system, *C. elegans* are retained in the cultivation chamber, and periodically, at a constant rate, the nutrient solution, *Escherichia coli (E. coli*) is introduced and waste is removed automatically. One important advantage of our system over current nematode culture systems used in space is the capability to apply a control 1xG centrifugal force to assess factors such as microgravity, radiation and vibration on nematode behavior and gene expression.

In addition to space flight applications, this system can be used on earth to investigate the effects of various parameters such as hypergravity and radiation. These parameters can cause physiological changes in organisms that have evolved in an 1xG gravity environment[4] including fluid redistribution, muscle atrophy and reduced immune response[5] and may influence physiological ageing. Manipulating space environmental parameters may represent an effective strategy for studying ageing and related changes in gene expression. This has profound biological implications, including applications to space exploration and extra-terrestrial habitation[4,6,7].

To enhance the versatility of our system, a hypergravity environment can be quantitatively applied to the cultivation chambers by manipulating angular frequency and disc geometry. This will allow us to investigate of the effects of gravitation. In this paper we describe the properties of this automated CD based platform, population growth patterns in the system.

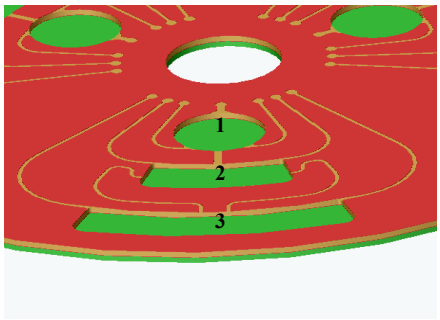
2 METHODS

Animal assay

The CNC-machined CD system consists of three polycarbonate discs and two pressure sensitive adhesive layers (Fig 1). The top plate (including venting holes), the middle plate (containing all chambers), and the bottom plate contain the alignment holes for easy assembly.

Between each of the plastic plates, pressure sensitive adhesive layers (FLEX mount DFM 200 clear V-95), cut by a vinyl cutter (Graphtec CE-2000 60), were aligned and the entire system was then bonded.

(a)



(b)

Figure 1 CNC machined CD assembly for the *C.elegans* hypergravity experiments **a,** A photo of assembled CD (12cm diameter and 4mm thickness) **b,** (a) Schematic illustration of the microfluidic structure employed for the CD cultivation system. The fluidic structure contains a nutrient reservoir (1), a cultivation chamber (2), and a waste reservoir (3).

Liquid nutrient is loaded in a nutrient reservoir (1). Upon increasing the rotation rate of the system, the nutrient solution is gated into the cultivation chamber and some of the waste from the cultivation chamber (150µl) can drain through the microchannels (50µm * 40µm). Fluidic flow between these reservoirs is controlled by balancing capillary and centrifugal forces. The capillary nature of microfluidic channels tends to resist flow because of surface tension effects. Rotation of the CD applies a force on the liquids in the direction from the center of rotation outward. This centrifugal force can be controlled by varying the rotational speed. At some rotational frequency the rotation induced force will overcome the restraining capillary force and flow will begin. The flow rate can be further increased by increasing the rotational speed of the CD. For further function and applications of CD technology the reader is referred to a review paper on CD technology[8]. Fluid gating, is accomplished using capillary valves in which capillary forces pin fluids at an enlargement in a channel until rotationally induced pressure is sufficient to overcome the capillary pressure (at the so-called burst frequency.) Thus, feeding and waste removal processes are achieved automatically by specified centrifugal forces and require no mechanical pumping or valving. By manipulating the microfluidic network design, fluidic design, and rotational speed of the disc, a wide range of flow rates can be achieved.

3 RESULTS

3.1 Population growth pattern of *C. elegans*

C. elegans is a non-parasitic free-living nematode worm. This species is partially dioecious, but normal reproduction occurs via a self-fertilizing hermaphrodite with a brood size of about 300 eggs produced during its lifetime[9]. *C. elegans* develops through four larval stages (L1-L4) into adulthood in about 3 days under optimal conditions. Under the stress of harsh environmental conditions such as heat, starvation and overcrowding, larvae developmentally arrest as morphological distinct dauer larvae and adults cease fertilizing eggs. Thus, population growth and larval development reflect cultivation conditions.

To evaluate our CD cultivation platform, a single adult hermaphrodite was transferred into each cultivation chamber, and number of worms in the chambers was counted every 24 hours for 14 days. The first wave of population increase in the CD cultivation chamber began around 3 days cultivation on the CD platform. The number of worms in each cultivation chamber reached about 300 on day 5.

The population continued to grow after 6 days in the cultivation chambers before reaching saturation on day 9, indicating that the second generation that were born in the CD developed and reproduced in this time. This three-day life cycle indicates that worms cultivated on the CD platform proceeded through normal larval development and reproduction processes as one could expect them under optimal growth conditions. The population growth did not change after day 9 as the cultivation chamber became over-crowded and the worms formed dauer larvae. These results indicated that one cultivation chamber on the CD platform would allow us to monitor three generations of living worms. The tempo of the development, the rate of the population growth and the brood size of the worms demonstrated that optimal conditions are maintained throughout the experiment on the CD platform.

3.2 Multi-generational cultivation system

The multi-generational cultivation system can allow us to observe the adaptation to the hypergravity environment through the multi-generations. We separated progeny generations of *C. elegans* on CD using the basic principles of size exclusion. We took advantage of the size difference between 4 larval stages and adult stages. The diameter of adult worms is 60-80µm and the 4 larval stage diameters range between 25 and 50µm. We designed the multigenerational cultivation system with nylon-based meshes. The mesh opening was 30µm. The multigenerational cultivation CD enabled us to successfully separate only progeny from the parents. (fig 2).

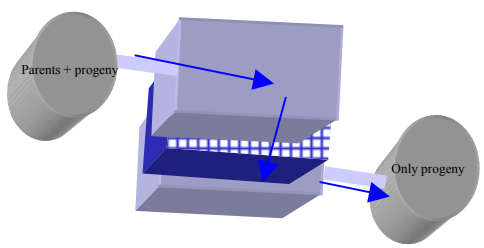


Figure 2 Schematic illustration of the filtration structure employed for the CD-based mutigenerational cultivation system. (a) This cultivation chamber has adult and various stages of *C. elegans*. (b) 30µm nylon mesh was implanted between two layers. (c) Only eggs and larvae were collected in the last cultivation chamber.

4 CONCLUSION

We have demonstrated an automated microfluidic cultivation system that allows behavioral observation of *C. elegans.* One advantage of this system is its versatility.

We have succeeded in breeding three generations of *C. elegans* in the current cultivation system in two weeks as a gauge of optimal growth conditions. By implanting filters with small pore sizes into our cultivation system, we could separate progeny from the parents, thereby future studies of the behavior and physiology of multiple generations. This feature will be particularly useful to investigate developmental responses and adaptation to a space environment.

Microfabricated centrifugal fluidic platforms have potential for a wide range of practical applications in addition to *C. elegans* cultivation. Given the malleability of fluidic flow rates, sequencing, valving, metering, mixing and switching functions on the CD and its ability to handle various biological samples such as blood, serum or even cells, this platform could be developed into a "Lab on a CD"[8]. The capacity of our CD system to change gravity levels by altering angular frequency and disc geometry opens up the opportunity to investigate genetic gravitational responses in a living system. Custom-design of the chambers for biochemical and genetic assays could minimize the risk of sample contamination and eliminate much labor. The possibility of sustaining identical and synchronous flow rates, to supply duplicate volume, to establish identical incubation times, mixing, and detection in a multitude of parallel assay elements makes the CD a promising platform for experimental molecular biology.

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