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A microgravity responsive synthetic genetic device in *Escherichia coli*

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ABSTRACT

Bioengineering solutions to human space travel must consider microgravity as an important component. Thus, one of the fundamental challenges of space bioengineering is to create cellular microgravity responsive device, which integrate microgravity as a signal within biochemical and cellular processes. Here, we designed, fabricated and characterized the first biochemical and cellular microgravity responsive device using an engineered genetic circuit in *E.coli*, which responded to microgravity by changing the expression of a target enhanced green fluorescent gene (EGFP). Our device design was based on the deregulation of HfQ protein in *E.coli* in microgravity, which was translated through HfQ mediated silencing of EGFP by anti-EGFP synthetic small regulatory RNAs. This resulted a reduced silencing (~28 times) of the EGFP in microgravity. We demonstrated that the basic design of the device is universal in nature for *E.coli*, by creating multiple successful devices, where target genes (EGFP, TdTomato, and FtsZ) and the promoters (inducible and constitutive) were altered. Further, we applied this device to control the cell division process by microgravity. Here we targeted the cell division regulator FtsZ, which resulted an elongated cell shape in normal gravity and this deformed cell shape got rescued to normal one by applying microgravity. The work showed for the first time, a way to integrate microgravity as a physical signal within biochemical processes of a living cell in a human designed way and thus, may have significance in space bioengineering and synthetic biology.

1. Introduction

Microgravity is a unique and ubiquitous property of space, which is not experienced by the biochemical world on the earth. Microgravity influence biological processes at the molecular, cellular and organism level, including astronauts' health and immunity (Wilson et al., 2007; Mukhopadhyay et al., 2016; Celen et al., 2019; Planel, 2004). Studies have been performed on multiple organisms to understand how they sense microgravity at the cellular and molecular level (Wilson et al., 2007; Mukhopadhyay et al., 2016; Celen et al., 2019; Zheng et al., 2015; Kohn et al., 2017; Häder et al., 2017; Roy et al., 2016; Vogel and Luisi, 2011; Ingber, 1999). Several mechanisms have been proposed including the presence of gravireceptor (Häder et al., 2017), the effect of low shear stress (Wilson et al., 2007; Nickerson et al. (2004), altered transport phenomena due to microgravity induced low fluid shear (Nickerson et al., 2004), alteration of membrane fluidity (Kohn et al., 2017) and the change in second messenger molecules (Mukhopadhyay et al., 2016). However, no definite mechanistic details were known.

On the other hand, rapid advancement of space technology, in recent years, inspires ambitious long-duration human space travel plan to space, Mars and asteroids (Binzel, 2014). To achieve such goals, it is suggested that the bioengineering solutions, based on engineered biochemistry of the cells, could be the key to the space technology challenges (Langhoff et al., 2011). Engineered cells with synthetic genetic circuits produce highly controlled, human-designed functions (Langhoff et al., 2011; Horneck et al., 2010; Bashor and Collins, 2018). Thus, it offers numerous projected applications for the near-future space technology, which spans from future space medicines, resource utilization, self-building habitats, space biomanufacturing, life support, programmable materials, and terraforming (Langhoff et al., 2011; Menezes et al., 2015a; Menezes et al., 2015b).

However, bioengineering solutions to space travel must consider microgravity as an important component. Thus, one of the fundamental challenges of space bioengineering is to create engineered biochemical systems to integrate microgravity as a physical signal within molecular and cellular processes. Engineered bacteria with synthetic genetic circuits have been shown to sense and integrate various environmental as well as intracellular chemical signals and compute complex human-

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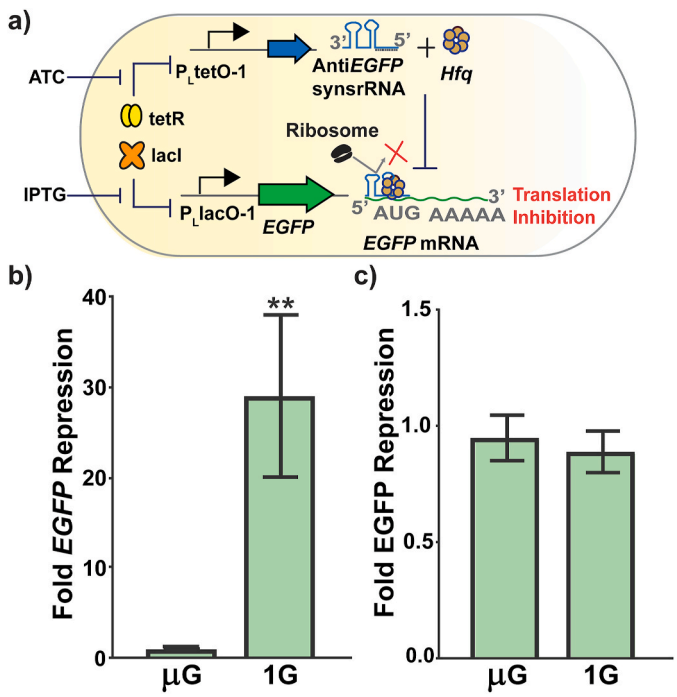

Fig. 1. a) The design of the microgravity sensor circuit with EGFP. b) The experimental behavior of microgravity sensor. Each bar represent the EGFP expression folds change between IPTG only (absence) and IPTG + ATC (presence of anti-EGFP SynsrRNA) conditions. c) Control experiments.

designed functions through designed molecular interactions and reactions (Gupta et al., 2019; Saltepe et al., 2018). Apart from the chemical signals, cellular devices have been created to sense and integrate physical signals like temperature (Bagh et al., 2011) and light (Liu et al., 2018; Olson and Tabor, 2014). However, no molecular or biological microgravity responsive device has been created.

The aim of this study is to create a synthetic genetic device in *Escherichia coli (E. coli)*, which integrates microgravity as a physical signal with biochemical processes in a human designed way and responds by changing the expression of a target protein. To create simulated microgravity in the lab, we ran the cells in High Aspect Ratio Vessels (HARV) in a rotating wall vessel (RWV) microgravity simulator, an important microgravity laboratory apparatus, designed by NASA (Nickerson et al., 2000). When the high aspect ratio rotating wall vessels were positioned in such a way that the axis of rotation was perpendicular to the gravity vector (Fig. S1a), the rotation offsets the effect of sedimentation of the cells of the gravity force. Thus, the cells are in continual suspension and in free-fall condition. This mimics the microgravity (Wilson et al., 2002; Gilbert et al., 2020). On the other hand, when the axis of the rotation was parallel to the gravity vector (Fig. S1b), this did not offset the gravity force and allowed the cells to settle down. This position provided a normal gravity environment (Wilson et al., 2002; Gilbert et al., 2020). Therefore, we used the same protocol to simulate the microgravity and the normal gravity condition in our experiment (Figs. S1a and b).

2. Results and discussions

2.1. Design and fabrication of microgravity responsive synthetic genetic device

Though the microgravity brings prominent phenotypic changes in organisms including bacteria, the molecular genetic studies showed that the microgravity-induced alterations of expression of individual genes at both the transcript and the protein level were low (Mukhopadhyay et al., 2016; Roy et al., 2016). Here we targeted a small change in specific biomolecules in response to microgravity and processed that signal through a molecular signal-processing device to get a bigger response and better control. Protein HfQ binds with a specific scaffold made with small regulatory RNAs (srRNA) and the resultant complex binds with target mRNAs to degrade it in bacteria including *E.coli* (Yoo et al., 2013). Using this principle, synthetic srRNA (SynsrRNA) based systems were created to repress gene expression (Yoo et al., 2013). The intracellular quantity of HfQ within bacterial cells including *salmonella*, *pseudomonas*, and *E.coli* was reduced in microgravity (Wilson et al., 2007; Roy et al., 2016; Aunins et al., 2018).

To test, if the HfQ was down regulated in *E.coli* cell strain DH5αZ1 (Lutz and Bujard, 1997), chassis of our synthetic genetic device, *E.coli* DH5αZ1 in our experiment showed 50% less expression of HfQ mRNA expression in microgravity compare to the earth's gravity control (Fig. S1c).

We connected those two facts, as our design hypothesis. The decrease in HfQ protein in *E.coli* DH5αZ1 in microgravity would alter the repression capability of a designed SynsrRNA against a target protein expression compare to the earth's normal gravity.

In that direction, we designed a SynsrRNA based molecular network (Fig. 1a), which might work as a microgravity sensing and responsive device in *E. coli*. Our designed SynsrRNA against enhanced green fluorescence protein (EGFP) mRNA was around 130 bp long (Fig. 2) and its expression was under the control of an anhydrotetracycline (ATC) induced promoter pLtetO-1 (Lutz and Bujard, 1997). The designed SynsrRNA consisted of an 87 bp MicC scaffold for HfQ binding (Yoo et al., 2013) and a 40 bp sequence to bind the mRNA of the target enhanced green fluorescent protein (EGFP). The DNA sequence of the full SynsrRNA has been shown in Supplementary Fig. 2 (Fig. S2). The EGFP is our machine-readable reporter protein, which was under the control of an Isopropyl β-d-1-thiogalactopyranoside (IPTG) regulated plLacO-1 promoter. The lacI and tetR proteins, which repress the plLacO-1 and pLtetO-1 promoter respectively, were produced constitutively in the *E.coli* cell strain DH5αZ1, the chassis of our device. Therefore, in presence of the IPTG only, the EGFP will produce and would give a high fluorescence signal. However, in the simultaneous presence of ATC and IPTG, the anti-SynsrRNA against EGFP mRNA would produce and reduce the EGFP expression and fluorescence signal.

The device was fabricated according to the design and incorporated in two different plasmid vectors, where plasmid L_eGFP_AC (Fig. S3a) carries EGFP under IPTG inducible pLlacO-1 promoter in low copy (p15Ori) and plasmid T_antieGFP_synsrRNA_UA (Fig. S3b) carries SynsrRNA against EGFP mRNA under ATC inducible pLtetO-1 promoter in a very high copy (PUC Ori). Both the plasmids of our system were transformed into DH5αZ1 cells. The details of the methods can be found in supplementary note 1.

2.2. The fabricated synthetic genetic device responds to microgravity by altering expression of a fluorescence protein

Next, the individual colonies of the engineered cells were subject to overnight growth with appropriate antibiotics at 37^{O} C in a shaker incubator. Overnight cell culture from a single colony was divided into two groups. One group of cells was treated with both the inducers ATC and IPTG. The other group of cells was treated with IPTG only. The overnight culture was diluted 100 times and re-suspended in microgravity simulator reactors filled with fresh media with appropriate antibiotics and inducers. The cells in the simulators were grown for 24 h at 37^{O} C, collected, washed, re-suspended in PBS (pH:7.4) and the expression of EGFP was measured in a multimode micro-plate reader. We calculated (see method section) the fold changes in EGFP expression between IPTG only (express EGFP but not anti-EGFP SynsrRNA) and IPTG + ATC condition (express both EGFP mRNA and anti-EGFP SynsrRNA). A substantial differences in fold change (28.2) values between microgravity and normal gravity was observed (Fig. 1b). Next, to test, if

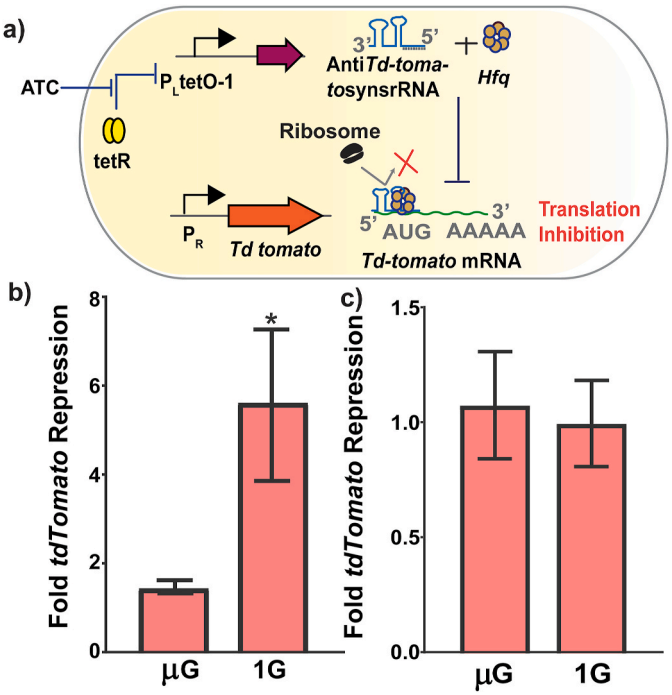

Fig. 2. a) Gene circuit design of TdTomato based microgravity sensor. b) Experimental behavior of the TdTomato based microgravity sensor. Experimental characterization and calculation are same as in the EGFP based sensor. c) Control experiment.

the difference was stemmed from the microgravity induced changes in inherent expression of EGFP, instead of information processing through our device, we ran similar experiments with cells having EGFP under $P_{Llaco-1}$ (Fig. S3a) but without the plasmid carrying anti-EGFP SynsrRNA (Fig. S3b), in two conditions (IPTG only and IPTG + ATC). We observed no significant differences (0.94 times, p value = 0.4) between microgravity and normal gravity (Fig. 1c).

This suggested that our genetic device integrated the microgravity signal and responded accordingly. The experimental methods can be found in supplementary note 1.

However, we observed a higher level of standard deviation in EGFP repression fold change in normal gravity than the microgravity (Fig. 1c). During IPTG + ATC induced state, the anti-EGFP SynsrRNAs were produced from the high copy number plasmid carrying a pUC origin of replication (pUC Ori). As this pUC Ori had a disrupted plasmid copy number regulation mechanism, it showed a higher degree of variation in plasmid borne gene expression (Bagh et al., 2008). Therefore, a moderate level of variation in the expression of anti-EGFP SynsrRNAs is highly probable, which was reflected in the higher standard deviation in normal gravity. However, in microgravity, Hfq was deregulated. This resulted a little or no repression of the EGFP expression by anti-EGFP SynsrRNAs. Therefore, the variation in anti-EGFP SynsrRNAs due to plasmid copy number variation had little or no effect. Hence, lower standard deviation was observed.

It was interesting to note that a 50% change in Hfq in microgravity (Fig. S1c) was good enough to create substantial changes in the synthetic genetic device, using HfQ mediated silencing of target genes with SynsrRNAs. The Hfq was found downregulated in microgravity in a few other bacteria (Wilson et al., 2007; Roy et al., 2016). This inspires the possibilities of creating microgravity responsive genetic devices in other bacteria too. However, its functionality beyond *E.coli* would depend on two facts. First the nature and degree of Hfq mediated regulation of gene expression by small regulatory RNAs and the degree of the HfQ repression in microgravity.

2.3. The basic device design is general in nature for E.coli

As the SynsrRNA can be designed potentially against any gene (mRNA), our designed device should serve as a general design for creating biochemical microgravity responsive device. To test, we created a new genetic device (Fig. 2a) by replacing the target gene EGFP to TdTomato, an orange fluorescence protein, derived from DsRed, whose DNA sequence was completely different from EGFP and GFP derived fluorescent proteins (Shaner et al., 2004). Further, we replaced the anti-EGFP SynsrRNA to anti-TdTomato SynsrRNA (Fig. S4, Fig. S3d) and the IPTG inducible PLacO-1 promoter to a constitutive lambda promoter P_R (Fig. S3c). We performed the same microgravity experiments with this new system and found that it worked as a microgravity responsive device too with a 3.77 times differences between 1G and microgravity (Fig. 2b).

Next, to test, whether the differences was due to the microgravity induced change in the inherent expression of TdTomato under P_R promoter, but not through microgravity integration in our device, we ran control experiments with cells having TdTomato under P_R promoter (Fig. S3c) but without the plasmid carrying anti-TdTomato SynsrRNA (Fig. 3d), in two conditions (without ATC and with ATC). We observed no significant differences (0.93 times, p value = 0.67) between microgravity and normal gravity (Fig. 2c). This suggested that the new genetic device responded against microgravity through integrating the microgravity as a signal within the genetic circuits.

However, the difference (3.77 times) is several times less than the EGFP based device (where the signal difference between 1G and microgravity was 28.2 times). This was due to the differences in the efficacy of the corresponding synthetic small regulatory RNA. It was evident from the fact that in case of EGFP there was a 30 times repression of EGFP expression, when SynsrRNA expression was fully induced (Fig. 1b, the column in 1G) compare to 5 times repression in TdTomato (Fig. 2b, the column in 1G). The inhibition efficiency of designed antisense domain of the SynsrRNA is a strong function of length, the position of target binding, and the target mRNA itself. In our design, we applied the rational strategy proposed by (Yoo et al., 2013), which suggested to use atleast the first 24 bases of the target mRNA. Though, this strategy was a good starting strategy, different efficiency was observed based on target mRNA and the scaffold region (Park et al., 2013; Sakai et al., 2014; Zhao et al., 2016). An efficient screening strategy may overcome this bias.

2.4. Application of the device to control native cellular properties with microgravity

As this biochemical device could integrate microgravity with it and potentially target any gene as a microgravity responsive gene, it may be applied to target inherent cellular biochemical process, which can be controlled in a microgravity responsive way. Therefore, we applied this device to control the cell division process with microgravity. The effect of microgravity on the *E. coli.* cell division and growth was studied in space flight and simulated microgravity condition. An increase in cell growth was observed in microgravity (Arunasri et al., 2013; Gasset et al., 1994; Nickerson et al., 2004; Kim et al., 2014). However, no mechanistic details are known. Here, using our device, we targeted a protein FtsZ, which directly involves in the cell division process. FtsZ is a tubulin family protein and its deregulation hinders the cell division process and shows elongated and filament-like shape (Sánchez-Gorostiaga et al., 2016). Here we targeted native FtsZ by creating a microgravity responsive biochemical device (Fig. S5). Here, we expressed anti-FtsZ SynsrRNAs under $P_{LtetO-1}$ promoter from plasmid T_anti-FtszsynsrRNA_UA (Fig. S3e) in DH5αZ1. We also co-transformed a constitutively expressing EGFP plasmid (Fig. S3f) for easy visualization of the cells under fluorescence microscope. In normal gravity, with the help of normal level of HfQ, in ATC induced condition, anti-FtsZ SynsrRNAs repressed the expression of the native FtsZ in the cell. The

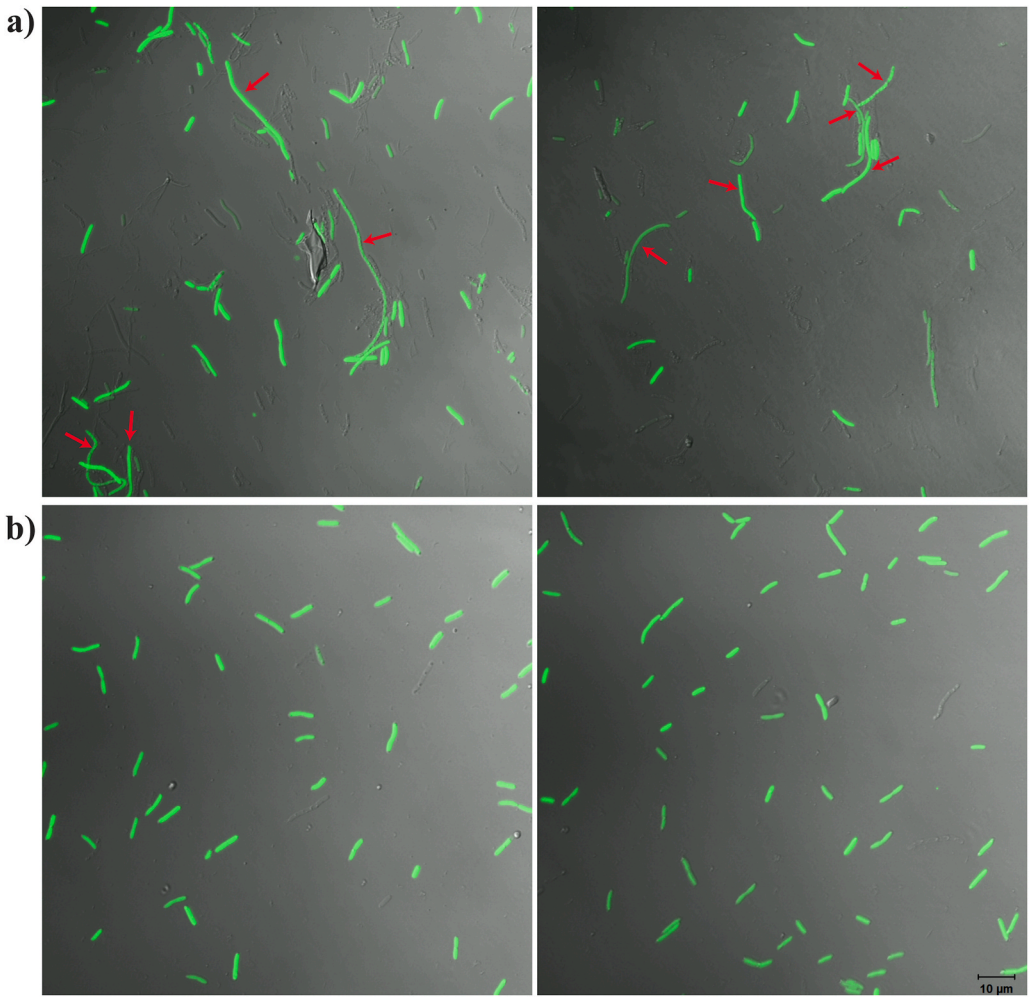

Fig. 3. Merged fluorescence and DIC image of *E. coli* DH5αZ1 cells expressing SynsrRNA against FtsZ from microgravity responsive synthetic genetic device were cultured in microgravity simulating reactor for 24 h in presence of inducer at saturated concentration in A) Earth's gravity condition (horizontal position), B) microgravity condition (vertical position). The elongated cells in the earth's gravity were shown with red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reduced repression of FtsZ resulted a deregulation in cell division process, which resulted deformed and elongated bacterial cell shapes (Fig. 3a). The HfQ mediated repression of FtsZ by anti-FtsZ SynsrRNAs (in ATC induced condition) was disrupted due to decrease in the Hfq, when the cells were under microgravity. This allowed the native FtsZ to function normally and brings back the elongated cells to normal size (Fig. 3b).

Thus, the E.*coli* with this device expressing SynsrRNA against FtsZ showed that the deformed and elongated cell shapes in earth gravity got rescued in microgravity as shown in Fig. 3 and Fig. S7a. However, the cell sizes in microgravity were still bigger than the control (Fig. S6 and Fig. S7b), where the engineered cells were grown in the absence of inducers in both gravity and microgravity conditions. We reasoned that in microgravity, the amount of hfQ decreased but not became zero. Thus, a basal level Hfq mediated repression was always present. This made a slight deregulation in FtsZ, which resulted a little increase in the cell shape in microgravity compare to the control (cells without FtsZ carrying plasmid, Fig. S6 and Fig. S7b). All the individual fluorescence and DIC images are shown in Fig. S7.

The human designed control of cell division processes and phenotype with microgravity suggested successful integration of microgravity as a physical signal with cellular biochemical processes and may have some applications in space bioengineering. However, in order to perform such experiments in space, like in international space station, specialized hardware is required. For example, the confocal microscopy experiment of this work may be done in space by using FLUMIAS-DEA, a miniaturized fluorescence microscopy platform (Thiel et al., 2019) and other cellular fluorescence measurements could be done with 'Microflow1', a sheath less fiber optic flow cytometer (Dubeau-Laramée et al., 2014) and Nanoracks fluorescence plate reader (Nimon, 2013). Further, innovative superwettable microchip platform was developed for biosensing applications in microgravity (Xu et al., 2017). Such platform would be useful to conduct the experiments on cellular microgravity responsive device at the single cell level in space.

3. Conclusion

In this work, we created the first biochemical and cellular microgravity responsive device using an engineered genetic circuit in *E.coli*, which responded to microgravity by changing the expression of a target gene. The underlying principle of the device was based on the down-regulation of HfQ protein in *E.coli* in microgravity, which was translated by coupling HfQ mediated silencing of target gene by synthetic small regulatory RNAs. This resulted a reduced silencing of the target gene in microgravity. Thus, the basic design of the device is general in nature for *E.coli*, which we demonstrated by altering its target genes (EGFP, TdTomato, and FtsZ) and the promoters (inducible and constitutive). We applied this device to control the cell division process, where the disrupted cell shape due to FtsZ deregulation in normal gravity was rescued in microgravity. However, for better results, we believe an extensive screening of SynsrRNA against target genes is required. As the device can integrate microgravity as a physical signal potentially with any downstream synthetic and native cellular processes in *E.coli*, it may have future space applications in microgravity responsive metabolic engineering and in developing cellular microgravity-health hazards monitoring systems. However, to assess its real usefulness, the device must be

experimented in space. Taken together, our work may have significance in space bioengineering and synthetic biology.

Credit author contribution statement

Sayak Mukhopadhyay: Experiments, Formal analysis, Data curation. **Sangram Bagh:** Concieved the study, Formal analysis, Data curation, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2020.112462.

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