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Counteracting Muscle Atrophy on Earth and in Space via Nanofluidics Delivery of Formoterol

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Skeletal muscle atrophy is a critical health problem that affects quality of life and increases morbidity and mortality. At present, exercise training remains the only intervention and pharmaceutical treatments remain elusive. Formoterol (FMT), a β2-adrenergic receptor agonist, has emerged as a potential therapeutic by triggering skeletal muscle anabolism with daily dosing. Here, the efficacy of sustained FMT release is investigated via a subcutaneously implanted nanofluidic delivery system (nF) to prevent muscle wasting. Pharmacokinetics of nF-mediated sustained FMT delivery (nF-FMT) in healthy mice is assessed for 56 days, which demonstrates an anabolic effect on skeletal muscles. Using a hind limb suspension unloading mouse model, it is shown that nF-FMT treatment attenuates soleus mass loss in comparison to control mice. Further, the very first study of an implantable drug delivery device in microgravity in vivo is launched. The microgravity environment aboard the International Space Station is leveraged to assess the atrophy prevention capability of nF-FMT in mice for 29 and 55 days. Muscle hypertrophy is observed in both ground control and spaceflight mice treated with nF-FMT compared to their respective vehicle controls. Overall, the nF system is presented as a viable platform for sustained delivery of FMT for therapeutic intervention of skeletal muscle atrophy.

1. Introduction

Skeletal muscle wasting is a debilitating pathological condition characterized by accelerated loss in muscle mass due to rates of protein degradation exceeding synthesis. Muscle wasting develops as a consequence of numerous factors, including 1) prolonged disuse such as immobilization, denervation, and exposure to microgravity, 2) aging (sarcopenia), or 3) systemic response to chronic medical conditions such as cancer, acquired immune deficiency syndrome (AIDS), diabetes, neuromuscular disorders, and organ failure (cachexia).[1,2] The progressive loss of muscle mass deleteriously affects quality of life, and amplifies the burden of existing diseases, leading to increased morbidity and mortality.[3] For example, cachexic muscle wasting occurs in 80% of patients with cancer, directly accounting for ≈30% of cancer mortality.[3,4] In patients with AIDS, muscle wasting is associated with increased risk of opportunistic infections and death.[5] Currently,

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there are no pharmacological therapies for muscle wasting, and physical exercise remains the only validated treatment.[2] However, exercise training is impractical for frail, bedridden, or physically confined individuals. In space, microgravity-induced muscle atrophy occurs rapidly in spite of the allocated 2.5 h of daily physical exercise.[6,7] Moreover, the strict requirement for adherence to vigorous in-flight exercise routine coupled with the physically restrictive environment aboard the International Space Station (ISS) United States National Laboratory (NL) poses significant challenges.[8,9] Muscle atrophy compromises astronaut health and imposes risks of injuries during space flight missions and upon return to Earth.[10] For these reasons, safe prevention and treatment of muscle wasting is of high clinical relevance but remains elusive.

β-adrenergic receptors (ARs) are key for maintaining muscle activity and function, including that of skeletal, smooth, and cardiac muscles. β2 subtype is the predominant AR expressed in smooth and skeletal muscles.[11,12] β2-AR agonists are approved for local administration through inhalation for bronchodilation of smooth muscles to treat respiratory ailments such as chronic obstructive pulmonary disease (COPD) or asthma.[13,14] In skeletal muscle, β2-AR signaling activation is central to muscle protein synthesis.[11] Preclinical studies demonstrated that systemic administration of β2-AR agonists supports anabolic and anticatabolic processes in skeletal muscles, resulting in progrowth and antiatrophic effects.[11,15,16] Specifically, formoterol (FMT) is a potent β2-AR agonist that triggers hypertrophy in both normal and perturbed skeletal muscles.[17,18] In addition to promoting muscle growth, FMT prevents muscle wasting by inhibiting ubiquitin-dependent proteolysis and decreasing muscle cell apoptosis.[17] FMT attenuation of muscle mass loss is demonstrated in preclinical and clinical studies.[18–22] In a recent phase I/II clinical study in cachexic cancer patients, FMT administered orally twice daily for 8 weeks along with megestrol (a progestin hormone used for appetite stimulation) resulted in augmented muscle mass and function.[22]

While FMT holds promise for preventing or reversing muscle loss, high-dose exposure could result in β2-AR downregulation and desensitization, thereby limiting therapeutic efficacy.[23,24] Further, clinical application of FMT for treating muscle wasting is currently limited due to concerns of cardiovascular side effects.[22,25,26] FMT treatment is implicated in cardiac hypertrophy due to the presence of β2-AR, albeit low, in the heart muscle.[18,19,22,24] Moreover, the relatively short plasma half-life[27] and requirement of daily dosing presents a clinical challenge in terms of medication adherence, which is a long-standing problem in medicine.[28] Taking these factors into consideration, we postulate that sustained low dose systemic FMT administration through a minimally invasive implantable drug delivery platform could be a viable therapeutic intervention for muscle wasting. This hypothesis is supported by preclinical studies of muscle-hypertrophy-inducing effects of FMT at systemic doses as low as 1/300 of conventionally administered dose in rodents.[14] Here, we present a subcutaneously implantable nanofluidic delivery system (nF) for sustained low dose FMT delivery. With the nF, sustained drug delivery occurs via diffusion through nanochannels, whereby controlled release is achieved by leveraging nanofluidics without requiring pumps, power supply, or physical manipulation. We posit that continuous release through the nF could address the challenge of medication adherence, while low dosage delivered could alleviate adverse side effects without compromising muscle anabolic effects.

In this study, we investigated sustained release of FMT via the nF through in vitro analysis and in vivo studies in mice. First, we conducted a pharmacokinetics study of nF-mediated sustained FMT administration (nF-FMT) in healthy mice for 56 days. Then, we performed a 21-day hind limb suspension study of muscle unloading in mice to assess efficacy of nF-FMT to prevent atrophy. Finally, we performed the first-of-its-kind investigation of implantable drug delivery devices in space. We leveraged the microgravity environment aboard the ISS NL and conducted a muscle atrophy prevention study using nF-FMT in mice for 29 and 55 days. Our studies demonstrated muscle hypertrophy in nF-FMT-treated mice, as well as feasibility of long-term sustained FMT release on Earth and in space. Overall, we present our nanofluidics delivery platform for sustained low dose FMT administration as a viable alternative to daily dosing for treatment of muscle atrophy. Beyond the potential clinical implications of alleviating muscle wasting in individuals on Earth, our nanofluidics delivery platform could conceivably be adopted by astronauts to supplement and concomitantly reduce the required intensive in-flight exercise countermeasures.[8,29,30]

2. Results

2.1. In Vitro Release Analysis of Formoterol via nF

In this study, we utilized our nF for controlled release of FMT (**Figure 1**A,B). Fundamental to the release mechanism is a nanofluidic membrane, permitting continuous, controlled drug elution (Figure 1A–C). As drug molecules traverse across the nanochannels, exposure to the enhanced electrostatic and steric interactions in the nanometrically confined setting generates a saturated diffusive transport that is quasi-independent from the concentration gradient.[31–33] Thus, constant and sustained drug release is achieved via passive diffusion, free of actuation, external components, or requirement for physical manipulation. Release rate is governed by the dimension and quantity of nanochannels that are perpendicularly interconnected to microchannels by which the drugs are ultimately released (Figure 1C).[31,32,34,35] In accordance with our previously established algorithm to achieve sustained release through the nanofluidic membrane,[32] we selected nanochannel size of 250 nm based on the physicochemical properties of FMT. To assess release kinetics of FMT from the nF, we performed an in vitro analysis where nF-FMT devices were submerged in a sink solution at 37 °C. Drug elution analysis demonstrated a linear cumulative release of 1.9 ± 0.6 μg FMT over 14 days (Figure 1D), corresponding to an average daily release of ≈136 ng per day. This in vitro release result validated sustained delivery of FMT with our selected nanochannel size.

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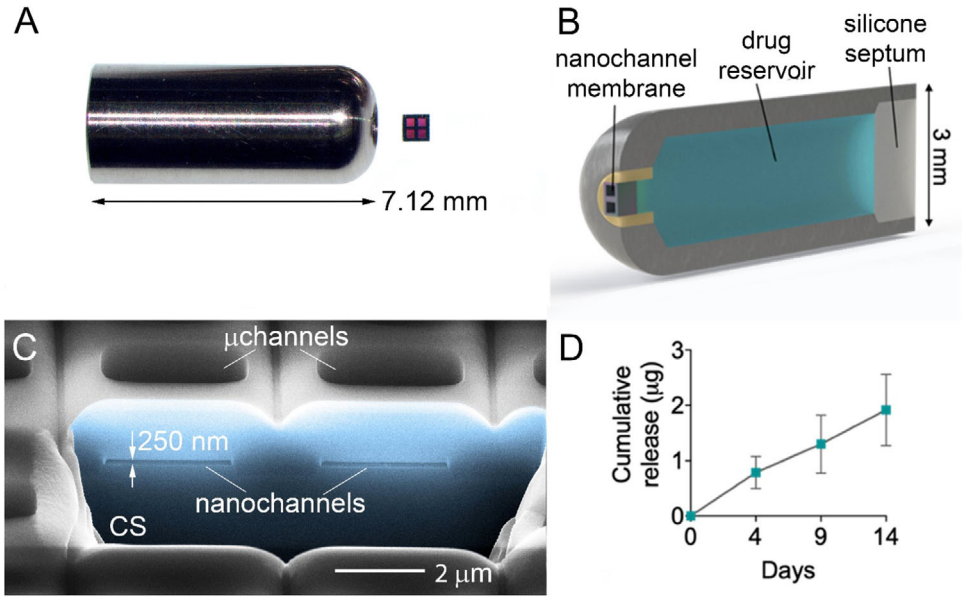

Figure 1. Nanofluidic delivery system (nF) for sustained formoterol (FMT) release. A) Picture of nF showing titanium cylindrical drug reservoir with 2 × 2 nanochannel membrane in comparison to a dime. B) Cross-section rendering of nF depicting nanochannel for controlled drug release, drug reservoir, and silicon septum for device sealing. C) FIB–SEM image of the 250 nm nanofluidic membrane showing microchannels (μchannels) and nanochannels cross-section (CS; colored in blue). Scale bar represents 2 μm. D) In vitro cumulative release of FMT from nF at 37 °C in Solutol solution over 14 days measured via HPLC. Data are shown as mean ± SD ($n = 3$).

2.2. Pharmacokinetic Analysis of Sustained FMT Delivery via nF

To evaluate the pharmacokinetics and effects of FMT achieved through sustained subcutaneous (SQ) delivery from the nF, we performed a 56-day study in healthy mice. We used nF loaded with either FMT or phosphate-buffered saline (PBS) as a vehicle control (nF-VEH), and subcutaneously implanted the devices in the dorsum of the mice. To determine effective doses of sustained nF-FMT administration, we used nF loaded with FMT at either low (1 mg mL^{-1}; nF-FMT_{low}) or high (10 mg mL^{-1}; nF-FMT_{high}) dose, for a nominal target of ≈35 or 350 ng release per day, respectively.

In humans and rodents, atrophy due to immobilization, unloading, or microgravity occurs primarily in antigravity muscles of the hind limb, mainly in the soleus followed by gastrocnemius muscles.[36,37] Therefore, we focused on FMT effects on the soleus and gastrocnemius. We also examined the tibialis anterior (TA), a dorsiflexor muscle less prone to unloading-induced atrophy, due to reported anabolic effects of FMT.[17,18,38,39] Sustained FMT treatment over 56 days increased absolute soleus weight above that of VEH at both doses tested in a dose-dependent manner (**Figure 2**A). Specifically, nF-FMT_{low} increased soleus weight by 9.6% above VEH ($p = 0.04$), while nF-FMT_{high} exerted the greatest hypertrophic effect of 24.5% ($p < 0.0001$) above VEH control and 13.6% ($p = 0.004$) above nF-FMT_{low}. As for the gastrocnemius, sustained release via nF-FMT_{high} increased absolute muscle weight by 7.1% ($p = 0.02$) and 7.6% ($p = 0.01$) compared to VEH and nF-FMT_{low}, respectively (Figure 2B). No hypertrophic effects of the gastrocnemius were observed with nF-FMT_{low}. Similar to gastrocnemius, nF-FMT_{low} did not induce hypertrophy of the TA. Instead, nF-FMT_{high} induced TA hypertrophy by 6.9% with respect to VEH ($p = 0.03$) and 8.1% compared to nF-FMT_{low} (Figure 2C). Importantly, nF-FMT treatment groups did not cause cardiac hypertrophy, which is a significant reported side effect of systemic FMT administration (Figure 2D).

Older generations of β2-AR agonists such as clenbuterol are associated with decreased body weight during the first 5 days of treatment.[14] This is attributable to appetite suppression, which could be detrimental to individuals in fragile physiological conditions. nF-FMT_{high} treatment group exhibited an increase in body weight, which was significantly higher than VEH on days 7, 14, 28, and 56 of the study (Figure 2E). When normalized to body weight, soleus (Figure S1A, Supporting Information), gastrocnemius (Figure S1B, Supporting Information), and TA (Figure S1C, Supporting Information) of nF-FMT_{high} mice were significantly increased by 22.6% ($p < 0.0001$), 4.7% ($p = 0.03$), and 5.2% ($p = 0.02$), respectively, compared to that of VEH. Thus, we attributed the increase in overall body weight at study endpoint to the hypertrophic effects of FMT on the muscles. Compared to the nF-FMT_{low} group, nF-FMT_{high} induced a 10.5% ($p = 0.008$), 4.6% ($p = 0.04$), and 5.1% ($p = 0.04$) increase in the soleus, gastrocnemius, and TA, respectively (Figure S1, Supporting Information), further substantiating the dose-dependent anabolic effect of FMT.

Throughout the study, we detected FMT levels in the blood, albeit declining from day 3 onward, with the exception of nF-FMT_{low} on days 42 and 56 (Figure 2F). Specifically, nF-FMT_{low} showed a median FMT blood concentration of 0.8×10^{-9} M (interquartile range (IQR): 0.4×10^{-9}–2.2×10^{-9} M) whereas nF-FMT_{high} was 2.3-fold higher with a median of 1.8×10^{-9} M (IQR: 0.9×10^{-9}–4.5×10^{-9} M). As a comparison, we performed a single-dose pharmacokinetic analysis of FMT in a separate cohort of mice via SQ injection of 1.0 mg kg^{-1} (Figure 2G). We observed that peak FMT blood concentration (C_{max}) of 561.0 ± 141.0×10^{-9} M was reached at 0.3 ± 0.0 h (T_{max}) with an area

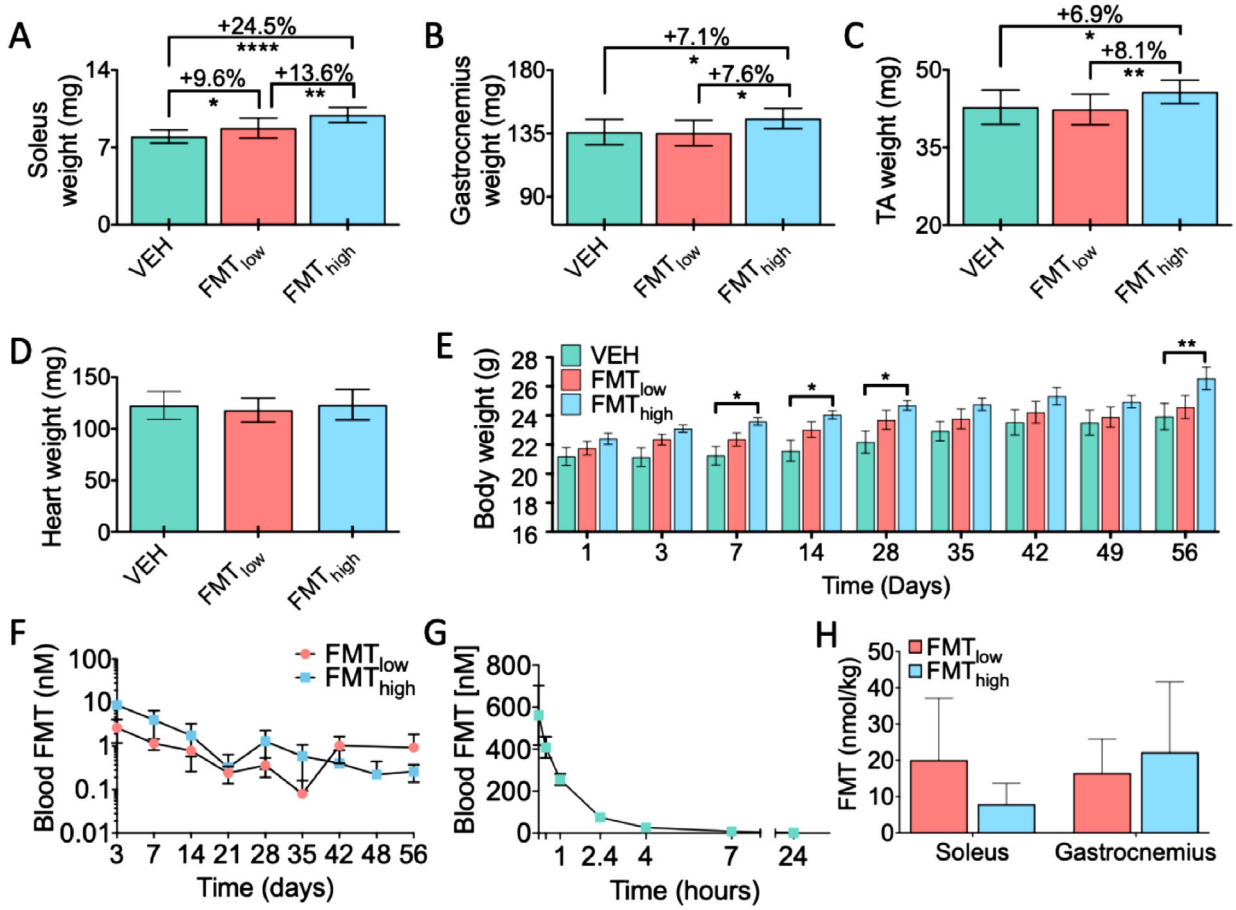

Figure 2. Effects and pharmacokinetics of sustained FMT delivery via nF in healthy mice over 56 days. Weights of A) soleus, B) gastrocnemius, and C) TA muscles, D) hearts, and E) body weights of mice treated with either nF-VEH or nF-FMT (FMT_{low} or FMT_{high}) for 56 days. F) Blood FMT analysis of nF-FMT groups (FMT_{low} or FMT_{high}) throughout the 56-day study was performed via LC-MS/MS. G) Blood FMT analysis after subcutaneous bolus injection (single dose of 1 mg kg^{-1}) in mice was performed via LC-MS/MS. H) LC-MS/MS analysis of FMT exposure in the soleus and gastrocnemius muscles of nF-FMT groups (FMT_{low} or FMT_{high}). All data are shown as mean ± SD. nF-VEH ($n = 9$), nF-FMT_{low} ($n = 10$), and nF-FMT_{high} ($n = 10$). Unpaired, two-tailed Student's *t*-test was used for comparisons between two groups with the exception of (E) where two-way ANOVA with Tukey's post hoc was used. $*p < 0.05$, $**p < 0.01$, and $****p < 0.0001$.

under the curve (AUC) of (760 ± 164.0) × 10^{-9} M h. We also analyzed FMT concentrations in the soleus and gastrocnemius at study termination (Figure 2H). We observed drug exposure in the muscles of both nF-FMT_{low} and nF-FMT_{high} treatment groups in spite of declining blood concentrations, indicating a potential accumulation of FMT in muscle tissue over time. Although FMT blood concentrations in nF-FMT_{high} had an inexplicable drop below that of nF-FMT_{low} after 42 days, there was no significant difference in muscle concentrations.

Overall, we showed that sustained release of FMT using nF induced anabolic effects on muscles without triggering cardiac hypertrophy in healthy mice.

2.3. Efficacy of nF-FMT to Prevent Muscle Atrophy in Hind Limb Suspension Unloading Rodent Model of Simulated Microgravity

To assess efficacy of nF-FMT to prevent muscle atrophy, we used the hind limb suspension unloading (HSU) model to simulate muscle disuse in rodents.[40] HSU is also a commonly used surrogate approach for microgravity simulation.[14] Mice were suspended by their tails, preventing their hind limbs from load bearing. HSU induces atrophy mainly in the soleus as well as gastrocnemius, and to a lesser extent, TA,[41] recapitulating skeletal muscle responses to unloading in humans.[42,43] One week prior to HSU, mice were implanted with either nF-VEH or nF-FMT and an additional cohort pretreated with daily SQ injection of FMT at a dose of 2 µg per injection. Based on our results in healthy mice (Figure 2), we filled the nF with 20 mg mL^{-1} of FMT, which corresponds to a release of ≈680 ng per day, in anticipation that a higher dose would be required for counteracting muscle atrophy in HSU mice. Nonsuspended untreated mice were established as ground control (GC), and allowed to roam freely within their environment.

After 21 days of HSU, soleus showed significant atrophy across all groups of suspended mice. nF-VEH had the largest decline in soleus weight (−26.9%; $p = 0.002$), followed by SQ FMT groups (−25.0%; $p = 0.002$), compared to GC (**Figure 3**A).

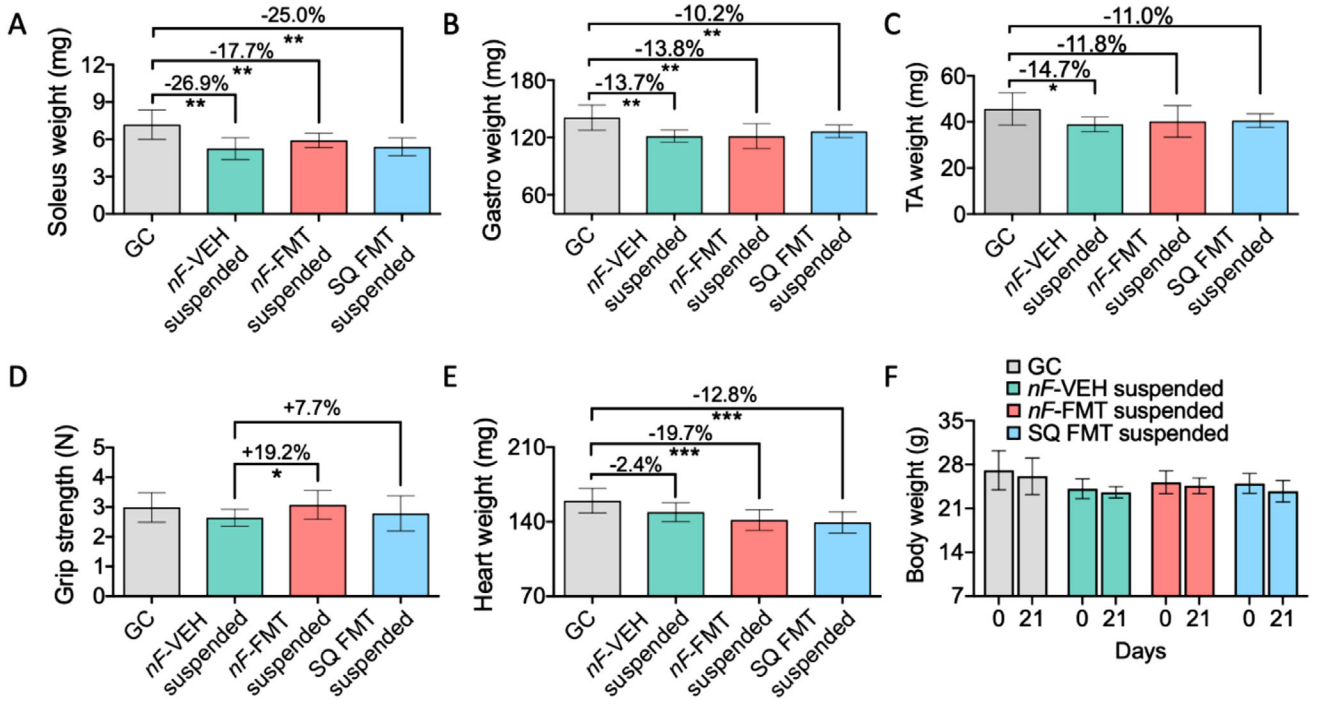

Figure 3. Effects of FMT on hind limb suspension unloaded mice for 21 days. A) Soleus, B) gastrocnemius (gastro), and C) TA weights of ground control (GC), and suspended VEH control, nF-FMT, and subcutaneous (SQ) FMT mice. D) Grip strength of hind limb for characterization of muscle strength after 21 days of HSU. Hind limb grip strength in each mouse was measured in triplicate and the average plotted. E) Heart weights at the end of the 21-day study. F) Body weights of the mice pre- and post-HSU at the end of the 21-day study. All data are shown as mean ± SD. GC ($n = 8$), VEH-suspended ($n = 8$), nF-FMT ($n = 11$), SQ FMT ($n = 9$). An unpaired, two-tailed Student's t-test was used for comparisons between two groups. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

nF-FMT-treated mice demonstrated the smallest degree of soleus weight loss (−17.7%; $p = 0.006$), suggesting that sustained FMT delivery attenuated atrophy as compared to the GC group. Gastrocnemius atrophy occurred in all suspended mice, similar to that of the soleus, albeit to a smaller extent (Figure 3B). Specifically, HSU induced gastrocnemius atrophy in nF-VEH (−13.7%; $p = 0.002$), nF-FMT (−13.8%; $p = 0.003$), and SQ FMT (−10.2%; $p = 0.009$). With respect to nF-VEH, FMT treatment either through nF or SQ administration did not attenuate gastrocnemius atrophy. Atrophy of the TA was significant only in VEH-treated suspended mice (−14.7%; $p = 0.02$), compared to GC (Figure 3C). We did not detect significant decline in TA weights of nF-FMT (−11.8%; $p = 0.09$) or SQ FMT (−11.0%; $p = 0.06$) relative to GC; suggesting atrophy attenuation effects of FMT on this muscle.

As a characterization of muscle strength changes during HSU, we assessed hind limb grip strength upon study termination on day 21 (Figure 3D). Grip strength of VEH-treated suspended mice (2.6 ± 0.5 N) decreased compared to GC mice (3.0 ± 0.5 N), albeit not significantly ($p = 0.1$). Of the suspended mice, nF-FMT-treated group had significantly higher grip strength (3.1 ± 0.3 N) than VEH-treated group (2.6 ± 0.5 N), corresponding to a 19.2% improvement ($p = 0.04$). SQ FMT (2.8 ± 0.6 N) treatment non-significantly increased grip strength by 7.7% compared to VEH group ($p = 0.5$). No significance difference in grip strength was detected in either nF or SQ FMT-treated suspended mice relative to GC ($p = 0.7$ and $p = 0.4$, respectively), suggesting that FMT treatment improved muscle strength.

The effects of HSU on heart are controversial, with mixed reports of either reduction or unaffected heart weight.[44] nF-VEH-suspended mice had 2.4% ($p = 0.06$) decrease in heart weight compared to GC mice (Figure 3E). FMT treatment in HSU mice caused significant decline in heart weight (Figure 3E); we detected 19.7% ($p = 0.001$) and 12.8% ($p = 0.0008$) decrease in heart weight of nF-FMT and SQ FMT mice, respectively. These results are unexpected considering that β2-agonists are typically implicated in cardiac hypertrophy.[14] Moreover, in the 56-day study of nF-FMT in healthy mice, no change in heart weight was detected (Figure 2D). Therefore, we attribute cardiac atrophy to inexplicable combinatorial effects of HSU and FMT treatments, which to our knowledge remains unreported in literature. Despite significant muscle loss, we did not observe a corresponding decline in body weights of HSU mice (Figure 3F). Further, food consumption was assessed as an indirect measure of stress experienced by animals subjected to HSU with or without FMT, whereby no substantial differences were noted between groups (Figure S2, Supporting Information).

Overall, these results indicate that while significant prevention of muscle wasting was not achieved, sustained FMT delivery via nF showed trends toward attenuated soleus and TA atrophy and a reversion of grip strength to that of GC mice. Importantly, nF-FMT was equivalently efficacious as SQ FMT

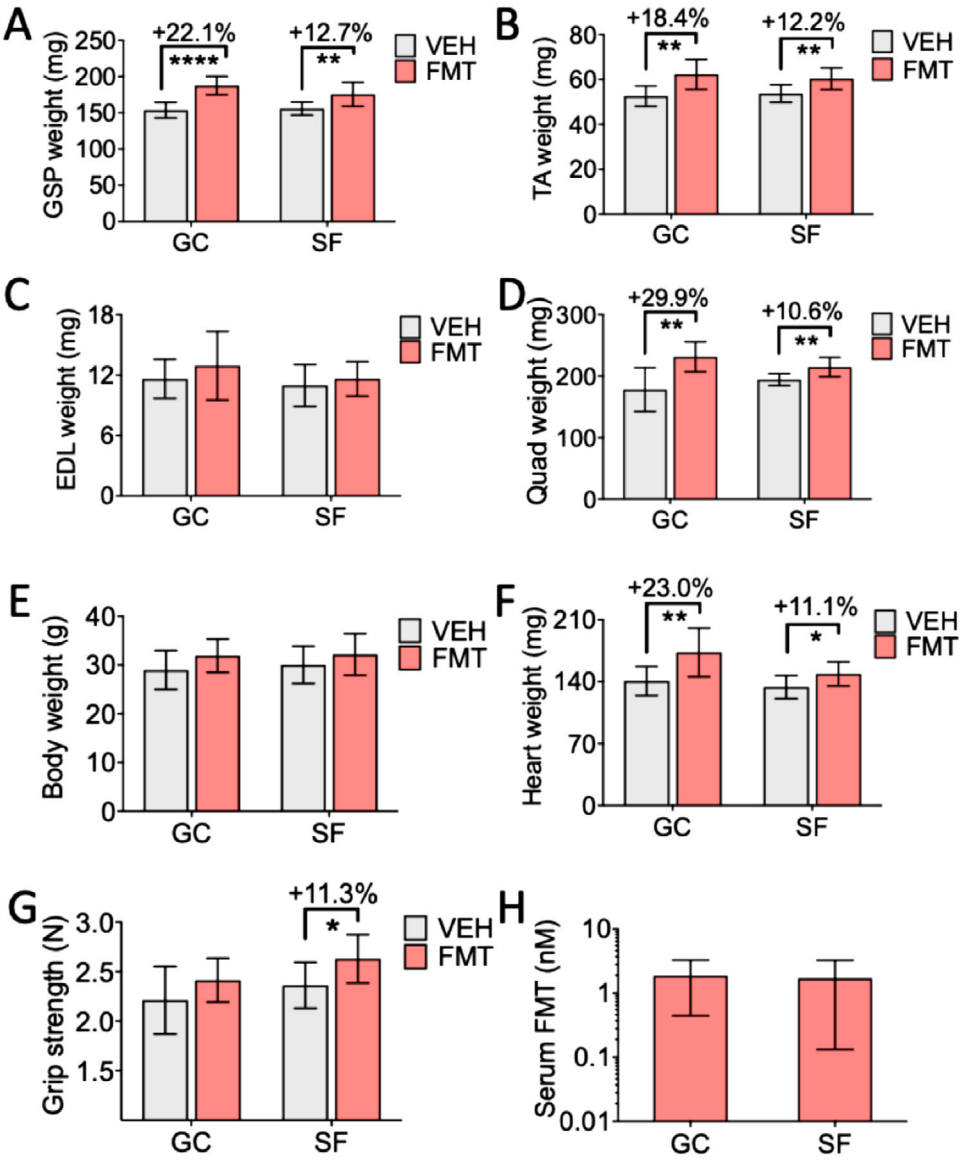

Figure 4. nF-FMT effects on mice in microgravity aboard the ISS NL for 29 days. Weights of A) GSP, B) TA, C) EDL, and D) quadriceps muscles, E) body, and F) heart of GC and spaceflight (SF) mice treated with either nF-VEH or nF-FMT treatment at the end of the 29-day study. G) Grip strength was measured upon live animal return to Earth. Hind limb grip strength in each mouse was measured 4 times and the average plotted. H) Serum FMT analysis of GC and SF collected at the end of the 29-day study was performed via HPLC. All data are shown as mean ± SD. $n = 10$ for all groups except GC VEH ($n = 9$). Unpaired, two-tailed Student's t-test was used for comparisons between two groups. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$.

(at 1/3 of the SQ dose) without the inconvenience of daily injection.

2.4. Effects of FMT on Muscles Exposed to Microgravity Conditions

Next, we evaluated the effects of nF-FMT on muscles of mice under microgravity conditions. We conducted a rodent spaceflight (SF) study known as Rodent Research-6 (RR-6) on SpaceX CRS-13 commercial resupply mission by National Aeronautics and Space Administration (NASA). Prior to SF, mice were subcutaneously implanted with either nF-FMT or nF-VEH, in which a cohort was designated as GC group. nF-FMT was prepared with the same dosage as in the HSU study in light of the atrophy attenuation results achieved (Figure 3). SF mice were exposed to microgravity conditions aboard the International Space Station National Laboratory for 29 or 55 days.

Microgravity affects mainly the plantar flexors (gastrocnemius, soleus, and plantaris; GSP), as well as quadriceps (quad).[6,41,43,45,46] In contrast to previously published studies, we did not observe muscle atrophy in the 29-day study, as demonstrated by lack of GSP muscle weight loss in nF-SF-VEH compared to nF-GC-VEH mice (**Figure 4**A, $p = 0.63$). It is conceivable that lack of atrophy could be attributable to age, environmental enrichment, or activity level of the mice during SF.[47,48]

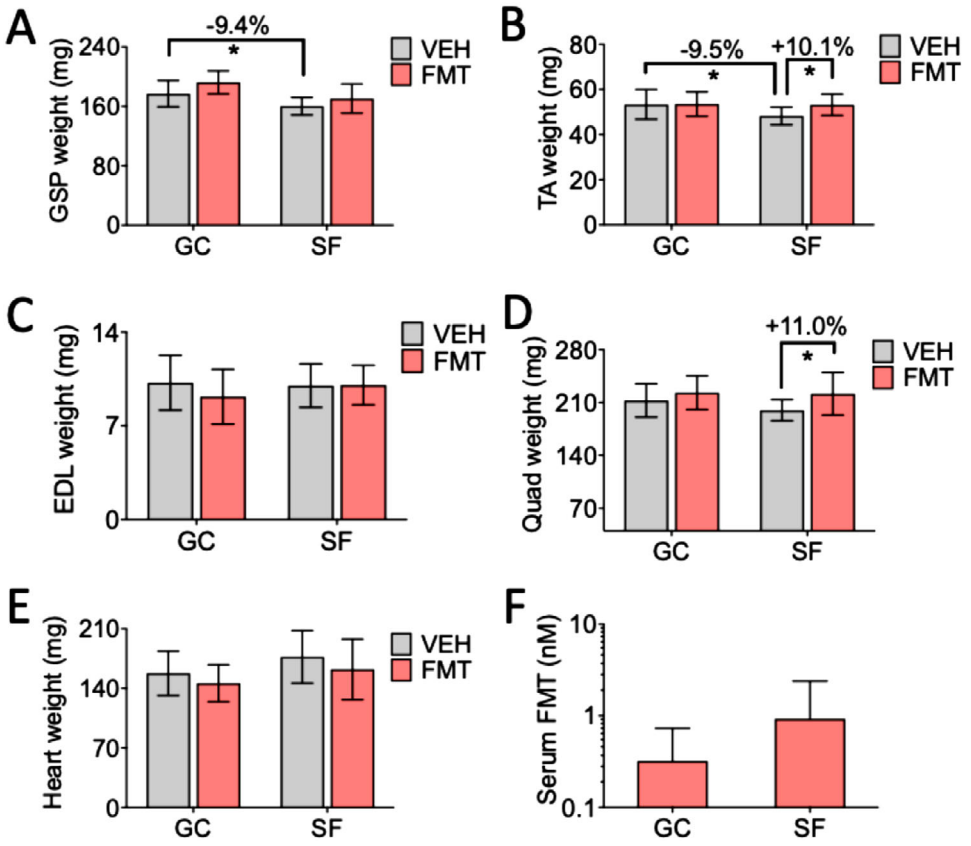

Figure 5. nF-FMT effects on weights of mice in microgravity aboard the ISS NL for 55 days. Weights of A) GSP, B) TA, C) EDL, and D) quad muscles, E) body, and F) heart of GC and SF mice treated with either nF-VEH or nF-FMT treatment at the end of the 55-day study. All data are shown as mean ± SD. $n = 10$ for all groups. An unpaired, two-tailed Student's t-test was used for comparisons between two groups. *$p < 0.05$ and **$p < 0.01$.

Nevertheless, we detected muscle hypertrophy across various muscle tissues of nF-FMT-treated mice. GSP weights in both nF-FMT GC and nF-FMT SF mice were significantly increased compared to their respective nF-VEH controls (Figure 4A). Specifically, nF-FMT induced 22.1% ($p < 0.0001$) increase in GSP mass in GC group and 12.7% ($p = 0.004$) in SF group. Upon further analysis, soleus weights of nF-FMT GC mice were augmented by 40.3% ($p = 0.01$) (Figure S3A, Supporting Information), whereas GP weights of nF-FMT GC and nF-FMT SF cohorts increased by 20.3% ($p < 0.0001$) and 10.0% ($p = 0.01$), respectively (Figure S3B, Supporting Information), compared to their respective nF-VEH controls.

In the dorsiflexors, hypertrophy due to nF-FMT treatment was detected in the TA of GC and SF mice with increase of 18.4% ($p = 0.002$) and 12.2% ($p = 0.003$), respectively (Figure 4B), but not in the extensor digitorum longus (EDL) (Figure 4C). Further, we observed anabolic effects of nF-FMT in the quad muscles, with 29.9% ($p = 0.001$) increase in GC group and 10.6% ($p = 0.002$) in SF group (Figure 4D). After 29 days of SF, body weights were comparable to GC mice; no significant difference was detected between nF-VEH or nF-FMT mice (Figure 4E). In contrast to literature, SF body weight was not impacted compared to GC.[49–51]

Microgravity exposure did not affect heart weight of nF-VEH-treated SF mice with respect to that of nF-VEH GC (Figure 4F; $p = 0.3$). However, compared to their respective VEH controls, nF-FMT induced cardiac hypertrophy in GC (+23.0%; $p = 0.007$) and SF mice (+11.1%; $p = 0.02$). The increase in cardiac mass is in contrast to our previous experiments (Figures 2D and 3E) but could be partially explained by the different housing and feeding used in the experiments. Microgravity environment causes muscle function decline,[6] however, nF-FMT SF mice had an 11.3% increase in grip strength when compared to nF-VEH SF ($p = 0.02$), whereas this effect was not apparent in nF-GC mice ($p = 0.14$) (Figure 4G). Analysis of serum FMT levels after 29 days of nF-FMT treatment showed similar levels between the different experimental conditions (Figure 4H), indicating that nF drug release function was not likely impacted by microgravity exposure.

While atrophy did not occur in mice during the 29-day SF, anabolic effect of nF-FMT on skeletal muscles in microgravity was discernible, specifically in GSP and TA muscles. It can also be noted that the effect of FMT treatment tended to be consistently lower in SF mice; indicating that in the absence of overt atrophy, there was some blunting of FMT-induced hypertrophy in microgravity. These results indicate that our device could sustainably deliver sufficient dose of FMT to stimulate muscle growth in mice on Earth and in microgravity environment.

In contrast to the 29-day study, mouse carcasses returned from 55 days in microgravity demonstrated significant atrophy in GSP (−9.4%; $p = 0.02$) (**Figure 5**A) and TA (−9.5%; $p < 0.05$) (Figure 5B), but not EDL ($p = 1.0$) (Figure 5C) or quad ($p = 0.7$)

(Figure 5D), when comparing muscle weights between nF-VEH in GC and SF mice. Further evaluation showed that compared to the nF-VEH GC group, nF-VEH SF mice had significant muscle mass loss in the soleus (slow twitch) muscles (−18.8%; $p = 0.04$) (Figure S4A, Supporting Information) and GP (predominantly fast twitch) muscles (−10.0%; $p = 0.03$) (Figure S4B, Supporting Information). Based on this, the soleus muscle exhibited the largest degree of atrophy induced by microgravity. However, with nF-FMT treatment, only TA and quad of SF mice demonstrated increase in muscle weight of 10.1% ($p = 0.02$) and 11.0% ($p = 0.04$), respectively, compared to nF-VEH GC. There was no substantial difference in heart weights of GC and SF mice, suggesting minimal effect of microgravity or treatment condition (Figure 5E). Similar to the 29-day study, serum FMT remained detectable at 55 days, confirming long-term drug release capability of the nF (Figure 5F).

3. Discussion

Skeletal muscle mass loss is a common manifestation in individuals with chronic diseases, bedridden, or those that are exposed to microgravity conditions, as well as in the aging population. Further, medications such as glucocorticoids, which are frequently prescribed for various inflammatory or malignant diseases, are implicated in mediating rapid muscle wasting as early as 7 days after initial dose.[52,53] Despite the prevalence of muscle loss conditions and compounding effect on disease morbidity and mortality, pharmacologic treatment for muscle wasting remains an unmet medical need.

β2-AR agonists have demonstrated protective or anabolic effects on muscles when administered daily.[16,18,21,22,54] However, requirement for daily dosing could present adherence challenges, which could impair therapeutic efficacy. Thus, we posit that an implantable device to systemically continuously deliver therapeutic levels of β2-AR agonists could alleviate the burden of daily dosing, increase compliance, and mitigate adverse side effects associated with fluctuations in drug concentration. In our study, we demonstrated that sustained low dose administration of FMT through subcutaneously implanted nF for up to 56 days significantly induces muscle hypertrophy in healthy mice. Further, these results are in agreement with previous literature reports of the muscle anabolic effects of FMT, at doses ranging from 1 μg kg^{-1} per day to 2 mg kg^{-1} per day administered systemically.[14,16,21] Ryall et al. demonstrated muscle hypertrophic effects of FMT in rats at doses as low as 1 μg kg^{-1} delivered via daily intraperitoneal (IP) injection for 4 weeks.[14] Here, we showed that sustained subcutaneous delivery of FMT resulting in systemic levels as low as $\approx 1 \times 10^{-9}$ M is sufficient for increasing muscle mass in healthy mice and in mice exposed to microgravity conditions.

While our primary focus was on antigravity muscles, we observed hypertrophic effect of FMT on non-antigravity muscles as well. On this note, it is possible that the variable hypertrophic effect of FMT across different muscles could be attributable to the density and distribution of β-adrenoceptors. For example, β2-ARs are evenly distributed over GSP muscles,[55] and in particular, the soleus has 61% more β-adrenoceptors than the EDL.[14,56] This could explain the observed hypertrophic effect of nF-FMT on the GSP (Figure 4A) and not on the EDL (Figure 4C) in the 29-day microgravity study. However, previous studies have shown that FMT administered via IP injection at a dose of 25 μg kg^{-1} per day induces significant hypertrophy in the EDL as well as the soleus in healthy young and old rats, and in healthy or dystrophic mice.[14,21,57] In studies of tumor-bearing cachectic and control nontumor-bearing mice and rats, daily FMT dosing either via IP (2 mg kg^{-1}) or subcutaneous (0.3 mg kg^{-1}) injection resulted in an increase in gastrocnemius, TA, and EDL muscles.[18,19] In a phase I/II study in cachectic patients with advanced cancers, 80 μg per day oral FMT for 8 weeks improved muscle mass and function, specifically in the quadriceps. Thus, we posit that FMT exerts anabolic effect on muscles regardless of delivery route, host environment (on ground vs microgravity), age, and across different physiological conditions or disease states; though, muscle-specific responses could be dependent on the aforementioned factors (i.e., receptor density). Our results are in accordance with that reported in literature, albeit without the cumbersome requirement of daily medication administration, which could be particularly advantageous for those in fragile conditions or physically challenged.

Our RR-6 SF study was conducted to assess efficacy of nF-FMT to prevent muscle atrophy in the microgravity environment such as that aboard the ISS NL. This aspect is of paramount importance considering increased human endeavors in space exploration and research for on-Earth benefits to healthcare and the biomedical field.[58–60] In contrast to literature where atrophy is demonstrated to occur as soon as 12 days in space,[46,47,50] we did not observe this phenomenon in our 29-day microgravity study (Figure 4). This result was unexpected as older animals (>24 months) are thought to be more susceptible to the atrophic effects of microgravity. In other space studies where relatively younger (i.e., 16-week old) mice were used,[47,51] it is conceivable that muscle loss may be a combination of atrophy and inhibition of further growth and development.[50] Lack of muscle atrophy could also be attributable to active behavior, as observed in the 33-day Rodent Research-1 (RR-1) mission,[48] which used a similar animal housing system. The Rodent Habitats used have grids that line the floor and walls, allowing for mice to grab and utilize to facilitate ambulation. In line with this, free-floating never exceeded 3% of the total physical activity during RR-1. Moreover, SF mice in RR-1 were observed to switch from forelimb to quadrupedal ambulation, indicative of adaptation to weightlessness in space. Importantly, it was noted that in the RR-1 mission, SF mice spent more time ambulating than GC mice, suggestive of increased physical activity in space.[48] Further, the environment enrichment in RR-6 involved addition of huts where mice tended to congregate in confined conditions for a large duration of the experiment, potentially allowing for various degrees of reloading throughout the course of the SF experiment. Despite the lack of muscle mass loss, nevertheless we demonstrated that nF-FMT induced muscle hypertrophy in both GC and SF mice. Although not successfully demonstrated in our HSU study (Figure 3), the 55-day microgravity study alludes to nF-FMT-mediated atrophy attenuation, specifically the TA muscles (Figure 5). In light of the lack of substantial atrophy in SF mice, we did not pursue further investigation of muscle fiber changes through histology, as our primary goal in which we achieved, was to demonstrate sustained FMT delivery through a nanofluidic device on Earth and in microgravity.

While promising for treating muscle wasting, the adverse cardiovascular effect of FMT is a potential clinical concern. Even though $\beta 1$ is the main AR subtype in cardiac muscles and FMT is selective for $\beta 2$, its effects on the heart are controversial. In preclinical assessments, cardiac hypertrophy upon FMT administration is reported in some studies,[18,19] but not others.[21] In rodents, cardiac hypertrophy was observed to be age- and dose-dependent;[14,21] young rats are more susceptible than adult and old rats, and an IP dose of 10 μg kg^{-1} per day and above led to increased heart mass. We postulated that low dose systemic delivery in a sustained manner could avert the cardiovascular effects. However, in our studies, effects of FMT on the heart was confounding. Ground studies showed no effect on the heart (Figure 2D), whereas cardiac atrophy occurred during HSU (Figure 3E) and hypertrophy after 29 days in space (Figure 4F). Thus, more studies are needed to better understand our perplexing results in this aspect. In humans, potential safety issues of FMT were noted in cachectic cancer patients.[22] One patient with pre-existing condition experienced supraventricular tachycardia, resulting in study withdrawal, while there were two reports of tachycardia in the seven patients who completed the 8-week study of 80 μg per day oral FMT.[22] Apart from cardiac effect, eight incidences of muscle tremors were reported, albeit none resulted in study discontinuation.[22] Considering the reported therapeutic effects of FMT at low doses on muscles, along with our results, a clinical study with substantially lower dosage warrants further investigation for efficacy and safety evaluation. Of relevance, no adverse cardiovascular events were observed when FMT was administered at a reduced dose of 12 μg twice daily for up to 12 weeks in COPD patients, albeit through inhalation.[61]

4. Conclusions

Overall, we show that continuous low dose subcutaneous administration of FMT through nF can produce significant augmentation of muscle mass and function via ground investigations. We have also demonstrated novel utility and efficacy of a nF drug delivery device in spaceflight through a pioneering study on the ISS NL.

While this was more evident for mice in normal ambulatory ground conditions, significant results were also obtained for HSU and SF animals. For context, a 10–15% increment in muscle mass in those affected by muscle wasting could translate to substantial improvement in quality of life.[14] Future studies such as that in cachectic models are needed to establish nF-FMT efficacy in treatment of those with pre-existing muscle wasting for clinical relevance. Nevertheless, our findings suggest that nF-mediated delivery of FMT could represent a transformative therapeutic intervention for muscle wasting conditions. As muscle wasting occurs in chronic or prolonged disuse conditions, long-term applicability of the nF is substantiated by an alternative iteration of the device, presenting with ports for transcutaneous drug refilling.[34] Transcutaneous drug refilling allows for straightforward, minimally invasive replenishment without the need for device replacement. Further, in the event that dose adjustment is required, the option for remote controlled drug release modulation through the nF is also feasible[62] and of value in the context of potentially reducing the risks of FMT-induced tachycardia. Taking these practical features into consideration, the nF-FMT could be a viable therapeutic countermeasure for astronauts to prevent muscle atrophy. In this context, in light of the benefits, it is plausible that implantable systems may play an important role as a countermeasure of space-related conditions for long-term space exploration such as bone density loss, muscle wasting, radiation exposure, and any other space-induced maladies that could benefit from a nF-delivered pharmaceutical.

In summary, given that FMT is already Food and Drug Administration approved for bronchodilation, and that the nF offers user independence eliminating the burden of daily dosing or physical manipulation, we envision a promising long-term clinical solution for treatment of muscle wasting conditions.

5. Experimental Section

nF Device Fabrication: nF devices were fabricated using titanium alloy Ti6Al4V in a bullet-like shape measuring 7.12 mm in length and 3 mm in diameter (Figure 1), with a reservoir volume capacity of 15 μL. One extremity of the capsule was closed with a resealable silicone rubber septum to permit drug loading, whereas a silicon nanofluidic membrane was affixed on the other extremity for drug elution. Silicon nanofluidic membrane microfabrication and characteristics are described in detail elsewhere.[31–34,63] Nanofluidic membranes (3 μm in width and 1 μm in length) used in this study housed 11 248 densely packed slit nanochannels measuring 250 nm, and were diced into 2×2 macrochannels, as previously described.[35,64,65] Drug loading into nF was performed using two 32 G needles, one for injection of FMT solution into the reservoir and one for venting air and excess drug.

Focused Ion Beam (FIB)–Scanning Electron Microscopy (SEM) Imaging: Silicon nanofluidic membranes were evaluated using a dual-beam ion beam (FIB) system FEI 235 (Nanofabrication Facility, University of Houston, TX) and imaged at a 52° angle via SEM in the FIB system.

In Vitro FMT Release Analysis: To assess the release kinetics of FMT from the nF, an in vitro drug elution study was performed. nF devices ($n = 3$) with 250 nm nanochannel membranes were loaded with 15 μL of 4 mg mL^{-1} FMT (Sigma-Aldrich, St Louis, MO, USA) and placed in a sink solution of 200 μL 10% Solutol (Kolliphor; Sigma-Aldrich) excipient in polystyrene microcuvettes at 37 °C. Sink solution was collected at predetermined intervals of days 4, 9, and 14 and analyzed via high performance liquid chromatography (HPLC). HPLC measurement of FMT was performed using Hitachi Chromaster. A Hypersil GOLD C18 Selectivity LC Column (4.6 × 250 mm, 5 μm) controlled at 30 °C was used as the stationary phase. A solution of 0.065% trifluoroacetic acid (TFA) in water was used as mobile phase preparation A, while 0.05% TFA in acetonitrile was used as mobile phase C to follow the gradient program listed in Table S1 (Supporting Information) with a flow rate of 0.75 mL min^{-1} and a detection wavelength of 242 nm. Injection volume was 20 μL, and total run time was 18 min. The retention time for FMT was 12.7 min.

Animals: All animal experiments were carried out according to the provisions of the Animal Welfare Act, Public Health Service Animal Welfare Policy, and the principles of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th ed.). All procedures for the pharmacokinetics and hind limb suspension experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Houston Methodist Research Institute (HMRI; AUP 0717-0040). Animals were housed in different scenarios per study design, which are described in the respective study design section below.

Pharmacokinetics Study in Healthy Mice (Ground Study): nF filled with 1× PBS as vehicle control (nF-VEH) and either 1 mg mL^{-1} (FMT_{low}) or 10 mg mL^{-1} FMT (FMT_{high}) were prepared. The experiment was performed with healthy 16-week old female C57BL/6NTac mice (Taconic Biosciences, Hudson, NY). Animals were housed under standard conditions, had ad libitum access to water and a standard laboratory diet. nF-VEH ($n = 9$) or nF-FMT ($n = 10$) were surgically inserted subcutaneously in

the mice dorsum (day 0) using sterile procedure. Blood samples were collected from the saphenous vein before implantation, on days 1, 2, 7, and once a week thereafter for the duration of the experiment. Concentrations of FMT in the blood were analyzed via liquid chromatography with tandem mass spectrometry (LC-MS/MS), in which samples below the lower limit of quantification of 0.3×10^{-9} M were designated as zero. Body weights were measured weekly (as a surrogate marker for muscle hypertrophy) at time of blood sampling to longitudinally assess the effects of FMT on muscle mass. At study termination on day 56, all animals were euthanized by CO_2 asphyxiation followed by blood collection through cardiac puncture. Hind limb muscles (gastrocnemius, soleus, plantaris, and tibialis anterior) and hearts were collected, and wet weights recorded. Values reported were the average of left and right hind limb muscle weights.

Single Dose Pharmacokinetic Analysis of FMT (Ground Study): A cohort of three 16-week old male C57BL/6 mice (Charles River Laboratories) were subcutaneously injected with one bolus dose of FMT at 1 mg kg^{-1} in 0.9% NaCl (administration volume of 5 mL kg^{-1}). Blood sampling was performed at 0.25, 0.5, 1, 2.4, 4, 7, and 24 h after FMT injection via saphenous vein. The pharmacokinetic parameters evaluated for FMT were the maximum blood concentration (C_{max}), area under the blood concentration versus time curve from time zero to time at 24 h (AUC; $\times 10^{-9}$ M h), time to reach the maximum blood concentration (T_{max}). C_{max} and T_{max} were calculated directly from the blood concentration time profiles of the mice.

Blood FMT Quantification via LC-MS/MS: Serum FMT levels were quantitated using LC-MS/MS. Briefly, 20 µL of serum was subjected to solid phase extraction (SPE) using OASIS MAX uElution SPE plates (Waters, Milford, MA, USA); analytes were ultimately separated via liquid chromatography using a Shimadzu Ultra Performance Liquid Chromatography system interfaced with a API6500 QTRAP mass analyzer (SCIEX, Foster City, CA). Chromatographic separation occurred at 60 °C using an ACE C18-PFP, 2.1 × 50 mm, 3.0 µm column (Advanced Chromatography Technologies Ltd., Aberdeen, UK). Chromatographic separation was achieved using a gradient program, as listed in Table S2 (Supporting Information) starting with 98% 10×10^{-3} M ammonium acetate/0.1% formic acid in water (eluent A) and 2% 0.1% formic acid in methanol (eluent B). Injection volume was 10 µL and total run time was 4.5 min. The retention time for FMT was 2.05 min. FMT was quantified using glyburide as an internal standard; transitions for FMT and the internal standard were m/z 345.1 → 149.0 and m/z 494.2 → 169.2, respectively. The lower limit of quantification was 0.025 ng mL^{-1}.

HSU Experiment (Ground Study): The HSU experiment was performed with healthy female C57BL/6NTac mice between 30 to 35 weeks of age for a duration of 21 days. On day 7, mice were implanted with a tail wire inserted in the intervertebral space ≈1 cm from the base of the tail.[40] At the same time, animals were subcutaneously implanted with nF consisting of either PBS as a VEH control ($n = 8$) or 20 mg mL^{-1} FMT ($n = 11$) or treated with daily SQ injection of 2 µg FMT ($n = 10$). Animals were allowed to recover for a week. Following the recovery period, on day 0, animals were suspended with a tail-ring in custom-designed cages using a modified version of the Morey-Holton method to unload the hind limb muscles and induce atrophy, adopting the procedure performed by Ferreira et al.[40] In this setup, hind limb-suspended animals had free 360° rotation and front limbs were able to contact the cage. Hind limb-suspended animals had access to water and food ad libitum with their forelimbs. Food was weighed and changed every other day. Animals were housed each individually to prevent tail entanglement. At the same time, a separate cohort of free-roaming mice within the cages was used as a weight-bearing GC ($n = 8$). The HSU experiment was performed for a duration of 21 days. At study termination, grip strength test of hind limbs was performed with a grip strength meter (Columbus Instruments, Columbus, OH), by allowing the animals to grasp a bar attached to a force gauge, followed by pulling the animal away from the gauge. The test was repeated 3 times and the readings recorded. All animals were euthanized by CO_2 asphyxiation and cardiac puncture, and thereafter muscles and heart collected, weighed, and snap frozen in liquid nitrogen for storage at −80 °C. Values reported were the average of left and right hind limb muscle weights.

RR-6 Spaceflight Mission: In all phases of the experiment (preflight, flight, and postflight), handling of animals was in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th ed.). Animal experiment protocols related to RR-6 were approved by the IACUCs of Kennedy Space Center (KSC), Novartis, and the spaceflight IACUC located at NASA Ames Research Center (Protocol #: FLT-17-110) prior to conducting experiments.

A total of 80 female C57Bl/6NTac at 36 weeks of age (time of launch) from Taconic Biosciences were used. Mice were implanted subcutaneously in the scapular region of the thorax with radiofrequency identification chips (BMDS, IMI-500) prior to shipment to the KSC Animal Care Facility (Annex). Four weeks prior to launch, animals were shipped to the KSC Annex and allowed to acclimate to NASA Type 12 Nutrient-upgraded Rodent FoodBar (NuRFB), autoclaved water via spring-loaded Lixit spouts, and raised cage grid floors, as well as 12:12 h dark/light cycle (lights on: 0600–1800 Greenwich Mean Time). Five days prior to launch (L−5 days), baseline blood samples were collected via tail vein and animals were surgically implanted with either PBS vehicle control (nF-VEH; $n = 40$) or 20 mg mL^{-1} FMT (nF-FMT; $n = 40$), for GC and SF groups.

At L−1 day, SF animals were moved into Transporters in preparation for launch day (L+0). Each Transporter housed 20 animals, ($n = 10$ per side, 2 sides per unit). At launch (December 15, 2017), 20 nF-VEH and 20 nF-FMT mice were launched in an unmanned SpaceX Dragon capsule (CRS-13) from KSC to the ISS. At L+2 days, the Dragon capsule arrived at the ISS and animals were transferred to Rodent Habitats (RH) by the astronauts. Each RH housed 10 mice, with five mice per side, each consisting of nF-VEH ($n = 5$) and nF-FMT ($n = 5$). Synchronous GC mice were treated identically to SF mice (with the exception of launch and SF) and housed in Transporter and RH units accordingly. Specifically, GC Transporter and RH units included NASA Type 12 NuRFB, autoclaved water via spring-loaded Lixit spouts, and raised cage grid floors, as well as 12:12 h dark/light cycle (lights on: 0600–1800 GMT). Environmental enrichment was provided in the form of huts for both GC and SF mice.

Twenty mice (nF-VEH and nF-FMT; $n = 10$ per group) were designated as a Live Animal Return group returned on schedule at L+29 days. After splashdown near Long Beach Port, CA on January 13, 2018, animals were transferred from KSC to the Genomics Institute of the Novartis Research Foundation facility in San Diego, CA on January 15, 2018 for analysis. Mice were weighed and tested for grip strength, as described in the previous section (HSU Experiment (Ground Study)). Mice were exsanguinated via CO_2 and whole blood collected via cardiac puncture. Skin was removed from the back down to the ankle of the animals. Hind limbs were dissected via cuts made through the pelvis of each mouse rostral to the hip joint, avoiding damage to femur and spine. Designated muscles and organs were collected, weighed, and stored accordingly in which some were fixed with 4% paraformaldehyde (stored at 4 °C) and some snap frozen in liquid nitrogen (stored at −80 °C). GC groups were processed using identical euthanasia and dissection procedures. Values reported were the average of left and right hind limb muscle weights.

For the 55-day (average) microgravity study, the remaining 20 mice (nF-VEH and nF-FMT; $n = 10$ per group) were anesthetized via IP injection of ketamine/xylazine/acepromazine (120/15/3 mg kg^{-1}), followed by euthanasia via cardiac exsanguination and cervical dislocation on orbit at L+53 to L+56 days. Whole blood collection and hind limb harvesting were performed by astronauts aboard the ISS over 4 days, with dissection protocol similar to the 29-day study. The remainder of the carcasses was wrapped in aluminum and stored at −80 °C. Synchronous GC groups for the 55-day time point were dissected at KSC during the same week as the SF animal dissections in an identical manner. Because the hind limbs of SF mice were removed and carcasses frozen in orbit, the body weights of the animals in this 55-day microgravity study were not reported.

A tissue sharing list of organs and tissues harvested from the RR-6 mice that were not used for the primary study objectives were established based on agreement by HMRI, Novartis, NASA Ames Research Center, and BioServe Space Technologies (University of Colorado at Boulder, CO).

Statistical Analyses: GraphPad Prism 8 (version 8.0a; GraphPad Software, Inc., La Jolla, CA) was used to plot all graphs and perform statistical analyses. Data were represented as mean ± standard deviation (SD) or as indicated, IQR between the first (25th percentile) and third (75th percentile) quartiles. Either Student's *t*-tests or two-way ANOVA with Tukey's

post hoc were performed to determine the significant difference between groups where indicated. p values of <0.05 were considered statistically significant, whereby symbols indicating statistical significance were as follows: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

G.L., D.L., and S.M.C. are employees of Novartis. All other authors declare no competing interests.

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[1] W. J. Evans, *Am. J. Clin. Nutr.* **2010**, *91*, 1123S.
[2] S. Cohen, J. A. Nathan, A. L. Goldberg, *Nat. Rev. Drug Discovery* **2015**, *14*, 58.
[3] S. Palus, S. von Haehling, J. Springer, *J. Cachexia Sarcopenia Muscle* **2014**, *5*, 193.
[4] P. E. Porporato, *Oncogenesis* **2016**, *5*, e200.
[5] W. D. Dudgeon, K. D. Phillips, J. A. Carson, R. B. Brewer, J. L. Durstine, G. A. Hand, *HIV Med.* **2006**, *7*, 299.
[6] R. H. Fitts, S. W. Trappe, D. L. Costill, P. M. Gallagher, A. C. Creer, P. A. Colloton, J. R. Peters, J. G. Romatowski, J. L. Bain, D. A. Riley, *J. Physiol.* **2010**, *588*, 3567.
[7] M. V. Narici, M. D. de Boer, *Eur. J. Appl. Physiol.* **2011**, *111*, 403.
[8] D. L. Feltz, L. Ploutz-Snyder, B. Winn, N. L. Kerr, J. M. Pivarnik, A. Ede, C. Hill, S. Samendinger, W. Jeffery, *BMC Psychol.* **2016**, *4*, 54.
[9] C. D. McKay, M. Standage, *Musculoskeletal Sci. Pract.* **2017**, *27*, S38.
[10] C. J. Gaffney, E. Fomina, D. Babich, V. Kitov, K. Uskov, D. A. Green, *Sports Med. - Open* **2017**, *3*, 40.
[11] S. Sato, K. Shirato, K. Tachiyashiki, K. Imaizumi, *J. Biomed. Biotechnol.* **2011**, *2011*, 729598.
[12] M. Johnson, *J. Allergy Clin. Immunol.* **2006**, *117*, 18.
[13] M. Cazzola, C. P. Page, P. Rogliani, M. G. Matera, *Am. J. Respir. Crit. Care Med.* **2013**, *187*, 690.
[14] J. G. Ryall, M. N. Sillence, G. S. Lynch, *Br. J. Pharmacol.* **2006**, *147*, 587.
[15] G. S. Lynch, J. G. Ryall, *Physiol. Rev.* **2008**, *88*, 729.
[16] J. G. Ryall, D. R. Plant, P. Gregorevic, M. N. Sillence, G. S Lynch, *J. Physiol.* **2004**, *555*, 175.
[17] S. Busquets, M. Toledo, E. Marmonti, M. Orpí, E. Capdevila, A. Betancourt, F. J. López-Soriano, J. M. Argilés, *Oncol. Lett.* **2012**, *3*, 185.
[18] S. Busquets, M. T. Figueras, G. Fuster, V. Almendro, R. Moore-Carrasco, E. Ametller, J. M. Argilés, F. J. López-Soriano, *Cancer Res.* **2004**, *64*, 6725.
[19] S. Busquets, M. Toledo, S. Sirisi, M. Orpí, R. Serpe, J. Coutinho, R. Martínez, J. M. Argilés, F. J. López-Soriano, *Exp. Ther. Med.* **2011**, *2*, 731.
[20] A. Salazar-Degracia, S. Busquets, J. M. Argilés, N. Bargalló-Gispert, F. J. López-Soriano, E. Barreiro, *Biochimie* **2018**, *149*, 79.
[21] J. G. Ryall, J. D. Schertzer, G. S. Lynch, *J. Gerontol., Ser. A* **2007**, *62*, 813.
[22] C. A. Greig, N. Johns, C. Gray, A. MacDonald, N. A. Stephens, R. J. E. Skipworth, M. Fallon, L. Wall, G. M. Fox, K. C. H. Fearon, *Support Care Cancer* **2014**, *22*, 1269.
[23] F. Beitzel, M. N. Sillence, G. S. Lynch, *Am. J. Physiol.: Endocrinol. Metab.* **2007**, *293*, E932.
[24] T. C. Conte, L. H. Silva, M. T. Silva, S. M. Hirabara, A. C. Oliveira, R. Curi, A. S. Moriscot, M. S. Aoki, E. H. Miyabara, *J. Gerontol., Ser. A* **2012**, *67*, 443.
[25] J. G. Ryall, G. S. Lynch, *Pharmacol. Ther.* **2008**, *120*, 219.
[26] P. Molenaar, L. Chen, W. A. Parsonage, *Br. J. Pharmacol.* **2006**, *147*, 583.
[27] B. T. van den Berg, M. C. Braat, C. J. van Boxtel, *Eur. J. Clin. Pharmacol.* **1998**, *54*, 463.
[28] M. Burnier, *Front. Pharmacol.* **2018**, *9*, 1540.
[29] N. Petersen, P. Jaekel, A. Rosenberger, T. Weber, J. Scott, F. Castrucci, G. Lambrecht, L. Ploutz-Snyder, V. Damann, I. Kozlovskaya, J. Mester, *Extreme Physiol. Med.* **2016**, *5*, 9.
[30] L. L. Ploutz-Snyder, M. Downs, E. Goetchius, B. Crowell, K. L. English, R. Ploutz-Snyder, J. W. Ryder, E. L. Dillon, M. Sheffield-Moore, J. M. Scott, *Med. Sci. Sports Exercise* **2018**, *50*, 1920.
[31] G. Bruno, N. D. Trani, R. L. Hood, E. Zabre, C. S. Filgueira, G. Canavese, P. Jain, Z. Smith, D. Demarchi, S. Hosali, A. Pimpinelli, M. Ferrari, A. Grattoni, *Nat. Commun.* **2018**, *9*, 1682.
[32] S. Ferrati, D. Fine, J. You, E. De Rosa, L. Hudson, E. Zabre, S. Hosali, L. Zhang, C. Hickman, S. S. Bansal, A. M. Cordero-Reyes, T. Geninatti, J. Sih, R. Goodall, G. Palapattu, M. Kloc, R. M. Ghobrial, M. Ferrari, A. Grattoni, *J. Controlled Release* **2013**, *172*, 1011.
[33] D. Fine, A. Grattoni, S. Hosali, A. Ziemys, E. De Rosa, J. Gill, R. Medema, L. Hudson, M. Kojic, M. Milosevic, L. B. Iii, R. Goodall, M. Ferrari, X. Liu, *Lab Chip* **2010**, *10*, 3074.
[34] C. Y. X. Chua, P. Jain, A. Ballerini, G. Bruno, R. L. Hood, M. Gupte, S. Gao, N. D. Trani, A. Susnjar, K. Shelton, L. R. Bushman, M. Folci, C. S. Filgueira, M. A. Marzinke, P. L. Anderson, M. Hu, P. Nehete, R. C. Arduino, J. K. Sastry, A. Grattoni, *J. Controlled Release* **2018**, *286*, 315.
[35] C. Y. X. Chua, P. Jain, A. Susnjar, J. Rhudy, M. Folci, A. Ballerini, A. Gilbert, S. Singh, G. Bruno, C. S. Filgueira, C. Yee, E. B. Butler, A. Grattoni, *J. Controlled Release* **2018**, *285*, 23.
[36] H. Akima, K. Kubo, H. Kanehisa, Y. Suzuki, A. Gunji, T. Fukunaga, *Eur. J. Appl. Physiol.* **2000**, *82*, 30.
[37] B. Ferrando, M. C. Gomez-Cabrera, A. Salvador-Pascual, C. Puchades, F. Derbré, A. Gratas-Delamarche, L. Laparre, G.

Olaso-Gonzalez, M. Cerda, E. Viosca, A. Alabajos, V. Sebastiá, A. Alberich-Bayarri, F. García-Castro, J. Viña, *Sci. Rep.* **2018**, *8*, 3549.
[38] Q. R. Joassard, A. Amirouche, Y. S. Gallot, M. M. Desgeorges, J. Castells, A.-C. Durieux, P. Berthon, D. G. Freyssenet, *Int. J. Biochem. Cell Biol.* **2013**, *45*, 2444.
[39] S. M. Gehrig, R. Koopman, T. Naim, C. Tjoakarfa, G. S. Lynch, *Am. J. Pathol.* **2010**, *176*, 29.
[40] J. A. Ferreira, J. M. Crissey, M. Brown, *J. Visualized Exp.* **2011**, *10*, 2467.
[41] D. Sandona, J.-F. Desaphy, G. M. Camerino, E. Bianchini, S. Ciciliot, D. Danieli-Betto, G. Dobrowolny, S. Furlan, E. Germinario, K. Goto, M. Gutsmann, F. Kawano, N. Nakai, T. Ohira, Y. Ohno, A. Picard, M. Salanova, G. Schiffl, D. Blottner, A. Musarò, Y. Ohira, R. Betto, D. Conte, S. Schiaffino, *PLoS One* **2012**, *7*, e33232.
[42] B. R. Macias, R. Minocha, A. A. Cutuk, J. Hill, J. Shiau, A. R. Hargens, *Aviat., Space Environ. Med.* **2008**, *79*, 749.
[43] R. H. Fitts, D. R. Riley, J. J. Widrick, *J. Appl. Physiol.* **2000**, *89*, 823.
[44] C. A. Ray, M. Vasques, T. A. Miller, M. K. Wilkerson, M. D. Delp, *J. Appl. Physiol.* **2001**, *91*, 1207.
[45] T. Lang, J. J. W. A. Van Loon, S. Bloomfield, L. Vico, A. Chopard, J. Rittweger, A. Kyparos, D. Blottner, I. Vuori, R. Gerzer, P. R. Cavanagh, *NPJ Microgravity* **2017**, *3*, 8.
[46] E. A. Radugina, E. A. C. Almeida, E. Blaber, V. A. Poplinskaya, Y. V. Markitantova, E. N. Grigoryan, *Life Sci. Space Res.* **2018**, *16*, 18.
[47] S. M. Cadena, Y. Zhang, J. Fang, S. Brachat, P. Kuss, E. Giorgetti, L. S. Stodieck, M. Kneissel, D. J. Glass, *Sci. Rep.* **2019**, *9*, 9397.
[48] A. E. Ronca, E. L. Moyer, Y. Talyansky, M. Lowe, S. Padmanabhan, S. Choi, C. Gong, S. M. Cadena, L. Stodieck, R. K. Globus, *Sci. Rep.* **2019**, *9*, 4717.
[49] D. S. Gridley, G. A. Nelson, L. L. Peters, P. J. Kostenuik, T. A. Bateman, S. Morony, L. S. Stodieck, D. L. Lacey, S. J. Simske, M. J. Pecaut, *J. Appl. Physiol.* **2003**, *94*, 2095.
[50] B. C. Harrison, D. L. Allen, B. Girten, L. S. Stodieck, P. J. Kostenuik, T. A. Bateman, S. Morony, D. Lacey, L. A. Leinwand, *J. Appl. Physiol.* **2003**, *95*, 2462.
[51] E. A. Blaber, N. Dvorochkin, C. Lee, J. S. Alwood, R. Yousuf, P. Pianetta, R. K. Globus, B. P. Burns, E. A. C. Almeida, *PLoS One* **2013**, *8*, e61372.
[52] P. O. Hasselgren, N. Alamdari, Z. Aversa, P. Gonnella, I. J. Smith, S. Tizio, *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13*, 423.
[53] A. Y. Sato, D. Richardson, M. Cregor, H. M. Davis, E. D. Au, K. McAndrews, T. A. Zimmers, J. M. Organ, M. Peacock, L. I. Plotkin, T. Bellido, *Endocrinology* **2017**, *158*, 664.
[54] A. B. Gomez-SanMiguel, C. Gomez-Moreira, M. P. Nieto-Bona, C. Fernández-Galaz, M. Á. Villanúa, A. I. Martín, A. López-Calderón, *Am. J. Physiol.: Endocrinol. Metab.* **2016**, *310*, E925.
[55] Y. S. Kim, R. D. Sainz, P. Molenaar, R. J. Summers, *Biochem. Pharmacol.* **1991**, *42*, 1783.
[56] C. McCormick, L. Alexandre, J. Thompson, G. Mutungi, *J. Appl. Physiol.* **2010**, *109*, 1716.
[57] L. J. Harcourt, J. D. Schertzer, J. G. Ryall, G. S. Lynch, *Neuromuscular Disord.* **2007**, *17*, 47.
[58] J. S. Alwood, A. E. Ronca, R. C. Mains, M. J. Shelhamer, J. D. Smith, T. J. Goodwin, *NPJ Microgravity* **2017**, *3*, 5.
[59] A. Bergouignan, T. P. Stein, C. Habold, V. Coxam, D. O'Gorman, S. Blanc, *NPJ Microgravity* **2016**, *2*, 16029.
[60] F. Karouia, K. Peyvan, A. Pohorille, *Biotechnol. Adv.* **2017**, *35*, 905.
[61] N. Xia, H. Wang, X. Nie, *PLoS One* **2015**, *10*, e0137904.
[62] N. Di Trani, A. Silvestri, G. Bruno, T. Geninatti, C. Y. X. Chua, A. Gilbert, G. Rizzo, C. S. Filgueira, D. Demarchi, A. Grattoni, *Lab Chip* **2019**, *19*, 2192.
[63] S. Ferrati, E. Nicolov, E. Zabre, T. Geninatti, B. A. Shirkey, L. Hudson, S. Hosali, M. Crawley, M. Khera, G. Palapattu, A. Grattoni, *J. Sex. Med.* **2015**, *12*, 1375.
[64] N. Di Trani, P. Jain, C. Y. X. Chua, J. S. Ho, G. Bruno, A. Susnjar, F. P. Pons-Faudoa, A. Sizovs, R. L. Hood, Z. W. Smith, A. Ballerini, C. S. Filgueira, A. Grattoni, *Nanomedicine* **2018**, *16*, 1.
[65] R. L. Hood, G. Bruno, P. Jain, J. Anderson, T. Wolfe, C. Quini, J. Schmulen, X. C. Li, E. B. Butler, S. Krishnan, A. Grattoni, *J. Biomed. Nanotechnol.* **2016**, *12*, 1907.

