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Hardware Article

A compact electromagnetic syringe stirrer and temperature controller for the reliable dispensing of living cells and microparticles

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ABSTRACT

Lab-on-chip technologies frequently require biological samples, such as cells or microorganisms, to be maintained inside a syringe for prolonged periods of time during operations. Challenges include preventing cell sedimentation, ensuring cell viability, and maintaining buffer rheological properties (i.e. viscosity and density) constant, particularly in applications like 3D bioprinting and diagnostic assays. To address these challenges, we have developed the Syringe Electromagnetic Controller (SEC), an integrated system capable of simultaneously stirring and thermoregulating samples inside a syringe. SEC prevents sedimentation through the cyclic movement of a magnet actuated by an electromagnetic field, while maintaining a stable temperature (within ± 0.5 °C from a set-point) with a feedback loop. The system is compact, cost-effective, and easily integrated into various setups. Experimental validation shows that SEC effectively keeps living cells in suspension and at a constant temperature without compromising cell viability. Thus, we have ultimately demonstrated the functionality of SEC as a versatile solution for enhancing the reliability of lab-on-chip applications.

1. Specifications table

Hardware name	Syringe Electromagnetic Controller (SEC)
Subject area	• Engineering and materials science • Medical (e.g., pharmaceutical science)

(*continued on next page*)

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(*continued*)

Hardware name	Syringe Electromagnetic Controller (SEC)
Hardware type	• Biological sciences (e.g., microbiology and biochemistry) • Biological sample handling and preparation • Mechanical engineering and materials science
Closest commercial analog	No commercial analog with the same capabilities of the proposed hardware is currently available. The proposed hardware can replace the capabilities of: • CETONI Nemix 50 syringe mixer • Syringe Heaters
Open source license	CERN-OHL-S 2.0
Cost of hardware	<50 €
Source file repository	Design files uploaded on OSF, https://doi.org/10.17605/OSF.IO/FB94X

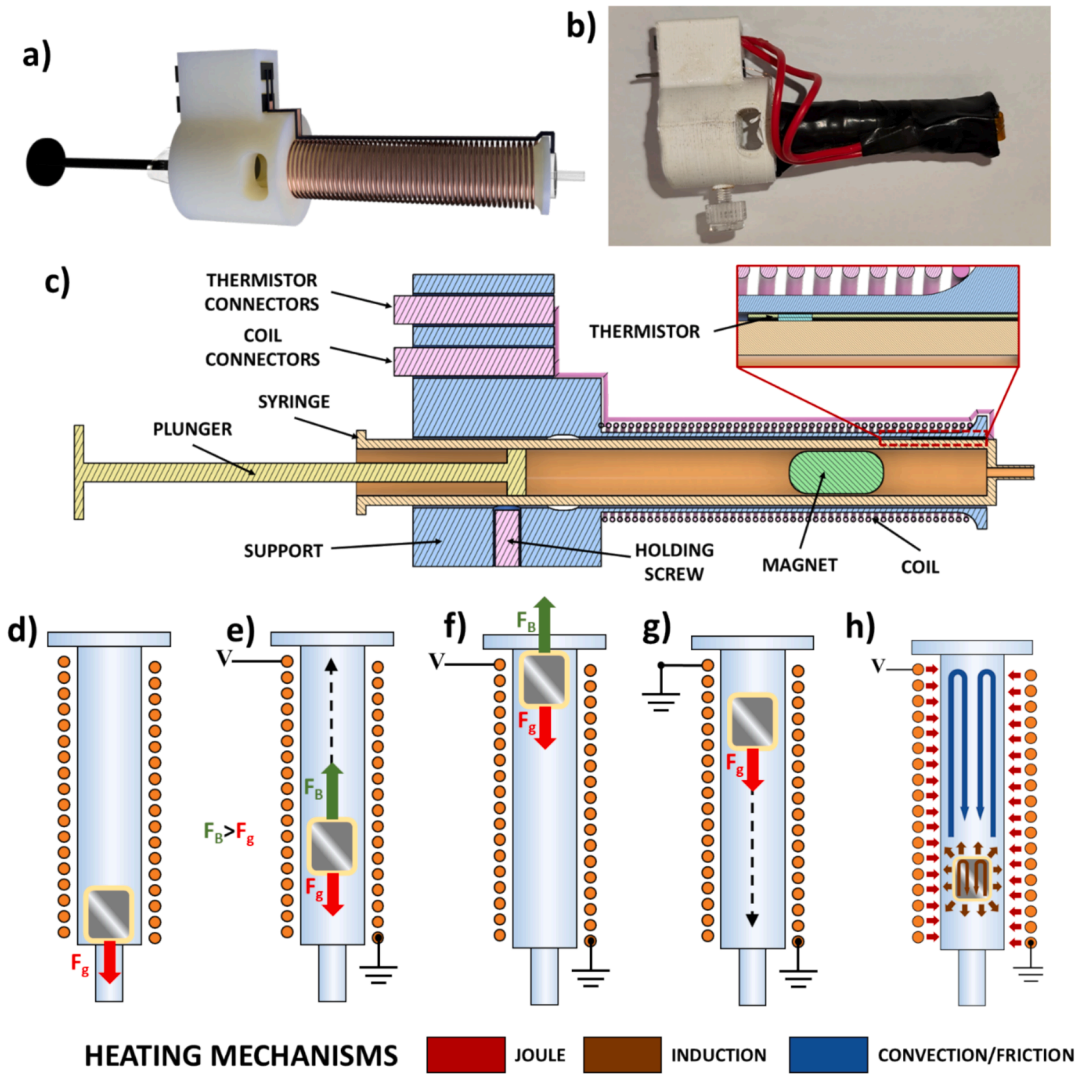

Fig. 1. Schematic drawing of the apparatus. a) Computer rendering of the device. b) Picture of the assembled device. c) Cross-section analysis of the device: The syringe slides inside the support and it's held in place by a holding screw. An enameled copper wire is winded around the support and soldered to connectors, and a magnet is inserted inside the syringe. A thermistor is glued inside the support, in close contact with the syringe. d-g) Stirring mechanism: (d) if no potential is applied, the magnet falls to the bottom of the syringe due to gravity. (e) If a current is applied to the induction coil, a magnetic force greater than the gravitational force is generated, and (f) the magnet moves towards the top of the syringe. (g) The cyclic application of the potential causes a cyclic movement of the magnet. h) Heating mechanisms: (red) Joule effect, from the coil towards the center of the syringe; (brown) Induction, from the magnet towards the outer syringe surface; (blue) Convection and Friction, from the liquid towards the outer syringe surface. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Hardware in context

Several lab-on-chip applications involve biological samples such as cells or microorganisms to be loaded inside a syringe for an extended period of time (e.g. up to 30 min), and require the sample to be maintained in suspension with high viability for the whole duration of the pumping operations [1,2]. For example, in 3D bioprinting applications, cells are suspended in bioinks to create complex tissue structures, and gravity-driven cell settling can lead to uneven cell distribution, resulting in regions with insufficient cell density, thus compromising the structural integrity and functionality of the 3D bioprinted tissue [3–7]. Additionally, cells must remain viable and functional after the 3D bioprinting process, thus they should be ideally kept at constant temperature to minimize potential thermal stresses which can lead to cell death or functional impairment [8]. Moreover, in 3D bioprinting thermal regulation ensures that the bioink maintains a constant viscosity over time, essential for accurate layer deposition and for the structural integrity of the printed constructs [9,10]. Similarly, in diagnostic lab-on-chip applications (e.g. cytometry), the precise control of cell suspensions is crucial for the accurate execution of processes like cell sorting, chemical assays, and diagnostic tests, which depend on the predictable behavior of cells within the fluidics [11]. Cell sedimentation can also lead to clogging (e.g. in tubing or in the microchannel), uneven flow, and inconsistent results, ultimately affecting the experimental reliability and reproducibility [12]. Moreover, these systems require an accurate temperature control, as temperature directly regulates the kinetics of biochemical reactions, the cell metabolism, as well as the buffer viscosity and the surface tension [13,14].

These applications would greatly benefit from a system able to simultaneously stir the syringe to maintain cells in suspension while providing heat to keep its temperature constant. While solutions to either problem separately are available, no available system exists to solve both aspects simultaneously (e.g. syringe stirring and thermoregulation). Solutions to address the issue of sedimentation in syringes include magnetic stirrers [15–19] and mechanical shakers [20]. However, these solutions tend to be bulky and cumbersome, thus posing significant integration challenges in compact and delicate setups typical of 3D bioprinting and lab-on-chip systems, limiting their applicability. Additionally, these platforms are often proprietary, making them expensive and inaccessible, and further limiting their customizability and adaptability [18]. Similarly, current available systems for syringe thermoregulation, including external heating jackets/pads (e.g. flexible coverings that wrap around the syringe with embedded heating elements) [21,22], heating blocks (e.g. blocks heated electronically with cavities to hold syringes) [21], or heating baths [23] can't be used in conjunction with a stirrer, and, with the exception of heating jackets/pads, are bulky and may not fit seamlessly into compact, automated, or portable setups.

3. Hardware description

We present an integrated system that can simultaneously heat and stir the cell/particle suspension loaded inside the syringe, named SEC (Syringe Electromagnetic Controller). SEC can prevent cell sedimentation for long–term measurements, heat the sample faster than purely convectional approaches, and maintain the temperature inside the syringe constant. This system will improve the quality and reproducibility of data coming from biological samples used in all those technologies that imply microfluidics/3D bioprinting systems. SEC is based on a rigid support which mounts itself around the syringe barrel (Fig. 1a–c). An induction coil is winded around the support and is used to actuate a magnet loaded inside the syringe.

3.1. Stirring

The stirring is based on the cyclic upward/downward movement of the magnet inside the syringe induced by the intermittent electromagnetic field generated by a current flowing in the external coil. When no voltage is applied to the coil, the magnet moves towards the bottom of the syringe due to gravity (Fig. 1d). When a voltage is applied to the coil, an upward magnetic field is generated inside the syringe which interacts with the natural magnetic field of the magnet (Fig. 1e). If the resulting magnetic force on the magnet is larger than the gravitational force, it starts moving upwards, towards the top of the syringe (Fig. 1f). When the voltage is removed, the magnet returns to the bottom of the syringe due to gravity (Fig. 1g). By applying a cyclic voltage, the magnet will cyclically move up/down. The frequency and intensity of the voltage can be controlled to achieve the desired stirring effect, thus preventing particle sedimentation. The magnet must be sufficiently large with respect to the syringe diameter in order to induce sufficient fluid recirculation during operations to achieve stirring. At the same time the magnet must be able to fit comfortably inside the syringe, slide without significant friction, and ensure there is sufficient space for cells to move without being damaged. In this work, we used HSW 1 ml syringes with d = 4.7 mm (similar to most 1 ml plastic syringes) and a 4 mm magnet resulting in a $\approx$ 350 mm gap which was sufficient to prevent sedimentation without affecting cell viability (cfr. Validation and characterization section). The system was operated with a cycle frequency of 0.5 Hz, which was sufficient for both heating and stirring. To minimize its cost, this version of the apparatus requires the syringe to be mounted vertically on a syringe pump (usually with the syringe output oriented downward), but it's straightforward to extend its application to horizontal syringes by using an electronic circuit able to cyclically reverse the current flow (instead of switching it ON/OFF).

3.2. Fast heating

The system exploits 4 synergic heating mechanisms to achieve fast syringe thermoregulation (Fig. 1h): (i) The current flowing in the coil around the syringe produces heat by Joule effect, resulting in heating from outside the syringe towards the center; (ii) The magnetic field induces eddy currents inside the magnet, also producing energy due to Joule effect, resulting in heating from inside the

syringe towards the surface; (iii) The cyclic movement of the magnet inside the syringe results in heat generation due to friction with the buffer; (iv) The movement of the fluid inside the syringe allows heat to be transferred in a forced convection regime, more efficient than the natural convection which would otherwise be present in absence of stirring.

3.3. Temperature control

The temperature is measured by a sensor located in close proximity to the syringe (the thermistor shown in the inset of Fig. 1c). A controller constantly compares the temperature reading with a set-point and switches the current ON/OFF accordingly in a feedback loop (Fig. 2). When the temperature is lower than the set-point, heating is required, and the system operates normally. When the temperature is equal to or greater than the set-point, heating is no longer required, and the controller reduces the time for which the voltage is applied. In this regime, the current is still able to move the magnet, but only a small amount of heat is generated, which is lower than the heat dissipated towards the environment resulting in a decrease in temperature. The feedback controller regulates the time for which each regime is applied, equalizing, on average, the heat generated and dissipated, resulting in a constant temperature. The main difference between the current contribution towards stirring and heating resides in the duration of the applied current pulse in each up/down cycle: to move the magnet, only a short current pulse is required, whereas the amount of heat provided to the sample is proportional to the duration of the current pulse. Thus, it's possible to operate at different heating/no-heating regimes while providing continuous stirring by changing the duration of the applied current pulse. It's worth noting that the SEC doesn't provide active cooling and relies on heat exchange with the external environment for cooling and thus can't cool the sample below room temperature. Additionally, the SEC is aimed at biological applications, therefore the typical operations temperature range is 20–37 °C (all tests were performed in that range). Theoretically, it should be possible to reach higher temperatures (e.g. to improve rheological properties of materials), but we envision that the upper temperature limit would be near the glass transition temperature of the polymer used to 3D print the support (50–60 °C in the case of PLA) which would lead to the SEC support losing its mechanical properties.

In summary, the Syringe Electromagnetic Controller (SEC):

- Provides stirring and heating to suspension of cells/particles loaded in a syringe.
- Prevents cell/particle sedimentation for long–term measurements.
- Can heat the sample quickly.
- Can maintain constant temperature inside the syringe.
- Does not affect cell viability.

4. Design files summary

Design file name	File type	Open source license	Location of the file
SEC_CAD	Autodesk CAD (.iam,.ipt)	CERN-OHL-S 2.0	Available at online repository [24]
SEC_STL	3D-print (.stl)	CERN-OHL-S 2.0	
SEC_CODE	Software (.ino)	CERN-OHL-S 2.0	

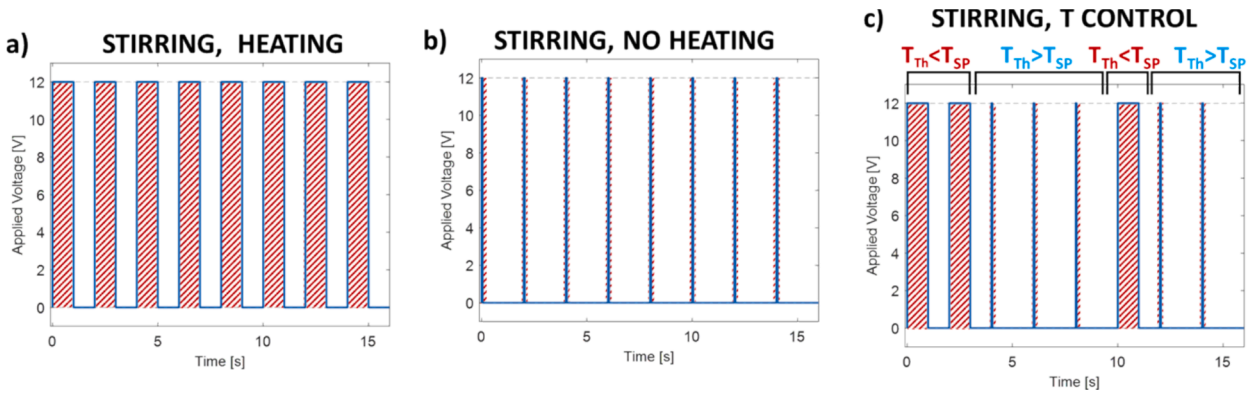

Fig. 2. Schematic voltage application protocol. The blue line represents the applied voltage, and the red dashed area represents the heating. Stirring is always enabled. a) If heating is required, a 12 V voltage is applied every 2 s for the duration of 1 s (0 V are applied for the remaining time). b) If heating is not required, the same voltage is applied every 2 s for the duration of 50 ms (0 V are applied for the remaining time). c) If the temperature must be regulated at T_{SP}, the heating protocol is applied while $T_{Th} < T_{SP}$, switching to the non-heating protocol when $T_{Th} > T_{SP}$, where T_{Th} is the temperature measured by the integrated thermistor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SEC_CAD: This is the CAD file of the SEC support structure for the actuator.
SEC_STL: This is the 3D print-ready version of the previous file.
SEC_CODE: This is the Arduino code that runs the control unit.

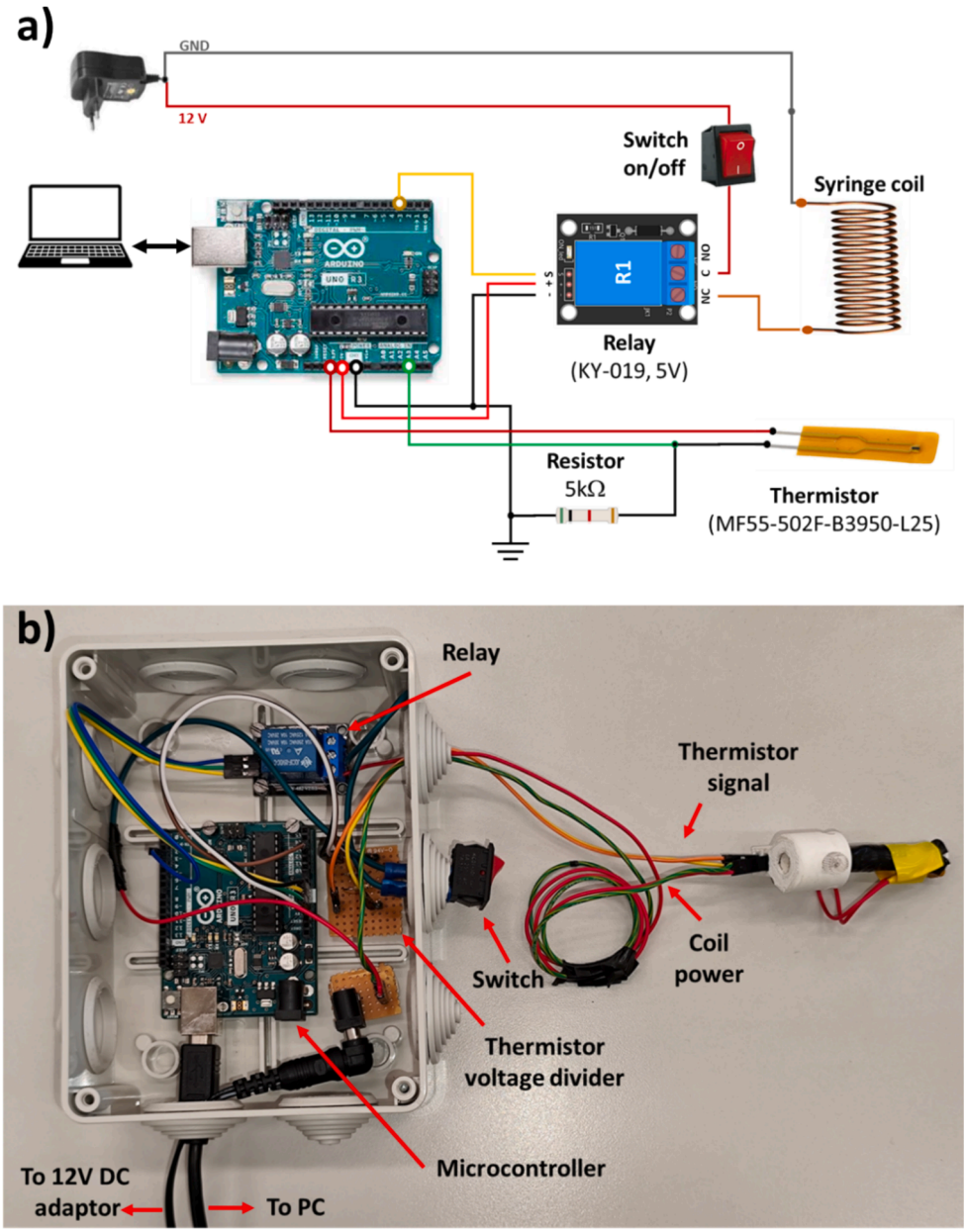

Fig. 3. SEC control unit: a) Electronic connections schematic. b) Hardware image. SEC is controlled using an Arduino UNO board, powered over a USB connection with the computer. The board is used to control the switching of a relay (KY-019, 5 V) using one of its digital output pin, and to perform the reading from the thermistor using an analog input pin. The relay controls the opening/closing cycles of the coil circuit, powered by a 12 VDC adaptor. One end of the coil is connected to the ground pin of the adaptor, while the other is connected to the normally closed pin of the relay. The positive pin of the adaptor is connected to the common pin of the relay. The thermistor is powered by the 3.3 V Arduino output, and a 5 kΩ resistor is used to achieve the voltage divider.

5. Bill of materials summary

Designator	Component	#	Total cost	Source of materials	Manufacturer number	Material type
ACTUATOR						
Syringe	Henke-Sass-Wolf Disposable syringe	1	<1 €	Sigmaaldrich.com	Z683531	– Polymer
Support	PLA 3D-printed support structure	1	<5 €	rs-online.com (us.rs-online.com)	832–0223	– Polymer
Coil	Enameled copper induction coil	1	<1 €	rs-online.com (us.rs-online.com)	304–09-375	– Metal
Thermistor	Integrated thermistor	1	<1 €	amazon.com	B0BMVXFG6F	– Semi-conductor
Holding screw	Nylon screw M3	1	<1 €	rs-online.com (us.rs-online.com)	232–6918	– Polymer
CONTROL UNIT						
Arduino board	Arduino Uno board	1	20 €	rs-online.com (us.rs-online.com)	715–4081	– Other
Relay	1-channel Relay 5 V Module High-Level-Trigger	1	2 €	az-delivery.de	KY-019	– Other
Power adaptor	Adaptor AC/DC 3–12 V, 600 mA	1	12 €	rs-online.com (us.rs-online.com)	206–4908	– Other

6. Build instructions

6.1. Actuator fabrication

The support is designed for a 1 ml plastic syringe (HSW) and fabricated by 3D printing a custom design developed using Fusion360 (Autodesk), which is available with this paper. The file can be edited for syringes with larger diameters. A step-by-step fabrication guide is provided in Fig. S1 of the Electronic Supplementary Information. An enameled copper wire with a diameter of 0.1 mm is winded 300 times around the support along the syringe housing forming the induction coil. The two wire ends are stripped of the insulating coating and soldered to Dupont-style connectors, which are glued to the support to provide quick connections to the electronics and power supply. The coil is fixed to the support using adhesive tape or adhesive. A parylene-coated magnet stir-bar (d = 4 mm, L = 8 mm, pull-force = 0.9 kg) is inserted inside the syringe. A thin film thermistor (MF55-502F-B3950-L25-5 K-3950) is glued to the inside of the syringe housing, and its ends are soldered to wire quick connectors. The support has a tapped hole that allows for an M3 polymeric screw to travel orthogonally to the syringe barrel and to hold the syringe in place (metallic screws should be eschewed to avoid affecting the magnetic field close the magnet).

6.2. Control unit

The SEC is controlled using an Arduino Uno board, powered over a USB connection with the computer (Fig. 3). The board controls the switching of a relay (KY-019, 5 V) using one of its digital output pins, and reads the thermistor voltage using an analog input pin.

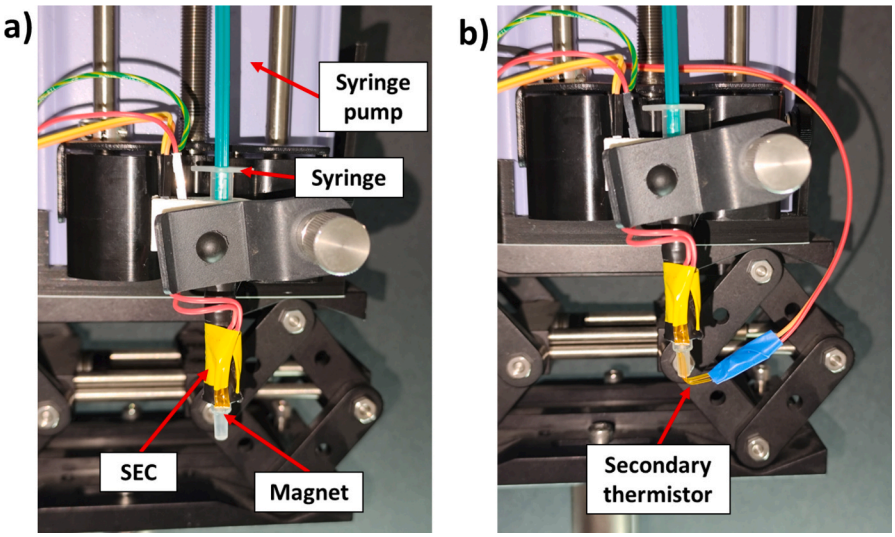

Fig. 4. Experimental setups used during SEC validation. a) Normal operations and sedimentation prevention: the SEC was used to heat a 1 ml plastic syringe filled with 650 µl of PBS. The system was mounted vertically on a syringe pump, and infused at 25 µl/min. b) Thermoregulation analysis: To measure the actual temperature inside the syringe, a second thermistor was inserted inside the syringe through its tip to be in contact with the liquid (for validation only).

The relay controls the opening/closing cycles of the coil circuit, powered by a 12 V DC adaptor. One end of the coil is connected to the ground pin of the adaptor, while the other is connected to the normally closed pin of the relay. The positive pin of the adaptor is connected to the common pin of the relay. The thermistor is powered by the 3.3 V Arduino pin, and a 5 kΩ resistor is used for the voltage divider.

7. Operation instructions

To prepare the system, the following operations are performed: (1) The magnet is loaded into the syringe; (2) the sample is loaded into the syringe; (3) the syringe is inserted inside the actuator; (4) the actuator and syringe are mounted on a vertical syringe pump (outlet pointing downwards) and, eventually connected to the tubing; (5) the control unit is connected to the PC, and the power supply to the mains; (6) the SEC switch is turned on. A feedback loop reads the thermistor temperature T_{Th} and regulates the duration of the coil current flow to maintain the temperature to a desired set-point T_{SP} (Fig. 2). If heating is required (i.e. $T_{Th} < T_{SP}$), 12 V are applied for 1 s every 2 s (pins are grounded for the remaining 1 s). If heating is not required (i.e. $T_{Th} > T_{SP}$), 12 V are applied for 0.05 s every 2 s (pins are grounded for the remaining 1.95 s).

8. Validation and characterization

A video showing the simultaneous stirring/thermoregulation operations is available in the supplementary material.

8.1. Thermoregulation analysis

The ability of the system to achieve stable temperature control was assessed by measuring the temperature over time as the liquid was pumped normally (Fig. 4). The SEC was used to heat a 1 ml plastic syringe filled with 650 µl of PBS. The system was mounted on a syringe pump, and infused at 25 µl/min. To measure the actual temperature inside the syringe, a second thermistor was inserted inside the syringe through its tip (in contact with the liquid) and connected to the Arduino board with a circuit in parallel to the first one. To demonstrate that multiple heating mechanisms are present in addition to the pure coil Joule heating, a measurement without the stir-bar inside the syringe was also performed. Each measurement was repeated 3 times.

8.1.1. Temperature control

The second temperature sensor was placed inside the syringe through its tip to measure the actual liquid temperature (Fig. 5b). The steady state temperature inside the syringe was 1–2 °C higher than the reading of the main sensor as a result of the induced internal heat generation. To account for this effect, the temperature set-point was reduced to 35.5 °C to ensure that the actual internal temperature would not significantly exceed 37 °C. With this configuration, after the system reached the steady state in ~ 200 s, the main sensor reading was 35.6 ± 0.08 °C and the internal sensor reading was 36.5 ± 0.38 °C for the whole measurement.

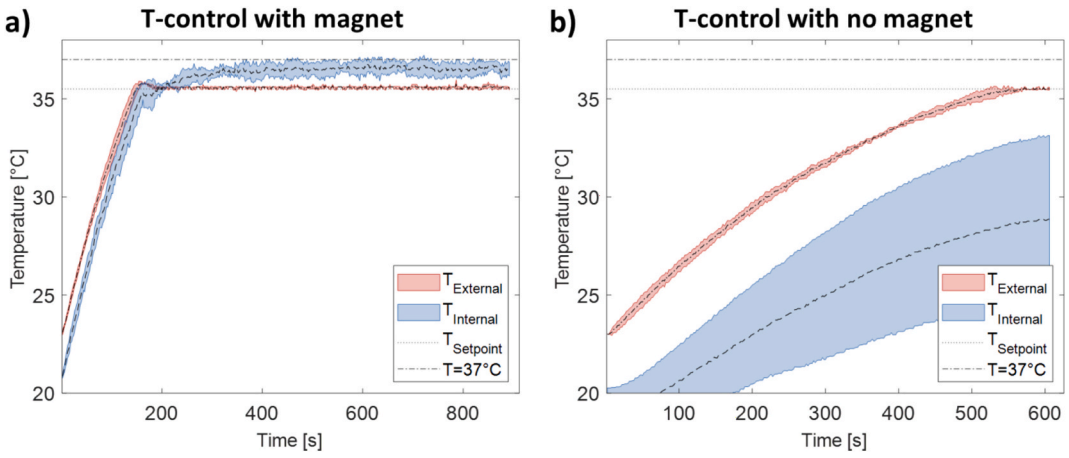

Fig. 5. The temperature of the system was measured with the integrated temperature sensor (red line) and with a second temperature sensor located inside the syringe tip in contact with the liquid buffer (blue line), as shown in Fig. 4.The setpoint was 35.5 °C. Lines represent mean values and shaded areas represent ± 1 standard deviation (n = 3). a) With the magnet inside the syringe, SEC achieved a heating rate of 0.08 °C/s. After the heating phase (~200 s), the readings over the measurements (900 s) were 35.6 ± 0.08 °C, and 36.5 ± 0.38 °C, for the integrated and internal sensors, respectively. b) When heated without the magnet being present inside the syringe, the heating rate decreased to 0.02 °C/s, thus demonstrating the synergistic multimodal heating. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

8.1.2. Multimodal heating

A comparison between the heating curve of the system in the presence and in the absence of the magnet inside the syringe was performed to demonstrate the synergistic multimodal heating (Fig. 5b). In the presence of the magnet, the system was able to heat the sample at 0.08 °C/s (0.09 °C/s according to the internal sensor) compared to 0.02 °C/s (0.01 °C/s according to the internal sensor) if the magnet was removed (pure Joule heating from the outside). These findings suggest that there are additional heating mechanisms present when the magnet is located inside the syringe, resulting in a higher heating rate. Additionally, a higher variability of the internal sensor reading was observed when the magnet was removed, suggesting that the lack of fluid mixing induced a lack of homogeneity of the temperature inside the syringe.

8.2. Sedimentation prevention

The SEC was used to stir a 1 ml plastic syringe filled with 650 µl of sample (cells or beads), as shown in Fig. 4. The sample was pumped at 25 µl/min and the liquid at the outlet of the syringe was collected and fractioned over 5 min intervals (0–5 min, 5–10 min, 10–15 min, 15–20 min, and 20–25 min), and the number of particles present in each fraction was quantified using a hemocytometer and normalized with respect to the initial value. For the cells experiment, a horizontal syringe without stirring was used as control. To demonstrate that the vertical syringe orientation alone isn't sufficient to prevent sedimentation, a vertical syringe without stirring was used as control in the beads experiment. Each measurement was repeated 3 times.

8.2.1. Sample preparation

Silica polymeric beads (Microparticles GmbH) with nominal diameter 10 µm were dispersed in Phosphate Buffered Saline (PBS) at a concentration of approximately 10^6 particles/ml. Samples were loaded into the 1 ml plastic syringe and driven using a Harvard Instruments syringe pump. HeLa cells purchased from ATCC (Manassas, VA, USA) were cultured in DMEM complete media containing 10 % FBS, penicillin/streptomycin plus glutamine at 37 °C in 5 % CO2.

8.2.2. Cell sedimentation

As shown in Fig. 6a, the normalized concentration of samples loaded in the horizontal syringe decreased constantly over time, reaching 7 ± 2 % of the initial concentration after 25 min, whereas the stirring system was able to prevent most of the sedimentation, with a normalized concentration 79 ± 1 % after 25 min. A 2-tailed unpaired *t*-test was performed to assess statistical significance and returned $p < 0.05$ for all conditions after the initial reference measurement.

8.2.3. Beads sedimentation

To verify if the sedimentation prevention was due to the horizontal/vertical syringe orientation or to the SEC–stirring, the measurement was repeated against a vertical syringe using silica beads (Fig. 6b). These beads have a higher density than cells, and thus are harder to maintain in suspension. Also in this case, the normalized concentration of samples not stirred decreased constantly over time, reaching 1 ± 1 % of the initial concentration after 25 min, whereas the stirring system was able to prevent most of the sedimentation, with a normalized concentration $\sim 73 \pm 12$ % after 25 min. The statistical analysis returned $p < 0.05$ for all samples collected after 15 min.

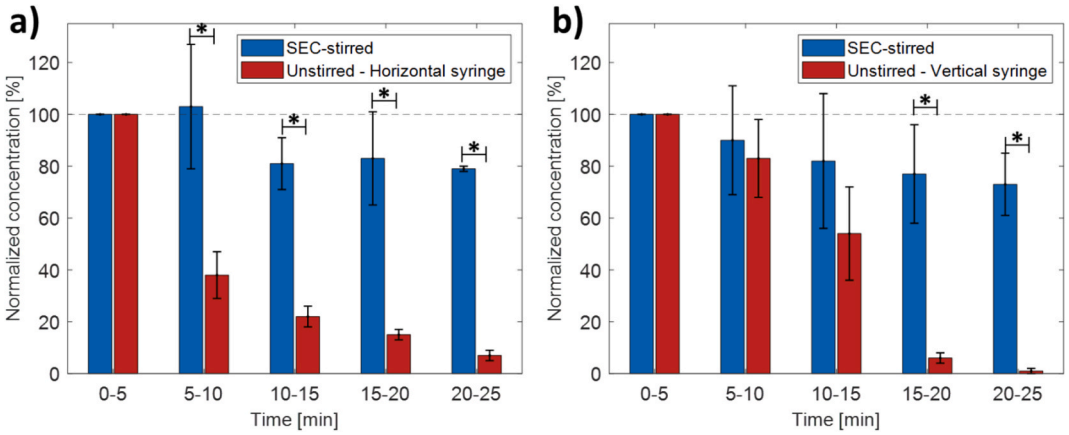

Fig. 6. Sedimentation prevention: concentrations of particle samples were measured over time when stirred with the SEC (blue bars) and under unstirred conditions (red bars). Concentrations are normalized with respect to the concentration of the first fraction. a) Hela cells, the unstirred syringe is horizontal. b) Silica beads, the unstirred syringe is vertical. Error bars represent $\pm$ 1 standard deviation ($n = 3$) and * denote statistical significance (*t*-test, $p < 0.05$, unpaired, 2 tails). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

8.3. Viability analysis

While low intensity magnetic fields are known to not cause any significant effect in cell cultures [25,26], the viability of cells samples was assessed after SEC stirring/heating to check for eventual detrimental effects on cell viability potentially caused by mechanical or thermal stresses. Cells were harvested and single cell suspension was used to test cells viability prior and upon 30 min experiment at 27 °C or 37 °C temperature and under stirring. A control sample was left at room temperature (RT) during the experiment. To evaluate viability, 10^6 cells from each sample were stained with Fixable Viability Stain 780 (FVS780, BD Horizon, BD Bioscience, USA), a dye able to discriminates viable from non-viable cells, used 1/1000 in PBS w/o salt for 10 min at room temperature in the dark. Upon extensive washing, cells were run at the flow cytometer Becton Dickinson (BD) instruments LSRFortessa (Becton Dickinson, BD Biosciences, USA) equipped, among others, with a 639 nm laser. Forward scattering (FSC), Side-scattering (SSC), and fluorescence intensity signals were acquired using FACSDiva software (BD Biosciences, version 6.1.3) and analyzed using a custom Matlab script. A total of 30,000 events/sample were collected. The viability of cells samples was assessed after SEC-stirring for 30 min by Live/Dead immunofluorescence staining followed by flow cytometry analysis. The percentage of live and dead cells (L/D) cells was assessed by gating flow-cytometry data on the bivariate Forward Scatter (FSC) – Fluorescence Intensity distributions (Fig. 7). Three samples were measured: (a) an un-stirred, un-heated control, yielding L = 89 % and D = 8 %, (b) a SEC-stirred sample thermo-regulated at 37 °C, yielding L = 85 % and D = 11 %, and (c) a SEC-stirred sample thermo-regulated at 27 °C, yielding L = 92 % and D = 6 %. The results shown minor variations on L/D percentages, thus indicating that SEC stirring doesn't affect cell population viability.

9. Conclusions

We reported the design of the SEC, a hardware apparatus for the stirring and thermoregulation of samples formed by cells/particles suspended in a syringe. SEC is capable of:

- Prevents sedimentation for up to 25 min.
- Can heat the sample faster than purely convectional approaches (0.08 °C/s).
- Can maintain the temperature inside the syringe constant within ± 0.5 °C.
- Does not affect cell viability.

10. Ethics statements

The authors have no conflicts to disclose. This work does not contain any studies with human or animal subjects performed by any of the authors.

CRediT authorship contribution statement

Maryamsadat Ghoreishi: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Giovanna Peruzzi:** Methodology. **Lucia Iafrate:** Investigation. **Gianluca Cidonio:** Writing – review & editing, Funding acquisition, Conceptualization. **Noemi D'Abbondanza:** Writing – review & editing, Investigation. **Giancarlo Ruocco:** Writing – review & editing, Funding acquisition, Conceptualization. **Marco Leonetti:** Writing – review & editing, Funding acquisition, Conceptualization. **Riccardo Reale:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization.

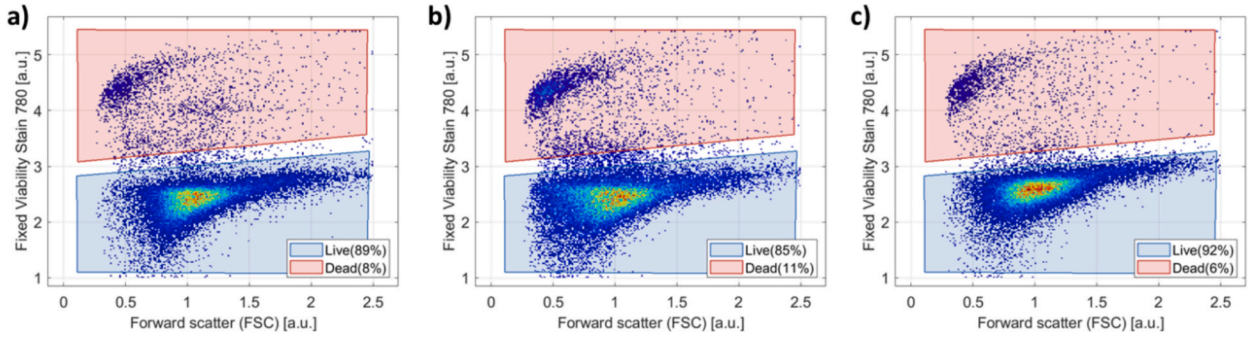

Fig. 7. Cell viability analysis: The percentage of Live/Dead cells after 30 min was assessed by flow-cytometry after immunofluorescence viability staining. a) No stirring, no temperature control (L = 89 %, D = 8 %). b) Stirring, temperature controlled at 37 °C (L = 85 %, D = 11 %). c) Stirring, temperature controlled at 27 °C (L = 92 %, D = 6 %). Gates are determined based on the control sample and are shown as blue and red shaded areas for Live and Dead cells, respectively. 30,000 events are shown for each dot plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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

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References

[1] G. Huang, et al., Applications, advancements, and challenges of 3D bioprinting in organ transplantation, *Biomater. Sci.* 12 (2024) 1425–1448.
[2] A. Khademhosseini, R. Langer, A decade of progress in tissue engineering, Nature Protocols 11 (10) (2016) 1775–1781.
[3] G. Janarthanan, et al., 3D Printing of Bioinspired Alginate-Albumin Based Instant Gel Ink with Electroconductivity and Its Expansion to Direct Four-Axis Printing of Hollow Porous Tubular Constructs without Supporting Materials, *Adv. Funct. Mater.* 31 (2021) 2104441.
[4] R.E. Saunders, J.E. Gough, B. Derby, Delivery of human fibroblast cells by piezoelectric drop-on-demand inkjet printing, *Biomaterials* 29 (2008) 193–203.
[5] H. Xu, J. Liu, Z. Zhang, C. Xu, Cell sedimentation during 3D bioprinting: a mini review, *Biodes Manuf* 5 (2022) 617–626.
[6] H. Xu, D.M. Martinez Salazar, C. Xu, Investigation of Cell Concentration Change and Cell Aggregation Due to Cell Sedimentation during Inkjet-Based Bioprinting of Cell-Laden Bioink, *Machines* 10 (2022) 315.
[7] C. D'Antoni, et al., Unlocking Neural Function with 3D In Vitro Models: A Technical Review of Self-Assembled, Guided, and Bioprinted Brain Organoids and Their Applications in the Study of Neurodevelopmental and Neurodegenerative Disorders, International Journal of Molecular Sciences 24 (2023) 10762.
[8] H. Xu, L. Jiachen, Zhang Zhengyi & Xu Changxue. A review on cell damage, viability, and functionality during 3D bioprinting, *Mil. Med. Res.* 9 (2022) 70.
[9] K. Hölzl, et al., Bioink properties before, during and after 3D bioprinting, *Biofabrication* 8 (2016) 032002.
[10] M.E. Cooke, D.H. Rosenzweig, The rheology of direct and suspended extrusion bioprinting, *APL Bioeng.* 5 (2021) 11502.
[11] M. D'Orazio, et al., Cells in the 3D biomatrix on-chip: better mimicking the real micro-physiological system, *Next Mater.* 5 (2024) 100229.
[12] B. Hammarström, M. Vassalli, P. Glynne-Jones, Acoustic focussing for sedimentation-free high-throughput imaging of microalgae, *J. Appl. Phycol.* 32 (2020) 339–347.
[13] X. Zhao, et al., A high-precision thermometry microfluidic chip for real-time monitoring of the physiological process of live tumour cells, *Talanta* 226 (2021) 122101.
[14] A.D. Radadia, Microfluidics for Biochemical and Chemical Reactions, *Encycl. Microfluidics Nanofluidics* 1195–1207 (2008).
[15] Nemix 50 syringe stirrer for homogeneous mixing inside the syringe. https://cetoni.com/nemix-50/.
[16] R. Sánchez, et al., Fully Automatic In-Syringe Magnetic Stirring-Assisted Dispersive Liquid-Liquid Microextraction Hyphenated to High-Temperature Torch Integrated Sample Introduction System-Inductively Coupled Plasma Spectrometer with Direct Injection of the Organic Phase, *Anal. Chem.* 89 (2017) 3787–3794.
[17] B. Horstkotte, R. Suárez, P. Solich, V. Cerdà, In-syringe-stirring: A novel approach for magnetic stirring-assisted dispersive liquid–liquid microextraction, *Anal. Chim. Acta* 788 (2013) 52–60.
[18] T. Puttrich, et al., Development of a programmable magnetic agitation device to maintain colloidal suspension of cells during microfluidic syringe pump perfusion, *PLoS One* 18 (2023) e0282563.
[19] S. Cremaschini, et al., Magnetic stirring device for limiting the sedimentation of cells inside microfluidic devices, *Sensors* 24 (2024) 5014.
[20] Dispense Systems Syringe Mixer, Agitator. https://gpd-global.com/dispense-systems-syringe-mixer-agitator/.
[21] Syringe Heater - Pad - SyringePumps. https://www.syringepump.eu/product/syringe-heater-pad/.
[22] Syringe Heater Jacket | Syringe Warmer - Heating Sleeve. https://chemyx.com/products/syringe-heating-sleeve/.
[23] Syringe block warmer — Arelo.dk. https://www.arelo.dk/shop/syringe-block-warmer-1.
[24] Reale, R. A compact electromagnetic syringe stirrer and temperature controller for the reliable dispensing of living cells and microparticles. https://osf.io/fb94x/ (2024) doi:10.17605/OSF.IO/FB94X.
[25] A. Rekena, E. Didrihsone, K. Vegere, The role of magnetic field in the biopharmaceutical production: Current perspectives, *Biotechnol. Rep,* 22 (2019) e00334.
[26] T. Norimura, H. Imada, N. Kunugita, N. Yoshida, M. Nikaido, Effects of strong magnetic fields on cell growth and radiation response of human T-lymphocytes in culture, *J. UOEH* 15 (1993) 103–112.

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