

Compact and tunable stretch bioreactor advancing tissue engineering implementation. Application to engineered cardiac constructs

Original

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1 **Compact and tunable stretch bioreactor advancing tissue engineering**
2 **implementation. Application to engineered cardiac constructs**

3

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16

17 **Keywords:** Bioreactor, Cyclic stretching, Mechanical stimulation, Tissues engineering, Cardiac
18 tissue maturation.

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21

22 **Abstract**

23 Physical stimuli are crucial for the structural and functional maturation of tissues both *in vivo* and *in*
24 *vitro*. In tissue engineering applications, bioreactors have become fundamental and effective tools
25 for providing biomimetic culture conditions that recapitulate the native physical stimuli. In addition,
26 bioreactors play a key role in assuring strict control, automation, and standardization in the
27 production process of cell-based products for future clinical application. In this study, a compact,
28 easy-to-use, tunable stretch bioreactor is proposed. Based on customizable and low-cost
29 technological solutions, the bioreactor was designed for providing tunable mechanical stretch for
30 biomimetic dynamic culture of different engineered tissues. In-house validation tests demonstrated
31 the accuracy and repeatability of the imposed mechanical stimulation. Proof of concepts biological
32 tests performed on engineered cardiac constructs, based on decellularized human skin scaffolds
33 seeded with human cardiac progenitor cells, confirmed the bioreactor Good Laboratory Practice
34 compliance and ease of use, and the effectiveness of the delivered cyclic stretch stimulation on the
35 cardiac construct maturation.

36 **1. Introduction**

37 Tissue engineering (TE) is a multidisciplinary research field whose primary purpose is the *in vitro*
38 development of functional tissue constructs used as models for basic research, drug testing, and
39 disease investigations, or ultimately aimed at repairing injured tissues or even organs [1,2].

40 According to the TE paradigm, the bioprocess for generating functional constructs is based on three
41 key elements: cells, scaffolds, and culture environmental cues [3,4].

42 Cells play a crucial role, since they generate the new tissue through proliferation, differentiation and
43 maturation. In particular, the use of human stem or progenitor cells, which can differentiate into
44 tissue-specific functional cell types, provides promising perspectives for patient-specific tissue
45 models and personalized TE [5–10].

46 Scaffolds substantially serve as active biochemical and structural support for cell growth. In
47 particular, decellularized extracellular matrix (ECM) is recognized as one of the most promising
48 biological scaffolds, because of its native biochemical and biomechanical features, and its three-
49 dimensional (3D) microarchitecture [11,12].

50 Lastly, biomimetic chemical and physical environmental cues have proven to be fundamental for
51 defining the fate and the functionality of the engineered constructs [13–16]. Focusing on strategies
52 for engineering tissues that *in vivo* are physiologically subjected to mechanical stimuli (e.g., tensile
53 or compressive load), several studies demonstrated that the use of dynamic culture devices
54 providing adequate *in vitro* mechanical stimuli leads to significant improvements in structural and
55 functional tissue maturation [17–20]. For example, it was observed that the controlled exposure of
56 engineered skeletal muscle tissues to mechanical cyclic stretch promotes their development, with
57 improved morphological, contractile and myogenic properties [21–24]. Furthermore, stretch was
58 successfully applied for cultivating *in vitro* tendon and ligament grafts, with several studies
59 demonstrating that mechanical stimulation is crucial for promoting tenocyte differentiation, tendon
60 matrix synthesis, and construct tensile strength [25–30]. Dynamic culture devices providing stretch
61 stimuli were also used for generating skin tissue models characterized by thick epidermal layers
62 with high levels of expressed basement membrane proteins [31], and for *ex vivo* expansion of skin
63 grafts, promoting dermal ECM synthesis [32,33]. Cyclic stretch plays a fundamental role in
64 bioprocesses designed for the *in vitro* maturation of cardiac tissue models. A large body of literature
65 demonstrated that the provision of cyclic stretch stimulation mimicking the cyclic diastolic filling of
66 the ventricles promotes cell proliferation, myocardium-like morphological arrangement and
67 maturation, and contractile performance of engineered cardiac tissues [34–42].

68 The need of TE bioprocesses to provide biomimetic physical stimuli in a strictly controlled manner
69 is faced using bioreactors. When equipped with advanced and programmable technological

70 solutions, these devices can guarantee control, automation, and standardization of the production
71 process [43,44], fulfilling the rigorous requirements for clinical translation of cell-based products.
72 Moreover, bioreactors represent useful platforms for generating *in vitro* tissue models, thus
73 addressing the need for providing investigation methods alternative to animal-based
74 experimentation.
75 However, bioreactor-based approaches have to cope with a series of drawbacks limiting their wide
76 spread. In particular, complex technology and high costs, often related to the high level of
77 customization required by the specific application, represent relevant limiting factors [45].
78 Moreover, difficulty of use is a critical aspect affecting both custom-made and commercial
79 bioreactor platform diffusion [46,47].
80 Nowadays, the availability of affordable open-source and low-cost electronic solutions for
81 bioprocess monitoring and control purposes and the diffusion of low-cost 3D printing technologies
82 give the opportunity to rethink the design phase as well as to develop highly customizable and
83 flexible bioprocess platforms at limited implementation costs [48–52]. In this perspective, we
84 present here a compact, easy-to-use, tunable stretch bioreactor platform for TE applications.
85 Customizable and low-cost technological solutions are adopted for the platform implementation.
86 Using a purpose-built test bench, in-house validation tests are performed to assess the motor motion
87 accuracy and repeatability. To demonstrate the bioreactor platform performance in a cell culture
88 laboratory and to investigate the impact of cyclic stretch on maturation of engineered cardiac
89 tissues, explanatory biological experiments on decellularized human skin (d-HuSk) scaffolds seeded
90 with human cardiac progenitor cells (hCPCs), performed within the bioreactor platform, are
91 presented. The hCPC-seeded d-HuSk scaffolds are subjected to controlled cyclic stretch, and the
92 effect of cyclic stretch conditioning is analyzed in terms of cell organization and gene expression of
93 typical cardiac markers.

94

95 **2. Materials and methods**

96 **2.1 Bioreactor platform**

97 The design of the bioreactor platform was guided by specific requirements. Firstly, the device
98 should provide tunable mechanical stretch for biomimetic dynamic culture of different engineered
99 tissues (e.g., myocardium, skeletal muscle, skin, tendon, and ligament tissue). Then, it should
100 accomplish general specifications of a bioreactor for TE strategies [17], particularly Good
101 Laboratory Practices (GLP) compliance in terms of ease of assembling, cleaning, and use in a cell
102 culture laboratory and with conventional laboratory equipment. Moreover, the bioreactor platform

103 should be modular for facilitating assembling/disassembling/cleaning procedures and
104 customization, and it should be characterized by small size, to be easily handled under laminar flow
105 hood and within the incubator. Lastly, for promoting the use of the system, the bioreactor platform
106 should be designed and produced with easy-to-use and low-cost hardware and software, and overall
107 it should guarantee reliability for long-term experiments within the incubator (37°C, 5% CO₂, and
108 90-95% humidity).

109 Based on these requirements, the bioreactor platform is designed consisting of three main units (Fig.
110 1A): (1) the culture unit, housing the constructs; (2) the stimulation unit, providing the biomimetic
111 mechanical stimuli; (3) the control unit, devoted to the control of the stimulation unit. Both the
112 culture unit and the stimulation unit are mounted on an aluminum planar base (342 mm x 128 mm)
113 to be incubated, while the control unit is located outside the incubator.

114 In detail, the culture unit, adapted from a previously developed device [53], is composed of a
115 polycarbonate culture chamber (140 x 80 x 75 mm³ with a priming volume of ~100 ml) designed to
116 house multiple constructs to be cultured simultaneously. Within the culture chamber, two opposite
117 polyoxymethylene (POM) clamps allow grasping the constructs during stimulation. One clamp is
118 mobile, coupled with a stainless steel through-shaft externally connected to the stimulation unit
119 motor, while the opposite clamp is fixed (Fig. 1B). Silicone bellows (J-Flex rubber, Retford, UK)
120 assure watertightness of the culture chamber. The culture chamber is inserted within an L-shaped
121 chassis, previously developed for guaranteeing a correct positioning of the culture chamber on the
122 planar base [54]. The stimulation unit consists of a watertight box (130 x 95 x 65 mm³), which
123 houses a captive stepper motor (NEMA 14, Nanotec Electronic GmbH & Co. KG, Feldkirchen, DE)
124 that generates a linear motion with a resolution of 10 µm/step. The motor provides the mechanical
125 stimulation to the cultured constructs, controlled by the control unit. The latter is made of a compact
126 box (170 x 150 x 60 mm³) containing a microcontroller board (Arduino Due, Arduino, Ivrea, IT),
127 selected because it is an open-source and low-cost electronics platform, which is coupled with a
128 small-sized motor driver (A4988, Allegro MicroSystems, Manchester, USA). The motor driver with
129 built-in translator and current regulator acts as bridge component between the microcontroller and
130 the motor, and enables motor control in open-loop configuration efficiently assuring the needed
131 power supply. A user-friendly interface, based on push buttons and a 1.8" LCD screen (Arduino),
132 allows the proper adjustment of the initial relative position between clamps and the setting of the
133 stimulation parameters (i.e., stretching amplitude and frequency). A schematic diagram of the
134 control unit implementation is reported in Figure 1C.

135 To perform the explanatory biological tests, dedicated to culture cardiac constructs under cardiac-
136 like cyclic stretch, the microcontroller is programmed to generate a sinusoidal motor motion with

137 tunable stretching amplitudes (0.1-3.0 mm, by 0.1 mm steps) and frequencies (1-3 Hz, by 1 Hz
 138 steps). Available combinations of stimulation parameters for culturing constructs are reported in
 139 Table 1.
 140

Amplitude range (mm)	Frequency (Hz)		
	1	2	3
2.1 - 3	●		
1.1 - 2	●	●	
0.1 - 1	●	●	●

141 **Table 1.** Stimulation parameter combinations. Black dots indicate the available combinations.
 142

143 All culture chamber components in contact with medium or constructs are made of cytocompatible
 144 and autoclavable materials [53,55]. The L-shaped chassis housing the culture chamber and the
 145 stimulation unit box are manufactured in ABS thermoplastic material by fused deposition modelling
 146 (FDM) for guaranteeing design flexibility and cost-efficiency [54].

147 **2.2 In-house tests**

148 The ease of use and the reliability of stimulation and control units were preliminarily tested in-
 149 house. In detail, the motion accuracy of the stimulation unit operated by the control unit was
 150 characterized using a purpose-built test bench. A linear variable displacement transducer (LVDT,
 151 AML/EU/±5/S, Applied Measurements Ltd., Aldermaston, UK), mounted on a chassis and
 152 connected to a dedicated data acquisition system (Personal computer equipped with a cDAQ-9174
 153 coupled with a NI 9218 module, National Instruments, Austin, TX, USA), was put in contact with
 154 the through-shaft connected to the stimulation unit motor (Supplementary Fig. S1), and all the 60
 155 combinations of motor amplitude and frequency parameters were tested. In detail, for each possible
 156 combination, the motor imparted displacement was acquired continuously over 30 cycles (sampling
 157 rate = 1652 Hz). The measured LVDT signals were acquired, filtered (Butterworth low-pass filter,
 158 order 8, cut-off frequency = 10 Hz), and analyzed in LabVIEW environment (LabVIEW, National
 159 Instruments) to evaluate the peak-to-peak amplitude as well as the frequency of the recorded
 160 displacement signals. All measurements were carried out in triplicate. The motor displacement
 161 waveforms were characterized by comparing the measured waveforms with the prescribed ideal
 162 sinusoidal waveforms. The mean percentage errors of measured amplitude and frequency values
 163 with respect to the prescribed nominal values were expressed as mean ± standard deviation (SD).
 164

165 **2.3 Biological tests**

166 *2.3.1 Bioreactor platform performance in a cell culture laboratory*

167 The bioreactor platform was then tested in a cell culture laboratory in order to assess its ease of use
168 and compliance with GLP procedures. In detail, the components of the culture chamber were
169 autoclaved and assembled under laminar flow hood, the culture chamber was filled with Dulbecco's
170 Modified Eagle's Medium/Ham's Nutrient Mixture F12 culture medium (Sigma-Aldrich, St. Louis,
171 MO, USA), and the assembled system was placed in incubator without constructs but with the
172 mechanical stimulation (1 mm, 1 Hz) switched on for 5 days.

173 *2.3.2 Preparation and culture of cardiac constructs*

174 To investigate the influence of biomimetic cyclic stretch on the maturation of cardiac constructs,
175 explanatory biological tests were carried out on decellularized human skin (d-HuSk) scaffolds
176 seeded with human cardiac progenitor cells (hCPCs) and hCPC-derived early cardiac myocytes.
177 Concerning the scaffold preparation, human skin samples were obtained from patients undergoing
178 abdominoplasty (n = 4, mean age 41.75 ± 2.36). Upon receipt, samples were washed in
179 physiological saline solution, then subcutaneous tissue was removed and multiple specimens were
180 cut (length = 20 mm, width = 10 mm) marking Langer's line orientation. For decellularization
181 treatment, specimens were enclosed in embedding cassettes housed in a purpose-built sample-
182 holder, put within a beaker filled with the decellularizing solution (700 ml) and placed on a
183 magnetic stirrer, and kept under constant stirring (150 rpm) for 24 h [56]. The decellularizing
184 solution contained 1% w/v sodium dodecyl sulfate (SDS) (Sigma-Aldrich) and 1% v/v Triton
185 (Sigma-Aldrich). The specimens were then rinsed for 24 h in antibiotic solution containing 0.25
186 $\mu\text{g/ml}$ Amphotericin B, 100 U/ml Penicillin, and 50 U/ml Streptomycin (all from Sigma-Aldrich) in
187 PBS, and lastly in sterile bidistilled water for additional 30 min [57,58]. The d-HuSk specimens
188 were snap-frozen, mounted on a cryostat chuck using Tissue Freezing Medium (Leica
189 Microsystems, Wetzlar, Germany), and sliced into 600- μm -thick sections by a Leica CM1950
190 cryostat (Leica Microsystems). Cryosections of d-HuSk were sterilized by exposure to ultraviolet
191 radiation for 40 min and rehydrated for one week with F12K medium in incubator (37°C , 5% CO_2).
192 The sterilized and rehydrated d-HuSk cryosections were then stored in standard culture conditions
193 with the same medium until use.

194 As regards the hCPCs, they were isolated from cardiac specimens derived from macroscopically
195 uninjured areas of the left ventricle of explanted hearts of patients undergoing heart transplant
196 because of end-stage heart failure (n = 10, mean age 49.5 ± 4.7). Specifically, following a
197 previously described protocol [59] cardiac specimens were washed in physiological saline solution,

198 dissected, minced, and enzymatically disaggregated by incubation in 0.25% trypsin (Sigma-
199 Aldrich) for 6 h at 4 °C and in 0.1% w/v collagenase II (Sigma-Aldrich) for 30 min at 37 °C. The
200 digestion was stopped by adding double volume of Hanks' Balanced Salt solution (Sigma-Aldrich)
201 supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich). Resulting fragments of tissue
202 were further disaggregated by pipetting. Tissue debris and cardiomyocytes were then removed by
203 sequential centrifugation at 100 g for 2 min, passage through a 40-µm cell strainer (BD Biosciences,
204 Franklin Lakes, NJ, USA), and centrifugation at 400 g for 5 min. The obtained cell population was
205 then incubated with anti-fibroblast MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) to
206 magnetically label fibroblasts that were then removed loading cells onto a MACS column (Miltenyi
207 Biotec) placed in the magnetic field of a MACS separator (Miltenyi Biotec). The negative fraction
208 of unlabeled hCPCs ran through the column was collected and plated at a density of 4×10^3 cells per
209 cm^2 in F12K medium, prepared from Nutrient Mixture F-12 Ham medium (Sigma-Aldrich)
210 supplemented with 10% FBS (Sigma-Aldrich), basic fibroblast growth factor (Peprotech, Rocky
211 Hill, NJ, USA), glutathione (Sigma-Aldrich), penicillin and streptomycin (Sigma-Aldrich). The
212 hCPCs were cultured in incubator (37°C, 5% CO₂) and observed daily by an inverted phase-contrast
213 microscope (Olympus, Tokyo, Japan). Medium was replaced every 3 days until the 75% confluence
214 was reached. Then, an unselected subpopulation of hCPCs was induced to differentiate towards
215 cardiac myocytes by adding 50 µg/ml of ascorbic acid (Sigma-Aldrich) and 10 ng/ml of Vascular-
216 Endothelial Growth Factor (Sigma-Aldrich) to the culture medium for 7 days.
217 Successively, the d-HuSk scaffolds (n = 24) were seeded with 2.5×10^6 hCPCs and 2.5×10^6 hCPC-
218 derived early cardiac myocytes. After 7 days of static culture in Petri dish, half of the constructs (n
219 = 12) were transferred, in pairs, into the bioreactor culture chamber with F12K medium and
220 subjected to dynamic conditions (i.e., sinusoidal cyclic stretch, 10% strain, 1 Hz) for additional 7
221 days (see Supplementary Movie 1) for mimicking the cyclic diastolic filling of the ventricles [60–
222 63]. As control experiment, the other half of the constructs (n = 12) were cultured statically in Petri
223 dishes for the entire duration of 14 days (Fig. 2). Finally, constructs were cut into smaller specimens
224 that were either fixed in 10% neutral-buffered formalin (Sigma-Aldrich) for morphological analyses
225 or processed for RNA extraction for gene expression profiling.

226 2.3.3 *Histochemistry analysis*

227 Following standard protocols, subsets of constructs cultured in static conditions (control) or in
228 bioreactor and fixed in 10% neutral-buffered formalin were dehydrated in a graded series of
229 alcohols, embedded in paraffin and sliced into serial 5-µm-thick sections [57,58]. Sections were
230 stained with Hematoxylin and Eosin (H&E) and with Mallory's trichrome staining using specific
231 kits (both from Bio-Optica, Milan, Italy). Stained sections were observed by at least three

232 independent researchers using a light microscope DM2000 Led (Leica Microsystems) equipped
233 with an ICC50HD camera (Leica Microsystems).

234 *2.3.4 Gene expression profile analysis*

235 Total RNA was extracted from hCPCs seeded on d-HuSk scaffolds, cultured both in static and
236 cyclic stretch conditions, using Trizol Reagent (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA,
237 USA), according to the manufacturer's instructions. RNA was dissolved in RNase-free water and its
238 final concentration was quantified at the NanoDrop 1000 spectrophotometer (Thermo Scientific,
239 Waltham, MA, USA). All RNA samples were checked for quality and resulted suitable for gene
240 expression profiling analyses. Analysis was performed as previously described [64]. Briefly, RNA
241 from each sample was reverse transcribed into cDNA with QuantiTect Reverse Transcription Kit
242 (Qiagen, Hilden, Germany) and gene expression was quantified by real-time qPCR using Power
243 SYBR Green PCR Master Mix (Applied Biosystem, Thermo Fisher Scientific). DNA amplification
244 was carried out using QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) and the
245 detection was performed by measuring the binding of the fluorescent dye SYBR Green I to double-
246 stranded DNA. The thermal cycling conditions included an initial enzyme activation at 95°C for 10
247 min and 40 cycles consisting of a denaturation step at 95°C for 15 s and an annealing step at 60°C
248 for 60 s. Melt curve analysis was performed to assess uniformity of product formation, primer
249 dimer formation and amplification of non-specific products. Primers used in this study were
250 designed with Primer3 software (<http://frodo.wi.mit.edu>) starting from the CDS (coding sequence)
251 of mature mRNA available on GeneBank (Supplementary Table S1). All samples were tested in
252 triplicate with the housekeeping gene (GAPDH) to correct for variations in RNA quality and
253 quantity. Comparative quantification of target gene expression in the samples was performed based
254 on cycle threshold (Ct) normalized to the housekeeping gene and using the $2^{-\Delta\Delta Ct}$ method.

255 *2.3.5 Statistical analysis*

256 Data from gene expression profiling were analyzed by GraphPad Prism 5.0 (GraphPad Software, La
257 Jolla, CA, USA) using Student's two-tailed unpaired t-test. All experiments were performed in
258 triplicate and data were averaged and expressed as the mean \pm standard error of the mean. The
259 statistical significance is denoted as * for p-value \leq 0.05, ** for p-value \leq 0.01, *** for p-value \leq
260 0.001.

261

262 **3. Results**

263 **3.1 *In-house tests***

264 In-house tests confirmed the ease of use and the reliability of the stimulation and control units. In
265 detail, tests on motor imposed displacement accuracy highlighted that real displacement waveforms
266 agree with the prescribed ideal sinusoidal waveforms for all the available combinations of working
267 conditions, as testified by the explanatory waveforms presented in Figure 3A. As regards the
268 comparison between the measured stretching amplitude values and the nominal ones, mean error
269 values up to $13.3\% \pm 7.3\%$ were observed, with the largest deviation from the nominal curve
270 corresponding to the combination characterized by minimum amplitude equal to 0.1 mm and
271 frequency equal to 1 Hz. For nominal amplitude values higher than 0.4 mm the observed mean error
272 values were lower than 4% (Fig. 3B). Concerning the nominal frequency, mean error values up to
273 10.5% (corresponding to the combination characterized by amplitude equal to 1.3 mm and
274 frequency equal to 1 Hz) were observed (Fig. 3C).

275 **3.2 Biological tests**

276 *3.2.1 Bioreactor platform performance in a cell culture laboratory*

277 Preliminary tests performed in a cell culture laboratory confirmed ease of use, sterility maintenance,
278 and functionality of the bioreactor platform in a standard incubator. During the explanatory cyclic
279 stimulation tests run for 5 days in incubator, the system did not present adverse issues, the
280 watertightness of the culture chamber and the stimulation unit was confirmed, and the culture
281 medium did not present any signs of contamination.

282 *3.2.2 Histochemistry*

283 The H&E and Mallory's trichrome staining of the cardiac constructs revealed that, under both static
284 (control) and dynamic (sinusoidal cyclic stretch, 10% strain, 1 Hz) culture conditions, hCPCs
285 organized into a structured multilayered tissue on the surface of the d-HuSk scaffolds (Fig. 4).
286 Noteworthy, the histochemical analysis highlighted that the dynamic culture promoted hCPC
287 migration towards the inner layers of the scaffolds (Figs. 4B and 4D).

288 *3.2.2 Gene expression profile analysis*

289 The gene expression profile analysis of hCPCs extracted from constructs cultured under either static
290 or dynamic conditions included genes typical of main cardiac cell lineages. In particular, in
291 bioreactor-cultured constructs, a significant up-regulation of cardiac alpha actin (ACTC1), a marker
292 typical of late differentiating and mature cardiac myocytes, was observed. On the opposite, markers
293 typical of undifferentiated hCPCs (like CD117) or of early stages of cardiac myocyte differentiation
294 (like TBX3 and TBX5) were significantly down-regulated with respect to control constructs (Fig.
295 5). The transcription of other markers typical of cardiac myocytes, like MEF2C, CX43, and
296 GATA4, did not differ significantly among constructs cultured in static or dynamic conditions, and

297 similarly happened for the transcription of the mesenchymal cell marker CD105 and of genes
298 typical of smooth muscle cells (GATA6, ACTA2) and endothelial cells (ETS1, FVIII)
299 (Supplementary Fig. S2).

300

301 **4. Discussion**

302 In TE research, a number of studies demonstrated that successful strategies for the *in vitro*
303 generation of functional engineered tissues require a synergistic combination of appropriate cells,
304 scaffolds, and biochemical and biophysical signals [65–67]. As specifically concerns mechanical
305 cues, in the last two decades a plethora of custom-made bioreactors providing *in vitro* biomimetic
306 mechanical stretch have been proposed [23,24,29–31,33,40,42]. In parallel, ready-to-use systems
307 have been developed by commercial companies (e.g., Tissue Train 3D Culture System from
308 FlexCell International, Hillsborough, USA; TC-3 from Ebers Medical Technology, Zaragoza,
309 Spain; MCT6 from CellScale, Waterloo, Canada; BioDynamic 5100 from TA Instruments, New
310 Castle, USA). All the developed culture devices substantially contributed to unravel the
311 fundamental role that mechanical stretch has on structural and functional development of biological
312 tissues and in regulating tissue homeostasis and pathophysiology. Moreover, their use increased the
313 knowledge on sensitivity of cells to mechanical stimuli, to which cells react activating specific
314 mechanotransduction pathways that can lead to phenotypic changes [68–71].

315 However, the proposed custom-made bioreactors were often based on complex technological
316 solutions, difficult to use by non-trained operators in a cell culture laboratory and typically
317 dedicated to highly specialized applications, while the commercial devices are generally expensive
318 and not fully customizable.

319 Taking into account these limitations, in this study we developed a compact, easy-to-use, tunable
320 stretch bioreactor platform for culturing *in vitro* 3D engineered constructs under biomimetic stretch
321 conditions. Particular attention was paid in developing reliable and affordable stimulation and
322 control units. As regards the stimulation unit, the use of a captive stepper motor enables the
323 provision of linear motion adopting an open loop control strategy ensuring high displacement
324 resolution without the need for additional and complex feedback sensing solutions. In combination,
325 a compact control unit, based on low-cost open-source hardware and freeware software, avoids the
326 use of cumbersome and expensive equipment (e.g., laptop, data acquisition module, and
327 commercial software). Moreover, the integrated user-friendly interface allows ease-of-use to not
328 experienced operators as well as system portability. In-house performance tests confirmed that the
329 bioreactor platform is reliable in providing accurate and repeatable stimulation within a range of

330 physiological interest. For imposed motor displacement values higher than 0.4 mm, the mean error
331 values between the measured amplitude values and the nominal ones were lower than 4%, thus
332 negligible, for all available stimulation parameter combinations. Conversely, for motor
333 displacement values in the range of 0.1 - 0.4 mm, higher amplitude errors were calculated (Fig. 3).
334 However, it should be noted that such small displacement values are not commonly adopted for
335 mechanical stimulation of macroscopic constructs. This inaccuracy could be ascribed to the axial
336 play of the motor shaft, and to inertial and vibrational phenomena that are intrinsic to stepper
337 motors. In addition, possible signal artefacts during the LVDT data acquisition, due to inductive and
338 capacitive electrical interference, could not be excluded. As concerns the stimulation frequency,
339 measurements revealed negligible errors, probably ascribable to intrinsic technical limitations of the
340 adopted low-cost microcontroller.

341 Preliminary tests in a cell culture laboratory demonstrated that the device is easy-to-use with GLP
342 compliant procedures, compact to handle and fit in a standard incubator, and guarantees
343 watertightness, sterility maintenance and functionality.

344 For investigating the effect of cyclic stretch on cardiac construct maturation, the biological
345 experiments were performed on decellularized human skin scaffolds seeded with hCPCs and
346 cultured for 7 days in static conditions, and then transferred into the bioreactor (sinusoidal cyclic
347 stretch, 10% strain, 1 Hz) for additional 7 days. The histochemical analysis showed cell engraftment
348 on the scaffold surface in both controls and dynamically cultured constructs (Fig. 4), but only when
349 subjected to cyclic stretch cells migrated towards the inner layers of the scaffolds, starting to
350 colonize their 3D structure (Figs. 4B and 4D). The gene expression analysis highlighted a
351 significant up-regulation of the ACTC1 marker, typical of late differentiating and mature cardiac
352 myocytes, concomitantly with a marked down-regulation of CD117, TBX3 and TBX5 markers
353 (Fig. 5), typical receptors for stem cells or early stage cardiac myocytes, suggesting that dynamic
354 culture likely promoted hCPC differentiation towards mature cardiac myocytes, in accordance with
355 previous studies [72–76].

356 Although further and longer experimental tests will be necessary for comprehensively
357 characterizing the effect of cyclic stretch on the maturation of d-HuSk scaffolds seeded with
358 hCPCs, the latter particularly sensitive to the microenvironment, the preliminary promising findings
359 provided evidence of the bioreactor platform reliability and suitability for cardiac tissue engineering
360 applications. In the future, the possibility to switch from stretching to compression mode will be
361 implemented in the bioreactor platform, and the device will be adapted to be equipped with an
362 electrical stimulation unit [77] to provide combinable mechanical and electrical stimulations for
363 mimicking the complex native cardiac environment.

364 In conclusion, adopting customizable and low-cost technological solutions, a compact, easy-to-use,
365 tunable stretch bioreactor platform for biomimetic dynamic culture of 3D engineered was
366 developed. Based on modular components and providing tunable stimulation, the proposed device is
367 versatile and adaptable for different tissue engineering applications. Moreover, the choice of the 3D
368 printing technology and low-cost hardware coupled with free and open-source software,
369 substantially limited the development costs and will support in the future the use of the system as
370 valuable tool for *in vitro* investigation and for future production of functional engineered constructs.
371

372 **Data Availability Statement**

373 Data associated with this study is available upon request to the corresponding author.
374

375 **Conflict of Interest**

376 The authors declare that the research was conducted in the absence of any commercial or financial
377 relationships that could be construed as a potential conflict of interest.
378

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382 Ethical approval: Patients provided written informed consent and samples were collected without
383 patient identifiers, following protocols approved by the Federico II University Hospital Ethical
384 Committee (ref. number 79/18) and in conformity with principles outlined in the Declaration of
385 Helsinki.
386

387 **References**

- 388 [1] Lanza R, Langer R, Vacanti J, editors. Principles of Tissue Engineering. 4th ed. Elsevier;
389 2014. <https://doi.org/10.1016/C2011-0-07193-4>.
- 390 [2] Vunjak Novakovic G, Eschenhagen T, Mummery C. Myocardial Tissue Engineering: In
391 Vitro Models. Cold Spring Harb Perspect Med 2014;4:a014076–a014076.
392 <https://doi.org/10.1101/cshperspect.a014076>.
- 393 [3] Goldstein AS, Christ G. Functional Tissue Engineering Requires Bioreactor Strategies.

- 394 Tissue Eng Part A 2009;15:739–40. <https://doi.org/10.1089/ten.tea.2009.0046>.
- 395 [4] Lyons F, Partap S, O’Brien FJ. Part 1: Scaffolds and Surfaces. *Technol Heal Care*
396 2008;16:305–17. <https://doi.org/10.3233/THC-2008-16409>.
- 397 [5] Kropp C, Massai D, Zweigerdt R. Progress and challenges in large-scale expansion of human
398 pluripotent stem cells. *Process Biochem* 2017;59:244–54.
399 <https://doi.org/10.1016/j.procbio.2016.09.032>.
- 400 [6] Chimenti I, Massai D, Morbiducci U, Beltrami AP, Pesce M, Messina E. Stem Cell
401 Spheroids and Ex Vivo Niche Modeling: Rationalization and Scaling-Up. *J Cardiovasc*
402 *Transl Res* 2017;10:150–66. <https://doi.org/10.1007/s12265-017-9741-5>.
- 403 [7] Bianco P, Robey PG. Stem cells in tissue engineering. *Nature* 2001;414:118–21.
404 <https://doi.org/10.1038/35102181>.
- 405 [8] Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative
406 medicine. *J Cell Physiol* 2007;213:341–7. <https://doi.org/10.1002/jcp.21200>.
- 407 [9] Kwon SG, Kwon YW, Lee TW, Park GT, Kim JH. Recent advances in stem cell therapeutics
408 and tissue engineering strategies. *Biomater Res* 2018;22:36. [https://doi.org/10.1186/s40824-](https://doi.org/10.1186/s40824-018-0148-4)
409 [018-0148-4](https://doi.org/10.1186/s40824-018-0148-4).
- 410 [10] Edri R, Gal I, Noor N, Harel T, Fleischer S, Adadi N, et al. Personalized Hydrogels for
411 Engineering Diverse Fully Autologous Tissue Implants. *Adv Mater* 2019;31:1803895.
412 <https://doi.org/10.1002/adma.201803895>.
- 413 [11] Badylak SF, Taylor D, Uygun K. Whole-Organ Tissue Engineering: Decellularization and
414 Recellularization of Three-Dimensional Matrix Scaffolds. *Annu Rev Biomed Eng*
415 2011;13:27–53. <https://doi.org/10.1146/annurev-bioeng-071910-124743>.
- 416 [12] Porzionato A, Stocco E, Barbon S, Grandi F, Macchi V, De Caro R. Tissue-Engineered
417 Grafts from Human Decellularized Extracellular Matrices: A Systematic Review and Future
418 Perspectives. *Int J Mol Sci* 2018;19:4117. <https://doi.org/10.3390/ijms19124117>.
- 419 [13] Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. *Nat Mater*
420 2014;13:547–57. <https://doi.org/10.1038/nmat3937>.
- 421 [14] Guilak F, Butler DL, Goldstein SA, Baaijens FPT. Biomechanics and mechanobiology in
422 functional tissue engineering. *J Biomech* 2014;47:1933–40.
423 <https://doi.org/10.1016/j.jbiomech.2014.04.019>.

- 424 [15] Almouemen N, Kelly HM, O’Leary C. Tissue Engineering: Understanding the Role of
425 Biomaterials and Biophysical Forces on Cell Functionality Through Computational and
426 Structural Biotechnology Analytical Methods. *Comput Struct Biotechnol J* 2019;17:591–8.
427 <https://doi.org/10.1016/j.csbj.2019.04.008>.
- 428 [16] Vunjak-Novakovic G, Tandon N, Godier A, Maidhof R, Marsano A, Martens TP, et al.
429 Challenges in Cardiac Tissue Engineering. *Tissue Eng Part B Rev* 2010;16:169–87.
430 <https://doi.org/10.1089/ten.teb.2009.0352>.
- 431 [17] Martin I, Wendt D, Heberer M. The role of bioreactors in tissue engineering. *Trends*
432 *Biotechnol* 2004;22:80–6. <https://doi.org/10.1016/j.tibtech.2003.12.001>.
- 433 [18] Bilodeau K, Mantovani D. Bioreactors for Tissue Engineering: Focus on Mechanical
434 Constraints. A Comparative Review. *Tissue Eng* 2006;12:2367–83.
435 <https://doi.org/10.1089/ten.2006.12.2367>.
- 436 [19] Ravichandran A, Liu Y, Teoh S-H. Review: bioreactor design towards generation of relevant
437 engineered tissues: focus on clinical translation. *J Tissue Eng Regen Med* 2018;12:e7–22.
438 <https://doi.org/10.1002/term.2270>.
- 439 [20] Mantero S, Sadr N, Riboldi SA, Lorenzoni S, Montevecchi FM. A new electro-mechanical
440 bioreactor for soft tissue engineering. *J Appl Biomater Biomech* 2007;5:107–16.
- 441 [21] Powell CA, Smiley BL, Mills J, Vandeburgh HH. Mechanical stimulation improves tissue-
442 engineered human skeletal muscle. *Am J Physiol Physiol* 2002;283:C1557–65.
443 <https://doi.org/10.1152/ajpcell.00595.2001>.
- 444 [22] Moon DG, Christ G, Stitzel JD, Atala A, Yoo JJ. Cyclic Mechanical Preconditioning
445 Improves Engineered Muscle Contraction. *Tissue Eng Part A* 2008;14:473–82.
446 <https://doi.org/10.1089/tea.2007.0104>.
- 447 [23] Somers SM, Spector AA, DiGirolamo DJ, Grayson WL. Biophysical Stimulation for
448 Engineering Functional Skeletal Muscle. *Tissue Eng Part B Rev* 2017;23:362–72.
449 <https://doi.org/10.1089/ten.teb.2016.0444>.
- 450 [24] Turner DC, Kasper AM, Seaborne RA, Brown AD, Close GL, Murphy M, et al. Exercising
451 Bioengineered Skeletal Muscle In Vitro: Biopsy to Bioreactor. *Methods Mol. Biol.*, vol.
452 1889, 2019, p. 55–79. https://doi.org/10.1007/978-1-4939-8897-6_5.
- 453 [25] Paxton JZ, Hagerty P, Andrick JJ, Baar K. Optimizing an Intermittent Stretch Paradigm

- 454 Using ERK1/2 Phosphorylation Results in Increased Collagen Synthesis in Engineered
455 Ligaments. *Tissue Eng Part A* 2012;18:277–84. <https://doi.org/10.1089/ten.tea.2011.0336>.
- 456 [26] Wang T, Gardiner BS, Lin Z, Rubenson J, Kirk TB, Wang A, et al. Bioreactor Design for
457 Tendon/Ligament Engineering. *Tissue Eng Part B Rev* 2013;19:133–46.
458 <https://doi.org/10.1089/ten.teb.2012.0295>.
- 459 [27] Youngstrom DW, Rajpar I, Kaplan DL, Barrett JG. A bioreactor system for in vitro tendon
460 differentiation and tendon tissue engineering. *J Orthop Res* 2015;33:911–8.
461 <https://doi.org/10.1002/jor.22848>.
- 462 [28] Burk J, Plenge A, Brehm W, Heller S, Pfeiffer B, Kasper C. Induction of Tenogenic
463 Differentiation Mediated by Extracellular Tendon Matrix and Short-Term Cyclic Stretching.
464 *Stem Cells Int* 2016;2016:1–11. <https://doi.org/10.1155/2016/7342379>.
- 465 [29] Dursun G, Tohidnezhad M, Markert B, Stoffel M. Effects of uniaxial stretching on tenocyte
466 migration behaviour. *Curr Dir Biomed Eng* 2018;4:313–7. <https://doi.org/10.1515/cdbme-2018-0076>.
- 468 [30] Talò G, D'Arrigo D, Lorenzi S, Moretti M, Lovati AB. Independent, Controllable Stretch-
469 Perfusion Bioreactor Chambers to Functionalize Cell-Seeded Decellularized Tendons. *Ann*
470 *Biomed Eng* 2020;48:1112–26. <https://doi.org/10.1007/s10439-019-02257-6>.
- 471 [31] Tokuyama E, Nagai Y, Takahashi K, Kimata Y, Naruse K. Mechanical Stretch on Human
472 Skin Equivalents Increases the Epidermal Thickness and Develops the Basement Membrane.
473 *PLoS One* 2015;10:e0141989. <https://doi.org/10.1371/journal.pone.0141989>.
- 474 [32] Jeong C, Chung HY, Lim HJ, Lee JW, Choi KY, Yang JD, et al. Applicability and Safety of
475 in Vitro Skin Expansion Using a Skin Bioreactor: A Clinical Trial. *Arch Plast Surg*
476 2014;41:661. <https://doi.org/10.5999/aps.2014.41.6.661>.
- 477 [33] Huh M-I, Yi S-J, Lee K-P, Kim HK, An S-H, Kim D-B, et al. Full Thickness Skin Expansion
478 ex vivo in a Newly Developed Reactor and Evaluation of Auto-Grafting Efficiency of the
479 Expanded Skin Using Yucatan Pig Model. *Tissue Eng Regen Med* 2018;15:629–38.
480 <https://doi.org/10.1007/s13770-018-0154-6>.
- 481 [34] Zimmermann W-H, Schneiderbanger K, Schubert P, Didié M, Münzel F, Heubach JF, et al.
482 Tissue Engineering of a Differentiated Cardiac Muscle Construct. *Circ Res* 2002;90:223–30.
483 <https://doi.org/10.1161/hh0202.103644>.

- 484 [35] Zimmermann W-H, Melnychenko I, Wasmeier G, Didié M, Naito H, Nixdorff U, et al.
485 Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts.
486 *Nat Med* 2006;12:452–8. <https://doi.org/10.1038/nm1394>.
- 487 [36] Birla RK, Huang YC, Dennis RG. Development of a Novel Bioreactor for the Mechanical
488 Loading of Tissue-Engineered Heart Muscle. *Tissue Eng* 2007;13:2239–48.
489 <https://doi.org/10.1089/ten.2006.0359>.
- 490 [37] Schaaf S, Shibamiya A, Mewe M, Eder A, Stöhr A, Hirt MN, et al. Human Engineered Heart
491 Tissue as a Versatile Tool in Basic Research and Preclinical Toxicology. *PLoS One*
492 2011;6:e26397. <https://doi.org/10.1371/journal.pone.0026397>.
- 493 [38] Mihic A, Li J, Miyagi Y, Gagliardi M, Li S-H, Zu J, et al. The effect of cyclic stretch on
494 maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes.
495 *Biomaterials* 2014;35:2798–808. <https://doi.org/10.1016/j.biomaterials.2013.12.052>.
- 496 [39] Salazar BH, Cashion AT, Dennis RG, Birla RK. Development of a Cyclic Strain Bioreactor
497 for Mechanical Enhancement and Assessment of Bioengineered Myocardial Constructs.
498 *Cardiovasc Eng Technol* 2015;6:533–45. <https://doi.org/10.1007/s13239-015-0236-8>.
- 499 [40] Mannhardt I, Breckwoldt K, Letuffe-Brenière D, Schaaf S, Schulz H, Neuber C, et al.
500 Human Engineered Heart Tissue: Analysis of Contractile Force. *Stem Cell Reports*
501 2016;7:29–42. <https://doi.org/10.1016/j.stemcr.2016.04.011>.
- 502 [41] Morgan KY, Black LD. Creation of a Bioreactor for the Application of Variable Amplitude
503 Mechanical Stimulation of Fibrin Gel-Based Engineered Cardiac Tissue, 2014, p. 177–87.
504 https://doi.org/10.1007/978-1-4939-1047-2_16.
- 505 [42] Valls-Margarit M, Iglesias-García O, Di Guglielmo C, Sarlabous L, Tadevosyan K, Paoli R,
506 et al. Engineered Macroscale Cardiac Constructs Elicit Human Myocardial Tissue-like
507 Functionality. *Stem Cell Reports* 2019;13:207–20.
508 <https://doi.org/10.1016/j.stemcr.2019.05.024>.
- 509 [43] Hambor JE. Bioreactor design and bioprocess controls for industrialized cell processing:
510 Bioengineering strategies and platform technologies. *Bioprocess Int* 2012;10:22–33.
- 511 [44] Hansmann J, Groeber F, Kahlig A, Kleinhans C, Walles H. Bioreactors in tissue engineering-
512 principles, applications and commercial constraints. *Biotechnol J* 2013;8:298–307.
513 <https://doi.org/10.1002/biot.201200162>.

- 514 [45] Ozturk S, Hu WS. Cell Culture Technology for Pharmaceutical and Cell-Based Therapies.
515 CRC Press; 2005.
- 516 [46] Martin I, Smith T, Wendt D. Bioreactor-based roadmap for the translation of tissue
517 engineering strategies into clinical products. Trends Biotechnol 2009;27:495–502.
518 <https://doi.org/10.1016/j.tibtech.2009.06.002>.
- 519 [47] Wendt D, Riboldi SA, Cioffi M, Martin I. Potential and Bottlenecks of Bioreactors in 3D
520 Cell Culture and Tissue Manufacturing. Adv Mater 2009;21:3352–67.
521 <https://doi.org/10.1002/adma.200802748>.
- 522 [48] Raveling AR, Theodossiou SK, Schiele NR. A 3D printed mechanical bioreactor for
523 investigating mechanobiology and soft tissue mechanics. MethodsX 2018;5:924–32.
524 <https://doi.org/10.1016/j.mex.2018.08.001>.
- 525 [49] Schneidereit D, Tschernich J, Friedrich O, Scharin-Mehlmann M, Gilbert DF. 3D-Printed
526 Reusable Cell Culture Chamber with Integrated Electrodes for Electrical Stimulation and
527 Parallel Microscopic Evaluation. 3D Print Addit Manuf 2018;5:115–25.
528 <https://doi.org/10.1089/3dp.2017.0103>.
- 529 [50] Smith LJ, Li P, Holland MR, Ekser B. FABRICA: A Bioreactor Platform for Printing,
530 Perfusing, Observing, & Stimulating 3D Tissues. Sci Rep 2018;8:7561.
531 <https://doi.org/10.1038/s41598-018-25663-7>.
- 532 [51] Rimington RP, Capel AJ, Chaplin KF, Fleming JW, Bandulasena HCH, Bibb RJ, et al.
533 Differentiation of Bioengineered Skeletal Muscle within a 3D Printed Perfusion Bioreactor
534 Reduces Atrophic and Inflammatory Gene Expression. ACS Biomater Sci Eng 2019;5:5525–
535 38. <https://doi.org/10.1021/acsbiomaterials.9b00975>.
- 536 [52] Tandon N, Taubman A, Cimetta E, Saccenti L, Vunjak-Novakovic G. Portable bioreactor for
537 perfusion and electrical stimulation of engineered cardiac tissue. 2013 35th Annu. Int. Conf.
538 IEEE Eng. Med. Biol. Soc., IEEE; 2013, p. 6219–23.
539 <https://doi.org/10.1109/EMBC.2013.6610974>.
- 540 [53] Pisani G, Massai D, Rodriguez A, Cerino G, Galluzzi R, Labate Falvo D'Urso G, et al. An
541 automated adaptive bioreactor – based platform for culturing Cardiac Tissue Models. Proc V
542 Meet Ital Chapter Eur Soc Biomech 2015.
- 543 [54] Putame G, Terzini M, Carbonaro D, Pisani G, Serino G, Di Meglio F, et al. Application of
544 3D Printing Technology for Design and Manufacturing of Customized Components for a

- 545 Mechanical Stretching Bioreactor. *J Healthc Eng* 2019;2019:1–9.
546 <https://doi.org/10.1155/2019/3957931>.
- 547 [55] Massai D, Pisani G, Isu G, Rodriguez Ruiz A, Cerino G, Galluzzi R, et al. Bioreactor
548 Platform for Biomimetic Culture and in situ Monitoring of the Mechanical Response of in
549 vitro Engineered Models of Cardiac Tissue. *Front Bioeng Biotechnol* 2020;8.
550 <https://doi.org/10.3389/fbioe.2020.00733>.
- 551 [56] Carbonaro D, Putame G, Castaldo C, Meglio F Di, Belviso I, Sacco AM, et al. A Novel 3D-
552 Printed Sample-Holder for Agitation-Based Decellularization - Human Cardiac Tissue
553 Application. Proceeding “25th Congr. Eur. Soc. Biomech. (ESB 2019),” Vienna (A), 7-10
554 July: 2019, p. 546.
- 555 [57] Di Meglio F, Nurzynska D, Romano V, Miraglia R, Belviso I, Sacco AM, et al. Optimization
556 of Human Myocardium Decellularization Method for the Construction of Implantable
557 Patches. *Tissue Eng Part C Methods* 2017;23:525–39.
558 <https://doi.org/10.1089/ten.tec.2017.0267>.
- 559 [58] Belviso I, Romano V, Sacco AM, Ricci G, Massai D, Cammarota M, et al. Decellularized
560 human dermal matrix as a biological scaffold for cardiac repair and regeneration. *Front*
561 *Bioeng Biotechnol* 2020;In Press. <https://doi.org/10.3389/fbioe.2020.00229>.
- 562 [59] Nurzynska D, Di Meglio F, Romano V, Miraglia R, Sacco AM, Latino F, et al. Cardiac
563 primitive cells become committed to a cardiac fate in adult human heart with chronic
564 ischemic disease but fail to acquire mature phenotype: genetic and phenotypic study. *Basic*
565 *Res Cardiol* 2013;108:320. <https://doi.org/10.1007/s00395-012-0320-2>.
- 566 [60] Schneck D. An outline of cardiovascular structure and function. In: Bronzino J, Peterson D,
567 editors. *Biomed. Eng. Handb. Four Vol. Set*, CRC Press; 2018.
- 568 [61] Klingensmith M, Ern Chen L, Glasgow S, Goers T, Melby S. *The Washington Manual of*
569 *Surgery*. 2008.
- 570 [62] Kuznetsova T, Herbots L, Richart T, D’hooge J, Thijs L, Fagard RH, et al. Left ventricular
571 strain and strain rate in a general population. *Eur Heart J* 2008;29:2014–23.
572 <https://doi.org/10.1093/eurheartj/ehn280>.
- 573 [63] Kensah G, Roa Lara A, Dahlmann J, Zweigerdt R, Schwanke K, Hegermann J, et al. Murine
574 and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in
575 vitro. *Eur Heart J* 2013;34:1134–46. <https://doi.org/10.1093/eurheartj/ehs349>.

- 576 [64] Sacco AM, Belviso I, Romano V, Carfora A, Schonauer F, Nurzynska D, et al. Diversity of
577 dermal fibroblasts as major determinant of variability in cell reprogramming. *J Cell Mol Med*
578 2019;23:4256–68. <https://doi.org/10.1111/jcmm.14316>.
- 579 [65] Cerino G, Gaudiello E, Grussenmeyer T, Melly L, Massai D, Banfi A, et al. Three
580 dimensional multi-cellular muscle-like tissue engineering in perfusion-based bioreactors.
581 *Biotechnol Bioeng* 2016;113:226–36. <https://doi.org/10.1002/bit.25688>.
- 582 [66] Schmid J, Schwarz S, Meier-Staude R, Sudhop S, Clausen-Schaumann H, Schieker M, et al.
583 A Perfusion Bioreactor System for Cell Seeding and Oxygen-Controlled Cultivation of
584 Three-Dimensional Cell Cultures. *Tissue Eng Part C Methods* 2018;24:585–95.
585 <https://doi.org/10.1089/ten.tec.2018.0204>.
- 586 [67] Piola M, Prandi F, Bono N, Soncini M, Penza E, Agrifoglio M, et al. A compact and
587 automated ex vivo vessel culture system for the pulsatile pressure conditioning of human
588 saphenous veins. *J Tissue Eng Regen Med* 2016;10:E204–15.
589 <https://doi.org/10.1002/term.1798>.
- 590 [68] Chen CS. Mechanotransduction - a field pulling together? *J Cell Sci* 2008;121:3285–92.
591 <https://doi.org/10.1242/jcs.023507>.
- 592 [69] Paluch EK, Nelson CM, Biais N, Fabry B, Moeller J, Pruitt BL, et al. Mechanotransduction:
593 use the force(s). *BMC Biol* 2015;13:47. <https://doi.org/10.1186/s12915-015-0150-4>.
- 594 [70] Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix
595 homeostasis. *Nat Rev Mol Cell Biol* 2014;15:802–12. <https://doi.org/10.1038/nrm3896>.
- 596 [71] Martino F, Perestrelo AR, Vinarský V, Pagliari S, Forte G. Cellular Mechanotransduction:
597 From Tension to Function. *Front Physiol* 2018;9:1–21.
598 <https://doi.org/10.3389/fphys.2018.00824>.
- 599 [72] Massai D, Cerino G, Gallo D, Pennella F, Deriu M, Rodriguez A, et al. Bioreactors as
600 Engineering Support to Treat Cardiac Muscle and Vascular Disease. *J Healthc Eng*
601 2013;4:329–70. <https://doi.org/10.1260/2040-2295.4.3.329>.
- 602 [73] Liaw NY, Zimmermann W. Mechanical stimulation in the engineering of heart muscle. *Adv*
603 *Drug Deliv Rev* 2016;96:156–60. <https://doi.org/10.1016/j.addr.2015.09.001>.
- 604 [74] Parsa H, Ronaldson K, Vunjak-Novakovic G. Bioengineering methods for myocardial
605 regeneration. *Adv Drug Deliv Rev* 2016;96:195–202.

606 <https://doi.org/10.1016/j.addr.2015.06.012>.

607 [75] Stoppel WL, Kaplan DL, Black LD. Electrical and mechanical stimulation of cardiac cells
608 and tissue constructs. *Adv Drug Deliv Rev* 2016;96:135–55.
609 <https://doi.org/10.1016/j.addr.2015.07.009>.

610 [76] Paez- Mayorga J, Hernández- Vargas G, Ruiz- Esparza GU, Iqbal HMN, Wang X, Zhang
611 YS, et al. Bioreactors for Cardiac Tissue Engineering. *Adv Healthc Mater* 2019;8:1701504.
612 <https://doi.org/10.1002/adhm.201701504>.

613 [77] Gabetti S, Putame G, Montrone F, Isu G, Marsano A, Audenino A, et al. Versatile electrical
614 stimulator for providing cardiac-like electrical impulses in vitro. *Biomed Sci Eng* 2020;3.
615 <https://doi.org/10.4081/bse.2019.111>.

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617