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2 and functionality of rat islets of Langerhans
3

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Abstract

Organ-on-chip technology is a promising tool for investigating physiological *in vitro* responses in drug screening development, and in advanced disease models. Within this framework, we investigated the behavior of rat islets of Langerhans in an organ-on-chip model. The islets were trapped by sedimentation in a biochip with a microstructure based on microwells, and perfused for 5 days of culture. The live/dead assay confirmed the viability of the islets in the biochips cultures. The microfluidic culture leads to upregulation of mRNA levels of important pancreatic islet genes: *Ins1*, *App*, *Insr*, *Gcgr*, *Reg3a* and *Neurod*. Furthermore, insulin and glucagon secretion were higher in the biochips compared to the Petri conditions after 5 days of culture. We also confirmed glucose-induced insulin secretion in biochips *via* high and low glucose stimulations leading to high/low insulin secretion. The high responsiveness of the pancreatic islets to a drug treatment (glucagon-like peptide 1) in the biochips was reflected by the upregulation of mRNA levels of *Gcgr*, *Reg3a*, *Neurog3*, *Ins1*, *Ins2*, *Stt* and *Glp-1r* and by increased insulin secretion. The results obtained highlighted the functionality of the islets in the biochips and illustrated the potential of our pancreas-on-chip model for future pancreatic disease modeling and anti-diabetic drugs screening.

Keywords: pancreas, islets of Langerhans, microfluidic biochips, glucose homeostasis, glucagon, insulin

75

76 **Introduction**

77

78 The pancreas is a gland organ that plays a key role in endocrine regulation.
79 The functional units of the endocrine system are the islets of Langerhans. These
80 islets are clusters of cells whose size varies between 20 and 500 μm , with five
81 different cell types: α , β , δ , ϵ , and γ cells (Jouvet and Estall, 2017; Kumar and Melton,
82 2003). The most abundant cells are the glucagon-producing α cells and insulin-
83 producing β cells. Blood glucose levels are obtained through the interaction of two
84 antagonistic hormones secreted by pancreatic α and β cells. During a period of
85 starvation, when glucose levels are low, glucagon is released by alpha cells to
86 promote glycogenolysis and gluconeogenesis in the liver in coordination with cortisol
87 (a hormone secreted by the adrenal gland). In contrast, insulin secretion from β cells
88 is stimulated by elevated glucose levels and activates glycogenesis in the liver and
89 glucose uptake by muscles and adipose tissues, thereby decreasing postprandial
90 blood sugar levels (Baker 2016; Jellali et al., 2020).

91

92 Diabetes mellitus is the most significant endocrine system dysfunction in the
93 pancreas. According to the latest estimates by the International Diabetes Federation
94 (IDF), 1 in 11 adults are living with diabetes in 2019 (463 million people worldwide,
95 IDF official web site, 2019). The incidence of diabetes is increasing dramatically, and
96 is predicted to reach more than 700 million people by 2045 (IDF official web site,
97 2019). Type 1 diabetes mellitus (T1DM) is an autoimmune disorder which leads to
98 the destruction of insulin-secreting β cells, resulting a lack of insulin production (Jellali
99 et al., 2020; Rogal et al., 2019). This pathology affects about 10% of diabetic patients,
100 mostly the young population (Aghamaleki et al., 2019; IDF official web site, 2019).
101 The daily insulin administration is currently the most common treatment of T1DM
102 (King and Bowe 2016). Type 2 diabetes mellitus (T2DM) is the most prevalent form
103 of diabetes, accounting for 90% of all adult diabetic patients, i.e. 20-79 years old
104 (Sardu et al., 2019; IDF official web site, 2019). T2DM is a metabolic disorder
105 characterized by insulin resistance coupled to impaired insulin secretion from the
106 pancreatic β cells (Jun et al., 2019; Zbinden et al., 2020). Insulin resistance is the
107 organs incapacity to respond properly to normal insulin levels (mainly the liver,
108 muscles and adipose tissue, Rogal et al., 2019). The treatment of T2DM involves

109 Lifestyle adjustments and drug therapy such as metformin, sulphonylureas,
110 glitazones and GLP-1 (King and Bowe 2016; Zbinden et al., 2020).

111

112 Nowadays, Diabetes is one of the most prevalent chronic diseases (Silva et al.,
113 2018). This raises a need for developing pancreatic models to increase knowledge of
114 the underlying mechanisms of diabetes and to screen and identify new anti-diabetic
115 drugs. Pancreatic disease modeling and pertinent models for pancreatic drug
116 screening involve considering the pancreas and its interaction with other organs,
117 such as the liver, muscle, adipose tissues, kidney, (Rogal et al., 2019) . This is the
118 reason why the disease models involve transgenic and knockout animals (King and
119 Bowe 2016). However, animal experimentation is ethically controversial, and often,
120 the animal models developed in other species lose relevance when extrapolating the
121 results to humans (Ghaemmaghami et al., 2012; Merlier et al., 2017; Rogal et al.,
122 2019). Concerning *in vitro* models, the cell cultures used for drug screening and
123 biomarker discovery are mainly performed in static 2D cultures using conventional
124 Petri dishes or multi-well plates. Although these models have significantly contributed
125 to medical research and drugs screening, they present certain limitations. Today, it
126 seems clear that 2D cultures are poorly representative of human *in vivo* physiology,
127 metabolism and toxicity, due to the physiological gap between the cells cultivated in
128 static 2D mode and human cells as they exist in their native state (3D, dynamic
129 mode) and the lack of physiological integration between cells and organs (Merlier et
130 al., 2017). As a result, it is essential to improve these basic plate cultures to
131 understand and model metabolic processes and human disorders. This is why many
132 groups are developing tissue-engineering and 3D culture processes in order to
133 provide a more appropriate micro-environment for tissue maintenance and
134 development. This environment must reproduce, as closely as possible, the
135 characteristics found *in vivo*.

136

137 The organ-on-chip approach is one way of mimicking organ physiology to help
138 in the development of therapeutic solutions and pharmacological studies (Huh et al.,
139 2012; Bathia and Ingber 2014). This approach has many advantages to reproduce
140 the characteristics of physiological microenvironments, including three-dimensional
141 architectures, cell-cell interactions and dynamic flow that ensures the transport and
142 exchange of culture medium, hormones, metabolic waste and other chemicals

143 (merlier et al., 2017; Lee et al., 2018; Zbinden et al., 2020). Unlike the static cultures
144 in Petri dishes, organ-on-chip approach provides the possibility to perform co-cultures,
145 where the different cell types can be cultivated in separated microreactors and the
146 cell-cell interactions are ensured by soluble factors exchange (Merlier et al., 2107).
147 The co-culture of two or more organ is a promising tool to study multi-organs
148 diseases such as T2DM (Rogal et al., 2019). In the last years, organ-on-chip
149 technology has been used to reproduce pancreas *in vitro* models (Schulze et al.,
150 2017; Li et al., 2017; Brooks et al., 2016; Lee et al; 2018; Zbinden et al., 2020). A
151 literature review highlighted that most of the current microfluidic platforms have been
152 designed mainly for islet quality assessment for subsequent *in vivo* implantation
153 (Rogal et al 2019). In parallel, some recent works have also demonstrated the
154 potential of organ-on-chip technology for more complex pancreatic physiopathology
155 analysis (Bauer et al., 2017; Zbinden et al., 2019).

156

157 In this work, we developed a simple microfluidic biochip for assessment of
158 pancreatic islet function and long-term cultures. We also presented and
159 demonstrated that the islets functionality was maintained in microfluidic biochips
160 when compared to conventional islet cultures inside polystyrene (TCPS) dishes.

161

162 **2. Material and methods**

163

164 **2.1 Device design and fabrication**

165

166 The microfluidic biochip consists of a cell culture chamber manufactured with
167 two polydimethylsiloxane (PDMS) layers (Fig. 1A). The micro-structured bottom layer,
168 used for trapping the islets, is composed of 600 micro-wells of 400 μm in diameter
169 (depth of 300 μm), and spaced 50 μm apart (Fig. 1B). The second PDMS layer, with
170 a reservoir (depth of 100 μm), is placed on top of the first layer and includes an inlet
171 and outlet for culture medium perfusion. A micro-channel network placed at the inlet
172 and outlet of each layer ensures homogeneous culture medium distribution within the
173 biochip (Fig. 1A).

174

175 The PDMS biochip was manufactured using the replica molding process. First,
176 photolithography was performed to create the mold masters of the bottom and top

177 layer of the biochips using SU-8 photosensitive resin. Then, the PDMS prepolymer
178 (in a mixture of 10:1 base polymer: curing agent; Sylgard 184, Dow Corning) was
179 poured on to the SU-8 master and cured for 2 h at 75°C. The surfaces of the PDMS
180 layers obtained were activated with reactive air plasma (1 min; Harrick Scientific) and
181 brought together immediately to form an irreversible seal.

182

183 **2.2 Pancreatic islets isolation**

184

185 Islets of Langerhans were isolated from male Wistar rats (8–9 weeks old, 200–
186 300 g) (CLEA Japan, Inc, Tokyo, Japan) following a slight modification to the protocol
187 described by Yonekawa et al. (2006) and Kiba et al. (2013). All animal
188 experimentation procedures were carried out in accordance with the guidelines of the
189 University of Tokyo and the Japanese Ministry of Education.

190

191 The rats were anesthetized with isoflurane inhalation solution (Pfizer). After
192 clamping of all irrigation blood vessels, the enzymatic solution (Liberase™ TL by
193 Roche) was injected through the bile duct, previously identified and clamped. After
194 the pancreatectomy, there was selective chemical digestion of the organ at 37°C for
195 30 min with Liberase TL/ ET-K solution (ET-Kyoto solution, Otsuka Pharmaceutical).
196 The digestion was followed by washing and purification steps using a discontinuous
197 OptiPrep® (Sigma-Aldrich) density gradient. The islets of Langerhans were then
198 identified, individually hand-picked with a Pasteur pipette under a stereomicroscope
199 (Leica S9 D), and transferred to a cold preservation solution made of UW solution
200 (University of Wisconsin, Kneteman et al., 1990) complemented with Miraclid
201 (Mochika pharmacy, Japan) and heparin (Mochika pharmacy, Japan). After
202 assessing and counting the islets, the tissue was stored at 4 °C until starting the
203 culture in order to maintain full functional properties as shown in Kimura et al. (2013).

204

205 **2.3 Pancreas on chip and Petri culture**

206

207 The biochips and perfusion circuits (silicone/Teflon tubing and bubble trap)
208 were sterilized by autoclaving and dried in an oven. The biochips were then
209 assembled with the perfusion system and filled with culture medium in order to
210 remove air bubbles and moisturize the circuits. The bubble trap was used as a

211 reservoir interconnected to the biochips by the silicone/Teflon tubing of 0.65 mm in
212 diameter. The preconditioning process was carried out for one hour at 37° C in the
213 incubator. The entire setup is presented in Fig.S1 (supplementary file).

214

215 The pancreatic islets in the preservation solution were washed with cold
216 culture medium and gently diluted in the appropriate amounts in order to ensure fair
217 and even distribution of the tissue in the biochips and Petri culture. The estimated
218 number of islets per biochip or well is ≈ 40 . In order to minimize damage to the islets,
219 wide orifice pipette tips with low binding were used throughout the handling process.
220 Once the islets were loaded in the biochips from the inlet port or seeded in the 24-
221 well plate, the counting step took place under the microscope in order to keep a
222 record of the islets per biochip and/or well. The cultures were continuously
223 maintained at 37 °C in a 5% CO₂ supplied incubator.

224

225 Two groups of study (biochip and Petri) and 4 conditions were established:
226 Petri control; Petri with GLP-1; biochip control and biochip with GLP-1. The basal
227 culture medium used in our study were the classic RPMI 1640 Medium (Gibco, 2.5
228 mM of glucose) supplemented with 10% FBS (Gibco), 100 units/mL of penicillin,
229 100 mg/mL streptomycin (Gibco) and GlutaMAX™ (Gibco™) at 10 mM. For the
230 hormone-stimulated media, GLP-1 (Peprtech, USA) was added for a final
231 concentration of 100 nM. The biochips were perfused in a circuit loop containing ≈ 2
232 ml of culture medium with a peristaltic pump (flow rate of 20 μ L/min). The medium
233 was renewed every 2 days. In static conditions, the islets were seeded in 1 mL of
234 medium/well and the culture medium was exchanged every day.

235

236 **2.4 Islets viability**

237

238 At the end of the experiment, the islets were incubated in a solution of
239 propidium iodide (PI) at 4.5 μ mol/L and calcein-AM at 2 μ mol/L (Cellstain kit, Dojingo)
240 in RPMI 1640 medium for 30 min in the dark. Then, the samples were washed with
241 RPMI 1640 medium and observed under an epifluorescence microscope (Olympus,
242 Japan). The size of necrotic core was quantified by ImageJ software (NIH, Bethesda,
243 Maryland) using the collected images. The area of the cells stained with PI was
244 measured and normalized by the islet area.

245 **2.5 RTqPCR assays**

246

247 Total RNAs were extracted and purified from samples using a hybrid protocol
248 that combines Trizol™ Reagent (Life Technologies) and RNeasy Mini Kit (QIAGEN
249 74104) following the manufacturer's instructions. The concentrations and qualities of
250 the RNAs extracted were assessed using a BioSpec-nano (Shimadzu Scientific
251 Instruments). Reverse-transcription into cDNA was performed from 0.5 µg of total
252 RNA using the ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO).
253 Real-time quantitative PCR was then performed with the THUNDERBIRD SYBR
254 qPCR Mix (TOYOBO) according to the manufacturer's protocol and a StepOnePlus
255 Real-Time PCR system (Applied Biosystems). The primer sequences of the genes
256 are shown in Table S1. β-Actin was used as the reference gene and fresh isolated
257 islets (days 0) as the reference sample for the normalization of gene expression data.

258

259 **2.6 Immunostaining**

260

261 Immunofluorescent detection was investigated at the end of the experiment
262 (day 5). We selected to stain the GCK because the coding protein glucokinase is a
263 key enzyme using glucose as substrate. We also stained two important markers in
264 the pancreatic islets: insulin and glucagon that are markers of β-cells and α-cells,
265 respectively.

266

267 After transfer to an untreated TCPS 24-well plate (not a plate treated for cell
268 cultures, to prevent the islet cells from attaching to the plate), the islets were washed
269 with phosphate buffer saline solution (PBS) and fixed in paraformaldehyde 4% at 4°C
270 for 24 hours. In order to perform the immunostaining in a 3D structure, the islets were
271 permeabilized with 1% Triton X100 in PBS for 3 hours at 4°C and washed 3 times
272 with PBS for 30 min. Then, the islets were blocked with a gelatin buffer for 24 hours
273 at 4°C. Primary antibodies (anti-insulin [ab6995, Abcam], anti-glucagon [ab167078,
274 Abcam] and anti-glucokinase [sc-17819, Santa Cruz]) were incubated for 48 hours at
275 4°C in a BSA/PBS solution. Secondary antibodies coupled with Alexa Fluor
276 fluorochromes (Glucagon: anti-rabbit Alexa Fluor 680 [A-21109, ThermoFisher],
277 Insulin: anti-mouse Alexa Fluor 594 [ab150116, Abcam] and Glucokinase GCK: anti-
278 mouse Alexa Fluor 647 [ab150107, Abcam]) were further incubated overnight in a

279 BSA/PBS solution at 4°C in the dark. Finally, the nuclei were stained with DAPI (342-
280 07431, Dojindo) at 1/1000 for 30 min at room temperature (RT) in the dark. All the
281 incubations and washing steps were carried out using a shaker. Observations were
282 made with an Olympus IX-81 confocal laser-scanning microscope.

283

284 **2.7 Functional assays**

285

286 **2.7.1. Glucose-stimulated insulin secretion (GSIS) assays**

287

288 After 4 days of culture, we performed a low high glucose stimulation. In
289 biochips, the culture medium was removed from the bubble trap and the circuit
290 (biochip, tubing and bubble trap) was washed with a 0-glucose solution (D-MEM, No
291 Glucose, Wako) for 2 hours. This washing 0-glucose solution was removed from the
292 bubble trap, and 1 mL of fresh 0-glucose was added and perfused for 2 hours. After
293 this low glucose perfusion, the islets were exposed to a high glucose culture medium
294 for 2 hours (D-MEM high Glucose, Wako, 25 mM of glucose). For that purpose, the
295 low glucose solution was removed from the bubble trap and replaced with 1 mL of
296 high glucose. In a Petri dish, this protocol led to 2h of 0-glucose exposure, followed
297 by another 2h of 0-glucose exposure and finally 2h of high glucose stimulation. At the
298 end of the assays, basal media were re-established for all conditions.

299

300 **2.7.2. GLP-1 stimulations**

301

302 In order to test the response of the islets to drug stimulations, we exposed the
303 cultures to GLP-1. For that purpose, we followed the same protocols described in
304 section 2.3, to which we added 100 nM of GLP-1 at each culture medium change
305 step.

306

307 **2.8 Insulin, glucagon and C-peptide measurements**

308

309 The hormones released into the culture medium were assessed using ELISA
310 assays, following the manufacturer's protocol. To measure each hormone
311 concentration, we used a rat Insulin ELISA kit (10-1250-01; Mercodia) for insulin, a
312 Glucagon DuoSet ELISA kit (DY1249; R&D Systems) with the DuoSet ELISA

313 Ancillary Reagent Kit 2 (DY008; R&D Systems) for glucagon, and a rat C-Peptide
314 ELISA kit (10-1172-01; Mercodia) for C-peptide.

315

316 **2.9 Glucose and lactate quantification**

317

318 Glucose and lactate were measured using an YSI 2950 Biochemistry Analyzer.
319 For that purpose, 150 μ L of culture medium were inserted into the analyzer.
320 Measurements were based on a direct reading of L-lactate (L-lactic acid) and glucose
321 in the culture medium by the YSI enzyme sensors, as the enzymes L-lactate oxidase,
322 and glucose oxidase are respectively immobilized in the lactate and glucose sensors.

323

324 **2.10 Statistics**

325

326 The rat islets were used in 6 to 12 biochips according to the experiments. The
327 RTqPCR were performed on three samples for each condition in three independent
328 experiments (n=3x3). The measurements of glucose, lactate, insulin, C-peptide and
329 glucagon were also performed in triplicate. The Kruskal Wallis test was performed for
330 the statistical analysis.

331

332 **3. Results**

333

334 **3.1 Characterization of islet biochip cultures when compared to Petri dish** 335 **cultures**

336

337 **3.1.1 Viability assay showed highly viable islets in the biochip**

338

339 After extraction (Fig. 2A), the islets were cultivated in Petri dishes (Fig. 2B)
340 and in biochip dynamic culture for 5 days (Fig. 2C). In the device, the islets
341 sedimented into the microwells located at the bottom of the culture chamber. Then,
342 we confirmed that the islets had not been washed away by the flow rate after 5 days
343 of perfusion. Indeed, the number of islets seeded at the beginning of the experiments
344 and collected at the end of the perfusion remained similar, as shown in Fig. 2D. In
345 addition, the islets presented a round shape at the end of the perfusion (Fig. 2B, 2C)

346 and Fig.S2, supplementary file). The typical size of the islets was about 150 ± 50
347 microns.

348

349 The fluorescent images of islets stained with calcein AM/PI are presented in
350 Fig. 2E. We found a different size to the necrotic core of the islets between static and
351 dynamic conditions after 5 days of culture. Nevertheless, the viability was higher in
352 the islets cultivated in the biochips inasmuch as the IP staining was weaker when
353 compared to Petri situations. The quantification of the necrotic core showed that the
354 proportion of dead cells in dynamic biochip was significantly lower when compared to
355 the Petri culture. The normalized size of necrotic core was of 0.34 ± 0.15 and
356 0.12 ± 0.03 for islets cultivated in Petri and biochip, respectively (Fig.S3,
357 supplementary file).

358

359 **3.1.2 RTqPCR analysis revealed higher mRNA levels of pancreatic islets** 360 **markers in biochip cultures**

361

362 At the end of the experiments, we compared the mRNA levels of the cells in
363 the islets cultivated in biochips and Petri dishes (Fig. 3). The markers related to
364 maintaining islet differentiation, such as *Reg3a* and *Neurod*, were upregulated in the
365 biochips when compared to the Petri cultures (fold change, FC, of 190 and 13,
366 respectively). In addition, the markers related to islet functions such as *App* (4.3 FC),
367 *Ins1* (1.7 FC), *Sst* (2.8 FC) and *Gcg* (2.1 FC) were also upregulated in the biochips.
368 Finally, the levels of several receptors and transporters such as *Gcgr*, *Insr*, and *Glut2*
369 were 3.2 to 5.5 times higher in the biochip compared to the Petri dishes (Fig. 3).

370

371 **3.1.3 Immunostaining confirmed the expression of pancreatic islets markers** 372 **and glucose regulators in both biochips and Petri dishes**

373

374 The immunostaining of the islets, prior to inoculation in the biochips,
375 demonstrated that the islets were positive for insulin and glucagon (day 0, Fig. 4A
376 and Fig. S4 in supplementary file). At the end of the experiments, the islets from the
377 Petri dishes and biochips were positive for insulin and glucagon as shown in Fig. 4A
378 and Fig. S4 (supplementary file). We observed three types of cell populations, insulin
379 positive cells, glucagon positive cells and a third subpopulation expressing both

380 insulin and glucagon (those bihormonal cells may reflect a partial switch from β -cells
381 to α -cells, supplementary file). We confirmed also that the islets in both culture
382 modes were positive for GCK (Fig. 4B and Fig. S5 in supplementary file), consistently
383 with the composition of the culture medium containing 2.5 mM of basal glucose (nb:
384 GCK, in the pancreas, plays a role in glucose-stimulated insulin secretion).

385

386 **3.1.4 Functional assays revealed higher insulin secretion in the islets in the** 387 **biochips**

388

389 The basal functionality of the islets was demonstrated by measuring insulin, C-
390 peptide and glucagon secretion. At the end of each experiment, the islets were
391 counted to normalize the data. We found that the insulin concentration in the culture
392 medium decreased in both biochips and Petri dishes. However, the biochip cultures
393 contributed to maintaining relatively high levels for all experiments until day 4,
394 whereas a drop occur between day 3 and day 4 in Petri (Fig 5A). Biochip cultures
395 contributed to maintaining insulin secretion close to 130 ng/islet/day until day 4, and
396 decreasing to 44 ng/islet/day at day 5. In the Petri dishes, we found a significant
397 reduction in days 3 and 4, leading to measured secretion close to 30 ng/islet/day
398 before dropping to 17 ng/islet/day at day 5. We also observed higher secretion of C-
399 peptide in the biochips when compared to the Petri dish cultures, as shown in Fig 5B.
400 Secretion was about 5 times higher in the biochip from days 3 to 5. C-peptide
401 secretion in Petri dish decreased significantly since day 2, whereas it decreased only
402 at day 5 in the biochips.

403

404 The glucagon levels were higher in the biochips than in the Petri dishes, as
405 shown in Fig. 5C. Furthermore, the levels remained constant in the biochip cultures
406 for 4 days of culture, close to 1000 pg/islet/day, before decreasing to 500 pg/islet/day
407 on day 5. In the Petri dishes, glucagon secretion decreased from day 3 to day 4 and
408 leading to a drop from 560 to 180 pg/islet/day between day 2 to day 5. The ratio
409 between insulin and glucagon secretion is shown in Fig 5D. In the biochips, the ratio
410 increased from 130 to 250 between days 2 and 4, before dropping to 85 on day 5. In
411 the Petri dishes, the ratio decreased continuously from 380 to 80, but with high intra
412 and extra experiment variability.

413

414 Finally, glucose and lactate levels were measured during culture. As the
415 culture medium was frequently changed during the experiments, we observed that
416 glucose levels remained relatively stable and very high in our experiments. Both in
417 the Petri dishes and biochips, we were unable to detect any significant glucose
418 depletion (Fig 5E). Furthermore, lactate production was higher in the biochips than in
419 the Petri dishes (Fig 5E).

420

421 **3.2 Pancreatic islet response to stimulations demonstrated functional and** 422 **active biochip culture conditions**

423

424 **3.2.1 Low-high glucose stimulations**

425

426 After 4 days of culture, we performed a low-high glucose stimulation. The
427 islets demonstrated lower insulin secretion in low glucose stimulation when
428 compared to high glucose stimulation. To evaluate islets responsiveness to low/high
429 glucose stimulation, we calculated the GSIS (glucose-stimulated insulin secretion)
430 index by dividing insulin measured in high-glucose and low-glucose media (Fig. 5F).
431 The GSIS index values were of 5 and 2.1 in the Petri dishes and biochip, respectively.
432 These results indicate that glucose response of islets was maintained in both culture
433 modes. However, we should mention that we observed wide variability in this assay
434 inasmuch as some Petri dishes or biochips were not induced, thus leading to a
435 significant error bar and dispersion.

436

437 **3.2.2 Effect of glucagon-like peptide-1, GLP-1, drug**

438

439 The GLP-1 stimulations contributed to the modification of mRNA levels, both in
440 Petri dishes and biochips (Fig 6A). GLP-1 increased the mRNA levels of the
441 receptors *Glp-1r*, *Gcgr*, and of the *Reg3a*, *Ins1*, *Ins2* and *Stt* genes in the biochips
442 (when compared to non-treated biochip controls) and downregulated *Neurod*. In well
443 plates, we also found upregulation of *Gcgr*, *Insr*, *Ins2*, and downregulation of *Neurod*
444 (Fig. S6, supplementary file). Furthermore, the mRNA levels in the GLP-1-treated
445 biochips were upregulated when compared to GLP-1-treated Petri dishes (data not
446 shown).

447

448 Fig. 6B shows the insulin immunostaining of islets treated by GLP-1. The data
449 demonstrated a high fluorescence intensity in GLP-1-treated Petri and biochip. We
450 also found that the levels of the GCK protein in the islets were not over-expressed by
451 the GLP-1 treatment (Fig. 6B). At the functional levels, insulin secretion was
452 increased in both Petri dishes and biochips by the GLP-1 treatments (Fig 7A). On
453 days 2, 3 and 4, the levels of insulin in the biochips were up to 3 times higher than
454 those attained in control. However, after 5 days, this induction was weaker (2 times).
455 We then performed the low-high glucose stimulation tests after pre-treatment with
456 GLP-1. As expected, the drug did not inhibit the effect of the high / low glucose
457 stimulations and maintained a high level of insulin production in the biochips under
458 high glucose stimulation (Fig 7B). In this assay, the low / high glucose stimulation led
459 to an insulin over production of 2.2 and 4 in biochips and Petri, respectively (GSIS
460 index of 2.24 and 4, respectively). Furthermore, we did not observe variability in the
461 assay when compared to the untreated conditions (cf. Fig 5F).

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Discussion

466 In this study, we investigated the performance of islets of Langerhans in a
467 closed loop microfluidic system. Several investigations have reported microfluidic
468 techniques for keeping islet or spheroid cultures on chip, including the pancreas (Tan
469 and Takeuchi 2007; Zbinden et al., 2019; Lee et al., 2018; Mohammed et al., 2009).
470 Those devices are based on a flow trapping process (Tan and Takeuchi 2007;
471 Zbinden et al., 2019). We built a simple culture biochip where the pancreatic islets
472 can be trapped by sedimentation in the bottom microstructure. This type of trapping
473 was also reported by Lee et al., 2019. In our biochip, the microstructure contributed
474 to create an array of 600 microwells allowing to trap an important quantity of islets.
475 The depth of microwells made it possible to protect the islets from flow circulation
476 and thus prevent them from being washed away and from mechanical damage. As a
477 result, we maintained a constant number of islets between the beginning and the end
478 of the perfusion. Furthermore, due to sedimentation in the microwells, the islets were
479 protected from the fluid shear stresses created by the laminar flow.

480

481 In biochip, we observed higher viability for the cells located in the center of the
482 pancreatic islets when compared to the Petri dish cultures. This was illustrated by the

483 viability assay in which a larger number of dead cells was observed in the center of
484 the islets when they were cultivated in Petri dishes. Central necrosis and apoptosis in
485 pancreas islets are due mainly to the high density of the tissue and the lack of irrigation
486 as has been widely reported in literature (Giuliani et al., 2005, Moritz et al., 2002).
487 Several hypotheses are proposed to explain central necrosis and apoptosis in
488 pancreatic islets (and also in cell spheroids), including deprivation of oxygen,
489 nutrients and serum as a result of limited diffusion (Giuliani et al., 2005). In the case
490 of the pancreatic islets, the blood micro-vessels usually reach the center of the
491 structure *in vivo* but the microvasculature gets closed in *ex vivo* tissue (Jansson et al.
492 2016). In parallel, microfluidic cultures have been shown to improve the viability of
493 spheroid tissue by reducing the central necrotic core thanks to control of local
494 glucose concentrations and local oxygenation through the dissolved oxygen provided
495 by the medium flows (Barisam et al., 2018, Baye et al., 2017). As a result, those
496 literature reports appeared consistent with our findings.

497

498 In our investigation, we found that the microfluidic culture maintained and
499 improved pancreatic regeneration and maturation markers of the rat islet cells. This
500 was illustrated by higher levels of mRNA in important pancreatic islet markers,
501 including receptor genes (*Gcr* and *Insr*), hormone secretion-related genes (*App*, *Sst*
502 and *Ins1*) and differentiation genes (*Neurod* and *Reg3a*). More particularly, we found
503 a 190-fold upregulation of *Reg3a*, which is an islet regeneration marker (Coffey et al.,
504 2014) involved in the pro-islet gene cascade and their protection against induced
505 diabetes mellitus (Xiong et al., 2011). In addition, *Neurod* was 13 times higher in the
506 biochips compared to Petri dish levels. *Neurod* is an important gene (an insulin trans
507 activator) required to maintain functional maturity in pancreatic beta cells, including
508 insulin production through *Ins1* (Gu et al., 2010). We also consistently found
509 upregulation of the *Ins1* gene in the biochips, which is consistent with *Ins1* silencing
510 in *Neurod* KO mice (Gu et al., 2010). *Neurod* KO-mice expressed the *Ins2* gene and
511 were thus able to produce insulin in a glucose stimulation test, which also appeared
512 consistent with our findings in which *Ins2* was commonly expressed in both the Petri
513 dishes and the biochips (Gu et al., 2010).

514

515 In parallel, an over-expression of the insulin receptor *Insr* was observed. *Insr*
516 is involved in controlling glucose-stimulated insulin secretion *via* its relationship with

517 insulin gene expression, *Glut2* and *Pdx1* (Wang et al., 2018). We consistently
518 observed a tendency for higher mRNA levels in the *Glut2* and *Pdx1* genes in the
519 biochips. Upregulation of *Gcgr*, the glucagon receptor, in the biochips also indicated
520 a probable beneficial effect of the microfluidic cultures on the alpha cells inasmuch as
521 the lack of glucagon receptors is correlated with several pancreas disorders and
522 alteration to glucose homeostasis, such as hyperplasia and hyperglucagonemia
523 (Charron et al., 2015).

524

525 At the functional level, we analyzed the performances of the pancreas islets
526 when they were cultivated in the biochips. At first, this was illustrated by the kinetics
527 of the secretion of insulin in the culture medium. Although the overall secretion
528 decreased between the first day and fifth day of culture in both culture conditions, the
529 biochip levels remained higher when compared to those of the Petri dishes. Secondly,
530 the high/low glucose stimulation assay demonstrated that islet response to stimuli
531 was preserved in our biochip culture. However, in the biochips, the protocol required
532 an extended washing process to remove the insulin and remaining glucose from the
533 basal culture medium in the perfusion circuit (mainly due to the dilution of high
534 glucose solution in washing solution that would lead to bias stimulation). This need of
535 washing process in the biochips, when compared to conventional Petri dishes, has
536 already been reported in pancreas-on-chip investigations (Zbinden et al., 2019).
537 Nevertheless, enhancements of basal islets functions such as insulin secretion and
538 glucose-induced insulin secretion in microfluidic devices have been observed
539 consistently in the literature (Sankar et al., 2011). In our dataset, this over-secretion
540 of insulin was not correlated with over-expression of *Gck*, as demonstrated by the
541 immunostaining. The glucose-stimulated insulin secretion was regulated by the rate
542 of glucose metabolism within β cells, and a key event in this process is the
543 phosphorylation of glucose by glucokinase (the coding gene is *Gck*, Wu et al.,
544 2004). Although we worked in a closed loop perfusion circuit, the flow rate led to
545 continuously renewing the culture medium locally in the biochips. Furthermore, the
546 level of glucose remained high in the medium, probably due to the number of islets.
547 The microfluidic culture completely modulated the chemical cellular
548 microenvironment (*via* a complex gradient of molecules, a balance between diffusion
549 and convection, cellular consumption) leading to complex signals (Young et al., 2010,
550 Halldorson et al., 2015). In parallel, the microwells almost certainly influenced the

551 local nutrient islets' microenvironment (a balance between glucose consumption,
552 glucose renewal, secreted insulin concentration, etc...). As a result, additional local
553 measurements (*via* integrated sensors) and numerical simulations are needed to
554 understand the complex kinetics of insulin secretion in relation to local glucose
555 concentrations in our biochips.

556

557 Finally, we tested the effect of the glucagon-like peptide-1 (GLP-1) drug in
558 both culture conditions. GLP-1 physiologically induces glucose-dependent insulin
559 secretion from β -cells and GLP-1 analogues improve hyperglycemia in T2D patients
560 (Drucker et al., 2006; DeFronzo et al., 2005, Vilsbol et al., 2007). Furthermore, GLP-1
561 treatment increases *Gck* activity (Ding et al., 2011). However, long term GLP-1
562 exposure (about 18h) also resulted in the promotion of the metabolic reprogramming
563 of β -cells through mTOR-dependent HIF signaling, and independently of *Gck* post-
564 translational activation (Carlessi et al., 2017). In the present study, GLP-1 stimulation
565 clearly upregulated several pancreatic islet genes such as *Glp-1r* and *Gcgr*, in both
566 Petri dishes and biochips. In addition, those mRNA levels were upregulated in the
567 biochips compared to the Petri dishes when the GLP-1 was loaded. At the functional
568 level, GLP-1 induced an increase in insulin secretion in both culture modes (as
569 confirmed by the immunostaining of insulin and the insulin extracellular
570 concentrations in the medium). The secretion levels in the culture medium were
571 found to be higher in the biochips. However, we did not find any of the inhibitory
572 effect we expected in the alpha cells of the islets of Langerhans (glucagon secretion)
573 (Tudori et al. 2016). In our biochips, we found mRNA *Glut2* over-expression
574 compared to the Petri dishes, leading to the suspicion of higher glucose transport,
575 and thus metabolism, in the islets. We did not find an effect of GLP-1 on the *Gck* at
576 the immunostaining fluorescent level. Although these findings seems consistent with
577 the observations made by Carlessi et al., 2017, more extensive investigations are
578 needed to confirm the underlying mechanism in biochips.

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582

583 **Conclusion**

584

585 In this work, we studied the behavior of rat islets of Langerhans when cultivated in
586 microfluidic biochips or in Petri dishes. The microfluidic biochips cultures maintained
587 high islet viability throughout the 5 days of culture. More particularly, several
588 important pancreatic islet genes, including *Reg2a*, *Neurod*, *Insr*, *Gcgr*, *Glut2*, *Ins1*,
589 *App* and *Stt* were overexpressed in biochips cultures (compared to islets cultivated in
590 Petri). The islets were able to secrete insulin and glucagon, as well as to respond to
591 GLP-1 drug stimulation and high-low glucose test. Furthermore, the levels of insulin
592 secretion appeared higher in biochips when compared to the Petri dishes. Our
593 dataset illustrated the fact that the microfluidic culture is beneficial for maintaining *in*
594 *vitro* maturation and functionality of islets of Langerhans. We believe that our results
595 are encouraging for the development of functional pancreas *in vitro* models using the
596 advantages of organ-on-chip technology.

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598

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600

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