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4 5 **An easy 3D printing approach to manufacture Vertical Diffusion Cells for *in*** 6 ***vitro* release and permeation studies**

7
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15 16 **ABSTRACT**

17 Vertical diffusion cells are utilized in the pharmaceutical and cosmetic fields to study the release and
18 permeation of active ingredients through polymeric or biological membranes. Nevertheless, the
19 commercially available glass-based systems are expensive and need to be carefully handled due to
20 their fragility. Fusion deposition modeling 3D printing is an additive manufacturing technique that
21 allows producing objects by printing layer over layer different thermoplastic materials. Among them,
22 polypropylene is a robust, flexible, and chemically inert polymer that can resist to many organic
23 solvents and to heating. In this work, we designed and printed a vertical diffusion cell following
24 pharmacopeia requirements by using polypropylene in a fused deposition modeling 3D printer. The
25 model was developed to fit in a heating block to avoid the use of warm water recirculating system.
26 The vertical diffusion cells were leak-free and presented chemical resistance and no interaction with
27 the tested molecules (*i.e.*, caffeine, diclofenac sodium, and glycyrrhetic acid). The 3D printed cells
28 were compared to commercially available glass cells and then two different types of synthetic
29 membranes (*i.e.*, PDMS and Strat-M[®]) were used to evaluate the permeation of a caffeine hydrogel.
30 The developed 3D printed testing system could represent an efficient alternative to the glass-based
31 equipment.

32
33 **Keywords:** 3DP; fusion deposition modeling (FDM); Franz cells; VDCs; polypropylene (PP);
34 polymeric membranes.

36 1. INTRODUCTION

37 During the last few years, 3D printing (3DP) continued to grow as an innovative additive
38 manufacturing (AM) technology with applications in many different areas including pharma
39 (Melocchi et al., 2020; Ngo et al., 2018). Recently, many pharmaceutical applications have been
40 published in the literature reporting the production of dosage forms (i.e., tablets, capsules,
41 suppositories, and vaginal rings), testing systems (i.e., ocular, nasal, and respiratory models), and
42 manufacturing devices (i.e., microfluidic chips) (Lim et al., 2018; Mathew et al., 2020; Tiboni et al.,
43 2020; Trenfield et al., 2020). Among the 3D printing techniques, fused deposition modeling (FDM)
44 presents several advantages including relatively inexpensive printers and materials, low maintenance
45 costs, a large selection of commercially available materials, the ease of initial use, and the ability to
46 start, stop, and integrate complexity on the fly (Romanov et al., 2018).

47 Taking advantage of this technology, the acronym DIY (Do-It-Yourself) is gaining attention over
48 research laboratories. The additive manufacturing techniques allow researchers to develop and
49 produce almost any kind of object needed in the laboratory from the simplest to even more complex
50 ones with a real decrease in costs (Boparai et al., 2016; Capel et al., 2018).

51 In the pharmaceutical and cosmetic fields, vertical diffusion cells (VDCs or Franz cells) are routinely
52 used for the study and analysis of both release and permeation of active molecules from different
53 formulations through the use of polymeric and biological membrane (Marques et al., 2009). These
54 kinds of studies are important since they can determine the feasibility of delivering the cargo to and
55 through the skin (Johal et al., 2016).

56 Conventional VDCs are typically manufactured from glass and they can be found in the market in
57 many shapes, sizes and may be modified depending on the required experimental conditions.
58 According to United States Pharmacopeia (USP, www.uspnf.com, Topical and transdermal drug
59 products), the VDC assembly consists of two chambers (donor and receptor), separated by a
60 membrane. Commonly, this system is used for testing the *in vitro* release rate of topical drug products
61 such as creams, gels, and ointments. Even alternative diffusion cells that conform to the same general
62 design can be used and can be made from any materials that do not react with or absorb the test
63 product or samples. Commercial VDCs are commonly made from borosilicate glass that results
64 fragile and require careful handling during their utilization.

65 Only one 3D printing approach was considered in the literature to produce VDCs using
66 stereolithography (SLA) (Sil et al., 2020). This additive manufacturing technique requires the
67 utilization of acrylate-based resins which are photopolymerized during the printing procedure and
68 then they need to be post-cured to obtain the final object. Moreover, the type of resin utilized

69 presented physical and chemical interactions with the tested drug, requiring a plastic coating to avoid
70 these problems.

71 FDM, in comparison to SLA, is easier to use, it has lower overall production costs and it does not
72 need post-curing after printing. Another important aspect to consider is that the selection of
73 thermoplastic FDM printing materials is very wide and the most appropriate one can be chosen
74 depending on the needs.

75 In this work, we developed an alternative 3DP vertical diffusion cell using polypropylene (PP) as
76 manufacturing material in a FDM printer. This material was selected since it is a robust, flexible, and
77 chemically inert polymer that can resist to many organic solvents and to heating (Price et al., 2020).
78 The 3DP VDCs were tested for leaking and then were compared to glass VDCs to evaluate the
79 potential applicability.

80 In the *in vitro* permeability studies, different membranes can be used including human skin, animal
81 skin as well as polymeric membranes. However, biological membranes have limitations such as cost
82 and availability of human skin and ethical consideration for the use of animal skins. Besides,
83 compared to synthetic membranes, biological models exhibit high variability that complicates the
84 experimental design, statistical significance, and number of replicates required (Haq et al., 2018a).
85 Moreover, biological models possess a short half-life, special storage requirements, higher costs, and
86 safety issues (Haq et al., 2018b).

87 For our work, we selected skin-mimicking membranes (Strat-M[®]) which comprise two layers of
88 polyethersulfone on top of one layer of polyolefine. These membranes possess a porous structure that
89 imparts additional skin-like properties by creating a gradient across the entire thickness (Uchida et
90 al., 2015).

91 Finally, Strat-M[®] membranes were compared with polydimethylsiloxane (PDMS) membranes (Ng et
92 al., 2012), using a caffeine hydrogel as model formulation since this active is a hydrophilic molecule
93 widely used in topical applications (Herman and Herman, 2012).

94

95 **2. MATERIALS AND METHODS**

96 **2.1 Materials**

97 Neutral polypropylene 3D printing filament was kindly provided from Verbatim (Italy). Caffeine was
98 kindly provided by BASF (Germany), diclofenac sodium was obtained from Farmalabor (Italy),
99 glycyrrhetic acid, and xanthan gum were purchased from A.C.E.F. (Italy). Strat-M[®] membranes
100 were obtained from Merck (Germany), 250 µm thick polydimethylsiloxane (PDMS) membranes were
101 kindly provided by Shielding Solutions Limited (Essex, UK), Spectra/Por[™] 7 Standard RC dialysis

102 membranes (6-8 kDa cut-off) were purchased by Spectrum Labs (USA). All the other solvents used
103 were HPLC grade.

104

105 **2.2 Design and development of the VDCs**

106 The original 3D model project was designed using the free online computer aided design (CAD) tool
107 Tinkercad® (Autodesk, USA). The cell was designed to fit in a heating block (IKA, Germany) used
108 to control the temperature during experiments. The cell is composed of a receptor part in which is
109 present a withdrawal window with its cap, two donor compartments depending on the origin of the
110 formulation, liquid or semisolid, and a stirring block useful to adjust the receptor volume. The 3D
111 printed stirring block presents a slot to insert a magnetic stirring bar. The 3DP VDCs present a
112 receptor compartment volume of 9 or 11.5 mL (with or without stirring block respectively) and an
113 effective diffusion area of 1.583 cm². The files were exported from the online CAD software as STL
114 (Stereolithography interface format) to be then converted into machine language with a computer
115 aided manufacturing (CAM) software (STL files provided in the supplementary material).

116

117 **2.3 Manufacturing process of the 3D printed VDCs**

118 3D-printed PP VDCs were produced via fused deposition modeling (FDM) using an Ultimaker 3
119 printer (Ultimaker, The Netherlands). The VDCs were printed at a print speed of 25 mm/s with a
120 nozzle temperature of 205 °C. The infill density was set at 100 % and the build plate was preheated
121 at 85 °C after the application of a polypropylene adhesion sheet (Ultimaker, The Netherlands). The
122 original STL file was converted to a print pattern using Ultimaker Cura 4.7 software (Ultimaker, The
123 Netherlands). Layer thickness was set to 150 µm enabling the production of leak-free PP VDCs. The
124 3DP VDCs were tested for leaks by filling both compartments with water. The receptor compartment
125 and the donor compartment were ulteriorly sealed with the application of laboratory sealing film. The
126 system was examined for leaks over a minimum of 24 hours and it was considered good if no water
127 was present on the outer wall after this period.

128

129 **2.4 Compatibility studies of the 3D printed VDCs**

130 To assess the compatibility of the VDCs with active compounds, three different model drugs were
131 evaluated, *i.e.*, caffeine (2 mg/mL water solution), diclofenac sodium (2 mg/mL water solution),
132 glycyrrhethinic acid (0.02 mg/mL 50% ethanolic solution). The solutions were prepared and used to
133 fill the receptor compartment of the cell that was then closed using laboratory sealing film and
134 warmed up at 32 °C together with 400 rpm magnetic stirring. After 24 hours, the concentration in the

135 receptor compartment was compared with the initial concentration to confirm that any amount of
136 drug was retained or adsorbed from the cell wall.

137 The amounts of the model drugs were measured by HPLC (1260 Infinity II, Agilent, USA) using a
138 mixture of 0.5% formic acid in water and methanol (ratio 60:40 for caffeine, 30:70 for diclofenac
139 sodium, and 5:95 for glycyrrhetic acid) as mobile phase, with a flow rate of 1 mL/min in an Agilent
140 Zorbax Eclipse Plus C18, 150 x 4.6 mm, 5 µm column (Agilent, USA). The injection volume was 20
141 µL and the detection signals were recorded at 275 nm (caffeine and diclofenac sodium) and 276 nm
142 (glycyrrhetic acid) keeping the analysis system at room temperature.

143

144 **2.5 *In vitro* release comparison: glass vs polypropylene 3D printed VDCs using a caffeine** 145 **hydrogel**

146 A comparison between commercial glass VDCs and 3DP VDCs was performed using a cellulose-
147 based dialysis membrane (6-8 kDa cut-off, Spectra/Por 7 Standard RC Dry Dialysis Tubing,
148 Spectrum Labs, USA). The selected model formulation was a caffeine hydrogel composed of caffeine
149 (5 mg/mL), xanthan gum (0.5% w/v), and water.

150 The glass VDCs (Teledyne Hanson Research, USA) presented a receptor compartment volume of 7
151 mL and an effective diffusion area of 1.766 cm² meanwhile the 3DP VDCs were utilized with the
152 stirring block presenting a receptor volume of 9 mL and an effective diffusion area of 1.583 cm².
153 Water was used as receptor medium in both cell types. The receptor medium was continuously stirred
154 at 400 rpm. The glass system was thermostated at 32 ± 1 °C with a circulating jacket meanwhile the
155 3DP system was thermostated at 32 ± 1 °C with a heating block positioned over a heating plate. The
156 efficacy of heat transfer and temperature control between the heating plate and the receptor medium
157 inside the 3DP cell was previously assessed by measuring the temperature with a thermometer. At
158 predetermined sampling intervals (0.5, 1, 2, 3, 4, 5, 6, and 24 h), samples were withdrawn from the
159 receptor compartment and replaced with an equal volume (0.2 mL) of fresh buffer. The content of
160 the active compound in each sample was then determined by HPLC as reported above. A calibration
161 curve of caffeine was performed with a concentration ranging from 0.01 to 0.5 mg/mL obtaining a
162 correlation coefficient (R²) of 0.9997.

163 The amounts of the active compound released at each time point (AR_{tn}) were obtained using the eq.
164 (1) for the first time point and eq. (2) for the subsequent time points:

165

$$166 \quad AR_{t_1} = \frac{C_{t_1} * 1000 * V_c}{A_o} \quad (1)$$

167

168
$$AR_{t_n} = \frac{C_{t_n} * 1000 * V_c}{A_o} + (AR_{t_{n-1}} * \frac{V_s}{V_c}) \quad (2)$$

169

170 where AR ($\mu\text{g}/\text{cm}^2$) is the amount released at t_n sampling interval, the C_t (mg/mL) is the concentration
171 of caffeine determined at t_n sampling interval, V_c (mL) is the volume of diffusion cell receptor
172 compartment, A_o (cm^2) is the cell diffusion area and V_s (0.2 mL) is the sampling aliquot volume.

173

174 **2.6 *In vitro* permeation studies using 3D printed VDCs with different membrane models**

175 Permeation studies using the PP 3DP VDCs were conducted using a caffeine hydrogel (5 mg/mL,
176 0.5% xanthan gum) applied to two different membranes, *i.e.*, skin mimicking Strat-M[®] membranes
177 and 250 μm thick PDMS membranes. The skin-mimicking Strat-M[®] membranes are composed of
178 two layers of polyethersulfone on top of one layer of polyolefine. These polymeric layers create a
179 porous structure with a gradient across the membrane in terms of pore size and diffusivity. The porous
180 structure is impregnated with a proprietary blend of synthetic lipids, imparting additional skin-like
181 properties to the synthetic membrane (Kaur et al., 2018). PDMS membranes were selected as model
182 membranes, already used in other studies, with a lower permeation compared to dialysis membranes
183 (Ilbasmiş Tamer and Değim, 2007; Jung et al., 2012; Sil et al., 2020).

184 The receptor chambers were filled with water kept continuously stirred at 400 rpm. The system was
185 thermostated at 32 ± 1 °C with a heating block positioned over a heating plate. At predetermined
186 sampling intervals (0.5, 1, 2, 3, 4, 5, 6, 24 h), samples were withdrawn from the receptor compartment
187 and replaced with an equal volume (0.2 mL) of water. The content of caffeine in each sample was
188 determined by HPLC with the method reported above. Equations 1 and 2 were utilized to calculate
189 the amount of active compound released at each time point.

190

191 **2.7 Statistics**

192 The data presented are the mean \pm standard deviation of triplicate measurements and are
193 representative of at least three independent experiments.

194

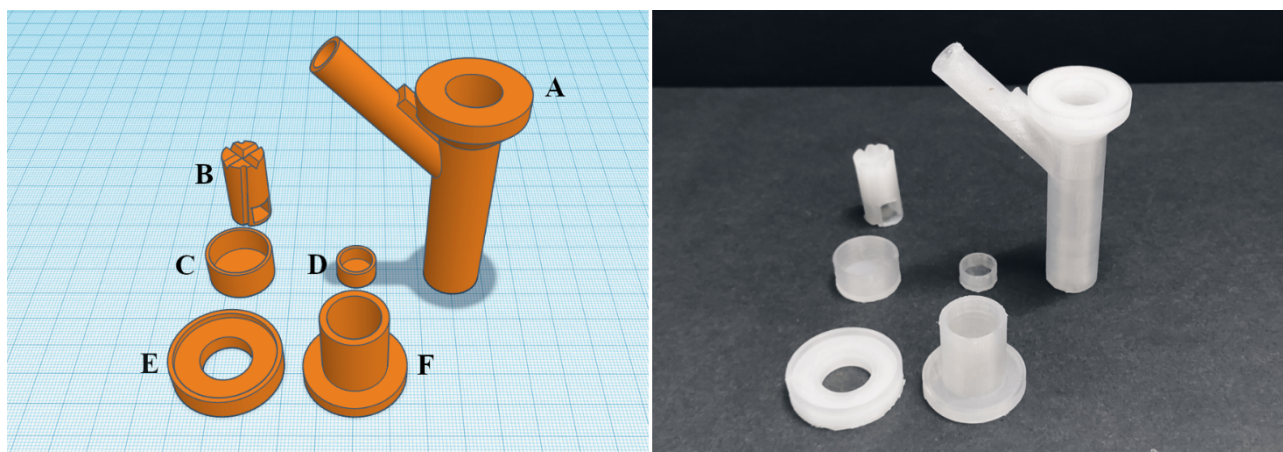
195 **3. RESULTS AND DISCUSSION**

196

197 **3.1 Design, 3D printing, and compatibility studies of the vertical diffusion cells (VDCs)**

198 The CAD design of the 3DP VDCs was developed following the USP guidelines presenting a receptor
199 and a donor compartment (Figure 1).

200



201

202 **Figure 1.** CAD design of the VDC parts and polypropylene 3D printed parts. A) Receptor compartment with withdrawal
203 window; B) Stirring block; C) Cap for donor compartment for liquid formulations; D) Cap for withdrawal window; E)
204 Donor compartment for semisolid formulations; F) Donor compartment for liquid formulations.

205

206 These two sections are separated by a membrane (*e.g.*, synthetic or biological) that allows the
207 permeation of the tested molecule. The material selected to print out the entire system was
208 polypropylene since it is a robust, flexible, and chemically inert polymer.

209 This last property is the most important to meet the pharmacopeia requirements since the cell material
210 does not have to interact chemically and/or physically with the compound analyzed. This is also the
211 reason because VDCs are traditionally made from glass that is a material known for its lack of
212 interaction with active ingredients (Skelly et al., 1987). The drawbacks of this material are mainly its
213 fragility and the high production costs. Taking advantage of FDM 3D printing, it was possible to print
214 a VDC with a low cost and without fragility since PP results robust and flexible. The printed cell
215 resulted semitransparent with the possibility to examine the receptor medium for the presence of air
216 bubbles. The printed layers fusion was evaluated to prevent eventual leakage. The receptor and the
217 donor compartments were filled to the top with water and sealed with laboratory film. After 24 hours
218 no leakages were detected from the VDCs confirming the effective fusion of the layers produced with
219 the FDM technique.

220 Then, another important step was to evaluate the compatibility of the material with active molecules
221 even if PP is already known for its chemical resistance. Authors were more worried about eventual
222 physical absorption into spaces between layers. We tested three different molecules varying their

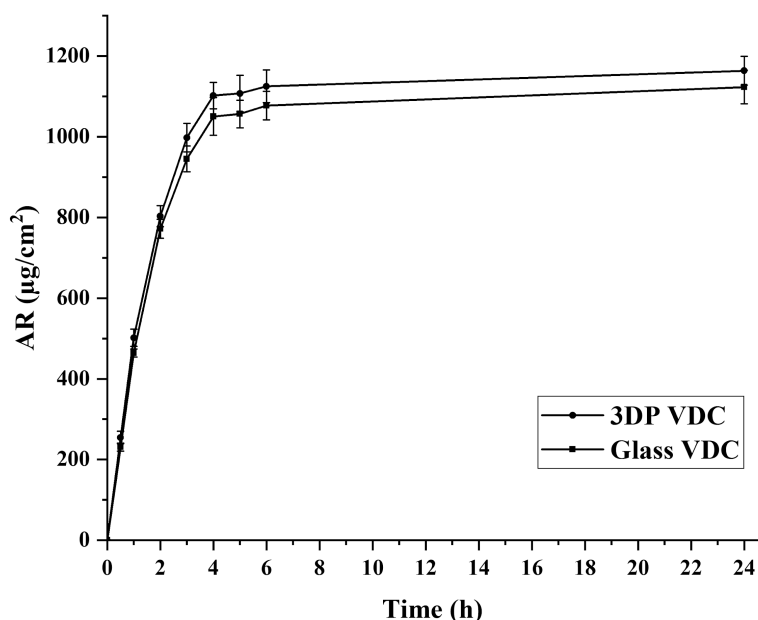
223 chemical nature: caffeine was selected as amphiphilic molecule, diclofenac sodium as salt, and
224 glycyrrhetic acid as hydrophobic molecule. These molecules in their respective solutions were used
225 to fill the receptor compartment for 24 hours and the analysis of concentration after this period
226 showed no differences with the initial concentration confirming the compatibility with these active
227 molecules. As it is impossible to test every type of molecule, we choose these three as models, but
228 we suggest assessing the compatibility of each specific active compounds before utilizing it in an *in*
229 *vitro* permeation test with the 3D printed VDCs.

230

231 3.2 *In vitro* release and permeation studies

232 *In vitro* release studies were performed first in both glass and 3DP VDCs to evaluate effective
233 comparability between the two systems. A commonly used cellulose dialysis membrane was applied
234 to divide the receptor from the donor compartment and a caffeine hydrogel was utilized. This
235 comparison showed no significant differences in the release of the active molecule with the 3DP
236 VDCs when compared with the glass VDCs as shown in figure 2 confirming the suitability of the
237 developed 3DP system. Release studies with the 3DP VDCs resulted in $1164 \pm 36 \mu\text{g}/\text{cm}^2$ of caffeine
238 permeated in the receptor compartment after 24 h meanwhile the release was $1123 \pm 41 \mu\text{g}/\text{cm}^2$ for
239 the glass homologues.

240



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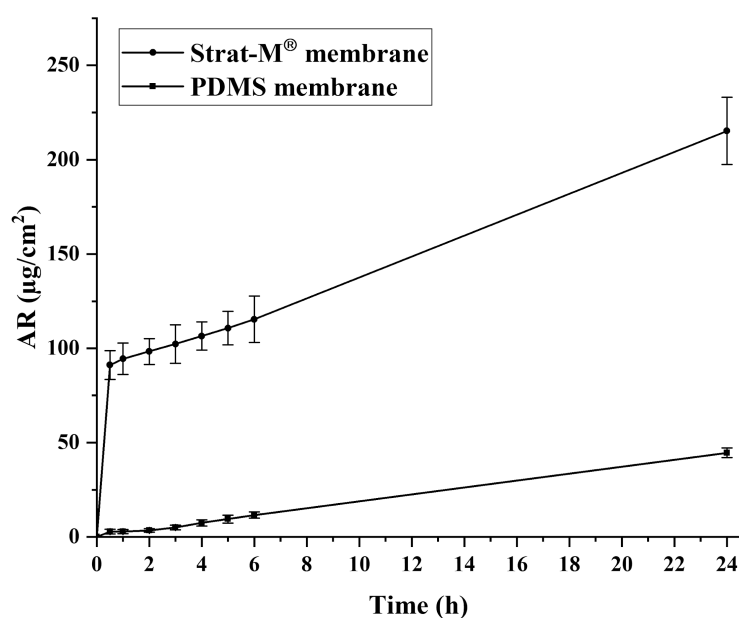
242 **Figure 2.** Comparison between glass VDCs and the 3DP VDCs using a cellulose dialysis membrane (6-8 kDa cut-off)
243 and a 5 mg/mL caffeine hydrogel.

244

245 After the assessed suitability of the 3DP VDCs, two different membranes were employed for a
246 permeation study using a caffeine hydrogel. Excised human and animal skins are often utilized to
247 study skin permeation profiles of topical formulations, however, they are expensive and possess
248 several drawbacks. Among them, there are variations of skin thickness, diseased skin states,
249 preparation complexity, age of the donor, the density of hair follicles, and skin storage conditions that
250 can hinder reproducibility data (Haq et al., 2018b, 2018a).

251 In this study, we decided to compare standardized synthetic Strat-M[®] membranes as a reproducible
252 alternative to excised human skin (Haq et al., 2018a) and 250 μm thick PDMS membranes as a low
253 permeability model membranes (Figure 3) (Ng et al., 2010).

254



255

256 **Figure 3.** Comparison between Strat-M[®] and PDMS membranes using the 3DP VDCs and a 5 mg/mL caffeine hydrogel.

257

258 The drug permeation resulted higher with the Strat-M[®] membranes with an amount permeated after
259 24 h of $215 \pm 18 \mu\text{g}/\text{cm}^2$ meanwhile for the PDMS membranes, the drug permeated was more than 4
260 times lower ($44.6 \pm 2.6 \mu\text{g}/\text{cm}^2$). Since this membrane is made with a hydrophobic material, the
261 permeation through it is influenced by the nature of the tested molecule. Since caffeine result
262 hydrophilic, its passage through this type of membrane resulted very low even after 24 hours.

263 In release studies, mathematical models play a crucial role in evaluating the drug release mechanism
264 (Siepmann and Peppas, 2011). In these studies, the release profile of the drug from the xanthan gum
265 hydrogel resulted linear with the time for the utilized membranes confirming zero-order kinetics
266 (Strat-M[®] R^2 0.9972; PDMS R^2 0.9974) (César dos Santos Nogueira et al., 2003).

267

268 **4. CONCLUSIONS**

269 In this work, we successfully developed a 3D printed VDCs model useful for the evaluation of *in*
270 *vitro* drug release and permeation. The design was in accordance with the pharmacopeia requirement
271 and the dimensions were studied to perfectly fit in a heating block to control the temperature avoiding
272 warm water recirculatory system. As the system has been developed for 3D printing it is possible to
273 continue the personalization based on the needs for example changing or reinventing the donor
274 compartment. The material employed for the manufacturing of the cell (*i.e.*, polypropylene)
275 confirmed its chemical resistance and the possibility to be used to produce leak-free FDM printed
276 objects. Moreover, compared to commercially available VDCs (usually made with glass), the 3D
277 printed VDCs require really low costs of production (less than 2 US \$ of material) and only a few
278 typical lab equipments such as a heating and stirring plate, a heating block and a magnetic stirring
279 bar.

280 VDC *in vitro* testing results fundamental to predict results from *ex vivo* or *in vivo* studies and the
281 possibility to have this testing system readily available in a research lab with a really low cost could
282 increase its diffusion and utilization.

283

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286

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289

290 **Conflicts of interest:** The authors declare no conflict of interest

291

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