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1 **Fluid dynamics characterisation of a rotating bioreactor for tissue**
2 **engineering**

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13
14 **ABSTRACT** : (155 words)

15
16 Biological scaffolds composed of extracellular matrix (ECM) derived from decellularised
17 tissue are increasingly used in regenerative medicine. In this project, a flow perfusion
18 bioreactor (the rotary cell culture system (RCCS), commercially available from Synthecon
19 (Houston, TX)) is used in order to obtain some esophageal extracellular matrix. A theoretical
20 mechanical characterisation of this experimental set-up is provided. Due to the combination
21 of rotation and perfusion, some spiral Poiseuille flow is created inside the tubular esophagus.
22 In a transverse section, a particle (or cell) experiences simultaneously gravitational,
23 Archimedes, centrifugal, Coriolis, and drag forces. In a frame of reference rotating with
24 angular velocity ω , the particle follows a periodic nearly circular path in the clockwise
25 direction, associated with a very slow centrifugal drift towards the esophagus wall. It appears
26 that moderate perfusion rate and rotation speed ($\omega < 20$ rpm and $Q < 30$ ml/min) are
27 appropriate experimental conditions for esophagus tissue engineering using the RCCS
28 Synthecon bioreactor.

29

30 **Keywords** : rotating bioreactor; spiral Poiseuille flow; esophageal substitute; tissue
31 engineering

32
33 *Paper word count (total)* : 7300 words.

34
35

36 **1. Introduction**

37 The concepts of tissue engineering and regenerative medicine are used to develop therapeutic
38 alternatives in order to provide viable solutions for patients waiting for a transplant of a
39 failing organ in terminal phase [1]. Increasingly used in regenerative medicine, biological
40 scaffolds composed of an extracellular matrix (ECM), derived from decellularised tissues, are
41 able to satisfy some clinical needs [2]. However, the decellularisation process must not
42 compromise the integrity of the native organ's overall three-dimensional architecture,
43 structural components and biomechanical properties. Different methods have been developed
44 to this aim [1].

45 Esophageal tissue engineering is a promising approach to the treatment of esophageal
46 pathologies such as esophageal atresia, which affects one newborn for every 3,500 births [3],
47 esophageal cancer, which is the eight most common cancer in the world [4], accidental or
48 intentional burns, and perforations. Currently, the restoration of digestive continuity after
49 esophagectomy is achieved through the interposition of a segment of the colon or by the
50 tubulation of the stomach (Lewis Santy intervention); however there are many post-operative
51 complications such as anastomotic leaks, infections, etc. [5]. There has been interest shown in
52 the development of a tissue-engineered esophageal substitute, constituted of an acellular
53 matrix and seeded cells, for the treatment of esophageal pathologies. It is necessary to develop
54 optimal decellularisation techniques in order to obtain a clinical grade esophageal ECM for
55 full thickness esophageal replacement. Previous studies have developed an acellular
56 esophageal substitute from skin, urinary bladder, intestinal submucosa from various animal
57 and human models; however, the use of porcine esophagus has been shown to be the most
58 adapted tissue engineering support for a full thickness esophageal replacement. The use of
59 decellularisation solutions in contact with native esophageal tissue ensures the removal of

60 cellular content and DNA. In recent years, different decellularisation solutions have been
61 explored such as sodium dodecyl sulfate, sodium deoxycholate, Triton X-100, and Chaps [6].
62 The most effective decellularisation procedure for engineering an acellular scaffold from a
63 porcine esophageal sample was found to be the use of sodium deoxycholate for cell lysis
64 coupled with DNase I for the removal of DNA [7]. The action of these chemical and
65 enzymatic solutions can be amplified by a mechanical action provided by immersion under
66 constant agitation, or by using the esophageal lumen as a perfusion way [8], [9]. In this study,
67 the decellularisation of a porcine esophagus sample is made possible by the perfusion of
68 sodium deoxycholate solutions coupled to DNase I, in order to provide biological scaffolds
69 able to be recellularised by human stem cells [9]. The functionalisation of an esophageal
70 substitute by a recellularisation method is a significant challenge in tissue regeneration after
71 transplantation of a tissue engineered organ [1]. It is possible to recellularise esophageal
72 decellularised matrices (DM) with several cell types: autologous or allogeneic, differentiated
73 or non-differentiated. The use of cell sheets [9] allows the recellularisation of DM under
74 static conditions, whereas recellularisation with suspended cells requires a dynamic
75 environment to overcome the sedimentation phenomenon [6]. For this purpose, a flow
76 perfusion bioreactor is used: the rotary cell culture system (RCCS), commercially available
77 from Synthecon (Houston, TX) [10]. This device allows liquid flow within the tubular
78 esophagus as well as a mechanical rotation in and around the tissue in two successive closed
79 chambers. The flow of this liquid is thus controlled according to a perfusion flow rate and a
80 rotation of the chamber. Thanks to this dynamic environment in the bioreactor the
81 distribution of the seeded cells in the DM is performed in a homogenous way [6]. The aim of
82 this paper is to provide a theoretical mechanical characterization of this experimental set-up
83 in order to determine: i) the velocity fields, pressures, shear stresses in the fluid without
84 suspended cells, ii) the forces that act on a suspended cell and determine its motion.

85 Although several papers [11-15] mention the basic principle of rotating wall bioreactors the
86 literature survey did not allow us to find a convenient detailed mechanical and mathematical

87 analysis of the combined rotation and perfusion movement in such devices. Pollack et al. [16]
88 provided the equations of motion for microcarriers in a rotating bioreactor. They validated
89 their analysis with some experimental and numerical results. However, they used a High
90 Aspect Ratio Vessel (HARV). This type of bioreactor has a large radius to depth ratio, it looks
91 like a disk and not like a cylinder. Their analysis was 2D (no longitudinal motion). The
92 HARV was also used by Mazzoleni et al. [17] as an in-vitro model of osteocytes'
93 differentiation and bone matrix formation, and by Ferrarini et al. [18] as a tool to study 3-D
94 tumor (myeloma) models. Varley et al. [19] used a more common type of bioreactor, named
95 RWV (Rotating Wall Vessel), to improve osteoblasts proliferation in floating scaffolds. They
96 proposed a dual-axis rotating system with rotation speeds of each axis in the range 5- 35 rpm.
97 They performed some numerical calculations but they did not provide information about their
98 numerical procedure. They considered the case where the bioreactor chamber is not 100%
99 filled with culture medium. Consequently, they had to take into account a fluid /air interface
100 with its specific boundary condition. Liu et al. [20] published an analysis on forces and
101 movement of cultivated particles in a rotating wall vessel bioreactor. They considered the case
102 where the rotating speeds (in the range 10 r.p.m. to 65 r.p.m.) of the inner and outer cylinders
103 are not the same. No longitudinal flow exists in their study and the sizes of the suspended
104 particles are in the range 100 μm to 1 mm. Their equations and mathematical solution seem
105 very questionable. Some longitudinal flow is considered in [21] but the bioreactor used by
106 these authors is very different from the one we use. Their bioreactor is composed of two
107 concentric cylinders that can be independently rotated (15-35 r.p.m.). The fluid is tangentially
108 perfused between the inner and outer cylinders. Since the inner cylinder wall is porous, the
109 fluid is collected in the inner cylinder and goes back to the external flow loop. Their perfusion
110 rate is typically 10 ml/min. They provide a numerical solution for the flow fields and shear
111 stresses but do not study the motion of suspended particles.

112 In 2014, Grimm et al. [22] published an extensive review of the existing devices that simulate
113 microgravity and can be used for various tissue engineering applications. A literature

114 synthesis on bioreactors and their utilisation in bone tissue engineering can also be found in
115 [23]. Some studies mention the use of a RCCS bioreactor and provide details about their
116 experimental procedures, but they do not give any mechanical analysis; for example, the
117 experimental conditions are: no longitudinal flow, low rotational speed (10 r.p.m.) in the
118 study of Morabito et al. [24] and no longitudinal flow, rotational speeds in the range 12 to
119 22 r.p.m. in the study of Lei et al. [25].

120 Other experimental studies demonstrate that the concept of rotating wall bioreactor associated
121 with longitudinal perfusion is pertinent for decellularisation and recellularisation of tissue
122 engineered tubular constructs. A double-chamber tracheal bioreactor is described in [26].
123 More recently, this device has been re-designed by Lee et al. [27] to improve the perfusion
124 cell seeding protocol in order to re-epithelialise de-epithelialised tracheal scaffolds. A tracheal
125 rotation along its longitudinal axis is allowed from 0 to 30 rpm, with flow rates range from
126 1.5 to 12 ml/min. This favours circulation and mixing of micronutrients and provides control
127 of the cell deposition patterns on the scaffold. Nayakawde et al. [7] also perform
128 recellularisation of acellular esophagus matrix in a perfusion-rotation bioreactor (Harvard
129 Apparatus) with very slow flow rate (3 ml/min) and rotation (0.5 r.p.m.). Urbani et al. [28]
130 use an Applikon bioreactor connected to a reservoir medium to create dynamic cell culture
131 conditions on their esophagus scaffolds. No rotation is possible with this device and the
132 medium flow rate is 5 ml/min. The flow loop is ensured by peristaltic pumps in [7, 27, 28]
133 and by a home-made motion unit in [26].

134

135

136 **2. Materials and methods**

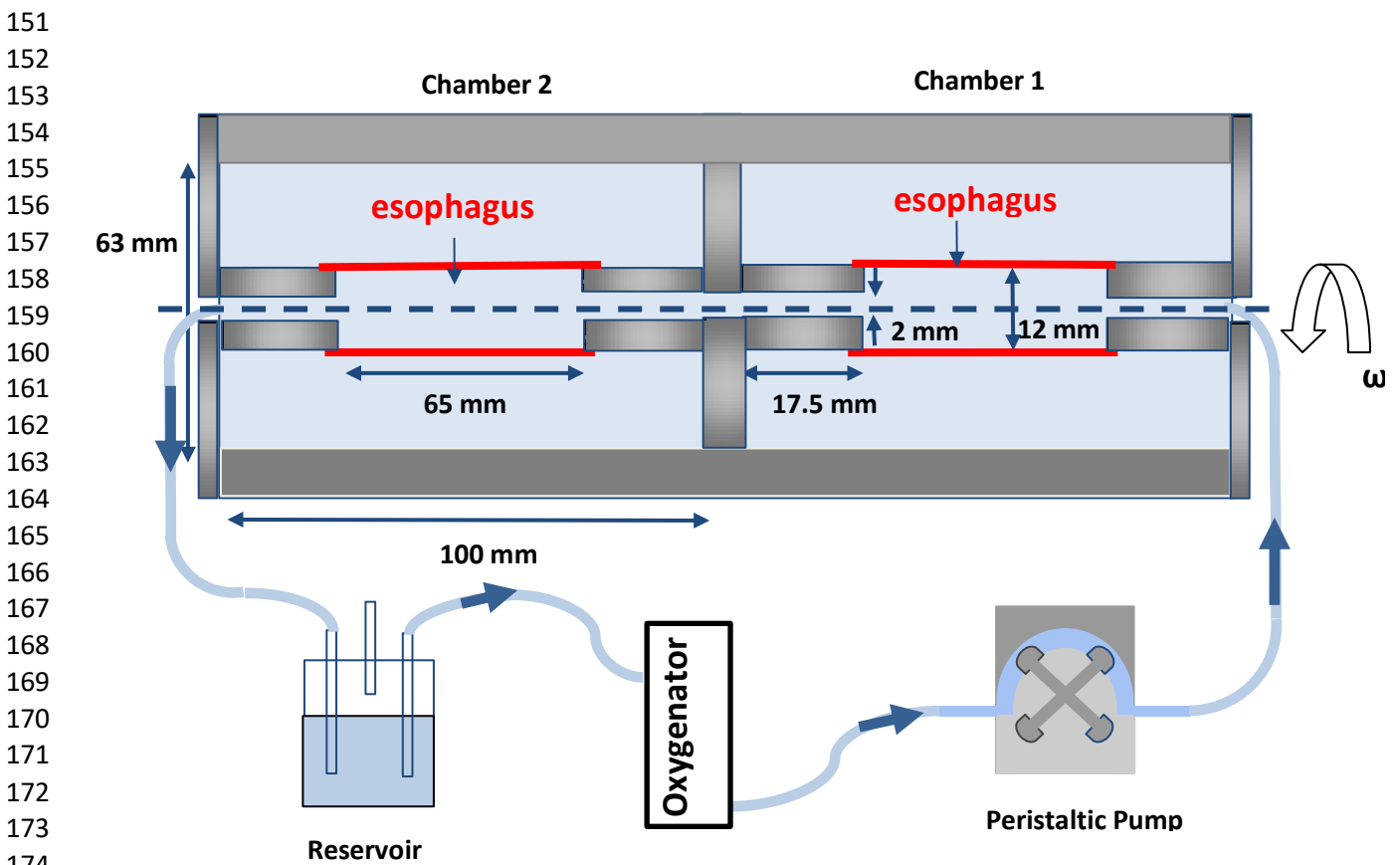
137

138 *2.1. Description of the perfusion bioreactor and closed flow loop*

139

140 A schematic representation of the RCCMax-dual esophagus bioreactor and of the flow bench
141 is reported in **Figure 1**. The bioreactor consists of two successive cylinder chambers that
142 rotate horizontally at the same constant angular speed. Some direct motor drive is used to

143 rotate the cylinders. In each chamber there are scaffold holders on which a tubular scaffold
 144 can be mounted and tied with a non-absorbable 2/0 USP suture. The scaffold thus rotates at
 145 the same angular velocity as the chamber wall. The chambers are connected to a media
 146 reservoir bottle, an oxygenator and a 4-rollers peristaltic pump (Watson Marlow 314D). One
 147 r.p.m. on the peristaltic pump provides a 0.5 ml/min flow rate through the tubing. Silicon
 148 tubing has a wall tube thickness 1.6 mm and internal diameter 1.6 mm. The oxygenator uses
 149 silicone membrane diffusion of gases. The reservoir is open to atmospheric pressure. The
 150 circulating medium contains Sodium Azide, Sodium Deoxycholate and DNaseI.



176 **Figure 1** - Schematic representation of the experimental set-up.

177
 178
 179
 180 *2.2. Analytical approach*

181
 182 *2.2.1. Fluid motion*

183 The fluid medium used for the decellularisation experiments is considered as newtonian with
 184 a viscosity $\eta_f = 1 \text{ mPas}$ and a density $\rho_f = 1015 \text{ kg/m}^3$. For the moment the esophagus wall is

185 assumed non-deformable and non-porous and its thickness (a few millimeters) is not taken
 186 into account. R_1 denotes the esophagus radius ($R_1 = 6$ mm) and R_2 the chamber radius ($R_2 =$
 187 31.5 mm). Both cylinders (esophagus and chamber) are supposed “infinitely” long with an
 188 axial symmetry.

189 In each chamber of the bioreactor two distinct parts will be considered: **Part A** will refer to
 190 the perfusion inside the esophagus and **Part B** will refer to the medium enclosed between the
 191 esophagus and the chamber wall. The scaffold is attached to some part of the chamber;
 192 consequently the esophagus wall rotates at the same angular velocity as the chamber wall.

193 In **Part B** there is no fluid circulation (no longitudinal fluid velocity). The fluid rotates as a
 194 rigid body with an angular velocity ω throughout the domain (Couette flow). In classical
 195 cylindrical coordinates (O, r, θ, z) this would yield: no radial velocity and an azimuthal
 196 velocity U_θ equal to ωr , r being the radial coordinate ($R_1 < r < R_2$). In this environment,
 197 shear stresses are null.

198 In **Part A** for a given value of the pump flow rate (Q) quantities of interest do not depend on
 199 time. The flow is driven by the combination of two factors: a constant axial pressure gradient
 200 (along Oz) and the rotation of the dual chamber. Velocity continuity prevails at the wall, due
 201 to the no-slip boundary conditions. This results in an exact superposition of an axial parabolic
 202 velocity profile U_z and an azimuthal solid-body rotation U_θ depending only on the radial
 203 coordinate r as:

$$204 \quad U_z(r) = 2 U_{mean} \left(1 - \frac{r^2}{R_1^2}\right), \quad \text{and} \quad U_\theta(r) = \omega r \quad (1)$$

205 where U_{mean} is the mean axial velocity associated with the axial pressure gradient $\left(-\frac{\partial P^*}{\partial z}\right)$
 206 according to:

$$207 \quad U_{mean} = \frac{Q}{\pi R_1^2} = \frac{\left(-\frac{\partial P^*}{\partial z}\right) R_1^2}{8\eta_f} \quad (2)$$

208 This type of flow is known as the rotating Hagen-Poiseuille flow or spiral-Poiseuille flow
 209 [29]. It is characterized by two non-dimensional control parameters: the streamwise Reynolds
 210 number:

$$211 \quad R_{ez} = \frac{\rho_f U_{mean} (2 R_1)}{\eta_f} \quad (3)$$

212 and the azimuthal (or rotational) Reynolds number:

$$213 \quad R_{e\omega} = \frac{\rho_f (\omega R_1) (2 R_1)}{\eta_f} \quad (4)$$

214 In such a flow, tangential shear stresses in the azimuthal direction are null since:

$$215 \quad \tau_{r\theta} = \eta_f r \frac{\partial}{\partial r} \left(\frac{U_\theta}{r} \right) \quad (5),$$

216 and tangential shear stresses in the axial direction may be calculated as:

$$217 \quad \tau_{rz} = \eta_f \frac{\partial U_z}{\partial r} = -\frac{r}{2} \left(-\frac{\partial P^*}{\partial z} \right) \quad (6).$$

218 They are maximal at the wall of the esophagus ($r = R_1$).

219 It is important to mention that both in **Part A** and **B**, the radial projection of Navier-Stokes
 220 equations can be simplified as:

$$221 \quad \frac{\partial P^*}{\partial r} = \rho_f \frac{U_\theta^2}{r} = \rho_f \frac{\omega^2 r^2}{r} = \rho_f \omega^2 r \quad (7),$$

222 thus demonstrating that a positive radial pressure gradient exists in each domain.

223

224 *2.2.2. Suspended particle motion*

225

226 In order to be able to describe the motion of a particle relative to the rotating fluid a rotating
 227 frame (O, x, y, z) is considered (**Figure 2**). Conversely (O, X, Y, Z) is a ground-based frame
 228 (fixed). Since the esophagus cylinder and the chamber cylinder are concentric, the (OZ) and
 229 (Oz) axis are the same. Unit vectors associated with (OXY) are denoted \mathbf{e}_X and \mathbf{e}_Y , and unit
 230 vectors associated with (Oxy) are denoted \mathbf{e}_x and \mathbf{e}_y . The (Oz) and (OZ) unit vectors are
 231 denoted \mathbf{e}_z , so that the frames are direct. The frame (O, x, y, z) rotates counter-clockwise
 232 about the (Oz) axis with a constant angular velocity ω . The rotating vector is thus: $\mathbf{\Omega} = \omega \mathbf{e}_z$.

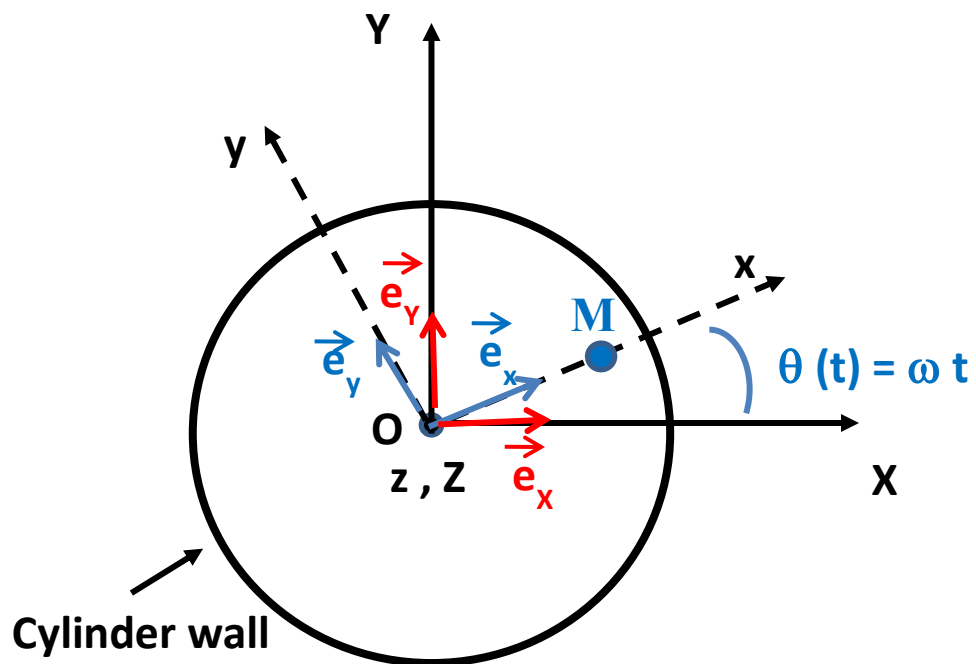
233 We consider a non-deformable spherical particle with radius a and density ρ_p (slightly higher
 234 than the fluid density ρ_f). The mass of the particle is thus:

235 $m_p = \rho_p V_p$, where V_p is the particle volume ($V_p = \frac{4}{3} \pi a^3$).

236 Since the particle is positively buoyant, it experiences sedimentation while the chamber and
 237 the fluid are rotating.

238

239



240

241

242

243 **Figure 2** – Ground-based and rotating cylindrical frames: definition of the notations. The

244 gravitational acceleration \mathbf{g} is perpendicular to the rotation axis (Oz); it is directed down the (OY) axis.

245

246

247 The particle position (\mathbf{OM}) , velocity (\mathbf{v}) and acceleration (\mathbf{a}) in the rotating frame are
 248 respectively :

249 $\mathbf{OM} = \begin{pmatrix} x \\ y \\ z \end{pmatrix}$, $\mathbf{v} = \begin{pmatrix} \dot{x} \\ \dot{y} \\ \dot{z} \end{pmatrix}$, $\mathbf{a} = \begin{pmatrix} \ddot{x} \\ \ddot{y} \\ \ddot{z} \end{pmatrix}$, where the dot denotes time derivative

250 of a quantity. Accordingly the entrainment velocity is obtained as:

251
$$\boldsymbol{\Omega} \wedge \mathbf{OM} = \begin{pmatrix} 0 \\ 0 \\ \omega \end{pmatrix} \wedge \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -\omega y \\ \omega x \\ 0 \end{pmatrix} \quad (8)$$

252 The entrainment acceleration is :

$$253 \quad \boldsymbol{\Omega} \wedge (\boldsymbol{\Omega} \wedge \mathbf{OM}) = \begin{vmatrix} 0 \\ 0 \\ \omega \end{vmatrix} \wedge \begin{vmatrix} -\omega y \\ \omega x \\ 0 \end{vmatrix} = \begin{vmatrix} -\omega^2 x \\ -\omega^2 y \\ 0 \end{vmatrix} \quad (9),$$

254 And the Coriolis acceleration is:

$$255 \quad 2 \boldsymbol{\Omega} \wedge \mathbf{v} = \begin{vmatrix} \mathbf{0} \\ \mathbf{0} \\ 2 \omega \end{vmatrix} \wedge \begin{vmatrix} \dot{x} \\ \dot{y} \\ \dot{z} \end{vmatrix} = \begin{vmatrix} -2 \omega \dot{y} \\ 2 \omega \dot{x} \\ 0 \end{vmatrix} \quad (10)$$

256

257 In **Part B** the particle experiences simultaneously gravitational, Archimedes, centrifugal,
258 Coriolis, and drag forces.

259 Introducing a buoyancy corrected mass, $m_b = (\rho_p - \rho_f) V_p$, the buoyancy corrected
260 weight of the particle is: $m_b \mathbf{g}$, where we have to consider the projection of the gravitational
261 acceleration \mathbf{g} in the rotating frame:

$$262 \quad \mathbf{g} = \begin{vmatrix} -g \sin(\omega t) \\ -g \cos(\omega t) \\ 0 \end{vmatrix} \quad (11).$$

263 Since the particle is small and the velocities are moderate, an appropriate estimation of the
264 viscous drag may be obtained using Stokes approximation:

$$265 \quad \mathbf{D} = -k \mathbf{v}, \text{ where the coefficient } k \text{ is given by : } 6\pi \eta_f a \quad (12).$$

266 Since the fluid is in solid body rotation the pressure gradient acting on $(\rho_f V_p)$ opposes the
267 the centripetal force on $(\rho_p V_p)$ and the resulting force will be written as:

$$268 \quad \begin{vmatrix} -m_b \omega^2 x \\ -m_b \omega^2 y \\ 0 \end{vmatrix} \quad (13)$$

269 Gathering all, in **Part B**, the motion of the particle in the rotating frame is governed by the
270 following differential equations:

$$271 \quad m_p \ddot{x} = -k\dot{x} + m_b \omega^2 x + 2m_p \omega \dot{y} - m_b g \sin(\omega t) \quad (14)$$

$$272 \quad m_p \ddot{y} = -k\dot{y} + m_b \omega^2 y - 2m_p \omega \dot{x} - m_b g \cos(\omega t) \quad (15)$$

273 Equations (14-15) indicate that particle motion may be affected by: density difference
274 between fluid and particle vessel rotation rate, fluid viscosity and particle radius.

275 In **Part A**, due to the fluid perfusion a longitudinal motion of the particle exists. The
 276 equation of motion for the relative particle displacement is:

$$277 \quad m_p \ddot{z} = -kz \quad (16)$$

278 Additionally the Poiseuille flow shear rate (radial variation of the longitudinal velocity)
 279 can induce a torque on the particle associated with a lift force and a radial migration [30].

280 This shear rate can be derived from Equ.(1) and (2) as follows:

281

$$282 \quad G = \frac{\partial U_z}{\partial r} = -\frac{4Qr}{\pi R_1^4} \quad (17).$$

283 A Reynolds number based upon the particle radius and the averaged velocity gradient,
 284 G_{mean} , can be defined as:

$$285 \quad Re_G = \frac{\rho_f G_{mean} a^2}{\eta_f} \quad (18),$$

286 where

$$G_{mean} = -\frac{2Q}{\pi R_1^3}.$$

287 **3. Results**

288

289 *3.1. Reynolds numbers, entry lengths and pressure losses in the bioreactor*

290

291 The effects of changes in operating conditions including rotation rates and fluid perfusion
 292 rates, are investigated. The chosen values are in agreement with the literature survey that is
 293 summarised in the ‘‘Introduction’’ Section. Slightly upper-range values are also selected in
 294 order to determine whether they would be acceptable or not for this type of tissue engineering
 295 applications.

296 The mean axial velocity in the esophagus, U_{mean} , is obtained from Equ. (2) with a diameter of
 297 the lumen $2R_1 = 12$ mm. The mean axial velocity in the lumen of the scaffold holder
 298 (diameter $d = 2$ mm) is denoted u_{mean} , and calculated as : $u_{mean} = 4Q/\pi d^2$. The streamwise
 299 Reynolds number in the esophagus and the azimuthal Reynolds number are deduced from
 300 Equ. (3) and (4). An estimation of the flow entrance length is provided by the classical
 301 formula:

$$302 \quad L_e \approx 0.05 Re_{ez} (2R_1) \quad (19)$$

303 Viscous pressure losses due to the singularities at the entrance and at the exit of the chamber

304 are respectively given by: $\Delta P_f^{ent.} = k_1 \frac{1}{2} \rho_f u_{mean}^2$, with $k_1 = \left(1 - \left(\frac{d}{2R_1}\right)^2\right)^2$ (20)

305 and : $\Delta P_f^{exit.} = k_2 \frac{1}{2} \rho_f u_{mean}^2$, with $k_2 = 0.5 \left(1 - \left(\frac{d}{2R_1}\right)^2\right)$ (21).

306 Since the flow rates are quite moderate, Equ. (22) provides a suitable evaluation of the
307 pressure losses along the silicone tubing ($l_{tube} = 0.5$ m, $d_{tube} = 1.6$ mm):

308 $\Delta P_f^{tube} = \frac{64}{R_e^{tube}} \frac{1}{2} \rho_f u_{tube}^2 \frac{l_{tube}}{d_{tube}}$, where $R_e^{tube} = \frac{\rho_f u_{tube} d_{tube}}{\eta_f}$ (22).

309 The numerical values of all these quantities are gathered in **Table1**.

310 Flow rates and corresponding velocities

Q (ml/min)	12,50	25,00	50,00
U_{mean} (mm/s)	1,84	3,68	7,37
u_{mean} (mm/s)	66,3	133	265

311

312 Reynolds numbers and entry lengths in the esophagus

R_{ez} (Equ.(3))	22,43	44,87	89,74
L_e (Equ.(19)) (mm)	13,5	26,9	53,8
L_e (% of the total esophagus length)	21	41	83

313

314 Pressure losses at the entrance and exit of the chamber

k_1 (Equ. (20))	0,9452		
k_2 (Equ. (21))	0,4861		
ΔP_f - Entrance (Pa) (Equ. (20))	2,11	8,44	33,74
ΔP_f - Exit (Pa) (Equ. (21))	1,085	4,34	17,35

315

316 Pressure losses along the tubing

u_{tube} (mm/s)	104	207	414
Reynolds-tubing (Equ. (22))	168,25	336,49	673,15
Pressure loss -tubing (Pa) (Equ. (22))	647,5	1 295	2 590,6

317

318 Azimuthal Reynolds number (Equ. (4))

ω (r.p.m.)	15	30
ωR_1 (mm/s)	9,42	18,85
Azimuthal Reynolds number	114,8	229,6

319

320

321

Table 1: Characteristic hydrodynamic data .

322

323 The data reported in [Table 1](#) indicate that:

- 324 - the Reynolds numbers are sufficiently small for the flow to be considered laminar
- 325 - depending upon the operating conditions, the azimuthal velocity may be slightly
- 326 higher than the longitudinal velocity
- 327 - pressure losses along the tubing are much more important than pressure losses at the
- 328 entrance and at the exit of the chamber
- 329 - in order to get a fully developed flow in the esophagus, one has to keep the flow at low
- 330 level. Otherwise the entrance length may represent a too important proportion of the
- 331 total esophagus length.

332

333 3.2. Analytical approach

334

335 3.2.1. Streamlines in Spiral-Poiseuille flow (without particle)

336

337 Equations for the velocity field inside the esophagus (**Part A** of the flow domain) are given in
338 Section 2.2.1 (Equations (1) and (2)). Reporting Equ.(2) in Equ.(1), the longitudinal velocity
339 turns out to be:

$$340 \quad U_z(r) = \frac{1}{4\eta_f} \left(-\frac{\partial P^*}{\partial z} \right) (R_1^2 - r^2) \quad (23)$$

341 Fluid particles pathlines are determined by:

$$342 \quad \begin{cases} dz = U_z(r) dt \\ d\theta = \omega dt \end{cases} \quad (24).$$

343 Integrating and eliminating the variable t (time) one gets the streamline equation associated
344 with the initial conditions $z = 0$ and $\theta = 0$ at $t = 0$:

$$345 \quad z = U_z(r) \frac{\theta}{\omega} \quad (25).$$

346 Equation (25) describes an helix curve whose constant radius is r and whose pitch is :

$$347 \quad h(r) = U_z(r) \frac{2\pi}{\omega} \quad (26).$$

348 This result may be illustrated with specified values of r , Q and ω .

349 For example: r is chosen as $R_1 / 2$ (that is 3 mm), $Q = 25$ ml/min, and $\omega = 15$ r.p.m.

350 With these numerical data,

351 $U_{\text{mean}} = 3,68 \text{ mm/s}$; $U_z (R_1/2) = 3 U_{\text{mean}} / 2 = 5,52 \text{ mm/s}$; $h (R_1/2) = 22,1 \text{ mm}$.

352

353 3.2.2. *Motion of a spherical particle suspended in the fluid*

354

355 *Order of magnitude of Coriolis force on the particle.*

356

357 Let us suppose that a cell can be represented by a spherical particle with diameter $2a = 15$

358 microns and density $\rho_p = 1070 \text{ kg/m}^3$ (the cell volume V_p will thus be $1767 \text{ }\mu\text{m}^3$ and its mass

359 $m_p = 1,89 \cdot 10^{-12} \text{ kg}$). We consider first the sedimentation equilibrium velocity, u_p , for such a

360 particle suspended in a non-rotating fluid. In these conditions the particle experiences drag

361 and Archimedes forces against gravity. All these forces are directed along (OY) (vertical).

362 The velocity u_p is given by the well-known Stokes formula:

$$363 \quad u_p = \frac{2g(\rho_p - \rho_f)}{9} a^2 \frac{1}{\eta_f} \quad (27)$$

364 With our numerical data this yields: $u_p = 6.74 \text{ }\mu\text{m/s}$, which is 3 orders of magnitude smaller

365 than the fluid velocities shown in [Table 1](#).

366 Similarly a Reynolds number based on the particle diameter $2a$ and terminal velocity u_p can

$$367 \quad \text{be evaluated as:} \quad R_e^p = \frac{\rho_f 2a u_p}{\eta_f} \quad (28)$$

368 Its value is: $R_e^p = 0.0001$ (five or six orders of magnitude smaller than the fluid

369 Reynolds).

370 Coming back to the rotating fluid and rotating frame (Oxyz) ([Figure 2](#)), the sedimentation

371 velocity u_p is the particle velocity relative to the rotating frame, and is thus involved in the

372 evaluation of Coriolis acceleration, for which the norm can be expressed as: $2 \omega u_p$.

373 For $\omega = 15 \text{ r.p.m.}$, Coriolis acceleration scales as: $2,12 \cdot 10^{-5} \text{ m/s}^2$. This has to be compared to

374 the centrifugal acceleration $\omega^2 r$. If the $\omega^2 r$ term is evaluated at a radial distance $r = 3 \text{ mm}$

375 (inside the esophagus) its value is $7,4 \cdot 10^{-3} \text{ m/s}^2$; if it is evaluated at $r = 2 \text{ cm}$ (between the

376 esophagus and the chamber wall) , its value is $49,3 \cdot 10^{-3} \text{ m/s}^2$. It may thus be concluded that

377 the ratio of Coriolis acceleration to centrifugal acceleration is very small (of order 10^{-3}), and

378 that Coriolis force may probably be neglected in Equ. (14-15).

379

380 *Importance of the lift effect on the particle in Poiseuille flow*

381

382 If the shear Reynolds number, Re_G , is computed from Equ. (18) in the case $Q = 25\text{ml/min}$

383 and for a 7.5 micron particle radius, one obtains $G_{\text{mean}} = 1.23 \text{ s}^{-1}$ and $Re_G = 7.01 \cdot 10^{-5}$. This

384 result has to be compared to the longitudinal (Re_z) or azimuthal Reynolds (Re_{θ}) numbers

385 presented in [Table 1](#) showing that the lift effect on the particle in Part A of the device is a

386 minor effect.

387 Consequently we do not consider it.

388

389 *Longitudinal motion of the particle inside the esophagus*

390

391 The longitudinal relative particle velocity, $v_z(t)$, is easily deduced from Equ. (16) since

392
$$v_z(t) = \dot{z} .$$

393 Equ. (16) becomes :

394
$$m_p \frac{dv_z(t)}{dt} = -k v_z(t) \quad (29),$$

395 indicating that inertial effects are balanced by the frictional force exerted on the sphere by

396 the fluid. The mathematical solution for such an equation involves an exponential term

397 decreasing with time: $\exp(-kt/m_p)$. Since $m_p = 1,89 \cdot 10^{-12} \text{ kg}$, and k (defined in Equ. (12) as

398 $6\pi \eta_f a$) equals $1.41 \cdot 10^{-7} \text{ Pa.s.m}$, the ratio $k/m_p = 7.48 \cdot 10^4 \text{ s}^{-1}$. The $\exp(-kt/m_p)$ term is thus

399 essentially transient and will decay very quickly. It can be ignored and the absolute

400 longitudinal velocity of the particle (in the laboratory frame) can be assumed to be roughly

401 the same as the fluid velocity. This result is consistent with the fact that the particle radius is

402 much smaller than the esophagus radius ($a / R_1 = 7.5 \cdot 10^{-6} \text{ m} / 6 \cdot 10^{-3} \text{ m} \approx 10^{-3}$). The particle

403 may be considered as a “tracer” and follows the fluid with the same speed as the local

404 Poiseuille velocity [31]. The longitudinal position of the particle increases linearly with

405 time:

406
$$z(r, t) = U_z(r) t \quad (30).$$

407 A key point for tissue engineering applications is the residence time of a suspended cell in

408 the bioreactor. Based on U_{mean} velocity, this residence time can be evaluated as: $\Delta t =$

409 esophagus length / U_{mean} . Since the esophagus length is roughly equal to 65 mm, for $U_{\text{mean}} =$
 410 3,68 mm/s, a 17.7 s residence time is found.

411
 412 *Rotating motion of the particle*

413
 414 Multiplying Equ. (15) by the complex number i ($i^2 = -1$) and adding Equ. (14), this
 415 coupled system can be transformed in one equation in the complex domain as follows:

$$416 \quad m_p \ddot{s} + (k + i 2m_p \omega) \dot{s} - m_b \omega^2 s = -m_b g i e^{-i\omega t}, \quad \text{where } s = x + iy \quad (31).$$

417 This equation is consistent with the work of Kessler et al. [32].

418 Equ. (31) may be re-written as:

$$419 \quad \ddot{s} + \left(\frac{k}{m_p} + 2 i \omega \right) \dot{s} - \frac{m_b}{m_p} \omega^2 s = -\frac{m_b}{m_p} g i e^{-i\omega t} \quad (32).$$

420 Solving Equ. (32) requires two steps:

421 * Solving the associated homogeneous equation:

$$422 \quad \ddot{s} + \left(\frac{k}{m_p} + 2 i \omega \right) \dot{s} - \frac{m_b}{m_p} \omega^2 s = 0 \quad (33).$$

423 Convenient solutions for Equ. (33), $s_h(t)$, are search as : $s_h(t) = \exp(\sigma t)$, with σ satisfying
 424 the condition:

$$425 \quad \sigma^2 + \left(\frac{k}{m_p} + 2 i \omega \right) \sigma - \frac{m_b}{m_p} \omega^2 = 0 \quad (34).$$

426 Equ. (34) has two complex σ solutions that can be developed in leading orders of the small
 427 quantity ($\omega m_p / k$):

$$428 \quad 2 \sigma_1 = -\left(\frac{k}{m_p} + 2i\omega \right) + \frac{k}{m_p} \left[1 + 2 \frac{m_b}{m_p} \frac{m_p^2 \omega^2}{k^2} + i \left(\frac{2\omega m_p}{k} - 4 \frac{m_b \omega^3 m_p^3}{k^3} \right) \right] \quad (35)$$

429 and

$$430 \quad 2 \sigma_2 = -\left(\frac{k}{m_p} + 2i\omega \right) - \frac{k}{m_p} \left[1 + 2 \frac{m_b}{m_p} \frac{m_p^2 \omega^2}{k^2} + i \left(\frac{2\omega m_p}{k} - 4 \frac{m_b \omega^3 m_p^3}{k^3} \right) \right] \quad (36).$$

431 Neglecting the term of order $\left(\frac{\omega^3 m_p^3}{k^3} \right)$ in Equ. (35) and (36), one obtains:

$$432 \quad \sigma_1 \approx \frac{m_b \omega^2}{k} \quad \text{and} \quad \sigma_2 \approx -\frac{k}{m_p} - \frac{m_b \omega^2}{k} - 2 i \omega \quad (37).$$

433 This yields:

434
$$s_h(t) = C_1 e^{\frac{t}{\tau}} + C_2 e^{-\frac{k}{m_p} t} e^{-\frac{t}{\tau}} e^{-2i\omega t} \quad (38),$$

435 where $\tau = k / m_b \omega^2$ has the physical meaning of a centrifugal time, and a numerical value
 436 of $5.9 \cdot 10^5$ s (obtained with $k = 1, 41 \cdot 10^{-7}$ Pa.s.m, $\omega = 1.57$ s⁻¹, $m_b = 9, 72 \cdot 10^{-14}$ kg).

437 C_1 and C_2 are integration constants.

438 As previously explained the $\exp(-kt/m_p)$ term will decay very quickly. Consequently all
 439 the C_2 term can be ignored and $s_h(t)$ may be approximated as:

440
$$s_h(t) \approx C_1 e^{\frac{t}{\tau}} \approx C_1 \left(1 + \frac{t}{\tau}\right) \quad (39).$$

441

442 * Searching a particular solution, $s_p(t)$, of the complete equation in the form $\beta \exp(-i\omega t)$.

443 One easily obtains:

444
$$s_p(t) = \beta e^{-i\omega t} \quad \text{with} \quad \beta = \frac{m_b g}{\omega k \left[1 - \frac{(m_b - m_p) i \omega}{k}\right]} \quad (40).$$

445 Observing that the term $\frac{m_b g}{k}$ is exactly the sedimentation velocity u_p defined in Equ.
 446 (27), and that the term $(m_b - m_p) \omega / k$ is of order 10^{-5} , the solution $s_p(t)$ can be reduced to:

447
$$s_p(t) = \frac{u_p}{\omega} (\cos(\omega t) - i \sin(\omega t)) \quad (41).$$

448 Finally $s(t) = s_h(t) + s_p(t)$. The integration constant C_1 is determined by the initial condition:
 449 $s(0) = s_0 = C_1 + u_p / \omega$. So that:

450
$$s(t) = \left(s_0 - \frac{u_p}{\omega}\right) e^{\frac{t}{\tau}} + \frac{u_p}{\omega} e^{-i\omega t} \quad (42).$$

451 Coming back to the real and imaginary part of $s(t) = x(t) + i y(t)$, the rotating trajectory of
 452 the particle is described by equations (43) and (44):

453
$$x(t) = \left(x_0 - \frac{u_p}{\omega}\right) e^{\frac{t}{\tau}} + \frac{u_p}{\omega} \cos(\omega t) \quad (43).$$

454
$$y(t) = y_0 e^{\frac{t}{\tau}} - \frac{u_p}{\omega} \sin(\omega t) \quad (44).$$

455 We thus confirm the solution proposed by Kessler et al. [32].

456 It is interesting to note that the terms associated with Coriolis force may be directly
 457 neglected in Equ. (14-15), so that the equations to solve become decoupled:

458
$$m_p \ddot{x} + k\dot{x} - m_b \omega^2 x = -m_b g \sin(\omega t) \quad (45)$$

459
$$m_p \ddot{y} + k\dot{y} - m_b \omega^2 y = -m_b g \cos(\omega t) \quad (46).$$

460 Solving separately Equ. (45) and (46) leads to:

461
$$x(t) = \left(x_0 - \frac{u_p}{\omega}\right) e^{\frac{t}{\tau}} + \frac{u_p}{\omega} \cos(\omega t) + \frac{u_p}{\omega} \frac{(m_p + m_b)\omega}{k} \sin(\omega t) \quad (47).$$

462
$$y(t) = y_0 e^{\frac{t}{\tau}} - \frac{u_p}{\omega} \sin(\omega t) + \frac{u_p}{\omega} \frac{(m_p + m_b)\omega}{k} \cos(\omega t) \quad (48).$$

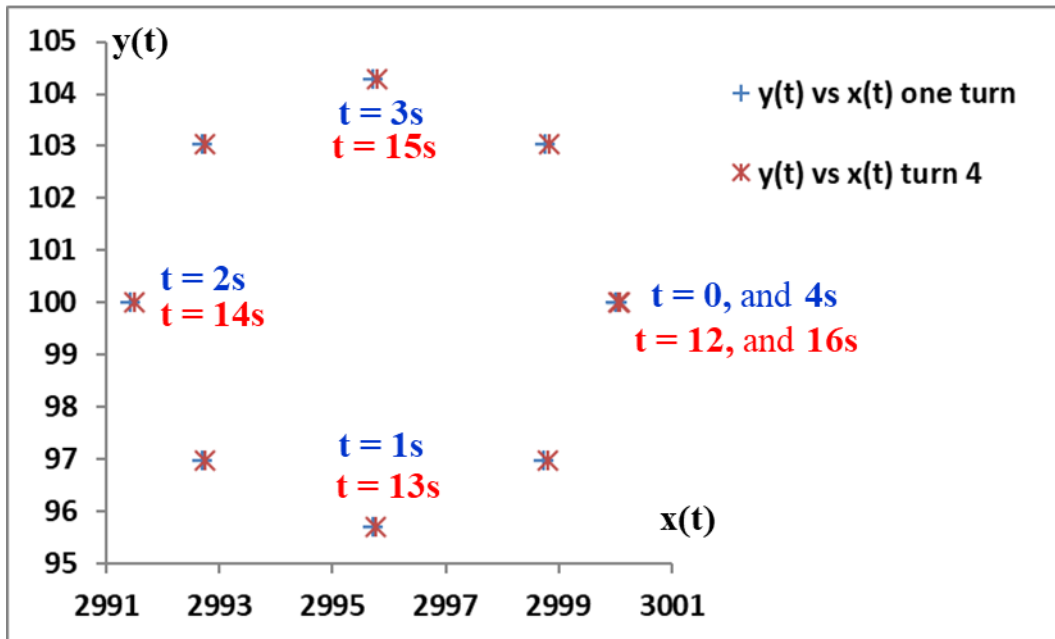
463

464 The quantity $(m_p + m_b) \omega / k$ being of order 10^{-5} , the associated terms can be dropped and
 465 one finds again expression (43) as a solution for $x(t)$ and expression (44) as a solution for
 466 $y(t)$.

467 An illustration of the trajectory described by Equ. (43) and (44) is shown in **Figure 3**, with
 468 $x_0 = 3000 \mu\text{m}$, and $y_0 = 100 \mu\text{m}$ as an initial position for the particle, $u_p = 6.74 \mu\text{m/s}$, $\tau =$
 469 $5.9 \cdot 10^5 \text{ s}$ and $\omega = 15 \text{ r.p.m.} = \pi/2 \text{ s}^{-1}$ ($\approx 1.57 \text{ s}^{-1}$). The particle follows a periodic nearly
 470 circular path in the clockwise direction, associated with a very slow centrifugal drift towards
 471 the esophagus wall. Since $\omega = \pi/2 \text{ s}^{-1}$, one turn is achieved each multiple of $t = 4\text{s}$. According
 472 to the estimation of its residence time in the esophagus (about 17.7s) the particle can execute
 473 only four complete turns before leaving the esophagus. The particle paths in the first turn and
 474 4th turn are represented in **Figure 3**.

475

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Figure 3 – Particle path in the rotating frame, as described by Equ. (43) and (44). The initial position for the particle is chosen as: $x_0 = 3000 \mu\text{m}$, and $y_0 = 100 \mu\text{m}$. Other numerical values are: $u_p = 6.74 \mu\text{m/s}$, $\tau = 5.9 \cdot 10^5 \text{ s}$ and $\omega = 15 \text{ r.p.m}$. Units for $x(t)$ and $y(t)$ are microns.

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Inside the esophagus (**Part A**) this nearly circular motion is combined with the longitudinal motion described by Equ. (30). This generates spiral trajectories within the esophagus.

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Equ. (43) and (44) show that the centrifugal drift of the particle is governed by the centrifugal time τ , and that the radius of the orbit is proportional to the sedimentation velocity u_p and inversely proportional to the rotation rate ω . The centrifugal shift is more and more negligible when τ increases. This may occur if the suspending medium viscosity η_f or the particle radius a increases. Conversely if the difference between the particle and suspending medium density increases or if ω increases, τ will decrease and the centrifugal shift will be more important. Increasing ω or η_f will also reduce the radius of the orbit whereas increasing the density difference $(\rho_p - \rho_f)$ and the particle radius a will increase u_p , and thus the circular path radius. A synopsis of the influence of the governing physical quantities on the particle motion described by Equ. (43) and (44) is presented in **Table 2**.

496

Parameter increase	Effect on the viscous drag coefficient k	Effect on the sedimentation velocity u_p	Effect on the centrifugal time τ	Effect on the orbit radius (u_p/ω)
$\eta_f \uparrow$	Increase	Decrease	Increase	Decrease
$\omega \uparrow$	-----	-----	Decrease	Decrease
$(\rho_p - \rho_f) \uparrow$	-----	Increase	Decrease	Increase
a \uparrow	Increase	Increase	Increase	Increase

497 **Table 2.** Influence of the main parameters of the problem on the quantities describing the particle
498 motion.

499
500 The particle motion $X(t)$ and $Y(t)$ in the ground-based frame may be easily obtained from
501 equ. (43) and (44):

$$502 \quad X(t) = \frac{u_p}{\omega} + \left(x_0 - \frac{u_p}{\omega}\right) e^{\frac{t}{\tau}} \cos(\omega t) - y_0 e^{\frac{t}{\tau}} \sin(\omega t) \quad (49).$$

$$503 \quad Y(t) = \left(x_0 - \frac{u_p}{\omega}\right) e^{\frac{t}{\tau}} \sin(\omega t) + y_0 e^{\frac{t}{\tau}} \cos(\omega t) \quad (50).$$

$$504 \quad \text{Since} \quad \left(X(t) - \frac{u_p}{\omega}\right)^2 + Y^2(t) = \left[\left(x_0 - \frac{u_p}{\omega}\right)^2 + y_0^2\right] e^{\frac{2t}{\tau}} \quad (51),$$

505 one can recognize a circle with an increasing radius and a stationary center. The rotation
506 along this circle is counter-clockwise. The physical parameters influencing the particle
507 trajectory remain the quantity (u_p/ω) and the centrifugal time τ .

508 **4 Discussion**

509
510
511
512 The particles trajectories predicted by Equ. (43) and (44) as well as Equ. (49)-(51) are in
513 excellent agreement with the experimental results of Pollack et al. [16] and Wolf and
514 Schwarz [33]. Pollack et al. [16] observed that in the rotating frame of reference,
515 microcarriers with density greater than the surrounding medium followed a circular motion
516 relative to the culture medium combined with a migration towards the outer wall of the
517 reactor. In the rotating frame, the direction of the gravitational force changes cyclically and

518 over a complete revolution of the chamber the particles experience an average gravitational
519 force about zero. Rotating bioreactors are thus said to simulate microgravity environment. In
520 their experiments polystyrene beads with a density of 1050 kg/m^3 and 0.5 mm diameter
521 radius were suspended in distilled water at 23°C . The bioreactor was rotated at 18 rpm. Their
522 results confirm that the microcarriers sedimentation velocity does not depend on ω , and is
523 the same as in free fall conditions.

524 Experiments by Wolf and Schwarz [33] examined parameters (gravitational strength, fluid
525 rotation rate, particle sedimentation rate, and particle initial position) within the useful range
526 for tissue cultures in NASA rotating wall culture bioreactors. They observed that the rotating
527 fluid effectively counters sedimentation. Biological tissue was simulated by nearly spherical
528 pieces of sponge suspended in water, with typical sizes of a few centimeters. The device
529 used was the NASA Slow Turning Lateral Vessel (STLV). Results from this group
530 demonstrate that the speed of the particle motion through the rotating fluid medium is the
531 same as its terminal sedimentation rate through a stationary fluid (for identical gravitational
532 conditions). They also demonstrate that the diameter of the nearly circular path is reduced
533 for the lower sedimentation rate and that it is increased for augmented gravitational
534 acceleration. They show that increasing the angular rotation rate from 8.64 r.p.m. to 17.7
535 r.p.m. induces a reduction of the diameter of the particle path.

536 In tissue engineering applications, the size of the suspended particles may change during the
537 culture due to cell proliferation and/or recruitment of additional cells into an aggregate,
538 causing an increase in sedimentation velocity by the square of the radius. To counteract the
539 increase in sedimentation velocity the speed of rotation may be augmented.

540 However from an experimental point of view, a low shear environment has to be maintained
541 during cell cultivation (especially in recellularisation experiments). From a theoretical point
542 of view, the mathematical descriptions presented in this paper are valid when the spheres and
543 the rotation rate are sufficiently small so that viscosity dominates and the Reynolds numbers
544 remain small. As explained in Section 3.2.2, for a 25ml/min perfusion flow and for a 7.5

545 micron particle radius, a representative value of the shear rate is $G_{\text{mean}} = 1.23 \text{ s}^{-1}$,
546 corresponding to 1.23 mPa shear stress. For the sake of comparison values reported by
547 Grimm et al. [22] are of order 180-320 mPa for 50 μm spherical beads, and 500 mPa for 3D
548 aggregates of BHK-21 cells, in a Rotating Wall Vessel (RWV).

549 In order to minimize mechanical damage to cultured cells optimal setting of the peristaltic
550 pump is required: choice of the tubing, low pump motor speed, minimized occlusion by the
551 roller heads. Complete filling of the chamber and solid body rotation of the culture medium
552 should also be achieved. The fluid thus rotates at the same angular velocity as the chamber
553 walls and thereby creates a laminar flow with minimal shear force. Complete filling of both
554 **Part A** and **Part B** of our device also minimizes the influence of the deformability and
555 porosity of the esophagus wall, that are not taken into account in the present theoretical
556 analysis. However Varley et al. [19] experimentally captured the flow velocity vectors in a
557 RWV bioreactor for cell culture under different speeds of rotation and different filling rates
558 (60%, 85%, 100%) and they concluded that 85% fill volume is an optimum condition as
559 regards cell oxygenation and proliferation . The presence of both fluid and air within the
560 chamber could increase the surface area for gas exchange. These authors do not address the
561 question of pressure and air compressibility.

562 One important limitation of the present study remains the entry length in the esophagus (
563 given in **Table 1**). If $Q = 25\text{ml}/\text{min}$, the entry length represents 41% of the esophagus total
564 length thus limiting the validity of the theoretical analysis to the remaining 59%. The length
565 of the tissue construct is thus important in such type of devices: the longer it will be, the
566 lower will be the relative importance of entry and exit flow perturbations. A numerical study
567 of the flow inside the RCCSmax bioreactor would allow to describe more precisely the
568 esophagus entry and outlet area (possible flow stagnation or cell accumulation).

569 **5 Conclusion**

570 The RCCS Synthecon bioreactor appears to be a convenient device for cell culture and
571 esophagus tissue engineering since it allows controlled mechanical stimulation, through the
572 combination of flow perfusion and rotation. Cells or particles are constantly maintained in
573 suspension in the media, which insures that nutrient, oxygen, and waste transfer will not be
574 limited by diffusion as they are in static culture systems. Forces that might damage cells are
575 minimized in this device and a low shear stress environment is created provided that the
576 perfusion rate and the rotation speed remain moderate ($\omega < 20$ rpm and $Q < 30$ ml/min).

577

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580

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582

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584

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586

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