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A Cellular Potts Model of single cell migration in presence of durotaxis

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*Abstract_* Cell migration is a fundamental biological phenomenon during which cells sense their surroundings and respond to different types of signals. In presence of durotaxis, cells preferentially crawl from soft to stiff substrates by reorganizing their cytoskeleton from an isotropic to an anisotropic distribution of actin filaments. In the present paper, we propose a Cellular Potts Model to simulate single cell migration over flat substrates with variable stiffness. We have tested five configurations: i) a substrate including a soft and a stiff region, ii) a soft substrate including two parallel stiff stripes, iii) a substrate made of successive stripes with increasing stiffness to create a gradient and iv) a stiff substrate with four embedded soft squares. For each simulation, we have evaluated the morphology of the cell, the distance covered, the spreading area and the migration speed. We have then compared the numerical results to specific experimental observations showing a consistent agreement.

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1. Introduction

Cell migration is a critical phenomenon occurring in several biological processes, such as morphogenesis [1], wound healing [2] and tumorogenesis [3]. It takes place in successive and cyclic steps [4] and it is triggered by specific interactions with the ExtraCellular Matrix (ECM). Actually, cell migration may occur in the absence of external signals thereby typically resulting in a random

walk. However, in most situations, cells are able to sense their surrounding environment and to respond for instance to chemical (i.e., chemotaxis) [5], electrical (i.e., electrotaxis) [6] or mechanical (i.e., mechanotaxis) [7] fields or yet to stiffness gradients (i.e., durotaxis) [8,9]. The latter mechanism consists of the cell preferential crawling from soft matrix substrates to stiffer ones, even in the absence of any additional directional cues [10,11]. By forming local protrusions (i.e., pseudopodia), the cells are in fact able to probe the mechanical properties of the surrounding environment and to more strongly adhere over stiff regions. Additionally, such behaviour results in a substantial reorganisation of the intracellular cytoskeleton. In fact, over soft substrates cells typically show an unstable and isotropic distribution of actin filaments, which are poorly extended and radially oriented, whereas over stiff substrates cell morphology is more stable and exhibits significant spreading and often anisotropic arrangements of actin filaments in the direction of migration (i.e., polarization) [12–16].

Although several computational models have been proposed in literature to investigate single cell migration, only few of them deal with durotaxis. Among others, it is worth to cite the work by Moreo et al. [17] who proposed a continuum approach based on an extension of the Hill's model for skeletal muscle behaviour to investigate cell response on two-dimensional (2D) substrates. They showed, in agreement with experimental observations, that cells seem to have the same behaviour when crawling on stiffer substrate and on pre-strained substrates. Harland et al. [18] instead represented a cell as a collection of stress fibres undergoing contraction and birth/death processes and showed that on stiff substrates cells exhibit durotaxis and stress fibres significantly elongate. Dokukina and Gracheva [19] developed a 2D discrete model of a viscoelastic

62	fibroblast cell using a Delaunay triangulation. At each node the balance of the
63	forces was determined by the contributions i) of the frictions between the cell and
64	the substrate, ii) of a passive viscoelastic force and iii) of an intrinsic active force.
65	The authors then evaluated cell behaviour over a substrate with a rigidity step in
66	good agreement with specific experimental observations. In fact, they found that
67	the cell preferentially moves on the stiffer substrate and turns away from the soft
68	substrate as reported by [8]. Stefanoni et al. [20] proposed a finite element
69	approach able to account for the local mechanical properties of the underneath
70	substrate and to analyze selected cell migratory determinants on two distinct
71	configurations: an isotropic substrate and a biphasic substrate (which consists of
72	two adjacent isotropic regions with different mechanical properties). Trichet et al.
73	[14] employed instead the active gel theory to demonstrate that cells preferentially
74	migrate over stiff substrates founding an optimal range of rigidity for a maximal
75	efficiency of cell migration. Further, in [21] a vertex-based approach (i.e., the so-
76	called Subcellular Element Model, SCE) was set to represent intracellular
77	cytoskeletal elements as well as their mechanical properties. In particular, the
78	dynamics of such subcellular domains were described by Langevin equations,
79	which account for a weak stochastic component (i.e., that mimic cytoplasmic
80	fluctuations) and elastic responses (i.e., modelled by generalized Morse
81	potentials) to both intracellular and intercellular biomechanical forces. The same
82	method was successfully applied in [22] for modelling substrate-driven bacteria
83	locomotion. Finally, in Allena and Aubry [23] a 2D mechanical model was
84	proposed to simulate cell migration over an heterogeneous substrate including
85	slipping regions and to show that over softer regions the cell slows down and is
86	less efficient.

87   In the present work, we describe a Cellular Potts Model (CPM, developed in

88   [24,25] and reviewed in [25–29]), which is a lattice-based stochastic approach

89   employing an energy minimization philosophy, to reproduce single cell migration

90   over flat substrates with different rigidity. In particular, we test four

91   configurations: i) a substrate including a soft and a stiff region, ii) a soft substrate

92   including two parallel stiff stripes, iii) a substrate made of successive stripes with

93   increasing stiffness to create a gradient and iv) a stiff substrate with four

94   embedded soft squares. For each scenario, we analyse cell behaviour in terms of

95   morphology, distance covered, spreading/adhesive area and migration speed in

96   order to capture the essential mechanisms of durotaxis. The computational

97   outcomes are then compared with specific experimental observations taken from

98   the existing literature.

99   The rest of this paper is organized as follows. In Section 2, we clarify the

100   assumptions on which our approach is based and present the model components.

101   The simulation results are then shown in Section 3. Finally, a justification of our

102   model choices as well as a discussion on possible improvements is proposed in

103   Section 4. Additionally, the article is equipped with an Appendix that deals with

104   statistics and parameter estimates.

105

106   ## 2. Mathematical model

107   The cell-substrate system is represented using a CPM environment [24,25].

108   The simulation domain is a three-dimensional (3D) regular lattice $\mathbb{W} \in \mathbb{R}^3$

109   constituted by identical closed grid sites, which are identified by their centre

110     $\mathbf{x} \in \mathbb{R}^3$ and labelled by an integer number $s(\mathbf{x}) \in \mathbb{N}$ (which can be interpreted as

111   a degenerate spin) [30,31]. The boundary of a generic site $\mathbf{x}$, one of its neighbours

112   and its overall neighbourhood are defined as $\P\boldsymbol{x}$, $\boldsymbol{x}^{\complement}$ and $\mathbb{W}'_{\boldsymbol{x}}$, respectively.

113   Subdomains with identical label ς form discrete objects S_{ς} (with border $\partial\Sigma_{\sigma}$),

114   which have an associated type $t\left(\mathsf{S}_{\varsigma}\right)$. In the case of our interest, $t = \boldsymbol{M}$ stands

115   for the medium, $t = \boldsymbol{C}$ for the cells and $t = \boldsymbol{S}_{i}$ for the i-th type of substrate. In this

116   respect, we anticipate that each type of matrix region will differ for stiffness and

117   therefore for adhesive affinity with moving individuals.

118   Cell dynamics result from an iterative and stochastic reduction of the energy of

119   the overall system, given by a Hamiltonian H (units: Kg·m^2/s^2), whose expression

120   will be clarified below. The employed algorithm is a modification of the

121   Metropolis method for Monte Carlo-Boltzmann dynamics [24,32], which is

122   particularly suitable to simulate the exploratory behaviour of biological

123   individuals as cells. Procedurally, at each time step t of the algorithm, called

124   Monte Carlo Step (MCS), a randomly chosen lattice site $\boldsymbol{x}_{source}$ belonging to a cell

125   tries to allocate its spin $\sigma(\boldsymbol{x}_{source})$ to one of its unlike neighbours $\boldsymbol{x}_{target} \in \mathbb{W}'_{\boldsymbol{x}}$,

126   which is also randomly selected. Then, the net energy difference $\mathsf{D}H$ due to the

127   proposed change of system configuration is calculated as

128
$$\left.\mathsf{D}H\right|_{\varsigma\left(\boldsymbol{x}_{source}\right)\to\varsigma\left(\boldsymbol{x}_{target}\right)} = H_{\left(after\ spin\ copy\right)} - H_{\left(before\ spin\ copy\right)} \qquad (1)$$

129   The trial spin update is finally validated by a Boltzmann-like probability function

130   defined as

131
$$P\left[\varsigma\left(\boldsymbol{x}_{source}\right)\to\varsigma\left(\boldsymbol{x}_{target}\right)\right](t) = \min\left\{1, e^{-\frac{\mathsf{D}H}{T_{C}}}\right\} \qquad (2)$$

132   where t is the actual MCS and $T_{C} \hat{\mathsf{I}} \ \mathsf{R}_{+}$ is a Boltzmann temperature, that has been

133   interpreted in several ways by CPM authors (see [33] for a comment on this

134   aspect). However, we here opt to give T_C the sense of a cell intrinsic motility (i.e.,

135   agitation rate), following the approach in [25]. Finally, it is useful to underline

136   that the matrix substrates are considered fixed and immutable.

137   As seen, the simulated system evolves to iteratively and stochastically reduce

138   its free energy, which is defined by a Hamiltonian function H which, for any

139   given time step t, reads

140
$$H(t) = H_{adhesion}(t) + H_{shape}(t) \quad (3)$$

141       $H_{adhesion}(t)$ is deduced from the Steinberg's Differential Adhesion Hypothesis

142   (DAH) [24,34] and is due to the adhesion between cells and extracellular

143   components (i.e., the medium or a given type of substrate). In particular, it reads

144
$$H_{adhesion}(t) = H_{adhesion}(t) = \sum_{(\partial \mathbf{x} \in \partial S_s) \subseteq (\partial \mathbf{x}' \in \partial S_{s'})} J_{t\left(S_{s(\mathbf{x})}\right), t\left(S_{s'(\mathbf{x})}\right)} \quad (4)$$

145   with $\mathbf{x}$ and $\mathbf{x}'$ two neighbouring sites and S_s and $S_{s'}$ two neighbouring objects

146   (with borders ∂S_s and $\partial S_{s'}$, respectively). $J_{t\left(S_{s(\mathbf{x})}\right), t\left(S_{s'(\mathbf{x})}\right)} \in R_+$ are constant and

147   homogeneous binding forces per unit area. They are symmetric with respect to

148   their indices and can be specified as follows:

149   - $J_{C,M}$ is the adhesive strength between the cells and the collagenous medium

150   which is constituted by a mixture of soluble adhesive ligands (i.e., carbohydrate

151   polymers and non-proteoglycan polysaccharides) and water solvent;

152   - J_{C,S_i} gives the adhesive strength between the cells and i-th type of substrate.

153   Recalling the minimization theory of the CPM, we assume that the stiffer the

154   substrate i, the lower the corresponding value J_{C,S_i} (i.e., the higher the adhesion

155   between the cells and the i-th type of substrate). This is a pivotal hypothesis of our

156   approach: it is consistent since it has been widely demonstrated in the

157 experimental literature that cells generate higher traction forces and generate more

158 stable focal adhesion points when migrating over stiffer substrates [16,35–38].

159 $H_{shape}(t)$ defines the geometrical attributes of each cell S_s, which are written

160 as elastic potentials as it follows:

$$H_{shape}(t) = H_{volume}(t) + H_{surface}(t)$$

161
$$= \sum_{S_s} \left[k_{S_s} \left(v_{S_s}(t) - V_C \right)^2 + n_{S_s}(t) \left(s_{S_s}(t) - S_C \right)^2 \right] \quad (5)$$

162 where $v_{S_s}(t)$ and $s_{S_s}(t)$ are the actual volume and surface of the cell S_s,

163 whereas V_C and S_C the corresponding cell characteristic measures in the initial

164 resting condition. k_{S_s} and $n_{S_s}(t)$ are instead two mechanical moduli in units of

165 energy. The former is linked to volume changes and, assuming that cells do not

166 significantly grow during migration, is considered constant with a high value (i.e.,

167 $k_{S_s} = k_C \gg 1$) for any individual S_s. The latter refers to the rigidity of a cell. As

168 we will explain in details later on, for each cell S_s, n_{S_s} is assumed to depend on

169 the underneath type of substrate. In particular, each cell decreases its initially high

170 (i.e., $\gg 1$) rigidity, thereby being more able to deform, if it comes in contact with

171 a stiff substrate. This assumption is consistent with experimental observations on

172 the fact that cell contact with stiff matrix regions activate downstream

173 intracellular pathways resulting in acto-myosin dynamics and therefore in

174 cytoskeletal remodelling [8,39]. More specifically, it seems that certain cells have

175 a binary sensor at their membrane junction sites that allows them to switch from a

176 relaxed and rounded morphology, when the substrate is softer than the cell's

177 elastic modulus [39–43], to a fan-shaped morphology with abundant stress fibres,

178 when the substrate is stiffer or as stiff as the cell itself [39]. Further, it has been

7

shown that cells tend to isotropically and poorly spread on soft substrates, whereas they form pseudopodia randomly distributed along the membrane on stiff substrates, resulting in a significant anisotropic spreading [16]. In this respect, according to several experimental observations [16,35–38,44], there exists a linear relationship between the adhesion forces exerted by the cell on the substrate and the spreading area of the cell. More specifically, the larger the contact area between the cell and the substrate, the higher the number of focal adhesion points that can be established. Nonetheless, the sequence of events is still unclear and two main processes may occur when a cell is seeded on a stiff substrate [45]:

i) the cell adheres because of the stiffness of the substrate, then it significantly spreads;

ii) the cell spreads because of the stiffness of the substrate, then it more strongly adheres.

Such uncertainty is the reason why in the present model both the adhesive parameters and the cell rigidity directly depend on the substrate stiffness, but are independent from each other.

The main components and the scales involved in the proposed model are summarized in the diagram in Fig. 1. Finally, all the parameters of the simulations are reported in Table 1, while the Appendix provides a careful explanation of how they have been estimated.

3. Numerical simulations

The characteristic size of each lattice site is 4 μm and the geometrical domain W is a 70x70x30 regular grid (280 μm x 280 μm x 120 μm) with no-flux boundary conditions in all directions. This choice mimics the situation of a

204   delimited experimental device, where cells are not able to overcome the physical

205   barriers. All our CPM cells are initially a hemisphere of a radius of 20 µm, whose

206   initial position will be specified for each simulation setting. A MCS is set to

207   correspond to 2 s of actual unit of time (see the Appendix for a comment on this

208   aspect), which results in simulations covering time intervals between 16 min to

209   5.5 h. This choice enables cells to migrate over sufficiently long paths in order to

210   compare numerical results and proper experimental observations. We have tested

211   several cell-matrix settings, which are presented in the followings. The resulting

212   simulations were performed on a modified version of the open source package

213   CompuCell3D (downloadable at www.compucell3d.org). In particular, a Phyton

214   script was specifically developed to account for substrate-dependent cell rigidity.

215

3.1   Cells preferentially crawl over stiff substrates

217   We first consider a substrate split into a soft and a stiff region, i.e., $t = S_1$ and

218      $t = S_2$ (see Fig. 2a). A cell S_1 is then seeded at the centre of the substrate and it

219   is allowed to move for 500 MCS (approximately 16 minutes). The rigidity of S_1,

220   n_{S_1}, has initially a high value $v_{\Sigma_1} = v_C = 25 \cdot 10^{-3}$ Kg/s^{2}m^2. However, it is allowed

221   to decrease, of 10^{-3} Kg/s^{2}m^2 for MCS until a threshold value n_t equal to 10^{-2}

222   Kg/s^{2}m^2, while the cell is in contact with the stiff region S_2, thereby leading to a

223   flattening of the initially rigid cellular hemisphere. In mathematical terms, we

224   indeed have that

$$v_{\Sigma_1}(t)$$

$$= \begin{cases} \max\left(v_{\Sigma_1}(t-1) - 10^{-3}; v_t\right) & \text{if } \exists(x, x' \in \Omega'_x): x \in \Sigma_1 \text{ and } x' \in S_2; \\ v_{\Sigma_1}(t-1) & \text{else,} \end{cases} \quad (6)$$

225   for each MCS.

226   We then study how cell behaviour is affected by variations in the ratio between

227   the adhesive affinity of the cell with either the soft or the stiff substrate region. In

228   particular, we keep fixed $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2 while decreasing the

229   value of J_{C,S_2} from $25 \cdot 10^{-15}$ Kg/s^2 to $1 \cdot 10^{-15}$Kg/s^2 (which is equal to J_{stiff},

230   the lowest value consistent with the case of our interest, see the Appendix). As

231   summarized in Fig. 3c, when J_{C,S_2} decreases, the cell is biased to crawl towards

232   the stiff domain, as it is confirmed by the plot of the trajectories of its center of

233   mass deriving from independent simulations. In fact, over a period of 500 MCS ($\approx$

234   16 minutes), the cell randomly moves around the substrate centre when $\frac{J_{C,S_1}}{J_{C,S_2}} = 1$

235   (Fig. 3a) while, when $\frac{J_{C,S_1}}{J_{C,S_2}} = 25$, the cell trajectories dramatically shift over the

236   stiff part of the substrate (Fig. 3b). Our numerical results are sustained and

237   consistent with the experimental observations according to which cells (i.e.,

238   fibroblasts, smooth muscle cells, Mesenchymal Stem Cells (MSCs)) crawl from

239   soft (1 to 5 kPa) to stiff (34 to 80 kPa) substrates (i.e., gels or polyacrylamide

240   sheets) [9–11,46]. Notably during motion towards the stiff substrate, our CPM

241   cell is also allowed to increase its remodeling ability, as its rigidity

242   ν_{Σ_1} progressively decreases upon contact with substrate S$_2$, according to Eq. (6).

243   In this respect, a further set of simulations evaluates cell morphological

244   differences due to the underneath type of substrate. Keeping the same domain as

245   in Fig. 2a, two cells, i.e., S$_1$ and S$_2$, are initially seeded in the middle of the soft

246   and the stiff regions, respectively. The rigidity of the two cells is then regulated

247   by Eq. (6). As reproduced in Fig. 4 (in particular, panel (a) represents the final cell

248   morphologies as resulted from a single representative simulation, whereas panel

249 (b) gives the mean final cell morphologies, as the plain ellipsoids derive from an

250 interpolation procedure of the cell adhesive areas coming from independent

251 simulations, see the Appendix for further details), both individuals do not

252 significantly move across the domain during a time lapse of 500 MCS

253 (approximately 16 min). However, the adhesive area of the cell located over the

254 soft region is almost 30% lower than the adhesive area of the cell that crawls over

255 the stiff substrate (Fig. 4c). Such a cell behaviour is consistent with the

256 experimental data by Lo and co-workers on 3T3 fibroblasts cultured on flexible

257 polyacrylamide sheets coated with type I collagen, where a transition in rigidity

258 was introduced by a discontinuity of the bis-acrylamide cross-linker, that resulted

259 in two substrate regions with Young's modulus equal to either 14 kPa and 30 kPa

260 [46]. In particular, on one hand, the value of the adhesive area of our CPM cell

261 seeded on the soft substrate is not surprisingly similar to the corresponding data

262 by Lo and co-workers [46], since we used such an experimental quantification for

263 our parameter estimate (see the Appendix). On the other hand, the adhesive area

264 of the CPM cell seeded on the stiff region is instead a completely independent and

265 self-emerging model outcome: therefore its consistency with the measurements by

266 Lo and colleagues [46] is relevant point of our work.

267

268 *3.2   Stiff vs soft substrate in the presence of an external cue*

269   For the second series of simulations we consider again a domain split into a

270 soft ($t = S_1$ such as $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2) and a stiff ($t = S_2$ such as

271 $J_{C,S_2} = J_{stiff} = 1 \cdot 10^{-15}$ Kg/s^2) region, but an additional external potential is

272 introduced. This results in an imposed artificial bias in the spin flip rate that is

273 able to affect the direction of cell migration. Entering more in details, the

274   expression of the Hamiltonian function presented in Eq. (3) is modified as it

275   follows

276    $$H(t) = H_{adhesion}(t) + H_{shape}(t) + H_{potential} \quad (7)$$

277   where $H_{potential} = -v_{ext}\left(\hat{x}_{target} - x_{source}\right)$ and v_{ext} is a vector whose components

278   determine the direction of the potential and whose modulus gives the relative

279   importance in the overall system energy. For the sake of simplicity, we assume

280   that the potential is constant in time and homogeneous throughout the entire

281   domain. As we will see later, it is in fact an artificial term that simply helps cells

282   to maintain a sustained directional movement. In this respect, what is relevant is

283   only its modulus, i.e., $|v_{ext}|$. We then test two configurations:

284   a) a cell S_1 placed at the south-east corner and the external potential directed

285   towards the north-west corner ;

286   b) the same cell Σ_1 placed at the south-west corner of the substrate and the

287   external potential directed towards the north-east corner.

288   In both cases, we set $|v_{ext}| = 7 \cdot 10^{-21} \text{Kg·m/s}^2$, which results in plausible cell

289   velocities (see later) and the simulations last 10000 MCS (approximately 5.5 h).

290   Further, cell rigidity is again regulated by Eq. (6).

291   In system configuration a) (see Movie 1 and Fig. 5a), the external cue guides the

292   cell towards the north-west corner of the domain. In particular, when a part of the

293   cell comes into contact with the stiffer substrate, it becomes the leading edge.

294   Further, the moving individual clearly accelerates as soon as it crosses the

295   boundary between the two matrix regions (3.6 µm/s versus 4.5 µm/s, Fig. 5c), as

296   experimentally observed in [8] for fibroblasts crawling over polyacrylamide

297   sheets. An increment of the adhesive area is observed as well when the cell shifts

298   over the stiff region.

299   In the case b), the external potential forces the cell to move towards the north-

300   east corner of the domain (see Movie 2 and Fig. 5b). However, as soon as the

301   individual approaches the soft region, it changes orientation, and starts moving

302   and elongating parallel to the boundary between the two substrate regions. These

303   outcomes may be compared to the experimental observations obtained by Lo et al.

304   in [46], who cultured fibroblasts on the already described substrate system, i.e.,

305   characterized by two areas with different Young's modulus. In particular, Lo and

306   colleagues seeded cells at low density to minimize the effects of intercellular

307   interactions and to avoid that pulling or pushing forces from neighbours

308   individuals may alter cell substrate probing processes (thereby impeding cells to

309   freely move across the soft and the stiff regions). Then, cell migration was

310   recorded over a time span of 10 h. Similarly to our numerical outcomes, the

311   authors found that as cells move towards a stiffer substrate, new lamellipodia are

312   formed in the direction of migration, thereby resulting in the dominant front end

313   of the individuals. On the opposite, local retractions occur when cells approach a

314   soft region, inducing therefore a change of direction. In a second series of

315   experiments, Lo and co-workers showed that mechanical inputs triggered by

316   substrate deformations might also control formation and retraction of

317   lamellipodia. In particular, they externally pulled or pushed the substrate away or

318   towards the cells centre to find that, due to the centripetal forces exerted by the

319   3T3 fibroblasts on the substrate [46], in the first case less motion is produced,

320   since cells experience a softening of the substrate, whereas in the second case the

321   overall motion is increased, since cells perceive the substrate as stiffer. In the

322   CPM model proposed here, the matrix substrates are not deformable, therefore the

323   numerical simulations are unable to capture the experimental observations coming

324   from this second set of assays.

325

326   *3.3   Two stiff stripes embedded in a soft substrate*

327   The third configuration that has been tested includes a soft substrate (again

328      $t = S_1$ with $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2) with two embedded stiff stripes (again

329      $t = S_2$ with $J_{C,S_2} = J_{stiff} = 1 \cdot 10^{-15}$ Kg/s^2), which are both 28 μm-wide (Fig. 2b).

330   A cell S_1 is initially seeded at the south-west corner, whose rigidity is allowed to

331   decrease following the constitutive law (Eq. (6)). An external potential is then

332   introduced towards the north-east corner of the domain: its intensity $|\boldsymbol{v}_{ext}|$ is

333   allowed to vary from a minimal value of $7 \cdot 10^{-21}$ Kg·m/s^2 to a maximal value of

334      $28 \cdot 10^{-21}$ Kg·m/s^2. All simulations last 10000 MCS, which correspond to nearly 5.5

335   h. In the case of a low $|\boldsymbol{v}_{ext}| = 7 \cdot 10^{-21}$ Kg·m/s^2 (see Fig. 6a and Movie 3), the cell

336   typically migrates towards the first stiff substrate stripe: then it remains stuck over

337   it and goes on migrating along such a matrix region. Furthermore, its morphology,

338   due to the dependency of its elasticity on the underneath type of substrate,

339   changes as the crawling individual acquires an elongated shape. Such a behaviour

340   is due to the fact that the external potential is too low to overcome the adhesive

341   interactions between the cell and the stiffest substrate: in particular, the individual

342   has not energetic benefits (deriving from the external bias) to move further in the

343   domain, i.e., to pass the first stiff stipe. The outcomes of our CPM are consistent

344   to that observed for cells (i.e., endothelial cells or fibrosarcoma cells) seeded on

2D substrates (i.e., maleic acid copolymer surfaces) structured with fibronectin stripes which orient their actin fibres along the stripe direction [47–49].

On the other hand, if the modulus of the external potential increases, we have a higher percentage of cells that are able to cross the entire domain (Fig. 6b). In particular, when $|v_{ext}|$ is maximal (i.e., $28 \cdot 10^{-21}$ Kg·m/s^2, Fig. 6c and Movie 4), the cells constantly migrate at the north-east corner of the domain passing also the second stiff stripe. In this case, the cell average velocity increases over the stiff stripes (about 4.4 µm/s) whereas it varies between 3.6 µm/s and 3.9 µm/s over the soft regions (Fig. 6d). With the maximal external potential, cell morphology does not significantly vary, as the moving individuals typically maintain an almost hemispheric shape, without substantial elongation or increments in the adhesive area during the entire motion. They in fact behave as translating rigid bodies, subjected to an external high force. This interesting behaviour is the consequence of the fact that the cells do not need to reorganize (nor have enough time to do it) to be able to crawl, as their motion is mainly due to the external bias: the specific substrate regions are only able to further accelerate (or partially slow down) cell movement, as previously commented. The numerical outcomes in the case of low or intermediate values of $|v_{ext}|$can be compared to those experimentally tested by Choi et al. [50] and Vincent et al. [51], where different cell phenotypes were seeded on micropatterned hydrogels with stiffness gradient. Although no external bias was introduced in such experimental configurations, a similar behaviour may be observed. In the former work [50], the authors proposed two mechanically-patterned hydrogels: one constituted by 100 µm stiff (10 kPa) and 500 µm soft (1 kPa) stripes and one containing 500 µm stiff (10 kPa) and 100 µm soft (1 kPa). First, Adipose-derived Stem Cells (ASCs) and C2C12 myoblasts were allowed to

370   adhere and both were able to sense the stiffness gradient and to migrate towards

371   the stiffer stripes (i.e., durotaxis) [46]. Such behaviour was also observed when

372   cells were far away from the stripe interface (around 250 µm). Nevertheless, since

373   cells only detect stiffness differences over short distances (around some microns)

374   [52], in this case the authors implied that the phenomenon was mostly due to

375   random walk towards the interface rather than to durotaxis itself. Regarding the

376   morphology of the cells, both ASCS and C2C12 myoblasts aligned in the

377   direction of the long axis of the stripe as we observe in our numerical simulations

378   (Fig. 5a and Movie 3) in the case of low intensity of the external potential.

379   Second, less contractile cells such as neurons were seeded on the hydrogels,

380   which did not show any preferential adhesion confirming previous experimental

381   observations according to which they prefer a softer niche [53].

382   In the latter work [51], the authors developed three types of polyacrylamide

383   (PA) hydrogel systems of stiffness gradients: physiological (1 Pa/µm),

384   pathological (10 Pa/µm) and step (100 Pa/µm). The step stiffness gradient, which

385   is the configuration of interest for the simulations presented above in this section,

386   was constituted by 500 µm wide regions of soft PA alternated with ~100 µm wide

387   stripes of stiff hydrogel producing a stripped stiffness profile. MSCs were plated

388   and they spread and attached independently of the gradient strength or the

389   stiffness within hours after the seeding, whereas after 3 days they started to

390   migrate towards stiffer regions. Additionally, cells crawled at 18 ± 0.7 µm/hr,

391   which is approximately 6-fold faster than on the other gradient configurations

392   discussed in the same paper (i.e. physiological and pathological) and confirms that

393   durotaxis velocity is influenced by gradient strength [11].

394

3.4   Stiffness gradient

In this section, we present the results for a simulation involving a substrate made of six successive stripes (i.e., $t = S_i$ where $i = 1,...,6$, each 46 μm-wide) which are organized to obtain a soft-to-stiff gradient from the left to the right side of the domain (from the red to the yellow subdomains). Such substrate regions are characterized by different cell adhesive affinity, i.e., J_{C,S_i}, which vary from $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2 to $J_{C,S_6} = J_{stiff} = 1 \cdot 10^{-15}$ Kg/s^2, respectively (see Fig. 2c and the corresponding caption for the specific details). A cell S_1 is initially seeded at the south-west corner and an external potential is introduced towards the north-east corner of the domain, whose magnitude $|\boldsymbol{v}_{ext}|$ is varied again from a minimal value of $7 \cdot 10^{-21}$ Kg·m/s^2 (Movie 5) to a maximal value of $28 \cdot 10^{-21}$ Kg·m/s^2 (Movie 6). The rigidity n_{S_1} of S_1 is allowed to decrease (from the usual initial high value of $v_C = 25 \cdot 10^{-3}$ Kg/s^{2}m^2) with a law analogous with Eq. (6), but which takes into account of the presence of different types of substrates, i.e.,

$$v_{\Sigma_1}(t) = \begin{cases} \max\left(v_{\Sigma_1}(t-1) - v_i; v_t\right) & \text{if } \exists(\boldsymbol{x}, \boldsymbol{x}' \in \Omega'_{\boldsymbol{x}}): \boldsymbol{x} \in \Sigma_1 \text{ and } \boldsymbol{x}' \in S_i; \\ v_{\Sigma_1}(t-1) & \text{else,} \end{cases} \quad (8)$$

where t is the actual MCS, n_t is the usual threshold value (equal to 10^{-2} Kg/s^{2}m^2) and $i = 2,...,6$. In this respect, $v_i = 0.05 \cdot 10^{-3}$, $0.06 \cdot 10^{-3}$, $0.1 \cdot 10^{-3}$, $0.2 \cdot 10^{-3}$, $1 \cdot 10^{-3}$ Kg/s^{2}m^2 while the cell is in contact with substrate S_2, S_3, S_4, S_5, S_6, respectively. n_{S_1} remains indeed constant and equal to v_C if the cell is located over the softest substrate S_1. All the resulting simulations last 10000 MCS (5.5 h). As reproduced in Fig. 7a, the percentage of cells able to reach the north-east corner increases

417   concomitantly with increments of $|\boldsymbol{v}_{ext}|$. Moreover, by fixing $|\boldsymbol{v}_{ext}| = 28 \cdot 10^{-21}$

418   Kg·m/s^2, it is possible to observe that the cell average velocity increases from 3.6

419   μm/s to 4.4 μm/s as they move from softer to stiffer substrates (Fig. 7b). This

420   result is coherent with the model outcomes of the previous set of simulations (i.e.,

421   see Fig. 6c and d), where we have observed that in the case of very high external

422   potential cells accelerate while crossing on stiffer matrix regions, even if they do

423   not significantly undergo morphological transitions.

424   A similar configuration was experimentally proposed by Cheung et al. [10]

425   who, using a microfluidics-based lithography technique, fabricated a

426   micropatterned cell-adhesive substrate made of a series of PEG-fibrinogen

427   hydrogels with uniform stiffness ranging from 0.7 to 50 kPa. Human Foreskin

428   Fibroblasts (HFFs) were then plated and their migratory trajectories were analysed

429   over 22 h. The authors found that the cells that were initially seeded on a stiffness

430   frontier tended to migrate towards the stiffer region, while cells plated on uniform

431   stiffness spread in both directions.

432

433   *3.5   Role of the characteristic dimension of the gradient stiffness*

434   The external potential introduced in most of the previous sets of simulations, is

435   an artificial term that is included in the Hamiltonial to bias and sustain cell

436   movement across the entire matrix substrate. In experimental assays, the

437   directional component in cell motion is typically established by geometrical cues,

438   such as microtracks and microchannels [54,55], or gradients of soluble or

439   insoluble chemical substances (chemotaxis and haptotaxis, respectively) or, in the

440   case of our interest, gradients of substrate stiffness [10,51]. However, we have

441   observed from our simulations that the sequence of different types of substrate

442   stripes employed in the previous section does not suffice to determine a persistent

443   cell movement across the entire matrix, since a high enough external potential has

444   to be included to allow cells reach the north-east corner of the domain (see the

445   plot in Fig. 7a). The reason of this discrepancy between computational and

446   experimental outcomes relies in the fact that "real cells", once established a

447   direction of movement, are able to dramatically orient their cytoskeleton (via the

448   polarization of actin filaments) and, eventually, start a persistent shape-dependent

449   locomotion. This way, real individuals are able to cross also large portions of

450   substrates without slowing down or changing direction. Such a cell behaviour can

451   not be captured in our approach since we do not include a proper model

452   component reproducing selected intracellular cytoskeletal dynamics (in this

453   respect, the interested reader may refer to [56,57], where polarization processes

454   and the subsequent cell persistent movements are simulated in CPMs either by

455   introducing an asymmetric correction to the Boltzman probability law or by

456   adding a further inertial term in the Hamiltonian). The CPM cells of our model are

457   only able to isotropically spread (due to decrements in their rigidity upon contact

458   with stiff substrates) or elongate following the geometry of the underlying matrix

459   region in order to maximize their adhesive interactions with the stiffer areas of the

460   domain (but only when the external potential is substantially low, see Fig. 6a and

461   c). However, the model presented in this paper can be used to predict if a

462   sustained cell motion can be achieved by only varying the geometrical

463   characteristics of the matrix substrate. With this purpose in mind, we employ the

464   same type of domain as in Section 3.4, but we progressively decrease the width of

465   the substrate stripes. We then evaluate the minimal magnitude of the external

466   potential needed by cells to reach the border of the domain opposite to their initial

467   position (again the south-west corner). Cell rigidity follows the law in Eq. [8] and

468   the simulations last 10000 MCS (5.5 h). As summarized in panel (a) of Fig. 8, we

469   can observe a tri-phasic behaviour. For sufficiently wide stripes (i.e., > 45 µm), a

470   cell sustained movement results only with very high external potentials (i.e.,

471   $>25 \cdot 10^{-21}$ Kg·m/s^2). Then, for lower stripe widths (i.e., in the range of 35-45 µm)

472   the critical value of the external potential modulus decreases almost linearly.

473   Finally, for low enough stripe widths (i.e., < 35 µm), the potential necessary to

474   have a sustained cell movement significantly drops, until becoming negligible for

475   stripe widths lower than 35 µm (Movie 7). Summing up, we can state that the

476   characteristic dimension of the stiffness gradient (here determined by the width of

477   the matrix stripes), which allows a persistent cell movement without the artificial

478   help of an external bias, is lower than the mean cell diameter (i.e., that in our

479   simulations is around 40-45 µm). From a computational viewpoint, the rationale

480   of this behaviour is that when a CPM cell is located on a given substrate stripe it

481   is however able to wandering its close proximity (due to the stochastic Metropolis

482   algorithm) which, if the stripe width is low enough, includes the neighbouring

483   matrix region. In this respect, the CPM cell simultaneously experiences the

484   adhesive affinity with a couple of neighbouring substrate stripes and then it moves

485   towards the stiffer one, thereby advancing across the domain. Such a process is

486   reiterated for all pairs of substrate stripes, thereby resulting in a sustained

487   directional movement. These results can be interpreted from an experimental

488   viewpoint as a prediction on the fact that cells may exhibit a persistent motion

489   also without an intracellular polarization, i.e., by only maintain an amoeboid

490   movement, if the substrate stiffness gradient is sufficiently fine-grained.

We finally conclude this section by analysing how cell velocity is affected by the wide of the substrate stripes, in the range of values sufficiently low to have a sustained cell crawling in the absence of an external potential (i.e., < 35 μm). As it is possible to see in panel (b) of Fig. 8, lower widths of the substrate regions (which means, as previously seen, more fine-grained stiffness gradients) results in increments in cell average velocity. From the computational viewpoint, this is due to the fact that the more the different stripes of the matrix are small, the more the previously described cell probing mechanism is facilitated and accelerated, thereby resulting in higher cell average velocities.

3.6   Soft squares embedded in a stiff substrate

As a final simulation, we test the substrate configuration in Fig. 2d, where four soft squares ($t = S_1$ with $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2) are embedded in a stiff substrate ($t = S_2$ with $J_{C,S_2} = J_{stiff} = 1 \cdot 10^{-15}$ Kg/s^2) at its three corners (north-west, north-east and south-east) and at the centre. A cell S_1 is initially seeded at the south-west corner and an external potential is introduced towards the north-east corner of the domain, whose magnitude $|v_{ext}|$ has been set equal to an intermediate $14 \cdot 10^{-21}$ Kg·m/s^2. As usual, cell rigidity is allowed to decrease according to Eq. (6) and the observation time is 10000 MCS (i.e., nearly 5.5. h). The cell starts moving in the direction determined by the potential with a trajectory of approximately 45° but, as soon as it encounters the central soft square, the cell avoids and circumvents it. As the original path is recovered, the cell needs to squeeze between the north-east square and the substrate frontier in order to achieve the target corner of the domain (Movie 8 and Fig. 9). The choice

of the migration track may depend on the initial position of the cell. In the present simulation, the cell is seeded along the substrate diagonal, thus the probability of circumventing the central soft square counter clockwise (as it happens here) or clockwise are the same. However, if the cell is seeded slightly downward and/or right, it will most certainly employ a counter clockwise trajectory, whereas if it is plated upward, it will probably follow a clockwise path. It is useful to notice that with a significantly higher modulus of the external potential the cell would have been able to pass across the soft regions, without deforming to avoid them, coherently with the simulations proposed in Fig. 6c.

This configuration is similar to that proposed in [23] where the cell must avoids two slipping regions in order to reach the external cue placed at 45°. Although the employed numerical approaches are substantially different, taken together the outcomes confirm the tendency of the cell to migrate over stiffer substrates where the higher adhesion forces may be developed.

4. Conclusions

In this paper we have proposed a three-dimensional CPM approach to simulate single cell migration over matrix domains in which soft and stiff regions are combined.

The CPM method is becoming an increasingly common technique for the mathematical modelling of a wide range of biological phenomena, including avascular and vascular tumor growth [58–61], gastrulation [62], skin pigmentation [63], yeast colony growth [64], stem cell differentiation [65], fruiting body formation of Dictyostelium discoideum [66], epidermal formation [67], hydra regeneration [66], retinal patterning [68], wound healing [69,70], biofilms [71],

chick limb-bud growth [72–74], cellular differentiation and growth of tissues, blood flow and thrombus development [75–77], angiogenesis [70,78–81], dynamics of vascular cells [82–85], cell scattering [86], cell migration on and within matrix environments [56,57,87]. Notably, in [88] the authors introduced a compartmentalized approach to subdivide a Myxococcus Xanthus into strings of subcellular domains with different rigidity, this in order to give the bacterium a particular geometry and to control its overall length. Further, in [89] a keratocyte has been represented with a set of undifferentiated hexagonal subunits, which has allowed to reproduce its polarization during motion. In this respect, it is useful to underline that, as commented in [25], although these approaches are correct, the fact that the proposed subcellular compartments do not have an immediate or direct correspondence with real subcellular elements, has limited the practicality and the usefulness of the relative models. The most accurate way of realistically reproducing different and extremely complex cell morphologies is to compartmentalize them according to the compartmentalization "suggested in nature", and thus to explicitly represent for instance the plasmamembrane (PM), the cytosolic region, the nucleus, and other intracellular organelles (e.g., mitochondria, ribosomes, Golgi apparatus, and secretory granules). This way is in fact possible, for example, to localize within the proper cell compartment selected biochemical pathways and/or to study the role play by the nucleus in cell movement, given its higher rigidity with respect to the surrounding cytoplasm [56,57,87].

Key benefits of the CPM energetic formalism are its simplicity and extensibility: almost any biological mechanism can in fact be included in the model, simply by adding an appropriate generalized potential term in the

Hamiltonian functional. For instance, it is possible to easily comprehend the importance of each mechanism involved in the simulated phenomenon by only altering the relative Potts parameter, so that the other terms in the Hamiltonian scale accordingly. In particular, by equating all the other terms to zero, it is possible to understand whether a mechanism is individually capable of producing the phenomenon of interest or whether it requires cooperative processes. Further critical features of the CPM (compared to alternative cell-based modelling approaches that represent biological individuals as point particles, such as Interacting Particle Systems or purely discrete models, or fixed-sized spheres or ellipsoids, such as Cellular Automata) is that i) it differentiates between bound and unbound regions of cell membranes and ii) morphological changes can be easily and realistically reproduced. These characteristics have been fundamental in our choice of using a CPM to describe the phenomenon of our interest since they are particularly suitable to implement our two main model assumptions, drawn according to the experimental observations reported in the literature: i) the adhesiveness of cells changes according to the substrate stiffness, that models the fact that higher traction forces and more stable focal points are generated over a stiffer substrate [16,35–38] and ii) each cell adapts its morphology as a function of the substrate stiffness so that over a soft region it maintains a rounded shape, whereas over a stiffer domain a significant spreading occurs [39–43]. The considerations above are in remarkable agreement with the scholarly dissertation proposed by Voss-Böhme in the conclusive section of her article [33]. She in fact argued that the application of CPMs is reasonable when the biological problem of interest involves "considerable variability in cell sizes and shapes", which is the case of the cell morphological transitions due to contact with soft/stiff substrates.

On the opposite, when "essentially isotropic, non-polarized cells of uniform size are considered", it would be preferable the use of more coarse-grained modelling approaches, like the already cited Cellular Automata or Interacting Particle Systems, which are better analysed both mechanistically and analytically.

Further, we have opted for a 3D setting since the adhesive interactions between cells and matrix substrates occur *under* the cell body (i.e., they are localized over the contact area between the cells themselves and the underneath substrate). In bi-dimensional CPMs cell-matrix interactions instead occur only "laterally", as the cells do not move *on* substrates but *within* the same plane as the matrix. Indeed, a three-dimensional domain is more appropriate to reproduce an adhesive-driven cell migration.

We have then used our CPM-based approach to test cell behaviour in different domain configurations, where soft and stiff substrates coexisted. In particular, the numerical outcomes have been consistently compared to specific experimental data, in terms of cell morphology, distance covered, spreading/adhesive area and migration speed. In this respect, following the dichotomy proposed in the already cited work by Voss-Böhme [33], we have interpreted our CPM as a *phenomenological* method. In particular, the resulting remarkable agreement (not only qualitative but also quantitative) between *in vitro* and *in silico* data has allowed us to conclude that our approach, although strongly simplified, was able to capture the main mechanisms underlying cell migration in presence of durotaxis. We have finally turned to use our model in a *predictive* manner, with the aim to analyse how the external potential and the critical dimensions of a substrate stiffness gradient (here represented by the width of the different types of matrix stripes) affect cell movement. In this respect, we have found that cells are

able to achieve a sustained cell migration in the absence of an external bias (and in the absence of intracellular polarization mechanisms) where the underneath matrix is characterized by a sufficient fine-grained gradient of rigidity.

However, our approach is not free of some serious shortcomings. First, it does not reproduce the active and continuous reorganization of the cytoskeleton, which provides the support for cells and mediates their coordinated and directed movements, mainly in response to mechanical tensions and stresses exchanged with the underneath substrate. In this respect, selected geometrical and mechanical properties of the cells, such their elongation and elasticity, should evolve according to a model of actin filament dynamics, which are powered, for example, by ATP (adenosine triphosphate) hydrolysis and controlled by inside-out signalling mechanisms transmitted from and by the extracellular matrix via focal adhesion points. Further, in our model, the substrates are not deformable and therefore it has not been possible to account how the matrix reacts to the probing processes exerted by crawling cells. Finally, it is useful to underline that our specific CPM application does not suffer of the limitation that Voss-Böhme proved to characterize most CPMs (see again [33]), i.e., cells die out in the long-run due to modifications in the original Metropolis algorithm. We have in fact focused on relatively short observation times: our model has indeed worked in a well-behaved parameter regime where the temporal evolution of the simulated system has been still directed towards the minimization of the Hamiltonian functional and the non-controlled, voter-like part of the lattice updates has been negligible.

Appendix

A.1   Morphological and migratory determinants

The *position* of a cell was established by the coordinate of its center of mass (CM). In particular, a cell was assumed to be located on a given type of substrate if its center of mass was located on that matrix region. In this respect, the migratory *trajectory* of a cell was generated by tracking the position of its center of mass at each time step (i.e., at each MCS).

The *adhesive area* of a cell was defined as the extension of its surface in contact with the substrate of interest at the final observation time.

The *average velocity* of an individual on a given type of substrate was measured as the ratio between the width of the substrate region itself (which is clarified for each simulation setting) and the time needed by the cell to cross it. In this respect, to obtain the amount of time spent by a cell to pass a given matrix region it is sufficient to multiply the corresponding average velocity for the width of the substrate of interest.

A.2   Statistics

In the plots, we represented cell trajectories coming from 10 independent and randomly chosen simulations. A number of 10 was chosen since we observed that it was sufficient to have a correct interpretation of the simulation outcomes but it was also low enough to have an acceptable graphical quality, as too many cell paths overlapped one to each other, thereby resulting undistinguishable.

Cell average velocity and adhesive area were instead given in the corresponding graphs as mean $\pm$ sd over 100 independent simulations.

662   In the plots representing the cell final distribution on the different types of

663   substrate, the relative frequency was given by the number of individuals that, over

664   100 independent simulations, were located over each matrix region at the end of

665   the observation time. Indeed, the sum of the relative frequencies is, in all cases,

666   equal to 100.

667   Finally, dashed and plain ellipsoids representing, respectively, initial and final

668   cell morphologies in a given simulation setting were established by interpolating

669   the cell adhesive areas coming from 10 independent simulations (typically the

670   ones used to track the cell trajectories for the same simulation setting). Obviously,

671   the initial cell position was the constant for each simulation setting, whereas the

672   initial cell shape was the same for all cases (i.e., an hemisphere of 20 μm of

673   radius).

674

675   *A.3   Parameter estimates*

676   Given the energetic nature of the CPM, a direct one-to-one correspondence

677   between model parameters and experimental quantities is not straightforward (as

678   commented also in [27] and in [90]). In particular, as explained in details in [33],

679   the CPM parameters can be subdivided in i) directly interpretable and measurable

680   quantities, such as cell geometrical dimensions ii) effective parameters that

681   subsume various cellular and subcellular properties, such as the adhesive strength

682   and the elastic moduli determining cell geometrical attributes iii) "merely

683   technical coefficients", such as the Boltzmann temperature, that has been

684   interpreted in different ways by CPM authors (in this work, we opted to link the

685   value of T_C to an intrinsic cell motility, i.e., the amplitude of cell boundary

686   fluctuations: consistently, we therefore added the subscript "C" to the coefficient

687   T_C). However, a plausible parameter setting was inferred by a proper comparison

688   with experimental findings, taking also advantage of selected sensitivity analysis

689   performed in other CPM-based works. First, the initial/target dimensions of our

690   virtual cells were consistent with the measures of NIH 3T3 mouse fibroblasts used

691   by Lo and colleagues [8] for their assays on durotaxis. Since, as previously seen,

692   we did not include in our model any nutrients and therefore cells were not allowed

693   to grow during migration, we set a high $\kappa_C = 25 \cdot 10^{-9}$ Kg/(s^{2}m^4) to keep cell

694   volume fluctuations within a few per cent. Such a specific value was taken from

695   other CPMs dealing both with single cell dynamics [82] and with multicellular

696   phenomena [91], where it was estimated after some trials. Further, observing from

697   the data by Lo et al. [46] that 3T3 cells seeded on soft enough substrates did not

698   significantly spread or undergo morphological transitions, we set a high value

699      $\nu_C = 25 \cdot 10^{-3}$ Kg/(s^{2}m^2) also for the intrinsic cell rigidity which, as previously

700   seen, can decreases (in our work) only upon cell contact with stiffer substrates.

701   This choice is consistent with other CPM-based approaches [57,70,82,91] that

702   employed similar values (i.e., ≥ 15) to model an initially low cell deformability.

703   We then turned to estimate both the Boltzmann temperature T_C and the cell-

704   substrate adhesiveness, denoted as J_{soft} thoughout the paper, in the case of the

705   softest matrix regions considered in this paper (i.e., the ones pseudo-coloured in

706   red in the simulations, typically identified by $\tau = S_1$). In particular, we looked for

707   the couple of coefficients (T_C, J_{soft}) that simultaneously best fitted the *in vitro*

708   results by Lo and co-workers in the corresponding experimental setting (i.e.,

709   collagen-coated polyacrylamide substrate properly manipulated to obtain a low

710   Young's modulus of 14 kPa) in terms of cell adhesive area (which was called by

711   Lo and colleagues "projected area" [46]). As it is possible to observe in Fig. 10,

there is a quite large range of values that matched experimental and computational

data (i.e., the yellow area of the graph): however, we opted for the intermediate

couple of coefficients $T_C = 50 \cdot 10^{-27}$ Kgm^2/s^2 and $J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2.

Decrements in cell-substrate adhesive strength and in cell rigidity, allowed in our

model as a consequence of cell contact with stiffer matrix regions, were then

performed until selected threshold values, i.e., $\nu_t = 10^{-2}$ Kg/s^{2}m^2 and $J_{stiff} = 1 \cdot$

10^{-15} Kg/s^2, respectively. In particular, ν_t was the lowest value of cell rigidity

that permitted to avoid unrealistic (often disconnected) cell shapes. J_{stiff}, i. e., the

adhesive force between cells and the stiffest substrates (the ones pseudo-coloured

in yellow in the simulation snapshots, typically labelled by $\tau = S_2$, except from

the case of the simulations dealing with the stiffness gradient) was instead the

lowest value for which cells did not start to slow down during migration. In fact,

as studied in details in [87], a too high cell-substrate adhesiveness partially

inhibits cell movement, as CPM cells are not able to detach from the matrix

component if the corresponding J-parameter is too low. Finally, the cell-medium

contact strength was set equal to J_{soft}(i. e., $= 25 \cdot 10^{-15}$ Kg/s^2). The rationale of

this choice relied in the fact that for lower values of $J_{C,M}$ cells detached from the

substrate and fluctuate in the middle of the medium, which was obviously an

unrealistic situation. On the opposite, too high values of $J_{C,M}$(i. e., $> 30 \cdot 10^{-15}$

Kg/s^2) forced cells to completely lay down on the matrix, in order to minimize

their contact surface with the medium, but also this situation was not plausible.

Finally, the correspondence between 1 MCS and 2 seconds of actual time was

taken from another CPM reproducing three-dimensional cell migration in matrix

environments [87]. Further, we observed that this setting resulted in a remarkable

accordance, in terms of cell velocity, between computational and experimental

737   results: our CPM cells in fact move at speeds in a range of (3, 5) μm/s, which is

738   consistent with the values measured by Vincent and colleagues [51] in the case of

739   MSCs plated on polyacrylamide (PA) hydrogels with selected stiffness.

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1097   **Figures, Movies and Tables**

1098   Fig. 1 Cell behaviour is determined by a modified Metropolis algorithm, which is based on a

1099   iterative and stochastic minimization of the cell-matrix system energy, defined by a Hamiltonian

1100   functional H. In particular, it includes energetic contributions for cell geometrical attributes and

1101   cell-substrate adhesive affinity. A Boltzmann-like law finally controls the likelihood of the

1102   acceptance of domain configuration updates, which is further biased by the intrinsic cell motility,

1103   established by parameter T_C.

1104   Fig. 2 Snapshots of the tested substrate configurations: (a) soft (red: t_{S_1} with $J_{C,S_1} = 25 \cdot 10^{-15}$

1105   Kg/s^2) and stiff (yellow: t_{S_2} with $J_{C,S_2} = 1 \cdot 10^{-15}$ Kg/ s^2) substrates, (b) soft (red: t_{S_1} with

1106 $J_{C,S_1} = 25 \cdot 10^{-15}$ Kg/ s^2) substrate with two stiff (yellow: t_{S_2} with $J_{C,S_2} = 1 \cdot 10^{-15}$ Kg/ s^2) stripes,

1107   (c) sequence of stripes with different stiffness (red: t_{S_1} with Kg/ s $J_{C,S_1} = 25 \cdot 10^{-15}$, dark orange:

1108 t_{S_2} with $J_{C,S_2} = 20 \cdot 10^{-15}$ Kg/ s^2, orange: t_{S_3} with $J_{C,S_3} = 15 \cdot 10^{-15}$ Kg/ s^2, light orange: t_{S_4} with

1109 $J_{C,S_4} = 10 \cdot 10^{-15}$ Kg/ s^2, dark yellow: t_{S_5} with $J_{C,S_5} = 5 \cdot 10^{-15}$ Kg/ s^2, yellow: t_{S_6} with $J_{C,S_6} =$

1110 $1 \cdot 10^{-15}$ Kg/ s^2), (d) stiff (yellow: t_{S_2} with $J_{C,S_2} = 1 \cdot 10^{-15}$ Kg/ s^2) substrate with embedded soft

1111   (red: t_{S_1} with $J_{C,S_1} = 25 10^{-15}$ Kg/ s^2) squares.

1112

1113   Fig. 3 Simulation for a substrate with soft (red) and stiff (yellow) regions. As the ratio $\frac{J_{C,S,1}}{J_{C,S,2}}$,

1114   increases, the cells are typically biased to migrate towards the stiff region (c). This is also

1115   confirmed by the trajectories of the cell centre of mass, which are relatively close to the centre of

1116   the substrate when $\frac{J_{C,S,1}}{J_{C,S,2}} = 1$ (a), whereas they are substantially shifted on the stiff region when

1117 $$\frac{J_{C,S,1}}{J_{C,S,2}} = 25 \text{ (b).}$$

1118

1119   Fig. 4 Two cells are initially seeded on a soft (red) and a stiff (yellow) substrate, respectively. (a)

1120   Simulation snapshot of the final positions (i.e., at MCS = 500 corresponding to nearly 16 minutes)

1121   of the two cells. (b) Initial (dashed) and final (plain) contour shapes give an idea of the position

1122   and the morphology of the two cells. (c) Cell adhesive area as a function of the type of substrate.

1123   The area is about 30% higher in the case of the cell seeded over the stiff substrate, due to the

1124   specific constitutive law given to cell rigidity (i.e., Eq. (6)).

1125

1126   Fig. 5 Simulation for a substrate with soft (red) and stiff (yellow) subdomains. The trajectories of

1127   the cell centre of mass as well as the initial (dashed) and the final (plain) cell contours are traced

1128 respectively for (a) a cell initially seeded at the south-east corner and an external potential

1129 introduced towards the north-west corner and (b) a cell initially seeded at the south-west corner

1130 and an external potential directed towards the north-east corner. (c) Cell average velocity over

1131 either the stiff and soft substrate.

1132

1133 Fig. 6 Configuration with a soft (red) substrate with two embedded stiff stripes (yellow). (a) and

1134 (c) Simulations with $|\boldsymbol{v}_{ext}| = 7 \cdot 10^{-21}$ Kg·m/s^2 and $|\boldsymbol{v}_{ext}| = 28 \cdot 10^{-21}$ Kg·m/s^2 respectively.

1135 Representative cell trajectories are plotted together with the initial (dashed) and the final (plain)

1136 cell contours. (b) Relative cell frequency as function of $|\boldsymbol{v}_{ext}|$. (d) Cell average velocity over the

1137 different substrate regions in the case of $|\boldsymbol{v}_{ext}| = 28 \cdot 10^{-21}$ Kg·m/s^2.

1138

1139 Fig. 7 Results for the simulation with a soft to stiff gradient (Sec. 3.4). (a) Relative cell frequency

1140 as $|\boldsymbol{v}_{ext}|$ increases. (b) Average cell velocity over the different substrate regions in case of $|\boldsymbol{v}_{ext}| =$

1141 $28 \cdot 10^{-21}$ Kg·m/s^2.

1142

1143 Fig. 8 Results for the simulations with a soft to stiff gradient (Sec. 3.5). (a) $|\boldsymbol{v}_{ext}|$ necessary to

1144 allow cells reach the opposite border of the domain vs width of substrate stripes. (b) Average cell

1145 velocity over the different substrate regions for different widths of the matrix stripes in case of a

1146 stiffness gradient sufficient fine-grained to have a cell persistent movement even in the absence of

1147 an external potential.

1148

1149 Fig. 9 Snapshots from a representative simulation dealing with a domain with four soft squares (red) embedded

1150 in a stiff substrate (yellow). The cell is initially seeded at the south-west corner and migrates in the direction of

1151 an external potential ($|\boldsymbol{v}_{ext}| = 14 \cdot 10^{-21}$ Kg·m/s^2), i.e., towards the north-east corner. Snapshots are taken at 2

1152 min (a), 30 min (b), 1.5 h (c), 2 h (d), 2.5 h (e), 3.5 h (f), 4.5 h (g) and 5.5 h (g).

1153

1154 Fig. 10 Cell adhesive area at 5.5 h, obtained from CPM simulations for different values both of the Boltzmann

1155 temperature T_C and of the adhesiveness between the cell and the softest substrate, i.e., J_{soft}. Values are given as

1156 the mean over 100 simulations. The experimental value measured by Lo and co-workers in corresponding

1157 conditions is $1.74 \cdot 10^3 \mu m^2$. From this plot, it was indeed possible to observe that the parameter region pseudo-

1158 colored in yellow gave the best fitting couples of coefficients. In particular, we opted for $T_C = 50 \cdot$

1159 10^{-27} Kgm^2/s^2 and $J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2.

1160

1161 Movie 1 Simulation of cell migration over a stiff-soft substrate (yellow = stiff region, red = soft

1162 region) in presence of an external potential directed towards the north-west corner (Sec. 3.2). The

1163 cell is initially seeded at the south-east corner.

1164

1165 Movie 2. Simulation of cell migration over a stiff-soft substrate (yellow = stiff region, red = soft

1166 region) in presence of an external potential directed towards the north-east corner (Sec. 3.2). The

1167 cell is initially seeded at the south-west corner.

1168

1169 Movie 3. Simulation of cell migration over a soft substrate (red) including two parallel stiff stripes

1170 (yellow) in presence of an external potential ($|\boldsymbol{v}_{ext}| = 7{\cdot}10^{-21}$ Kg·m/s^2) directed towards the north-

1171 east corner (Sec. 3.3). The cell is initially seeded at the south-west corner.

1172

1173 Movie 4. Simulation of cell migration over a soft substrate (red) including two parallel stiff stripes

1174 (yellow) in presence of an external potential ($|\boldsymbol{v}_{ext}| = 28{\cdot}10^{-21}$ Kg·m/s^2) directed towards the

1175 north-east corner (Sec. 3.3). The cell is initially seeded at the south-west corner.

1176

1177 Movie 5. Simulation of cell migration over a substrate with a soft-to-stiff gradient from the left to

1178 the right part of the domain (the cell-substrate adhesive affinities vary from $J_{soft} = 25{\cdot}10^{-15}$ Kg/s^2

1179 to $J_{stiff} = 1{\cdot}10^{-15}$ Kg/s^2) (Sec. 3.4). A low external potential ($|\boldsymbol{v}_{ext}| = 7{\cdot}10^{-21}$ Kg·m/s^2) is directed

1180 towards the north-east corner of the substrate, whereas the cell is seeded at the south-west corner.

1181

1182 Movie 6. Simulation of cell migration over a substrate with a soft-to-stiff gradient from the left to

1183 the right part of the domain (the cell-substrate adhesive affinities vary from $J_{soft} = 25{\cdot}10^{-15}$ Kg/s^2

1184 to $J_{stiff} = 1{\cdot}10^{-15}$ Kg/s^2) (Sec. 3.4). A high external potential ($|\boldsymbol{v}_{ext}| = 28{\cdot}10^{-21}$ Kg·m/s^2) is

1185   directed towards the north-east corner of the substrate, whereas the cell is seeded at the south-west

1186   corner.

1187

1188   Movie 7. Simulation of cell migration over a substrate with a soft-to-stiff gradient from the left to the right part

1189   of the domain (the cell-substrate adhesive affinities vary from $J_{soft} = 25 \cdot 10^{-15}$ Kg/s^2 to $J_{stiff} = 1 \cdot 10^{-15}$ Kg/s^2).

1190   Here, the width of the stripes has been highly reduced (i.e., = 28 μm) and no external potential is introduced

1191   (Sec. 3.5).

1192

1193   Movie 8. Simulation of cell migration over a stiff substrate (yellow) with four embedded soft

1194   squares (red) (Sec. 3.6). The cell is initially seeded at the south-west corner and an external

1195   potential ($|\boldsymbol{v}_{ext}| = 14 \cdot 10^{-21}$ Kg·m/s^2) is directed towards the north-east corner of the domain.

1196

1197   Table 1 Main parameters of the model.