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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.

Keywords_ Cell migration, Durotaxis, Cell polarity, Anisotropy, CPM

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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 tumorigenesis [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

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

51 Although several computational models have been proposed in literature to
52 investigate single cell migration, only few of them deal with durotaxis. Among
53 others, it is worth to cite the work by Moreo et al. [17] who proposed a continuum
54 approach based on an extension of the Hill's model for skeletal muscle behaviour
55 to investigate cell response on two-dimensional (2D) substrates. They showed, in
56 agreement with experimental observations, that cells seem to have the same
57 behaviour when crawling on stiffer substrate and on pre-strained substrates.
58 Harland et al. [18] instead represented a cell as a collection of stress fibres
59 undergoing contraction and birth/death processes and showed that on stiff
60 substrates cells exhibit durotaxis and stress fibres significantly elongate.
61 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 $W \in \mathbb{R}^3$
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 $s(\mathbf{x}) \in N$ (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 $\mathfrak{N}(\mathbf{x}, \mathbf{x}')$ and $W'_{\mathbf{x}}$, respectively.
 113 Subdomains with identical label S form discrete objects S_s (with border $\partial\Sigma_\sigma$),
 114 which have an associated type $t(S_s)$. In the case of our interest, $t = M$ stands
 115 for the medium, $t = C$ for the cells and $t = S_i$ 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: $\text{Kg}\cdot\text{m}^2/\text{s}^2$), 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 \mathbf{x}_{source} belonging to a cell
 125 tries to allocate its spin $\sigma(\mathbf{x}_{source})$ to one of its unlike neighbours $\mathbf{x}_{target} \in W'_{\mathbf{x}}$,
 126 which is also randomly selected. Then, the net energy difference DH due to the
 127 proposed change of system configuration is calculated as

$$128 \quad DH \Big|_{S(\mathbf{x}_{source}) \rightarrow S(\mathbf{x}_{target})} = H_{(after\ spin\ copy)} - H_{(before\ spin\ copy)} \quad (1)$$

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

$$131 \quad P[S(\mathbf{x}_{source}) \rightarrow S(\mathbf{x}_{target})](t) = \min \left\{ 1, e^{-\frac{DH}{T_c}} \right\} \quad (2)$$

132 where t is the actual MCS and $T_c \hat{=} R_+$ 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

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

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

$$144 \quad H_{adhesion}(t) = H_{adhesion}(t) = \frac{\dot{a}}{(\|\mathbf{x}_i\| \|\mathbf{S}_s\|) \zeta (\|\mathbf{x}_i\| \|\mathbf{S}_{st}\|)} \mathbf{J}_{t(S_s(\mathbf{x}), t(S_{st}(\mathbf{x}))} \quad (4)$$

145 with \mathbf{x} and \mathbf{x}^c two neighbouring sites and S_s and S_{st} two neighbouring objects
 146 (with borders $\|\mathbf{S}_s$ and $\|\mathbf{S}_{st}$, respectively). $\mathbf{J}_{t(S_s(\mathbf{x}), t(S_{st}(\mathbf{x}))}$ and $\hat{\Gamma} R_{\mp}$ 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:

$$\begin{aligned}
 H_{shape}(t) &= H_{volume}(t) + H_{surface}(t) \\
 &= \frac{1}{2} k_{S_s} (v_{S_s}(t) - V_C)^2 + n_{S_s}(t) (s_{S_s}(t) - S_C)^2 \quad (5)
 \end{aligned}$$

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

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

- 188 i) the cell adheres because of the stiffness of the substrate, then it significantly
189 spreads;
- 190 ii) the cell spreads because of the stiffness of the substrate, then it more strongly
191 adheres.

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

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

199

200 **3. Numerical simulations**

201 The characteristic size of each lattice site is $4\ \mu\text{m}$ and the geometrical domain
202 W is a $70 \times 70 \times 30$ regular grid ($280\ \mu\text{m} \times 280\ \mu\text{m} \times 120\ \mu\text{m}$) with no-flux
203 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 Python
 214 script was specifically developed to account for substrate-dependent cell rigidity.

215

216 *3.1 Cells preferentially crawl over stiff substrates*

217 We first consider a substrate split into a soft and a stiff region, i.e., $t = \mathcal{S}_1$ and
 218 $t = \mathcal{S}_2$ (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 $v_{\Sigma_1} = v_C = 25 \cdot 10^{-3} \text{ Kg/s}^2\text{m}^2$. However, it is allowed
 221 to decrease, of $10^{-3} \text{ Kg/s}^2\text{m}^2$ for MCS until a threshold value n_t equal to 10^{-2}
 222 $\text{Kg/s}^2\text{m}^2$, 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

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

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 $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$ while decreasing the
229 value of J_{C,S_2} from $25 \cdot 10^{-15} \text{ Kg/s}^2$ to $1 \cdot 10^{-15} \text{ Kg/s}^2$ (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 v_{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 ($t = \mathfrak{S}_1$ such as $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$) and a stiff ($t = \mathfrak{S}_2$ such as
271 $J_{C,S_2} = J_{stiff} = 1 \cdot 10^{-15} \text{ Kg/s}^2$) 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

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

277 where $H_{potential} = -v_{ext}(\mathbf{x}_{target} - \mathbf{x}_{source})$ 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 $|v_{ext}| = 7 \cdot 10^{-21} \text{Kg}\cdot\text{m}/\text{s}^2$, 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 $\mu\text{m}/\text{s}$ versus 4.5 $\mu\text{m}/\text{s}$, 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 $t = \mathfrak{S}_1$ with $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$) with two embedded stiff stripes (again
329 $t = \mathfrak{S}_2$ with $J_{C,S_2} = J_{stiff} = 1 \cdot 10^{-15} \text{ Kg/s}^2$), 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 $|\mathbf{v}_{ext}|$ is
333 allowed to vary from a minimal value of $7 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$ to a maximal value of
334 $28 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$. All simulations last 10000 MCS, which correspond to nearly 5.5
335 h. In the case of a low $|\mathbf{v}_{ext}| = 7 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$ (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

345 2D substrates (i.e., maleic acid copolymer surfaces) structured with fibronectin
346 stripes which orient their actin fibres along the stripe direction [47–49].

347 On the other hand, if the modulus of the external potential increases, we have a
348 higher percentage of cells that are able to cross the entire domain (Fig. 6b). In
349 particular, when $|\boldsymbol{v}_{ext}|$ is maximal (i.e., $28 \cdot 10^{-21}$ Kg·m/s², Fig. 6c and Movie 4),
350 the cells constantly migrate at the north-east corner of the domain passing also the
351 second stiff stripe. In this case, the cell average velocity increases over the stiff
352 stripes (about 4.4 $\mu\text{m/s}$) whereas it varies between 3.6 $\mu\text{m/s}$ and 3.9 $\mu\text{m/s}$ over the
353 soft regions (Fig. 6d). With the maximal external potential, cell morphology does
354 not significantly vary, as the moving individuals typically maintain an almost
355 hemispheric shape, without substantial elongation or increments in the adhesive
356 area during the entire motion. They in fact behave as translating rigid bodies,
357 subjected to an external high force. This interesting behaviour is the consequence
358 of the fact that the cells do not need to reorganize (nor have enough time to do it)
359 to be able to crawl, as their motion is mainly due to the external bias: the specific
360 substrate regions are only able to further accelerate (or partially slow down) cell
361 movement, as previously commented. The numerical outcomes in the case of low
362 or intermediate values of $|\boldsymbol{v}_{ext}|$ can be compared to those experimentally tested by
363 Choi et al. [50] and Vincent et al. [51], where different cell phenotypes were
364 seeded on micropatterned hydrogels with stiffness gradient. Although no external
365 bias was introduced in such experimental configurations, a similar behaviour may
366 be observed. In the former work [50], the authors proposed two mechanically-
367 patterned hydrogels: one constituted by 100 μm stiff (10 kPa) and 500 μm soft (1
368 kPa) stripes and one containing 500 μm stiff (10 kPa) and 100 μm soft (1 kPa).
369 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 $\mu\text{m/hr}$,
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].

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3.4 Stiffness gradient

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In this section, we present the results for a simulation involving a substrate made of six successive stripes (i.e., $t = \mathcal{S}_i$ where $i = 1, \dots, 6$, 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 $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$ to $J_{C,S_6} = J_{stiff} = 1 \cdot 10^{-15} \text{ Kg/s}^2$, 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 $|\mathbf{v}_{ext}|$ is varied again from a minimal value of $7 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$ (Movie 5) to a maximal value of $28 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$ (Movie 6). The rigidity η_{S_1} of S_1 is allowed to decrease (from the usual initial high value of $\nu_C = 25 \cdot 10^{-3} \text{ Kg/s}^2\text{m}^2$) with a law analogous with Eq. (6), but which takes into account of the presence of different types of substrates, i.e.,

$$\nu_{\Sigma_1}(t) = \begin{cases} \max(\nu_{\Sigma_1}(t-1) - \nu_i; \nu_t) & \text{if } \exists (\mathbf{x}, \mathbf{x}' \in \Omega'_x): \mathbf{x} \in \Sigma_1 \text{ and } \mathbf{x}' \in S_i; \\ \nu_{\Sigma_1}(t-1) & \text{else,} \end{cases} \quad (8)$$

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where t is the actual MCS, η_t is the usual threshold value (equal to $10^{-2} \text{ Kg/s}^2\text{m}^2$) and $i = 2, \dots, 6$. In this respect, $\nu_i = 0.05 \cdot 10^{-3}, 0.06 \cdot 10^{-3}, 0.1 \cdot 10^{-3}, 0.2 \cdot 10^{-3}, 1 \cdot 10^{-3} \text{ Kg/s}^2\text{m}^2$ while the cell is in contact with substrate S_2, S_3, S_4, S_5, S_6 , respectively. η_{S_1} remains indeed constant and equal to ν_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 $|\mathbf{v}_{ext}|$. Moreover, by fixing $|\mathbf{v}_{ext}| = 28 \cdot 10^{-21}$
418 $\text{Kg}\cdot\text{m}/\text{s}^2$, it is possible to observe that the cell average velocity increases from 3.6
419 $\mu\text{m}/\text{s}$ to 4.4 $\mu\text{m}/\text{s}$ 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 Hamiltonian 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., $> 45 \mu\text{m}$), a
470 cell sustained movement results only with very high external potentials (i.e.,
471 $> 25 \cdot 10^{-21} \text{ Kg}\cdot\text{m}/\text{s}^2$). 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., $< 35 \mu\text{m}$), 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.

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

500

501 3.6 *Soft squares embedded in a stiff substrate*

502 As a final simulation, we test the substrate configuration in Fig. 2d, where four
503 soft squares ($t = \mathfrak{S}_1$ with $J_{C,S_1} = J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$) are embedded in a stiff
504 substrate ($t = \mathfrak{S}_2$ with $J_{C,S_2} = J_{stiff} = 1 \cdot 10^{-15} \text{ Kg/s}^2$) at its three corners (north-
505 west, north-east and south-east) and at the centre. A cell S_1 is initially seeded at
506 the south-west corner and an external potential is introduced towards the north-
507 east corner of the domain, whose magnitude $|\mathbf{v}_{ext}|$ has been set equal to an
508 intermediate $14 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$. As usual, cell rigidity is allowed to decrease
509 according to Eq. (6) and the observation time is 10000 MCS (i.e., nearly 5.5. h).
510 The cell starts moving in the direction determined by the potential with a
511 trajectory of approximately 45° but, as soon as it encounters the central soft
512 square, the cell avoids and circumvents it. As the original path is recovered, the
513 cell needs to squeeze between the north-east square and the substrate frontier in
514 order to achieve the target corner of the domain (Movie 8 and Fig. 9). The choice

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

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

529

530 **4. Conclusions**

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

534 The CPM method is becoming an increasingly common technique for the
535 mathematical modelling of a wide range of biological phenomena, including
536 avascular and vascular tumor growth [58–61], gastrulation [62], skin pigmentation
537 [63], yeast colony growth [64], stem cell differentiation [65], fruiting body
538 formation of *Dictyostelium discoideum* [66], epidermal formation [67], hydra
539 regeneration [66], retinal patterning [68], wound healing [69,70], biofilms [71],

540 chick limb-bud growth [72–74], cellular differentiation and growth of tissues,
541 blood flow and thrombus development [75–77], angiogenesis [70,78–81],
542 dynamics of vascular cells [82–85], cell scattering [86], cell migration on and
543 within matrix environments [56,57,87]. Notably, in [88] the authors introduced a
544 compartmentalized approach to subdivide a *Myxococcus Xanthus* into strings of
545 subcellular domains with different rigidity, this in order to give the bacterium a
546 particular geometry and to control its overall length. Further, in [89] a keratocyte
547 has been represented with a set of undifferentiated hexagonal subunits, which has
548 allowed to reproduce its polarization during motion. In this respect, it is useful to
549 underline that, as commented in [25], although these approaches are correct, the
550 fact that the proposed subcellular compartments do not have an immediate or
551 direct correspondence with real subcellular elements, has limited the practicality
552 and the usefulness of the relative models. The most accurate way of realistically
553 reproducing different and extremely complex cell morphologies is to
554 compartmentalize them according to the compartmentalization “suggested in
555 nature”, and thus to explicitly represent for instance the plasmamembrane (PM),
556 the cytosolic region, the nucleus, and other intracellular organelles (e.g.,
557 mitochondria, ribosomes, Golgi apparatus, and secretory granules). This way is in
558 fact possible, for example, to localize within the proper cell compartment selected
559 biochemical pathways and/or to study the role play by the nucleus in cell
560 movement, given its higher rigidity with respect to the surrounding cytoplasm
561 [56,57,87].

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

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

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

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

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

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

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

638 **Appendix**

639 *A.1 Morphological and migratory determinants*

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

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

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

653

654 *A.2 Statistics*

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

660 Cell average velocity and adhesive area were instead given in the
661 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 $\kappa_C = 25 \cdot 10^{-9} \text{ Kg}/(\text{s}^2\text{m}^4)$ 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 $\nu_C = 25 \cdot 10^{-3} \text{ Kg}/(\text{s}^2\text{m}^2)$ also for the intrinsic cell rigidity which, as previously
700 seen, can decrease (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} throughout 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,

712 there is a quite large range of values that matched experimental and computational
713 data (i.e., the yellow area of the graph): however, we opted for the intermediate
714 couple of coefficients $T_C = 50 \cdot 10^{-27} \text{ Kg m}^2/\text{s}^2$ and $J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$.
715 Decrements in cell-substrate adhesive strength and in cell rigidity, allowed in our
716 model as a consequence of cell contact with stiffer matrix regions, were then
717 performed until selected threshold values, i.e., $\nu_t = 10^{-2} \text{ Kg/s}^2 \text{ m}^2$ and $J_{stiff} = 1 \cdot$
718 10^{-15} Kg/s^2 , respectively. In particular, ν_t was the lowest value of cell rigidity
719 that permitted to avoid unrealistic (often disconnected) cell shapes. J_{stiff} , i. e., the
720 adhesive force between cells and the stiffest substrates (the ones pseudo-coloured
721 in yellow in the simulation snapshots, typically labelled by $\tau = S_2$, except from
722 the case of the simulations dealing with the stiffness gradient) was instead the
723 lowest value for which cells did not start to slow down during migration. In fact,
724 as studied in details in [87], a too high cell-substrate adhesiveness partially
725 inhibits cell movement, as CPM cells are not able to detach from the matrix
726 component if the corresponding J-parameter is too low. Finally, the cell-medium
727 contact strength was set equal to J_{soft} (i. e., $= 25 \cdot 10^{-15} \text{ Kg/s}^2$). The rationale of
728 this choice relied in the fact that for lower values of $J_{C,M}$ cells detached from the
729 substrate and fluctuate in the middle of the medium, which was obviously an
730 unrealistic situation. On the opposite, too high values of $J_{C,M}$ (i. e., $> 30 \cdot 10^{-15}$
731 Kg/s^2) forced cells to completely lay down on the matrix, in order to minimize
732 their contact surface with the medium, but also this situation was not plausible.
733 Finally, the correspondence between 1 MCS and 2 seconds of actual time was
734 taken from another CPM reproducing three-dimensional cell migration in matrix
735 environments [87]. Further, we observed that this setting resulted in a remarkable
736 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) $\mu\text{m/s}$, 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 $J_{C,S_2} = 1 \cdot 10^{-15} \text{Kg/s}^2$) substrates, (b) soft (red: t_{S_1} with
 1106 $J_{C,S_1} = 25 \cdot 10^{-15} \text{Kg/s}^2$) substrate with two stiff (yellow: t_{S_2} with $J_{C,S_2} = 1 \cdot 10^{-15} \text{Kg/s}^2$) stripes,
 1107 (c) sequence of stripes with different stiffness (red: t_{S_1} with $\text{Kg/s} J_{C,S_1} = 25 \cdot 10^{-15}$, dark orange:
 1108 t_{S_2} with $J_{C,S_2} = 20 \cdot 10^{-15} \text{Kg/s}^2$, orange: t_{S_3} with $J_{C,S_3} = 15 \cdot 10^{-15} \text{Kg/s}^2$, light orange: t_{S_4} with
 1109 $J_{C,S_4} = 10 \cdot 10^{-15} \text{Kg/s}^2$, dark yellow: t_{S_5} with $J_{C,S_5} = 5 \cdot 10^{-15} \text{Kg/s}^2$, yellow: t_{S_6} with $J_{C,S_6} =$
 1110 $1 \cdot 10^{-15} \text{Kg/s}^2$), (d) stiff (yellow: t_{S_2} with $J_{C,S_2} = 1 \cdot 10^{-15} \text{Kg/s}^2$) substrate with embedded soft
 1111 (red: t_{S_1} with $J_{C,S_1} = 25 \cdot 10^{-15} \text{Kg/s}^2$) squares.

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 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 $|\mathbf{v}_{ext}| = 7 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$ and $|\mathbf{v}_{ext}| = 28 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$ 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 $|\mathbf{v}_{ext}|$. (d) Cell average velocity over the
1137 different substrate regions in the case of $|\mathbf{v}_{ext}| = 28 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$.

1138

1139 Fig. 7 Results for the simulation with a soft to stiff gradient (Sec. 3.4). (a) Relative cell frequency
1140 as $|\mathbf{v}_{ext}|$ increases. (b) Average cell velocity over the different substrate regions in case of $|\mathbf{v}_{ext}| =$
1141 $28 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$.

1142

1143 Fig. 8 Results for the simulations with a soft to stiff gradient (Sec. 3.5). (a) $|\mathbf{v}_{ext}|$ 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 ($|\mathbf{v}_{ext}| = 14 \cdot 10^{-21} \text{ Kg}\cdot\text{m/s}^2$), 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 $1.74 \cdot 10^3 \mu\text{m}^2$. 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 $10^{-27} \text{ Kg m}^2/\text{s}^2$ and $J_{\text{soft}} = 25 \cdot 10^{-15} \text{ Kg/s}^2$.

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 ($|\mathbf{v}_{ext}| = 7 \cdot 10^{-21} \text{ Kg} \cdot \text{m/s}^2$) 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 ($|\mathbf{v}_{ext}| = 28 \cdot 10^{-21} \text{ Kg} \cdot \text{m/s}^2$) 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 $J_{\text{soft}} = 25 \cdot 10^{-15} \text{ Kg/s}^2$
1179 to $J_{\text{stiff}} = 1 \cdot 10^{-15} \text{ Kg/s}^2$) (Sec. 3.4). A low external potential ($|\mathbf{v}_{ext}| = 7 \cdot 10^{-21} \text{ Kg} \cdot \text{m/s}^2$) 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 $J_{\text{soft}} = 25 \cdot 10^{-15} \text{ Kg/s}^2$
1184 to $J_{\text{stiff}} = 1 \cdot 10^{-15} \text{ Kg/s}^2$) (Sec. 3.4). A high external potential ($|\mathbf{v}_{ext}| = 28 \cdot 10^{-21} \text{ Kg} \cdot \text{m/s}^2$) 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 $J_{soft} = 25 \cdot 10^{-15} \text{ Kg/s}^2$ to $J_{stiff} = 1 \cdot 10^{-15} \text{ Kg/s}^2$).

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

1196

1197 Table 1 Main parameters of the model.