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A three dimensional model of multicellular aggregate compression.

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Multicellular aggregates are an excellent model system to explore the role of tissue biomechanics, which has been demonstrated to play a crucial role in many physiological and pathological processes. In this paper, we propose a three-dimensional mechanical model and apply it to the uniaxial compression of a multicellular aggregate in a realistic biological setting. In particular, we consider an aggregate of initially spherical shape and describe both its elastic deformations and the reorganisation of cells forming the spheroid. The latter phenomenon, understood as remodelling, is accounted for by assuming that the aggregate undergoes plastic-like distortions. The study of the compression of the spheroid, achieved by means of two parallel, compressive plates, needs the formulation of a contact problem between the living spheroid itself and the plates, and is solved with the aid of the augmented Lagrangian Method. The results of the performed numerical simulations are in qualitative agreement with the biological observations reported in the literature and can also be used to estimate quantitatively some fundamental aggregate mechanical parameters.

1 Introduction

Multicellular aggregates, and specifically multicellular 2 3 spheroids (MCS), represent one of the most valid in vitro systems to study the dynamics of multicellular three-dimensional systems, 4 being an intermediate step between monolayer growing cells and 5 tissue culture^{1,2}. In particular, living spheroids, made up of ei-6 ther healthy or malignant cells up to a size of 100-600 μm , are 7 rather simple to prepare and well mimic in vivo phenomena oc-8 curring inside tissues and organs, encompassing growth, struc-9 tural reorganisation, cell-cell and cell-extracellular environment 10 interactions, response to external and endogenous stimuli, em-11 bryogenesis, malignant invasion, wound healing, and tissue en-12 gineering^{1,3–8}. Furthermore, multicellular aggregates are an ex-13 cellent model system to explore the role of tissue biomechanics, 14 which has been demonstrated to play a crucial role in many phys-15 iological and pathological conditions. For instance, even though 16 embryogenesis and morphogenesis (i.e. the complex set of events 17 through which a living organism acquires its final shape) are un-18 der genetic control, genes by themselves do not create forms and 19

shapes. This is achieved by physical forces, which drive structure formation in a delicate interplay of genetic, molecular and 21 physical factors⁹. In the same way, cancer cell invasion and the 22 formation of metastasis is controlled by genetic mutations and al-23 tered patterns of gene expression, but the physical motion of cells 24 in the surrounding environment is determined by the mechanical 25 properties of the cells and the extracellular environment and by 26 their complex interactions^{10–16}. Therefore, the development of 27 three dimensional cell culture models to bridge the gap between 28 cell-based assays and animal studies has gained the attention in 29 the last decades, with the intent of reducing experimental uncer-30 tainties arising from monolayer cell cultures and hence the costs 31 of subsequent in vivo drug screening processes. However, the cor-32 rect interpretation of the experimental results obtained in these 33 living multicellular settings requires the thorough understanding 34 of the overall biophysical and mechanical properties of such sys-35 tems, which emerge in a complex manner from the properties of 36 the individual constituents (i.e. cells, extracellular matrix, liquid, 37 vessels, etc.) forming the system and from the interplay among 38 them, possibly mediated by subcellular molecules and organelles. 39 This task is really challenging since cells and biological tissues are 40 complex media, made of multiple subelements, with different me-41

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chanical properties and with various biological functions¹⁷: each 42 cell is bounded by the plasma membrane to form a closed object 43 containing the nucleus and a fluid, the cytosol (made of water, 44 soluble proteins, sugar and salt), in which numerous organelles 45 are immersed. Each subcellular element is different from the oth-46 ers and mechanical properties are non-homogenously distributed 47 inside each of them¹⁸. This high heterogeneity in cell compo-48 sition and in subcellular properties makes mechanical and bio-49 logical response difficult to be modelled even for a single cell. 50 Furthermore, cells are able to actively interact with each other to 51 form tissues and MCSs, containing both cells, fluids (embedded 52 inside each cell and in the intracellular space) and possibly extra-53 cellular matrix (ECM). The rheological properties of such materi-54 als are quite uncommon and are characterized by the occurrence 55 of many phenomena at the subcellular, cellular and macroscopic 56 scales. The mechanical properties of the cytoskeleton, the cell 57 membrane, the cell cytoplasm and the nucleus determine the me-58 chanical response of an individual cell in isolation, whilst the me-59 chanical behaviour of an ensemble of cells or a tissue is not merely 60 the sum of each single contribution. Rather, it arises through 61 the association and disassembling of adhesion molecules between 62 the cells and the extracellular matrix^{19,20} and through articulate 63 mechanisms of communications and transduction of both exter-64 nal and internal stimuli. In general, the elastic or elasto-plastic 65 behaviour of a MCS results from a complex interplay between cell 66 bulk, mainly represented by cytoskeleton and organelles, and cell 67 surface, which involves, in particular, its actomyosin cortex²¹. On 68 the other hand, viscous effects are mainly due to the presence of 69 the liquid. In particular, the ability of an aggregate to behave as 70 an elasto-plastic material or as a viscous fluid depending on the 71 experimental conditions, is related to the cell adhesion properties, 72 to the type of interactions among the cells and to the contraction 73 of the cell cortex. All these phenomena can lead, in general, to 74 the presence of stress thresholds (which can be viewed as "energy 75 barriers"²¹) that have to be overcome for the activation of the ag-76 gregate's dynanics to occur²¹. 77

This variety of behaviours has le to the definition of many dif-78 ferent mathematical models of multicellular aggregates and living 79 tissues, each of them focusing on different biological aspects at 80 different time and space scales (see Table 1 for a non-exhaustive 81 review of previous modelling effort of quiescent multicellular ag-82 gregates and the works of Gonzalez-Rodriguez et al.²² and Stirbat 83 et al.²³ and Khalifat et al.²⁴ for a more comprehensive review of 84 rheological properties of multicellular aggregates). Inspired by 85 cell sorting experiments on embryonic aggregates, in most cases, 86 tissues have been described as liquids, characterized by a viscosity 87 and a surface tension^{3,22,25}. Consequently, fluid-like constitutive 88 equations have been advocated to model the mechanical response 89 of growing living systems^{26-28,28-34} and quiescent multicellular 90 aggregates 22,25,35-37. However, biological experiments 21 show 91

that the behaviour of an aggregate can strongly deviate from the 92 one of a liquid. Thus, this approach gives back a not completely 93 satisfying approximation of the by far more complex behaviour 94 of cellular aggregates, which also display solid-like properties re-95 lated to the adhesive characteristics of the cells^{21,38} and to the 96 mechanical properties of the single cell in a cluster³⁹. In par-97 ticular, because of the occurrence of residual stresses, the stress 98 asymptotic plateau can be sensibly higher than the one predicted 99 by the pure surface tension in liquid models^{35–37}; some aggre-100 gates (e.g. Chinese hamster ovary (CHO) cell aggregates) are not 101 always able to fuse and round up within the time of experiments 102 or simulations; the aggregate shape after relaxation sometimes 103 displays a strong deviation from that of a liquid $drop^{21}$. Thus, 104 in some cases, cell aggregates are better described as solids with 105 linear or nonlinear elasticity $^{21,40-48}$. At the same time, it is not 106 correct to consider MCSs as elastic solids, because they are com-107 posed of living material: the cells forming the aggregates dupli-108 cate and die continuously, the ECM constantly remodels because 109 of cell reorganisation and, even in absence of growth and death, 110 cells can rearrange their relative adhesion complexes in response 111 to external mechanical stimuli. Moreover, living systems mani-112 fest anelastic reorganisation of the internal structure and resid-113 ual stresses^{41,44,49}, two unique features with no analogy in liq-114 uids 50-53. In particular, the description of such phenomena can 115 be achieved by assuming a plastic-like behaviour of the biological 116 structures under study^{51,54}. Thus, the debate about the best me-117 chanical modelling approach is still open and a comprehensive 118 model of multicellular aggregates and living tissues is far from 119 being developed. Then, a specific MCS mechanical model should 120 be chosen depending on the phenomena we are interested in and 121 on the time and length scale of the observation, recalling that 122 cell aggregates behave as fluids on the timescales of cell division 123 (mitosis) and apoptosis, which characterise growth (many hours 124 or few days)^{9,21,23,37,55,56}, and as elasto-plastic solids on shorter 125 timescales of the order of some minutes or few hours. Therefore, 126 if we want to focus only on the description of cell compression 127 during the time lapse of a biological experiment of the type stud-128 ied in the sequel, elasto-plastic models will better describe cell 129 behaviour, whereas the long-time fluid-like behaviour is more ap-130 propriate for capturing cell proliferation and death^{55,57}. 131

In this paper, in order to move a step towards a more re-132 alistic description of multicellular aggregate mechanical proper-133 ties, we focus on the typical uniaxial compression of a living 134 spheroid^{9,25,35–37}. In this test, an initially spherical aggregate 135 is placed on a lower compression plate, made of non-sticking 136 (glass or steel) material, in an inner chamber filled with tissue-137 culture medium (maintained at 37°C by a circulating water bath 138 through the outer chamber). The spheroid is rapidly compressed 139 against fixed upper compression plate by a stepping motor, which 140 is programmed to produce a deformation of a definite magni-141

Table 1 Mathematical models of quiescent multicellular aggregates

Scale	Model	Constitutive behaviour	Reference
Continuous	1D adhesion energy model	liquid with surface tension	25
Continuous	1D adhesion energy model	viscous liquid with surface tension	58
Continuous	1D spring and dashpot model	visco-elastic liquid with surface tension	35–37,59
Continuous	1D continuous mechanical model	incompressible visco-elastic liquid	6
Continuous	1D continuous mechanical model	visco-elasto-plastic solid	50,53,60,61
Continuous	2D phase-field model	complex fluids	62
Continuous	dynamic network of bounded/unbounded springs	elastic	23
Hybrid	1D macroscopic model+ 2D Cellular Potts Model	visco-elasto-plastic material	21
Discrete	2D Cellular Potts Model	area and volume elastic constraint	63
Discrete	3D lattice model with kinetic Monte Carlo (KMC) method	surface tension constraint	64

tude $^{9,25,35-37}$. Then, to perform a *stress relaxation test*, the force 142 exerted by the aggregate on the upper plate is recorded (by mea-143 suring the apparent weight of the upper compression plate with 144 a Cahn-Ventron electrobalance, connected to the upper compres-145 sion plate 9), while maintaining the deformation constant, until it 146 reaches a constant stationary value. When this state is reached, 147 the compression plates are separated and the aggregate is let 148 free to possibly regain its initial shape (shape recovery test). Dur-149 ing the release phase, the aggregate shape is continuously video 150 recorded: it is observed that, if the compression is maintained 151 for a very short time, the aggregate will bounce back to its initial 152 shape, thus behaving as an elastic (or viscoelastic) solid; on the 153 other hand, if the compression is maintained for a longer time, 154 and if it is sufficiently high to induce the reorganization of the 155 cellular structure, the initial configuration is no longer recovered 156 after the compression plate is removed (at least for the times for 157 which other phenomena, such as growth and apoptosis, do not 158 occur). This denotes an elasto-plastic (or visco-elasto-plastic) be-159 haviour of the living structure, which cannot be captured by the 160 pure fluid model, based essentially on the existence of a surface 161 tension holding together the cell aggregate 35-37. 162

Starting from the elasto-plastic model proposed in ^{50,60,65} and the elasto-visco-plastic model presented in ^{53,61}, we here propose a three-dimensional model of multicellular aggregate compression at constant deformation, supported by three-dimensional numerical simulations of the problem in a realistic geometry in order to overcome some limitations of previous works. Specifically, in 50,60 it was proposed to apply the theory for materials 169 with evolving natural configurations, introduced in^{66–69}, to suc-170 cessfully investigate cell aggregate growth and remodelling, by 171 coupling the visco-elastic behaviors with a yield condition, gener-172 ating a plastic reorganization inside the structure, when the stress 173 becomes too high. The viscous contribution of the liquid, em-174 bedded inside the cells and filling the voids of the multicellular 175 structure, was then introduced ^{53,61} in order to fit the stress-free 176 evolutions of spheroids observed in^{35–37} when the constant de-177 formation is removed. However, in all these works^{50,53,61}, the 178 representation of the whole experimental setting is highly simpli-179 fied, postulating a homogeneous and constant cell density and a 180 homogeneous deformation inside the whole body. The deforma-181 tion on the normal plane to the applied force or displacement is 182 then imposed in order to guarantee the conservation of the total 183 aggregate volume and mass. Under these simplifying assump-184 tions, the model was reduced to a set of two ordinary differential 185 equations^{53,61}, that can be easily studied analytically. This is of 186 course a simplification of the real phenomenon, since, even when 187 the aggregate compression is directed only along one direction, 188 the deformation of the living body and the cell density inside are 189 not homogeneous and the determination of the correct shape of 190 the system is determined by solving the mass and momentum bal-191 ance inside the whole structure in the fully three dimensional set-192 ting, with proper boundary conditions. In this regard, we resort 193 to other works^{54,70–72}, in which three dimensional visco-elasto-194 plastic models for biomechanical problems are presented. Such 195

works have been conceived to address totally different kind of 196 197 biological tissues and biomechanical tests. Hence, they do not allow to obtain information on the mechanical behaviour of a MCS 198 under uniaxial compression. Furthermore, those models do not 199 tackle contact boundary conditions, which naturally occur when 200 the aggregates boundaries come in contact with the upper and 201 lower plates. This is a non trivial problem to solve when elasto-202 plasticity is involved and it causes a series of technical difficulties 203 both in commercial softwares and in user-defined codes. 204

In this work we present a fully three dimensional model for cell aggregate compression and the numerical simulations considering the real biological setting. In particular the mathematical model is introduced in Section 2, then the model is numerically solved to reproduce stress relaxation experiments and shape recovery tests in Section 3. Finally, the main outcomes of this work and future improvements are discussed in Section 4.

212 2 Materials and Methods

Even though we do not perform the biomechanical tests on 213 living aggregates, in order to understand the chosen modelling 214 and numerical set-up, we here briefly report the standard pro-215 tocol of the parallel-plate compression technique introduced by 216 Steinberg and co-workers^{25,35–37}, which is one of the most widely 217 used to characterize tissue properties. In this method, as already 218 described in Section 1, an aggregate is placed inside a thermally 219 isolated chamber filled with tissue-culture medium between two 220 non-adhering parallel plates and compressed with a fixed defor-221 mation. A force sensor measures the evolution of the compres-222 sion force, whereas the aggregate's profile is continuously video 223 recorded. 224

In this section, referring to the biomechanical experiments reported in the literature, we first present the general mathematical model for a living aggregate and we then introduce the boundary conditions necessary to describe the uniaxial compression test at constant deformation. Finally we show how the proposed model can be numerically implemented.

231 2.1 The aggregate model

To derive the in-silico three-dimensional model of cell aggre-232 gate compression and release tests at the macroscopic scale, we 233 refer to experimental procedures based on multicellular aggre-234 gates with radii of hundreds micrometers^{25,35–37} up to some mil-235 limiters (e.g. some kind of avascular tumour spheroids). Consid-236 ering a cell radius in the range of 5-10 μ m, such kind of multi-237 cellular spheroids contain a number of cells of the order of thou-238 sands up to hundreds thousand of cells^{1,21}. Given this high num-239 ber of cells, MCS can be computationally expensive to be nu-240 merically simulated by means of discrete models and previous 241 discrete models focused on a smaller number of cells^{21,73}. Fur-242 thermore, the scale of the imposed displacements is comparable 243

4 |



Fig. 1 Geometry of the in-silico model of cell aggregate compression. (a) Three dimensional numerical domain: Ω_{s0} , Ω_{u0} and Ω_{b0} represent the spheroid, the upper and lower plates in their reference and, in this case, initial configurations, respectively. (b) Two dimensional geometry obtained exploiting the axial symmetry of the original problem. (c) Current configurations $\Omega_s(t)$, $\Omega_u(t)$, $\Omega_b(t)$ for the spheroid, the upper and lower plates, respectively. In the picture, we sketched the Dirichlet boundary $\Gamma^d = \Gamma^d_u \cup \Gamma^d_b$, the free traction boundary $\Gamma^t(t) = \Gamma^t_u(t) \cup \Gamma^t_b(t) \cup \Gamma^t_s(t)$, and the contact boundary $\Gamma^c(t) = \Gamma^c_u(t) \cup \Gamma^c_b(t)$.

with the scale of the spheroids used in the numerical simulations 244 and, thus, well-separated from the cell scale. Therefore, in order 245 to obtain a general model of multicellular aggregates mechanical 246 behaviour, continuous model could be more appropriate. Thus, 247 we define the three regions of space at time t, $\Omega_{s}(t)$, $\Omega_{u}(t)$ and 248 $\Omega_{\rm b}(t)$ occupied, respectively, by the cellular spheroid, the upper 249 and the bottom plates of the compressing apparatus (see Fig. 1). 250 The boundaries of these three regions at time t are denoted with 251 $\Gamma_{\rm s}(t)$, $\Gamma_{\rm u}(t)$ and $\Gamma_{\rm b}(t)$. The mass and momentum balance laws in 252 the three regions $\Omega_{\rm s}(t)$, $\Omega_{\rm u}(t)$ and $\Omega_{\rm b}(t)$ read 253

$$\partial_t \rho_{\alpha} + \nabla \cdot (\rho_{\alpha} \mathbf{v}_{\alpha}) = 0,$$
 with $\alpha = s, u, b,$ (1)

$$\rho_{\alpha} \dot{\boldsymbol{\nu}}_{\alpha} = \rho_{\alpha} \left(\partial_t \boldsymbol{\nu}_{\alpha} + \boldsymbol{\nu}_{\alpha} \cdot \nabla \boldsymbol{\nu}_{\alpha} \right) = \nabla \cdot \mathbf{T}_{\alpha}, \quad \text{with } \alpha = s, u, b, \quad (2)$$

where ρ_{α} is the mass density, \mathbf{v}_{α} is the velocity and \mathbf{T}_{α} is the Cauchy stress tensor of the material in the α -domain. We remark that, in the present setting, where a deformation is rapidly imposed to the MCS, inertial effects are not negligible. To close the equation of motion (2), together with the balance of mass (1), we need to prescribe proper constitutive equations that account for the behavior of the materials in each domain. 260

Mechanical response of living aggregates. As stated in Sec-261 tion 1, the description of the mechanical response of living sys-262 tems is still an open problem. In this work, we decided to focus 263 on the occurrence of plastic behaviours at the macroscopic scale, 264 neglecting cell growth, viscous effects (due to the presence of the 265 liquid encapsulated inside the structure) and other phenomena 266 related to possible cellular heterogeneity and to mechanotrans-267 duction (i.e., the ability of cells to transform mechanical external 268 stresses into biochemical signals and vice versa)⁷⁴. Even in this 269 simplified setting, living media, when subjected to external loads, 270

undergo an internal reorganization due to the rupture and for-271 mation of bonds among the different cells composing the aggre-272 gates³⁸. This aspect poses a series of theoretical difficulties that 273 can be adressed resorting to the theory of evolving natural con-274 figurations^{41,66–68}, which enables to separate the contributions 275 related to elastic distortions from the ones related to anelastic 276 distortions (e.g. growth and remodelling) and to model each of 277 them individually, through a multiplicative decomposition of the 278 deformation gradient tensor⁷⁵. Calling Ω_{s0} and Ω_s , respectively, 279 the reference and the actual configuration of the cellular aggre-280 gate, we introduce the smooth motion 281

$$\chi_{s}(t, \cdot): \Omega_{s0} \longmapsto \mathbb{R}^{3}, \mathbf{X} \longmapsto \mathbf{x} = \chi_{s}(t, \mathbf{X}) \in \Omega_{s}(t) \subset \mathbb{R}^{3},$$

where **X** denotes the material coordinates associated with Ω_{s0} , whereas **x** denotes the spatial coordinates associated with Ω_s . The material gradient of the map χ_s defines the deformation gradient tensor $\mathbf{F}_s := \text{Grad} \chi_s$.

Because of the occurence of remodeling in the living medium, 286 the global undeformed configuration Ω_{s0} is generally not stress-287 free^{70,75}. It is then possible to introduce the so called "natural" 288 state Ω_{sn} of the tissue under study, understood as a collection of 289 relaxed, or stress free, body pieces^{66,75}. In this way, it is possible 290 to decouple the deformation of the medium under study from Ω_{s0} 291 to Ω_s into two components: the first one describes how material 292 body pieces are distorted and relaxed towards the natural state, 293 whereas the second one refers to the accommodating part of the 294 deformation gradient tensor⁷⁵. The structural changes of the 295 MCS are modelled by means of a second-order distortion tensor, 296 denoted with F_p, and describing incompatible strains and mate-297 rial inhomogeneities triggered by cellular re-organisation^{61,70,75}. 298 On the other hand, the accommodating distortions, determining 299 the actual configuration of the multicellular aggregate from the 300 relaxed natural state, are represented by the second-order tensor 301 \mathbf{F}_{n} . We remark that, although the body pieces in the natural state 302 do not generate a configuration in the standard sense, they can 303 be still thought of as a configuration if this is intended as a Rie-304 mannian manifold characterized by the curved metric induced by 305 \mathbf{F}_{p} ^{76,77}. Hence, the multiplicative decomposition of the deforma-306 tion gradient F reads 75,78 (see Fig. 2) 307

$\mathbf{F}=\mathbf{F}_{n}\mathbf{F}_{p}\,.$

We remark that neither \mathbf{F}_{p} nor \mathbf{F}_{n} is necessarily the gradient of a 308 deformation. Rather, they should be regarded as primitive kine-309 matic entities that define, together with the motion, the basic 310 kinematic parameters that are necessary and sufficient for de-311 scribing the kinematics of a remodeling living tissue⁷⁵. In anal-312 ogy with ^{53,54,70,75}, we assume that the mechanical response from 313 Ω_{sn} to Ω_s is hyperelastic. Of course, this is a simplification of the 314 behaviour of a biological medium, which, in principle, would be 315 better approximated by using a viscoelastic constitutive model. 316



Fig. 2 Diagram of the multiplicative decomposition of the deformation gradient tensor **F** in the framework of evolving natural configurations: the reference configuration, Ω_{s0} , the current configuration, Ω_s , the natural state Ω_{sn} .

Nevertheless, since in the case of not growing living media, the 317 characteristic times of the rate dependent response of the ma-318 terial are much less than the characteristic times of remodelling 319 and of mechanical loading (expect for the loading and unloading 320 phases)^{18,79,80}, the material can be thought of as hyperelastic, 321 without introducing a significant error. The variations of volume 322 due to the elastic and the anelastic distortions are denoted by 323 $J_n := \det(\mathbf{F}_n)$ and $J_p := \det(\mathbf{F}_p)$, respectively, and the multiplica-324 tive decomposition of **F** implies $J := det(\mathbf{F}) = J_n J_p$. Then, we will 325 assume \mathbf{F}_p to be isochoric, so that $J_p = 1$ and $J = J_n$ 326

To close the description of the living aggregate behaviour we have to define the strain energy density of the system per unit volume of the natural state and prescribe a proper evolution law for $\mathbf{F}_{\rm p}$. For what concerns the strain energy density we assume that the cellular aggregate can be considered to behave like an isotropic hyperelastic solid with a strain energy density of the Holmes&Mow type⁸¹, i.e. 333

$$\mathscr{W}_{sn} = \alpha_0 \left[\exp(\Psi) - 1 \right], \tag{3a}$$

$$\Psi = \alpha_1 [I_1 - 3] + \alpha_2 [I_2 - 3] - \beta \log(I_3), \qquad (3b)$$

where $I_1 := \operatorname{tr}(\mathbf{C}_n)$, $I_2 := \frac{1}{2} \left[(\operatorname{tr} \mathbf{C}_n)^2 - \operatorname{tr}(\mathbf{C}_n^2) \right]$ and $I_3 := \operatorname{det}(\mathbf{C}_n)$ 334 represent the three orthogonal invariants of the elastic 335 right Cauchy–Green deformation tensor $\mathbf{C}_n = \mathbf{F}_n^T \mathbf{F}_n$, whereas 336 $\alpha_0, \alpha_1, \alpha_2, \beta$ are the coefficients related to material properties and 337 are related to the mechanical parameters of the tissue, the shear 338 modulus μ and the Poisson's ratio ν , by 339

$$\alpha_0 = \frac{\mu(1-\nu)}{2\beta(1-2\nu)}, \quad \alpha_1 = \beta \frac{1-3\nu}{1-\nu}, \quad \alpha_2 = \beta \frac{\nu}{1-\nu}, \quad \beta = \alpha_1 + 2\alpha_2.$$
(4)

340 Then, the Cauchy stress tensor reads

$$\mathbf{\Gamma}_{\rm s} = J_{\rm n}^{-1} \mathbf{F}_{\rm n} \left(2 \frac{\partial \mathscr{W}_{\rm sn}}{\partial \mathbf{C}_{\rm n}} \right) \mathbf{F}_{\rm n}^{T} \,. \tag{5}$$

We remark that the strain energy function (3) implies a com-341 pressible multicellular aggregate. Indeed, even though, to our 342 knowledge, quantitative measurements are not available in the 343 literature⁸², during compression, single cells inside the aggregate 344 can highly change their volume, thanks to an exchange of liquid 345 through the cellular membrane and even through compaction of 346 the nuclear material⁸³. Then, when we deal with incompressible 347 media, we need to partially reformulate the continuum problem 348 at hand, by considering incompressibility as a kinematical con-349 straint, appended to the balance of linear momentum by means 350 of a suitable Lagrange multiplier. In general, such method or, in 351 the same way, penalty methods, may lead to numerical issues⁸⁴. 352 Thus, we preferred to consider a compressible spheroid, with a 353 strain energy density largely employed for biological porous me-354 dia⁸¹. 355

The last equation needed to close the MCS mechanical de-356 scription is the one governing the time evolution of the plastic-357 like distortions^{53,54}. Because of the huge quantity of cross-links 358 among the cells and of the low amount of extracellular matrix 359 embedded in cellular aggregates²², the mechanical properties 360 of cellular aggregates results to be isotropic. In light of these 361 considerations, the structural reorganisation of cellular spheroids 362 relies on an isotropic description and its evolution law can be 363 conveniently written as a time differential equation in the ten-364 sor field $\mathbf{B}_{p} = \mathbf{F}_{p}^{-1}\mathbf{F}_{p}^{-T}$, which is the inverse of the right Cauchy-365 Green tensor $\mathbf{C}_{\mathbf{p}} = \mathbf{F}_{\mathbf{p}}^T \mathbf{F}_{\mathbf{p}}$, associated with the plastic-like distor-366 tions (see^{85,86} for a review on this topic). In this context, the 367 evolution law representing plastic-like behaviour of cellular ag-368 gregates, previously proposed by Preziosi et al.⁶⁰ and Giverso and 369 Preziosi⁶¹ can be recast in the form^{70,87} 370

$$\dot{\mathbf{B}}_{\mathrm{p}} = -\frac{2}{\lambda_{\mathrm{p}}} \left[1 - \frac{\tau_{\mathrm{y}}}{f(\mathbf{T}_{\mathrm{s}}')} \right]_{+} \mathbf{B}_{\mathrm{p}} \mathbf{M}_{\mathrm{s}}'$$
(6)

where $[\cdot]_+$ denotes the positive part of its argument, λ_p is a ma-371 terial parameter related to the reorganization time due to remod-372 elling, τ_v is the yield stress of the aggregate, $f(\mathbf{T}'_s)$ is a frame-373 invariant equivalent measure of the stress \mathbf{T}_{s}' , $\mathbf{M}_{s} = J\mathbf{F}^{T}\mathbf{T}_{s}\mathbf{F}^{-T}$ 374 is the Mandel stress tensor of the cell aggregate and the apex 375 $(\cdot)'$ denotes the deviatoric part of the tensor field to which it is 376 applied. We remark that eq. (6) assumes that remodelling mani-377 fests itself as a rate-dependent plasticity model of Perzyna-type 75, 378 which means that remodelling occurs only when $f(\mathbf{T}'_{s})$ exceeds 379 the threshold stress value τ_v , and is modulated by the timescale 380 $\lambda_{\rm p}^{53,61,70,75}$. This assumption captures the essential phenomena 381 occurring inside the aggregate at the cell scale: if we consider a 382 cluster of cells subjected to a sufficiently high stress, some of the 383

adhesive bonds among the cells may break and eventually reform 384 in other places. Finally, we notice that, while the left-hand-side 385 of eq. (6) is symmetric by definition, the right-hand-side is sym-386 metric only for isotropic media, for which the Mandel stress ten-387 sor \mathbf{M}_{s} satisfies the symmetry condition ⁸⁸ $\mathbf{B}_{p}\mathbf{M}_{s} = (\mathbf{M}_{s}\mathbf{B}_{p})^{T}$, as in 388 this case. For anisotropic materials, eq. (6) is no longer valid, 389 and the way in which remodelling is conceived must take into ac-390 count the evolution of the anisotropy 71,72 . For example, this is 391 the case of fibre-reinforced tissues, whose macroscopic mechani-392 cal properties and remodelling are substantially influenced by the 393 distribution of the fibres embedded in the extracellular matrix. 394

Mechanical response of the compressive apparatus. The up-395 per and bottom compressive plates are made of inert material, so 396 that no biological remodelling might occur. Furthermore, their 397 deformation is so small that plastic distortions cannot be trig-398 gered. Therefore, in the regions Ω_u and Ω_b the introduction of 399 virtual natural configurations is not needed and we assume that 400 the compressive apparatus behaves as a linear elastic solid, which 401 implies that the Cauchy stress tensor can be constitutively pre-402 scribed as 403

$$\mathbf{T}_{\alpha} = \mathbb{C}_{\alpha} : \boldsymbol{\varepsilon}_{\alpha} \quad \text{in} \quad \Omega_{\alpha} \text{ with } \alpha = u, b \tag{7}$$

409

where $\mathbb{C}_{\alpha} = \mathbb{C}_{\alpha}(E_{\alpha}, v_{\alpha})$ is the fourth-order stiffness tensor, which depends on the Young's modulus E_{α} and the Poisson's ratio v_{α} dos of the plates, because of the isotropy of the plates, with $\boldsymbol{\varepsilon}_{\alpha} = 406$ $1/2 \left[\nabla \mathbf{u}_{\alpha} + (\nabla \mathbf{u}_{\alpha})^T \right]$ being the infinitesimal strain tensor, given \mathbf{u}_{α} dos the displacement vector field inside the plates.

2.2 Boundary conditions of the aggregate model.

In order to fulfil the definition of the aggregate model, we 410 have to assign proper conditions at the boundaries. In general, 411 we can divide the boundary of a body into the following different regions: 413

- 1. the Dirichlet boundary $\Gamma^{d}(t)$, on which displacements are 414 prescribed; 415
- 2. the traction boundary $\Gamma^{t}(t)$, on which the surface traction 416 $\mathbf{t} = \mathbf{T}_{s}\mathbf{n}$ is prescribed; a special case of this condition is a free 417 surface, when $\mathbf{t} = \mathbf{0}$ is imposed; 418
- 3. the contact boundary $\Gamma^{c}(t)$, on which the boundaries of 419 the two adjacent domains are in contact, moving with the 420 same normal velocity, and with the normal component of 421 the traction continuously transferred. For frictionless con-422 tact boundaries, only the normal force is transferred, on the 423 other hand, when friction is accounted for, the additional 424 friction force is calculated from the relative motion of the 425 two bodies and the contact pressure. 426

In particular, to describe the uniaxial compression test of cellular aggregates, we impose a null displacement on the upper side



Fig. 3 Spatio-temporal evolution of the normalized spheroid density $\tilde{\rho} := \rho_s / \rho_{s0} = J^{-1}$ (top row) and von Mises stresses $T_{\alpha}^{mises} := \sqrt{3/2} ||\mathbf{T}'_{\alpha}||$, with $\alpha = \{s, u, b\}$ (bottom row) inside each domain. The loading and unloading ramp time is equal to 5 s, whereas the compression is maintained for 30 s. The simulations are obtained using $\tilde{u}_z^{max} = \tilde{u}_z^{max} / (2R) = 0.3$ and the set of parameters reported in Table 2.

of the upper plate $\Gamma_{u}^{d}(t)$ and a vertical displacement ramp $u_{z}(t)$ 429 on the lower boundary of the bottom plates, $\Gamma_{\rm b}^{\rm d}(t)$, such that 430 $\Gamma^{d}(t) = \Gamma^{d}_{u}(t) \cup \Gamma^{d}_{b}(t)$ (see Fig. 1-(c)). Frictionless contact bound-431 ary conditions apply on the two contact regions between the ag-432 gregate and the upper and lower plates, $\Gamma^{c}(t) = \Gamma^{c}_{u}(t) \cup \Gamma^{c}_{b}(t)$, with 433 $\Gamma_{\rm u}^{\rm c}(t)$ and $\Gamma_{\rm b}^{\rm c}(t)$ being the contact boundary between the spheroid 434 and the upper and bottom plate, respectively (see Fig. 1-(c)). 435 Free surface boundary conditions are imposed on the remaining 436 portions of the domains, $\Gamma_{\alpha}^{t}(t)$ with $\alpha = s, u, b$. We observe that, 437 whilst the location of the boundaries Γ^d_u and Γ^d_b is known, the 438 boundaries $\Gamma_{u}^{c}(t)$, $\Gamma_{b}^{c}(t)$ and $\Gamma_{\alpha}^{t}(t)$ change in time, depending on 439 whether the surfaces of the two bodies come in contact or detach, 440 and that the sets $\Gamma_{\rm u}^{\rm c}(t)$ and $\Gamma_{\rm b}^{\rm c}(t)$ can possibly be empty. 441

Therefore, the following set of boundary conditions (BCs) de-scribes the uniaxial compression test

$$\mathbf{u}|_{\Gamma_{u}^{d}} = \mathbf{0},\tag{8}$$

 $\mathbf{u}|_{\Gamma_{\mathbf{b}}^{\mathrm{d}}} = u_{z}(t)\mathbf{e}_{z},\tag{9}$

 $(\mathbf{T}_{\alpha}\mathbf{n})|_{\Gamma_{\alpha}^{t}} = \mathbf{0},$ with $\alpha = s, u, b,$ (10)

$$(\mathbf{v}_{s} \cdot \mathbf{n} - \mathbf{v}_{\alpha} \cdot \mathbf{n})|_{\Gamma^{c}} = 0, \qquad \text{with } \alpha = u, b, \qquad (11)$$

$$(\mathbf{n} \mathbf{T}_s \mathbf{n} - \mathbf{n} \mathbf{T}_{\alpha} \mathbf{n})|_{\Gamma_{\alpha}^c} = 0, \quad \text{with } \alpha = u, b.$$
 (12)

2.3 Finite element numerical simulations

Equations (1), (2) and (6) with the constitutive assumptions 445 (5) and (7) and the BCs (8)-(12) were numerically solved to re-446 produce an unconfined uniaxial compression test of a cellular 447 spheroid. Exploiting the symmetry of the cellular spheroid and 448 of the compressive apparatus, the model equations can be rewrit-449 ten in cylindrical coordinates and solved into the two dimensional 450 domain of Fig. 1-(b). The top boundary of the upper plate was 451 fixed, accordingly to the BC (8), while on the bottom bound-452 ary of the lower plate the controlled vertical displacement $u_7(t)$ 453 was imposed. The analytical expression of $u_z(t)$ is specified later, 454 in the context of the uniaxial compression and release test. On 455 the remaining boundaries, stress-free boundary conditions (10) 456 are applied when the plates and the spheroid are not in contact, 457 while frictionless contact conditions (11)-(12) are imposed when 458 the plates and the aggregate come into contact. We remark that 459 the presence of contact surfaces is a source of complexity for the 460 performed simulations. Indeed, the extent of the contact region 461 evolves in time and is an unknown of the problem calculated 462 from the relative displacement, which, in turn, depends on the 463 momentum balance. Therefore, boundary conditions depend on 464 the solution itself, so that portions of the boundary that are free 465 with BCs of the type (10) may come in contact, thereby acquir-466 ing BCs of the type (11) and (12). In this respect, the contact 467

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BCs (11) and (12) can be conveniently accounted for by employ-468 ing the "augmented Lagrangian method"^{89,90}. This method imple-469 ments a penalty regularization of the standard Lagrangian multi-470 plier method by incorporating both a Lagrange multiplier and a 471 penalty term to solve the contact constraints and impose the con-472 ditions (11) and (12). Indeed, while in the standard formulation 473 of the Lagrange multiplier method, the Lagrange multiplier is an 474 unknown, in the augmented Lagrangian method, it is computed 475 algorithmically and its initial estimation is iteratively improved, 476 until the constraint violation is small enough (or equivalently, un-477 til the multiplier stops changing appreciably). 478

The Lagrangian numerical simulations have been ob-479 tained using the finite element software (FEM) COMSOL 480 Multiphysics® (version 5.3a). The contact boundary conditions 481 (11) and (12) are imposed by combining the routine expressly 482 written to solve the evolution law of plastic deformations with 483 the built-in environment for contact constraints in COMSOL Mul-484 tiphysics, developed by taking inspiration of the work by Simo 485 and Laursen⁹⁰. We then choose a segregated approach, where 486 the contact pressures are solved in a separate lumped step, which 487 is the default solver when the augmented Lagrangian formulation 488 is used. Given the numerical issues arising from the treatment of 489 contact boundary conditions in the case of an elasto-plastic model 490 of cellular aggregates, we have performed several tests to choose 491 a proper mesh for solving the aggregate compression problem 492 presented in our work. The dimension of the mesh elements at 493 the border of the cell aggregate should be at least half the typical 494 dimension of the elements at the upper and lower plates bound-495 aries, to give a good resolution of the contact patch and stress 496 state in the contact regions. In particular, computations involv-497 ing a spheroid of 100 μ m in radius were performed using a mesh 498 of 7978 triangular elements in the spheroid bi-dimensional sec-499 tion and 380 triangular elements inside the 2D sections of each 500 plate, for a total of 8736 elements. The number of mesh elements 501 have then been adapted to the cases of greater spheroid radii. 502 To verify the quality of our mesh, we have used a functionality 503 implemented in COMSOL Multiphysics® and we have obtained a 504 positive response. As last step, before using the mesh described so 505 far, we have performed several refinements and solved the same 506 benchmark tests. In doing this, we have noticed no significant 507 changes in the results, with an increasing of the time needed to 508 509 complete the simulations (i.e., few hours of computation instead of one or even more days). The choice of this kind of mesh has 510 represented, for us, the best compromise between computational 511 efficiency and accuracy, also in the light of running several sets of 512 simulations. 513

514 **3** Results and discussion

In this section, we apply the aggregate model presented in
 Section 2 to reproduce the uniaxial compression-release test of a
 MCS and the stress relaxation and shape recovery curves. The

Table 2 Values of the material parameters used in the numerical simulations.

Parameter	Value in the simulations	Reference
R	100 µ m	1,3–5
λ	$0.001 (kPa \cdot s)^{-1}$	54
$\tau_{\rm v}$	2 kPa	87
	20kPa	43,54,91,92
μ V	0.2	54,91,92

numerical results are discussed on the basis of available experimental data reported in the literature. 519

3.1 Typical compression-release test

We first study the case in which the aggregate is compressed at a given deformation maintained for a certain amount of time and then released. We impose the following vertical displacement

$$u_{z}(t) = \begin{cases} \bar{u}_{z}^{max} \frac{t}{t_{ramp}}, & \text{for } t < t_{ramp}, \\ \bar{u}_{z}^{max}, & \text{for } t_{ramp} \leq t < t_{end} - t_{ramp}, \\ -\bar{u}_{z}^{max} \frac{t - t_{end}}{t_{ramp}}, & \text{for } t_{end} - t_{ramp} \leq t < t_{end}, \end{cases}$$
(13)

where t_{ramp} is small compared to the compression time $t_c =$ 524 $t_{end} - 2t_{ramp}$. Figure 3-top reports the normalized spheroid den-525 sity $\tilde{
ho}$:= $ho_{
m s}/
ho_{
m s0}$ = J^{-1} in the case in which remodelling is 526 triggered: after the sudden imposition of the deformation, the 527 cellular density highly increases in the region close to the upper 528 and lower plates and decreases close to the middle point of the 529 outer boundary, where J > 1, as a consequence of the volumet-530 ric expansion of the spheroid along the radial direction. As the 531 compression is maintained, the cells reorganize and redistribute 532 inside the aggregate and the compaction of the cells inside the 533 spheroid decreases. When the compression is released the density 534 of cells inside the deformed aggregate continues to be inhomoge-535 neous and different from the initial one (see last picture in the 536 top row of Fig. 3). We remark that the total mass of the cellular 537 spheroid is preserved during the compression and release of the 538 cellular aggregate. Finally, we note that, the plates being slightly 539 deformable, their normalized density is almost constant. 540

Looking at the distribution of the stress inside the cellular 541 aggregate, we plot the von Mises stress $T_s^{Mises} = \sqrt{3/2} ||\mathbf{T}'_s||$ in-542 side the spheroid (see Fig. 3-bottom). In this case it is possi-543 ble to observe that the maximum of the stress occurs not in the 544 contact area but inside the spheroid, at some distance from the 545 contact boundaries. We remark that this result recalls Hertz's the-546 ory of contact. Although this comparison may be worth further 547 investigations, here we do not examine possible analogies with 548 Hertz's theory because it is developed under the hypothesis of 549 perfect elastic materials, absence of friction forces and moder-550 ate area of the contact materials. It is also possible to see that, 551 as the compression is maintained, the stress inside the spheroid 552

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Fig. 4 Spatio-temporal evolution inside Ω_s of the components of the remodelling tensor $\mathbf{B}_p = \mathbf{C}_p^{-1}$ where $\mathbf{C}_p = \mathbf{F}_p^T \mathbf{F}_p$ is the plastic Cauchy-Green deformation tensor. The parameters in the simulations are the same used for the results in Fig. 3. Notice that the in the lower and upper plate no remodelling occurs (gray regions).

decreases and, when the compression is removed, the spheroid 553 returns stress-free at the boundary (see the blue line, reporting 554 the normal stress, on the spheroid boundary in the last picture 555 of Fig. 3-bottom), while residual stresses appear inside the ag-556 gregate (see last picture of Fig. 3-bottom). The amount of von 557 Mises stress inside the multicellular structure is the chosen frame-558 invariant measure of the stress $f(\mathbf{T}'_{s})$ that drives cell reorgani-559 zation. Therefore, no remodelling occurs in the regions where 560 T_s^{Mises} is below the threshold $\tau_y = 2$ kPa for plastic reorganization. 561 In particular, if $T_s^{Mises} < \tau_v$ everywhere in Ω_s , the spheroid de-562 forms elastically and no residual stresses and deformations can be 563 observed when the imposed deformation is removed and the den-564 sity of the cellular aggregate returns equal to the initial one (not 565 shown in the figures). Furthermore, when the spheroid deforms 566 purely elastically, no decreases in the stresses inside the multicel-567 lular structure (stress relaxation) can be observed. In fact, the 568 decreasing of the amount of stress is due to the onset of plastic-569 like distortions. In this case, indeed, the stress contributes to the 570 change of internal structure of the medium under study. 571

To quantify the amount of remodelling triggered by $T_{\rm s}^{Mises}$, in Fig. 4 we report the radial, axial and shear component of the remodelling tensor $\mathbf{B}_{\rm p} = \mathbf{C}_{\rm p}^{-1}$. We observe that the radial component $B_{\rm p}^{\rm RR}$ is less than 1 almost everywhere in the aggregate, 575 since, when the aggregate is compressed, remodelling in the ra-576 dial direction occurs due to the expansion of the structure along 577 the radial axis and only a small region close to the middle point of 578 the outer boundary experiences compressive radial remodelling. 579 The axial component B_p^{ZZ} of **B**_p is bigger than 1 everywhere since 580 remodelling occurs due to compression in the axial direction. Fi-581 nally $B_{\rm p}^{\rm RZ}$ is a measure of the remodelling due to shear. The sign 582 of the shear remodelling is in agreement with the convention used 583 for shear stresses: positive shear stresses act clockwise, while neg-584 ative shear stresses act counter-clockwise. The point delimiting 585 the contact area between the spheroid and the upper plate de-586 fines the starting point of the 45° plane that identifies a change 587 of sign in the shear remodelling. Close to the lower plate, in the 588 region below the 45°-plane, B_p^{RZ} is negative since shear is nega-589 tive there, while in the region above the 45°-plane, B_p^{RZ} is positive 590 since the shear is positive there. Similar reasoning applies to the 591 region close to the upper plate, with a change of sign due to the 592 convention on the sign of shear stresses. 593



Fig. 5 Stress relaxation curves for different values of the parameters (a) τ_y , (b) λ_p , (c) μ and ν , (d) *R* and (e) normalized imposed deformation, i.e., $\tilde{u}_z^{max} = \bar{u}_z^{max}/(2R)$. The curves are obtained integrating the stress exerted by the aggregate over the surface of contact with the upper plate, while maintaining the compression of the aggregate at a constant deformation. (f) Contact area between the spheroid and the upper plate for different values of normalized imposed deformation, $\tilde{u}_z^{max} = \bar{u}_z^{max}/(2R)$.

594 3.2 Stress relaxation curves

In order to compare the predicted numerical results with 595 the available stress relaxation curves reported in the litera-596 ture^{9,35–37,93}, we integrate the normal stress exerted by the ag-597 gregate on the surface of contact with the upper plate to compute 598 the total force acting on the plate, when the compression is main-599 tained. The numerical results show that, when remodelling is 600 triggered, the initial force transferred to the upper plate by the 601 compressed aggregate is relaxed as the compression at constant 602 deformation is maintained. Furthermore, the amount of relax-603 ation of the initial force depends on the threshold stress set for 604 the activation of plasticity, since the force exerted on the upper 605 plate at the equilibrium depends on the value of τ_v (see Fig. 5-606 a). This behaviour, which is not observed when the aggregate 607 behaves elastically, is in agreement with the results obtained in 608 the one dimensional analysis reported by Giverso et al.⁵³. The 609 time required to relax the initial stress is related to the inverse 610 of the parameter λ_p (see Fig. 5-b). Indeed it is possible to de-611 fine the plastic reorganization time as $t_p = (\mu \lambda_p)^{-1}$, where μ is 612

the shear modulus of the cellular aggregate. We remark that the parameter τ_y does not affect the value of the initial force exerted by the aggregate on the upper plate as shown by the maxima in Fig. 5-a, while it determines the equilibrium force on the contact areas. Conversely, λ_p does not affect the initial and the final value of force exerted on the upper plate.

In order to take into account of the variety of tissues, we have 619 then exploited the effect of varying the cell mechanical parame-620 ters μ and v on the MCS response. The mechanical parameters 621 are strongly dependent on the cell type considered and a wide 622 range of parameters can be found in the literature. Specifically, 623 supported by biological evidences, we take the shear modulus μ 624 varying between 3 kPa and 40 kPa43,94 and the Poisson's ratio 625 v ranging between 0.2 and $0.45^{43,92}$. The resulting stress re-626 laxation curves (Fig. 5-c) show that the mechanical parameters 627 mostly affect the value of the initial stress exerted on the upper 628 plate, with higher initial stresses for increasing value of μ . Then, 629 for the same value of μ , the Poisson's ratio v further magnifies the 630 initial stress exerted on the upper plate. We also observe that, for 631

increasing values of the parameter μ , the plastic reorganization 632 time decreases, accordingly to its definition, i.e., $t_{\rm p} = (\mu \lambda_{\rm p})^{-1}$. On 633 the other hand, the size parameters of the model, i.e., the radius 634 of the spheroid R (Fig. 5-d) and the normalized imposed defor-635 mation $\tilde{u}_{z}^{max} = \bar{u}_{z}^{max}/(2R)$ (Fig. 5-e), significantly influence both 636 the initial force and the one at the stationary condition, with in-637 creasing contact forces for both increasing MCS radius (keeping 638 \tilde{u}_{max}^{z} fix) and imposed normalized deformations (at fixed τ_{y}). The 639 increase in the normalized force exerted by the aggregate on the 640 upper plate, in the case of increasing imposed deformations, is 641 mainly due to the increase in contact area (Fig. 5-f). Then, for 642 very small deformations, such as for the blue curve of Fig. 5-e, 643 the stress is slightly above the threshold value required to induce 644 the internal reorganization of the cellular spheroid, so that the 645 stress relaxation is less perceivable. On the other hand, as the 646 imposed deformation increases, the stress inside the aggregate 647 rises and the rearrangement of the cells inside the structure leads 648 to an intense relaxation of the initial load exerted on the upper 649 plate. Furthermore, the increase in the imposed vertical displace-650 ment leads to a higher deformation of the multicellular structure 651 and to an almost linear increase in the contact area between the 652 spheroid and the upper plate (Fig. 5-f). 653

We remark that the reported stress relaxation curves are qual-654 itatively in agreement with the experimental curves reported in 655 the works of Forgacs et al. 35-37, Jakab et al. 93 and Andolfi et al. 93. 656 Indeed, by compressing multicellular spheroids composed by ei-657 ther limb bud mesoderm, or heart ventricles, or livers cells taken 658 from chicken embryos, they observed that the initial force (nor-659 malized with respect to the gravitational acceleration) exerted 660 by the aggregate on the upper plate which is in the range 7-8 661 mg is relaxed to a load in the range 2.5-4 mg for a compres-662 sion at constant deformation maintained for 160 s. On the other 663 hand, in the experimental work of Jakab et al.⁹ on Chinese Ham-664 ster Ovary (CHO) cells, stress relaxation is achieved on longer 665 timescale ($\approx 400 - 1000$ s). In this work, without performing a 666 quantitative analysis and without conducting a direct validation 667 (which would require further biological data and details on the 668 mechanical tests), we showed in Fig. 5 that our model is able to 669 reproduce a sufficiently wide range of normalized stresses. We 670 achieved that by varying the parameters of the model, so that dif-671 ferent cellular populations can be described by varying the com-672 673 bination of the parameters of our model, possibly supported by other mechanical tests. Specifically, in contrast to what has been 674 done in^{35–37}, where viscous effects are included, we reproduce 675 here the typical stress relaxation curves reported in the literature 676 677 by resorting solely to the reorganization of the cells inside the structure. Indeed, as anticipated in the Introduction, as long as 678 relatively short timescales are considered, this process seems to 679 be the fundamental mechanism occurring in the biomechanical 680 tests addressed in this work. In fact, upon a detailed analysis of 681

the biological experiments in which the displacement of fluores-682 cently labeled cells is followed by confocal microscopy during ag-683 gregate compression⁹, it is possible to see that tissue relaxation 684 is driven predominantly by cell shape changes, a unique prop-685 erty of living systems with no analogy in liquids. Furthermore, 686 using field emission scanning electron microscopy (FESEM) to 687 visualize individual cells in a precompressed, compressed, and 688 postcompressed equilibrated aggregate⁹, it is possible to see that 689 after compression a pressure gradient is set up. This is put in 690 evidence by the fact that cells in the vicinity of the compressive 691 plates and toward the vertical axis of symmetry of the compressed 692 aggregate are deformed more strongly than those near the equa-693 tor and side boundary, which denotes a solid-elastic behaviour 694 of the cellular aggregate under compression, in accordance also 695 with our simulations (before the occurrence of plasticity). Then, 696 whilst when the aggregate is described as a viscoelastic material, 697 any internal stress created by the initial compression is dissipated 698 by the time the system reaches equilibrium and the remaining 699 stresses are encapsulated only at the interface between the ag-700 gregate and the surrounding tissue culture medium^{35–37}, in the 701 case of an elasto-plastic model, such as the one proposed here, 702 residual stresses may appear inside the structure, in accordance 703 with^{41,44,49}. Therefore, even though stress relaxation curves can 704 be reproduced only accounting for viscosity, elasto-plastic models 705 might be more adequate to capture the biological phenomenon. 706

For the sake of completeness, we point out that a quantitative 707 differences between the experimental curves^{9,37,93} and the ones 708 that can be obtained with our model are due to the possible pres-709 ence of more than one relaxation time for living tissues, such as 710 seems to be recorded in the biomechanical tests^{9,37}. However, 711 the smaller relaxation time is of the order of very few seconds^{9,37} 712 and it is probably related only to the recording of the elastic re-713 sponse⁹, whereas the biggest relaxation time, which is of the 714 order of 20-40 seconds in^{37,93} and 70-120 seconds in⁹, reflects 715 the global cellular rearrangement. Therefore, in the present pa-716 per, being interested in modelling anelastic behaviour in living 717 systems, we have chosen to incorporate only the longer relax-718 ation phenomenon which is due to the reorganization occurring 719 inside the structure. The presence of more than one relaxation 720 time and its origin, should be further investigated and clarified 721 before being properly included in a MCS model. 722

3.3 Shape recovery curves

In this Section, we study the shape recovery behaviour of 724 cellular aggregates after the release of a constant deformation 725 maintained for different compression times $t_c = t_{end} - 2t_{ramp}$. As 726 observed in the previous subsection, whilst the shape relaxation 727 curve reported in the literature can be reproduced even without 728 resorting to the plastic-like behaviour of the aggregate, the capability of the multicellular structure to maintain an amount of the 730

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Fig. 6 (a) Height-width ratio chart for different values of imposed deformation and (b) corresponding aggregate deformed shapes. The height-width ratio is obtained dividing the height by the width of the deformed aggregate, after release of the imposed deformation (normalized with respect to the initial diameter of the spheroid, i.e., $\bar{u}_z^{max}/(2R)$), for different values of compression time $t_c = t_{end} - 2t_{ramp}$. On the right the displacement inside the spheroid is plotted.



Fig. 7 Height-width ratio at the stationary state for different values of μ and ν , for an imposed deformation of $\tilde{u}_z^{max} = 0.3$.

imposed deformation when the compression is released cannot 731 be explained using a simple viscoelastic model. Indeed, as ob-732 served in the work of Forgacs et al. ^{36,37}, when the compression is 733 released, cell aggregates that were subjected to a very brief com-734 pression spring back almost to their original shapes, whereas mul-735 ticellular structures compressed for a longer time do not. In par-736 ticular, if the shape of the aggregates after deformation released 737 is observed for 10-15 minutes, the MCSs maintain their deformed 738



Fig. 8 Height-width ratio chart for different values of $\lambda_{\rm p}$, for an imposed deformation of $\bar{u}_z^{max} = 0.29$. The numerical results are compared with the experimental results extrapolated from the work of Forgacs et al.³⁷.

shape. Only incubating the aggregates for 24 hours will lead to 739 cell spheroids rounding up again, which is probably due to the 740 occurrence of other reorganization inside the structure and pos-741 sibly cell proliferation⁵⁷. The capability of the MCS to maintain 742 an amount of the deformation is due to the rearrangement of the 743 internal structure, as experimentally observed on both chick95 744 and amphibian embryonic cells⁹⁶. The rearrangement of the cell 745 internal structure and of the bonds among the cells should be con-746 verted in the model in the existence of a plastic behaviour.

748 Therefore, we have focused on the results obtained by means of the employment of our elasto-plastic model in the case in which 749 the compression is maintained for different times and we report in 750 Fig. 6-a the spheroid height over width ratio for increasing values 751 of the cumulative time under compression, t_c , and for different 752 values of normalized imposed deformations, $\tilde{u}_z^{max} := \bar{u}_z^{max}/(2R)$. 753 From the reported curves it is possible to see that, in accordance 754 with the biological evidences, if the compression is maintained 755 for few minutes, the aggregate will bounce back to almost its ini-756 tial shape, since in this case the extent of the plastic rearrange-757 ment is not consistent. On the other hand, if the compression 758 is maintained for a longer time, the aggregate remains flattened 759 after subsequent releases from compression, signifying the attain-760 ment of the stationary state. This is also clear from the deformed 761 configurations reported in Fig. 6-b, where the aggregate's shape 762 and the spatio-temporal evolution of the displacement inside the 763 aggregate are reported for different values of cumulative time un-764 der compression and for different imposed deformations. We also 765 show that, for the same value of normalized imposed deforma-766 tion and yield stress, the total plastic deformation of the MCS is 767 highly influenced by the mechanical parameters μ and v (see Fig. 768 7), whilst it is not affected by the initial radius of the spheroid 769 (not shown in the paper). We then compare the curves obtained 770 from the numerical simulations for different values of λ_p with the 771 experimental data reported in the work of Forgacs et al.³⁷ (see 772 Fig. 8). It is possible to see that the best fitting for the height 773 over width ratio curves occurs for $\lambda_p = 0.0005 (kPa \cdot s)^{-1}$, given 774 an imposed deformation of 29% of the spheroid initial size. This 775 observation is also in agreement with the experimental observa-776 tion reported in the work of Jakab et al.⁹, in which they observe 777 that aggregates can be compressed up to a maximum of $\approx 30\%$ 778 of their original diameter, in order to avoid irreversible damage 779 to the cells and intense shape modification of the cellular struc-780 ture. For the sake of completeness, we point out that the numer-781 ical height over width ratio curves have been obtained without 782 allowing the relaxation of the MCS after each compression step, 783 differently from what done in the experimental work of Forgacs 784 et al.³⁷. However, the difference between the numerical and the 785 experimental protocols does not significantly affect the plastic de-786 formation of the aggregate, since in our model we do not include 787 788 viscous effects and the plastic reorganization mainly occurs during the compression phases. To confirm this theoretical expecta-789 tion, we have also run a simulation in which the compression is 790 removed at intervals (corresponding to the data points reported 791 in Forgacs et al. 37) and the spheroid is let free to relax for 11 s: 792 the discrepancy between the two protocols leads to height over 793 with ratios that differ less than 0.4% (results not shown here). 794 Finally, we remark that in our numerical tests, the spheroid de-

Finally, we remark that in our numerical tests, the spheroid deformation is maintained even for very long time, after the release of the imposed deformation. In order to reproduce the long-time 797 recovery of the initial spherical shape, other factors should be included in the model, such as the presence of the external liquid 799 and the proliferation of cells, that have not been accounted for in 800 the present model. 801

802

4 Conclusions

Biological tissues show complex mechanical responses and 803 their mechanical behaviour is still far from being completely un-804 derstood. In this work, we aim to move a step towards this in-805 volved purpose by defining a setting to simulate the mechanical 806 behaviour of cell aggregates when they are subjected to a uniax-807 ial compression test. In particular, we consider an elasto-plastic 808 model and we numerically solve it through finite element simu-809 lations by imposing contact boundary conditions to simulate the 810 experimental set-up. With respect to previous mechanical models 811 on aggregates^{53,61}, we here numerically solve the real three di-812 mensional problem, with inhomogeneous deformation and com-813 plex shape changes. By doing this, we have provided the visu-814 alisation of a compression test on multicellular spheroids that re-815 quires the formulation of a contact problem to extract information 816 on MCSs inelastic behaviour. We have observed, for instance, the 817 redistribution of the spheroid's mass density in response to ap-818 plied compressive loads, the reorganisation of the spheroid's in-819 ternal structure, described through the inelastic variable $B_{\rm p}$, and 820 the time evolution of the height-to-width ratio of the spheroid. 821 From the point of view of numerical simulations, within a fully 822 nonlinear regime, the contact boundary conditions are combined 823 with the evolution law of plastic deformations, with the latter 824 ones being determined by means of a routine expressly written for 825 the works 54,70,71, and without having recourse to standard COM-826 SOL packages. The numerical results demonstrate that the stress 827 relaxation curves reported in the literature could be explained by 828 assuming an elasto-plastic behaviour of the spheroids, i.e., with-829 out taking into account viscous effects, differently from previous 830 models $^{35-37}$. At the same time, they show that the permanent de-831 formation observed after the application of the load/deformation 832 can be resolved in terms of plastic deformations. The results pre-833 dicted by the numerical simulations are qualitatively in agree-834 ment with the results of biological experiments and we have also 835 proposed some quantitative comparisons in order to estimate the 836 parameters of the model, by fitting available experimental data. 837

Future works will be devoted to the definition of a multiphase 838 model of cell aggregate compression, taking into account the vis-839 cous contribution related to the presence of the culture medium 840 liquid inside the whole structure, to account for the description of 841 MCS non instantaneous recovery after release^{35–37}. Some previ-842 ous attempts to couple viscous effects with elasto-plasticity have 843 been done, for example, in 53,61 where the viscous contribution 844 related to the intracellular liquid is considered. However, in that 845 case, the liquid motion is constrained to the one of the cellular 846

phase, whereas when an aggregate is compressed between a par-847 848 allel plate apparatus, the liquid exudes from the lateral boundaries of the MCS. Conversely, when the compression is removed, 849 the liquid will slightly fill the porous cellular structure, leading to 850 a viscous recovery of the cell shape after compression, in agree-851 ment with the biological observation. This phenomenon can be 852 accurately described only considering a multi-phase model, with 853 a cellular constituent responsible of the elasto-plastic behaviour 854 and a liquid phase carrying the viscous contribution. Further-855 more, the definition of a multi-phase model will allow to investi-856 gate the mechanical contribution of the extracellular matrix that 857 in the present model has been neglected and that can be possibly 858 encapsulated inside living spheroids. 859

Another point to investigate in future works could be the role of 860 spheroid heterogeneous shapes^{97,98}, that could lead to different 861 quantitative results and to the intensification of the stresses in cor-862 respondence of bumps or, alternatively, to possible detachments 863 in correspondence of pits in the contact region. However, het-864 erogeneous shapes will not alter the capability of the aggregate 865 of partially relaxing the initial stress and of giving rise to plastic 866 deformations, as long as remodelling is triggered. In the same 867 way, heterogeneity in the composition could lead to regions with 868 higher/lower remodelling and to more complex MCS shapes dur-869 ing the compression and release processes. For moderate hetero-870 geneities, the main results of the present work are not expected 871 to vary significantly, although a rephrasing of the model might be 872 necessary. Indeed, when a medium is heterogeneous, the multi-873 plicative decomposition of the deformation gradient tensor is not 874 sufficient, alone, to describe the structural reorganisation of the 875 medium itself. In fact, different material responses are possible 876 at different points of the same medium, so that the strain energy 877 density of the spheroid must depend explicitly on the material 878 point at which it is evaluated. Accordingly, with reference to the 879 spheroid's natural state, one has to write $\mathscr{W}_{sn}(\mathbf{C}_n, \mathbf{X})$, where the 880 explicit dependence of \mathscr{W}_{sn} on X must be prescribed and, in prin-881 ciple, in a heterogeneous material it is also possible that different 882 plastic evolution laws apply at different body points. 883

Future efforts will also be addressed to the definition of the ac-884 tive behaviour of living systems. Indeed, in spite of similarities 885 of living tissues with inert soft materials and liquids, multicel-886 lular systems additionally display active responses that are not 887 observed in inert soft materials²². In particular, the accurate de-888 scription of MCS compression cannot encompass the character-889 ization of cell active response when subjected to stresses. This 890 response is due to mechanotransduction, which is the ability of 891 cells to transform mechanical stresses into biochemical signals 892 (and vice versa) in order to transfer information to and from the 893 nucleus^{18,28,74}. This ability of cells to deform and generate forces 894 in an active manner, coupled with their extreme complexity and 895 their non linear response to mechanical stimuli, outlines the need 896

of a specific mathematical model to describe aggregate dynamics.

Finally, the development of specific mathematical models to 898 describe living system responses should be supported by exper-899 imental tests. In particular, it would be interesting to perform ad 900 hoc biological experiments in order to quantify the anelastic be-901 haviour of such systems, determining the tissue yield stress, which 902 physically arises from the critical force required to break intercel-903 lular bonds and induce cellular reorganization. A definitive an-904 swer to the debate of characterizing tissues as either viscoelastic 905 fluids or visco-elasto-plastic solids could arise from measuring the 906 frequency response of tissues to a periodic forcing²², which is a 907 much-needed experiment that, to our knowledge, has not been 908 previously reported. Then, until today, models of tissue mechan-909 ics have often focused on partial descriptions of tissue behaviour 910 that are successful in explaining specific features at a certain scale 911 and under certain conditions. Future modelling efforts should ad-912 dress the general applicability of theoretical models to different 913 tissues and various phenomena, as well as link the physics at dif-914 ferent scales, by connecting the macroscopically measurable tis-915 sue properties to the biomolecular and intracellular mechanisms, 916 to provide a comprehensive view of tissue mechanics 22 . 917

In conclusion, studying tissue mechanics provides the basis to understand many physiological and pathological phenomena and to foster tissue engineering, which aims to develop new strategies of medical treatment based on artificial tissue regeneration^{7,22}. Proposing a three-dimensional elasto-plastic model of living system behaviour aims at moving a step towards this ambitious goal.

Conflicts of interest

There are no conflicts t	o declare.	925
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Notes and references

- 1 R.-Z. Lin and H.-Y. Chang, *Biotechnology Journal*, 2008, **3**, 1172–1184.
- 2 A. Mgharbel, H. Delanoë-Ayari and J.-P. Rieu, *HFSP journal*, 2009, **3**, 213–221.
- 3 J. Holtfreter, Journal of Experimental Zoology, 1943, 94, 261–318.
- 4 A. Moscona and H. Moscona, *Journal of anatomy*, 1952, **86**, 287–301.
- 5 W. Mueller-Klieser, Journal of Cancer Research and Clinical Oncology, 1987, **113**, 101–122.
- 6 M. Yu, A. Mahtabfar, P. Beelen, Y. Demiryurek, D. I. Shreiber, J. D. Zahn, R. A. Foty, L. Liu and H. Lin, *Biophysical Journal*, 2018, **114**, 2703–2716.

- 7 V. Mironov, R. P. Visconti, V. Kasyanov, G. Forgacs, C. J. Drake and R. R. Markwald, *Biomaterials*, 2009, **30**, 2164–2174.
- 8 L. V. Garmanchuk, E. M. Perepelitsyna, M. V. Sydorenko and L. I. Ostapchenko, *Cytology and Genetics*, 2010, **44**, 19–22.
- 9 K. Jakab, B. Damon, F. Marga, O. Doaga, V. Mironov, I. Kosztin, R. Markwald and G. Forgacs, *Developmental Dynamics*, 2008, 237, 2438–2449.
- P. Friedl and K. Wolf, *Nature Reviews Cancer*, 2003, 3, 362– 374.
- 11 P. Friedl, Current Opinion in Cell Biology, 2004, 16, 14-23.
- E. Sahai, Current Opinion in Genetics & Development, 2005, 15, 87–96.
- 13 A. R. Skovoroda, A. N. Klishko, D. A. Gusakyan, Y. I. Mayevskii, V. D. Yermilova, G. A. Oranskaya and A. P. Sarvazyan, *Biophysics*, 1995, 40, 1359–1364.
- 14 L. A. Taber, ASME Appl. Mech. Rev., 1995, 48, 487-545.
- 15 M. Lekka, P. Laidler, D. Gil, J. Lekki, Z. Stachura and A. Hrynkiewicz, *Eur. Biophys. J.*, 1999, **28**, 312–316.
- 16 B. S. Winters, S. R. Shepard and R. A. Foty, *International Journal of Cancer*, 2005, **114**, 371–379.
- 17 A. Vaziri and A. Gopinath, Nat. Materials, 2008, 7, 15–23.
- 18 C. Verdier, J. Etienne, A. Duperray and L. Preziosi, *Comptes Rendus Physique*, 2009, 10, 790–811.
- 19 A. Blumlein, N. Williams and J. J. McManus, *Scientific Reports*, 2017, 7, 7346.
- 20 L. D. Muiznieks and F. W. Keeley, *Biochimica et Biophysica Acta* (*BBA*) *Molecular Basis of Disease*, 2013, **1832**, 866–875.
- 21 P. Marmottant, A. Mgharbel, J. Käfer, B. Audren, J.-P. Rieu, J.-C. Vial, B. van der Sanden, A. F. M. Marée, F. Graner and H. Delanoë-Ayari, *Proceedings of the National Academy of Sciences*, 2009, **106**, 17271–17275.
- 22 D. Gonzalez-Rodriguez, K. Guevorkian, S. Douezan and F. Brochard-Wyart, *Science*, 2012, **338**, 910–917.
- 23 T. Vasilica Stirbat, S. Tlili, T. Houver, J. P. Rieu, C. Barentin and H. Delanoë-Ayari, *The European Physical Journal E*, 2013, 36, 1–14.
- 24 N. Khalifat, G. Beaune, U. Nagarajan, F. M. Winnik and F. Brochard-Wyart, *Japanese Journal of Applied Physics*, 2016, 55, 1102A8.
- 25 M. Steinberg, Science, 1963, 141, 401-408.
- 26 D. Ambrosi and L. Preziosi, *Mathematical Models and Methods in Applied Sciences*, 2002, **12**, 737–754.
- 27 D. McElwain and G. Pettet, Bulletin of Mathematical Biology, 1993, 55, 655–674.
- 28 C. Chen, H. Byrne and J. King, Journal of Mathematical Biology, 2001, 43, 191–220.
- 29 K. A. Landman and C. P. Please, Mathematical Medicine and Biology, 2001, 18, 131–158.
- 30 H. Byrne and M. Chaplain, Mathematical Biosciences, 1995,

130, 151 – 181.

- 31 V. Cristini, J. Lowengrub and Q. Nie, *Journal of Mathematical Biology*, 2003, 46, 191–224.
- 32 A. Friedman and F. Reitich, EJ. Math. Biol., 1999, 38, 262-84.
- 33 H. P. Greenspan, Studies in Applied Mathematics, 1972, 51, 317–340.
- 34 C. Giverso and P. Ciarletta, *European Physical Journal E*, 2016, 39, year.
- 35 R. Foty, G. Forgacs, C. Pflegerand and M. Steinberg, *Physical Review Letters*, 1994, **72**, 2298–2301.
- 36 R. Foty, C. Pfleger, G. Forgacs and M. Steinberg, *Development*, 1996, **122**, 1611–1620.
- 37 G. Forgacs, R. Foty, Y. Shafrir and M. Steinberg, *Biophysical Journal*, 1998, 74, 2227—2234.
- 38 L. Preziosi and G. Vitale, Mathematical Models and Methods in Applied Sciences, 2011, 21, 1901–1932.
- 39 C. Giverso, A. Arduino and L. Preziosi, Bulletin of mathematical biology, 2018, 80, 1017–1045.
- 40 M. A. J. Chaplain and B. D. Sleeman, *Journal of Mathematical Biology*, 1993, **31**, 431–473.
- 41 R. Skalak, S. Zargaryan, R. K. Jain, P. A. Netti and A. Hoger, *Journal of Mathematical Biology*, 1996, **34**, 889–914.
- 42 D. Ambrosi and F. Mollica, Journal of Mathematical Biology, 2004, 48, 477–499.
- 43 T. Roose, P. A. Netti, L. L. Munn, Y. Boucher and R. K. Jain, *Microvascular Research*, 2003, **66**, 204–212.
- 44 C. Voutouri, F. Mpekris, P. Papageorgis, A. Odysseos and T. Stylianopoulos, *PLoS ONE*, 2014, **9**, e104717.
- 45 R. Vandiver and A. Goriely, *Journal of Biological Dynamics.*, 2009, **3**, 180–195.
- 46 D. Ambrosi and F. Mollica, Journal of Mathematical Biology, 2004, 48, 477–499.
- 47 R. Araujo and D. McElwain., European Journal of Applied Mathematics, 2004, 15, 365—384.
- 48 J. Humphrey, Proceedings of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, 2003, 459, 3–46.
- 49 T. Stylianopoulos, J. D. Martin, V. P. Chauhan, S. R. Jain, B. Diop-Frimpong, N. Bardeesy, B. L. Smith, C. R. Ferrone, F. J. Hornicek, Y. Boucher, L. L. Munn and R. K. Jain, *Proceedings of the National Academy of Sciences*, 2012, **109**, 15101–15108.
- 50 D. Ambrosi and L. Preziosi, Biomechanics and Modeling in Mechanobiology, 2009, 8, 397—413.
- 51 C. Giverso, M. Scianna and A. Grillo, Mechanics Research Communications, 2015, 68, 31–39.
- 52 C. Giverso and L. Preziosi, *International Journal of Non-Linear Mechanics*, 2019, **108**, 20–32.
- 53 C. Giverso and L. Preziosi, International Journal of Non-Linear Mechanics, 2013, 56, 50–55.

- 54 P. Mascheroni, M. Carfagna, A. Grillo, D. Boso and B. Schrefler, *Mathematics and Mechanics of Solids*, 2018, 23, 686–712.
- 55 J. Ranft, M. Basan, J. Elgeti, J.-F. Joanny, J. Prost and F. Jülicher, Proceedings of the National Academy of Sciences, 2010, 107, 20863–20868.
- 56 B. Aigouy, R. Farhadifar, D. Staple, A. Sagner, J. Röper, F. Jülicher and S. Eaton, *Cell*, 2010, **142**, 773–786.
- 57 A. Grillo, S. Di Stefano, A. Ramírez-Torres and M. Loverre, *GAMM-Mitteilungen*, 2019, 1–30.
- 58 S. Douezan, K. Guevorkian, R. Naouar, S. Dufour, D. Cuvelier and F. Brochard-Wyart, *Proceedings of the National Academy of Sciences*, 2011, **108**, 7315–7320.
- 59 K. Guevorkian, M.-J. Colbert, M. Durth, S. Dufour and F. m. c. Brochard-Wyart, *Phys. Rev. Lett.*, 2010, **104**, 218101.
- 60 L. Preziosi, D. Ambrosi and C. Verdier, *Journal of Theoretical Biology*, 2010, 262, 35–47.
- 61 C. Giverso and L. Preziosi, *Mathematical Medicine and Biology: A Journal of the IMA*, 2010, **29**, 181–204.
- 62 X. Yang, Y. Sun and Q. Wang, *Journal of Biomechanical Engi*neering, 2013, **135**, year.
- 63 F. m. c. Graner and J. A. Glazier, *Phys. Rev. Lett.*, 1992, **69**, 2013–2016.
- 64 Y. Sun and Q. Wang, Soft Matter, 2013, 9, 2172–2186.
- 65 D. Ambrosi and F. Mollica, *International Journal of Engineer ing Science*, 2002, **40**, 1297–1316.
- 66 J. D. Humphrey and K. R. Rajagopal, *Mathematical Models and Methods in Applied Sciences*, 2002, **12**, 407–430.
- 67 J. D. Humphrey and K. R. Rajagopal, *Biomechanics and Modeling in Mechanobiology*, 2003, **2**, 109–126.
- 68 E. K. Rodriguez, A. Hoger and A. D. McCulloch, Journal of Biomechanics, 1994, 27, 455–467.
- 69 L. A. Taber and J. D. Humphrey, *Journal of Biomechanical Engineering*, 2001, **123**, 528–535.
- 70 A. Grillo, R. Prohl and G. Wittum, Continuum Mechanics and Thermodynamics, 2016, 28, 579–601.
- 71 S. Di Stefano, M. Carfagna, M. Knodel, K. Hashlamoun, S. Federico and A. Grillo, *Computing and Visualization in Science*, 2019.
- 72 E. Crevacore, S. Di Stefano and A. Grillo, *International Journal* of *Non-Linear Mechanics*, 2019, **111**, 1–13.
- 73 G. W. Brodland, J. Yang and J. Sweny, *HFSP Journal*, 2009, **3**, 273–281.
- 74 M. J. Paszek, N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, D. A. Hammer and V. M. Weaver, *Cancer Cell*, 2005, 8, 241–254.
- 75 M. Mićunović, *Thermomechanics of Viscoplasticity*, Springer New York, 2009.
- 76 S. Di Stefano, A. Ramírez-Torres, R. Penta and A. Grillo, In-

ternational Journal of Non-Linear Mechanics, 2018, **106**, 174–187.

- 77 A. Goriely, *The Mathematics and Mechanics of Biological Growth*, Springer New York, 2017.
- 78 E. H. Lee, Journal of Applied Mechanics, 1969, 36, 1-6.
- 79 Y. H. Chim, L. M. Mason, N. Rath, M. F. Olson, M. Tassieri and H. Yin, *Scientific Reports*, 2018, **8**, 14462.
- 80 S. Moreno-Flores, R. Benitez, M. dM Vivanco and J. L. Toca-Herrera, *Nanotechnology*, 2010, **21**, 445101.
- 81 M. Holmes and V. Mow, Journal of Biomechanics, 1990, 23, 1145–1156.
- 82 F. Piccinini, A. Tesei, M. Zanoni and A. Bevilacqua, *BioTechniques*, 2017, 63, 227–229.
- 83 M. T. Doolin, T. S. Ornstein and K. M. Stroka, *Cells*, 2019, 8, 427.
- 84 J. Bonet and R. D. Wood, *Nonlinear continuum mechanics for finite element analysis*, Cambridge university press, 1997.
- 85 J. Simo and T. Hughes, *Computational inelasticity.*, Springer Science & Business Media, 1988.
- 86 J. Simo, Computer Methods in Applied Mechanics and Engineering, 1988, 66, 199–219.
- 87 A. Grillo, R. Prohl and G. Wittum, *Mathematics and Mechanics* of Solids, 2017, **22**, 502–527.
- 88 G. Maugin and M. Epstein, *International Journal of Plasticity*, 1998, 14, 109–115.
- 89 P. Wriggers, J. Simo and R. Taylor, *Proceedings of the NUMETA, Swansea*, 1985, 85, year.
- 90 J. Simo and T. Laursen, Computers & Structures, 1992, 42, 97–116.
- 91 P. A. Netti, D. A. Berk, M. A. Swartz, A. J. Grodzinsky and R. K. Jain, *Cancer Research*, 2000, **60**, 2497–2503.
- 92 T. Stylianopoulos, J. D. Martin, M. Snuderl, F. Mpekris, S. R. Jain and R. K. Jain, *Cancer Research*, 2013, **73**, 3833–3841.
- 93 L. Andolfi, S. L. Greco, D. Tierno, R. Chignola, M. Martinelli, E. Giolo, S. Luppi, I. Delfino, M. Zanetti, A. Battistella, G. Baldini, G. Ricci and M. Lazzarino, *Acta Biomaterialia*, 2019, 94, 505 – 513.
- 94 P. A. Netti, D. A. Berk, M. A. Swartz, A. J. Grodzinsky and R. K. Jain, *Cancer Research*, 2000, **60**, 2497–2503.
- 95 H. M. Phillips and M. S. Steinberg, Proceedings of the National Academy of Sciences of the United States of America, 1969, 64, 121–127.
- 96 H. Phillips, Integrative and Comparative Biology, 1978, 18, 81– 93.
- 97 M. Zanoni, F. Piccinini, C. Arienti, A. Zamagni, S. Santi, R. Polico, A. Bevilacqua and A. Tesei, *Scientific Reports*, 2016, 6, 19103 EP –.
- 98 J. C. Mombach, D. Robert, F. Graner, G. Gillet, G. L. Thomas, M. Idiart and J.-P. Rieu, *Physica A: Statistical Mechanics and*

its Applications, 2005, **352**, 525 – 534.