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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 spheroids (MCS), represent one of the most valid in vitro systems to study the dynamics of multicellular three-dimensional systems, being an intermediate step between monolayer growing cells and tissue culture^{1,2}. In particular, living spheroids, made up of either healthy or malignant cells up to a size of 100-600 μm , are rather simple to prepare and well mimic in vivo phenomena occurring inside tissues and organs, encompassing growth, structural reorganisation, cell-cell and cell-extracellular environment interactions, response to external and endogenous stimuli, embryogenesis, malignant invasion, wound healing, and tissue engineering^{1,3-8}. Furthermore, 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 conditions. For instance, even though embryogenesis and morphogenesis (i.e. the complex set of events through which a living organism acquires its final shape) are under genetic control, genes by themselves do not create forms and

shapes. This is achieved by physical forces, which drive structure formation in a delicate interplay of genetic, molecular and physical factors⁹. In the same way, cancer cell invasion and the formation of metastasis is controlled by genetic mutations and altered patterns of gene expression, but the physical motion of cells in the surrounding environment is determined by the mechanical properties of the cells and the extracellular environment and by their complex interactions¹⁰⁻¹⁶. Therefore, the development of three dimensional cell culture models to bridge the gap between cell-based assays and animal studies has gained the attention in the last decades, with the intent of reducing experimental uncertainties arising from monolayer cell cultures and hence the costs of subsequent in vivo drug screening processes. However, the correct interpretation of the experimental results obtained in these living multicellular settings requires the thorough understanding of the overall biophysical and mechanical properties of such systems, which emerge in a complex manner from the properties of the individual constituents (i.e. cells, extracellular matrix, liquid, vessels, etc.) forming the system and from the interplay among them, possibly mediated by subcellular molecules and organelles. This task is really challenging since cells and biological tissues are complex media, made of multiple subelements, with different me-

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chanical properties and with various biological functions¹⁷: each cell is bounded by the plasma membrane to form a closed object containing the nucleus and a fluid, the cytosol (made of water, soluble proteins, sugar and salt), in which numerous organelles are immersed. Each subcellular element is different from the others and mechanical properties are non-homogeneously distributed inside each of them¹⁸. This high heterogeneity in cell composition and in subcellular properties makes mechanical and biological response difficult to be modelled even for a single cell. Furthermore, cells are able to actively interact with each other to form tissues and MCSs, containing both cells, fluids (embedded inside each cell and in the intracellular space) and possibly extracellular matrix (ECM). The rheological properties of such materials are quite uncommon and are characterized by the occurrence of many phenomena at the subcellular, cellular and macroscopic scales. The mechanical properties of the cytoskeleton, the cell membrane, the cell cytoplasm and the nucleus determine the mechanical response of an individual cell in isolation, whilst the mechanical behaviour of an ensemble of cells or a tissue is not merely the sum of each single contribution. Rather, it arises through the association and disassembling of adhesion molecules between the cells and the extracellular matrix^{19,20} and through articulate mechanisms of communications and transduction of both external and internal stimuli. In general, the elastic or elasto-plastic behaviour of a MCS results from a complex interplay between cell bulk, mainly represented by cytoskeleton and organelles, and cell surface, which involves, in particular, its actomyosin cortex²¹. On the other hand, viscous effects are mainly due to the presence of the liquid. In particular, the ability of an aggregate to behave as an elasto-plastic material or as a viscous fluid depending on the experimental conditions, is related to the cell adhesion properties, to the type of interactions among the cells and to the contraction of the cell cortex. All these phenomena can lead, in general, to the presence of stress thresholds (which can be viewed as “energy barriers”²¹) that have to be overcome for the activation of the aggregate’s dynamics to occur²¹.

This variety of behaviours has led to the definition of many different mathematical models of multicellular aggregates and living tissues, each of them focusing on different biological aspects at different time and space scales (see Table 1 for a non-exhaustive review of previous modelling effort of quiescent multicellular aggregates and the works of Gonzalez-Rodriguez et al.²² and Stirbat et al.²³ and Khalifat et al.²⁴ for a more comprehensive review of rheological properties of multicellular aggregates). Inspired by cell sorting experiments on embryonic aggregates, in most cases, tissues have been described as liquids, characterized by a viscosity and a surface tension^{3,22,25}. Consequently, fluid-like constitutive equations have been advocated to model the mechanical response of growing living systems^{26–28,28–34} and quiescent multicellular aggregates^{22,25,35–37}. However, biological experiments²¹ show

that the behaviour of an aggregate can strongly deviate from the one of a liquid. Thus, this approach gives back a not completely satisfying approximation of the by far more complex behaviour of cellular aggregates, which also display solid-like properties related to the adhesive characteristics of the cells^{21,38} and to the mechanical properties of the single cell in a cluster³⁹. In particular, because of the occurrence of residual stresses, the stress asymptotic plateau can be sensibly higher than the one predicted by the pure surface tension in liquid models^{35–37}; some aggregates (e.g. Chinese hamster ovary (CHO) cell aggregates) are not always able to fuse and round up within the time of experiments or simulations; the aggregate shape after relaxation sometimes displays a strong deviation from that of a liquid drop²¹. Thus, in some cases, cell aggregates are better described as solids with linear or nonlinear elasticity^{21,40–48}. At the same time, it is not correct to consider MCSs as elastic solids, because they are composed of living material: the cells forming the aggregates duplicate and die continuously, the ECM constantly remodels because of cell reorganisation and, even in absence of growth and death, cells can rearrange their relative adhesion complexes in response to external mechanical stimuli. Moreover, living systems manifest anelastic reorganisation of the internal structure and residual stresses^{41,44,49}, two unique features with no analogy in liquids^{50–53}. In particular, the description of such phenomena can be achieved by assuming a plastic-like behaviour of the biological structures under study^{51,54}. Thus, the debate about the best mechanical modelling approach is still open and a comprehensive model of multicellular aggregates and living tissues is far from being developed. Then, a specific MCS mechanical model should be chosen depending on the phenomena we are interested in and on the time and length scale of the observation, recalling that cell aggregates behave as fluids on the timescales of cell division (mitosis) and apoptosis, which characterise growth (many hours or few days)^{9,21,23,37,55,56}, and as elasto-plastic solids on shorter timescales of the order of some minutes or few hours. Therefore, if we want to focus only on the description of cell compression during the time lapse of a biological experiment of the type studied in the sequel, elasto-plastic models will better describe cell behaviour, whereas the long-time fluid-like behaviour is more appropriate for capturing cell proliferation and death^{55,57}.

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

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

142 tude^{9,25,35–37}. Then, to perform a *stress relaxation test*, the force
 143 exerted by the aggregate on the upper plate is recorded (by mea-
 144 suring the apparent weight of the upper compression plate with
 145 a Cahn-Ventron electrobalance, connected to the upper compres-
 146 sion plate⁹), while maintaining the deformation constant, until it
 147 reaches a constant stationary value. When this state is reached,
 148 the compression plates are separated and the aggregate is let
 149 free to possibly regain its initial shape (*shape recovery test*). Dur-
 150 ing the release phase, the aggregate shape is continuously video
 151 recorded: it is observed that, if the compression is maintained
 152 for a very short time, the aggregate will bounce back to its initial
 153 shape, thus behaving as an elastic (or viscoelastic) solid; on the
 154 other hand, if the compression is maintained for a longer time,
 155 and if it is sufficiently high to induce the reorganization of the
 156 cellular structure, the initial configuration is no longer recovered
 157 after the compression plate is removed (at least for the times for
 158 which other phenomena, such as growth and apoptosis, do not
 159 occur). This denotes an elasto-plastic (or visco-elasto-plastic) be-
 160 haviour of the living structure, which cannot be captured by the
 161 pure fluid model, based essentially on the existence of a surface
 162 tension holding together the cell aggregate^{35–37}.

163 Starting from the elasto-plastic model proposed in^{50,60,65} and
 164 the elasto-visco-plastic model presented in^{53,61}, we here propose
 165 a three-dimensional model of multicellular aggregate compres-
 166 sion at constant deformation, supported by three-dimensional
 167 numerical simulations of the problem in a realistic geometry in
 168 order to overcome some limitations of previous works. Specif-

ically, in^{50,60} it was proposed to apply the *theory for materials*
 with *evolving natural configurations*, introduced in^{66–69}, to suc-
 cessfully investigate cell aggregate growth and remodelling, by
 coupling the visco-elastic behaviors with a yield condition, gener-
 ating a plastic reorganization inside the structure, when the stress
 becomes too high. The viscous contribution of the liquid, em-
 bedded inside the cells and filling the voids of the multicellular
 structure, was then introduced^{53,61} in order to fit the stress-free
 evolutions of spheroids observed in^{35–37} when the constant de-
 formation is removed. However, in all these works^{50,53,61}, the
 representation of the whole experimental setting is highly simpli-
 fied, postulating a homogeneous and constant cell density and a
 homogeneous deformation inside the whole body. The deforma-
 tion on the normal plane to the applied force or displacement is
 then imposed in order to guarantee the conservation of the total
 aggregate volume and mass. Under these simplifying assump-
 tions, the model was reduced to a set of two ordinary differential
 equations^{53,61}, that can be easily studied analytically. This is of
 course a simplification of the real phenomenon, since, even when
 the aggregate compression is directed only along one direction,
 the deformation of the living body and the cell density inside are
 not homogeneous and the determination of the correct shape of
 the system is determined by solving the mass and momentum bal-
 ance inside the whole structure in the fully three dimensional set-
 ting, with proper boundary conditions. In this regard, we resort
 to other works^{54,70–72}, in which three dimensional visco-elasto-
 plastic models for biomechanical problems are presented. Such

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

205 In this work we present a fully three dimensional model for
 206 cell aggregate compression and the numerical simulations consid-
 207 ering the real biological setting. In particular the mathematical
 208 model is introduced in Section 2, then the model is numerically
 209 solved to reproduce stress relaxation experiments and shape re-
 210 covery tests in Section 3. Finally, the main outcomes of this work
 211 and future improvements are discussed in Section 4.

212 2 Materials and Methods

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

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

231 2.1 The aggregate model

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

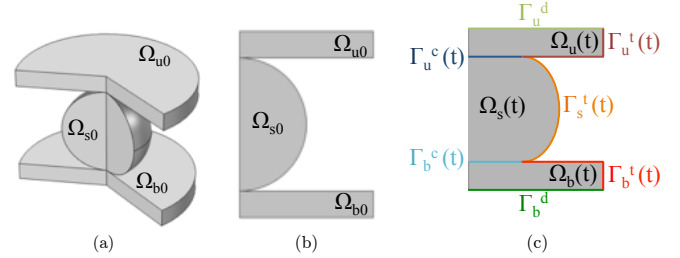


Fig. 1 Geometry of the in-silico model of cell aggregate compression. (a) Three dimensional numerical domain: Ω_{s0} , Ω_{u0} and Ω_{b0} represent the spheroid, the upper and lower plates in their reference and, in this case, initial configurations, respectively. (b) Two dimensional geometry obtained 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 $\Gamma^d = \Gamma_u^d \cup \Gamma_b^d$, the free traction boundary $\Gamma^t(t) = \Gamma_u^t(t) \cup \Gamma_b^t(t) \cup \Gamma_s^t(t)$, and the contact boundary $\Gamma^c(t) = \Gamma_u^c(t) \cup \Gamma_b^c(t)$.

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

$$244 \quad \partial_t \rho_\alpha + \nabla \cdot (\rho_\alpha \mathbf{v}_\alpha) = 0, \quad \text{with } \alpha = s, u, b, \quad (1)$$

$$245 \quad \rho_\alpha \dot{\mathbf{v}}_\alpha = \rho_\alpha (\partial_t \mathbf{v}_\alpha + \mathbf{v}_\alpha \cdot \nabla \mathbf{v}_\alpha) = \nabla \cdot \mathbf{T}_\alpha, \quad \text{with } \alpha = s, u, b, \quad (2)$$

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

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

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

$$\chi_s(t, \cdot) : \Omega_{s0} \mapsto \mathbb{R}^3, \mathbf{X} \mapsto \mathbf{x} = \chi_s(t, \mathbf{X}) \in \Omega_s(t) \subset \mathbb{R}^3,$$

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

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

$$\mathbf{F} = \mathbf{F}_n \mathbf{F}_p.$$

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

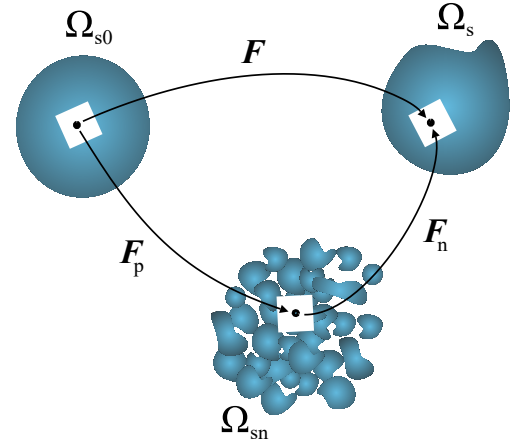


Fig. 2 Diagram of the multiplicative decomposition of the deformation gradient tensor \mathbf{F} in the framework of evolving natural configurations: the reference configuration, Ω_{s0} , the current configuration, Ω_s , the natural state Ω_{sn} .

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

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}_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.

$$\mathcal{W}_{sn} = \alpha_0 [\exp(\Psi) - 1], \quad (3a)$$

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

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

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

340 Then, the Cauchy stress tensor reads

$$\mathbf{T}_s = J_n^{-1} \mathbf{F}_n \left(2 \frac{\partial \mathcal{W}_{sn}}{\partial \mathbf{C}_n} \right) \mathbf{F}_n^T. \quad (5)$$

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

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

$$\dot{\mathbf{B}}_p = -\frac{2}{\lambda_p} \left[1 - \frac{\tau_y}{f(\mathbf{T}'_s)} \right]_+ \mathbf{B}_p \mathbf{M}'_s \quad (6)$$

371 where $[\cdot]_+$ denotes the positive part of its argument, λ_p is a ma-
 372 terial parameter related to the reorganization time due to remodel-
 373 ling, τ_y is the yield stress of the aggregate, $f(\mathbf{T}'_s)$ is a frame-
 374 invariant equivalent measure of the stress \mathbf{T}'_s , $\mathbf{M}_s = J \mathbf{F}^T \mathbf{T}_s \mathbf{F}^{-T}$
 375 is the Mandel stress tensor of the cell aggregate and the apex
 376 $(\cdot)'$ denotes the deviatoric part of the tensor field to which it is
 377 applied. We remark that eq. (6) assumes that remodelling mani-
 378 fests itself as a rate-dependent plasticity model of Perzyna-type⁷⁵,
 379 which means that remodelling occurs only when $f(\mathbf{T}'_s)$ exceeds
 380 the threshold stress value τ_y , and is modulated by the timescale
 381 λ_p ^{53,61,70,75}. This assumption captures the essential phenomena
 382 occurring inside the aggregate at the cell scale: if we consider a
 383 cluster of cells subjected to a sufficiently high stress, some of the

384 adhesive bonds among the cells may break and eventually reform
 385 in other places. Finally, we notice that, while the left-hand-side
 386 of eq. (6) is symmetric by definition, the right-hand-side is sym-
 387 metric only for isotropic media, for which the Mandel stress ten-
 388 sor \mathbf{M}_s satisfies the symmetry condition⁸⁸ $\mathbf{B}_p \mathbf{M}_s = (\mathbf{M}_s \mathbf{B}_p)^T$, as in
 389 this case. For anisotropic materials, eq. (6) is no longer valid,
 390 and the way in which remodelling is conceived must take into ac-
 391 count the evolution of the anisotropy^{71,72}. For example, this is
 392 the case of fibre-reinforced tissues, whose macroscopic mechani-
 393 cal properties and remodelling are substantially influenced by the
 394 distribution of the fibres embedded in the extracellular matrix.

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

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

404 where $\mathbb{C}_\alpha = \mathbb{C}_\alpha(E_\alpha, \nu_\alpha)$ is the fourth-order stiffness tensor, which
 405 depends on the Young's modulus E_α and the Poisson's ratio ν_α
 406 of the plates, because of the isotropy of the plates, with $\boldsymbol{\varepsilon}_\alpha =$
 407 $1/2 [\nabla \mathbf{u}_\alpha + (\nabla \mathbf{u}_\alpha)^T]$ being the infinitesimal strain tensor, given \mathbf{u}_α
 408 the displacement vector field inside the plates.

409 2.2 Boundary conditions of the aggregate model.

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

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

427 In particular, to describe the uniaxial compression test of cellu-
 428 lar aggregates, we impose a null displacement on the upper side

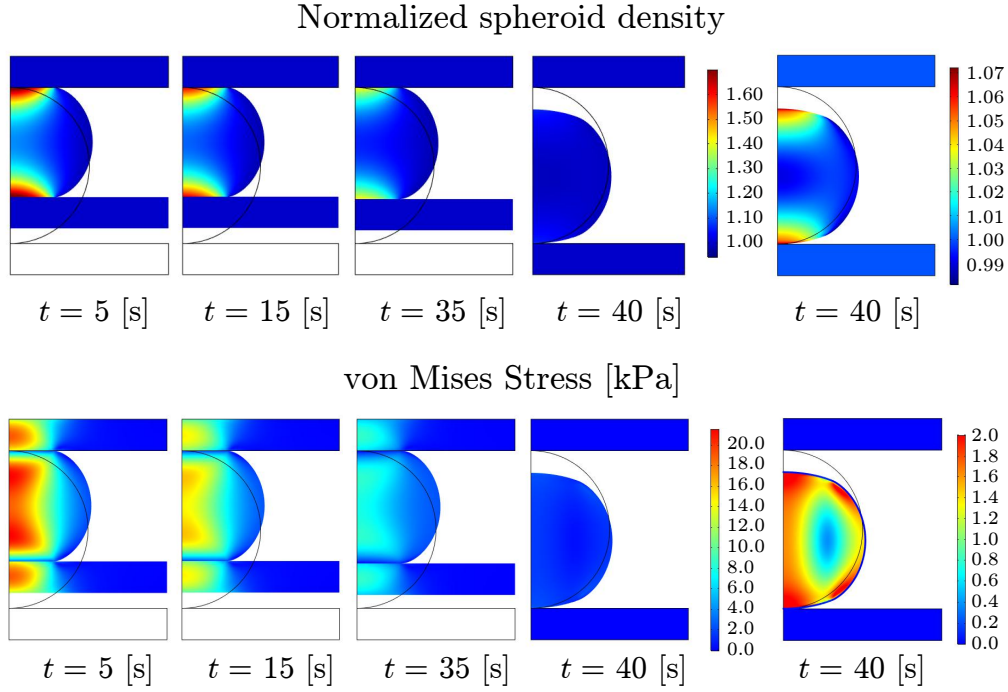


Fig. 3 Spatio-temporal evolution of the normalized spheroid density $\bar{\rho} := \rho_s/\rho_{s0} = J^{-1}$ (top row) and von Mises stresses $T_\alpha^{mises} := \sqrt{3/2} \|\mathbf{T}'_\alpha\|$, with $\alpha = \{s, u, b\}$ (bottom row) inside each domain. The loading and unloading ramp time is equal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

429 of the upper plate $\Gamma_u^d(t)$ and a vertical displacement ramp $u_z(t)$
 430 on the lower boundary of the bottom plates, $\Gamma_b^d(t)$, such that
 431 $\Gamma^d(t) = \Gamma_u^d(t) \cup \Gamma_b^d(t)$ (see Fig. 1-(c)). Frictionless contact bound-
 432 ary conditions apply on the two contact regions between the aggre-
 433 gate and the upper and lower plates, $\Gamma^c(t) = \Gamma_u^c(t) \cup \Gamma_b^c(t)$, with
 434 $\Gamma_u^c(t)$ and $\Gamma_b^c(t)$ being the contact boundary between the spheroid
 435 and the upper and bottom plate, respectively (see Fig. 1-(c)).
 436 Free surface boundary conditions are imposed on the remaining
 437 portions of the domains, $\Gamma_\alpha^f(t)$ with $\alpha = s, u, b$. We observe that,
 438 whilst the location of the boundaries Γ_u^d and Γ_b^d is known, the
 439 boundaries $\Gamma_u^c(t)$, $\Gamma_b^c(t)$ and $\Gamma_\alpha^f(t)$ change in time, depending on
 440 whether the surfaces of the two bodies come in contact or detach,
 441 and that the sets $\Gamma_u^c(t)$ and $\Gamma_b^c(t)$ can possibly be empty.
 442 Therefore, the following set of boundary conditions (BCs) de-
 443 scribes the uniaxial compression test

$$\mathbf{u}|_{\Gamma_u^d} = \mathbf{0}, \quad (8)$$

$$\mathbf{u}|_{\Gamma_b^d} = u_z(t)\mathbf{e}_z, \quad (9)$$

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

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

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

2.3 Finite element numerical simulations

444 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 $u_z(t)$
 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

BCs (11) and (12) can be conveniently accounted for by employing the “augmented Lagrangian method”^{89,90}. This method implements a *penalty regularization* of the standard Lagrangian multiplier method by incorporating both a Lagrange multiplier and a penalty term to solve the contact constraints and impose the conditions (11) and (12). Indeed, while in the standard formulation of the Lagrange multiplier method, the Lagrange multiplier is an unknown, in the augmented Lagrangian method, it is computed algorithmically and its initial estimation is iteratively improved, until the constraint violation is small enough (or equivalently, until the multiplier stops changing appreciably).

The Lagrangian numerical simulations have been obtained using the finite element software (FEM) COMSOL Multiphysics® (version 5.3a). The contact boundary conditions (11) and (12) are imposed by combining the routine expressly written to solve the evolution law of plastic deformations with the built-in environment for contact constraints in COMSOL Multiphysics, developed by taking inspiration of the work by Simo and Laursen⁹⁰. We then choose a segregated approach, where the contact pressures are solved in a separate lumped step, which is the default solver when the augmented Lagrangian formulation is used. Given the numerical issues arising from the treatment of contact boundary conditions in the case of an elasto-plastic model of cellular aggregates, we have performed several tests to choose a proper mesh for solving the aggregate compression problem presented in our work. The dimension of the mesh elements at the border of the cell aggregate should be at least half the typical dimension of the elements at the upper and lower plates boundaries, to give a good resolution of the contact patch and stress state in the contact regions. In particular, computations involving a spheroid of 100 μm in radius were performed using a mesh of 7978 triangular elements in the spheroid bi-dimensional section and 380 triangular elements inside the 2D sections of each plate, for a total of 8736 elements. The number of mesh elements have then been adapted to the cases of greater spheroid radii. To verify the quality of our mesh, we have used a functionality implemented in COMSOL Multiphysics® and we have obtained a positive response. As last step, before using the mesh described so far, we have performed several refinements and solved the same benchmark tests. In doing this, we have noticed no significant changes in the results, with an increasing of the time needed to complete the simulations (i.e., few hours of computation instead of one or even more days). The choice of this kind of mesh has represented, for us, the best compromise between computational efficiency and accuracy, also in the light of running several sets of simulations.

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 μm	1,3-5
λ_p	0.001 (kPa · s) ⁻¹	54
τ_y	2 kPa	87
μ	20 kPa	43,54,91,92
ν	0.2	54,91,92

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

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

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

where t_{ramp} is small compared to the compression time $t_c = t_{\text{end}} - 2t_{\text{ramp}}$. Figure 3-top reports the normalized spheroid density $\bar{\rho} := \rho_s / \rho_{s0} = J^{-1}$ in the case in which remodelling is triggered: after the sudden imposition of the deformation, the cellular density highly increases in the region close to the upper and lower plates and decreases close to the middle point of the outer boundary, where $J > 1$, as a consequence of the volumetric expansion of the spheroid along the radial direction. As the compression is maintained, the cells reorganize and redistribute inside the aggregate and the compaction of the cells inside the spheroid decreases. When the compression is released the density of cells inside the deformed aggregate continues to be inhomogeneous and different from the initial one (see last picture in the top row of Fig. 3). We remark that the total mass of the cellular spheroid is preserved during the compression and release of the cellular aggregate. Finally, we note that, the plates being slightly deformable, their normalized density is almost constant.

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

Remodelling inside the spheroid

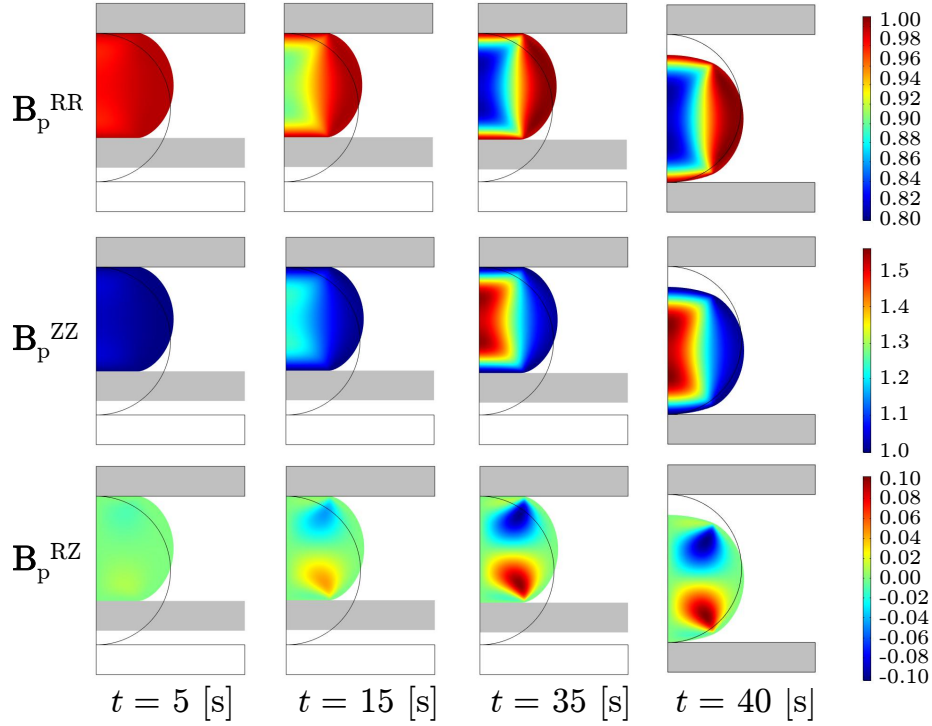


Fig. 4 Spatio-temporal evolution inside Ω_s of the components of the remodelling tensor $\mathbf{B}_p = \mathbf{C}_p^{-1}$ where $\mathbf{C}_p = \mathbf{F}_p^T \mathbf{F}_p$ is the plastic Cauchy-Green deformation tensor. The parameters in the simulations are the same used for the results in Fig. 3.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

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

572 To quantify the amount of remodelling triggered by T_s^{Mises} , in
 573 Fig. 4 we report the radial, axial and shear component of the
 574 remodelling tensor $\mathbf{B}_p = \mathbf{C}_p^{-1}$. We observe that the radial com-

ponent B_p^{RR} is less than 1 almost everywhere in the aggregate,
 since, when the aggregate is compressed, remodelling in the ra-
 dial direction occurs due to the expansion of the structure along
 the radial axis and only a small region close to the middle point of
 the outer boundary experiences compressive radial remodelling.
 The axial component B_p^{ZZ} of \mathbf{B}_p is bigger than 1 everywhere since
 remodelling occurs due to compression in the axial direction. Fi-
 nally B_p^{RZ} is a measure of the remodelling due to shear. The sign
 of the shear remodelling is in agreement with the convention used
 for shear stresses: positive shear stresses act clockwise, while neg-
 ative shear stresses act counter-clockwise. The point delimiting
 the contact area between the spheroid and the upper plate de-
 fines the starting point of the 45° plane that identifies a change
 of sign in the shear remodelling. Close to the lower plate, in the
 region below the 45° -plane, B_p^{RZ} is negative since shear is nega-
 tive there, while in the region above the 45° -plane, B_p^{RZ} is positive
 since the shear is positive there. Similar reasoning applies to the
 region close to the upper plate, with a change of sign due to the
 convention on the sign of shear stresses.

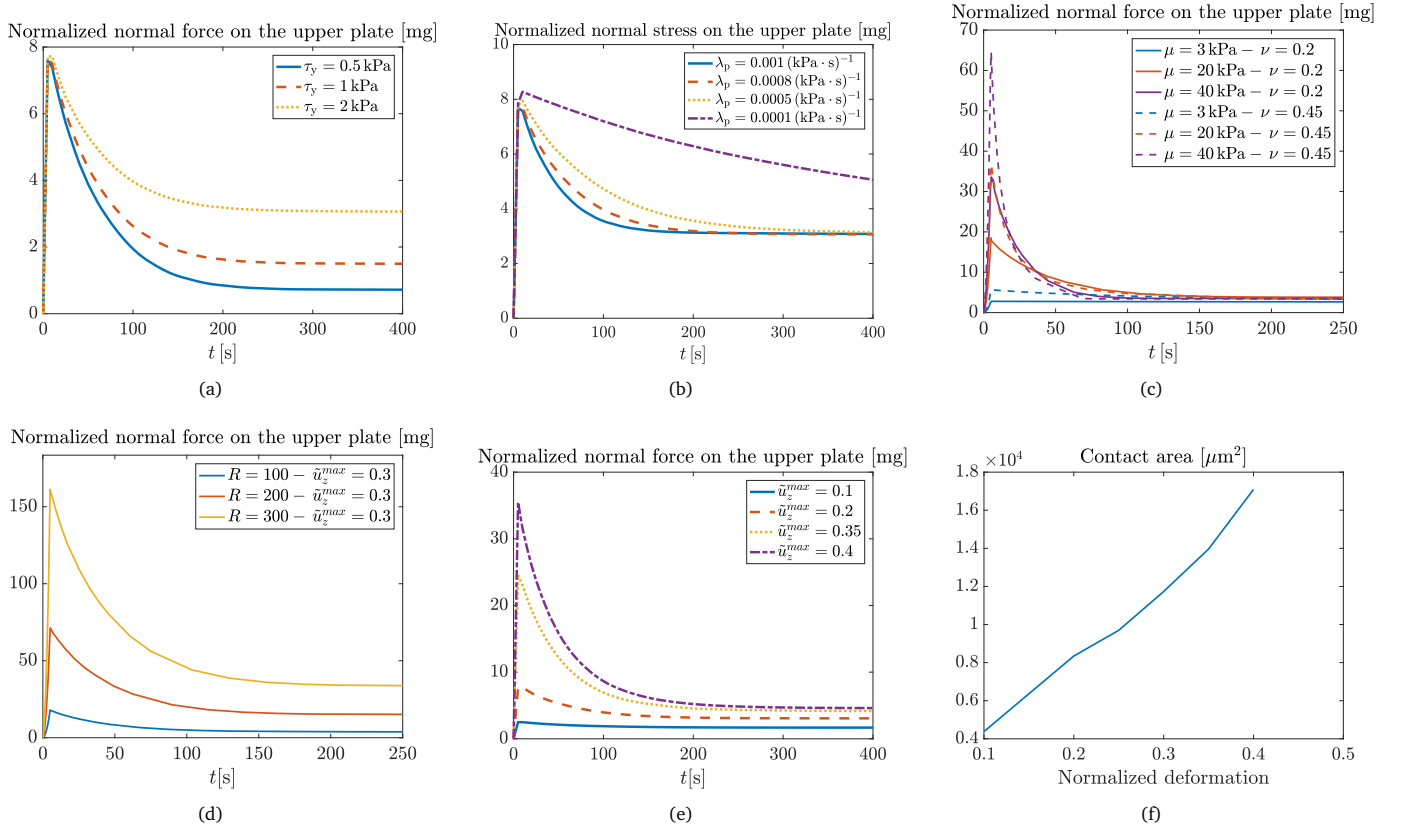


Fig. 5 Stress relaxation curves for different values of the parameters (a) τ_y , (b) λ_p , (c) μ and ν , (d) R and (e) normalized imposed deformation, i.e., $\bar{u}_z^{max} = \bar{u}_z^{max}/(2R)$. The curves are obtained integrating the stress exerted by the aggregate over the surface of contact with 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, $\bar{u}_z^{max} = \bar{u}_z^{max}/(2R)$.

594 3.2 Stress relaxation curves

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

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
 then exploited the effect of varying the cell mechanical paramet-
 ers μ and ν on the MCS response. The mechanical parameters
 are strongly dependent on the cell type considered and a wide
 range of parameters can be found in the literature. Specifically,
 supported by biological evidences, we take the shear modulus μ
 varying between 3 kPa and 40 kPa^{43,94} and the Poisson's ratio
 ν ranging between 0.2 and 0.45^{43,92}. The resulting stress relax-
 ation curves (Fig. 5-c) show that the mechanical parameters
 mostly affect the value of the initial stress exerted on the upper
 plate, with higher initial stresses for increasing value of μ . Then,
 for the same value of μ , the Poisson's ratio ν further magnifies the
 initial stress exerted on the upper plate. We also observe that, for

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

654 We remark that the reported stress relaxation curves are qual-
655 itatively in agreement with the experimental curves reported in
656 the works of Forgacs et al.³⁵⁻³⁷, Jakab et al.⁹ and Andolfi et al.⁹³.
657 Indeed, by compressing multicellular spheroids composed by either
658 limb bud mesoderm, or heart ventricles, or livers cells taken
659 from chicken embryos, they observed that the initial force (nor-
660 malized with respect to the gravitational acceleration) exerted
661 by the aggregate on the upper plate which is in the range 7-8
662 mg is relaxed to a load in the range 2.5-4 mg for a compres-
663 sion at constant deformation maintained for 160 s. On the other
664 hand, in the experimental work of Jakab et al.⁹ on Chinese Ham-
665 ster Ovary (CHO) cells, stress relaxation is achieved on longer
666 timescale ($\approx 400 - 1000$ s). In this work, without performing a
667 quantitative analysis and without conducting a direct validation
668 (which would require further biological data and details on the
669 mechanical tests), we showed in Fig. 5 that our model is able to
670 reproduce a sufficiently wide range of normalized stresses. We
671 achieved that by varying the parameters of the model, so that dif-
672 ferent cellular populations can be described by varying the com-
673 bination of the parameters of our model, possibly supported by
674 other mechanical tests. Specifically, in contrast to what has been
675 done in³⁵⁻³⁷, where viscous effects are included, we reproduce
676 here the typical stress relaxation curves reported in the literature
677 by resorting solely to the reorganization of the cells inside the
678 structure. Indeed, as anticipated in the Introduction, as long as
679 relatively short timescales are considered, this process seems to
680 be the fundamental mechanism occurring in the biomechanical
681 tests addressed in this work. In fact, upon a detailed analysis of

682 the biological experiments in which the displacement of fluores-
683 cently labeled cells is followed by confocal microscopy during ag-
684 gregate compression⁹, it is possible to see that tissue relaxation
685 is driven predominantly by cell shape changes, a unique prop-
686 erty of living systems with no analogy in liquids. Furthermore,
687 using field emission scanning electron microscopy (FESEM) to
688 visualize individual cells in a precompressed, compressed, and
689 postcompressed equilibrated aggregate⁹, it is possible to see that
690 after compression a pressure gradient is set up. This is put in
691 evidence by the fact that cells in the vicinity of the compressive
692 plates and toward the vertical axis of symmetry of the compressed
693 aggregate are deformed more strongly than those near the equa-
694 tor and side boundary, which denotes a solid-elastic behaviour
695 of the cellular aggregate under compression, in accordance also
696 with our simulations (before the occurrence of plasticity). Then,
697 whilst when the aggregate is described as a viscoelastic material,
698 any internal stress created by the initial compression is dissipated
699 by the time the system reaches equilibrium and the remaining
700 stresses are encapsulated only at the interface between the ag-
701 gregate and the surrounding tissue culture medium³⁵⁻³⁷, in the
702 case of an elasto-plastic model, such as the one proposed here,
703 residual stresses may appear inside the structure, in accordance
704 with^{41,44,49}. Therefore, even though stress relaxation curves can
705 be reproduced only accounting for viscosity, elasto-plastic models
706 might be more adequate to capture the biological phenomenon.

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

723 3.3 Shape recovery curves

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

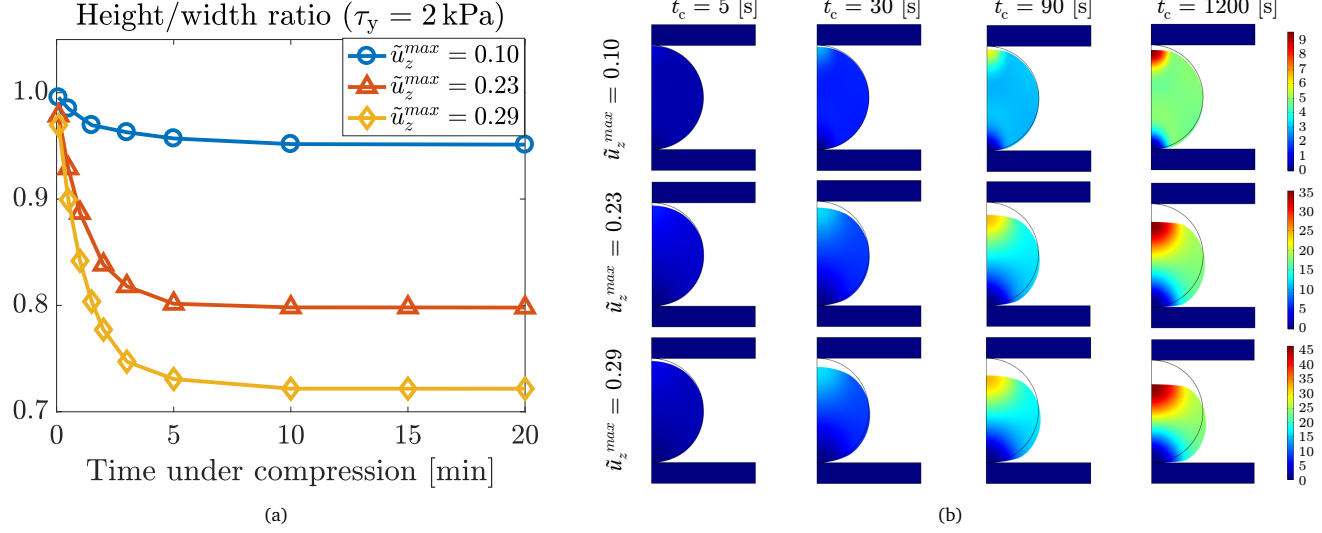


Fig. 6 (a) Height-width ratio chart for different values of imposed deformation and (b) corresponding aggregate deformed shapes. The height-width ratio is obtained dividing the height by the width of the deformed aggregate, after release of the imposed deformation (normalized with respect to the initial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

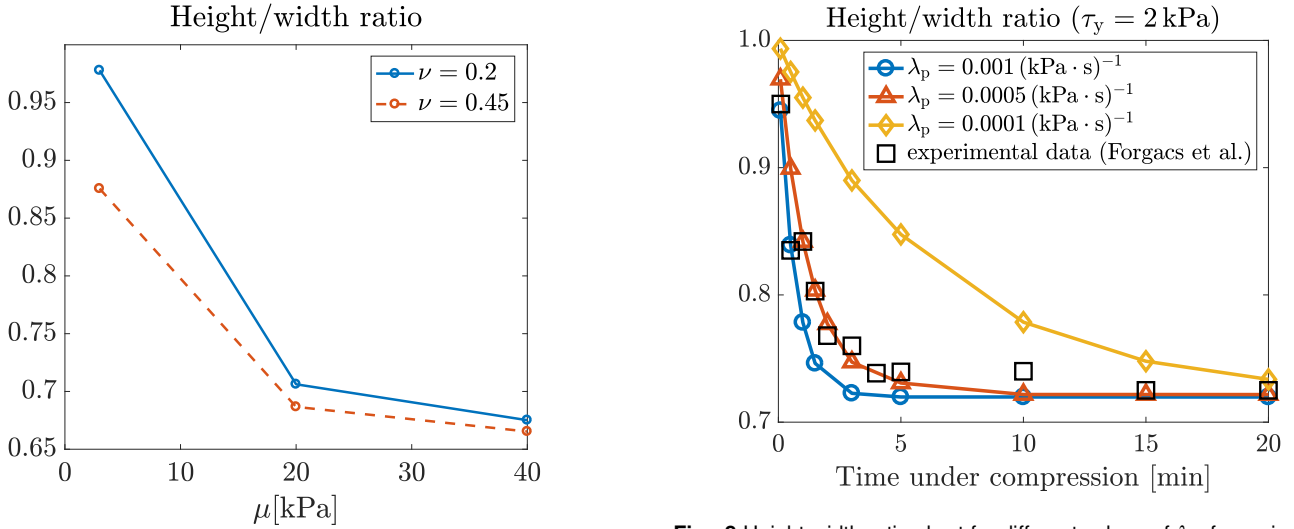


Fig. 7 Height-width ratio at the stationary state for different values of μ and ν , for an imposed deformation of $\tilde{u}_z^{max} = 0.3$.

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

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

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

747 verted in the model in the existence of a plastic behaviour.

748 Therefore, we have focused on the results obtained by means of

749 the employment of our elasto-plastic model in the case in which

750 the compression is maintained for different times and we report in

751 Fig. 6-a the spheroid height over width ratio for increasing values

752 of the cumulative time under compression, t_c , and for different

753 values of normalized imposed deformations, $\tilde{u}_z^{max} := \tilde{u}_z^{max}/(2R)$.

754 From the reported curves it is possible to see that, in accordance

755 with the biological evidences, if the compression is maintained

756 for few minutes, the aggregate will bounce back to almost its initial

757 shape, since in this case the extent of the plastic rearrangement

758 is not consistent. On the other hand, if the compression

759 is maintained for a longer time, the aggregate remains flattened

760 after subsequent releases from compression, signifying the attainment

761 of the stationary state. This is also clear from the deformed

762 configurations reported in Fig. 6-b, where the aggregate's shape

763 and the spatio-temporal evolution of the displacement inside the

764 aggregate are reported for different values of cumulative time under

765 compression and for different imposed deformations. We also

766 show that, for the same value of normalized imposed deformation

767 and yield stress, the total plastic deformation of the MCS is

768 highly influenced by the mechanical parameters μ and ν (see Fig.

769 7), whilst it is not affected by the initial radius of the spheroid

770 (not shown in the paper). We then compare the curves obtained

771 from the numerical simulations for different values of λ_p with the

772 experimental data reported in the work of Forgacs et al.³⁷ (see

773 Fig. 8). It is possible to see that the best fitting for the height

774 over width ratio curves occurs for $\lambda_p = 0.0005 \text{ (kPa} \cdot \text{s)}^{-1}$, given

775 an imposed deformation of 29% of the spheroid initial size. This

776 observation is also in agreement with the experimental observation

777 reported in the work of Jakab et al.⁹, in which they observe

778 that aggregates can be compressed up to a maximum of $\approx 30\%$

779 of their original diameter, in order to avoid irreversible damage

780 to the cells and intense shape modification of the cellular structure.

781 For the sake of completeness, we point out that the numerical

782 height over width ratio curves have been obtained without

783 allowing the relaxation of the MCS after each compression step,

784 differently from what done in the experimental work of Forgacs

785 et al.³⁷. However, the difference between the numerical and the

786 experimental protocols does not significantly affect the plastic

787 deformation of the aggregate, since in our model we do not include

788 viscous effects and the plastic reorganization mainly occurs during

789 the compression phases. To confirm this theoretical expectation,

790 we have also run a simulation in which the compression is

791 removed at intervals (corresponding to the data points reported

792 in Forgacs et al.³⁷) and the spheroid is let free to relax for 11 s:

793 the discrepancy between the two protocols leads to height over

794 width ratios that differ less than 0.4% (results not shown here).

795 Finally, we remark that in our numerical tests, the spheroid

796 deformation is maintained even for very long time, after the release

of the imposed deformation. In order to reproduce the long-time

recovery of the initial spherical shape, other factors should be included

in the model, such as the presence of the external liquid and the

proliferation of cells, that have not been accounted for in the

present model.

4 Conclusions

Biological tissues show complex mechanical responses and their

mechanical behaviour is still far from being completely understood.

In this work, we aim to move a step towards this involved purpose

by defining a setting to simulate the mechanical behaviour of cell

aggregates when they are subjected to a uniaxial compression test.

In particular, we consider an elasto-plastic model and we numerically

solve it through finite element simulations by imposing contact

boundary conditions to simulate the experimental set-up. With

respect to previous mechanical models on aggregates^{53,61}, we here

numerically solve the real three dimensional problem, with

inhomogeneous deformation and complex shape changes. By

doing this, we have provided the visualisation of a compression

test on multicellular spheroids that requires the formulation of

a contact problem to extract information on MCSs inelastic

behaviour. We have observed, for instance, the redistribution of

the spheroid's mass density in response to applied compressive

loads, the reorganisation of the spheroid's internal structure,

described through the inelastic variable \mathbf{B}_p , and the time

evolution of the height-to-width ratio of the spheroid. From

the point of view of numerical simulations, within a fully

nonlinear regime, the contact boundary conditions are combined

with the evolution law of plastic deformations, with the latter

ones being determined by means of a routine expressly written

for the works^{54,70,71}, and without having recourse to standard

COMSOL packages. The numerical results demonstrate that the

stress relaxation curves reported in the literature could be

explained by assuming an elasto-plastic behaviour of the

spheroids, i.e., without taking into account viscous effects,

differently from previous models³⁵⁻³⁷. At the same time,

they show that the permanent deformation observed after the

application of the load/deformation can be resolved in terms

of plastic deformations. The results predicted by the numerical

simulations are qualitatively in agreement with the results of

biological experiments and we have also proposed some

quantitative comparisons in order to estimate the parameters

of the model, by fitting available experimental data.

Future works will be devoted to the definition of a multiphase

model of cell aggregate compression, taking into account the

viscous contribution related to the presence of the culture

medium liquid inside the whole structure, to account for the

description of MCS non instantaneous recovery after release³⁵⁻³⁷.

Some previous attempts to couple viscous effects with elasto-

plasticity have been done, for example, in^{53,61} where the

viscous contribution related to the intracellular liquid is

considered. However, in that case, the liquid motion is

constrained to the one of the cellular

847 phase, whereas when an aggregate is compressed between a par- 897
848 allel plate apparatus, the liquid exudes from the lateral bound- 898
849 aries of the MCS. Conversely, when the compression is removed, 899
850 the liquid will slightly fill the porous cellular structure, leading to 900
851 a viscous recovery of the cell shape after compression, in agree- 901
852 ment with the biological observation. This phenomenon can be 902
853 accurately described only considering a multi-phase model, with 903
854 a cellular constituent responsible of the elasto-plastic behaviour 904
855 and a liquid phase carrying the viscous contribution. Further- 905
856 more, the definition of a multi-phase model will allow to investi- 906
857 gate the mechanical contribution of the extracellular matrix that 907
858 in the present model has been neglected and that can be possibly 908
859 encapsulated inside living spheroids. 909

860 Another point to investigate in future works could be the role of 910
861 spheroid heterogeneous shapes^{97,98}, that could lead to different 911
862 quantitative results and to the intensification of the stresses in cor- 912
863 respondence of bumps or, alternatively, to possible detachments 913
864 in correspondence of pits in the contact region. However, het- 914
865 erogeneous shapes will not alter the capability of the aggregate 915
866 of partially relaxing the initial stress and of giving rise to plastic 916
867 deformations, as long as remodelling is triggered. In the same 917
868 way, heterogeneity in the composition could lead to regions with 918
869 higher/lower remodelling and to more complex MCS shapes dur- 919
870 ing the compression and release processes. For moderate hetero- 920
871 geneities, the main results of the present work are not expected 921
872 to vary significantly, although a rephrasing of the model might be 922
873 necessary. Indeed, when a medium is heterogeneous, the multi- 923
874 plicative decomposition of the deformation gradient tensor is not 924
875 sufficient, alone, to describe the structural reorganisation of the 925
876 medium itself. In fact, different material responses are possible 926
877 at different points of the same medium, so that the strain energy 927
878 density of the spheroid must depend explicitly on the material 928
879 point at which it is evaluated. Accordingly, with reference to the 929
880 spheroid's natural state, one has to write $\mathcal{W}_{sn}(\mathbf{C}_n, \mathbf{X})$, where the 930
881 explicit dependence of \mathcal{W}_{sn} on \mathbf{X} must be prescribed and, in prin- 931
882 ciple, in a heterogeneous material it is also possible that different 932
883 plastic evolution laws apply at different body points. 933

884 Future efforts will also be addressed to the definition of the ac- 934
885 tive behaviour of living systems. Indeed, in spite of similarities 935
886 of living tissues with inert soft materials and liquids, multicel- 936
887 lular systems additionally display active responses that are not 937
888 observed in inert soft materials²². In particular, the accurate de- 938
889 scription of MCS compression cannot encompass the character- 939
890 ization of cell active response when subjected to stresses. This 940
891 response is due to mechanotransduction, which is the ability of 941
892 cells to transform mechanical stresses into biochemical signals 942
893 (and vice versa) in order to transfer information to and from the 943
894 nucleus^{18,28,74}. This ability of cells to deform and generate forces 944
895 in an active manner, coupled with their extreme complexity and 945
896 their non linear response to mechanical stimuli, outlines the need

of a specific mathematical model to describe aggregate dynamics. 897

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 mechanics²². 917

In conclusion, studying tissue mechanics provides the basis to 918
understand many physiological and pathological phenomena and 919
to foster tissue engineering, which aims to develop new strategies 920
of medical treatment based on artificial tissue regeneration^{7,22}. 921
Proposing a three-dimensional elasto-plastic model of living sys- 922
tem behaviour aims at moving a step towards this ambitious goal. 923

Conflicts of interest 924

There are no conflicts to declare. 925

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