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Morphogenesis in space offers challenges and opportunities for soft matter and biophysics

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The effects of microgravity on soft matter morphogenesis have been documented in countless experiments, but physical understanding is still lacking in many cases. Here we review how gravity affects shape emergence and pattern formation for both inert matter and living systems of different biological complexities. We highlight the importance of building physical models for understanding the experimental results available. Answering these fundamental questions will not only solve basic scientific problems, but will also enable several industrial applications relevant to space exploration.

The physical study of morphogenesis is the investigation of the key features driving the emergence of shape in both living and non-living systems.

Recent research in morphogenesis has focused on classical pattern-forming instabilities in inert matter[1–3] as well as problems related to the mechanobiology and the biophysics of development[4–6]. Complex shapes may arise through morphological changes driven by internal processes, such as active changes of the cytoskeletal structure within a living cell[7] and phase transitions in oil droplets that acquire polygonal shapes during cooling[8]. Complex shapes may also arise from collective effects, such as geometrically constrained growth in biological tissues[9].

Common to these seemingly disparate systems are the two central questions of morphogenesis:

(1) What are the minimal physical and mechanical ingredients for shape formation?
(2) What are the mechanochemical feedbacks[10] out of which emerge collective structures with mechanical properties at the scale of their aggregates?

In particular, predicting the morphogenesis of biological systems requires understanding the interplay between biological rules and physical and mechanical laws.

In this context, the effects of microgravity on soft matter systems have been documented in countless experiments, but physical understanding is still lacking in many cases. Answering these fundamental questions will not only solve basic scientific problems, but will also enable several industrial applications relevant to space exploration.

This review therefore focuses, from a physics point of view, on two key open issues for morphogenesis in space: the fundamental physical understanding both of pattern-forming instabilities of inert matter, and of shape emergence in living systems in microgravity conditions, by discussing qualitative experiments on selected biological systems relevant to space exploration. We will first review the main recent achievements for inert matter before discussing recent advances and open questions for living systems of different biological complexities.

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Pattern-forming instabilities in microgravity

The interplay of geometrical and material nonlinearities in soft matter gives rise to a wide variety of complex pattern forming instabilities that are well understood for inert materials at terrestrial gravity conditions[1]. Their onset and full nonlinear development are typically controlled by dimensionless parameters representing a competition between the gravitational energy and the constitutive energy of the material at a given length scale. These dimensionless parameters can change by several orders of magnitude in microgravity conditions, whence the control of shape for soft matter in space has radically different features[11] that strongly affect the effectiveness of key technologies for space exploration, such as additive manufacturing[12,13] and fuel injection and combustion[14,15]. Moreover, the investigation of morphogenesis in reduced gravity allows exploring new regimes of these pattern-forming instabilities, paving the way not only to answering fundamental questions about the effect of gravity on spontaneous shape formation in soft matter, but also to transforming these results into new paradigms of material design in space by controlling such topological transitions. In the following we summarise the main achievements on pattern formation in microgravity conditions for liquid matter, highlighting particularly their relevance for industrial applications.

Fluid jets and droplets. The gravitational potential is a stabilising factor for the motion of fluids in terrestrial environments. However, related pattern formation phenomena are strongly influenced by a change of gravity conditions especially when the relative strength of gravity becomes negligible compared to forces of a different physical nature, such as inertia or capillarity[16]. We consider in the following a few fundamental effects of microgravity in pattern formation.

First, it is useful to remark that a reduction of gravity intuitively causes retardation of the threshold of those instabilities in which gravity is the main driving force. For example, a regular pattern of convection cells can appear when a horizontal layer of fluid is heated from below, due to a competition between buoyancy and viscous forces. This phenomenon is known as the Rayleigh–Bénard instability and occurs at a critical value of the dimensionless Rayleigh number[17]

$$\mathrm{Ra} = \frac{g\Delta T H^3 \alpha}{\nu\beta}, \tag{1}$$

where g is the acceleration due to gravity, H is the layer thickness, α is the thermal diffusivity, β is the thermal expansion coefficient, ν is the kinematic viscosity, and ΔT is the temperature difference between the bottom and the upper layer. Since the onset of the instability is at a critical value of Ra, the critical temperature difference is inversely proportional to g. Thus, the Rayleigh–Bénard instability is suppressed in microgravity conditions. However, experiments have shown convection rolls in space flights triggered by a similar interplay between a magnetic force and a magnetisation gradient in ferrofluids[18], also when thermo-capillary forces are taken into account[19].

Second, even in those instabilities that are not directly caused by gravity, gravitational effects play a dominant role in pattern formation far from equilibrium. For example, the interface between two fluid layers with densities ρ_t, ρ_b becomes dynamically unstable when the dimensionless Atwood parameter[17]

$$\mathrm{At} = \frac{\rho_\mathrm{t} - \rho_\mathrm{b}}{\rho_\mathrm{t} + \rho_\mathrm{b}}, \tag{2}$$

becomes positive, i.e. when the top layer is heavier than the bottom one, $\rho_\mathrm{t} > \rho_\mathrm{b}$. This phenomenon is known as the Rayleigh–Taylor instability, and it is controlled by a competition between the acceleration due to gravity g and surface tension γ. In particular, there are a typical capillary length ℓ_c and characteristic time τ_c given by[17]

$$\ell_\mathrm{c} = \sqrt{\frac{\gamma}{g(\rho_\mathrm{t} - \rho_\mathrm{b})}};\ \tau_\mathrm{c} = \sqrt{\frac{\ell_\mathrm{c}}{g}} = \left(\frac{\gamma}{g^3(\rho_\mathrm{t} - \rho_\mathrm{b})}\right)^{1/4}, \tag{3}$$

with ℓ_c and τ_c respectively defining a cut-off for the wavelengths of the unstable perturbation of the interface and the relative time-scale of its growth. Accordingly, a reduction of gravity selects larger interface undulations that grow much more slowly than in terrestrial conditions, and hence dynamically stabilises the interface, even when heat and mass transfer are considered[20]. Understanding these interface mixing properties enables space industry applications, especially related to fuel atomisation processes[21] and dusty plasmas[22].

Third, in the absence of gravity, the capillary and wetting forces of fluids become relevant at much bigger length scales than in terrestrial conditions. For example, wall-bounded droplets are characterised by the Bond number[23]

$$\mathrm{Bo} = \frac{L^2}{\ell_\mathrm{c}^2} = \frac{\Delta\rho g L^2}{\gamma}, \tag{4}$$

where L is the characteristic length of the droplet, and $\Delta\rho$ is the density difference between the liquid and the vapour phases. In microgravity conditions the buoyancy force becomes negligible compared to surface tension, so the droplet acquires the shape of minimal curvature as Bo is very small[24]. However, since the volume V of the droplet scales as ℓ_c^3, i.e. $V \sim g^{-3/2}$, and that of a free drop scales as $V \sim g^{-3}$, both wall-bounded and free droplets have a significantly bigger volume in microgravity conditions[25] (Fig. 1). Moreover, since the droplets are much bigger, the characteristic time scale $t_\mathrm{i} \sim \rho V/\gamma$ of the inertial-capillary dynamics is much longer than in terrestrial conditions[23]. The inertial-capillary motion is therefore much slower; thus, more accurate measurements of droplet dynamic wetting can be made in microgravity conditions[26,27]. Characterisation of these inertial-capillary dynamics for wettability, coalescence, cavitation[28], and evaporation[29] properties is fundamental for several applications in space exploration, e.g. droplet deposition 3D printing[30,31] and atomisation and combustion of liquid fuels[32,33].

Last, the small vibrations that are inherently present in spacecraft, induced for example by aerodynamic forces, crew activities, or on-board machinery, create a time-dependent residual gravity effect known as g-jitters that may have a surprising effect in suppressing some instabilities and promoting others. For example, g-jitters can enable Rayleigh–Bénard instabilities, allowing thermal convection rolls[34] that would be unattainable otherwise. Moreover, such residual vibrations can induce parametrically forced waves.

For example, the Faraday instability occurs at the interface between two immiscible liquids[35] with densities ρ_1, ρ_2 and dynamic viscosities μ_1, μ_2 subjected to a controlled periodic vibration. The wavenumber k of the standing subharmonic waves and the critical acceleration a are related to the frequency of vibration 2ω by[36]

$$\omega^2 = \frac{\rho_1 - \rho_2}{\rho_1 + \rho_2} gk + \frac{\gamma}{\rho_1 + \rho_2} k^3;\ a \sim 8\omega k \frac{\mu_1 + \mu_2}{\rho_1 - \rho_2}. \tag{5}$$

Since gravity and capillarity are restoring forces that resist topological changes of the interface, one could naively expect that this parametric instability would be favoured by g-jitters in microgravity. On the contrary, the reduced gravity selects an increased critical wavenumber k that in turn imposes an increased critical acceleration a, whence the onset of Faraday waves gets delayed[37]. However, since the restoring gravity forces become negligible in microgravity, the fully nonlinear patterns

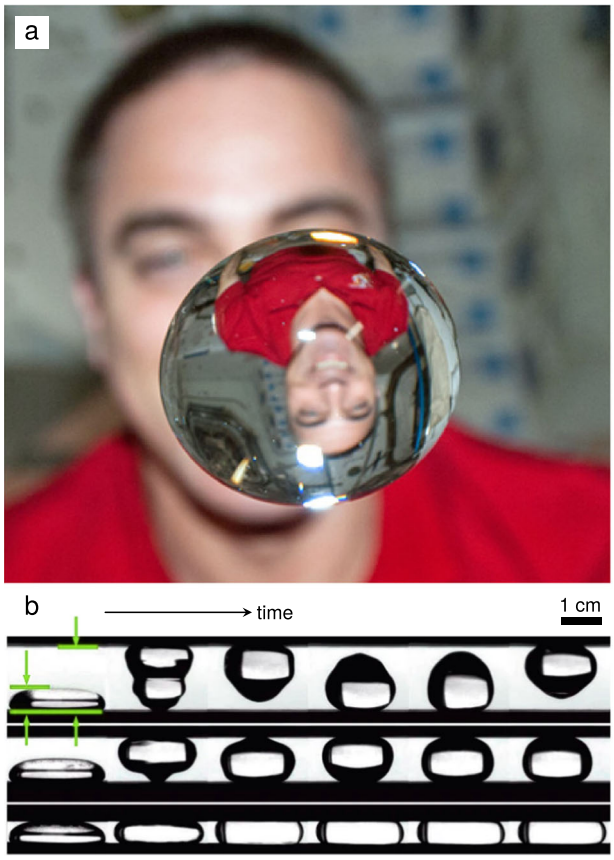

Fig. 1 Water drop physics in microgravity. a A NASA astronaut on the International Space Station observes a water drop floating freely between him and the camera. His image is refracted in the water drop (public domain image credit: NASA). **b** 10 Hz images from drop tower tests of 2 mL water drops between parallel hydrophobic surfaces with varying separation height (vertical arrows). Adapted from Ref. [25] under a CC BY license. Scale bar: 1 cm.

develop faster far from the instability threshold, being more sensitive to cross-wave excitations[11].

Another marked effect of g-jitters in microgravity concerns the Kelvin–Helmholtz instability at the flat interface between two immiscible fluids moving horizontally with a velocity difference V. If both fluid layers are sufficiently deep, the critical V for instability is given by[38]

$$V^2 = 2\frac{\rho_1 + \rho_2}{\rho_1 \rho_2}\sqrt{\gamma g(\rho_1 - \rho_2)}, \qquad (6)$$

and the critical mode is $k \sim 1/\ell_c$. Thus, the instability occurs immediately in microgravity conditions and its critical mode tends to zero. Like in terrestrial gravity, fast oscillations of the container favour the onset of standing waves, also known as frozen waves, over rolling waves[39]. Nonetheless, in microgravity conditions the interface oscillations grow much more rapidly in the normal direction, forming columnar structures that interact nonlinearly with the domain boundaries[40]. Accordingly, g-jitters force immiscible fluids to be segregated laterally in columnar domains the typical length scale of which is controlled non-trivially by a competition of viscous and capillary forces and geometrical constraints[41].

Gravity and morphogenesis in biology

In biological systems, the effect of gravity on morphogenesis need not be the mere direct mechanical effect of gravity discussed in the previous section. Indeed, mechanical forces or their absence can stress organisms, affect gene expression, and cascade via the regulatory biochemistry[42] to indirect changes of the morphogenesis of biological systems with changes of the magnitude and orientation of gravity. These direct and indirect effects of microgravity in biological systems are thus linked inextricably. Elucidating their contributions to morphogenesis will therefore require close integration of biological experiments and physical modelling, perhaps even more so than in the "classical" biophysics in normal gravity conditions because of the considerable added logistic complexity of biological experiments in space. Yet, physical models that can capture the multiscale interplay between such direct and indirect effects are few and far between.

In this section, we will therefore present the challenges and opportunities for physical models to answer the puzzles that biological experiments in microgravity have uncovered. We will base our discussion on a selection of such biological puzzles that we have made based on their impact on space exploration.

Gravitropism. Gravitropism, the tendency of plant stems to grow away from gravity and of plant roots to grow towards gravity[43], is perhaps the most well-known instance of the influence of gravity on biological systems. Understanding its physical basis is crucial because of the role of plants in nutrient production during long space missions[44] and hence space exploration. The most basic model of gravitropism is the so-called "sine law"[45] (Fig. 2a), that bending (by differential growth) is proportional to the sine of the angle of inclination to gravity and hence to the component of gravity perpendicular to the plant axis.

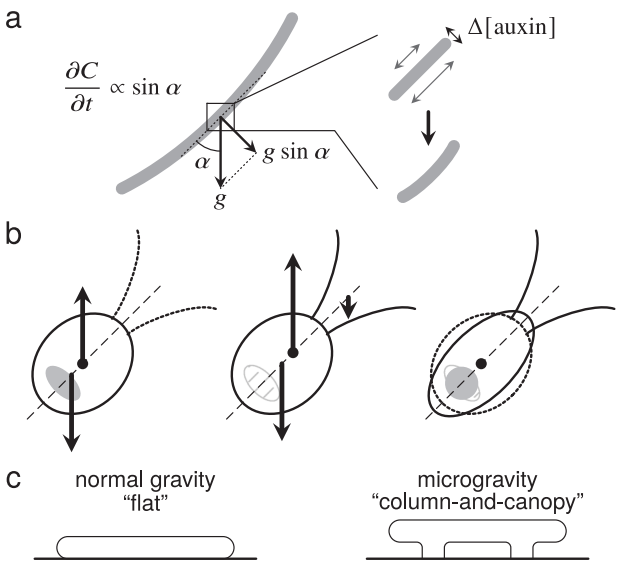

Fig. 2 Examples of the effect of gravity on biological systems. a The "sine law" of gravitropism[45]: the rate of bending, i.e. the rate of change $\partial C/\partial t$ of curvature, is proportional to the component of gravity perpendicular to the plant axis, i.e. to the sine of its inclination angle α. Bending results from the differential growth due to differential auxin concentrations[43]Δ[auxin]. **b** Direct[56] and indirect[63] gravitaxis mechanisms illustrated for the biflagellated alga *Chlamydomonas* (upward arrows: buoyancy; downward arrows: gravitational forces): An inhomogeneous mass distribution (grey area) leads to torques due to "bottom-heaviness" (left); shape asymmetries, e.g. due to the flagellar apparatus, also lead to torques (middle); active regulation of the shapes and density distribution (right) affects gravitactic behaviour. **c** Biofilm structures of wild-type *P. aeruginosa* in normal gravity ("flat") and microgravity ("column-and-canopy") conditions[78].

The bending of stems or shoots required for gravitropism is driven by differential growth resulting, at the molecular scale, from differential concentrations of the hormone auxin[43] (Fig. 2a). The sine law emerges from a multiscale model[46] that couples the elasticity of a plant shoot to the diffusion and production of auxin in the shoot through a morphoelastic growth law. This growth law must also include a contribution of autotropism[47,48] (the tendency to grow parallel to the plant axis), which is known to be required mathematically for wavy plant stems to straighten[49,50], although the biomolecular basis of autotropism remains poorly understood[47,48].

More is known about the mechanism that translates gravitational forces into auxin production: Specialised cells in the plant contain so-called statoliths, starch granules that sediment as an active granular fluid[51]. While one might expect this sedimentation to activate mechanosensitive ion channels[43], experiments suggest that the gravitropic response only depends on the inclination, but not on the magnitude of gravity[52]. This is consistent with a "position-sensing" model[53] in which statolith positions affect the trafficking of certain membrane proteins[43]. While the detailed molecular pathways in this mechanism remain to be determined and tested experimentally[43], a recent model of gravitropism[54] including a detailed description of these statolith dynamics and this protein trafficking suggests a more complex growth law beyond the sine law, mathematically different from, but qualitatively consistent with the latter[54].

In microgravity conditions, the direct gravitational stimulus that induces gravitropism is absent, leading to profound morphological changes, but quantitative understanding of the reorganisation of the distribution of auxin and other hormones and of the gene expression changes associated with microgravity conditions[44] is still lacking. In particular, can such indirect effects rescue gravitropism, or do other tropisms (such as phototropism, i.e. growth towards light) become dominant in microgravity conditions? The mechanical interplay of gravitropism and phototropism, although still poorly explored, is complex[46], and phototropism is itself modified in microgravity conditions[55]. For these reasons, physical models addressing these questions are still absent.

Gravitaxis. Gravitaxis is the tendency of microswimmers, such as the unicellular alga *Chlamydomonas*[56], the protist *Euglena*[57], or the nematode *C. elegans*[58], to align with gravity. Combined with hydrodynamic torques, this gravitational alignment gives rise to focusing of cells into plumes[59] and hence "bioconvection" patterns[60] that are the biological analogue of the classical Rayleigh–Bénard patterns discussed earlier. Two physical mechanisms for gravitaxis have been proposed[56] (Fig. 2b): (i) "bottom-heaviness", i.e. torques resulting from density inhomogeneities and hence differences of the positions of the centres of gravity and buoyancy of the microswimmers, and (ii) torques associated with shape asymmetries of the body and flagellar apparatus of the microswimmers. For *Chlamydomonas*, both of these effects contribute to gravitaxis, but the second effect dominates[61,62]. By contrast, gravitaxis in *Euglena* is dominated by active gravisensing through mechanosensitive channels[57]. On top of this, recent experiments have emphasised that phytoplankton can even regulate their shape and density asymmetries based on external cues in turbulent flows[63].

It remains unclear how this shape regulation or active gravisensing compare to the direct mechanical gravitaxis mechanisms in general. In particular, can such effects overcome the loss or reduction of the magnitude of gravitactic reorientation torques in microgravity conditions? The answers to these questions are relevant for bioreactors in microgravity which contain microswimmers; such bioreactors with self-generated flows could complement bioreactors of non-motile microalgae like *Chlorella vulgaris* that are being developed as life support systems for space exploration[64]. Biological and especially physical understanding of these problems is still lacking.

If the density of microswimmers in such bioreactors is to be high, an additional hydrodynamic effect arises: the motion of one microswimmer is affected by the flow fields produced by the others. Because of the small density differences of the microswimmers and the surrounding fluid medium, the flow field of a single microswimmer is commonly approximated by a so-called stresslet[65], but the slower decay of the so-called Stokeslet flow field[65] (associated with the gravitational forces resulting from these small density differences) means that the Stokeslet flow dominates sufficiently far away from the microswimmer[66]. Strikingly, the distance at which the Stokeslet starts to dominate over the stresslet can be quite small even for organisms with a tiny density excess such as the green alga *Volvox*[66], allowing for the collective "minuetting" phenomena in *Volvox*[67] that are driven by this Stokeslet contribution to the flow. Since density-matching the surrounding fluid medium cannot cancel gravitational torques resulting from inhomogeneous mass distributions of microswimmers, only microgravity conditions could therefore allow true experimental realisations of the theoretical idealisation of stresslet flows.

Biofilms. Biofilms are self-organised collectives of microbes that adhere to surfaces and to each other by matrix secreted by the microbes themselves[68]. Because of their association with pathologies and the possibility of increased virulence of pathogens in space[69–71], the formation of biofilms is an important contamination issue for spacecraft, especially for long space missions, given their potential deleterious effect on the immune system of their crews[72]. Biofilms are thus of interest to the biophysical and medical communities alike. In this context, recent physical models of biofilm formation, in combination with experimental visualisations of their structure at the single cell level[73,74], have begun to capture the formation of three-dimensional biofilm morphologies (in bulk or on surfaces). A particular focus has been the so-called verticalisation transition in which a single-layered biofilm becomes multilayered[75,76], but the intriguing role that topological defects in the nematic alignment of the microbes play in the formation of new layers[74,77] is not yet resolved fully.

Interestingly, the three-dimensional morphologies of biofilms can be different in normal gravity and microgravity conditions[78], with wild-type *P. aeruginosa* biofilms grown in space displaying a "column-and-canopy" structure different from the flat structure observed in normal gravity (Fig. 2c)[78]. This may be associated with a stress response of the microbes[69–71] or with modified surface accretion in microgravity conditions, but it remains completely unclear how such effects translate to the observed morphological changes[78] through direct or indirect physical mechanisms.

Cells and tissues in microgravity

After this brief discussion of gravitropism, gravitaxis, and the effect of gravity on biofilms, we turn to a more detailed discussion of cells and tissues in microgravity. Indeed, the structures formed by cellular assemblies of different cell types and the dynamics of their growth are modified dramatically in microgravity[79–81]. Here, we therefore discuss the physics of such cellular assemblies based on simple biological systems presenting qualitative results on these effects related to gravity.

The physics underlying the growth and proliferation of cellular assemblies are far from trivial, especially when they are composed

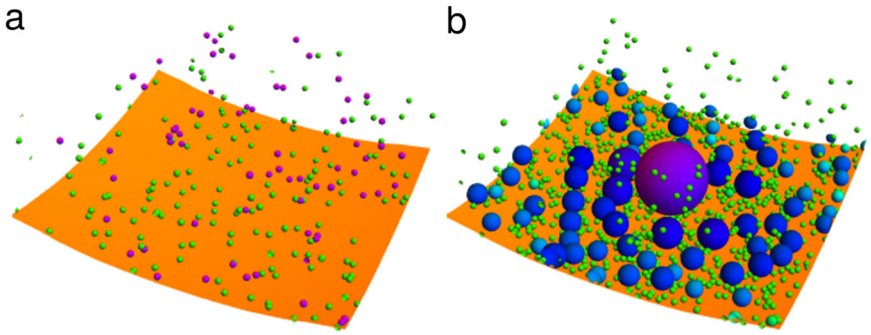

Fig. 3 Physics of cell aggregation. Simulations of cellular aggregation of macrophages (green / light) and proliferative cancer cells (purple or blue/dark) at the bottom of a well (orange surface). **a** Cells injected at the top of the well fall to the bottom with a rather weak Archimedean force just after the onset of the simulations and experiments[109]. **b** After 60 h, aggregates have formed and coalesced into bigger aggregates. The process will ultimately lead to a single aggregate, with ejection of most of the macrophages. The simulations, realised with a particle-based model, incorporate cell-cell interactions, adhesion, friction with the liquid bath, cells, and substrate, cell proliferation, and nutrient diffusion. (Simulation images provided by Joseph Ackermann.)

of different cell types. Their coexistence depends of course on their relative proliferation rates and on their mutual attractions but also on physical laws involving friction and adhesion between them or with the environment[82]. The organisation of animal cell communities is thus not only the result of individual cell behaviour and both biological and physical aspects of this organisation are modified in microgravity experiments[83]. These cellular changes in space offer new opportunities for tissue bioengineering and hence in medicine, for example in cancer and organ regeneration research. Further progress in cellular bio-reactors, tissue engineering, and organogenesis may make such research cost-effective in the future[83]. Moreover, cellular changes in space and the resulting tissue damage also affect the health of the crews of space missions who experience several pathologies which continue long after their return to Earth: Examples include bone loss, musculoskeletal disorders[84,85], and post-flight disc herniation[86]. All of this emphasises that many biophysical aspects of cells and tissues must be revisited in microgravity.

In the following, we present recent progress on these questions, focusing successively on nucleation physics in cell aggregates, tissue stresses in cysts, and tissue dynamics in epithelia. More biologically inclined readers may point out that our selection of systems cannot begin to give a complete account of the myriad cell and tissue systems, but we emphasise that it allows us to discuss the key physics at play, while briefly setting out relevant biology for our more physically inclined readers.

Aggregates of cells: nucleation physics. Experimentally, aggregates of cells for pharmacological applications are realised mostly in microfluidic chambers or in microplates both involving a large number of cells or wells. In some of these devices, the initial physical cue for aggregation is gravitational accumulation at the bottom due to weak density differences between cells and water. Aggregation may occur as cell-cell adhesion is established, for example by accumulation of cadherins at cell surfaces in contact[82,87–89]. Other biological mechanisms not covered here include responses to external physical forces such as fluid flows, and membrane stress during cell growth and cell division.

A recent experimental model has reported that the aggregates grow due to cell division but saturate at a size of about 150 μm, above which limit restriction of oxygen and nutrient diffusion leads to necrotic cores[82]. This initial gravitational accumulation depends on cell density (i.e. on the number of cells per unit volume) and is slower the weaker the gravitational force, whence we speculate that microgravity may increase the attachment maturation time and the timescale for compaction of aggregates dramatically. This is why bioengineering of tissues in microgravity and terrestrial conditions are rather different. This has prompted the development of rotating bioreactors by NASA[90] that have enabled progress in different tissues of high interest in precision medicine such as bones, cartilage, and pancreas or liver tissue[91–94].

Cell aggregation in this experimental platform therefore promises further insights into key questions about the mixing of active cells, such as cancerous cells and (innate or adaptive) immune cells. In vitro aggregates of cancerous cells and cells of the immune system (macrophages and fibroblasts first and lymphocytes later[95]) have already enabled better understanding of the role of immune cells in vivo, complementing immunotherapy results[96,97]. Cell aggregation in a platform of wells in series has addressed the forces which lead to nucleation of mini-aggregates and the coalescence and final composition of the aggregates compared to their initial composition[98]. In microgravity conditions, this mixing will be affected by changes of cell proliferation rates and apoptosis: Early experiments on human lymphocytes flown on the Soviet space station Salyut 6 suggested a decreased proliferation rate in microgravity[99], while a more recent study suggested the opposite for a human colorectal carcinoma cell line[100]. Moreover, mammalian cells cultured in bioreactors[101] that mimic fluid shear effects associated with microgravity[102–104] undergo physiological changes including not only changes of cell proliferation and apoptosis[102], but also changes of differentiation and cell morphology[105]. Multiple publications have also reported that human cells grown in simulated microgravity culture conditions (for example, rotating bioreactors that simulate the optimised suspension and low fluid forces associated with spaceflight) exhibit well-developed tight junctions and other junctional structures, as well as enhanced polarity compared to the same cells grown as flat monolayers[105–107].

Interestingly, the physics of the mixing and aggregation of active cells have surprising links to inhomogeneous nucleation processes of inert molecules in out-of-equilibrium thermodynamics[108]: a recent study[109] has shown that macrophages can serve as nucleation sites located at the centre of tiny aggregates which coalesce. Ultimately the macrophages are ejected towards the periphery, as seen in experiments and simulations of the system[109] (Fig. 3). Importantly, this ejection allows full access to nutrients during aggregation, which increases cell proliferation rates. This behaviour of macrophages during the expansion of in vitro aggregates may of course simply be specific to the particular in vitro system[109]; it is nonetheless surprising because it favours the aggregation of cancer cells.

All of this shows how, in spite of clear progress in tissue bioengineering over the last decades[110–112], in vitro studies, both

in normal and in reduced gravity, and physical models continue to reveal how novel cell aggregation behaviours arise from the cell biology of individual systems and common physical principles.

Cysts of pluripotent stem cells: tissue stress and growth. Human pluripotent stem cells (hPSCs) have emerged as the most promising source of cells for cell therapies[113–115], organ engineering[116–118], and drug testing[119,120]. Reprogramming terminally differentiated cells of a patient into human induced pluripotent stem cells (hiPSCs)[121] is, however, but the first step of these biomedical applications: they rely on the culture and proliferation of hPSCs to clinically-relevant numbers (typically $10^8 - 10^{10}$ cells) and hence maintenance of stemness during proliferation is crucial[103,104]. Standard methods for cell proliferation use 2D tissue culture and suspension culture systems such as micro-carriers[122]. In all methods, pluripotency markers are maintained but the proliferation rate depends on the method: scaffold or suspension method, nature of the scaffold, initial cell density, seeding strategy (single cell or clusters), and the composition of the culture medium. Some methods also involve physical forces induced by shear flows or stretching of substrates that can alter the cell proliferation and cell density of the cultures. Proliferation results in the formation and growth of spheroids that ultimately reach a maximal size of about 150 μm, a limit already mentioned earlier, above which restriction of oxygen and nutrient diffusion leads to the formation of necrotic cores[82].

Pluripotent stem cells spontaneously self-organise into cysts reminiscent of the lumenal epiblast stage[123]. Starting from a two-cell assembly[124] or with a single hPSC with a perinuclear apicosome structure[125], hPSCs seem to be programmed to form lumina and thus cysts upon growth. A recently developed microfluidic technique allows growth and differentiation of pluripotent stem cells in permeable micro-compartments or capsules with a micro-engineered extracellular matrix-like environment[126,127]. The approach is a priori compatible with the large production requirements in regenerative medicine. In each capsule, the growth of a 3D hiPSC culture starts from an initially small cell density (between 1 and 10 cells per capsule) and yields cysts that then become pseudo-stratified epithelia closed around a lumen over a time scale of one or two weeks. This self-organised growth is anisotropic, with strong extension of the cells in the radial direction[128]. Interestingly, this growth can be "stress-free", i.e. avoid geometric incompatibilities between the local growth and the global constraints of the tissue and hence eschew stress generation[128]. This may not however be possible in systems in which cell-substrate interactions dominate the mechanics. Since it is now recognised that mechanical cues are important regulators of the self-organisation and fate of tissues[129–134], stresses may alter the stemness of the cells by inducing unintended mutations or differentiation[135]. The possibility of such stress-free cyst growth[128] therefore suggests that bio-processing conditions recapitulating hiPSC self-organisation as cysts that grow under reduced mechanical stress could allow more efficient cell proliferation. This question is of course important for the health of space crews and organ and cancer stem cells alike. These concepts may extend to other alterations of cell integrity, such as changes of proliferation[136], differentiation[137], and tissue growth[138,139] which are clearly influenced by microgravity[139,140].

Epithelia: tissue dynamics. Epithelia are constituted by a single layer of tightly attached cells or by several such layers on top of each other, as in the case of skin[141]. These cells are rather geometrical and in vivo, they are attached to a basement membrane[142]. The physiological role of epithelia is mostly the protection of our organs from physical hazards and from pathogens or chemical compounds entering the underlying tissues[143]. This is why they surround most of our organs. Epithelia have been studied extensively by the biophysics community in embryogenesis for example, model systems being the *Drosophila* imaginal disc[144] or the *C. elegans* embryo[145]. Growth of a cell monolayer in a flask in vitro has also been analysed in detail to evaluate the subtle collective coordination of proliferative cells[146]. A special focus has been a front instability supposed to mimic wound-healing. These experiments only concern specific cancerous cells in practice (MDCK cells[147] or melanoma cancer cells[148] for example), so they give rather little information of what may happen in embryogenesis or in physiology. Modelling these tissue dynamics invokes either discrete models such as the vertex model[149] or continuum models of tissue mechanics involving a viscous[150] or elastic[151] approach to growth, but a physical description of the multiscale three-dimensional geometry of complex tissues and organs[152] is still elusive.

Space crews face the particular problem that epithelia need to maintain their barrier function in microgravity. The effect of microgravity on barrier function remains unclear, however, with different studies reporting an increase[153,154] or decrease[155,156] of tight-junction protein expression and localisation and epithelial barrier function in different systems and different experimental conditions. In particular, the observed differences in epithelial barrier function may have resulted partly or wholly from the dissociation of three-dimensional cell aggregates into planar structures, rather than constituting an inherent property of cell culture under simulated microgravity conditions.

Similarly, microgravity has variously been reported to upregulate, downregulate, or not to affect cell-cell or cell-substrate adhesion in different systems[157,158], suggesting a strong dependence on the cell type or organ. Further experiments, in particular in physiologically relevant three-dimensional culture systems[105,159,160], will therefore be needed to understand the effect of microgravity on epithelial integrity. Physical models that could shed light on these questions are still absent.

In addition, wound healing which is a concern for the crews of long space missions may be modified in microgravity: the first stage of repair is re-epithelialisation[161] which lasts for one or two days on Earth and corresponds to the covering of the wound by the neighbouring skin layers. This may be compromised in space if the epithelium looses its connection with the basal layer. Indeed, this stage seems to be delayed or imperfect in microgravity, leading to a perturbation of skin homoeostasis[162], but again, physical models addressing this effect of microgravity are lacking.

Outlook

We have reviewed recent progress on the effect of gravity on living and non-living systems and have highlighted the importance of building physical models for understanding the experimental results available. Describing the complex underlying nonlinear phenomena requires not only addressing fundamental physical questions, but also a close interaction with biologists to define the multi-scale models that will reveal how the effect of gravity on living systems arises from a combination of biological rules and physical laws at different scales.

Indeed, these approaches, in particular when coupled to data-driven approaches, will allow more general progress, not confined to the specific problem of the effect of gravity on inert or living systems, on bridging scales of physical and mechanical descriptions, from discrete scales to continuum scales. For example, how does the stochastic discrete scale of individual cells in living systems link to the continuum scale of tissues in such systems?

What is more, the approaches that allow understanding the effect of gravity on biological systems promise to spawn further insight into the effect of other environmental factors on these systems. In the context of space exploration, the most relevant of these is perhaps radiation[163].

Beyond these questions of basic science, their relevance to space exploration promises technological advances, for example in the realm of bioreactors, that will likely translate to terrestrial industrial benefits, too.

We close by noting that the open questions discussed in this review will likely push further experiments in microgravity conditions. We believe that it would be most efficient to use Earth-based experiments (e.g. drop towers, parabolic flights, sounding rockets) to address the fundamental physical questions. The questions of morphogenesis in living systems will also require experiments on longer time scales that are only accessible on the ISS, the Moon, or the proposed Deep Space Gateway. Here, we believe that close collaborations with biologists will be key to design quantitative experiments that can also give new biological insights. We have discussed these recommendations in more detail in our contribution to the ESA SciSpace White Papers[164].

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