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# Physics of liquid crystals in cell biology

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The last decade has witnessed a rapid growth in understanding of the pivotal roles of mechanical stresses and physical forces in cell biology. As a result an integrated view of cell biology is evolving, where genetic and molecular features are scrutinized hand in hand with physical and mechanical characteristics of cells. Physics of liquid crystals has emerged as a burgeoning new frontier in cell biology over the past few years, fueled by an increasing identification of orientational order and topological defects in cell biology, spanning scales from subcellular filaments to individual cells and multicellular tissues. Here, we provide an account of most recent findings and developments together with future promises and challenges in this rapidly evolving interdisciplinary research direction.

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## Glossary

- **Active liquid crystal**, Liquid crystal driven constantly away from equilibrium by local injection of energy from its constituent elements.
- **Mechanotransduction**, Conversion of mechanical cues received by cells into biochemical signals within the cells.
- **Liquid crystal**, *Intermediate phase between solid and liquid that flows like a liquid but maintains some of the ordered structure of crystals.*
- **Polar alignment**, *Alignment of particles with a distinct head and tail orientations along the same direction.*
- 15 • **Nematic alignment**, *Alignment of rod-like particles which present head-tail symmetry in their orientation. Nematic ordering can be obtained in two ways, either in systems where polar objects are parallel with random head-tail orientations or in systems where particles are themselves head-tail symmetric.*
- **Topological defects in orientational alignment** , Imperfections in liquid crystalline organisation.
- **Topological transition**, *Cell shape remodeling within tissues based on cellular forces that lead to fluid/solid, jammed/unjammed behaviors.*
- **Reynolds Number**, *Dimensionless quantity used in hydrodynamics that represent the ratio between inertial forces and viscous forces. Laminar flows occur at low Reynolds number, whereas turbulent flows appear at high Reynolds number.*

## THE RISING (ORIENTATIONAL) ORDER IN CELL BIOLOGY

17 From the earliest stages of development, where few stem cells self-organise into delicate well-  
 18 structured aggregates to form various organs [1], all the way to the organ repair and wound healing,  
 19 where cells actively coordinate their behavior to close gaps and scars in the tissue [2–4], multiple  
 20 genetic, chemical, and mechanical factors work together to control the spatio-temporal order in  
 21 cells [5]. Importantly, the breakdown of the order is at the root of tissue malfunctioning, impaired  
 22 healing, and disease initiation [6, 7]. The order is not only in the spatial arrangement of cells,  
 23 but also comprises meticulous orientational alignment to govern the direction of cell division [8, 9]  
 24 and cell migration [10], as well as directional transmission of mechanical forces and biochemi-  
 25 cal signals between the cells [11]. For example, coordinated cellular migration and directed cell  
 26 division events determine the elongation of embryos at the early stages of morphogenesis [12],  
 27 deciding where particular parts of the body will be located. Similarly, force transmission through

cell-cell contacts provides a directional guidance for the transfer of biochemical signals into specific locations within the tissue [13–16]. *These processes rely on the active nature of biological systems composed of living particles capable of continuously converting chemical energy into mechanical work [17].* Interaction of active particles can give rise to collective motion and the emergence of order. Remarkably, the orientational order is established and maintained across a range of scales, from multicellular organisation at the tissue level [18–20], to the alignment of individual cells [21, 22], and all the way down to subcellular filaments that form the skeleton of the cells as exemplified by both *in vitro* and *in vivo* experiments [23–25]. *These emergent properties led to an analogy with a well-known phase of physical systems called “liquid crystals” (Box 1) and in particular, nematics [26]. Other types of liquid crystals including cholesterics are important to describe biological processes (reviewed elsewhere [27]) but we mainly focus here on nematics. Passive liquid crystals thus display the general features of organization order, the local order breakdown through the formation of topological defects (Box 2), whereas other aspects are inherent to active liquid crystals including the emergence of active stresses, the formation of those defects and the movement of the defects. Here, we review how the emergence of orientational order and its breakdown are generic themes in fundamental processes in cell biology and provide an universal mechanism for choreographing the directional passage of mechanical and biochemical information in time, and across different scales.*

### BOX 1. Primer on Liquid Crystals

Liquid crystals are an intermediate phase of matter between solids and liquids. In a solid constituent atoms organise in well-structured crystalline lattices giving them high degree of positional and orientational ordering. In a liquid both positional and orientational ordering are absent. As an intermediate phase of matter, the constituent elements of a liquid crystals organise in such a way that orientational order is maintained, but the positional order is lacking. This structural arrangement endows liquid crystals with peculiar properties: liquid crystals can flow like a liquid, but resist deformations like a solid.

The liquid crystal phase itself can exist in different sub-classes. In a *nematic* (from the Greek word for ‘thread-like’) liquid crystal the molecules align (on average) along a common axis, but are free to drift around while keeping this orientational order (Fig. 1). In a *smectic* (‘soap-like’) liquid crystal additional degree of order emerges: the molecules organise into layers along a particular direction such that they are liquid-like within each layer, but they all point (on average) parallel to the plan normal to the layer (Fig. 1). A *chiral* (nematic or smectic) liquid crystal is formed when molecules possess helicity and periodically rotate along the alignment axis (Fig. 1).

## PHYSICS OF LIQUID CRYSTALS IN SUBCELLULAR ORGANISATION

Within an individual cell, complex networks of filamentous protein structures shape the cell cytoskeleton. The cytoskeleton extends from the nucleus to the cell membrane and is integral in

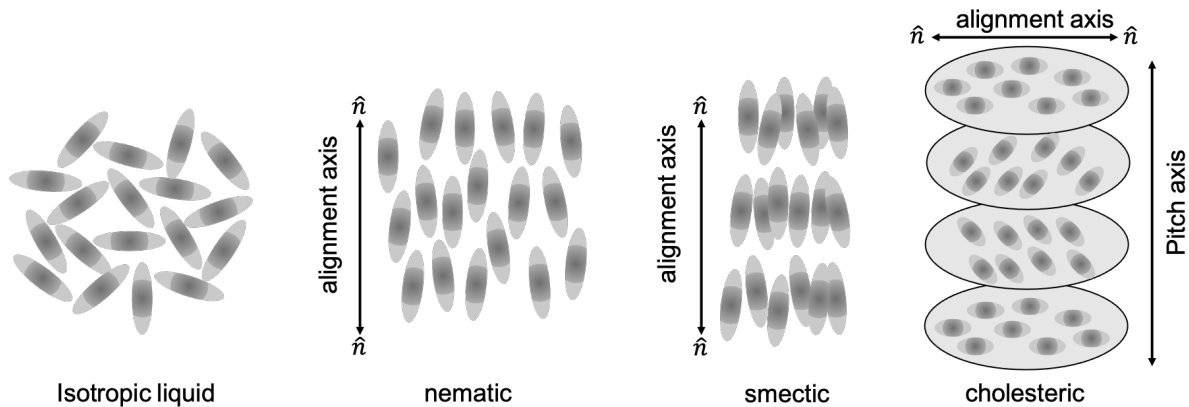


FIG. 1. Different liquid crystalline phases. [BOX 1]

53 maintaining cell shape and modulating its response to external stimuli [28]. The main building  
 54 blocks of the cell cytoskeleton are *actin filaments*, microtubule (made of protein tubulin), and inter-  
 55 mediate filaments. As groups of these elongated filaments assemble together within a cell, various  
 56 forms of orientational order emerge, sharing ordering properties of liquid crystals (see **Glossary**).  
 57 For example, *in vitro* reconstitution of microtubule [29] and actin filament networks [30, 31] both  
 58 demonstrate the emergence of nematic orientational order. Moreover, three-dimensional organ-  
 59 isation of actin filaments-myosin motor protein mixtures in filopodia show chiral ordering that  
 60 contributes to the helical rotation of the filopodia [32]. Recently, the emergence of smectic or-  
 61 der has also been observed in stacks of myosin-II filaments in epithelial cells [33]. Finally, the  
 62 mechanical interplay between cell shape and cytoskeleton organization relies on nematic ordering  
 63 of actin filaments at the single cell level, particularly important for rigidity sensing mechanisms  
 64 [21, 34–36]. The physical laws governing phase transitions and dynamics of liquid crystals have  
 65 thus been reported in various subcellular processes, *not only in vitro but also in living cells, as*  
 66 *described below.*

### 67 *Isotropic-nematic transition in actin filaments as a hallmark of cell polarization*

68 It is shown through *in vitro* experiments that culturing cells on substrates with incrementally  
 69 increased stiffness results in a sharp transition from circular, non-motile phenotype on soft sub-  
 70 strates to elongated cells with prominent actin stress fibers on stiffer substrates [34, 37]. A similar  
 71 transition has been observed on substrates with modulated viscoelastic properties [38]. The sharp  
 72 transition coincides with the isotropic-to-nematic transition of the actin cytoskeleton at a criti-  
 73 cal stiffness, below which the actin organisation is isotropic leading to circular shape of the cell,  
 74 while above the transition point actin stress fibers self-organise into nematically ordered struc-  
 75 tures resulting in polarised elongated cells [36]. Importantly, the isotropic-to-nematic transition is  
 76 shown to crucially depend on the activity of the cell cytoskeleton and its ability to generate con-  
 77 tractile stresses. This distinguishes the transition from isotropic-to-nematic transition in passive

78 liquid crystals, where thermodynamic ordering mechanisms such as increased density or reduced  
 79 temperature govern the transition to a nematically ordered state. As such, the cell cytoskeleton  
 80 shows properties of active liquid crystals [34, 35], where continuous injection of energy by the  
 81 constituent elements - here through motor proteins - constantly drives the filaments away from  
 82 thermodynamic equilibrium and the activity-induced ordering governs the transition from isotropic  
 83 to nematic phase [17].

#### 84 *Polar versus Nematic Order in Subcellular Filaments*

85 The presence of nematic order demarcates an emergent orientational order that is predominated  
 86 by the alignment of elongated filaments along a particular axis without any preferable head-to-tail  
 87 direction (polarity). In other words, nematic order along the direction  $\hat{\mathbf{n}}$  is head-tail symmetric  
 88 and thus does not change by the transformation  $\hat{\mathbf{n}} \rightarrow -\hat{\mathbf{n}}$  (*Fig. 2*), *meaning that the orientation*  
 89 *order is equivalent along the head or tail direction*. This implies that even polar filaments such  
 90 as actin or microtubule that possess positive and negative ends can in principle self-organise into  
 91 nematic structures if on average they align together without pointing into a specific direction, as has  
 92 been reported in various cytoskeletal constructs including actin stress fiber organization [31, 39–  
 93 41]. However, such polar entities can in principal also self-organise into structures with polar  
 94 orientational order, in which not only they align together, but also they predominantly point  
 95 towards their positive ends as shown, for instance, in protrusive cellular structures such as *filopodia*  
 96 and lamellipodia [42]. This immediately raises a question of when and how one form of order,  
 97 namely polar or nematic, dominates, whether it is even possible to form structures with mixed  
 98 symmetry [43] and eventually, how one structure can dynamically evolve into the other one to  
 99 drive cell migration processes [44].

100 Recent works have indeed taken first steps towards addressing these questions. It is shown  
 101 that tuning the strength of nematic interactions between actin filaments by adding small amounts  
 102 of PEG molecules can result in the emergence of polar waves co-existing with nematic bands in  
 103 actomyosin motility assays [45]. Interestingly, experiments on microtubule-kinesin motor systems  
 104 have also demonstrated the possibility of co-existing phases of polar and nematic alignment of  
 105 microtubules with a governing role in forming the bipolar mitotic spindle in cells [46]. Importantly,  
 106 such polar and nematic ordering of microtubules are shown to be controlled by two active features:  
 107 microtubule growth speed to motor speed ratio, and the motor number to microtubule number  
 108 ratio, which again highlights the importance of active, non-equilibrium factors in determining  
 109 cytoskeletal structure. As such, a new perspective on subcellular network organisation is surfacing,  
 110 in which the orientational order is an emergent property of the cell cytoskeleton that controls the  
 111 network structure by switching between various forms of orientational order [43, 45, 46].

113 An almost inevitable consequence of the establishment of orientational order is the possibility  
 114 of order breakdown. *Indeed, the domains of parallel orientation in any nematic material cannot*  
 115 *indefinitely extend in space and are characterized by finite orientational correlation lengths. Bends*  
 116 *and splays can thus emerge in the material that distort the orientations, and high bend and splay can*  
 117 *lead to the local misalignment of the anisotropic particles. Such singularities in the orientation field*  
 118 *are termed topological defects (see BOX. 2). Defects can form diverse patterns grouped into distinct*  
 119 *classes based on the degree of rotation of directors around the defect cores (BOX. 2). In the absence*  
 120 *of confinements and external topological constraints the defects with the lowest degree of distortions*  
 121 *for nematic materials are the half-integer,  $+1/2$  (comet-like) and  $-1/2$  (three-fold symmetric),*  
 122 *defects (see Fig. 2a) and are energetically favored over higher order defects as defects come with*  
 123 *elastic energy [47, 48]. On the other hand, to minimize the elastic energy, polar materials (see*  
 124 *Fig. 2a) favor formation higher order full-integer defects, i.e.  $+1$  and  $-1$  defects. Although, they*  
 125 mark imperfections in the alignment, topological defects are emerging as important organisation  
 126 centres of cell cytoskeleton. A striking example is the full-integer polar topological defects in  
 127 the form of aster-like structures in microtubule orientation field, which determine the location  
 128 of bipolar spindles in the cell [46]. Moreover, in agreement with the predictions from models of  
 129 active liquid crystals [49], recent *in vivo* experiments have revealed topological defects in the actin  
 130 filaments of the regenerating animal *hydra* as the organisation centres for animal morphogenesis,  
 131 showing that full- and half-integer topological defects determine the location of foot, mouth, and  
 132 tentacles of the animal during regeneration [50].

## BOX 2. Topological defects

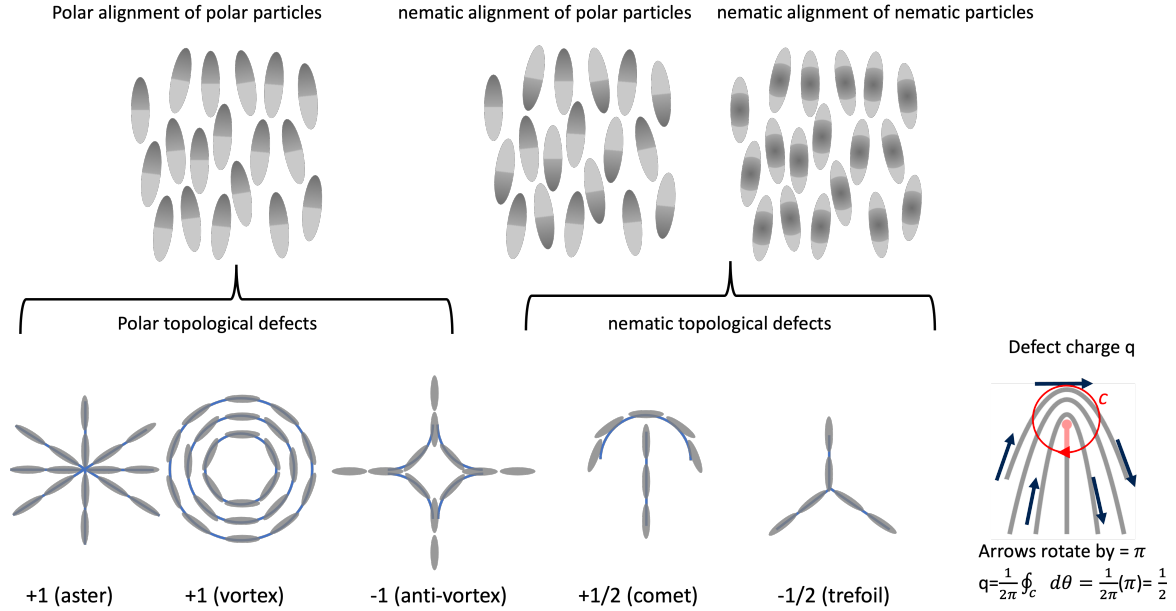
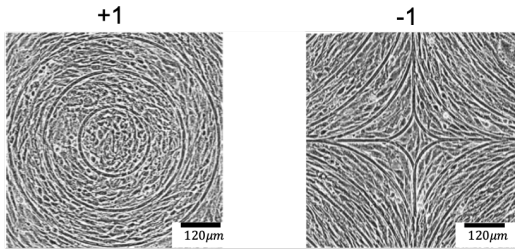
Topological defects mark singular points in the orientation field of the particles. They are topological because no smooth rearrangement of the particles can remove them, and they are defects because they correspond to imperfections in the alignment of particles, where domains with alignment mismatch meet each other. In two-dimensional orientation fields different kinds of topological defects can be formed that are distinguished based on their charge, which determines how much orientation of particles changes when a full rotation around the defect point is considered. For example, if during a full clock-wise turn (+360 degrees) around the defect point, orientation of particles rotates by  $\pm 180$  degrees, that defect has an half-integer charge of  $\pm 1/2$  ( $\pm 180 / + 360$ ), while  $\pm 360$  degrees rotation of particle orientations would correspond to a full-integer defect with  $\pm 1$  charge (see Fig. 2a-c).

In unconfined, two-dimensional systems with nematic symmetry formation of full-integer defects is energetically costly and half-integer defects *preferentially* form. On the other hand two-dimensional polar systems allow full-integer defects. As such the charge of the defect can be considered as an indicator of the prevalent symmetry of that system [41, 51].

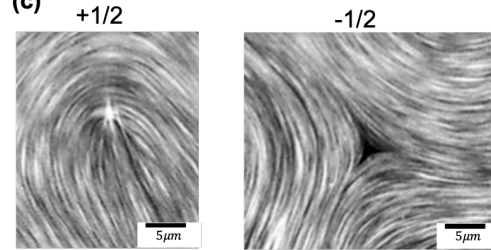
In active systems with nematic symmetry, the  $+1/2$  defects are motile and their direction of motion can be used as an indicator of the form of forces created by active particles. For extensile particles that exert pushing forces along their elongation direction,  $+1/2$  defects move towards their comet head, while  $+1/2$  defects in contractile systems, which exert pulling forces towards their elongation direction, move towards their comet tail (Fig. 2d) [26, 41].

The structural form of topological defects is additionally important in determining the dominant symmetry of the system: while full-integer topological defects imply polar symmetry, the appearance of half-integer topological defects in the form of comet- and trefoil-like structures is the hallmark of the dominant nematic symmetry in any given system with orientational order (Fig. 2a). Indeed, both full- and half-integer topological defects have been reported in subcellular constructs comprising microtubule-kinesin motor mixtures [46, 52, 53], and actin filaments-myosin motor assemblies [54]. Strikingly, the activity of these subcellular constructs renders topological defects as hotspots of flow generation. This is best characterised by the velocity field around half-integer topological defects in active nematics [29, 55], that show stark contrast with passive liquid crystals. As such, because of their comet-like shape and continuous injection of energy by activity, positive half-integer defects can self-propel in active systems. This self-propulsion feature of comet-like defects recently revealed the role of defects in establishing chaotic dynamics at low Reynolds number due to the active nature of biological fluids [56]. *Moreover, the defect shape can also be altered by the activity of the system. A study on actin filaments and myosin motors [54] revealed that the elastic properties of this active nematic system can be tuned by increasing the activity, leading to a decrease of the bending elasticity and changes in  $+1/2$  defect morphology.*



**(a) Topological defects and symmetry****(b)**

3T6 fibroblasts in the vicinity of prepatterned full integer defects

**(c)**

Microtubule-kinesin motor mixtures

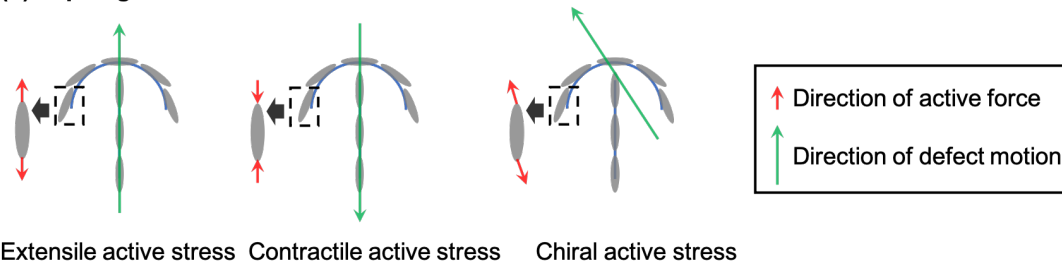
**(d) Topological defects and the nature of active stresses**

FIG. 2. The form of topological defects is related to (a-c) the symmetry of the alignment of constituent particles of the system, and (d) the nature of active stresses that particles generate. *In (a) an example of the calculation of defect charge around a +1/2 defect is shown: over a full,  $2\pi$  clockwise rotation around the defect core (marked by the closed path  $c$ ) the arrows that follow the orientation field rotate by  $\pi$  in the clockwise direction and as such the defect has the charge of +1/2.* Figures in (b) and (c) are adapted from [51, 57], respectively. [BOX 2]

## EMERGENCE OF LIQUID CRYSTALLINE FEATURES IN CELL MONOLAYERS

The power of applying concepts from physics to biological processes is in the universality of physical predictions. The physics of liquid crystal is no exception to this rule. As such, several liquid crystalline features found in subcellular filaments and cytoskeletal constructs within individual cells have been identified within cellular tissues that operate at completely different time and length scales compared to subcellular filaments. Here, in the tissue, the orientation field corresponds to the elongation direction of cells as a result of the anisotropic shape of the cells - either due to a naturally elongated shape such as in fibroblast [58], aggressive human breast cancer cell (MDA-MB-231) [59, 60] or because of the cell deformation due to forces from neighbouring cells such as in MDCK and MCF-7 cells [61, 62].

### *Polar or Nematic Cells in Collective Cell Migration*

There is now a growing list of cellular systems for which topological defects and liquid crystalline order have been identified. This includes epithelial cells such as Madine-Darby Canine Kidney (MDCK) cells, human skin keratinocyte (HaCaT) cells, luminal human breast cancer cells (MCF-7) [61, 62], Human Bronchial Cells (HBC) [63], *human* breast cancer cells (*MDA-MB-231*) [60], *mouse* myoblasts (*C2C12*) [64], *human retinal epithelial cells (RPE1)* [58], human fibrosarcoma (*HT1080*) [65], and neural progenitor stem cells [66, 67]. Interestingly, with the exception of spatially confined monolayers in which a full-integer topological charge is imposed by the circular confinement [64], all the existing works have reported half-integer (nematic) topological defects in cell monolayers. Similarly, a three-dimensional reconstruction of the orientation field in mouse liver tissues has also identified nematic order in the cellular alignment [68]. These emergent nematic features - and the absence of polar features such as full-integer defects - in cell layers are particularly alluring since they suggest the generic predominance of nematic rather than polar symmetry in cellular monolayers. It is well-established that at an individual level cells are often endowed with polarity, yet, when multicellular layers are formed nematic features arise. Recent cell-based models of monolayers have suggested a possible explanation for this mismatch between polar symmetry at the single cell level and the nematic symmetry at multicellular level as the dominance of nematic alignment between the cells due to cell-cell adhesion: rather than aligning their polarity, upon cell-to-cell contacts neighboring cells simply deform such that they are elongated along a same axis [62, 69]. Similar mechanistic description has been also proposed in theoretical models of cell layers based the physics of liquid crystals [43]. Nevertheless, it is not completely clear if such a mismatch in symmetry or the emergent nematic features serve any physiological significance. Interestingly, collective cell migration can also result from polar order at the single cell level within multicellular clusters under certain conditions as exemplified by the formation of multicellular structures at the front of migrating tissues [70] or during collective cellular rotation *in vivo* or *in vitro* [18, 20]. Overall, the interplay between polar and nematic orders in cellular assemblies

187 remains unclear and could be a property reminiscent of the one observed at the scale of cytoskeleton  
 188 inside a single cell within various actin structures. The ability to switch between polar and nematic  
 189 organizations may contribute to the mechanical plasticity of tissues [71].

190 *Biological Functions of Active Nematics in Tissues*

191 Concerning the active nematic behavior of biological tissues, recent studies have definitely  
 192 demonstrated the high potential of such approaches to better understand biological processes.  
 193 For instance, topological defects have been described as mechanotransduction hotspots (Fig. 3a).  
 194 Comet-like, positive half-integer defects govern cell death and consequent cell extrusion from ep-  
 195 ithelial monolayers (MDCK, MCF-7, HaCaT) [61]. Modeling and experiments have demonstrated  
 196 that compressive mechanical stresses build up at the head of the comet-shaped defect, resulting  
 197 in the translocation of the transcriptional co-activator YAP to the cytoplasm. Since YAP sup-  
 198 presses pro-apoptotic genes, deactivation of YAP by compression may result in the activation of  
 199 cell death signal (Caspase-3) and simultaneous extrusion of cells from the tissue. Remarkably, it is  
 200 further shown that inducing comet-like defects by engineering the topology of the cell layer can be  
 201 employed to localise cell extrusion events. In addition, topological defects have been described as  
 202 centers of cellular accumulation/depletion (Fig. 3b). Experiments on neural progenitor stem cells  
 203 have reported accumulation of cells at comet-like, positive half-integer defects and their depletion  
 204 from trefoil-like, negative half-integer defects [66]. It is further shown that cellular accumulation  
 205 results in three-dimensional mound formation at the comet-like defects. The mechanism for this  
 206 accumulation/depletion has been associated with anisotropic friction in cell layers, easing cellular  
 207 motion along the nematic axis and resisting perpendicular motion to it. *Recently it has been demon-*  
 208 *strated that the change in density of cells around topological defects in mesothelium can result in*  
 209 *the suppression of the ovarian cancer cells clearance from the mesothelium [72].* Furthermore, re-  
 210 cent experiments in confined myoblasts have also demonstrated the formation of three-dimensional  
 211 helical mounds at spiral full-integer defects that are formed by merging two half-integer defects  
 212 due to the imposed circular confinement [64].

213 *Importantly, a large number of non-equilibrium biological processes take place in confined envi-*  
 214 *ronments. This is particularly true during tissue morphogenesis [73]. As such, boundary effects*  
 215 *may influence the behaviours of active nematics. In particular, a spherical topology ensures that*  
 216 *the total charge of the surface-bound topological defects is +2. Along this line, a recent study has*  
 217 *shown that during hydra regeneration, the system folds into spheroids with a total charge of +2.*  
 218 *Importantly, the emergence of two +1 topological defects during Hydra morphogenesis visualized*  
 219 *by the pluricellular actin organization coincides with the apex of the head and the base of the foot,*  
 220 *thus defining the body axis of the animal [50]. The further development of the animal leads to*  
 221 *the formation of tentacles that are again associated with topological defects of charge +1 at the*  
 222 *tip leaving behind two  $-1/2$  at the base (Fig. 3c), that is consistent with earlier predictions from*  
 223 *continuum modeling of active nematic shells [49].*

224 In line with earlier predictions from models of active liquid crystals [74], recent experiments on  
 225 epithelial cells (MDCK) have shown that the self-propulsion of comet-like defects are correlated  
 226 with the protrusion formation and elongation of initially circular cell colonies (Fig. 3d) [75]. Under  
 227 cellular confinement, topological defects have been identified in cellular monolayers as pumps of  
 228 cellular flows. Indeed, recent experiments on confined human fibrosarcoma have reported, in  
 229 agreement with models of active liquid crystals, the emergence of chiral currents of cells at the  
 230 edges of confinement, identifying comet-like defects close to the boundaries as the sources of cell  
 231 currents (Fig. 3e) [67].

232 Finally, topological defects and nematic activity can reflect the collective cellular self-organisation  
 233 modes. It is recently found that weakening cell-cell adhesion by removing E-cadherin protein (E-  
 234 cad KO) in epithelial cells (MDCK and MCF-7) results in the switching of the self-propulsion  
 235 direction of comet-like defects, upending their behavior from extensile (see Fig. 2d and BOX 2)  
 236 in wild-type layers to contractile in E-cad KO cells (Fig. 3f) [62]. The mechanism for this switch  
 237 was explained based on the competition between contractile cell-substrate stresses and extensile  
 238 cell-cell contact stresses, showing that sufficient reduction of cell-substrate interaction in E-cad  
 239 KO cells by adding blebbistatin or culturing cells on soft substrates rescues the extensile behavior.  
 240 Since the loss of E-cadherin is a hallmark of epithelial-to-mesenchymal transition (EMT) the  
 241 dynamics of mixtures of wild-type and E-cad KO cells was explored showing autonomous cell  
 242 sorting governed by the competition between extensile and contractile stresses [62] *and providing*  
 243 *alternative mechanisms to the existing models of differential adhesion or tension [76–78].*

## 245 EXPLOITING PHYSICS OF LIQUID CRYSTALS FOR GUIDING CELLULAR 246 SELF-ORGANISATION

247 The increasingly emerging physics of liquid crystals in cellular systems and the growing list  
 248 of biological functionalities for topological defects, provide a fertile ground for exploiting physics  
 249 of liquid crystals in designing advanced biomaterials for improved tissue regeneration and for  
 250 biomimetic inspirations. Beyond the applications of liquid crystals in optical biosensors and ac-  
 251 tuators [79], the design and engineering of liquid crystal based biomaterials for tissue repair and  
 252 regeneration is still in its infancy. Nevertheless, recent experiments have provided early evidence  
 253 on applicability of the physics of liquid crystal principles in controlling cellular organisation. It is  
 254 shown that surfaces pre-patterned with liquid crystal elastomers (LCE) can be used for controlling  
 255 the orientation of human dermal fibroblasts (HDF) [80]. Upon swelling in an aqueous medium, the  
 256 elastomers form nano-scales ridges that guide alignment of elongated fibroblast cells in a mono-  
 257 layer. Using this approach it is shown that pre-designed patterns of half- and full-integer defects  
 258 can be employed to induce accumulation and depletion centers for cells, suggesting a means of  
 259 controlling cell density in space. Moreover, such a pre-imposed pattern is shown to be useful in  
 260 stabilising cellular orientation field to allow inferring elastic properties of the cell monolayers based  
 261 on theories of liquid crystals. Similar inference of cell layer material properties based on theories

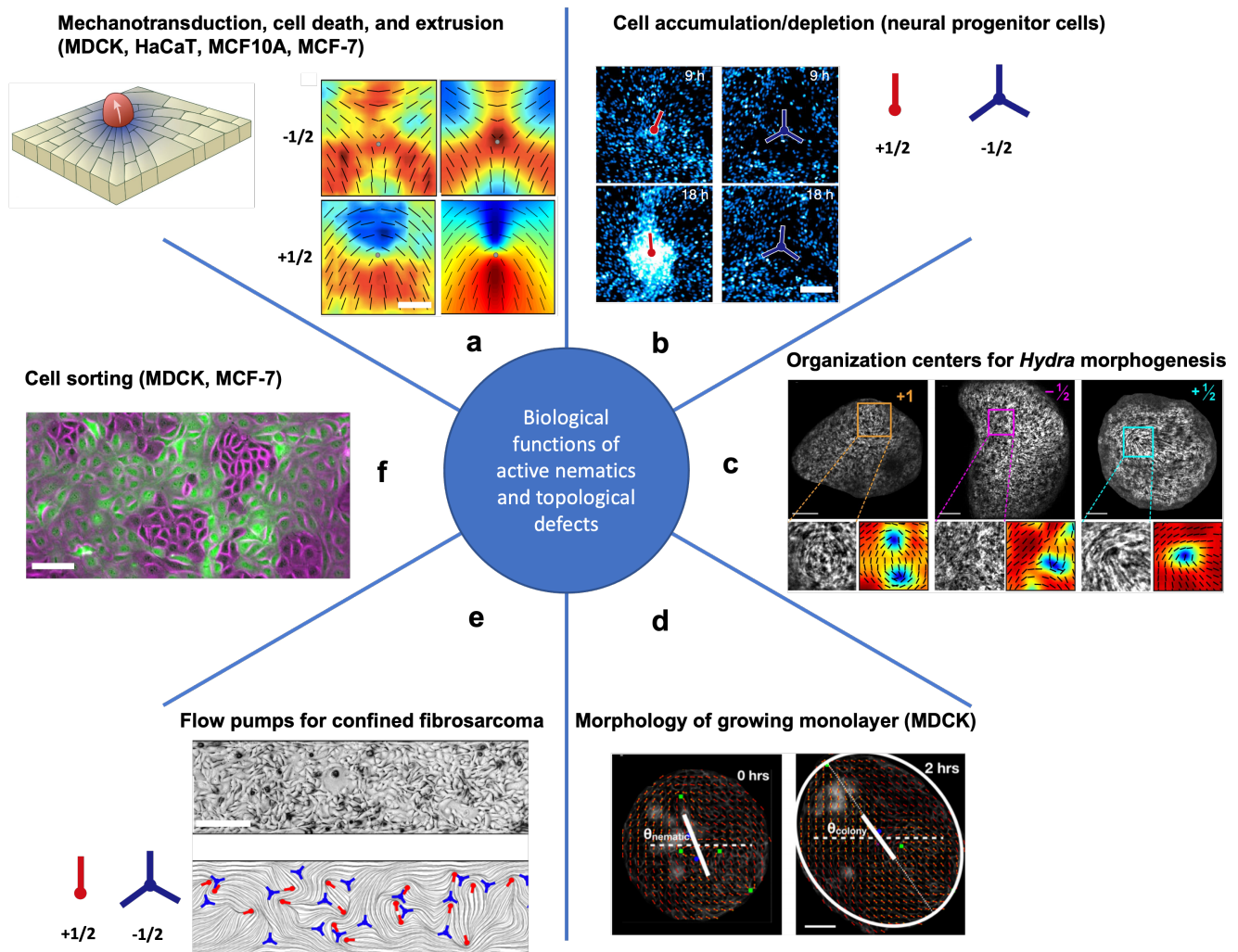


FIG. 3. Biological significance of topological defects. Figures in (a)-(f) are adapted from [50, 61, 62, 65, 66, 75], respectively. *In (a) the colormaps represent isotropic stresses, ranging from negative compressive stresses (blue) to positive tensile stresses (red), overlaid on the director field (solid black lines) for experiments on MDCK cells (left column) and continuum model of the monolayer (right column). The scale bars in (a)-(f) are  $50\mu\text{m}$ ,  $100\mu\text{m}$ ,  $100\mu\text{m}$ ,  $50\mu\text{m}$ ,  $200\mu\text{m}$ , and  $100\mu\text{m}$ , respectively.*

262 of liquid crystals are also proposed by confining myoblasts in small circular adhesive islands to  
 263 stabilise spiral topological defects at the centre of the confinement [81]. More recently, it is further  
 264 shown that pre-patterned micron-sized ridges can be exploited to control alignment of fibroblast  
 265 (3T6) and epithelial (EpH-4) cell layers to form stable full-integer topological defects [57]. Re-  
 266 markably, it was shown that such pre-imposed patterns can control the activation/deactivation of  
 267 mechanotransduction by triggering nuclear/cytoplasmic YAP translocation at topological defects  
 268 with positive and negative charges and in different cell types.

## CONCLUDING REMARKS

269

270 *In this review, we provide examples of active nematic liquid crystals, highlighting how they drive*  
271 *self-organization of biological systems at multiple length-scales.* Nematic ordering and topological  
272 defects have been identified in large variety of systems including cytoskeletons, various cell lines and  
273 tissues and even others such as bacterial colonies not reviewed here [82, 83]. From these studies,  
274 it emerges that topological defects are hotspots of mechanical signals [61, 66]. They can also serve  
275 as organization centers to shape tissues and organisms as shown in the Hydra development [50]. In  
276 addition, recent studies have highlighted the capacity of using LCE to control the organization of  
277 multicellular assemblies. Such approaches may help to further understand the interplay between  
278 extra-cellular matrix properties and tissue dynamics. Further understanding of liquid crystal-  
279 based approaches will also require a more profound insight into polar versus nematic organisation  
280 at subcellular and multicellular scales. How can both structures coexist? Could biological systems  
281 switch from one structure to the other? How such dynamics may impact on single cell polarity  
282 through cytoskeleton regulation as well as on large-scale polarization of tissues? *Additionally, cell*  
283 *layers establish collective patterns of motion on length scales that are often larger than the scale of*  
284 *individual cells [84]. It is not clear how these collective migration length scales are correlated with*  
285 *length scales associated with orientational order and liquid crystalline features in the tissue.* We  
286 anticipate that answering these questions will shed new light on developmental and pathological  
287 processes. Additionally, majority of studies so far have focused on liquid-crystalline features in  
288 two-dimensional systems. Recent experiments on reconstituted microtubule-motor protein mix-  
289 tures stabilised by filamentous viruses have provided first *in vitro* realisation of three-dimensional  
290 active topological defects in the form of lines and loop [85], as predicted from the theory of active  
291 nematics [86, 87]. The existence and possible functionality of such three-dimensional features  
292 in cell assemblies presents an intriguing venue for further studies of active liquid crystals in cell  
293 biology. Finally, an interesting perspective to further understand tissue mechanics concerns the  
294 links between active nematics and the physics of jamming-unjamming [88]. Similar parameters  
295 such as cell density, shape, cell-cell and cell-matrix adhesions [62, 89, 90] are at play in both  
296 descriptions but the interplay between these approaches remains to be established. *Particularly,*  
297 *it is suggested that the jamming-unjamming transition coincides with a topological transition in*  
298 *cell shape as the ratio of the cell perimeter to the square-root of its area exceeds a threshold [91].*  
299 *Interestingly, recent theoretical work [69] suggests that the emergence of orientational order and*  
300 *topological defects is also correlated with the cellular shape changes leading to the elongation of*  
301 *otherwise isotropic circular cells. It is an attractive idea to connect such topological transitions in*  
302 *cell shape changes to the cellular elongation and the establishment of orientational order in cell*  
303 *aggregates.*

304

## Highlights

- Various forms of liquid crystalline order including nematic, smectic and chiral features have been established in cytoskeletal constructs *in vitro* and in subcellular filaments *in vivo*.
- Nematic ordering and topological defects are identified in an unprecedented speed in tissues of various cell types.
- Topological defects are emerging as hotspots of mechanotransduction and organisation centres for cellular flows in tissues.
- Topological design through pre-patterned surfaces and geometric constraints is appearing as a promising tool for exploiting physics of liquid crystals for guiding cellular self-organisation.

## Outstanding Questions

- Can passive liquid crystals be used to pre-organize biological tissues?
- Can polar and nematic organizations coexist in collective cell behaviors?
- What are the links between the physics of liquid crystals and the physics of jamming-unjamming?
- What could be the role and structure of topological defects in 3D cellular tissues and multi-layered assemblies?
- How can topological defects influence *in vivo* morphogenetic processes?

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