A morphospace for synthetic organs and organoids: the possible and the actual

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Synthetic biology thinking has been dominated by the
picture of predictable engineering practices.
In some cases, when a modular design is feasible, such predictable
designs can be successful. However, many other factors interact
in complex ways leading to unexpected (or undesired) outcomes.
This is specially relevant when dealing with synthetic tissues and organs,
we face causal feedbacks between genetic regulation and physical
interactions. As a consequence,
design will be limited by self-organisation and emergence, thus
questioning the standard view that emergent properties must be
ignored or avoided. Here we present a global picture of the problem by
introducing the concept of organ morphospace, i. e. an abstract
space defined by three axes: development, cognition and physical
state. It is shown that this space is largely empty, indicating that
many potential designs are not observable. This void in the universe of
organ designs might contain many potentially unexplored solutions
that could be achieved by using synthetic biology in novel ways.

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A morphospace for synthetic organs and organoids: the possible and the actual

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Efforts in evolutionary developmental biology have shed light on how organs are developed and why evolution has selected some structures instead of others. These advances in the understanding of organogenesis along with the most recent techniques of organotypic cultures, tissue bioprinting and synthetic biology provide the tools to hack the physical and genetic constraints in organ development, thus opening new avenues for research in the form of completely designed or merely altered settings. Here we propose a unifying framework that connects the concept of *morphospace* (i. e. the space of possible structures) with synthetic biology and tissue engineering. We aim for a synthesis that incorporates both our understanding of evolutionary and architectural constraints and can be used as a guide for exploring alternative design principles to build artificial organs and organoids. We present a three-dimensional morphospace incorporating three key features associated to organ and organoid complexity. The axes of this space include the degree of complexity introduced by developmental mechanisms required to build the structure, its potential to store and react to information and the underlying physical state. We suggest that a large fraction of this space is empty, and that the void might offer clues for alternative ways of designing and even inventing new organs.

1 Introduction

The twenty-first century has witnessed the advent of synthetic biology, a new spin on biological engineering tasked with developing strategies into the purposeful modification of biological components in order to obtain novel and useful functions or behaviours in living organisms. This discipline has been enabled by several technological innovations that help synthetic biologists systematically alter biological information, reorganising genes, coding and regulatory sequences, guided by the extensive knowledge gathered by cellular and molecular biologists.

Synthetic biology has been inspired by traditional engineering principles such as predictability, modularity and scalability. When these properties are available to the engineer, a bottom-up approach to the design and building machines (living or otherwise) can be pursued. This means, as with other long-established engineering disciplines like electronics, that a collection of parts with well-defined functionality and behaviour can be used to construct more complicated machines by means of appropriately connecting and combining these components. This view has been repeatedly articulated as an implicit design approach to biological systems within the synthetic biology community: artificial constructs can be obtained from collections of lower-level components in such a way that their performance and behaviour are essentially modular and predictable [1].

Such expected predictability needs to be confronted with the existence of the so-called *emergent properties* [3–11]. These properties relate to higher-order phenomena that result from the interactions among components belonging to the low-level scale [3] that cannot be reduced to the properties of the lower-scale elements. Examples of emergent phenomena include cellular and molecular assemblies [12], morphogenesis [13,14], collective behavior in social animals [15–17], cancer cell patterns [18], microbial population growth [19] or neural networks and brains [20,21]. In a nutshell, neither the construction of a nest nor the cognitive complexity of a neural network can be reduced to the dynamics of single ants or neurones, yet they are brought about by the countless interactions among the lower-level entities.

At the level of tissues and organs, self-organisation processes provide multiple illustrations of how higher-order patterns emerge [22–24]. Similarly, many of the outcomes of development

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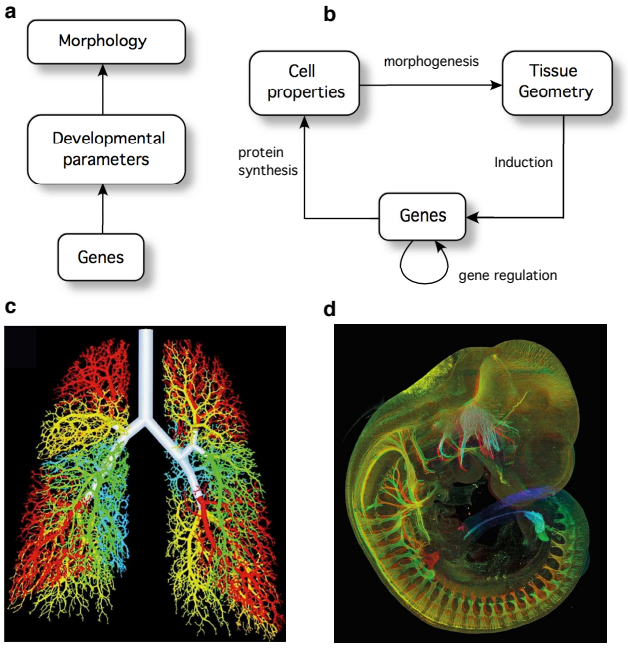

Fig. 1 Linear versus closed causality in development, from genotype to phenotype and back. Early views of development (a) suggested that the relationship between genes and the processes they affect would involve a linear causal chain where genes affect key parameters of development which ultimately would tune morphogenesis. A more realistic, integrated view of development (b) consider the effects of genes on cell properties that, due to cell-cell interactions influence tissue geometry and feedback to gene expression patterns (figures adapted from Alberch 1989[2]). Synthetic biology and tissue engineering techniques are capable of extensive modifications of some of the steps and consequences of this causal loop.

escape explanations confined to the gene or gene network levels. Morphogenetic mechanisms rely on tightly regulated chains of events and a dynamical change of boundaries resulting from the growth, deformation and chemical modification of tissues. This feedback between agents and their environment, also known as *stigmergy*[25,26], is a crucial feature of many collective phenomena. The presence of emergence does not limit our understanding of nature, but clearly defines a limit to what can be predicted from a detailed understanding of the component parts. In fact, the physics of phase transitions shows that most examples of collective dynamics can be accurately explained by means of models that largely ignore these details. Instead, interactions and boundary conditions play the leading role[5,7].

Nonetheless, despite the common acceptance of the interrelation between environmental constraints, cellular self-organisation and gene regulation[27], some controversy persists today. Most research has been traditionally focused on a reductionist program dealing almost exclusively with genes, treating the organism as a mere epiphenomenon[28] (Fig. 1a). Models of pattern formation based on positional information would illustrate this idea: a given signal gradient provides a cue to differentiation that is easily interpreted by cells. A more complete view was provided by the late Pere Alberch (Fig. 1b), linking gene regulation and transcription to tissue geometry. Once again, gene expression can only be seen as a necessary condition for morphogenetic dynamics, but it is far from sufficient. Even simple models of interaction between pattern formation and tissue/organ geometry reveal complex interactions that are not reducible to linear mappings[29–32].

The lack of a simple genotype-phenotype mapping is a consequence of two key components. One is the information associated to cell interactions, geometry and boundary conditions in embryogenesis. The second, the widespread use of tinkering made by evolution[33], which defines in fact a crucial difference between evolution and engineering. Whereas the human inventor can create novel structures out of new designs or materials, evolution does not. Sometimes, tinkering leads to optimality, and natural designs fit the expectation of the engineer. This is the case of most branching patterns that can be found within large living systems (Fig. 1c). In this case, the system displays a self-similar organisation of nested trees that can be fully understood as a problem of embedding the tree structure in a given finite space. But in most developmental processes (Fig. 1d) tinkering leads to tangled networks of interactions[34–36].

How is this connected with the potential to create synthetic organs? Engineers aim to construct functional objects given a repertoire of available components and design rules, often inspired in the scalability of electronic designs. What are the limits of our potential for engineering living tissues? Are there multiple solutions to design organs to solve a given problem? A tentative answer might come from the widespread presence of convergent evolution[37]: the same solutions to given problems are found independently. This is illustrated by the camera eye, which has evolved multiple times in different lineages to essentially the same design. This includes the broadest range of groups (Fig. 2a-b) from marine snails, squids or some types of jellyfish to the vertebrate eye and even the ocelloids of some single-celled dinoflagellates[38,39] (Fig. 2c). The widespread emergence of this structure might result from a combination of optimal design (there is one solution that humans also discovered while inventing cameras) as well as self-organisation processes associated to the morphogenesis of eyes. Yoshiki Sasai's classic experiments[40–42] on the formation of primordial eyes from embryonic stem cells (Fig. 2) illustrates this. Here structural motifs are generated from interactions between genetic and cellular-level mechanisms. Embodiment, self-organisation and system-level constraints play a leading role. This gives an important message to engineers aiming at create new organs: self-organisation an emergence are likely to be both inevitable (but perhaps helpful) components of future bioengineering procedures.

Here we aim to present a new picture of the design space of organs and organoids. Our goal is to provide a balanced view of the space of possible structures that include both natural as well as some artificial systems. This is known as the *morphospace*[45–49]. The morphospace has been extensively used within evolutionary biology as a way of describing the spectrum of physically possible forms available to a given group of organisms. But the approach can be made general and applied to networks[50–53]. Two main lessons can be extracted from the analysis of these spaces. One is that the occupied regions indicate accessible solutions for evolved systems as well as potential trade-offs that constrain the structural designs achievable. The second is that the presence of

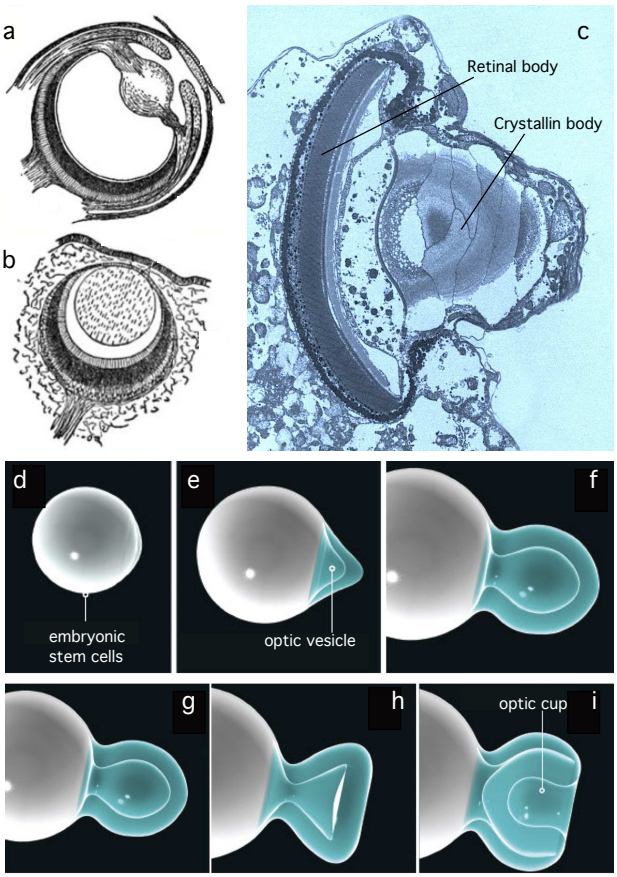

Fig. 2 The evolution of a complex organ: Convergent evolution of eye designs, from comparative anatomy to stem cells and self-organisation. In (a-b) we display two examples of the visual organs of different living species (redrawn from Gregory 2008[43]) and in (c) an electron microscope section of the ocelloid of a single-celled dinoflagellate species, where assemblies of endosymbiotic bacteria form a retina and a crystallin (modified from Gavelis *et al.*[39]). (d-i) Using stem cell-based methods, it has been shown that a whole eye cup gets formed when the right culture conditions are met (redrawn from Sasai 2012[44]). It was shown that a whole retinal structure gets self-organised in cell cultures, somewhat recapitulating the morphogenetic process that takes place during embryogenesis. These results indicate that an important part of the construction process is controlled by cell-cell interactions and boundary conditions beyond the purely genetic control metaphor.

empty spaces can indicate: (a) impossible designs or evolutionarily improbable designs or (b) designs that might not be accessible under some conditions but can be achieved through engineering approaches. Any of these possibilities is relevant when dealing with design principles for synthetic biology. Empty spaces might indicate that strong constraints forbid us from achieving solutions in that domain. However, they might be simply unlikely to be generated by evolution, but accessible to our engineering skills. If that is the case, we can obtain valuable lessons and guidance concerning our potential for engineering biological systems following orthogonal schemes. Moreover, if synthetic biology can be understood as a way to interrogate nature[54], the morphospace can also be a powerful guideline to ask ourselves about the logic of biological organisation and its limits.

The morphospace that we want to build must include both natural organs as well as artificial, designed systems. In order to define the right axes for our organ space, we will first review the different ways in which engineers and synthetic biologists have been able to tame cells and their environments, thus potentially expanding the space of possible designs. This will provide us a general picture of the different approaches aimed to change both functional and structural patterns in living tissues and organs, but also of the twilight zone that separates a standard definition of organ from what can be obtained by artificial means.

2 Setting boundaries

To developmental biologists, an organ is a collection of tissues joined in a structural unit to perform a specific function, being a tissue an ensemble of similar cells with a common origin that carry out a function[55]. However, the boundaries of what defines an organ are blurred once we enter the artificial domain, where organs can be constructed from cells of different origin and might harbour differently altered genotypes for instance. An organ then, could be redefined as a set of agents able to implement a common function, but also able to communicate and be coordinated in order to build temporal and spatial order. In this context, microbial biofilms living in our teeth or gut could be seen as an organ, but also bioprinted tissues, chip encapsulated organs, as well as tumours or organoids, since they are functionally coherent structures. Moreover, artificial organs can incorporate non-biological materials and communicate with electronic components, thus pushing the boundaries of the possible (Fig. 3). Those readers already familiar with these techniques can go straight to section 3.

2.1 Taming cells

Let us start with different ways to manipulate cellular properties by engineering gene regulatory networks affecting developmental pathways[56] like cell-cell signalling[57–62], epigenetic modification[63–66] or signal transduction and interpretation[67–69]. Progress here has been particularly significant, showcasing the construction of prosthetic gene networks that can serve as computation or interpretation devices for developmental signals, from switches or oscillators to complex computational devices[68,70–79].

Genome editing tools allow high specificity, low interference genomic modifications[80,81]. The engineering of programmable DNA nucleases, like Zinc-Finger Nucleases (ZFN), TALENs or CRISPR-Cas9, allows the introduction of double strand breaks on specific sites of the DNA. ZFN and TALENs are chimeric proteins which consist of DNA binding domains (ZFs or TALEs) and a nuclease[82–84], while the CRISPR-Cas9 platform[85] consist of a small RNA sequence, namely 'guide RNA' (CRISPR), able to recognise the DNA sequences of interest and Cas9, which can display nuclease activity, among others[86]. Nonetheless, there are still challenges that need to be confronted to have a widespread use of these techniques in mammalian cells[87–90]. An alternative is the use of artificial chromosome structures able to incorporate whole synthetic gene networks[91].

The control of epigenetic processes allows to write new developmental programs (Fig. 4a)[65,66]. Contributions range from the

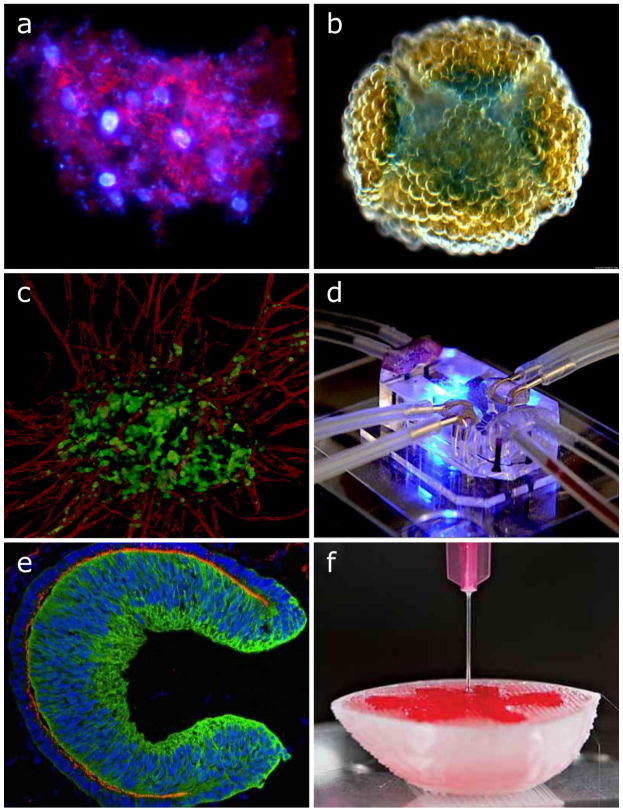

Fig. 3 Deviations from the organ definition. (**a**) Set of microorganisms structured in a biofilm consortia. (**b**) Applying 3D bioprinting techniques, cells can be ordered and shaped in a particular structure. In this case, aqueous droplets are used instead of cells. (**c**) Vascularised tumour spheroid. Mechanisms of cell sorting and cell communication allow differentiated cells be ordered in space, adopting functional structures. (**d**) Lung on a chip. Taking advantage of the mechanisms of cell sorting and providing a mechanical support, is possible to mimic organ structures on a chip. (**e**) Optic-cup organoid. Stem or iPS cells can, in the appropriate microenvironment, develop all the different kinds of cells needed to develop an organ and self-organise in the appropriate structure. (**f**) Printed kidney. Stem cells can also be printed in a complex extra cellular matrix scaffold to recreate whole organs substitutions.

use of optogenetics[64] to the direct efficient targeted demethylation through TALEs fused to a catalytic domain[92]. TALEs fused to histone demethylase[63] and CRISPR-Cas9 fused to a repressor[93], have been used for the inactivation of enhancer chromatin down-regulating proximal genes. Importantly, RNA-mediated epigenetic regulation[94] provides another path to alter gene expression.

Synthetic biology allows to alter transcriptional regulation by activating or repressing gene transcription through transcription factors (TF) appropriately bound to DNA binding domains, being the Zinc-fingers, TALEs and CRISPR-Cas9 the ones currently used. Several works, such as the building of a library of orthogonal synthetic TF using artificial ZF[95], a library of TALE repressors that bind newly designed hybrid promoters[69] or the coupling of a catalytically inactive dCas9 to a transcriptional repressor domain able to robustly silence expression of multiple genes, among others[86,96–98], show the great potential to alter regulatory networks. Moreover, optogenetic methods enable a dynamical control of this process[99].

Control of gene expression can be also engineered at the post-transcriptional level. Non-coding RNAs (ncRNAs) have a relevant role in this process, as RNA modules are engineered to respond to several types of molecular inputs, such as small molecules, metabolites or proteins (i.e. aptamers). Ligand-responsive riboregulators enable post-transcriptional gene expression with an external control[100,101], while ribozymes and riboswitches have also shown direct implications in gene expression[102], becoming targets of synthetic modification at an ever-increasing rate. Programmable sensing-actuation devices have also been designed[67]. Importantly, the engineering of RNA molecules is not limited to post-transcriptional regulators, as they display sensing, regulatory, information processing and scaffolding activities with great potential to reprogram cellular behaviour at other levels[103].

Signalling networks can be rewired to change cellular responses either to endogenous[67] or externally applied stimuli[68]. This requires unraveling protein-protein interactions (PPI) with engineering prospects[104], or taking into consideration the problem of crosstalk to design and create orthogonal (non-crossreacting) protein-protein interfaces[105,106]. Extracellular signals being transduced to the nucleus through a receptor's intracellular domain[107] allow for a controlled behaviour at a nuclear level in a ligand-dependent fashion[108]. Furthermore, directed molecular evolution can be a powerful tool to receptor design[109]. On the other hand, engineered systems controlling protein splicing[110–112] as well as optogenetic tools[113] are being developed due to the regulatory opportunities they offer.

2.2 Taming the environment

The final fate of a cell in a tissue is influenced by environmental interactions, which also participate in the self-organisation of tissues and organs. Most efforts within tissue-engineering have been focused on reproducing as reliably as possible the cellular microenvironment[116]. Since the environment can be engineered in ways that are forbidden to real development, it provides a crucial element to expand our morphospace. A clear example involves the dimensionality of the environment. Cells cultured in a 3D microenvironment exhibit a gene expression profile, cell morphology and migration activity more similar to physiological conditions than cells of 2D cultures. The scaffolds or hydrogels used in such cultures are polymers that mimic the native extracellular matrix (ECM)[117–120]. Several works have tackled this issue, achieving promising results[121–125]. Appropriate scaffold designs can improve diverse conditions affecting cell viability, stem cell differentiation and cell migration[126–129].

Using the correct mix of progenitor, ES or iPS cell cultures in the appropriate 3D context and biochemical conditions, a 3D structure similar to physiological tissues emerge, with self-organisation playing a critical role in this process. The resulting so-called *organoids* resemble real organs and can even display some of its characteristic functions. Several organoids that resemble organs originating from the ectoderm, such as optic cups[40], storable stratified neural retina[42], polarised cortical tissue[130], cerebral[131]

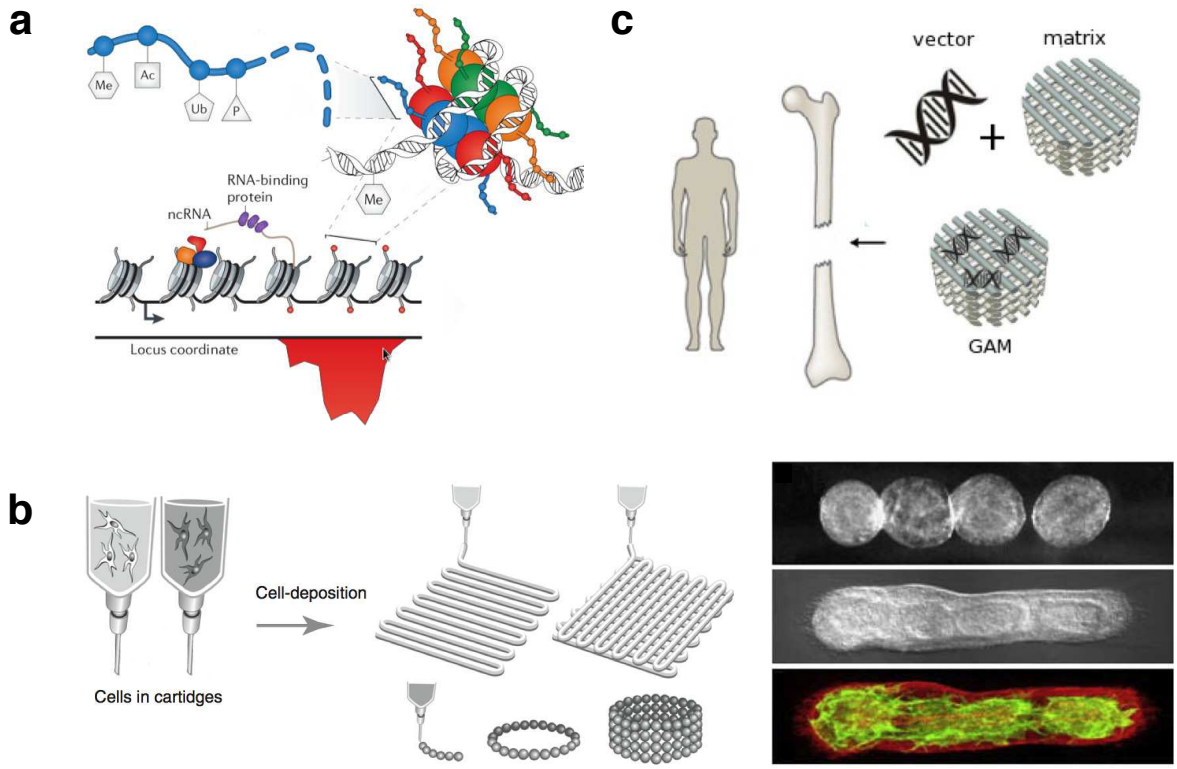

Fig. 4 Some techniques outlined in the sections of taming cells and taming the environment. **a)**The positioning of nucleosomes on DNA influences the accessibility of transcription factors to regions such as the promoter. The amino termini of histone proteins can be biochemically modified. By means of synthetic modifications of heterochromatin structure is possible to influence the binding of DNA and regulatory proteins. Adapted from Keung *et al.*[65]. **b)**Scheme of bioprinting of cell-laden hydrogels by extrusion. Deposition of spheroids in a circular structure are able to fuse to form a tubular structure with two layers. Adapted from Mironov *et al.*[114]**c)** GAMs consist of a matrix with associated DNA vectors that can be released in a controlled way. Once implanted, the colonising cells receive the DNA and promote differentiation. Adapted from Evans *et al.*[115]

and inner ear[132]. On the other hand, we find organoids recreating digestive system organs, such as the human liver[133], intestinal tissue[134] and crypt villus structures[135], gastric tissue[136] and pancreas[137], aimed at modelling human disease. Embryonic kidney structures have also been developed[138]. Although the potential of building organoids represents a major advance, some of them lack key cell types and others do not progress beyond an early developmental or embryonic stage in culture[133,138], although some can progress in development after *in vivo* implantation[139,140].

A very important advance has been provided by microfluidic devices and micropatterning[148] to control the microenvironment, creating stable molecular gradients and flows[149,150] and allowing cell compartmentalisation[151,152]. They provide a repertoire of possible environments way beyond the natural counterparts[153–155]. A major outcome of microfluidics is provided by the so-called *organs-on-a-chip*[156–158]. The cells cultured inside the chip are monitored by the digital components that control the inflow of nutrients, gases or chemical signals, overcoming the limitations of the conventional 3D culture[159]. They allow reproducing the biology of organs and the analysis of their responses under highly controlled conditions[160–162].

A final approach to engineering organs while preserving some key structural constraints is to keep their original extracellular matrix. These decellularised organs provide a cell-free scaffold[163], guiding cells into the desired organ structure. Several attempts have already been pursued in trying to rebuild a heart[164], a liver[165] or a lung[166,167]. An important issue here are the limits imposed by reconstructing these large structures outside from the whole organism context. Additionally, it is possible to use bioprinting to produce tissues and organoids[114,168]. Bioprinters relying on the inkjet technology, with ejected drops that can either contain individual cells or clusters of them, still lack the capacity to ensure appropriate cell density and survival[169,170] and rely on cellular self-assembly properties[171–173]. Alternative bioprinters use mechanical extruders to deliver multicellular aggregates of specific composition on a support following the desired topology[174]. In this approach, the cells inside the aggregates are in a 3D environment that allows cells to self-reassemble forming small organoids that will then fuse to one another[175,176] (see Fig. 4b). Endothelial cells, when co-cultured in an appropriate 3D environment, organise themselves into tubular forms in-between and throughout structures[177]. It is essential to simultaneously build a vascular network[178] and great efforts have been pursued in this direction[172,173,179–181]. For example, the most modern bioprinters allow for direct extrusion of angiogenic tubular networks at the same time than the tissue[182–184].

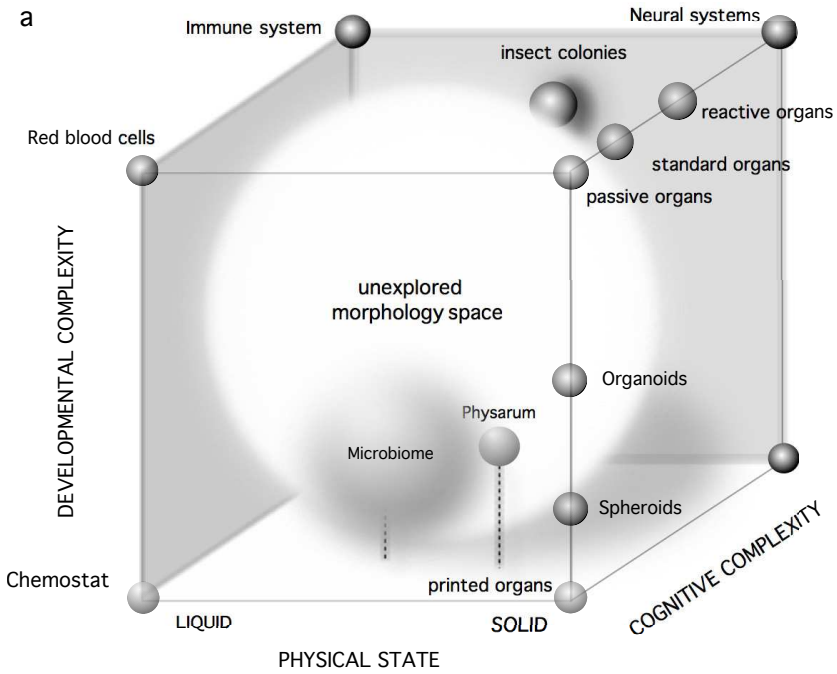

Fig. 5 The universe of organs and organoids. (a) A schematic representation of tissue distribution in our proposed three dimensional organ morphospace Ω. Different classes of organs, organoids and designed multicellular systems and other entities occupy particular regions in terms of relative developmental and physical complexity as well as physical state. An unexplored (or at least underpopulated) region of this morphospace is shown as a shaded volume which offers new opportunities to unravel the possible and the actual (see text). We have added to this domain three systems that are typically not within the definition of organ or organoid. These are insect nests, the unicellular mold *Physarum* and the microbiome. The later appears as a large, fuzzy sphere to highlight its diverse and complex nature. Additional elements could be included, such as tumours and biological-artificial interfaces, but they would not match the underlying definition of spatially-defined, functional structures capable of displaying autonomous behaviour (under a given biological context). An example of a system (sometimes labelled as organoid) that is solid, has little developmental complexity and no cognition is provided by cellular aggregates experiencing cell sorting [141–145] (b-c). Here a set of dissociated cells (two types of retinal cells) evolves to a segregated structure (d) that is stable (modified from Mombach *et al.* [146]). In this manner, an initially homogeneous mixture of cells can create discrete domains with well defined boundaries, which some authors suggest is an easy way of constructing layers in emerging animal embryos [145,147].

3 Merging synthetic biology, complexity and tissue engineering

The idea that synthetic biology should take advantage of the self-organising properties is supported by different approaches. The field of *morphogenetic engineering* [185] , for example explicitly considers the self-formation capabilities of biological systems and how can these be integrated with technological design. In similar lines, the work by Liu *et al.* [186]showed that *E. coli* cells can be engineered to form periodic stripes, being one of the few examples of an engineered self-organised process in synthetic biology. Interestingly, there is a lack of similar examples involving engineered mammalian cell lines. On the other hand, self-organisation plays a decisive role in many of the approaches used in tissue engineering when trying to build organ and tissue-level structures. However, this is achieved without proper control on the cellular behaviour, as all the currently used techniques rely on the natural cellular developmental programs, resulting in a field still dominated mostly by a trial and error strategy [187].

This suggest that synthetic biology and tissue engineering need to be taken together [58,188,189] when aiming to design organs and organoids. Jamie Davies *et al.* [190,191], who coined the term *synthetic morphology*, first proposed the application of synthetic biology to the problem of regenerating structures, which would have implications in regenerative medicine. This involves a systems engineering perspective that includes modelling and developing a modular library of synthetic morphogenetic driver genes to control different processes typically related with morphology, i.e. cell adhesion, locomotion or fusion, among others. Other related works in the field of synthetic biology aim to control cell density [192], cell migration [193,194], cell adhesion [195] and cellular communication [57,108], all key processes in the development of organ structures.

Some works have already shown the potential of genetically modifying cells in a tissue engineering framework. Notably, several works addressed the problem of proper engraftment of cells in composite tissue matrices [115,196–198]. The proposed solution involves using gene activated matrices (GAMs), which consist of a matrix with associated DNA vectors, which are slowly released and delivered into reparative cells (see Fig. 4c). By expressing the transgene, the infiltrating cells enhance migration and promote differentiation. The safety and efficiency of printing DNA vectors in GAMs for transfecting target cells surpasses the benefits of direct delivery of growth factors. Protein-protein interactions designed for synthetic switches in mammalian cells [199] have been successfully used to control the release of drugs from hydro-

gels[200]. For example, vascular endothelial growth factor (VEGF) loaded into the hydrogel can be released in a dose-dependent manner by oral administration of the trigger compound[201], opening new interesting avenues for research and therapy.

Most of our previous examples rely in a way or another in either reproducing or imitating natural organs or partial functionalities associated to them. However, we are here addressing the problem of the possible and the actual and the definition of a space of organs and organoids. And there is no reason to only build organs and tissues as they exist in nature. Novel organs that can still perform, but also expand, the functions of their natural counterparts can be envisioned. Such enhanced physiology could entail including completely new functions or even the capacity to diagnose and cure diseases. This perspective no longer remains in the domain of speculation[202]. Similarly, the fusion of organic and electronic systems have also drawn a great deal of attention. The first steps towards cyborg organs have already been undertaken. A striking example is the bioprinted bionic ear integrating condrocytes in alginate along with printed silver nanoparticles in the form of an inductive coil antenna[203]. Bioprinting organs to provide electric power, which is inspired on the myogenic electric organ that fish use to produce electric fields to communicate, navigate or defend themselves[204], could be a reality in a near future. Such novel electric organs could be used in humans as batteries for pacemakers, cochlear implants or as powering for prosthetic devices[205]. It is likely that the advances in genome editing will be the key to create these 'human-fish' cells.

What these examples clearly show is that a wealth of structures and potentially useful functions lie outside the paths taken by evolution. Freed from the constraints of developmental processes, new rules of engineering biological matter can be found. As a first step, we need to build a comprehensive theoretical framework based on the design space already explored by evolution.

4 Organ morphospace Ω

With the goal of guiding the exploration of new design spaces not found in natural development here we present a putative organ/organoid morphospace Ω (Fig. 5a) defined by three essential (ideally orthogonal) properties. An interesting example of a class of systems that is included in this space is provided by cell sorting (Fig. 5b-d). Starting from a mixture of cells (Fig.5b) that can be part of a given organ or belong to completely different, even engineered, cell types, a final stable configuration (Fig. 5d) forms. This particular spatial assembly would be located close to the right, low corner of the front face of our space (see below). The axes of Ω should be taken as abstract dimensions that allow us to locate our candidate systems. Here we define three basic axes:

1. *Developmental complexity*, i.e. the degree of complexity introduced by developmental mechanisms required to build the structure.

2. *Physical state*, namely the level of cellular interaction and physical coherence of the structure under consideration.

3. *Cognitive complexity*, described as the amount of information that a system is able to learn, store and process.

Following the standard approach of evolutionary morphospaces, the way (and how much of) this space of the possible is filled can reveal relevant features of the generative potential of these structures. It can also help to define the common features displayed by natural systems and explore the potential constraints placed on them. We should note that, in the morphospace proposed here, the location of objects in each axis is not quantified, although we will comment on this problem and potential approximations for each axis. However, it is worth mentioning that other studies involving these spaces often rely on statistically aggregated quantities, such as principal components. On the other hand, some well defined regularities can be established even when the morphospace is a purely qualitative one, as for example done in relation to cellular computation[206]. More importantly, each of the examples discussed here can be easily ordered relative to each other within a given axis. Moreover, although the axes are not fully orthogonal (as it happens too with many other case studies), they contain clearly different qualitative features that justify their status as morphospace "dimensions". Let us consider each one separately.

4.1 Developmental Complexity

This axis provides a way of ordering our systems from simple mixtures of unrelated cells to fully developed organs. Intuitively, a bioreactor provides the bottom line of minimal complexity, while fully formed structures resulting from embryogenesis would be located at the other side of the spectrum. The presence or absence (and degree) of developmental processes implies the existence of different levels of dynamical organisation and feedback controls, including size and form[207]. This property is sometimes maintained in the adult organism as exemplified by the human liver, which adapts in size in case one of the lobes becomes damaged or even in the event of transplant to a host of different body mass, recruiting either processes of growth or apoptosis accordingly[208]. In our organ space (Fig. 5a) we can see that systems with low scores in this axis include printed organs and cell sorting-related cell assemblies. Both typically involve some amount of differential adhesion, which is a mechanism also used in most other solid systems inhabiting the right face of the space.

Developmental complexity includes both the presence of a developmental program (that is completely or partially unfolded) and the resulting structural complexity at the steady state. Several complexity measures can be defined, and in biology they need to take into account cellular and functional diversity. Simpler measures of complexity have been used[210–212] and good candidates should take into account both spatial and temporal complexity. The number of different transcriptome profiles (considering coding and non-coding RNAs)[213] or, alternatively, the amount of configurations a gene regulatory network can adopt[214] could be used for such a measure. If we only attend to morphological complexity, the number of cell types of a multicellular system could be the simpler candidate[215–217] but more adequate measures should account for the presence of multiple scales and

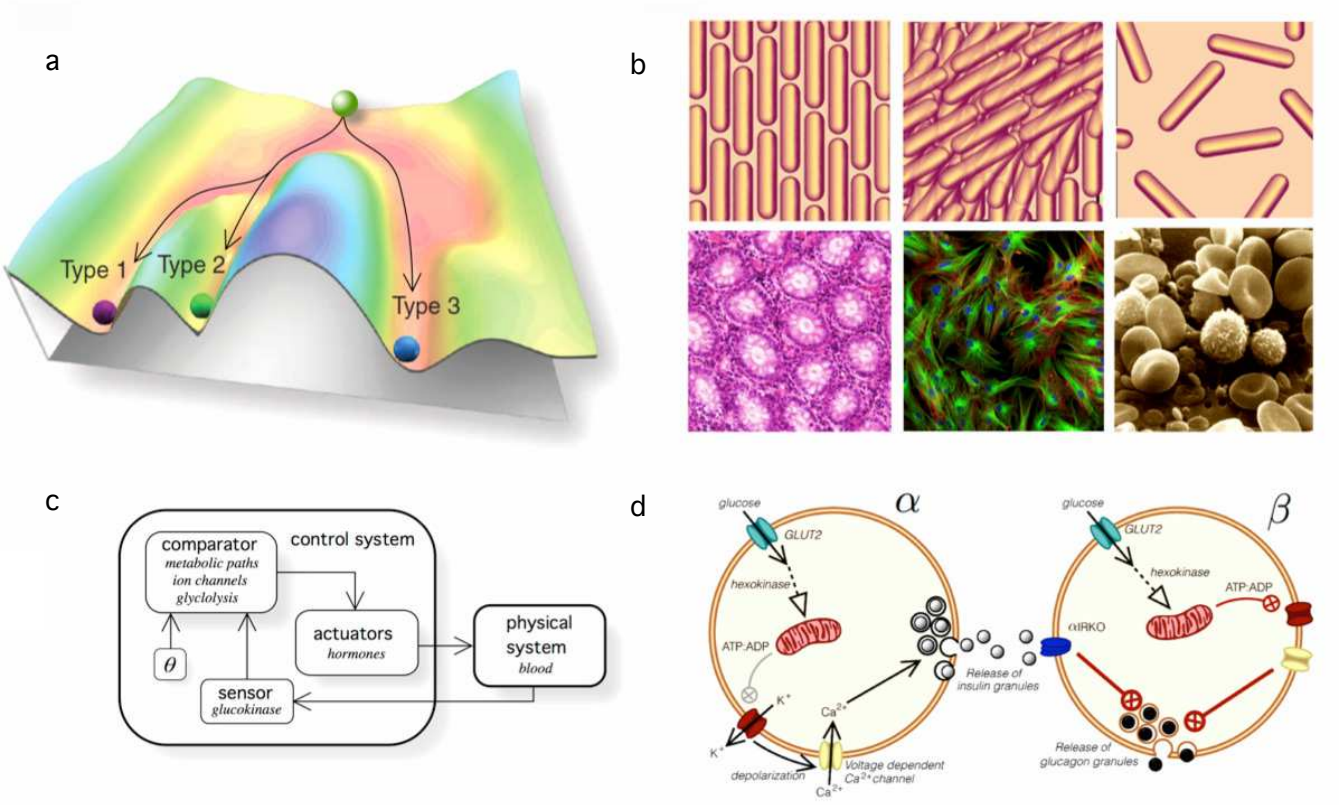

Fig. 6 Defining dimensions for the organ morphospace. Developmental complexity (**a**) in the form of cell types in differentiation lineages has been tackled by several authors, typically defined as a potential or Waddington landscape of differentiation. The physical state of matter (**b**) in the first row, including solid, nematic and liquid phases (left to right) along with different tissue architecture that roughly correspond to these ideal conditions: epithelial crypt, fibroblast cell culture and blood cells (second row, left to right). Cognitive complexity (**c**), as proposed in this article, is related to the information management capabilities of a tissue or organ. Here (left) a schematic representation of the glucose homoeostasis system coming from classic cybernetics and control systems theory (here θ incites internal thresholds). On the right we also offer the molecular counterpart, including the actual implementation that involves several signalling pathways, transcription control mechanisms and transport phenomena (adapted from Gaisano *et al.*[209]).

hierarchy[218]. A simpler but more complete measure could be to take into consideration the spatial distribution that the different cells can adopt, measured as the mutual information between cell types in a positional lattice[219]. Regarding cell lineages, they could also be used as a measure of developmental complexity[220–223]. On the other hand, the use of Waddington's developmental *epigenetic landscape*[224] could also provide ways of incorporating a quantitative measure combining both cell diversity and cell lineages (see Fig. 6a).

4.2 Physical State

The second axis introduces a well known concept from physics: states of matter, here reduced to two of them (solid and liquid). Matter can reside in multiple phases depending on external parameters like pressure or temperature. The main differences between these phases can be found in the mobility of the elementary particles and the mean distance between them. These are also related to how frequently and strongly the molecules conforming a material do interact among each other and, indirectly, how stable these pairwise interactions will be. In a biological setting (Fig. 6b) organs and tissues can also be characterised by different degrees of mobility in their constituent elements, with cells in the place of molecules. Biological assemblies of course differ from these physical systems, since they are far-from equilibrium structures, display cell turnover, heterogeneity and typically belong to a nested hierarchy of systems. On the one hand, typical solid organs like heart, lungs or kidneys display fairly static cell configurations and accordingly stable neighbourhoods of interacting cells. As we can see in our space, most relevant examples fall in the right wall of the cube. Blood on the other hand can be more readily mapped to a liquid phase. But despite the differences, several studies on the collective behaviour of multicellular assemblies clearly indicate that the physical analogies with states of matter are correct[225–227] and in some cases it is even possible to properly define the phases of tissue spreading in aggregates with varying adhesion parameters[228]. Because of the presence of universal behaviour, it is also possible to define rigorous measures associated to the phase state.

What can be found in the middle of this two potential extremes? On one hand, although we have located the immune system (strictly speaking it is not an organ) as a member of the "liquid" wall, it provides a potential example for an intermediate

liquid-solid scenario, since the immune cells are known to undergo a complex development that involves their circulation in blood but also a maturation process in specific tissue locations. In general, the vast majority of tissues and organs (Fig. 5a) belong to the "solid" boundary (the right wall of Ω) and this might be a consequence of the way development proceeds, instead of a truly strong constraint. We will discuss this particular point below.

4.3 Cognitive Complexity

Our third dimension involves the information processing capabilities displayed by different organs and organoids. This axis is connected to the complexity exhibited by these systems as computational structures (Fig. 6c). Here the key idea is that organs and organoids should be also considered as cybernetic systems incorporating the three elements of any homeostatic structure: sensors, comparators and actuators[229,230]. Sensing components provide the connection with the external world. This information (sometimes after a digital transformation through cooperative molecular mechanisms) is then internalised and used to evaluate the state of the system (the comparator plays this role). Finally, a response is generated by the actuators, often creating a feedback loop that restores previous states.

We use the term *cognition* as a general form of introducing different levels of information processing. Organs operate in a distributed fashion, since they consist of many components (ECM or cells) that interact locally to provide a global function. This computation is highly dynamic, as the components are continually being created, self-arranged and destroyed[231–233]. For a lower cognition system, these self-sustaining cell cultures exhibit no particular class of behavioural response beyond cell maintenance. At the other end of the spectrum, the immune system or the brain are able to process information from multiple sources, using very rich loops involving multiple scales (upper right corner in figure 5a). For instance, the immune system detects and responds to either new or previously experienced threats, while at the same time is a highly dynamical system (new lymphocytes are constantly being created and destroyed). On the other hand, the brain is able to process an astonishing wide spectrum of sensory information. Plasticity is a striking feature of the brain[234], being able to create new connections and reinforce them.

A simpler cybernetic system that displays some information processing capabilities is the pancreas (Fig. 6d). Its operating program includes determining the blood glucose concentration, evaluating the state in comparison with a target and making hormonal secretion.The endocrine effect of insulin and glucagon on blood glucose levels close the feedback loop, keeping the glucose homoeostasis[192,235]. The liver is another example, which is responsible for several hundreds of functions being critical to keep the metabolic homoeostasis of the whole body and indeed is capable of regenerating itself[236,237].

It is not difficult to order known systems within this axis, but a general measure of cognitive complexity would be desirable. One possibility is to use of transcriptomic high-throughput data, since it has been proved that the function of an organ can be re-predicted from the gene expression profiles[238]. Alternatively, information theory measures can provide a way to categorise the organs by its gene expression diversity[239].

5 Navigating the void

One of the most interesting features of Ω is the presence of a large void (Fig. 5a). Natural biological systems occupy the upper part of Ω, most on the right side associated to solid structures and resulting from embryogenesis. We have also included, close to this upper boundary, insect colonies, which are also known to display some traits closely related to developmental processes[240,241]. This is an interesting example, given their combination of a "fluid" phase associate to mobile, interacting agents, and a stable scaffold (the solid phase) given by the nest structure. Actually, the nests of army ant colonies are made of the living bodies of individuals, forming a large ball where ants attach to neighbours by holding onto each other's legs[242]. In most ant colonies there is a constant interaction (particularly through its development) between both phases, which would include both a static, solid scaffold (the next structure) as well as a "fluid" phase constituted by the ants. This reminds us the ways bone structures are build and rebuild by the interactions between the solid scaffold and the cells occupying it. These similarities could provide new perspectives within the context of synthetic biomaterials[243].

We also have included in our morphospace two additional examples that occupy a special place. These correspond to the microbiome and the slime moulds belonging to the genus *Physarum*. The first example is very relevant within our discussion, since it defines a complex multicellular assembly that cannot be considered neither liquid nor solid, and displays some level of cognitive complexity[244]. Its developmental complexity is connected to an ecological assembly process where a network of mostly cooperative interactions maintains its species diversity and stability[245]. *Physarum* on the other hand is a very interesting case study that deviates from the rest in several ways. It is a unicellular structure that achieves a macroscopic spatial organisation and is capable of remarkable computational tasks[246]. Despite its lack of multicellular complexity, it does experience a predictable developmental process and its enormous plasticity locates the system in a state that can be identified as "solid" but with an internal fluid structure. Since it is highly plastic and adaptive, it cannot be classified as solid nor liquid, but it does experience a developmental process and has a small cognitive complexity.

The previous examples depart from the standard biological architectures. Does this suggest that organs cannot fill parts of this empty space? We can argue that organisms are final-state outcomes of development and that the unfolding of information through differentiation and proliferation events occurs in a spatial context defined by the changing embryonic boundaries. This process needs to be regulated and under several controls that guarantee, in particular, that organ size and integration occur in the right sequence. Through this process, we could say that the physical plasticity of developing organs varies through time, thus transiently occupying parts of the void within Ω. The clever solution to maintain the right order of events and a robust final outcome is to create modular structures (organs) that can develop separately from others within spatially localised domains while interact with

the whole system in order to achieve a proper integration. It is thus likely that fluid or partially fluid organs are not expected to be common. But one way of engineering alternative organs might involve redesigning organ-specific functionalities into a fluid (liquid) system or perhaps creating hybrid systems combining both phases.

Different potential scenarios can be considered as tentative ways of moving away from the natural locations of the examples shown in figure 5a. As a first example, imagine that we want to engineer an endocrine gland aimed to deliver the a natural or a synthetic hormone into the blood stream. Is there a real need for a solid and thus spatially located gland? Why not "liquefy" the gland into single cells that can travel in the blood? In theory, this distributed gland would be able to perform required the function if the cells are able to properly sense the triggering signal (i. e. high glucose) and secrete in response the desired amount of hormone (i. e. insulin). To engineer those cells, we have to consider the level of the cognitive complexity of the design required to ensure the maintenance of the system's homeostasis, since the extend of oscillations within the healthy values depends on the strength of control of the (information) feedback and the dynamics of the response. Pursuing with the pancreas example, the blood glucose levels should remain within a well-defined range, and these limits should not be overstep either in fasting or after eating. If our engineered cells must only sense the high levels of glucose in the blood or it has to take also in to account early warning from metabolic hormones, and if an active shooting system is required, further engineering might be required.

A second example could be engineering micro-organs obtained from cell sorting processes leading to a multilayered system. We have already mentioned this kind of structure, which actually defines the lower level of complexity of organoids. These *spheroids* can then be engineered to perform artificial computations by designing a set of sensors and actuators that can be distributed between the internal and the external layer. These spheroids could easily incorporate decision-making circuits, thus moving towards the high cognitive complexity wall. Such increased cognition would make closer to organoids and can be achieved by using non-standard computational engineering, such as so called distributed multicellular computation (DMC)[247] where a given functionality is defined through a Boolean table. The different parts of the computation can be distributed over different cell types with no need for a communication among different engineered cells. This allows to easily and reliably build a class of *organoid computation* where we can exploit both DMC as well as spatial modularity[248] to increase the computational capacity of these simple multicellular aggregates. This also helps to store information and thus create a micro-organ with memory potential.

Finally, a third class of hybrid systems could exploit the microbiome, which has been shown to act as an interface between environmental signals and our body's responses. The microbiome defines a powerful layer of living complexity that has co-evolved with our bodies and that interacts closely with our cells[249]. A plethora of candidate microorganisms can be used to engineer synthetic interactions with their host organs and thus obtain a novel system that once again gets located within the empty domain of Ω. Such hybrid system can include on one hand a target organ and, on the other, the spatially less defined population of the chosen microorganisms. Since it is possible to engineer learning circuits[250] with memory[251] using synthetic biology designs, we could also achieve higher cognitive complexity levels. In this context, spheroids, organoids and organs could all be moved towards the end wall of the morphospace.

All these possibilities are open to serious inspection and are promising ways of finding potential, novel solutions to existing problems. But again we must remember that the lack of real systems occupying the void might also indicate intrinsic difficulties. Future work should allow to find out wether developmental constraints are the cause of its empty space of designs (and thus we can exploit the many open possibilities it opens) or instead such designs are suboptimal or forbidden solutions. In this context, the use of mathematical and computational models offers alternative ways of searching for answers and explore tentative designs. If organs only visit this domain transiently during their development, this can also be due to the stabilising role played by spatially patterned interactions[252]. In this context, it would be important to develop a theoretical understanding of our morphospace that includes other factors beyond the ones covered by our chosen axes. Just to mention an example, it has been shown that the spatial organisation of some tissues (such as the colonic crypts) can provide tumour suppressor properties[253]. If designed organoids have to be designed for long-term interactions within their host organisms, these type of properties should be taken into account.

6 *In silico* morphospaces

Experimental exploration of developmental processes has been widely successful in identifying the key events and genes underlying the construction of form. However, the *in vivo* approach has been usually constrained by the impossibility of modifying several variables or genes at a time while keeping track of the consequences and generate organised knowledge. High-throughput experiments allow the modification of multiple variables but again one by one, using an essentially reductionistic approach that remains largely oblivious to the higher order interactions of genes and their epistatic relations. Fortunately, a complementary approach exists in the use of computer assisted research, particularly in the formulation of theoretical models that allow the systematic analysis of complex variables and landscapes. This approach naturally complements experiments by providing new predictions that can be validated in experimental setups. The power of simple models has been widely proved by models involving differential adhesion, a phenomenon that we have already discussed above. As early suggested by Steinberg, the spontaneous dynamics associated to cell sorting and the formation of spheroids and other types of organ-like structures can be described by an energy minimisation process[141,142]. Given its importance and widespread use to model a wide range of developmental processes, it is worth briefly presenting it.

The model is described by a finite population of cells that occupy given positions within a discrete lattice. Their state (what type of cell we have) is indicated by means of a discrete number σ_n. Cells can move to neighbouring sites, provided that this is

consistent with a spontaneous, energy-minimisation process. Let us restrict here to a system with only two cell phenotypes namely yellow cells (σ_1) and red cells (σ_2), while σ_0 represents empty space.

Different cells have different forms of interacting with others as well as with the external environment. The strength of interactions among different states can be defined by means of an adhesion matrix $\mathscr{J}$:

$$\mathscr{J} = \begin{pmatrix} \mathscr{J}_{(\sigma_0,\sigma_0)} & \mathscr{J}_{(\sigma_0,\sigma_1)} & \mathscr{J}_{(\sigma_0,\sigma_2)} \\ \mathscr{J}_{(\sigma_1,\sigma_0)} & \mathscr{J}_{(\sigma_1,\sigma_1)} & \mathscr{J}_{(\sigma_1,\sigma_2)} \\ \mathscr{J}_{(\sigma_2,\sigma_0)} & \mathscr{J}_{(\sigma_2,\sigma_1)} & \mathscr{J}_{(\sigma_2,\sigma_2)} \end{pmatrix}. \quad (1)$$

Each term $J_{(a,b)}$ in this matrix provides a measure of how likely is a given pair of contacts given the state of the two sites. For obvious reasons, the matrix is symmetric, i.e. $\mathscr{J}_{(a,b)} = \mathscr{J}_{(b,a)}$, and $\mathscr{J}_{(\sigma_0,\sigma_0)} = 0$.

The energy associated to this system is defined as a function $\mathscr{H}$ that is determined at each lattice site μ i. e. :

$$\mathscr{H}_\mu = \sum_{S_\eta \in \Gamma_\mu} \mathscr{J}_{S_\mu, S_\eta} \quad (2)$$

where Γ_μ is the set defined by the nearest neighbours of a cell in position μ each of which occupies a position η, and has a defined state S_η. If we try to swap one cell to one of its nearest locations, we first determine the new energy H' using the same expression. The energy difference between the original and the new (potential) configuration is:

$$\Delta\mathscr{H} = \mathscr{H}' - \mathscr{H}^* \quad (3)$$

When the difference is negative, the cell is likely to swap to the chosen new position, whereas no such change should be expected if an increase in energy is at work. The probability associated to this is given by the so called Boltzmann rule:

$$P(S_\mu \to S_\eta) = \frac{1}{1 + e^{\Delta\mathscr{H}/T}} \quad (4)$$

where the parameter T is a noise factor tuning the degree of randomness associated to our model. It is easy to se that the Boltzmann factor $e^{\Delta\mathscr{H}/T}$ acts in such a way that if $\Delta\mathscr{H} = 0$, the probability of swapping is $1/2$. In figure 7 we display three examples of the distinct spatial arrangements of cells obtained from this simple (but rather realistic) model. The relative weights of the matrix elements provide clues for what to expect in each case, and thus can be used as a guideline to predict some of the possible patterns that can result from a given engineering design associated to adhesion properties.

Steinberg's model and other related efforts towards understanding and predicting the formation of simple organoids support the idea that simple models not incorporating the low-level molecular interactions can help to gain insight. Moreover, the model can be expanded in multiple directions, by incorporating (in particular) the three levels of complexity associated to the three axes of our morphospace. By increasing the temperature, for example, we can shift from a well-organised (solid) spheroid to a disordered (liquid) mixture. By incorporating additional components involving phenotypic transitions, a much richer spectrum of structures can be obtained. Finally, as we discussed in the previous section, simple and complex computational synthetic circuits can be added in order to introduce learning or adaptation to increase cognitive complexity. Some of the models discussed below rely precisely in adding other components to the original model. Here we present a few examples of theoretical avenues that can be followed to explore and predict potential outcomes of designed or evolved organs. They have been selected according to their relevance with the three axes previously described, outlining several theoretical approaches that can be useful in the exploration of new possibilities.

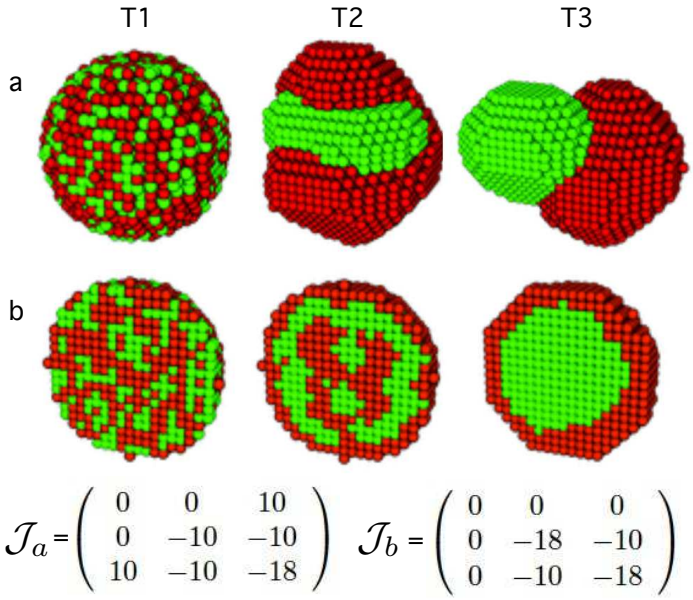

Fig. 7 Generating organoid complexity from differential adhesion. Three examples of the time evolution of an initially random set of mixed cells of two types and exhibiting different adhesion matrices (indicated below for each case). Here three snapshots are shown, corresponding to three different times (their values differ between examples). Adapted from Sun *et al.* 2013[254].

6.1 Developmental Complexity related models

Several attempts to understand developmental mechanisms from a theoretical perspective have been carried out throughout the past decades, including remarkable works on optimality of hierarchical tissue architecture[253], the developmental robustness origins[258] and limb development[259]. Regarding the study of the segmentation process, several works have tackled the question of how they arise highly coordinated both spatially and temporally[260–263]. The basic mechanism underlying its formation is the clock-and-wavefront model proposed by Cooke and Zeeman[264]: sub-cellular (gene regulation), cellular (synchronisation and-self organization) and tissular (signaling and gradient formation) scales are involved[265], exemplifying the difficulty of modelling developmental processes.

Theoretical tools can be used to model existing processes of development, however they can also be aimed at the exploration of the landscape of possibilities, following an approach similar to

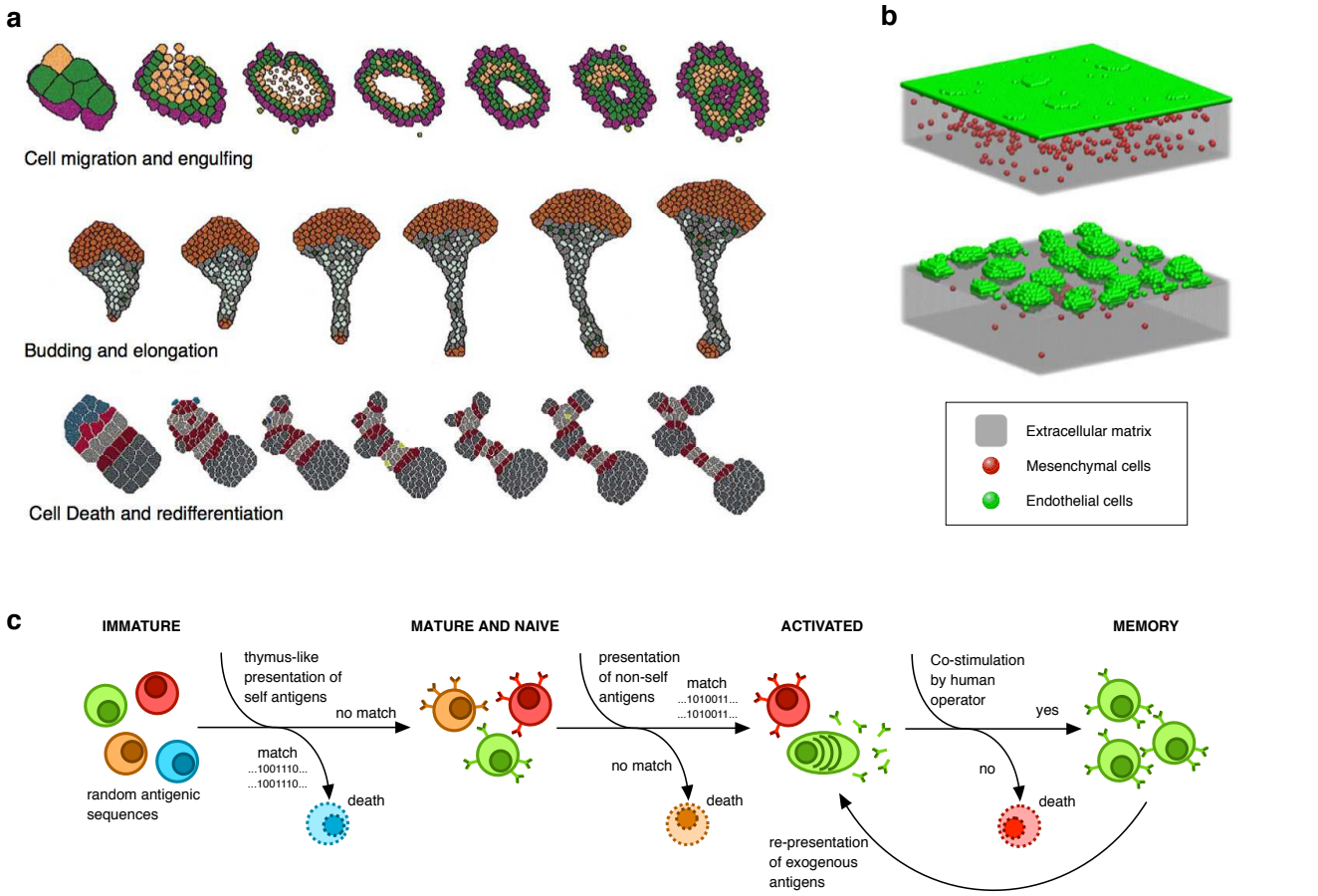

Fig. 8 Some examples of theoretical model outcomes related to the morphospace axes. (**a**) Developmental dynamics of three *embryos*, exemplifying the involvement of typical morphogenetic mechanisms, from Hogeweg *et al.*[255]. (**b**) Physical state axis. Simulations results from EMT behaviour adapted from Neagu *et al.*[256], illustrating the effect of cell-cell and cell-ECM interactions on the emergent morphology. Top: uniform invasion of the ECM by mesenchymal cells, while the endothelium remains stable. This situation is due to the negative interfacial tension between the ECM and mesenchymal cells. Bottom: the endothelial layer breaks down leading to the rounding up of the fragments and mesenchymal cells aggregated, due to the small interfacial tension between the medium and the ECM. (**c**) The lifecycle of a detector in the Artificial Immune System architecture, adapted from Forrest *et al.*[257] to include its immune system counterparts. When immature, the bit-strings (detectors) go through a process of negative selection, the so-called tolerisation period, in the same way thymocytes experience this phenomenon in the thymus or B cells in the bone marrow. Through proper co-stimulation, discerning between a false and a true positive when a detector gets activated, the system can attain the well-known property of memory typical of the adaptive immune system.

the one proposed here. In this vein, Hogeweg *et al.*[255] analysed the interplay between developmental mechanisms in virtual embryos. The subjacent hypothesis is that there is a natural drive for organisms to become more internally diverse. The results show that, despite no explicit search for particular spatial arrangements or specific developmental programs, morphogenetic behaviours appear as a side effect of selecting for cell heterogeneity, with the remarkable establishment of engulfment, budding and intercalation among others. A wide variety of 'life-like' forms similar to those observed in development were reported (Fig. 8a) offering new insights to the discussion of the role of complexity in development. This strategy has also been put forward in other works[266], obtaining interesting results that connect the evolution of complex patterns of gene expression and developmental toolkits: once a threshold of genetic complexity is achieved, the number of accessible patterns explodes.

6.2 Physical state related models

Regarding the interplay between physical properties and developmental processes, several examples can be found (from growth rates to cellular shapes) where the regulated recruitment of physical forces acts as a morphogenetic driver[145]. Albeit generally controlled by the expression of one or multiple genes, they introduce relevant epigenetic interactions beyond the gene level, key to the establishment of functional structures. In particular, one of the most well documented examples, also related to our proposed liquid-solid dichotomy, is the Epithelium Mesenchymal Transition (hereafter EMT)[256,267] (Fig. 8b). EMT is a reversible transdifferentiation process by which cells in the primitive streak, the endocardial cushion and the heart valves among others switch back and forth between more mobile and more adhesive phenotypes. This prompts the reorganisation of cell distribution, linked to proper functionality in the mentioned structures[256], but also related to tumour progression and invasion[268]. Models of metas-

tasis exploring the cell fate landscape have shown that cancer cells can make use of mixed phenotypes with epithelium and mesenchymal properties[269], a feature that was previously observed in experimental systems[270].

Another interesting theoretical model involving alterations in the physical state of cells is the one from Marie and Hogeweg, where they modelled the mechanisms behind the formation of a fruiting body in *Dictyostelium discoideum*. It is well known from *Dictyostelium* biology that, in the face of starvation, the solitary amoebas aggregate and form a collective structure composed of multiple cell types with the purpose of creating resistance forms (spores) and disseminating them. When trying to model this process, the authors introduced in different cell types with a specific function (either stalk or spore forming) and a differential adhesion pattern between them. This simple mechanism was shown to be of utmost importance in maintaining the general spatial relations between structures of the fruiting body when appropriately regulated. Moreover, the physical properties of the virtual cells and their environment where also modulated by an extracellular matrix, enabling the self-organisation and self-correcting behaviour of the fruiting body formation.

6.3 Cognitive complexity related models

We have already mentioned potential examples of re-engineering organs or organoids in order to increase their computational/cognitive complexity. A computational approach to these system could provide very useful insights about what to expect in terms of the robustness and novelty of the designed changes. In this context, we have recently suggested to explore the landscape of collective intelligence using computational models of engineered synthetic microbial systems[271]. There we suggest that some universal principles of detecting and sensing signals found in microbial communities (such as the quorum sensing circuitry) are very close to the switching systems found in intelligent swarms. Let us consider here a different scenario, that illustrates the implications in a given technological framework of mimicking the features of a biological system with high cognitive complexity.

Hofmeyr and Forrest described the architecture of an Artificial Immune System (AIS) able to solve the network intrusion detection problem in broadcast local-area networks (LANs)[257], of practical significance in network security. Natural immune systems are diverse, distributed, dynamic, self-protecting and adaptable, and these properties were taken as basic design principles to build a robust artificial distributed adaptive system. Broadly speaking, the AIS consists of a protected computer system (*self*) and infectious agents (*non-self*), which are both represented by dynamically changing sets of bit strings. These are continuously processed by detector programs that incorporate properties from different cells of the immune system. During the lifecycle of a detector (Fig. 8c) a *negative selection* process takes place, filtering some immature detectors in order to avoid *autoimmune reactivity*. Once a detector has survived the negative selection process it can become activated by means of presentation of *non-self* bit strings and human co-stimulation. This way, a memory of interaction is created and the threshold for further *non-self* detection is lowered. This constitutes a compelling example of how to implement a useful, cognitively complex process that is inspired in a natural system. Moreover, it lays the groundwork for further inquiry into artificial distributed information-processing.

7 Discussion

A central theme in our paper is the presence of constraints that can be unavoidable while dealing with living tissues and organs, where structural constraints might sometimes play a role that cannot be reduced to gene regulatory paths. This does not mean that we are necessarily limited in engineering complex cellular structures, and the existing literature on synthetic biological designs is a success story. What we try to highlight here, however, is the need for considering the potential constraints associated to the systems, organ-level of organisation. As we discussed above, evolution very often displays a surprising level of convergence, thus indicating that the same solutions are generated independently through different paths. Many examples of this process, taking place in different species often separated both in space and time, can be provided. These include among others: light-producing organs in squids[272], electric organs in teleost fish[273], neural circuits underlying communication and speech[274] and even technological networks[275,276]. Convergence is an indication of a limited repertoire of design rules, and more importantly the existence of limitations to what can be achieved using a bottom-up, standard engineering perspective. That includes both evolved and designed systems. The presence of constraints often imply a top-down causality that might be difficult (or even impossible) to modify. Bottom-up designs will succeed provided that they are capable of exploiting modular properties of the organ/organoid, thus avoiding unexpected or undesirable effects.

In this article we have reviewed some of the most recent advances in the fields of synthetic biology and tissue engineering, with the intent of exploring how far could we go in designing new non-standard organs. With that purpose, we address the idea that self-organising principles, a keystone component in tissue engineering, should be viewed as an advantage (instead of an obstacle) by synthetic biologists. These self-organisation principles are consistently used to obtain organ-like structures or organoids[24], exploiting the natural tendency of cells to differentiate and spatially arrange themselves in an orderly manner. These designed forms could potentially open the door to new functionality, but also offer invaluable information regarding the evolutionary potential that current structures bear (in the same way that Alberch's monsters do). Guidance to explore the generative landscape of organ design can be found in our proposed morphospace (Fig. 5), where we show that some regions remain underpopulated. These might be reached with current synthetic biology techniques, which really allow to build novel designed morphologies[190,191,277,278].

The morphospace of organs and organoids presented here provides a unifying picture of the known universe of possible structures. In this simplified universe of possibilities, we have a populated area inhabited by known organs and some designed organoids. The limit cases are also obvious, but a large space of design is essentially (as far as we know) empty. These empty

spaces in the maps of biological complexity require an explanation. What is the rationale for their presence? Their absence either indicate impossibility or simply that evolution within an embryogenesis context is unable to fill this space. But engineering deals with the exploration of what can be designed no matter if evolution would allow that to happen. Understanding the nature of this void is also a way of interrogating nature about its generative potential, and might shed light into novel ways of designing organs beyond the natural counterparts.

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TABLE OF CONTENTS ENTRY

Guiding synthetic organ exploration through acknowledging self-organisation and evolutionary constraints in the morphospace of the possible and the actual.