The wisdom of the body: future techniques and approaches to morphogenetic fields in regenerative medicine, developmental biology and cancer

"*The restoration of shape is a central goal of regenerative medicine – rebuilding a complex structure such as a hand or eye requires more than stem cells driven towards individual tissues – the placement of all components in the right arrangement is crucial and cannot be micromanaged by direct bioengineering.*"

KEYWORDS: bioelectricity ■ birth defect ■ developmental biology ■ morphogenetic field ■ patterning ■ regeneration ■ regenerative medicine ■ repair

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Birth defects, traumatic injury, aging and cancer are addressed by distinct disciplines, journals and funding bodies. This article discusses an unconventional perspective: morphogenetic fields (information-bearing global patterns in chemicoelectrical properties that guide growth and form) as a profound unifying concept central to biology and medicine [1,2]. It reviews several unconventional approaches to regenerative biology and discusses data from a range of model species that point to several areas for tractable, exciting future work that may have a transformative impact on our ability to control shape and restore complex organs:

- An information-centered understanding of the morphogenetic field as a fundamental, high-level regulator of shape along the regenerative repair–cancer continuum;
- An understanding of nonlocal (long range, perhaps neurally mediated) instructive patterning signals;
- The hypothesis of 'target morphology', having important implications for where and how shape-modulating signals should be applied for interventions;
- Incorporation of bioelectrical controls of patterning, which involves development of physiomic datasets and technologies to understand how patterning information is stored in dynamic physiological networks (beyond protein and gene regulatory network profiling);
- Application of techniques from computer science to develop algorithmic (constructivist) models – a bioinformatics of shape that will drastically increase the level of insight drawn from high-resolution genetic and functional data.

All cells in the body are immersed (Figure 1) in physical, chemical and electrical cues containing a rich field of information (attempts to formulate this as a mathematical field go back to Child, Driesch, Gurwitsch, Needham, Weiss, Waddington and Spemann [3–5], as well as more recent studies [2,6–9]). These signals provide cues about a cell's position within the host, and enable individual cell behaviors to be orchestrated into the exquisitely complex 3D structure of organs and appendages. Pattern (on many size scales, from subcellular organelles to organ systems) is a central concept in almost all aspects of biomedicine. The initial establishment of body structures (morphogenesis) is accomplished during embryonic development; errors in this process manifest as birth defects. Morphostasis (the maintenance of appropriate form) allows organisms to resist aging and tumorigenesis for decades while individual cells senesce or undergo DNA damage. The restoration of shape is a central goal of regenerative medicine – rebuilding a complex structure such as a hand or eye requires more than stem cells driven towards individual tissues – the placement of all components in the right arrangement is crucial and cannot be micromanaged by direct bioengineering. Groups of tissues that lack such overall global organization are teratomas – tumors with, for example, teeth or hair. Indeed, cancer has been described as a disease of geometry – a defection of cell groups from the normal patterning plan of the host [6,10–13]: cancer is the result of "an inexorable process in which the organism falls behind in its ceaseless effort to maintain order" [11]. Thus, the ability to understand and control shape in its most general form offers the opportunity to address a wide range of biomedical problems and restore complex structures damaged by injury, cancer, disease or age.

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future medicine part of fsg

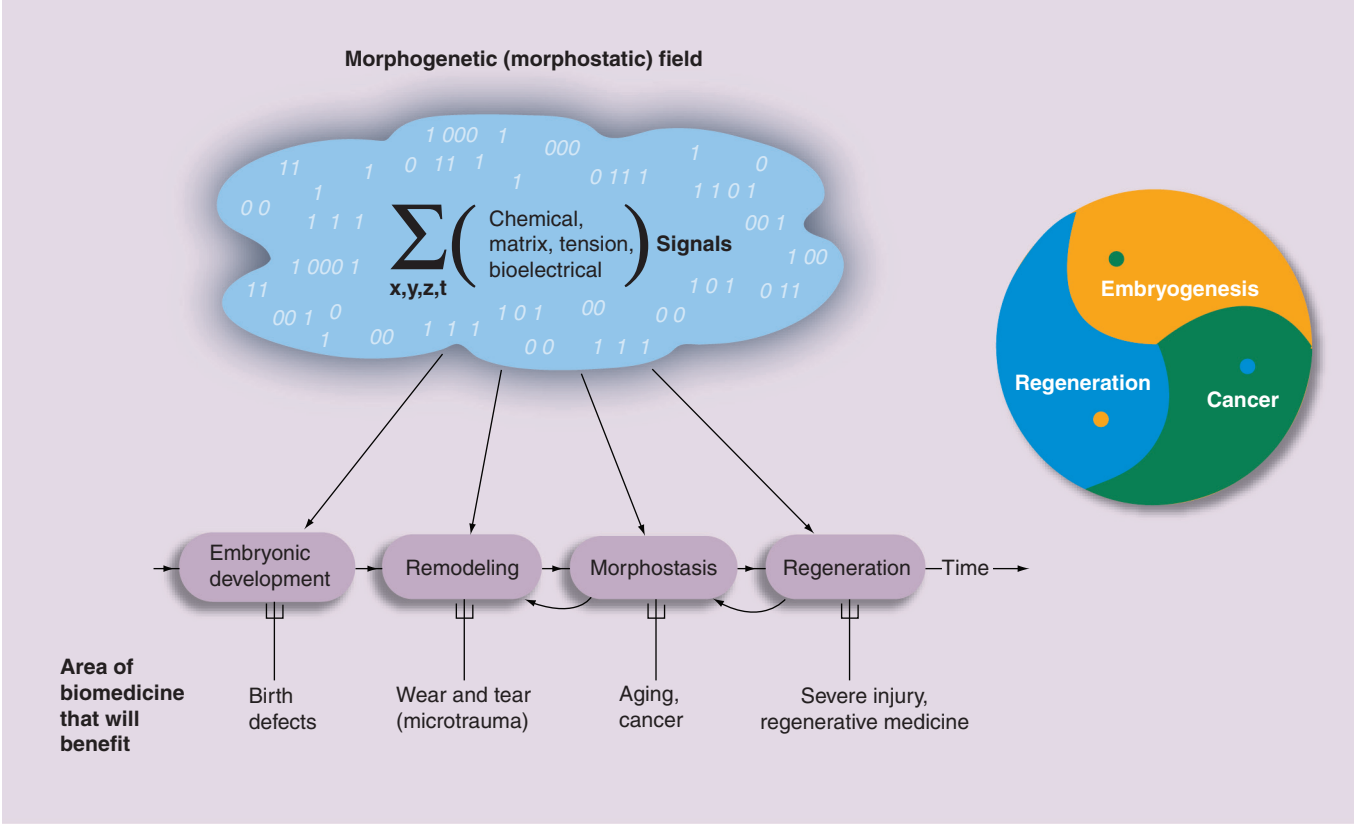

Figure 1. The morphogenetic field and pattern formation during the lifespan.

"Regeneration, development and cancer can all be seen as different aspects of the same fundamental question: how is the shape of complex large-scale structures specified, and what signals can be capitalized upon to integrate individual cell behaviors into a higher order pattern desired in biomedical repair?"

Regenerative biology seeks to explain how some organisms (e.g., salamanders) are able to perfectly restore entire limbs, eyes, jaws, hearts and portions of the brain. Reimposition of patterning information upon new cells after severe injury is a key goal of regenerative medicine. Planarian flatworms, complex organisms with bilateral symmetry and a true brain can regenerate any bodypart [14]; they show no evidence of aging at the organism level – while individual cells senesce and die, they are regenerated and the animal lives indefinitely. Even deer – a large, adult mammal – regenerate meters of bone and associated tissues when the same antler pattern is rebuilt year after year.

Regeneration, development and cancer can all be seen as different aspects of the same fundamental question: how is the shape of complex large-scale structures specified, and what signals can be capitalized upon to integrate individual cell behaviors into a higher order pattern desired in biomedical repair? This is a top-down view, focused on information flow (what do cells need to know in order to build or repair a structure? In what form and by what mechanism is the final morphology of any given organ or bodyplan encoded?) and distinct from the more popular bottom-up molecularly focused approach (what does protein X do?). The difference between these approaches has practical implications. For example, a focus on cell cycle controls and TGF-β signals leads to the prediction that cancer susceptibility and regenerative potential should go together: animals with ready access to plastic, highly proliferative cells should be prone to neoplasia and long-lived humans would be forever barred from powerful regenerative pathways because of the need to suppress cancer. Conversely, regeneration and cancer could be inversely related, as robust morphogenetic pathways necessary for regeneration would also keep cells within a coherent patterning plan and away from tumorigenesis.

In fact, the most highly regenerative animals tend to have the lowest incidence of cancer [15–18], suggesting a highly optimistic view of the potential for regenerative pattern control in human medicine. If a tumor is induced on the limb of a salamander and the limb is amputated through the tumor, the remaining cancer tissue becomes part of the newly regenerating limb. This readily illustrates the profound relationship between cancer and regeneration, and the importance of large-scale patterning mechanisms to what is often thought of as a cellular- or gene-level process. The current paradigm of killing cancer cells (which can often activate

undesired compensatory proliferation [19]) could be augmented by normalization or rebooting strategies [20] that reconnect the cells to the normal patterning signals of the host [17,21,22]. Developmental environments (loci of strong morphogenetic control) are known to reverse the cancer phenotype [23,24], although molecularly tractable models of the relationship between cancer and regeneration remain to be developed.

While developmental biologists are somewhat more inclined to try to explain and control higher order (systems-level) properties such as intercellular coordinate systems (positional information) and organ size determination [25,26], regenerative medicine and cancer biology are currently focused on the cell-level mechanisms of proliferation control, metastasis and differentiation. High-impact advances require understanding of how organisms exert patterning control on a large scale and the synthesis of molecular genetic data into information-based models of morphogenesis [27].

While much progress has been made in characterizing mechanisms operating at the site of injury, we largely lack understanding of remote signals that allow newly regenerated tissues and organs to be properly oriented, scaled and patterned with respect to the rest of the organism. When a planarian is bisected, the wound on the posterior half builds a new head, while the wound on the anterior half make a tail. Two completely different structures are formed by cells that, until the cut occurred, were sharing all aspects of the local environment. Thus, still poorly understood long-range signals allow the wound cells to know where they are located, which direction the wound is facing and what other structures are still present in the fragment and do not need to be replaced.

One interesting source and conduit for long-range patterning information is the CNS [28]. Denervated amphibian limbs do not regenerate [29], while injury to specific parts of the brain or spinal cord results in abnormal patterning [30] of regenerated appendages. The integrity of CNS connections near injured regions of planaria determines what structures are regenerated [31], and denervation of body regions causes disorganization of already existing structures [32] and promotes tumorigenesis [33,34], suggesting a role for the nervous system in morphostasis and *de novo* morphogenesis. Important advances in regenerative control are likely to come from understanding the contribution of the brain and nervous system as instructive patterning cues, not only as permissive trophic signals.

Exactly what is communicated and computed during morphogenesis has major implications for the design of biomedical strategies. The current paradigm holds that 3D pattern is emergent from the interaction of cells following purely local rules. By contrast, older models hypothesize that a map of the entire structure is available to each component. Much development of techniques and conceptual tools for understanding the encoding of large-scale order is needed. Inducing desired changes as a final outcome of a complex dynamical system (e.g., a patterning appendage) by manipulating low-level rules is an incredibly difficult 'inverse problem', because there is no way to determine how repeatedly executed cell regulation rules need to be changed to result in a particular desired patterning outcome. However, if a morphogenetic plan is more or less directly encoded, we will ultimately be able to provide external signals to activate or modify this plan in biomedical settings (e.g., change the shape of the face in the case of birth defect syndromes, or dictate the growth of needed structures after injury).

A 'target morphology' is the stable pattern to which a system will develop or regenerate after perturbation. Although not yet understood mechanistically, regeneration ceases when precisely the right size structure has been rebuilt, indicating a coordination of local growth with the size and scale of the host. However, morphostatic mechanisms require the body to process more information than simply size metrics. Consider what happens when an amphibian tail blastema is grafted to the side of a host animal. A tail results at first; however, over the subsequent few months, this tail is reshaped into a limb, illustrating that the control of local regions' fate is integrated into the large-scale morphology appropriate to the host animal even if their structure has to be remodeled [35]. In trophic memory in deer [36], antler injuries (if made in the absence of CNS-inhibiting anesthetic) result in ectopic tines growing at that same spot in several subsequent years (after the whole rack is shed and regenerated). The growth plate at the scalp senses the position of damage within the branched structure, remembers this location for several years and uses this information to guide cell growth towards the corresponding change in the regenerated appendage. Similarly, recent molecular work on the role of gap junctional communication in planarian regeneration [37] revealed that a transiently induced change in the physiological (not genetic) state of cells can permanently reset the target morphology:

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two-headed planaria produced by a 48-h inhibition of gap junctional communication during regeneration will subsequently continue to regenerate as double-headed forms through multiple rounds of regeneration with no more exposure to gap junction blockers. Thus, the target morphology can be permanently changed, and is stored at least partially in the dynamic patterns of direct small molecule exchange among cells. These fundamental results highlight a tremendous and now realistic opportunity for the field to dissect the mechanisms of morphogenetic memory.

What physiological processes could encode pattern, and thus be capitalized upon for restorative therapies? Steady-state endogenous ion currents, voltage gradients and electric fields are produced by ion channel and pump proteins, and control orientation and positioning of migratory cell types, differentiation of muscle and nerve progenitor cells into mature tissues, and proliferation rates of neoplastic cells [38–42]. Bioelectrical activity is important for determination of fate and other key properties in a stem and progenitor cells [43–46], including adult human mesenchymal stem cell [47,48] and induced pluripotent stem cells [49]. More generally, low voltage gradients demarcate stem, cancer and embryonic cells, while hyperpolarized potentials belong to mature, highly differentiated somatic cells [50]. Using fluorescent voltage-reporter dyes and targeted misexpression of well-characterized ion transporters [51–53], the instructive signaling roles of transmembrane voltage gradients have been linked with downstream molecular genetic effector pathways such as redistribution of signaling molecules, integrin pathways, phosphatase cascades and chromatin modification [42,54]. These data suggest exciting applications in the noninvasive imaging and control of many important cell types. For example, mature CNS neurons can be driven to re-enter mitosis by sustained depolarization [55], and even the long-distance electrical properties of host tissue play a role in neoplastic transformation [56,57]. More importantly, such bioelectrical signals are also crucial determinates of shape during patterning processes, such as determination of visceral organ positioning in vertebrate embryogenesis [58], amphibian tail regeneration [59], anterior–posterior anatomical polarity of regenerates in planaria [60] and craniofacial patterning during vertebrate embryogenesis [61].

While traditional electric field applications are being used in the clinic (e.g., spinal cord injury [62]), novel molecular-level techniques have shown endogenous voltage gradients to serve as 'master regulators' – simple signals that activate complex, highly coordinated downstream patterning cascades. For example, even when older animals are amputated, resulting in nonpermissive (scar-like) wound epithelium, treatment with a sodium modulator cocktail for just 1 h induces the regeneration of a normal tail (complete with spinal cord, musculature, peripheral innervation and vascular system) [63]. Given the complementary nature of cancer and regeneration discussed previously, it is not surprising that several ion transporters are now recognized as oncogenes [64,65], while ion channel drugs are being tested as cancer treatment modalities [66]. Advances in understanding the role of bioelectric isolation in carcinogenesis will have direct bearing on induction of regenerative repair by pharmacological and genetic modulation of ionic patterning cues.

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Numerous transformative applications await tools for the precise characterization and control of physiological state; novel physiomic techniques are needed because bioelectrical states are controlled post-translationally and are, therefore, invisible to popular proteomic/mRNA profiling strategies. The exciting recent developments of optogenetics (control of ion channels by means of light signals) [67] must be extended beyond ultra-fast spike generation in nerves and muscles to the control of long-term voltage properties of nonexciteable cells. Incorporation of light-emitting elements into scaffolds and bioreactors will enable unprecedented levels of control over cell fate and growth rates for bioengineered constructs *in vitro* [68] and for regenerative sleeves used for organ regeneration *in vivo* [69]. Similarly, the known aberrantly low voltage potential [50,70–72] (and the ability to disrupt long-range electrical properties of the host [73]) of tumor cells can be exploited for novel target drug delivery vehicles [74], while their bioelectric signature [75] can be used to detect cancer cells and tumor margins using noninvasive fluorescent ion-reporter dyes *in vivo* [76].

A major conceptual challenge holding back progress is the lack of a bioinformatics of shape. Despite excellent tools for working with gene sequences and networks, no accepted formalism exists for linking the ever-growing deluge of high-resolution functional molecular pathway data to the shapes (and shape-regulatory properties) encoded by these data. The kinds of 'model' figures that commonly appear in published papers represent connections among

genes or proteins but such network diagrams do not reveal the shape they encode. It is impossible to know what geometry will result from such a pathway diagram, or whether the cell behavior that is encoded by the model gives rise to a self-repairing structure. Results of molecular perturbations that alter shape cannot readily be entered into a searchable database (e.g., the National Center for Biotechnology Information), nor is there any standardized way of simulating a pathway model to know whether or not it produces the shape to be explained or controlled. "There is an obvious discrepancy between the single-cell genetic input and the multicellular geometrical output" [77]. True control over biological patterning will involve the development of computerized tools that enable algorithmic or generative models of patterning [78–81] (showing, at each step, how cells make the decisions that guide their behavior), and help biologists to infer stable candidate models from complex molecular and functional datasets. Only then will it be possible to know what signals to provide and when, to achieve the desired change in organ pattern or cell behavior *in vivo*.

Conclusion

Creation and maintenance of correct patterning of tissues and organs is the cornerstone of health; many biomedical interventions ultimately entail an attempt to restore the body's 'goal state' with respect to shape. This means we must learn to understand the key aspects of the morphogenetic field that controls pattern formation, including its biochemical [82], bioelectrical [83,84], physical [78] and planar polarity [85] aspects, as well as develop technology to facilitate the organism's use of this information during repair. Fortunately, theoretical tools and molecularly tractable model systems are now within reach.

Heroic piecemeal interventions that address individual failing systems to extend the last years of a body are fundamentally a strategy of patching up a sinking ship. The cost of increasingly more complex interventions required for each success (increasing the number and age of individuals in need of the next patch) is a positive feedback loop that no society can afford in the long term. Regenerative medicine promises a break from this cycle, by activating programs for continuous organ repair that already exist within the host. By learning the most profound tricks of existing model species (which clearly show that perfect regeneration throughout the adult lifespan is possible for complex animals), we will ultimately revolutionize the concept of health and treatment of disease.

Dedication

This paper is dedicated to Alexander G Gurwitsch – a pioneer of the morphogenetic field concept.

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