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Developmental engineering: Design of clinically efficacious bioartificial tissues through developmental and systems biology.

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Abstract

A major transition has been recently realized in tissue engineering towards the acceleration of clinical translation with the exploitation of the mechanisms of in vivo tissue development that can assure the formation of authentic, complex tissue structures (developmental engineering). The use as implants of intermediate developmental tissue forms, developmental modules that can autonomously progress in development and be transformed to mature tissue forms has improved the clinical efficacy, and equally important gave the ability for its prediction. Interestingly, critical concepts for the properties of developmental modules that are now studied in systems biology were developed by ancient philosophers with impressive subtlety and prescience.

Keywords

Tissue engineering; clinical efficacy; developmental biology; systems biology; developmental engineering; complexity; modularity; autonomy; networks

“We work with being, but non-being is what we use.”

Lao Tzu, Tao Te Ching

The creation of a new research field: Fung’s idea on engineering tissues.

Tissue engineering emerged three decades ago as a distinct research field with the goal to radically change the clinical practice by fabricating in vitro cell-biomaterial assemblies, bioartificial tissues, that could be used as biological tissue substitutes to restore, maintain or improve the functions of diseased or damaged tissues and to eventually solve the problem of organ shortage for transplantations. It was proposed as a new discipline in 1985 by Yuan-Cheng Fung, born in Jiangsu Province, China, Professor of the University of California at San Diego (UCSD) and founder of modern biomechanics, who also coined the term “tissue engineering” (Viola et al., 2003). In a proposal to the National Science Foundation (NSF) for the establishment of a research institute in UCSD entitled “Center for the Engineering of Living Tissues”, Fung recognized the gap of knowledge between the level of organs, studied

by physiologists and physicians, and the level of cells, studied by cell biologists and biochemists. He proposed that “a clear understanding of phenomena at the tissue level is a prerequisite to the engineering of tissues” (Viola et al., 2003). In the following years, Fung reiterated his unconventional for that time idea to an NSF meeting and finally motivated NSF to recognize tissue engineering as a distinct research field in 1988. Thanks to the official recognition, funding from governmental organizations ensued which allowed rapid and organized progress in the field. At the same time, the achievement of the groups of Robert Langer (MIT) and Joseph P. Vacanti (Harvard Medical School) to grow cells inside three-dimensional porous biomaterials, scaffolds, signaled the inception of methodological research activities in tissue engineering (Vacanti et al., 1988). The pore network of scaffolds resembles the body’s vein network, cells attached on the pores are in contact with medium that fills them which assures cell access to nutrients and oxygen and the removal of toxic metabolic by-products.

The challenge of tissue engineering: The lack of life of bioartificial tissues.

Despite the intense research efforts until now, the clinical translation of tissue engineering was not to the expected extent because the field still lacks a complete understanding of the phenomena taking place at the tissue level related with the cell organization to tissue structures with tissue functions, which Fung had proposed as its basis. On the other hand, the worldwide intensification of cell and tissue therapy clinical trials has raised concerns for the appropriate state of cell-biomaterial constructs implanted to patients that can assure clinical safety and efficacy that urgently necessitate an answer. The name that Fung had chosen for the research center in UCSD, referring to “living tissues”, points directly to the major deficiency of bioartificial tissues that limits their clinical efficacy, i.e., the lack of life as integral, coherent wholes. According to systems biology, living systems (e.g., tissues) are not a collection of unrelated component parts (e.g., cells) but they exhibit novel functions at the system level arising from the interaction of their parts (Editorial, 2005). For example, the functions of the liver tissue (e.g., glucose homeostasis), are not exhibited at the hepatocyte level but are novel, global, collective functions appearing at the level of the tissue. They arise from the metabolic cooperation of, unequally distributed in the liver lobules, hepatocytes that perform different but complementary metabolic functions (glycolytic and gluconeogenic) (Jungermann and Sasse, 1978). Such functions are not exhibited by bioartificial tissues because the cell cooperation required for their appearance must be spatially coordinated as in the authentic tissue structures that however are difficult to be recreated in biomaterial cell supports. Such properties, self-established from interactions among tissue cells, are characteristic of complex (and not complicated, i.e., many non-interacting components) systems (Editorial, 1999). Aristotle gave the first definition of a complex system as one in which its collective properties make the whole system something more than the sum of its parts (Aristotle, 1924). Accordingly, the liver (whole) is more than the sum of hepatocytes (parts) because it exhibits novel functions at the tissue level that are absent at the hepatocyte level.

The missing property of bioartificial tissues: Chuang Tzu's idea of modularity.

At the same time with Aristotle, the Chinese philosopher Chuang Tzu elaborated a critical characteristic of complex systems which is their modular organization from distinct, integral and coherent, subunits-modules (Chuang Tzu, 1968). In the story “Cook Ding Cuts Up an Ox”, Chuang Tzu referred to a conversation between a prince and his cook. The prince was impressed by the perfection and rapidness of separation of the meat of a sacrificed ox by the cook and asked him how he could achieve it. The cook answered that the body of the ox is composed from coherent parts that can be easily separated while any other separation across these parts is difficult because of their coherence. Not only adult tissues but also intermediate forms during tissue development, such as limb buds, tooth germs, cell condensations during embryogenesis or bone formation, and the growth plate (figure 1), called “developmental modules”, have the global property of coherence, integrity or wholeness, as Chuang Tzu had described it, that is reflected in the robustness of their structure against environmental disturbances (Bolker, 2000). The story of Chuang Tzu is further particularly revealing for developmental biology. When the prince asked the cook how he learned to accomplish this task, the cook replied that he relied on his intuition by experience and not on any kind of taught rules. Similarly, in the absence of a theory of development, there has not been yet any formal definition of developmental modules. Researchers use, as the cook in Chuang Tzu's story, various operational descriptions depending on the particular experimental system (Bolker, 2000).

Developmental modules, as mature tissues, are live entities exhibiting global, collective properties at the multi-cellular level of the whole module. Such a property is their ability to progress with (high semi-) autonomy along defined developmental pathways leading to the formation of complex tissue structures even ectopically. For instance, the cartilage template in endochondral ossification during axial skeleton and long bone development is evolutionary pre-disposed to be transformed to bone providing the required osteoinductive and angiogenic signals (figure 1). According to studies of systems biology, the autonomy of developmental modules is due to the autonomous dynamics of gene/protein interaction networks established throughout their cells from cell-to-cell interactions that, without external factors, activate the necessary signaling pathways for the establishment of morphogenetic patterns that lead to tissue formation (von Dassow et al., 2000).

New developments in tissue engineering: Making use of the autonomy of developmental modules.

The concepts of ancient philosophers related to the complexity of living systems have recently reached tissue engineering. In a workshop sponsored by the National Institutes of Health (NIH), the tissue engineering research community has officially proposed as a new research direction in the field the recapitulation of in vivo developmental mechanisms in bioartificial tissue formation in order to make use of the inherent capacity of cells to self-organize to complex tissue structures (Kaplan et al., 2005). In this new research line, developmental modules, because of their robustness and autonomy, have been proposed as

the prime targets of tissue engineering and their design rules have been analyzed in a new methodology named “developmental engineering” from the point of view of developmental biology (empirical concepts of development, modularity, robustness, autonomy (Lenas et al., 2009a)), as well as of systems biology (properties of gene interaction networks of developmental mechanisms that explain the empirical concepts (Lenas et al., 2009b)). The autonomous progression of developmental modules along developmental pathways has been recently validated in tissue engineering applications with clinically-relevant cell sources. For instance, instead of the commonly used method of direct differentiation of mesenchymal stromal cells (MSCs) to osteoblasts for fabrication of bioartificial implants for long bone defects (resembling intramembranous ossification that forms the craniofacial skeleton), a developmentally relevant approach has been attempted. In this approach, MSCs differentiate first towards the chondrogenic pathway forming a cartilage template which is subsequently replaced by bone, to emulate indirect bone formation in endochondral ossification. Indeed, human MSCs undergoing chondrogenic differentiation in vitro on biomaterials operated as developmental modules when implanted ectopically, by triggering autonomously (in the absence of instructive external signals) the whole phenomenon of endochondral ossification providing all necessary signals for bone formation, vascularization, remodeling, and establishment of a functional bone marrow (Scotti et al., 2013). This study shows that what has to be engineered in vitro in implants is not a fully developed tissue. Implants have to reach in vitro a stage that will allow them to autonomously proceed in development after implantation. The incorporation of the capacity of developmental modules to progress along established developmental trajectories into bioartificial implants not only improves clinical efficacy by installing in situ natural developmental processes of tissue formation but it also allows predictions of clinical efficacy based on information from developmental biology. Therefore developmental engineering is bound to bring a radical improvement in clinical translation and it has been characterized as an important milestone in the field (Martin 2014).

The future of tissue engineering: On Lao Tzu’s path for the use of ‘non-being’ of developmental tissue forms.

The re-focus of tissue engineering from the final tissue forms to intermediate in development modular ones, that possess the potential (“entelechy” according to Aristotle (Aristotle 1924)) of transformation to these forms, is also a striking example of the applicability of the philosopher Lao Tzu ideas to natural phenomena. According to Lao Tzu a pot is useful for its emptiness (Lao Tzu, 1963). Similarly, developmental engineering, makes use of the “non-(yet)-being” though it works with the “being”, i.e., cells, biomaterials and growth/differentiation factors. The use of developmental modules as implants requires the isolation and characterization of developmental modules and their properties in a variety of tissue systems. Ancient philosophy can assist in this endeavor too. As seen in figure 1, the transition through developing tissue forms during endochondral ossification, similarly to cell fate conversions during development (Zheng et al., 2014), seem to follow the process of differentiation from unity to multiplicity in nature described by Lao Tzu, as a generation of two entities from one, then generation of three by these two, and finally of all the remaining forms (Lao Tzu, 1963). This intriguing concept deserves further analysis, because it can

provide a physiological way to distinguish stages and developmental modules in tissue development that when identified can revolutionize clinical translation.

Though the use of developmental modules as implants has been proposed for several tissues, it is, at present, limited because the knowledge of the modular organization of developing tissues is not sufficient, especially for human tissues. A critical point, therefore, has been reached where the insufficiency of scientific knowledge required for this type of new engineering design calls for a substantial contribution of tissue engineers to developmental biology (Fung and Tong, 2001). The determination of modularity in biology involves the isolation of the putative developmental module and observation of the process in the absence of surrounding co-developing tissues and therefore it cannot be applied to human tissues (Bolker, 2000). However, the modularity of human developing tissues can be studied with the use of human pluripotent stem cells in the in vitro three-dimensional culture systems developed in tissue engineering. The resulting organoids offer a versatile tool to dissect the formation of human developmental modules as well as tissue-tissue interactions, when multilineage differentiation takes place (Clevers, 2016). The identification of developmental modules is therefore a central research theme for both tissue engineering and developmental biology, and argues for collaborative research activities which will overcome the clinical limitations of bioartificial tissues.

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Figure 1. Stages of endochondral ossification.

a. Mesenchymal condensation. Aggregation of a disperse population of mesenchymal stromal cells takes place through juxtacrine and paracrine interactions. Because vascular regression is a pre-requisite for condensation, condensates progress autonomously in development. **b. Chondrocyte differentiation.** The tissue entity formed in the previous stage (condensate) gives rise to two new ones (chondrocytes, perichondrium). Chondrocytes secrete a matrix forming the cartilaginous template of the future bone. Cells at the periphery of the condensation form the perichondrium which demarcates the developing tissue from the surrounding mesenchyme. **c. Chondrocyte maturation.** The two previously formed entities (chondrocytes, perichondrium) give three new types of progressively maturing chondrocytes (pre-hypertrophic, hypertrophic, and terminally hypertrophic that undergo apoptosis). Degraded chondrocytes leave behind cartilage matrix that serves as a scaffold for further bone matrix deposition. Hypertrophic chondrocytes are osteoinductive and angiogenic setting the stage for subsequent developmental events. **d. Cartilage vascularization.** The full spectrum of processes for the replacement of cartilage by bone takes place with the induction of vascular invasion by hypertrophic chondrocytes. Blood vessels bring in the space cartilage- and bone-matrix resorbing cells as well as osteoprogenitor cells that lay down bone matrix on the partially-degraded cartilage matrix.

This structure, in the long bones of mammals, divides in two areas of parallel columns of progressively more mature chondrocytes (growth plate), from which the bone formation toward the two directions of the cartilage template long axis takes place. Growth plate is a dynamic structure that continuously provides dying hypertrophic chondrocytes that are replaced by osteoblasts and bone matrix leading to bone elongation. It exhibits structural stability because the rate of chondrocyte differentiation, and consequently bone elongation, is timed (through a negative feedback loop of Indian hedgehog (Ihh)/parathyroid hormone-related protein (PTHrP)) with other processes of matrix secretion and organization that provide structural support to chondrocyte columns. The characterization of the properties of developmental modules appearing in endochondral ossification will allow for the fabrication of bioartificial analogues as implants for bone defects. Markedly, it seems that the generation of tissue forms in endochondral ossification follows Lao Tzu's concept "One produced Two, Two produced Three, Three produced All things" which may assist in the detection of modular tissue forms in various tissue systems.