25 Y(ears) On: The Present and Future of Tissue Engineering

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In 1997, a segment of the BBC docu-series *Tomorrow's World* aired footage that could have been mistaken for a clip from a Cronenberg film: a white-coated scientist handling a hairless mouse with what looked to be a human ear growing from its back. The "Vacanti Mouse," named for the physicians Charles and Joseph Vacanti, wasn't some miraculous fusion of genetic engineering and developmental biology – it was a rudimentary, yet innovative step in the nascent field of tissue engineering (1).

Tissue engineering as a concept had been around for some time by this point, but the rapid proliferation of images of the Vacanti Mouse undeniably cemented it within the popular imagination. Robert Langer and Joseph Vacanti's seminal 1993 paper in *Science* defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" (2). This definition hints at the central clinical shortage that spurred the birth of the field: healthy tissue and organs for those in need of grafts or transplants. What if there were off-the-shelf solutions to regrow failing articular cartilage in patients with osteoarthritis, or replace compromised skin in burn victims? **Even more ambitiously, what if we could grow entire lungs, livers, kidneys, or hearts de novo in the lab for patients in need?**

Most approaches to tissue engineering in the early aughts adopted a "top-down" approach, in which macroscopic scaffolds consisting of polymers or decellularized material were seeded with cells to recapitulate a mature tissue. This approach follows the mantra of "cells, scaffolds, and signals", in which cultured primary cells are coaxed to take on tissue-specific behaviors through extracellular matrix-mimicking structural blueprints (scaffolds) and a combination of physical, chemical, and electromechanical cues (signals). Indeed, the Vacanti Mouse's signature dorsal ear was little more than a molded scaffold of PLGA (poly(lactic co-glycolic acid)) seeded with bovine chondrocytes and implanted under the skin of an athymic host animal (1). Despite their relative simplicity, top-down approaches have yielded several FDA-approved therapies using biomaterials with or without the addition of exogenous cells for use in burn wounds, bone grafts, cartilage patches, and peripheral nerve repair (3-6).

Despite these successes, the top-down approach to tissue engineering is inherently limited by its differences from the carefully coordinated developmental processes that form tissue *in vivo*. Seeding cells and cytokines into a scaffold circumvents the orchestrated co-development and co-maturation of the cell populations comprising adult tissue, and so these constructs generally lack innervation, vascularization, and higher-order functions. While advances in 3D printing technologies have yielded a newer generation of tissue constructs with increasing levels of hierarchical complexity and predesigned vasculature for nutrient supply, **these techniques can be likened to trying to build an oak tree from wood, roots, and leaves instead of planting an acorn (7).**

These limitations gave rise to "bottom-up" tissue engineering, which seeks to use the guiding principles of developmental biology to generate macroscopic tissues through the modular assembly of micro-scale scaffolds and progenitor cell populations into discrete functional units of tissue (8). The fundamental difference from top-down tissue engineering is the attempt to recapitulate embryonic tissue morphogenesis, instead of skipping to the adult stage. These approaches were made feasible by the invention of induced pluripotent stem cell (iPSC) technology by Yamanaka et al. in 2007 and became increasingly sophisticated after the commercialization of CRISPR-based gene editing a few years later. Coordinated differentiation of pluripotent or multipotent cell populations by treatment with morphogens and genetic modifications represents a far more straightforward approach to generating heterogeneous functional tissues with complex populations of parenchymal, stromal, vascular, nervous, and immune cells.

While bottom-up approaches towards large-scale tissue replacements are still in their infancy, this paradigm shift towards capturing developmental morphogenesis has led to an explosion of microtissue and organoid-based models, often termed "organ-on-chip" systems. In just over a decade, these technologies have been adapted for a dizzying number of clinically salient research questions including high-throughput screening of cardiac drugs, modeling of tumor metastasis, and mechanistically defining viral infections (9-11). Organoid-based systems are also beginning to emerge in clinical trials databases for applications such as pancreatic islet transplantation and patient-specific testing of chemotherapeutic regimens (12-13). In fact, regulatory agencies have begun establishing standards for organoid research within the last few years as they become increasingly important adjuncts to animal-based disease modeling (14-15).

At present, the most pressing issues facing the generation of large-scale tissue and organ constructs can be divided into two general areas: complexity and scale.

Looking ahead into the next 25 years, I expect to see a convergence of top-down and bottom-up tissue engineering approaches that capitalize on the advantages of each approach to clear these respective hurdles.

First, the micro-scale hierarchical complexity of functional tissue continues to limit the size of most organoid models. Without a functioning vasculature to supply oxygen and nutrients and remove waste, organoids can typically only be sustained at scales of hundreds of microns. Fully-vascularized and perfusable tissue constructs have long been considered the holy grail of tissue engineering, and recent reports of vascular networks and vascularized organoids show a great deal of promise for solving this problem in the coming years (16-17). In terms of parenchymal function, progress in bottom-up tissue engineering has also made great strides in identifying tissue-specific combinations of morphogenic and temporal signaling present during embryonic development, allowing for

defined culture systems to generate functional organotypic subunits (18-20). The next set of challenges will involve determining how to induce further maturation: for example, iPSC-derived cardiac organoids that beat with the strength and regularity of the adult heart, or liver organoids that can produce bile, detoxify culture medium, and carry out their metabolic roles in parallel.

Second is the issue of scale. Concerted efforts in a laboratory setting can generate thousands of organoids for high throughput-experiments, or tens of macroscopic 3D-printed grafts for characterization and animal testing, but traditional monolayer cell culture is incredibly inefficient, especially considering the hundreds of billions of cells comprising an adult-sized human organ. In this arena, we will likely see widespread adoption of industry-style bioreactors for the mass expansion of human cells at cGMP standards. Several groups are tackling both problems at once, leading the charge towards the next generation of tissue engineering by using sophisticated, multi-nozzle 3D printers loaded not with individual cells, but with suspensions of tissue-specific organoids in order to print functional, vascularized subunits that can readily integrate with their neighbors (21-23). Similarly, the use of benchtop bioreactors to massively scale up organoid generation and differentiation is a first step towards whole-organ printing (24).

Just over a quarter-century ago, our most cutting-edge tissue engineering techniques relied on cartilage cells taken from cows, loaded into a primitive mold of an ear, and awkwardly saddled to the back of a mouse host to keep it alive. **Today, we can 3D print bespoke tissue grafts and generate organoids using a patient's own cells to model their disease.** Insights into developmental biology and morphogenesis, paired with efforts to massively scale up the biomanufacturing of human cells, mean that we are hurtling towards a future in which we can generate functional transplants without the need for long organ registries or lifelong immunosuppression.

There are no fundamental technological barriers that have yet to be overcome in the same way that the invention of iPSCs overcame the problem of cell sources – but there are great strides to be made in efficiency and sophistication. By 2050, we may not quite be at the point where industry representatives are present in the OR helping transplant surgeons select appropriately sized off-the-shelf hearts or lungs, but at the current rate that the field is developing, we can hope to at least see FDA approvals or late-stage clinical trials for engineered, transplantable hepatic lobules, cardiac patches, and renal pyramids.

Oh, and even better engineered ears (25).

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