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A perspective on the clinical translation of scaffolds for tissue engineering

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Abstract

Scaffolds have been broadly applied within tissue engineering and regenerative medicine to regenerate, replace, or augment diseased or damaged tissue. For a scaffold to perform optimally, several design considerations must be addressed, with an eye toward the eventual form, function, and tissue site. The chemical and mechanical properties of the scaffold must be tuned to optimize the interaction with cells and surrounding tissues. For complex tissue engineering, mass transport limitations, vascularization, and host tissue integration are important considerations. As the tissue architecture to be replaced becomes more complex and hierarchical, scaffold design must also match this complexity to recapitulate a functioning tissue. We outline these design constraints and highlight creative and emerging strategies to overcome limitations and modulate scaffold properties for optimal regeneration. We also highlight some of the most advanced strategies that have seen clinical application and discuss the hurdles that must be overcome for clinical use and commercialization of tissue engineering technologies. Finally, we provide a perspective on the future of scaffolds as a functional contributor to advancing tissue engineering and regenerative medicine.

Keywords: stem cells, scaffolds, engineering constraints, FDA approval, entrepreneurial biotechnology, material properties

1. Introduction

The challenges presented by tissue loss and organ dysfunction inspired the emergence of the field of tissue engineering as a means of applying a basic engineering approach to restore form and function to diseased, damaged, and lost tissue. From its beginnings, tissue engineering has included a scaffold component. The substance of this scaffold has ranged from naturally derived components to synthetic biodegradable polymers. Next generation approaches to scaffold design have incorporated signaling elements, provided tunable and dynamic mechanical properties, enabled control via external stimuli, and demonstrated new techniques for precise patterning of

spatial and geometric properties. Tissue engineering is subject to various design constraints that demand properties including mass transport, scaffold degradation, and biocompatibility be considered in scaffold design. The manufacturing of scaffolds for these purposes must also occur on a clinically relevant timeline, with appropriate assurances of safety, sterility, and stability, and be cost-efficient. As scaffolds are developed for tissue engineering applications, a delicate balance must be struck between efforts for highly engineered “smart” and “superfunctional” scaffolds and robust, scalable, and affordable production methods, with additional assurances for safety. Success for the field of tissue engineering is also reliant on advances in other disciplines, as seemingly disparate fields such as stem cell biology, materials science, robotics, medicine, chemical engineering, and manufacturing must be leveraged for design, production, and integration of functional tissue constructs.

When designing scaffolds for tissue engineering, the tissue to be augmented or replaced often dictates its form and function. Scaffolds must be designed with an appreciation of the chemical and material properties of native tissue, as well as an understanding of complex mechanisms surround cell interaction. The extracellular matrix (ECM), which forms the basis of native tissue scaffolds, has been increasingly appreciated as a bioactive entity that directs the fate and function of cells in both direct and nuanced ways.[1] The composition, density, nanostructure, and microstructure of ECM varies dramatically between different tissue types. As such, recapitulating the complex mechanical and biological roles of native ECM inspires the generation of both synthetic and naturally derived scaffolds for tissue engineering.

In terms of the clinical applications of tissue engineering, planar tissues and organs that act in a barrier or transport role, such as skin, bladder, cornea, trachea, and blood vessels, have seen the most progress.[2-7] The engineering of connective tissues, such as bone and cartilage,[8] as well as nervous tissue,[9] and muscle, have also been demonstrated in a variety of preclinical and clinical evaluations, and each have their own requirements for scaffold design. The ability to expand this repertoire to include the engineering of complete organs for applications in transplantation remains

a vision for the future of tissue engineering. Complicated and hierarchically organized tissues and organs, including multifunctional systems like liver, kidney, heart, and pancreas, still present a significant challenge at present. Scaffolds and tissue engineered constructs have been evaluated clinically in the context of replacing specific functions of these organs, such as hydrogels that facilitate survival and immunoisolation in transplantation of pancreatic islets,[10] along extracorporeal hepatocyte constructs to support detoxification in patients with acute liver failure.[11] A less distant vision could involve building interconnected function-specific modules to recreate bioartificial organ systems, which could replicate the function, if not the specific form, of complex organ systems. However, a scaffold for a completely tissue engineered organ requires spatial control over different tissue types or tissue layers, and incorporation of perfused vascularization to promote tissue health and function. Emerging approaches toward "self-organization" of tissues may alleviate some of the complexity in engineering a complete organ through cell-based direction of tissue formation with minimal manipulation.

This perspective will highlight the engineering challenges and emerging technologies in the area of scaffolds for tissue engineering. We will outline the engineering design constraints for scaffold development, along with progress toward the development of engineered "smart" and "superfunctional" (i.e. materials with multifaceted biological function) scaffold technologies. Finally, we hope to provide insight into the clinical translation of technologies rooted in tissue engineering, including lessons that have been learned by our group and others along the way, the hurdles that emerge on the road to translation, and the path forward for promising technologies in development.

2. Design Consideration and Engineering Constraints

When approaching a problem in tissue engineering, it is important to maintain an engineering mindset. This entails an understanding of the problem to be solved, the range of solutions available, and the unique manufacturing and economic considerations associated with the problem. These constitute the design constraints of an engineered system. For tissue

engineering, an appreciation of the chemical and mechanical properties of a tissue construct, its interaction with host tissue in the body, the mass transport requirements for the tissue, and the requirements for manufacturing a construct in an economical way are all important. In this section, we will detail these design constraints and highlight some considerations that a tissue engineer should remain mindful of when developing a tissue engineered construct.

2.1 Chemical, mechanical, and material properties

In designing a scaffold for tissue engineering, it is critical to understand the material properties of native tissue. Each basic tissue type serves a unique function and purpose which can be attributed in large part to its specific chemical and material composition. With materials, function follows form. Thus, emulating the mechanical and chemical characteristics of native tissue could improve the performance and integration of the scaffold in its biological role. Connective tissue, which includes bones, cartilage, and tendons, provide structural support to the body and tends to have a low cellular content relative to other tissue types. Muscle tissue, responsible for dynamic movement, includes skeletal, smooth, and cardiac muscle and subtle differences between these three types give rise to different functional roles. Epithelial tissue, including that of the skin, lungs, intestines, gastrointestinal tract, and endothelium of blood vessels, is cellularly dense with tight cell junctions that are critical in providing structural support and serving a barrier function to protect the underlying tissue and control transport of gases, nutrients, and waste across the barrier. Nervous tissue is responsible for transmitting electrical signal in response to stimuli and for functional control of muscle tissue. Since native tissues are characterized by a wide array of functions, compositions, and characteristics, the assumption that the same materials can be applied to the task of tissue engineering for all tissue types is impractical. We should therefore strive to consider the complexity and chemistry of these native tissues when selecting and developing new materials for tissue engineering.

In engineering a synthetic tissue scaffold, mimicking the broad chemical diversity of native tissue is a synthetically rigorous endeavor. The ECM, which is the main scaffold of native tissue, is

comprised of structural biopolymers, namely proteins, polysaccharides, glycoproteins, and proteoglycans. Proteins, specifically, are the major structural component of native scaffolds in the body. They are composed of amino acid building blocks linked together through peptide bonds. Filamentous protein fibrils, including collagen, keratin, and elastin, provide tissues with structure, guide cell morphology, and help to protect cells and tissues. Cytoskeletal proteins, such as myosin and actin in muscle, provide tissue elasticity, enable contractility, and facilitate cell motility and mitosis. Within proteins, covalent crosslinks can occur through the side chains of amino acids that can increase the stability and durability of structural proteins, such as through the formation of disulfide bonds via cysteines and imines via lysine by lysyl oxidase.[12] However, much of the structure and function of proteins arises from non-covalent interactions such as hydrophobic interactions, metal chelation, and hydrogen bonding. Hydrogen bonds within the peptide backbone of proteins facilitate complex and organized protein structure and enable specific function. In particular, collagen is a key structural component of native tissue. It is, therefore, not surprising that collagen has been routinely used with great success as a scaffold in tissue regeneration. Chemically, the sequence of collagen contains many domains of repeating tripeptide blocks rich in proline, hydroxyproline, and glycine. The positioning of the glycine and constrained backbone from the proline and hydroxyproline residues allows the protein to take on a characteristic left-handed helix,[13] which spontaneously self-assembles into a right-handed triple helix with other collagen molecules,[14] known as the collagen triple helix. While the collagen triple helix itself governs the structural and mechanical properties of many tissues, composites of collagen with other components can further enhance its mechanical properties. For example, in bone an inorganic material, hydroxyapatite (HAP) is integrated within the fibrous collagen structure, while in cartilage glycosaminoglycans (GAGs)[15] form composites with collagen. Composites of proteins with inorganic or other polymeric components are, therefore, important to the chemical and mechanical properties of a tissue.

The overall chemical composition in native tissue can vary dramatically depending on tissue type and tissue site. It is important that a tissue engineer remain mindful of the composition of native tissue when developing strategies to engineer new tissues or augment existing ones. Native tissue is comprised of varying amounts of water (10-75%) with the dry mass being mostly collagen (40-60%). Connective tissues can contain GAGs (25% in cartilage) and HAP (80% in bone).[16, 13] Myelin, the supporting tissue in the nervous system, is comprised of water (40%) with the dry balance being 75% lipids and 25% proteins.[17] Epithelial tissue is a dense network of cells, connected by keratin-rich junctions assembled on top of a basement membrane matrix of collagen, glycoproteins, and proteoglycan fibers. Sarcomeres, the basic units of contractility in muscle, are comprised of fibrous proteins actin and myosin.[18] The ratio of actin to myosin is between 2:1 and 10:1 in smooth muscle, compared to 6:1 in skeletal muscle and 4:1 in cardiac muscle.[18] In striated muscle, titin, a giant multi-domain protein functions as a molecular spring and is responsible for the elasticity of the tissue.[19]

From a materials perspective, all tissues are complex composites of natural biopolymers, inorganic components, and cells. The mechanical properties of such composites are similarly complex. An understanding of mechanical properties in native tissue is a critical design parameter for successful scaffold-based tissue engineering, as material stiffness is known to provide substantial feedback in cell-scaffold interactions and dictate cell phenotype.[20] Furthermore, an implant that is too stiff can result in degradation of the formerly healthy tissue surrounding the implant, as is the case for bone resorption surrounding femoral implants.[21]

Tissues in the body have a wide range of mechanical properties, as illustrated in **Table 1**, which contributes to their form and function. Connective tissue is largely responsible for the structure of the body and has among the strongest material properties; bone is the strongest material in the body with compressive strength and modulus ranging up to 300 MPa and over 1 GPa for adult femurs.[22] Cartilage has a high percentage of collagen, but has a much lower compressive modulus than bone. Instead, cartilage behaves like a classic viscoelastic material,[23]

with the viscous properties dominated by the water present in this well-hydrated tissue. However, under sustained loads, the polymeric collagen fibers dominate its mechanical properties. Epithelial tissue, with a high percentage of elastin, behaves like a synthetic elastomer. The high elastin content is responsible for the recoiling properties after deformation has been applied.[24] The structural components of nervous tissue are provided in part by myelin sheaths of Schwann cells, which create electrically insulating structures. Peripheral nerves also contain a significant amount of fibrous connective tissue,[25] which supplements their mechanical properties, making them quite strong. For example, an adult peripheral nerve trunk, such as the sciatic nerve, can sustain 61 MPa in tension before failure.[17] Muscle tissue is strong, but also elastic. It is unique among tissue types as its purpose it to generate forces rather than to resist extrinsic forces.[26]

Selecting materials that emulate the complex chemical and mechanical properties of native tissues is a significant engineering challenging. Many synthetic materials vastly underestimate the complexity of natural scaffold materials, which can prove detrimental to cell-material interactions and functional tissue integration.[27-30] However, using natural materials introduces attendant concerns of a possible immune response[31] if materials are not properly decellularized[32] or if there is contamination by endotoxins.[33] A number of naturally derived and synthetic scaffold materials have been evaluated in the field of tissue engineering. Most attempt to emulate certain aspects of the original tissue (shape, mechanical properties, water content, etc.). However, there have been limited efforts to develop materials that are able to mimic both the chemical and mechanical properties of native tissues, which contributes to a continued gap in translation. Furthermore, native ECM has a complex, organized three-dimensional structure that is difficult to recreate with synthetic materials. Even naturally derived materials assembled *ex vivo* with the exact physiological ratios do not recapitulate these properties, as biological interactions and cell rearrangement shape the scaffold such that the tissue properties match physiological need. For this reason, decellularized native tissues have found success in tissue engineering, as the chemical, mechanical, and biological infrastructure is primarily in place.

There are a number of natural materials that have secured FDA-approval for tissue engineering, and several approved devices have been based on these materials. Common materials of this class include collagens[34-36] and glycosaminoglycans, including hyaluronic acid,[36, 37] chondroitin sulfate,[35, 36, 38] and chitosan.[39] The source of these natural materials also influences their potential biocompatibility and function. Commonly, collagens are extracted from bovine or porcine tissues, and GAGs are derived from animal sources or bacterial synthesis. While these materials may elicit an enhanced immune response in some cases,[33] on the whole, these naturally synthesized and purified materials have been found to be safe. There are also a number of FDA-approved synthetic polymers that have been incorporated as structural components in tissue engineering scaffolds. These include polyethylene glycol (PEG),[40] poly(lactic-co-glycolic acid) (PLGA),[41] polycaprolactone (PCL), and ultra-high molecular weight polyethylene (UHMWPE).[42] These materials have found wide clinical application in adhesives, sutures, devices, and joint replacement. Although there is nothing biomimetic in the chemistry of these materials, matching mechanical properties and water content can be enough to stimulate tissue-appropriate responses from cells. It is assumed that this is because when a biomaterial is implanted, native proteins adsorb to the surface, which then modulate the cell-material interactions. Modulation of the mechanical properties of both natural and synthetic polymers and materials, in order to match the properties of native tissues, requires a basic understanding of polymer theory. Certain polymers, such as rigid rod peptides with a capacity for hydrogen bonding, are innately stiffer than a flexible, hydrophilic PEG chain that takes on a random coil configuration. Furthermore, using the same material system, but increasing the molecular weight of the polymers, incorporating shorter cross-linkers, or introducing more cross-linking junctions can greatly increase the modulus and ultimate strength of the material.[43]

2.2. Cell-material interactions

Scaffolds for tissue engineering must be designed to interact optimally with cells in order to promote tissue regeneration. The interaction between cells and scaffolds can promote distinct

changes in cell phenotype. Initially, these effects were thought to be strictly dominated by interactions at the cell-material interface. However, it is increasingly clear that scaffold architecture and three-dimensional geometry play a crucial role. Depending on the nature of the scaffold, cells can enter into a proliferative state, change the types of ligands and receptors presented on their surface, or even change function. Receptors on the surface of cells interact directly with the extracellular milieu, which includes structural proteins, glycoproteins, and the fluid and solutes trapped within the extracellular matrix.[44] Depending on the cues received from this environment, cells can change their phenotype. For example, without the correct cues from a scaffold, cells can undergo anoikis whereby they detach from their matrix and undergo apoptosis.[45] Endothelial cells grown on many polymers are known to shift to an inflamed and activated phenotype, but coating polymer surfaces with extracellular matrix proteins results in a more quiescent phenotype.[46, 47] These effects can be transient and change over time, as the cell responds to changes in its substrate or produces its own ECM.

In light of the significance of a substrate on cell behavior and health, it is important to characterize scaffold materials for their effects on cells, with a particular focus on both short- and long-term influence on cell phenotype. Traditionally, this has meant vetting the material in planar, two dimensional experiments where tissue culture plastic is coated with the material being investigated and then seeded with the cells of interest. The viability and phenotype of these cells are monitored to determine their cytocompatibility. However, the information gained from this type of study may be confounded by the physical geometry of the system. Specifically, many cells exhibit differences in phenotype when cultured on a planar surface compared to culture in three-dimensional environments. For example, mammary epithelial cells form normal acinus structures when encapsulated in a three dimensional material but develop a cancerous phenotype when cultured on a two dimensional substrate.[48] These geometric influences likely extend beyond the macroscale geometry of the device to include its nano- and micro-scale features as well. Therefore, the consideration of scaffold geometry across multiple length-scales is important to truly

understand the influence on cell phenotype, and to recapitulate normal cell-material interactions. Strategies that control these interactions in a predesigned, instructive, and purposeful way may improve scaffold design.

Once a scaffold is implanted, its biocompatibility and tissue reaction is another important feature that must be evaluated and understood. Typically, implanted materials become opsonized by proteins upon transplantation. This marks the implant as “non-self” and triggers a foreign body response from the host immune system to destroy and clear the material. Contaminants in the scaffold, such as endotoxins, can also contribute to recognition as a foreign body. Immune cells, typically macrophages, will first attempt to phagocytose smaller materials or break the material apart by secretion of proteases. Larger materials that cannot be broken down or phagocytosed are often walled off from the body through formation of an avascular fibrotic capsule. This capsule is predominantly made of collagen and is produced by myofibroblasts. Fibrosis may diminish the therapeutic function of an implant, as its margins become encased in a non-functional fibrotic scar tissue. This limits vascular integration of the device, resulting in hypoxia and apoptosis of encapsulated cells. Strategies to combat fibrosis include incorporating zwitterionic chemistry at the surface,[49] controlled release of antifibrotic drugs,[50] changes in surface geometry,[51] and the inclusion of fibrosis-modulating cells.[52] The immune response to a scaffold could also prove detrimental to strategies utilizing stem or progenitor cells, and may result in unintended outcomes in cell fate and phenotype should this process shift the microenvironment of an engineered cell niche.

2.3. Mass transport considerations

A multitude of techniques exist for the creation of cellularized scaffolds, but generating constructs that recapitulate the cellular density of native tissues remains a focus in tissue engineering. With increased cellular density comes an increased need for infrastructure capable of delivering oxygen and nutrients while removing cell products and waste, thus introducing mass transport considerations in scaffold design. These needs cannot be met by diffusion alone. For

example, to regenerate cell-dense tissues such as muscle, seeding a high density of cells within a scaffold is likely to result in a core that becomes hypoxic, leading to cell dysfunction and death. Diffusion is also inadequate when regenerating tissues that function through secreting or metabolizing compounds in order to maintain homeostasis in the body, such as endocrine functions. These cells must have proximity to a blood supply in order to sense physiologic indicators and secrete endocrine molecules, and also to allow access to oxygen and nutrients required for their long-term survival. To overcome diffusion limitations in constructs and support a larger number of cells, inclusion of convective mass transport must be addressed. In native tissue, cells exist no more than 150 μm away from a flowing blood supply.[49] Beyond this distance, there is insufficient diffusive mass transport. Therefore, for constructs with dimensions on this order or greater, especially those with high cell density, mass transport considerations dictate the incorporation of vasculature as a critical design parameter.

One strategy to address mass transport limitations for large, cell-dense engineered tissues is to promote pre-vascularization of the construct prior to implantation. Toward the pre-formation of blood vessels, strategies have been explored to include endothelial cells as part of cell-laden constructs. Using porous scaffolds and pre-seeding endothelial cells or endothelial progenitor populations, tubular structures can be generated *in vitro* which then form functional vascular elements once implanted.[54] This could be done with endothelial cells alone, or in combination with other functional cell populations.[55] An alternative method to incorporate endothelial cells and other cell populations at high density throughout a scaffold could be achieved through first mixing cells within a monomer or oligomer solution and then polymerizing or crosslinking these compounds. Ideally, the chemistries used to produce these types of scaffolds are bio-inert and do not compromise the viability of encapsulated cells. Other approaches that hold promise for developing pre-vascularized scaffolds include layer-by-layer construction and bottom-up assembly, which could provide control over the spatial arrangement of cells to build internal blood vessels.[56-59] These methods could be advantageous because they are scalable and the internal structure of the

scaffold can be controlled. Moreover, this method of large-scale scaffold construction is amenable to the organization of multiple cell types simultaneously, enabling functional therapeutic cells to be incorporated along with patterned endothelial cells to promote spatially organized vasculature.

Another strategy to address mass transport limitations would mimic nature using a more developmental approach. Here, scaffolds could be designed to guide and instruct the surrounding host tissue to form blood vessels within the scaffold. Cells recruited from the host migrate throughout the scaffold and supplement any cells that may have been pre-seeded within the scaffold. Thus, an instructive scaffold encourages the growth and maturation of the tissue, specifically in terms of blood vessel development, by leveraging natural mechanisms to promote vascularization. This can include leveraging the pro-angiogenic function of invading macrophages, along with engineered release of pro-angiogenic signaling molecules such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2).[60] Incorporation of chemical moieties and soluble cues can facilitate blood vessel growth, such as through presentation of heparan sulfate-like GAGs to modulate the activity of endogenous angiogenic factors.[61] The presentation of such factors could also be hidden and exposed in response to a specific stimulus, such as infiltrating cells that remodel the scaffold through cleavage of a caged bioactive domain to reveal new bioactive cues.[62-64] There have also been several examples of scaffolds engineered for controlled release or presentation of pro-angiogenic signals.[65-67] Release in response to a specific stimulus enables on-demand activation so that the appropriate signal is displayed at the appropriate time, avoiding possible risk of pathological angiogenesis from overexposure to potent angiogenic factors. For example, as cells infiltrate into the scaffold, angiogenic cues, such as VEGF, could be released to encourage blood vessel growth at a rate equal to cell infiltration. As cells migrate and proliferate deeper within the scaffold, a concomitant development of blood vessels can be formed allowing the tissue to grow in a controlled and biomimetic manner alongside the supportive vasculature.

2.4 Time required to prepare a functional construct

The time required to generate full-sized replacement tissues depends on the production of both the scaffold material and the generation of sufficient quantities of therapeutic cells, as well as the effort required to prepare a mature construct. This time frame must match clinical need for success of the technology to be realized. Also, more time and complexity associated with producing a scaffold translates to higher cost of the eventual product. Several advancements have been made in the areas of rapid or high-throughput manufacturing of therapeutic scaffolds, such as in-line production methods or automated printing. However, the pace of production for tissue-specific primary cells still presents an issue. Bioreactor systems have been produced to generate a large number of stem cells, with some also capable of further differentiating cells towards tissue-specific lineages. However, the specific culture conditions to reliably produce fully differentiated cells on a large scale remains a challenge. Once sufficient cells can be generated, new issues arise with regards to cell storage and transport. Unlike immortalized cell lines common in early research studies, primary cells have a limited time frame of use and continuous culture and passage can result in eventual senescence. For cases where cells must proliferate repeatedly in order to seed a scaffold, this can become a problem. In addition, the production of primary cells *en masse* may require that they be frozen prior to use. However, the freeze-thaw process can dramatically reduce the viability of cells. In the case where donor cells are used, on-demand production of the cells may therefore be required. This means the cells must be used immediately, which adds inflexibility to the overall process and could introduce lag times between patient need and an available construct. Otherwise, cells must be generated in excess to compensate for the reduced viability that results from long-term cryogenic storage.

The most obvious challenge to large-scale manufacturing of engineered tissues is the requirement for such a construct to be specifically tailored to a patient. A *"one size fits all"* mentality toward tissue engineering is unrealistic in the context of heterogeneous anatomy and physiology along with site-specific considerations. Moreover, the cell source presents a bottleneck in

manufacturing. For autologous cell-laden constructs, a tissue sample must be harvested from each patient individually, following which the cells of interest must be isolated, expanded, and seeded onto a scaffold. This entails cell production resembling a batch, rather than continuous, process. Additionally, depending on the disease state of the patient, there may not be a sufficient source of available cells, or there may be not be time to wait for a functional construct to be produced. If an allogeneic or xenogeneic cell source is used, the manufacturing may be more scalable, but the attendant risk for immune rejection could necessitate life-long immunosuppressive drug regimes. One possible solution to address manufacturing and immune rejection and produce patient-invariant constructs is to use allogeneic cells that express an immune escape phenotype to evade host immune detection, which is known to occur for certain types of cancer cells.[68] Technology or strategies for mass-production of tissue-relevant non-immunogenic cells could significantly streamline the manufacturing procedures to produce cell-laden tissue engineered constructs. Additionally, significant efforts focused on promoting tolerogenicity in organ transplant, specifically by dendritic cell reprogramming, could be adapted to cell-based tissue engineering to modulate the immune response to donor cells.[69, 70]

3. Emerging Technologies for Superfunctional Scaffold Materials

In recent years, a number of new strategies have emerged to generate scaffolds that are highly controlled, extremely tunable, and highly functional. This could provide future routes to address several of the design criteria to produce functional scaffolds and tissue constructs. Emerging work has demonstrated the feasibility of building patient-specific organs from decellularized donor organs, using native ECM as the scaffold. Additionally, advances in printing technologies that can precisely pattern a scaffold and its cellular components along with methods to engineer temporal control of soluble factors to influence cell differentiation and fate have been explored. Here we highlight some exciting emerging strategies that have demonstrated promise and will impact future scaffold development.

3.1. Decellularized organs

In recent years, organ decellularization has gained increased attention for its ability to generate scaffolds that retain native ECM structure and conduits for microvasculature that could be seeded with a patient's own cells to create a functional organ. As mentioned previously, decellularized tissues including basement membrane and small intestinal submucosa, have seen broad use as scaffolds for tissue engineering. However, decellularized total organs could point to a new direction to produce customized scaffolds with much greater hierarchical complexity. Specifically, the decellularization of organs with reseeding of cells has been utilized to generate a variety of functional whole organs, including bladder, heart, lung, liver, skin, trachea, esophagus, and kidney.[71-77] While decellularized organs have potential as highly controlled tissue scaffolds, several considerations must be addressed before these become clinically viable options for organ transplant. Scale-up and translation of current methods to human-sized organs is beginning to be explored.[78] The availability of donor organs and the time to fully recreate a functional organ need to be considered for this to be clinically viable. There is already a very limited availability of donor organs, and so procedures must ensure that all organs can lead to usable constructs in an acceptable time. Donors must be carefully screened to minimize any adverse reactions due to incomplete decellularization, or contamination by infection, which may leave behind virus particles or endotoxins. Additionally, the lifetime of these organs and their advanced functionality in disease state remain unclear.[79] Finally, an appropriate patient derived stem or progenitor cell source would need to be developed, requiring these methods to be optimized alongside functional organ creation. This work does, however, point to the enormous role of ECM in dictating the structure and phenotype of organs and inspires future efforts to make highly controlled three-dimensional bioactive scaffolds that more closely mimic native ECM as tissue engineering scaffolds.

3.2. Advances in printing and patterning

Rapid prototyping and additive manufacturing practices may enable the eventual production of highly controlled scaffolds and organs.[80] Additive manufacturing, the process by which 3D

objects are generated from a computer model through deposition of materials, has gained popularity of late with the emergence of 3D printers with z-stage control. This contrasts with conventional manufacturing, where undesired materials are removed to generate the final structure. A number of printing and patterning technologies have been evaluated, including 3D inkjet printing, stereolithography, laser sintering, and electrospinning. Computer-aided deposition of scaffold materials, cells, drugs, and growth factors have also been demonstrated, toward the production of hybrid or bioactive constructs. Additive manufacturing of biomaterials requires additional development before it can be considered a robust technology for large-scale production of functional scaffolds.[81] Methods to increase the throughput and strategies to scale-up printing to human-sized organs will be important. An improved understanding of tissue and organ structure, and its microstructure, will also result in better models for computer-aided deposition. New technologies, methods, and equipment also must be developed to handle a broad range of available materials and cells, with production procedures that are highly cytocompatible without sacrificing material properties.

3.3 Dynamic chemical signaling

Native ECM, while having specifically positioned bioactivity, also controls the temporal presentation of bioactive cues. One example of this is the exposure of cryptic sites in ECM proteins such as collagen, which have been found to play a role in directing cell fate.[82] These signals are generally hidden within the ECM and become exposed upon structural or conformational changes in order to direct cell migration, differentiation, and phenotype. Temporal control of bioactive cues, such as morphogens, in scaffolds has been explored using a variety of material and chemical approaches. For example, HAP and BMP-2 coated onto the surface of a bone implant using a biodegradable polymer afforded 7-day release of soluble factors, promoting enhanced implant integration compared to standard bone cement.[83] Combinations of factors may also be released with varying kinetic profiles to achieve synergistic effects.[84] For additional control of morphogen release, some have incorporated cleavable linkages tethering these molecules to the scaffold. In

one report, an MMP-13 cleavage site was utilized to release the fibronectin-derived RGD binding eptiope during mesenchymal stem cell differentiation into chondrocytes, mimicking the temporal profile of native adhesion signals during differentiation.[85] This temporal display resulted in improved cellular functionality, evidenced by increased cellular collagen deposition and glycosaminoglycan secretion. Release of molecules and control of scaffold properties via an external trigger, such as light, have also been investigated. To promote cellular attachment and differentiation, as well as angiogenesis, the release of various soluble factors and molecules from scaffolds has been explored. Soluble factors are known to elicit different effects on tissue regeneration when compared to attached factors, which are present at the scaffold-tissue interface exclusively.[86-88] This is thought to be due, in part, to the natural mechanism, by which cells are recruited to the site of factor release by following a chemokine gradient. Thus, engineering scaffolds with dynamic and temporal control over bioactivity could be a future strategy to produce more chemically biomimetic scaffolds for enhanced tissue regeneration.

3.4 Real-time scaffold monitoring

Next generation scaffolds may incorporate multiple technologies that provide advanced functionality to engineered tissues. This might include the ability to monitor tissue and scaffold performance over the lifetime of the transplant, or the ability to monitor disease state.[89] Real-time monitoring of scaffolds would enable the therapy to be fine-tuned using an analytics-based approach to modify the course of treatment. For example, a layered polyelectrolyte scaffold coating composed of hyaluronic acid and glycol-chitosan was demonstrated to provide real-time, label-free monitoring of cell growth and death.[90] Stem cells transfected with luciferase were also implanted within scaffolds to track cell retention and differentiation using bioluminescence imaging.[91] Additionally, hypoxia-responsive green fluorescent protein has been transfected into cells to provide a real time oxygen sensor,[92] which could have applications in assessing the oxygen perfusion of a tissue engineered construct. Optical coherence tomography, clinically used in ophthalmology, could provide a useful tool for mapping cells in scaffolds, enabling micron-scale

resolution at millimeter transplant depths.[93] Advances in MRI, PET and multiphoton imaging techniques could facilitate the tracking of cells within scaffolds, as well as assessment of overall scaffold performance.[94-96] These imaging techniques would enable monitoring of non-transfected native cells, but sensitivity and resolution would need to be improved before this becomes clinically viable. Nevertheless, combining real-time monitoring within a therapy through leveraging advanced imaging strategies could enable more personalized and tunable therapies, along with "theranostic" approaches to sense disease state over the lifetime of the construct.

4. Highlights and Challenges in Clinical Translation

In spite of several decades of intense research focused in tissue engineering, there are limited clinically approved therapeutics rooted in tissue engineering principles. Widespread use of tissue engineering to treat disease remains encumbered by a number of regulatory hurdles, which, in combination with the biological and engineering design constraints highlighted previously, provide technological limitations. An appreciation of these regulatory hurdles, though not always the first consideration of a scientist developing new technologies in a lab, is nevertheless critical when aspiring to design new therapies for the clinic.

4.1 Clinical success stories: skin regeneration

Some of the greatest advances in applying tissue engineering to the clinic have come in the area of skin regeneration. One of the first cell-based tissue engineering strategies to reach the clinic, Epicel® (Genzyme), consists of thin sheets (2-3 cell layers thick) of autologous keratinocytes cultured on a xenogeneic mouse feeder layer.[97] This approach does not rely specifically on a scaffold, as a layer of irradiated immortal mouse fibroblasts provides the support matrix for cell attachment and growth. As such, the transplanted product contains residual xenogeneic materials and cells, resulting in its classification as a xenotransplantation product. The time to expand autologous cells from a biopsy to obtain a sufficient graft is on the order of 3-4 weeks for Epicel®,

with this process occurring at a localized facility and necessitating shipping to bring the completed graft to the patient.

Other strategies of note in the area of skin regeneration have examined allogeneic cells cultured within scaffolds. Dermagraft® (Organogenesis, Inc) consists of neonatal fibroblasts seeded onto a bioabsorbable polyglactin scaffold that has specifically demonstrated promise in healing of diabetic foot ulcers.[98] Another allogeneic product Apligraf® (also produced by Organogenesis, Inc) utilizes a two-layered construct, with a layer of neonatal karitinocytes seeded onto a second layer consisting of a collagen matrix containing neonatal fibroblasts, which has received approval for healing both diabetic foot ulcers and venous leg ulcers.[99] The use of an allogeneic cell source for these two approaches dramatically reduces the time required to treat a patient with a functional construct as compared to autologous strategies that require biopsy, cell isolation, and *ex vivo* expansion and seeding. The caveat is that these constructs offer only temporary coverage before immune rejection of the graft. It is thought that the graft efficacy for allogeneic approaches arises from reducing the likelihood of infection at the site and stimulating the host wound healing response to promote wound closure, as opposed to the graft itself contributing to functional tissue.

An alternative is to use an acellular scaffold-based approach, such as that commercialized under the name of INTEGRA® Dermal Regeneration Template (Integra Life Sciences, Inc). This scaffold has two layers: a cross-linked matrix made up of bovine type-1 collagen and shark chondroitin-6-sulfate that is coated on one side with a layer of silicone. The cross-linked matrix is intended to recruit endogenous cells to reconstitute functional tissue, while the silicone layer is intended to provide a synthetic dermis to protect the wound bed from infection and reduce heat and moisture loss.

The pace of development for these approaches to skin regeneration is particularly noteworthy. The first published works forming the basis for these technologies were reported between 1979 and 1982.[100-102] This serves to highlight the lengthy process between initial development of a technology, its evaluation, and the final clinically approved product. It is

reasonable to expect this entire process to take two to three decades, and certainly a very high percentage of technologies fail to advance along the way. In addition to demonstrating efficacy as a technology advances through clinical trials, it also must demonstrate appropriate safety with low risk of side-effects, secure sufficient financing for this long journey, have a dedicated and capable team pushing it forward, and demonstrate superiority relative to other technologies in development within the sector.

4.2. The regulatory hurdles

There are several regulatory hurdles that must be considered when translating tissue engineering scaffolds to the clinic which depend on the nature of the particular technology. It stands to reason that an acelluar scaffold should face less regulatory scrutiny than approaches utilizing allogeneic or xenogeneic cells, iPS cells, embryonic stem cells, or even significant *ex vivo* manipulation of autologous cells. The introduction of cells as a component in tissue engineering introduces attendant risks associated with possible immunogenicity, teratoma formation, cell culture adaptation/morphogenesis, or contamination which must be addressed to assure safety.

The scaffold material itself can affect the regulatory process for a new tissue engineering strategy. The 510(k) procedures used by the U.S. Food and Drug Administration (FDA) allows devices characterized as “substantially equivalent” to an existing approved device to be brought to market more quickly. Examples of this are bone void fillers prepared from either preformed calcium sulfate pellets or methylmethacrylate.[103] Conversely, development of highly bioactive scaffolds with poorly defined degradation products may require significant effort to establish the safety of the material as well as its degradation products, increasing time and cost for pre-clinical and clinical evaluations. This can lead to a dichotomy, as efforts to create novel systems that interact with biology in a fundamentally new way are often distinct from efforts towards the rapid translation of technologies through focusing on 510(k) and GRAS (generally regarded as safe) materials. The former may enable groundbreaking academic research, but oftentimes the cost and effort required to translate fundamentally new technologies can be a significant barrier. Ideally, the development

of novel materials for use in tissue engineering will give rise to some innovative functionality that cannot be recreated with existing materials, and then these transformational technologies can be advanced based on their ability to perform a specific function. It is, therefore, imperative that efforts toward novel transformational technologies continue through support and funding for innovate research, in spite of the additional hurdles presented in their clinical translation.

Prior to beginning the regulatory approval process through the filing of an Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, a substantial body of pre-clinical data must be obtained for the proposed systems. This includes theoretical modeling, *in vitro* characterization, and *in vivo* (i.e. in animals) studies. One concern raised in particular for tissue engineered scaffolds is the extent to which these studies are predictive of eventual function and performance in humans. For example, interspecies variability in the immune system introduces concerns that studies in rodents may not be predictive of the immune response to an implanted construct in human, as there are many differences between mouse and human immunology.[104, 105] Evidence suggests that the sex of laboratory animal chosen may also affect demonstrated efficacy.[106] Efforts have been taken to prepare humanized mice with immune systems that more closely resemble that of a human,[107] which may eventually improve the predictive ability of small rodent models. For genetic disease models, otherwise healthy inbred mice may not be ideal in terms of recapitulating the complexities of human disease in a heterogeneous patient population. Additionally, for strategies evaluating autologous cell approaches, sourcing cells from a typically young healthy mouse may not resemble the case where cells are harvested from older patients with associated co-morbidities.[108] Taken together, this would caution against relying on a single animal model to collect pre-clinical results, and demonstrating efficacy in multiple animal models across multiple species, including in large animals, may be desirable. However, this can take several additional years of pre-clinical development and require substantial funding.

As part of the regulatory approval process through the U.S. FDA, a new therapeutic entity is traditionally routed along one of four distinct pathways: tissues, biological products, drugs, or

medical devices. Tissue engineering introduces unique challenges at this stage. Depending on its manifestation, many tissue engineering approaches may fall into two, three or even all four of these categories. A simple scaffold used for a tissue filler, for example, could be characterized purely as a medical device if it "*does not achieve its primary intended purposes through chemical action and is not dependent upon being metabolized for the achievement of its primary intended purposes*" (Section 201(h) of the U.S. Food, Drug, and Cosmetic Act). However, once additional scaffold complexity is incorporated, the construct may no longer be classified purely as a medical device. In fact, a majority of tissue engineering approaches combine cells seeded onto a scaffold, and may additionally incorporate signaling elements, chemical functionalities, or the release of small molecule drugs or proteins from the scaffold. Thus, from the onset the precise regulatory pathway of a tissue engineered therapy becomes less clear, and oftentimes aspects of the regulatory processes for each of these pathways must be addressed in order to develop a single therapy. This can significantly increase the time and cost to achieve regulatory approval, as each pathway has its own accepted methods for demonstrating safety. At present, pre-clinical and early clinical evaluations of technologies are addressed on a case-by-case basis, often times requiring innovation in the regulatory framework by companies and regulatory officials in order to chart the best course of action to conclusively assure safety. This process is therefore undertaken with extreme caution, and likely ends up requiring that additional verifications be performed. It is assumed that, as more tissue-engineered therapies move into clinical evaluation and establish a precedent for production and use, the precise regulatory pathway and requirements for these approaches will be clarified. The FDA has several places where those interested in commercializing a tissue engineered product can go in search of guidance, and those interested are encouraged to review the publication by Lee et. al.[109] along with resources available through the FDA Tissue Reference Group and the FDA Office of Combination Products.

4.3. The cost of development

The costs associated with bringing a tissue engineering technology to market can be quite high, and may require several rounds of financing in addition to cooperation in development from large companies. It is estimated that a pharmaceutical company spends, on average, over $850 million to bring a new drug or biologic to the market, increasing to roughly $1.8 billion after capitalization.[110] It is less clear what the cost may be for a tissue engineered therapy, but it could be considerably higher when additional components (i.e. cells, scaffold, proteins), complex or individualized production methods, and multiple regulatory pathways must be negotiated in the development. Also, an increasingly cost-constrained healthcare system with limited insurer reimbursement, both in the U.S. and abroad, adds additional pressures on complicated technologies to remain cost-efficient in production. This enormous cost means that a technology must have demonstrated convincing efficacy in a broad market to have a chance at being profitable. These are considerations not often made by a scientist seeking to develop new technologies and therapies. However it is imperative that, should the vision be to eventually translate a technology, sights remain set on the most efficient way to streamline its production and development. The good news is that, in spite of escalating costs in the development of new therapies, the tissue engineering sector is experiencing a boom. Data as of 2011 showed commercial sales of tissue engineering products had increased three-fold since 2007, amounting to $3.5 billion in sales that year.[111] Meanwhile, the industry is spending $3.6 billion annually and employing almost 14,000 people.[111] This is promising for the future of tissue engineering strategies, and it is lends hope to eventual profitability of the entire sector as more advanced strategies begin to emerge from R&D pipelines.

4.4. Strategies for commercialization of promising technologies

The formula used to evaluate technology from the Langer Lab to determine whether starting a company makes sense has been described by "*The three Ps*": platform, paper, and patent.[112] A technology that is a good candidate for commercialization ideally should be a platform technology,

meaning it has the potential to be used in many different applications. A great technology that only has niche applicability has less chance for success than one that could lead to products in several different areas. The next step in this process has traditionally been publication of this technology in a high-profile journal. Demonstration of the scientific aspects of the technology through publishing a high-impact paper can be very beneficial to securing scientific and investment partners to aid in the development. Finally, a good technology for commercialization must have a very strong patent behind it, which is ideally a blocking patent written broadly to protect the platform from other technologies being developed. A strong patent often flows directly from high-impact publications. In addition to these three elements of a successful company, the technology must demonstrate strong proof-of-principle results in relevant preclinical studies. Finally, an aspect that is often more important than the technology itself is the people behind its development. For a platform to be successful, that technology needs a champion (or several) who are willing to do whatever it takes to see that technology succeed. Therefore, the team trusted with developing the technology must be carefully selected, and it often helps if these individuals have a vested interest in seeing the technology succeed.

To surpass the various hurdles discussed here, there are many aspects that must be addressed for successful translation of a technology. This begins with a strong scientific foundation for the technology and a strong patent. The technology must be thoroughly established in pre-clinical studies, and must navigate multiple regulatory pathways in its development. The company founded around the technology must be able to secure enough financing for its development, have a clear vision for the best path forward, and be comprised of champions of the technology. As the product is developed, it must be reproducibly manufactured, and clinical trials must be carefully constructed to ensure reliable efficacy and safety. The market potential of the technology, including its cost balanced with its potential for reimbursement, must also be considered. No matter how novel a technology may be, for investors and partners to be enthusiastic and for eventual clinical success, all of these facets of the scientific and business development must be addressed.

5. Conclusions and Outlook

When designing scaffolds for tissue engineering, adopting the mindset of an engineer is an important perspective. Information from many areas of biology and medicine must be combined with knowledge in materials synthesis and development to produce functionally relevant constructs that could augment or replace native tissue. The engineering design constraints of a tissue engineered scaffold include its chemistry and mechanical properties, its source of origin, an understanding of how it interfaces with cells and tissues, an appreciation of mass transport limitations, and reliable and scalable solutions to its unique manufacturing and production requirements. For clinical translation of these technologies, an assurance of safety is paramount, and many regulatory hurdles must be navigated in order to demonstrate safety and succeed in translation. The efficacy and versatility of the technology must be robustly established as well, and a dedicated team must be on board to drive the technology forward. Strong scientific and financial partnerships are a requirement on this journey, which can take many years and cost hundreds of millions of dollars.

Even with all of these considerations, and in spite of only limited clinical success thus far, the future of the field of tissue engineering is bright. Technologies across various stages of development hold enormous promise for the treatment of diseased and damaged tissue. In addition, teams of brilliant and dedicated scientists and engineers are striving every day to improve the quality of life for patients worldwide through tissue engineering. The journey is long and arduous, and many technologies will fail along the way. However, a commitment to the development of tissue engineering constructs by scientists and engineers in partnership with academic institutions, government agencies, financial partners, and large corporations will lead to both a fruitful and impactful future for tissue engineering in the coming decades.

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Table:

Table 1. Comparative table of most relevant mechanical properties and compositions for major tissue types. This table is not meant to be exhaustive, but to demonstratively compare tissue mechanics and compositions. Col: collagen, HAP: hydroxyapatite, GAG: glycosaminoglycan, PG: proteoglycan

Tissue Type	Modulus	Composition	Major Chemical Constituents
Bone[22]	10 GPa (tensile) 8.7 GPa (buckling)	70% HAP, 18% Col, 10% water	HAP, Col I
Cartilage[16]	300 kPa (shear)	75% water, 15% Col, 6% GAG	Col II, CS
Tendon[113]	500- 660 MPa (tensile)	60% water, 30% Col, 3% PG, 2% elastin	Col I, elastin
Peripheral nerve,[114]	1.3- 2.9 MPa (tension)	78% water, 11% lipids, 8% protein	Galatocerebroside, cholesterol
Brain[17,115]	8-15 kPa (shear)		
Muscle[116]	25- 100 kPa (indentation)	Cellularly dense, striated arranged in sarcomeres	Myosin, actin, titin
Skin[117]	1-2 Mpa (indentation)	Cellularly dense	Col I, keratin, Col IV