Advanced Fabrication Techniques to Engineer Cell-Laden Multilayered Hollow Tubular Structures

*Tess De Maeseneer, Fang Zhou, and Nasim Annabi**

Hollow tubular tissues play a vital role in the human body, facilitating the transport of nutrients and waste. Damage to these structures can lead to luminal narrowing or complete dysfunction, necessitating medical intervention. While stents remain the gold standard for repair, they are associated with significant complications. Tissue engineering offers a promising alternative by encapsulating cells within biomaterial scaffolds to regenerate native-like tubular tissues. Given the multilayered complexity of these structures, advanced fabrication techniques are essential to achieve precise spatial and temporal control over cell distribution and scaffold architecture. While traditional methods rely on post-fabrication cell seeding, biofabrication techniques are utilized to enable direct cell integration within scaffolds during fabrication. This review explores cutting-edge strategies for engineering multilayered cell-laden tubular constructs, including 3D bioprinting, cell-embedded electrospinning, gel casting, self-folding cell sheets, and microfluidic-assisted assembly. Bioreactors play a crucial role in establishing dynamic culture conditions to further improve cell differentiation, extracellular matrix (ECM) deposition, and tissue remodeling and maturation. Special emphasis is placed on the application of these fabrication techniques and bioreactor systems in regenerating tubular structures such as the urethra, trachea, blood vessels, and esophagus, highlighting their potential to revolutionize tissue-engineered tubular constructs.

1. Introduction

For optimal function and survival, the human body relies on tubular tissue structures to efficiently exchange essential substances, transport nutrients, and remove toxins.[1] Key structures involved in this system include the vasculature, gastrointestinal tract, respiratory tract, and urethra.[2] Each tubular tissue construct has its distinct function, with the main similarity being the transportation of fluids through the lumen of the tube. The vascular system, a complex hierarchical network of blood vessels of varying sizes, is responsible for transporting blood throughout the body.[3] Similarly, the tubular structures of the gastrointestinal tract, composed of the esophagus and intestines, manage the intake, digestion, and processing of food and liquids.[1] In the respiratory tract, the main tubular structure is the trachea, through which air flows to support gas exchange.[1] Finally, the urethra plays a dual role in the urinary and reproductive systems, serving as a passage for both urine and semen.

For a variety of reasons, including congenital defects, trauma, and disease, these tubular structures can be damaged, typically resulting in a narrowing of the lumen, making efficient fluid transport difficult.[4–7] A widely applied solution to maintain integrity of the open luminal space and prevent tubular collapse, is the placement of stents, which are used to provide internal support to the walls of natural tubular structures including blood vessels,[4] the gastrointestinal tract,[8] airways,[9] and the urethra.[10] However, foreign body reactions to stents, typically made of synthetic polymers or metals, may create issues such as infections and negative immune responses. In addition, in-stent restenosis[11] and encrustation[12] remain major complications for coronary and urethral stents, respectively. In-stent restenosis is an example of a negative immune response, in which scar tissue forms inside the stent, leading to its re-narrowing. While this can occur when placing stents anywhere in the body, a rate of in-stent restenosis as high as 10 to 15% has been reported for coronary stents.[11] Overcoming this issue resulted in the development of drug-eluting stents (DES). Early DES designs utilized metallic stents with polymeric drug coatings to deliver antiproliferative medicines to prevent in-stent restenosis following stent implantation.[13] However, the permanent metallic backbone of these stents could still trigger adverse body responses, including chronic inflammation and impaired tissue healing.[14] To address these limitations, efforts were made to replace metallic components with more biocompatible materials.[14] Building upon these advances, a new generation of bioresorbable stents emerged as an alternative strategy to DES, aiming to prevent restenosis while eliminating long-term foreign material in the body.[15,16] These stents gradually degrade within the body over a period of 6 months to 2 years

T. De Maeseneer, F. Zhou, N. Annabi
Department of Chemical and Biomolecular Engineering
University of California, Los Angeles
Los Angeles, CA 90095, USA
E-mail: nannabi@ucla.edu

N. Annabi
Department of Bioengineering
University of California, Los Angeles
Los Angeles, CA 90095, USA

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after implantation, minimizing the risk of long-term chronic inflammation.[17] Critical considerations in their development include achieving sufficient mechanical strength, controlling corrosion rates, and ensuring that degradation byproducts are biocompatible, as maintaining mechanical integrity during the healing period remains a challenge.[18] Additionally, hybrid approaches have been developed in which bioresorbable stents are combined with drug coatings, offering the benefits of controlled drug delivery alongside gradual scaffold resorption.[14] Encrustation occurs naturally in urethral stents as minerals in the urine are deposited on the stent surface and leads to serious complications such as increased risk of infection, stent blockage, and difficulty removing the stent.[12,19] Despite previous innovation in stent design, such as introducing antirestenotic drugs and incorporating biocompatible or biodegradable materials, more focus has recently been given to tissue engineering of the damaged tubular structures as an enhanced repair strategy. In addition, tissue engineering can be used to replace not only stents, but also entire pieces of tissue removed during (cancer) surgery. Currently, autologous grafts from other parts of the body are used for replacement, but these tissues are scarce and they often do not mimic the exact structural properties for effective repair.[5] This challenge is further compounded by the limited availability of structures such as small diameter arteries. Furthermore, even if available, these structures are difficult to harvest without causing significant donor site morbidity.[20] In addition, many grafts have limited long-term patency. For example, although saphenous vein grafts are considered the clinical gold standard, they have shown a failure rate close to 50% within 10 years.[20,21]

Tissue engineering offers a promising alternative by combining cells with supportive biomaterials, typically hydrogels, which mimic the natural extracellular matrix (ECM). However, engineering tubular tissue structures remains challenging due to their multilayered composition, where each layer supports distinct cell types with unique microenvironmental needs.[1] Successfully replicating these structures requires precise control over hydrogel composition and spatial deposition of cells. Advanced fabrication techniques, such as 3D bioprinting,[22] electrospinning,[23] and gel casting,[24] have been used to generate these complex multilayered structures due to their high spatial and temporal control. Despite these advancements, these review articles mainly focus on scaffold fabrication followed by post-seeding of cells onto their surfaces, which often leads to poor cellular distribution and limited tissue integration.[22–24] A more effective strategy involves 3D cell encapsulation, where cells are mixed with the prepolymer solution and subsequently polymerized, forming a tubular structure with a uniform distribution of embedded cells. This method better replicates the natural 3D microenvironment of native tissues,[25] preventing cell aggregation and ensuring more homogeneous scaffold composition.[26] Moreover, cell encapsulation offers protective benefits by shielding the cells from external stressors, thereby minimizing immune responses.[26] In addition to enabling cell distribution, certain fabrication techniques using cell-laden hydrogels can further enhance cellular behavior by promoting cell alignment through controlled micropatterning and spatial structuring.[27] Aligned cells exhibit improved morphology, enhanced communication, and a more physiologically relevant phenotype, making this a crucial factor in engineering functionally mature tubular tissues.

Advanced fabrication techniques enhance scaffold architecture and promote cell alignment. However, the successful implementation of tissue engineering also depends heavily on the growth and cultivation of cells within the scaffolds. Bioreactors are valuable tools for fostering cell growth by replicating the native physiological environment.[28] For example, perfusing a tubular tissue construct mimics natural fluid flow through the lumen, whereas mechanical stretching replicates its native contraction behavior.

This review focuses on the reconstruction of cell-laden multilayered tubular structures, emphasizing how advanced fabrication strategies enable the precise spatial organization of distinct cell types across layers. Unlike traditional approaches that rely on surface cell seeding post-fabrication, we highlight techniques that integrate cells within hydrogels during scaffold formation, ensuring more physiologically relevant constructs. A comprehensive overview is provided on how these cutting-edge fabrication methods are applied to engineer vasculature, the esophagus in the gastrointestinal tract, the trachea in the respiratory tract, and the urethra. We first examine the anatomical composition of these structures to identify the unique cellular arrangements essential for functional tissue engineering. This is followed by an in-depth discussion of various fabrication methods used to construct complex tubular scaffolds. Additionally, we explore the critical role of bioreactors in mimicking physiological conditions to enhance cell viability, organization, and tissue maturation. By integrating scaffold fabrication with dynamic in vitro conditioning, this review offers a holistic perspective on the engineering of functional tubular tissues. Finally, we address existing challenges and outline key advancements required for successful clinical translation of these bioengineered constructs.

2. From Native Anatomy to Scaffold Design for Engineering Multilayered Tubular Tissue

2.1. Anatomy of Multilayered Hollow Tubular Structures

Although each tubular tissue construct is unique, they share common structural features. The innermost layer, the mucosa, is always lined with an epithelium, serving as a barrier to prevent fluids from passing through the tubular wall into the surrounding organs.[29] The specific type of epithelium varies between tubular structures and can even change along the length of a single tube. For example, the male urethra contains five different types of epithelium along its entire length.[30] The epithelium primarily consists of epithelial cells, whereas blood vessels have a specialized inner lining called the endothelium, composed predominantly of endothelial cells (ECs).[31] For blood vessels specifically, this layer is referred to as the tunica intima. Beyond its role as a selective barrier, the endothelium actively promotes hemostasis and contributes to the formation of new blood vessels, a process termed angiogenesis.[31] In some tubular structures, the epithelial layer adopts a unique 3D shape to accommodate functional demands. For example, the urethra has a star-like shape to facilitate the expansion and contraction of the tube during voiding.[32] The mucosa is typically surrounded with a

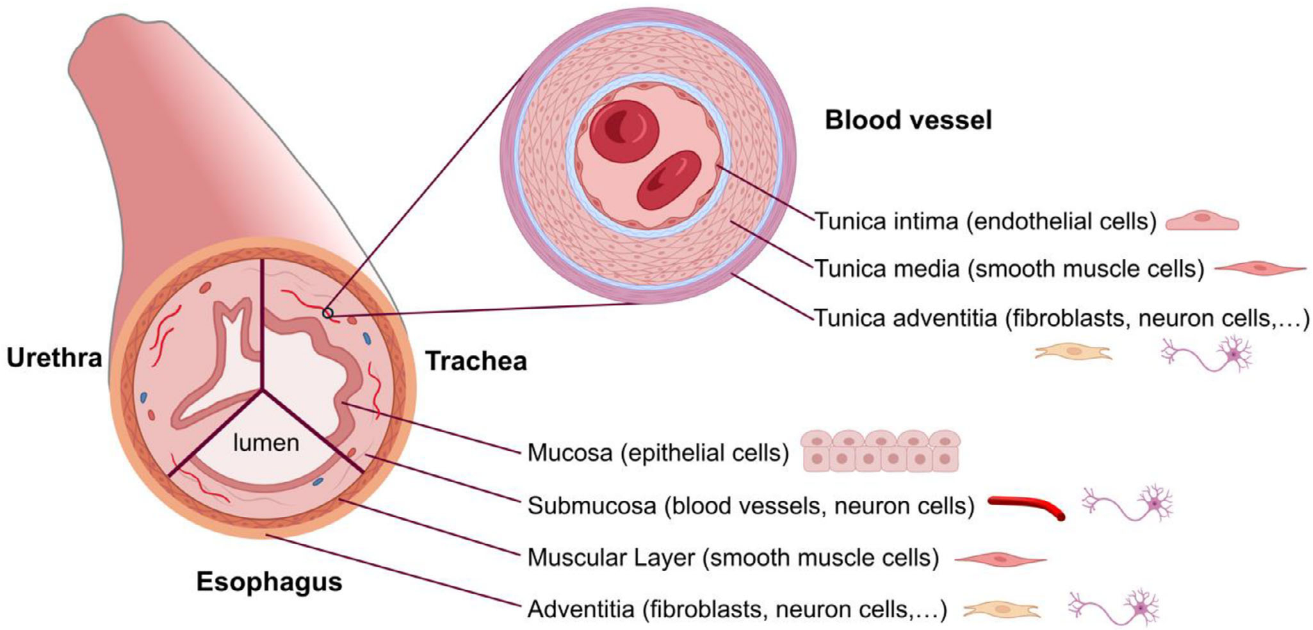

Figure 1. Schematic representation of the multilayered structure and diverse cell types found in hollow tubular tissues, including the trachea, esophagus, urethra, and blood vessels.

submucosa layer which provides vascularization and innervation to the tissue construct.[1]

The second key layer in tubular structures is the elastic and muscular layer (tunica media for blood vessels), composed primarily of smooth muscle cells (SMCs). This layer is essential for generating contractile forces to propel fluids through the lumen. The muscle composition can vary along the length of certain tubes. For instance, the esophagus transitions from skeletal muscle in its upper region to smooth muscle in its lower part.[33] Skeletal muscles, which are under voluntary control, initiate the movement of food down the esophagus, while smooth muscles take over to continue the process autonomously.[34] The main cell type responsible for skeletal muscle growth are myoblasts.[35]

Finally, a third structural component is a connective tissue layer termed adventitia or tunica adventitia in vascular terminology, providing mechanical support and integration with surrounding tissues.[33] This layer is typically vascularized and innervated, hosting a diverse mixture of cell types, including nerve cells, ECs, SMCs, and immune cells. In the case of the trachea, there is a very specific additional layer of C-shaped hyaline cartilage rings, which surround the smooth muscle layer, offering structural rigidity to prevent airway collapse.[1] The main cellular component of these rings are chondrocytes.

While these three layers (epithelium, smooth muscle layer, and connective tissue layer) form the fundamental framework of multi-layered tubular structures, real anatomical structures are far more complex. Variations in layer compositions, specialized cell types, and microarchitectural adaptations reflect the diverse functional demands of each tubular organ. A schematic representation of the simplified anatomical structure of tubular structures is shown in **Figure 1**.

2.2. Rational Design of Scaffolds for Effective Tissue Integration

As described in the previous section, tubular tissues consist of multilayered architectures with distinct cell types and mechanical needs. Bioengineering efforts must reflect this complexity and hierarchy. Typically, tubular tissue constructs include an inner epithelial layer, a smooth muscle layer, and an outer connective tissue layer, each encapsulated with specific cell types to recapitulate native structure and function. Each layer must be carefully designed in terms of material selection to ensure effective tissue integration and functionality. To support epithelial cell adhesion, polarization, and tight junction formation, biomaterials with high water content and bioactive motifs are preferred for the epithelial layer. Due to their biocompatibility and capacity to replicate the natural ECM, soft hydrogels such as tissue-specific decellularized ECM (dECM), fibrin, and gelatin methacryloyl (GelMA) have been extensively utilized for the construction of this layer.[29,36,37] Although dECM offers vital biochemical signals, it has to be carefully processed to eliminate immunogenic epitopes and cellular remains that may trigger an immune response.[38] Synthetic hydrogels such as polypropylene and poly(lactic-co-glycolic acid) (PLGA) have also been used for the inner epithelial layer. However, to reduce inflammation and diminish protein adsorption, they are typically conditioned with natural ECM such as collagen.[39,40]

Moderate stiffness and the ability to support cell alignment and mechanical conditioning are important design considerations for the smooth muscle layer. To achieve this, natural materials are typically supplemented with synthetic components that enhance the mechanical strength and induce electrical stimulation to the SMCs. For example, GelMA-alginate blends have been integrated with heparin-doped polypyrrole.[41,42] Alternatively, synthetic elastomeric materials such as poly(ester urethane)urea can be used to increase elasticity.[43,44] However, synthetic polymers may cause a foreign body reaction if not properly surface modified.[43,44] Otherwise, SMCs might lose their contractile phenotype which contributes to restenosis and graft failure.

The connective tissue layer offers mechanical strength, helps maintain shape, and integrates the scaffold with the host tissue. Electrospun polycaprolactone (PCL), PLGA or silk fibroin scaffolds have been extensively used to reconstruct the fibrous architecture of this tissue layer.[44–46] These materials have a high tensile strength and adequate breakdown rates to mirror normal tissue remodeling. However, cell adhesion is sacrificed due to hydrophobic and bioinert surfaces of synthetic PCL/PLGA polymers.[38]

In addition to scaffold design, effective tissue integration can be enhanced by encapsulating cells within hydrogels rather than seeding them on the surface. Encapsulation provides a protective hydrogel barrier that shields cells from direct attack by immune cells while still allowing the diffusion of nutrients and oxygen, thereby promoting immune acceptance and integration.[47] Moreover, the 3D microenvironment of the hydrogel offers essential mechanical and biochemical cues that support appropriate phenotypic behavior and functional tissue formation, which are often lost in 2D cultures.[48] An additional benefit of hydrogel encapsulation is the ability to introduce a co-culture of cells that are physically separated, but still close enough to have beneficial cell communication.[49] Co-culturing multiple cell types within 3D multilayered scaffolds, such as ECs or epithelial cells in the inner layer, SMCs in the middle layer, and fibroblasts or macrophages in the outer layer, has been shown to enhance phenotypic expression and promote functional tissue maturation.[50] Therefore, it is highly encouraged to utilize fabrication strategies and scaffold materials that support spatially organized cocultures within multilayered constructs. For example, fabrication strategies that enable precise cell deposition, such as bioprinting and microfluidic patterning, are ideal candidates to achieve these structured architectures. Encapsulation also enables spatial control over cell distribution within the constructs, preventing cells from randomly migrating and forming clusters. This ensures structural homogeneity and uniform integration with the host tissue.[25,26]

To summarize, the production of functionally biomimetic tubular constructs is dependent on a layer-specific material approach that strikes a balance between mechanical integrity, cellular compatibility, and immunological safety. Integrating natural and synthetic polymers in a spatially ordered way enables the reproduction of complex tissue interfaces while lowering the risk of rejection or chronic inflammation. Importantly, 3D cellular encapsulation within multilayered scaffolds not only replicates the native architecture for each cell type but also facilitates beneficial communication between co-cultured cell types, promoting phenotypic stability and functional tissue maturation. Fabrication strategies that support precise spatial organization are highly encouraged to maintain structured cellular distribution and prevent uncontrolled migration or clustering. Continuous developments in hybrid fabrication methods, scaffold formulation, and immune engineering are essential for enhancing the functioning and translational potential of created tubular tissues.

3. Fabrication Techniques for Engineering Multilayered Hollow Tubular Tissue Constructs

Creating complex multilayered tubular tissue constructs requires precise deposition of cell-laden hydrogels in specific patterns and locations, which can be achieved by using advanced fabrication techniques with specialized temporal control. This section highlights recent progress in 3D bioprinting, cell-embedded electrospinning, gel casting, and microfluidic technologies, which enable the construction of single tubular structures with multiple layers, each containing distinct encapsulated cell lines. The general methodology for developing multilayered tubular tissue constructs is discussed, along with specific applications of these techniques in engineering the esophagus, trachea, vasculature, and urethra.

3.1. 3D Bioprinting

3.1.1. Extrusion-Based 3D Bioprinting

3D bioprinting is the layer-by-layer deposition of cell-laden biomaterials to fabricate complex 3D structures that mimic the architecture and function of natural tissues and organs. There are several types of bioprinting techniques, with extrusion-based bioprinting being the most widely used for engineering tubular structures due to its versatility in depositing a wide range of biomaterials. In addition, multilayered tubular constructs can be created using a bioprinter equipped with multiple nozzles, each loaded with a distinct bioink. By alternating between nozzles, different layers are precisely deposited one after another. However, direct extrusion of soft hydrogels often leads to poor structural integrity, particularly when fabricating large tubular constructs of human size. To address this challenge, several strategies have been developed to maintain mechanical stability during and after printing. One approach is the integration of a synthetic support structure. For example, Zhang et al. used this approach to 3D bioprint a rabbit urethra consisting of a middle polymeric support layer based on PCL/poly(l-lactide-co-ϵ-caprolactone) (PLCL), an inner urothelial cell (UC)-laden fibrin hydrogel, and an outer SMC-laden fibrin hydrogel (**Figure 2A**).[51] Although high cell viability was reported immediately after printing, a marked decrease in viability was observed after seven days, likely due to insufficient oxygen diffusion to the encapsulated UCs. From a clinical translation perspective, the construct was not evaluated in an in vivo model, which may limit current insights into its structural stability, host integration, and immunogenicity.

A similar approach, combining a 3D-printed synthetic support structure with cell-laden bioinks, was also employed by Nam et al. to engineer an esophageal tissue construct (Figure 2B).[52] They first 3D printed a medical-grade PCL support structure with specific structural design characteristics to mimic esophageal functionality. This support featured a three-layered structure with void spaces between the layers. To allow for peristaltic movement, the outer layer was shaped like a bellow, while the inner and middle layers were designed with a wrinkled pattern to closely resemble the native esophageal mucosa. Instead of regular printing, the PCL was deposited using a dragging technique to generate pores, which enhanced cell proliferation by improving

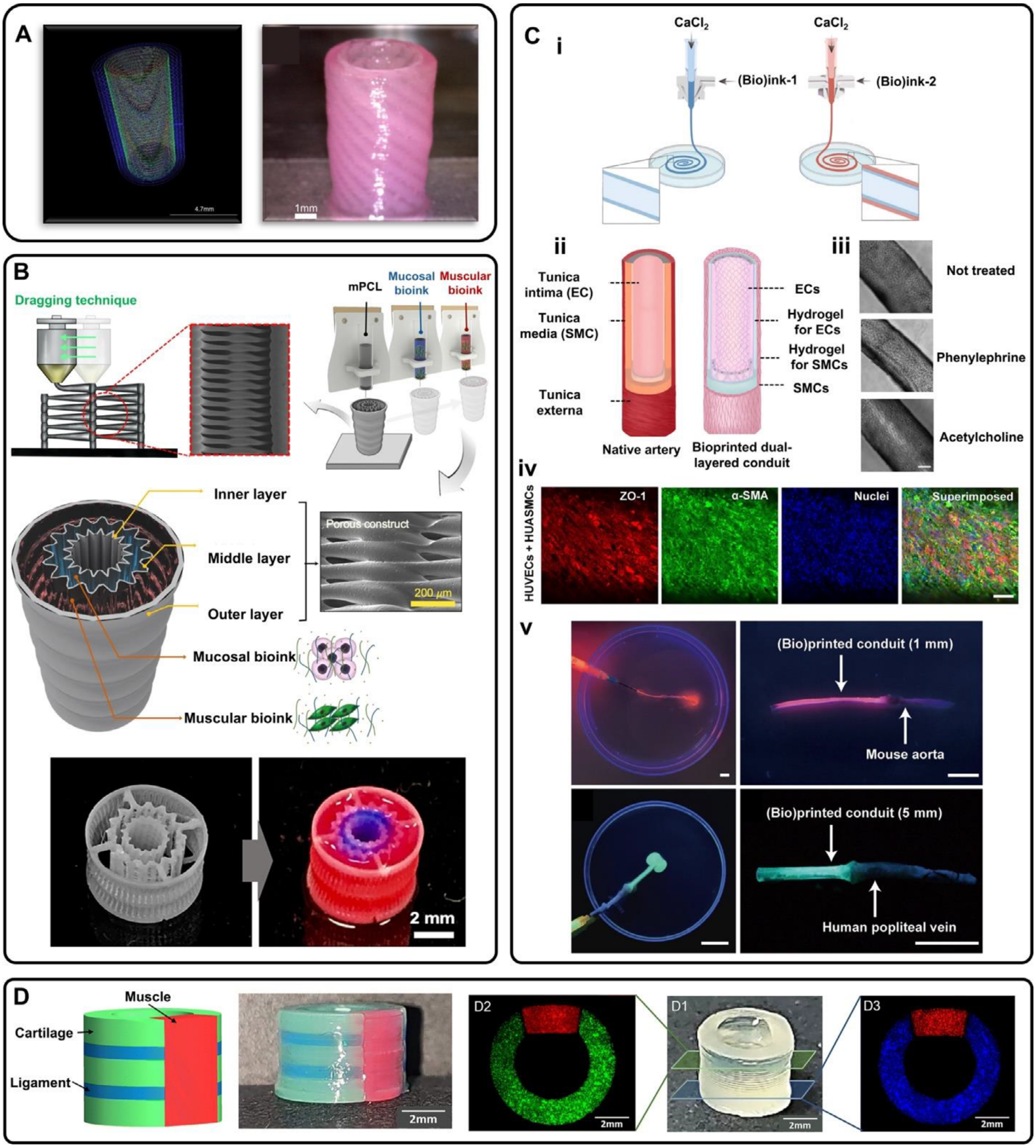

Figure 2. 3D bioprinting of cell-laden multilayered hollow tubes. A) A 3D bioprinted rabbit urethra consisting of a middle polymeric support layer (polycaprolactone (PCL)/poly(l-lactide-co-ϵ-caprolactone) (PLCL)), an inner urothelial cell (UC)-laden fibrin hydrogel, and an outer smooth muscle cell (SMC)-laden fibrin hydrogel. Reproduced with permission.[51] Copyright 2025, Elsevier. B) A 3D printed PCL support featuring a 3-layered structure designed to mimic esophageal functionality. Bioinks containing decellularized esophageal tissue, SMCs, and epithelial cells were bioprinted into the scaffold's voids, ensuring uniform cell distribution. Reproduced with permission.[52] Copyright 2025, Springer Nature. C) Coaxial extrusion-based bioprinting of biomimetic blood vessels and their ex vivo applications. i) The use of double and triple coaxial nozzles to bioprint a single- or multi-layered hollow tube; ii) 3D bioprinting of vein and artery mimics using double and triple coaxial nozzles with alginate-based bioinks and calcium (Ca)-induced crosslinking; iii) functional vessel responses to acetylcholine-induced dilation and phenylephrine-induced contraction; iv) engineered artery featuring a SMC-laden outer layer and endothelial cell (EC)-seeded inner lining expressing zonula occludens-1 (ZO-1) and α-smooth muscle actin (α-SMA); v) ex vivo anastomosis and perfusion of bioprinted vessels with mouse and human vasculature. Reproduced with permission.[60] Copyright 2025, Science Advances. D) Bioprinting of the trachea featuring C-shaped cartilage rings, ligaments, and muscles with fluorescently labeled mesenchymal stem cells (MSCs) (D1), showing (D2) alternating green- and red-labeled cells, and (D3) alternating blue- and red-labeled cells in adjacent layers. Reproduced with permission.[65] Copyright 2025, Royal Society of Chemistry.

media and oxygen infiltration. Next, two bioinks composed of decellularized esophageal tissue encapsulating SMCs and epithelial cells, respectively, were 3D bioprinted inside the voids of the PCL framework. This method of printing ensured effective cell placement and homogeneous cell distribution. Despite demonstrating promising in vitro results, questions regarding the immunogenicity of the synthetic scaffold remain unanswered due to the limited in vivo evaluation, which was restricted to only two mice.

Alternatively, freeform reversible embedding of suspended hydrogels (FRESH) printing offers a strategy to overcome collapse during bioprinting without relying on synthetic materials. FRESH printing prints soft cell-laden constructs in a hydrogel support bath that can be easily removed after crosslinking or completion of the 3D bioprinted structures. For example, gelatin can be used as a support bath and then washed away by increasing the temperature.[53] However, if the print bed is heated to promote cell survival, gelatin is not a good option. To address this issue, Lee et al. used Carbopol as the support bath to print a single layer blood vessel in cardiac tissue.[54] Removal of Carbopol was easily achieved by dissolution in Dulbecco's Phosphate Buffered Saline (DPBS). While FRESH offers better biocompatibility, its constructs often lack the mechanical strength required for load-bearing or dynamically active tissues such as the trachea or esophagus.[55] This mechanical constraint limits their solitary use in tubular organ engineering applications. As a result, FRESH printing is more suited as a supplementary method than a total replacement for synthetic scaffolds. Hybrid techniques combining FRESH-printed bioinks with biodegradable polymers or composite materials present a viable option that balances biocompatibility and mechanical support.[56,57]

Besides structural integrity, adhesion between layers can be a second challenge in extrusion-based bioprinting. To overcome this problem, coaxial nozzles can be used to form multilayered hollow tubular structures. By concentrically assembling multiple needles, a multichannel nozzle can be created. Gao et al. used a double coaxial nozzle with alginate and decellularized ECM containing endothelial progenitor cells as an outer ink and Pluronic F-127 supplemented with calcium (Ca) ions as the inner ink.[58] The Ca ions ensured alginate gelation upon layer contact on the print platform and Pluronic F-127 was removed afterwards by increasing the temperature to create a hollow tubular construct. This approach successfully generated a functional blood vessel that promoted neovascularization and preserved limb viability in a murine ischemic model. However, the construct more closely resembled a capillary vessel rather than a larger artery or vein, which would require additional structural support provided by secondary or tertiary layers. While a double coaxial nozzle only allows for the fabrication of double-layered constructs, many tubes in nature consist of at least three layers (see Section 2). A triple coaxial nozzle can be designed to create more complex multilayered tissue constructs.

Pi et al. developed a nozzle with three individual channels to generate a biomimetic urethra and blood vessel.[59] A cell-laden hydrogel mixture consisting of GelMA, alginate, and poly(ethylene glycol) with tripentaerythritol (PEGOA) core was extruded through the outer two channels of the triple coaxial nozzle. The inner layer of the nozzle was flushed with Ca ions to physically crosslink the alginate component of the bioink and maintain an open lumen. For both urethral and vascular reconstruction, SMCs were encapsulated within the outer layer. UCs and human umbilical endothelial cells (HUVECs) were encapsulated in the middle layer for urethral and vascular reconstruction, respectively. Excellent cell viabilities of at least 89% after seven days were achieved. Another remarkable application of coaxial bioprinting for fabricating hollow, multilayered blood vessels was demonstrated by Wang et al.[60] Using both double and triple coaxial nozzles, they extruded alginate-based bioinks through the outer channels while introducing a Ca solution in the inner channel to facilitate crosslinking (Figure 2Ci). This approach successfully replicated both veins and arteries (Figure 2Cii). To assess the dynamic functionality of the construct, the bioprinted vessel was exposed to pharmacological stimulants that regulate dilation and contraction. Acetylcholine induced vessel dilation, while phenylephrine caused contraction as evident from diameter measurements (Figure 2Ciii). Notably, the artery mimicking tube was 3D-bioprinted with a SMC-laden outer hydrogel layer, followed by EC seeding to form the inner lining. The engineered artery exhibited key cell-specific markers indicative of successful biomimicry. Zonula occludens-1 (ZO-1) confirmed the formation of a tight endothelial barrier, while α-smooth muscle actin (α-SMA) validated the development of a functional muscular outer layer (Figure 2Civ). In addition, ex vivo experiments highlighted successful anastomosis of both small (1 mm) and larger (5 mm) bioprinted vessels with mouse and human vasculature, ensuring seamless perfusion (Figure 2Cv). Neither study[59,60] included in vivo evaluation, so their clinical translation potential remains untested at this stage. However, the structural design and functional layering of the constructs were promising. It would be valuable to explore their performance in relevant animal models to assess biocompatibility, integration, and functional outcomes, which are essential steps toward future clinical application.

The use of extrusion-based 3D bioprinting with regular and coaxial nozzles to engineer hollow tubular structures has shown promising results due to its ease of use and the ability to utilize a variety of materials. The concept of bioprinting is easily expandable to a variety of tissues upon design changes of hydrogels. One of the most transformative innovations of bioprinting in tubular tissue engineering is its capacity to fabricate patient-specific constructs derived directly from clinical imaging data, such as computed tomography or magnetic resonance imaging (MRI) scans.[61] This enables the creation of anatomically accurate and personalized grafts tailored to a patient's unique physiology, marking a major advancement toward personalized regenerative medicine. Despite the ease and personalized potential of this method, there are limited studies reporting on the development of cell-laden hollow tubular tissue constructs with more than one or two layers. For example, Lee et al. only reported on the 3D bioprinting of monolayer blood vessels containing ECs, which were more representative of capillaries.[54] Nevertheless, this work has great potential to be extended to multilayer blood vessels with a biomimetic structure closer to arteries and veins. This could be achieved by 3D bioprinting a second and third layer around the first, designed for the growth of SMCs and fibroblasts, respectively. These additional outer support layers are extremely important, as shown by the work of Gao et al.[58] Although ECs were able to proliferate well on the construct, the tube was too weak for suturing to the existing blood vessels. This could be overcome by designing a multilayered tube with an additional layer for structural support.[58]

Another significant drawback of extrusion-based bioprinting is the necessity for high pressures to achieve continuous extrusion of the hydrogels. These elevated pressures often generate substantial shear stress on cells, which can be detrimental to cell viability. Moreover, the resolution of the printed structures tends to be limited, as using smaller needles to enhance precision may result in even higher shear stresses to the cells. Interestingly, while these stresses lead to a high initial rate of cell death, they may promote cell interactions and network formation over prolonged culture periods.[62] However, the substantial initial cell loss reduces cost-effectiveness, posing a challenge for broader application. Furthermore, cell death after multiple days has been reported due to the lack of oxygen supply to the cells.[51] This could potentially be overcome by using dynamic culturing conditions in a bioreactor (see Section 4).

In addition to these biological challenges, extrusion-based bioprinting using coaxial nozzles also faces physical limitations. The maximum size of the printed construct is inherently constrained by the diameter of the needle assembly, which presents a major hurdle when attempting to fabricate human-scale tubular structures such as the trachea, esophagus, or urethra. Overcoming both the cellular and structural limitations of this method is crucial for advancing its clinical relevance.

3.1.2. Digital Light-Based Bioprinting

Digital light-based bioprinting (DLP) has emerged as an interesting tool for multi-material printing with high resolutions of 1–50 µm, significantly higher than extrusion-based bioprinting, which has a resolution of only 200 µm at best.[63] DLP uses light, usually in the UV range, to project 2D patterns onto a curing surface, initiating localized photopolymerization of the biomaterial resin. Kim et al. developed a biomimetic trachea tissue construct by DLP printing cell-laden methacrylated silk fibroin sheets that were then folded into a 3D structure.[64] A smooth inner layer containing turbinate-derived MSCs made up the base tube. This was surrounded by a patterned outer layer containing chondrocytes to replicate the structural cartilage rings of the native tracheal tube. After culturing the scaffold for three days, the tube was implanted in rabbits. The engineered tube successfully formed an intact epithelial layer and neo-cartilage within eight weeks, demonstrating promising results for this approach. However, the 3D printing device could only print one material at a time. To address this limitation, researchers have explored modifying standard DLP printers to enable simultaneous printing of multiple materials.

By integrating multiple material vats and a cleaning system into a standard DLP printer, materials can be easily interchanged, facilitating the creation of gradient structures with greater complexity.[65] Su et al. used this technology to create a three-layered trachea construct, with the individual layers mimicking the muscle, ligament, and cartilage (Figure 2D).[65] The mechanical properties of polyethylene glycol diacrylate (PEGDA) hydrogels were tuned for each layer by varying the polymer concentration and crosslinking time. MSCs were encapsulated within each layer and labeled with different colors for visualization. The spatial distribution of the cells was excellent and cell viability was > 90% after seven days. Nevertheless, the concentration of the light absorber R1800 was required to be low because of its cytotoxicity at higher concentrations, which compromised structural stability and resolution. A similar approach was also used by Yang et al. who developed a vascular network consisting of monolayer tubes with varying mechanical properties.[63] ECs were successfully cultured on the construct. While these two recent studies have introduced the potential of multimaterial DLP bioprinting for fabricating complex tissue constructs, neither has yet incorporated in vivo evaluation. As such, the clinical translation of this approach remains unassessed. Nonetheless, the ability of DLP to achieve microscale resolution and precise spatial control highlights its strong potential for developing anatomically accurate and scalable tissue-engineered grafts.

A key innovation of DLP-based printing is its exceptional resolution, which enables the fabrication of highly complex and finely detailed geometries that are not achievable with extrusion-based approaches. This precision facilitates the creation of intricate multilayered architectures essential for engineering functional tubular tissues. However, despite its potential, relatively few studies have demonstrated its use for fabricating cell-laden multilayered tubular structures. One major limitation is the challenge of implementing multiple materials within the same print. Multimaterial DLP printers are costly and require thorough cleaning between material changes to avoid cross-contamination, significantly increasing fabrication time. Additionally, DLP printing relies on photopolymerization to crosslink hydrogels, which can introduce cytotoxicity if photoinitiators are not sufficiently biocompatible. Therefore, further advancements are required in developing cell-friendly photocrosslinkable materials and absorbers to preserve high cell viability throughout the printing process. Once the cell viability concerns are adequately addressed, the next critical step should be the integration of in vivo studies to evaluate functional performance and host response. While recent studies[63,65] have demonstrated the feasibility of multimaterial DLP for generating anatomically relevant multilayered constructs, no in vivo evaluation has yet been reported, and clinical translation remains elusive. Another limitation of current DLP strategies is the difficulty to directly fabricate 3D tubular geometries. Most existing approaches rely on printing flat, layered constructs followed by a separate folding or rolling step to achieve a tubular shape.[64] To address this, future efforts should focus on enabling direct in situ tubularization within the DLP platform. Innovations such as volumetric 3D bioprinting or multiphoton polymerization may be exciting avenues to explore to successfully engineer multilayered hollow tubular tissue constructs using light-based bioprinting.[66,67]

3.2. Cell-Embedded Electrospinning

Electrospinning is a widely used technique for fabricating multilayered tubular structures. Tubularization is typically achieved by depositing a polymer solution onto a mandrel or needle under high voltage. The rotation of the mandrel provides an additional advantage by aligning polymer fibers, which not only enhances cell alignment, spreading, and viability but also mimics the anisotropic architecture of native tissues.[68,69] An alternative approach to achieving fiber alignment involves using oppositely charged electrodes, which pull fibers into an aligned direction (**Figure 3A**).[70] By adapting flow rate, voltage, electrode distance and needle-to-electrode distance, fiber properties can be adapted. The ability to replicate the aligned topography found in native tubular tissues makes fiber orientation a valuable tool for creating biomimetic structures. This precise architectural replication sets electrospinning apart from other fabrication methods, offering unparalleled control over microstructural mimicry of native tissue.

Beyond fiber alignment, researchers have also explored innovative mandrel modifications to enhance structural biomimicry. Since native blood vessels exhibit longitudinal grooves that contribute to their biomechanical properties, Rodriguez-Soto et al. adapted their mandrel design by first creating a microgrooved PCL scaffold using lithography (Figure 3Bi).[71] This scaffold was then wrapped around the mandrel before electrospinning two distinct fiber layers: PCL/salt and PCL/gelatin (Figure 3Bii). Although this design was primarily intended to promote SMC alignment for blood vessel reconstruction, it was not evaluated in vitro with SMCs, providing limited insight into its effectiveness. Similarly, to replicate the cartilage rings necessary for tracheal repair, Townsend et al. 3D printed PCL rings and affixed them to the mandrel, allowing nanofibers to be deposited onto the structured surface (Figure 3C).[72] This scaffold demonstrated sufficient tissue integration following implantation in a rabbit model, indicating promise for clinical translation. However, incorporating a natural component was necessary to maintain an open tracheal lumen comparable to that of an uninjured trachea, as fully synthetic scaffolds alone resulted in a reduced airway diameter. Similarly, the encapsulation of relevant cell types within the scaffold could further enhance integration and functional outcomes by better replicating the native cellular environment. These innovative mandrel designs, which generate important topographical features, demonstrate the great potential of electrospinning as a fabrication technique for engineering tubular tissue constructs. Further development of these techniques could enable the fabrication of cell-laden electrospun multilayered tubular scaffolds for applications in urethral, esophageal, vascular, and tracheal tissue engineering, an approach that has only been achieved using conventional electrospinning followed by post-seeding.

To bridge the gap from post-seeding to cell-embedding, the critical challenge of limited cell viability, one of the major barriers to the widespread adoption of this approach, must be addressed. It was found that a drastic drop in cell viability occurred when the voltage applied during cell-embedded electrospinning exceeded a critical threshold.[70] This limitation is a major drawback, as high voltages are typically required for effective fiber formation. In addition, in most electrospinning applications, synthetic polymers must first be dissolved in organic solvents,[73] which limits their use in engineering cell-laden constructs. Green electrospinning was developed to use safer solvents such as acetic acid or aqueous-based systems that comply with FDA Class 3 solvent guidelines, mitigating toxicity concerns.[74] However, water-soluble systems may lead to rapid scaffold degradation in aqueous cell culture conditions.[75] Protection of cells throughout the manufacturing process can be used to address the issue of solvent toxicity.[44] For example, Weidenbacher et al. utilized microfluidic encapsulation combined with cell electrospinning to protect cells from organic solvent exposure by first depositing them in polymer capsules before fiber formation.[76] In addition, simple post-processing procedures can be applied to remove remaining solvents prior to cell culture.[73] To improve cell viability, Zhao et al. developed an innovative co-electrospinning technique (Figure 3D), in which cells were encapsulated inside alginate microgel beads before being deposited within an electrospun fibrous scaffold.[77] The cells could be released on demand by treating the scaffold with a dilute sodium citrate solution to dissolve the microspheres. This strategy maintained high cell viability (96%) across different cell densities (1×10^7 and 5×10^6 cells mL^{-1}), while also maintaining reactive oxygen species levels low, indicating minimal cellular stress. This strategy highlights how electrospinning can be adapted to address key translational barriers, such as maintaining high cell viability during scaffold fabrication. Ensuring high cell viability is a fundamental prerequisite, since without it, further translational progress remains out of reach.

While maintaining cell viability remains a primary hurdle for cell-embedded electrospinning, achieving precise spatial organization of cells within multilayered constructs presents an additional challenge compared to other advanced biofabrication methods such as 3D bioprinting or microfluidic patterning. This lack of spatial accuracy limits the capacity to design large, multicellular structures that closely resemble biological tissue organization.[78] Strategies developed for post-seeding offer promising insights that could be adapted to overcome this limitation in cell-embedded approaches. For instance, Soliman et al. developed a multilayered electrospun scaffold for esophageal regeneration by designing a three-layered construct with large pores on the inner and outer layers and small pores in the middle.[79] SMCs were seeded onto the outer layer, while MSCs were introduced into the inner layer. The nanoporous middle layer served as a barrier to prevent unwanted cell migration between layers. A similar approach could be translated to cell-embedded electrospinning by tuning fabrication parameters to generate porous fiber architectures capable of supporting precise spatial organization of encapsulated cells. Nevertheless, this approach enables compartmentalization of different cell types but does not permit precise spatial alignment at the single-cell level, which is more effectively achieved through techniques such as 3D bioprinting.

3.3. Gel Casting

One of the earliest methods for hydrogel production is gel casting, in which a prepolymer solution is injected into a mold and crosslinked into a hydrogel using physical, chemical, or enzymatic crosslinking techniques. The simplest way to obtain cell-laden tubular scaffolds is to inject a cell-laden prepolymer solution into a mold with a glass rod in the center to create the open lumen.[80] However, to develop multilayer tubular scaffolds, a separation between the mold chambers should be achieved to ensure a clear separation of the prepolymer solutions used to fabricate different layers.

To create a cell-laden multilayered tubular structure via gel casting, Van Velthoven et al. used a polydimethylsiloxane (PDMS) mold containing three separate chambers to create a cell-laden gelatin hydrogel sheet with each chamber holding different cell

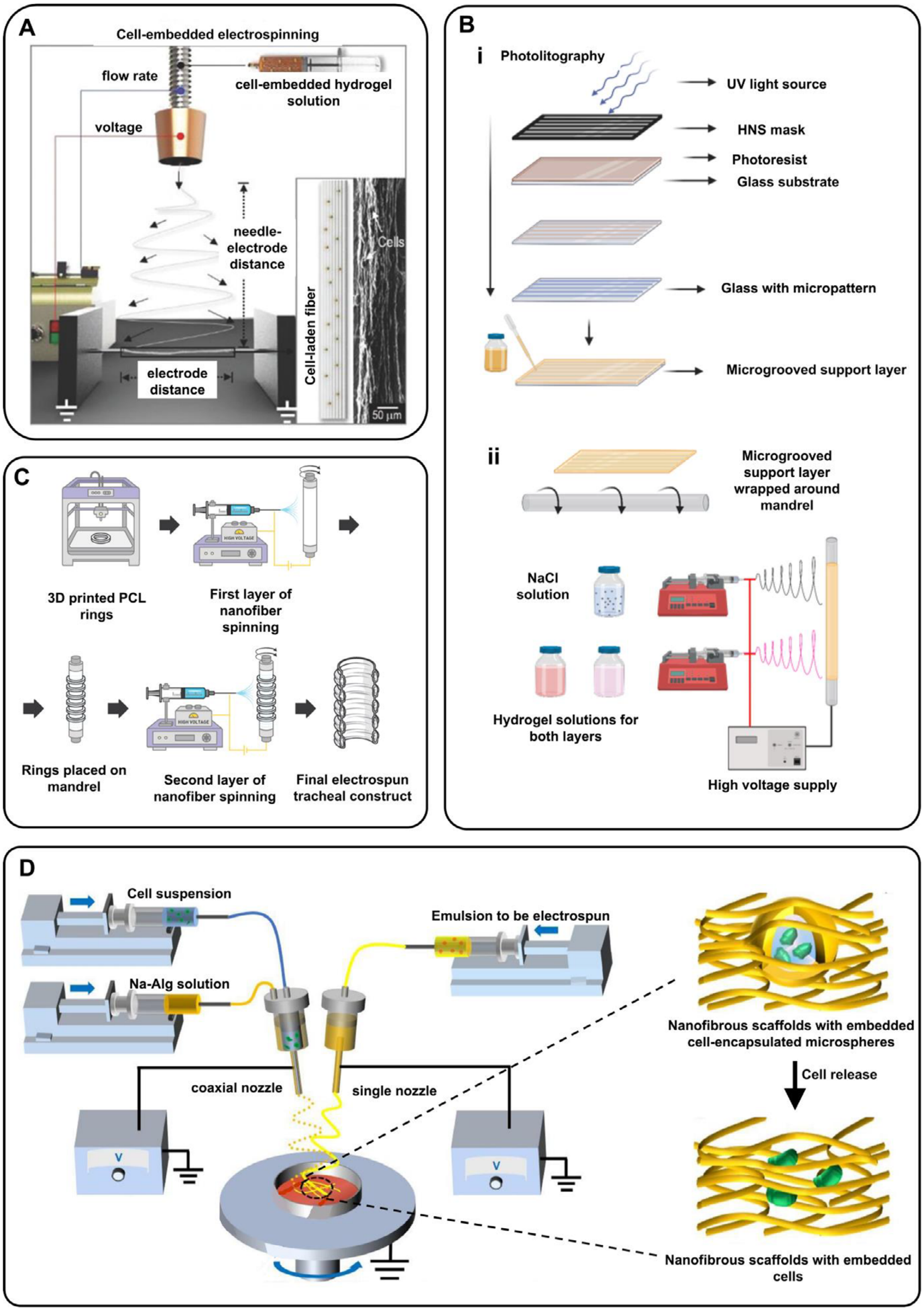

Figure 3. Cell-embedded electrospinning to generate biomimetic hollow tubular tissue constructs. A) Schematic of the cell-embedded electrospinning process to fabricate anisotropically aligned cell-laden nanofibers. Reproduced with permission.[70] Copyright 2025, John Wiley and Sons. B) The fabrication of blood vessel inspired three-layered scaffolds. i) Fabrication of a microgrooved support layer using photolithography; (ii) electrospinning of hydrogels on top of the support layer to fabricate the second and third layers. Reproduced with permission.[71] Copyright 2025, MDPI. C) Adaptation of a mandrel with 3D printed rings to form a tracheal construct by electrospinning. Reproduced with permission.[72] Copyright 2025, IOP Publishing. D) A combined emulsion and coaxial cell-embedded electrospinning process to fabricate nanofibrous scaffolds with cell-encapsulated microspheres. Reproduced with permission.[77] Copyright 2025, Elsevier.

types.[24] First, a mesh was placed over the PDMS mold, followed by the addition of the cell-laden gelatin prepolymer solutions. The smallest chamber remained acellular, the middle chamber contained SMCs, and the largest chamber was filled with a co-culture of HUVECs and pericytes. Once crosslinked and removed from the mold, the hydrogel sheet was rolled into a hollow tubular construct, with the acellular layer forming the inner surface, the SMC-laden layer in the middle, and the HUVEC/pericyte layer on the outer side. The construct was sutured and cultured for seven days before seeding the inner layer with epithelial cells. While this approach enabled the relatively simple fabrication of a multilayered hollow tube with distinct cell layers, the vasculature failed to survive after UC seeding, which may limit the overall functionality of the engineered construct.

A more advanced version of the latter technique used an innovative PDMS mold that allowed the cell-loaded sheets to self-roll as demonstrated by Yuan et al. (**Figure** 4Ai).[81] This specific PDMS mold consisted of a fully cured stretched layer and a semi-cured PDMS membrane that created internal stress in the mold. The combination of these two layers was referred to as the stress-induced rolling membrane (SIRM). PDMS channels containing cell-laden solutions were attached to these layers. After culturing the cells, the channels were removed and the internal stress of the bottom layers caused the cell sheets to roll up. Biomimetic blood vessels were then created by casting and rolling up HUVEC (inner, red), SMC (middle, green), and fibroblast (outer, blue) solutions (Figure 4Aii–iv).[81] While the co-culture of cells within the scaffold design is well-suited for promoting blood vessel regeneration, no in vivo functional assessments were conducted to evaluate its efficacy. Instead of using different PDMS channels, various cell layers were also directly cultured on top of a SIRM device to form multilayered structures.[82] The development of compartmentalized PDMS molds and SIRM devices represents a significant innovation in gel casting, enabling the fabrication of multilayered structures using straightforward, cost-effective techniques that are easily implemented in standard laboratory or clinical settings.

Another approach for engineering multilayered cellular tubular constructs is the use of cell sheets instead of cell-laden hydrogels. For example, Williams et al. engineered cell sheets that were subsequently folded into a tubular construct (Figure 4B).[83] First, cell sheets were attached to a structural support and rolled inside a tubular mold. Next, a gelatin hydrogel was poured and crosslinked into the negative space formed by the rolled-up cell sheets to ensure the structural integrity of the lumen. The entire construct containing hydrogel and cell sheets was then removed from the mold, followed by removal of the support, resulting in a tubular construct containing cell sheets and hydrogel. This method demonstrated its versatility by successfully generating smooth muscle, cardiac, and skeletal cell sheets, as evidenced by in vitro cell markers, but not yet by in vivo testing.

Instead of using a mold in which the cell sheets are folded into a tubular construct, a glass capillary assembly can be used (Figure 4C).[84] In this method, cell or cell-laden hydrogel sheets were first generated in vitro and systematically wrapped around a structured capillary assembly, consisting of an inner glass capillary positioned within two outer capillaries (Figure 4Ci,ii). To enhance structural stability, a collagen gel was applied along with assistive jigs that maintained alignment during fabrication (Figure 4Ciii). In the final step, the inner capillary was carefully removed, leaving the cell or hydrogel sheets supported externally by the two outer capillaries (Figure 4Civ). The resulting tubular construct was then perfused to facilitate cell maturation and tissue development.

Centrifugal gel casting is another variation of gel casting that eliminates the need for molds, thereby simplifying the fabrication process. In this technique, cell-laden hydrogels are centrifuged inside a cylindrical vial, where centrifugal forces cause the hydrogel to coat the inner surface of the vial. This method was used to fabricate a hollow hyaluronic acid (HA)/ECM-based hydrogel structure encapsulating epithelial cells (Figure 4D).[85] The gel required a centrifugation speed of 2000 rpm to successfully coat the inner surface (Figure 4Di,ii). Furthermore, the study demonstrated that the choice of hydrogel is critical for success. While HA/ECM yielded uniform tubular constructs (Figure 4Diii,iv), collagen and agarose formulations failed under the same conditions. Importantly, epithelial cells tolerated the centrifugal forces, however, other cell types may be more sensitive, particularly with repeated spinning cycles. Using this technique, different cell-laden hydrogels can be sequentially centrifuged to create multilayered structures. By adjusting the hydrogel volume, the thickness of each layer can be controlled.

Although gel casting techniques are relatively simple and do not require hydrogels with specialized properties (e.g., photoresponsiveness, extrudability, or voltage sensitivity), their main limitation is their lower resolution compared to other microfabrication methods. It could be interesting to look at hybrid approaches in the future that combine gel casting with higher-resolution techniques such as extrusion-based bioprinting. For example, cell-laden hydrogels could be printed inside the casting chambers to improve cellular placement. Another limitation is that the advantage of ease comes at the expense of time efficiency, as the gel casting process typically involves multiple casting and gelation steps, making it labor-intensive and time-consuming. To make gel casting more viable for clinical and industrial-scale applications, future research should focus on automating the layering process, possibly through integration with robotic systems or programmable mold systems to reduce manual labor and ensure reproducibility. Finally, advances in hydrogel formulations that allow for enhanced interlayer adhesion could also improve the structural fidelity and mechanical stability of the final constructs.

3.4. Self-Folding Cell Sheets

Instead of relying on manual folding of cell sheets or the use of molds (as discussed in Section 3.3), the intrinsic contractile forces of the cells can be harnessed to drive their self-assembly into hollow tubular constructs.[86–89] This automation significantly reduces labor and enables the formation of complex, biomimetic geometries that are difficult to achieve using conventional approaches representing a paradigm shift toward scalable and mold-free tissue fabrication. This process involves the creation of a three-layered porous film containing a sacrificial Ca-alginate layer, a middle layer, and a parylene-C layer. Dissolving the sacrificial layer triggers the contraction of the remaining two layers, leading to spontaneous rolling into a tubular structure.

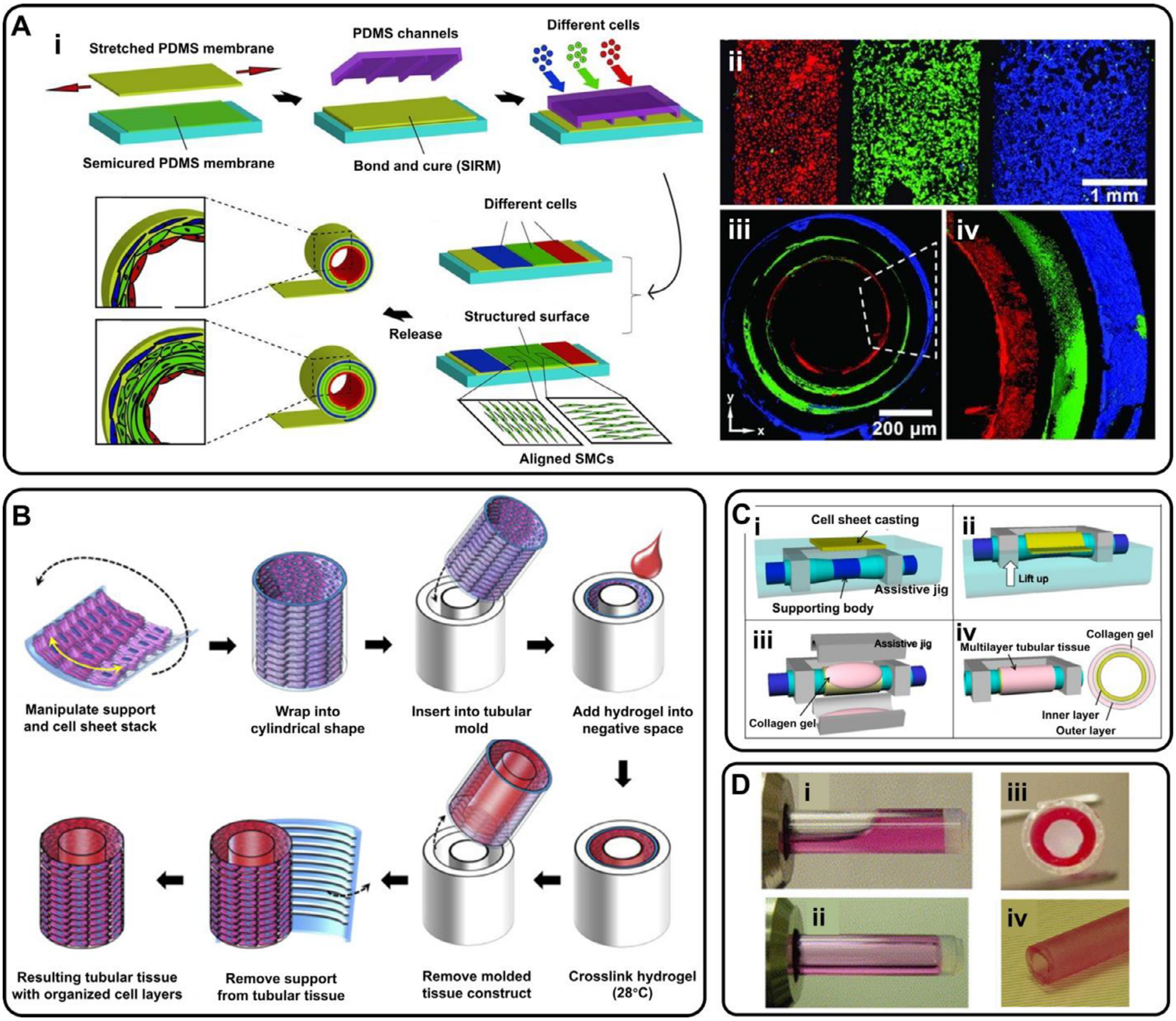

Figure 4. Gel casting for fabrication of multilayered tubular structures. A) A stress-induced rolling membrane (SIRM) used to fabricate multilayered cell-laden tubular structures. i) Schematic of the SIRM fabrication, showing the use of a bilayer polydimethylsiloxane (PDMS) mold with built-in stress to induce spontaneous rolling of cell sheets into tubular structures after channel removal; ii) the PDMS chambers of the SIRM were filled with three distinct cell types (human umbilical vein endothelial cells (HUVECs) in red, smooth muscle cells (SMCs) in green, and fibroblasts in blue); iii) upon rolling, cells distributed in 3D, forming tube walls similar to blood vessels, with endothelial cells (ECs), SMCs, and fibroblasts arranged from inside out; iv) magnified side view of the dashed region in (iii). Reproduced with permission.[81] Copyright 2025, John Wiley and Sons. B) A schematic of the tubular tissue casting process using multilayered cell sheet stacks. Reproduced with permission.[83] Copyright 2025, Elsevier. C) Cell sheet casting using a glass capillary assembly; i, ii) cell sheets are deposited layer-by-layer on a glass capillary setup; iii) a supporting collagen hydrogel and assistive jigs are placed; iv) the inner glass capillary is removed and the multilayered tubular construct is matured into a hollow tissue tube. Reproduced with permission.[84] Copyright 2014, AIP Publishing. D) A centrifugal gel casting technique to create a tubular construct based on hyaluronic acid (HA)/extracellular matrix (ECM) hydrogel encapsulating epithelial cells. i) The lack of tubular hydrogel formation at 1000 rpm; ii) the concentric redistribution of tubular hydrogel at 2000 rpm; iii) cross-section of the crosslinked tubular hydrogel following 10 min of rotation; iv) the crosslinked tubular hydrogel removed from the glass tube. Reproduced with permission.[85] Copyright 2025, Elsevier.

The tube's curvature can be finely tuned by varying the thickness of the parylene-C layer, with thinner layers yielding smaller diameters. A variety of materials, such as silk fibroin,[89] graphene,[87] hexagonal boron nitride,[86] and molybdenum disulfide,[86] have been used to generate the middle layer. For example, Sakai et al. used graphene in the middle layer to construct arterial-like structures (**Figure 5Ai**).[87] Following the dissolution of the Ca-alginate layer, the film self-rolled into a tube, with curvature controlled via the parylene-C thickness (t_p) (Figure 5Aii). To promote cellular attachment, the film was coated with fibronectin and seeded with HUVECs. Other coatings including polyethyleneimine (PEI) and laminin have also been reported to enhance cell attachment.[90]

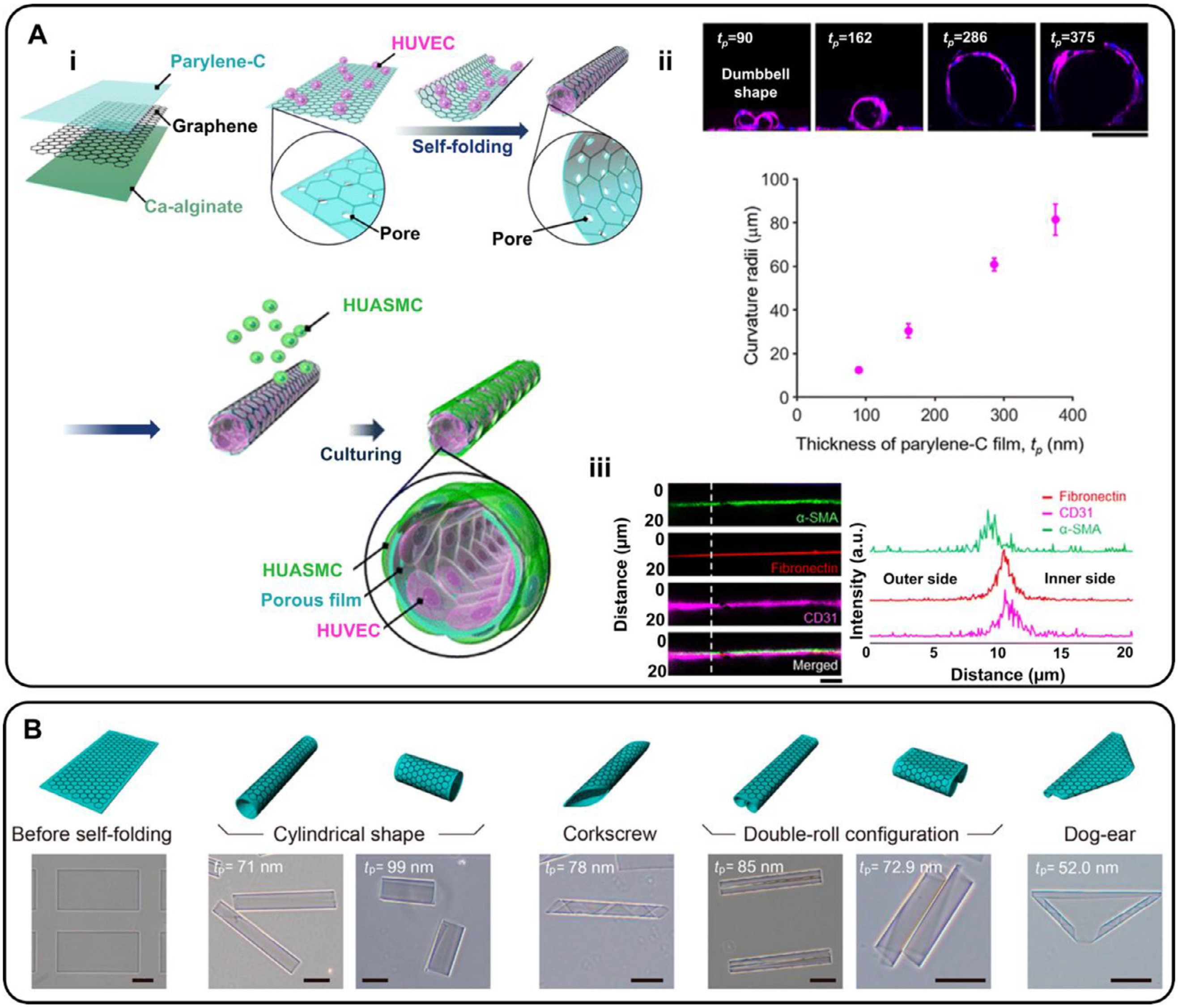

Figure 5. Self-folding cell sheets for the fabrication of multilayered hollow tubes. A) Multi-layered 3D self-folded tube that mimics small arteries. i) The fabrication of a self-folded tube by generating a three-layer porous film containing a sacrificial calcium (Ca)-alginate layer, a middle layer, and a parylene-C layer. Dissolution of the sacrificial layer initiates the contraction causing the two-layer membrane to self-fold into a hollow tube. Human umbilical vein endothelial cells (HUVECs) and smooth muscle cells (SMCs) are incorporated in the tube's inner and outer surfaces, respectively; ii) cross-sectional images of HUVECs in tubes with different parylene-C thickness, t_p, stained for CD31 (magenta) and nuclei (Hoechst 33 342, blue), as well as curvature radius plots showing increased curvature with thicker parylene-C layers (scale bar: 100 µm); iii) immunocytochemical images of the tube wall with an intensity profile along the white dotted line, showing HUVECs (CD31, magenta) and SMCs (α smooth muscle actin (α-SMA), green). The boundary between the outer and inner sides is marked with rhodamine-conjugated fibronectin (red). Reproduced with permission.[87] Copyright 2025, Royal Society of Chemistry. B) Illustration of the variety of 3D self-foldable tubes with accompanying optical pictures (scale bar: 100 µm). Reproduced with permission.[88] Copyright 2025, American Chemical Society.

CD31 staining confirmed the formation of a continuous HUVEC monolayer inside the tube (Figure 5Aiii), while SMCs seeded on the outer surface were identified using α-SMC markers, demonstrating the creation of a multilayered arterial construct. Interestingly, the porous film can also be rolled into a variety of geometries, as demonstrated by Teshima et al., who used a similar three-layered membrane incorporating graphene as the middle layer to fabricate diverse tubular configurations (Figure 5B).[88] This geometric versatility makes the technique particularly promising for applications in tracheal or urethral tissue engineering, where the native anatomy features distinct wrinkled or star-like morphologies.

Future advancements in multilayered tubular tissue engineering may increasingly leverage smart, responsive materials to enhance construct functionality and adaptability. One promising avenue in self-folding sheets involves the local functionalization with temperature-sensitive polymers, such as poly(N-isopropylacrylamide), to enable spatially controlled self-folding of porous layers.[91] The functionalized regions exhibit an enhanced degree of folding when the temperature is increased to 35–45 °C, which falls within the physiological range. As a result, controlled folding can occur during cell culture or even post-implantation. Moreover, the folding process is reversible, allowing the structure to unfold upon cooling, providing dynamic tunability for biomedical applications. Expanding on this, incorporating pressure- or compression-sensitive polymers could further refine control over scaffold behavior, particularly for applications in tracheal or urethral tissue engineering where mechanical adaptability is critical. In addition, the integration of electrically conductive components within the scaffold's middle layer has shown potential for both functional enhancement and biosensing.[86] For instance, such conductive materials can support the real-time monitoring of physiological activity like the beating of embedded cardiomyocytes or facilitate neural regeneration by aiding signal transmission. These multifunctional materials offer exciting opportunities to bridge regenerative medicine with smart diagnostics, setting the stage for the next generation of responsive, therapeutically active tubular constructs.

3.5. Microfluidic Technologies for Tubular Tissue Engineering

Microfluidic techniques have revolutionized the fabrication of hollow tubular constructs by offering precise control over micro- and nano-scale architectures. As a result, these approaches have gained significant attention in physiological modeling.[92–94] Despite their primary use in modeling, microfluidic strategies for tissue engineering are emerging as highly relevant tools for constructing biomimetic tubular structures.[95–98] However, most current reviews focus on material design[97,98] or are highly specific to bio-actuation[95] or blood vessel network formation[96] without focusing on the design of multilayered hollow tubular structures. Therefore, the current section focuses specifically on the use of different microfluidic fabrication techniques for the generation of multilayered tubes.

3.5.1. Microfluidic Spinning

Microfluidic spinning emerged in the early 2000s as a novel technique for fabricating hollow microfibers.[99] Unlike electrospinning, it does not require high voltages or organic solvents, making it more suitable for cell-embedded structures.[100] Two primary setups exist, whereby one utilizes a microfluidic chip[99] and the other employs a nested capillary approach.[101,102] In both setups, coaxial flow patterns are used to shape the fiber. Hollow architectures are formed by introducing an additional core solution. The geometry and complexity of the resulting microfibers can be finely tuned by manipulating the flow dynamics and channel arrangements.[103] Moreover, integrating fluid flows with pneumatic valves enables extreme spatial control, allowing the creation of multi-compartmental structures.[104] After microfiber production using either one of these techniques, microfiber stabilization is achieved through polymer-specific crosslinking methods, such as photo-induced curing for GelMA or Ca ion crosslinking for alginate.[105] The produced microfibers could be further processed by weaving or knitting to generate higher-order structures.[106] Hollow microfibers hold significant potential for integration into larger tissue-engineered constructs to mimic vascular networks.

Examples of both setups are provided in **Figure 6A–C**.[99,102] In the microfluidic chip setup (Figure 6A), a PDMS chip housed a tapered sample capillary through which the sample solution flowed, surrounded by a larger capillary that delivered a sheath solution to maintain hydrodynamic stability of the sample solution. To produce hollow microfibers, an additional core capillary introduced a core solution in the center of the sample solution. Complex architectures were generated (Figure 6C) by arranging multiple capillaries in parallel.[103] The nested capillary approach (Figure 6B) involved directly connecting various capillaries, including inlet (iv), outlet (iii), and spindle injection capillaries (vi-vii), to a glass slide (i) and needles (v) for fluid delivery.[102]

Cheng et al. reconstructed blood vessels using microfluidic spinning with nested capillaries.[107] They encapsulated HUVECs in a mixture of alginate and GelMA, which was used as the sample solution. A 2% calcium chloride ($CaCl_2$) solution and a mixture of 0.2% $CaCl_2$ and 10% polyvinylalcohol were used as sheath and core solution, respectively. UV crosslinking was applied to stabilize the fibers. The cells were well distributed on the inside of the hollow microfiber and a high cell viability of 85% was achieved after five days. In this study, the successful formation of a cell-laden structure was demonstrated, but the generation of multilayered structures was not yet achieved. In another study, Paradiso et al. fabricated a multilayered microfiber.[108] They used an MSC/HUVEC encapsulated fibrin hydrogel as the core layer and an alginate solution as the surrounding layer. Although the co-culture was effective and showed good cell survival rates, no cells were encapsulated in the alginate hydrogel, therefore, no multilayered cell-laden microfibers were formed. In addition, the microfiber was not hollow. Nevertheless, the microfibers were collected on a rotating mandrel, demonstrating the possibility of post-processing the microfibers to generate higher-order fiber bundles. An example of multilayered cell-laden hollow microfibers is presented by Yue et al.[109] They utilized a microfluidic chip to construct a PEGDA microfiber with fibroblasts encapsulated in the inner and outer layer. The cells had a viability higher than 80% despite their exposure to UV light. This study demonstrated the success of using microfluidic spinning to fabricate multilayered hollow tubular tissue constructs. However, as mentioned before, the main advantage of regular electrospinning is the ability to generate topographical features during the fabrication of these tubes. To generate these features in microfluidic spinning, novel techniques have been integrated.

Recently, the rope-coiling technique has been integrated with microfluidic spinning to fabricate hollow, helically structured conduits.[110–112] In this approach, a hydrogel precursor solution is injected into a capillary containing a sheath fluid. By increasing the ratio of inner to outer flow, the hydrogel precursor forms a helical stream that compacts along the capillary walls. This packed hydrogel is then stabilized through light- or ion-induced crosslinking. By fine-tuning the inner and outer flow rates, various stream patterns and distinct topographical features have been achieved (Figure 6Di).[112] Additionally, placing multiple capillaries in parallel allowed for the use of multicomponent hydrogels as inlet streams, enabling the fabrication of multicompartment tubes (Figure 6Dii).[112] These helically structured tubes with longitudinal compartments hold great potential for tracheal tissue engineering, as they closely mimic the native topography and provide defined compartments for cartilage ring

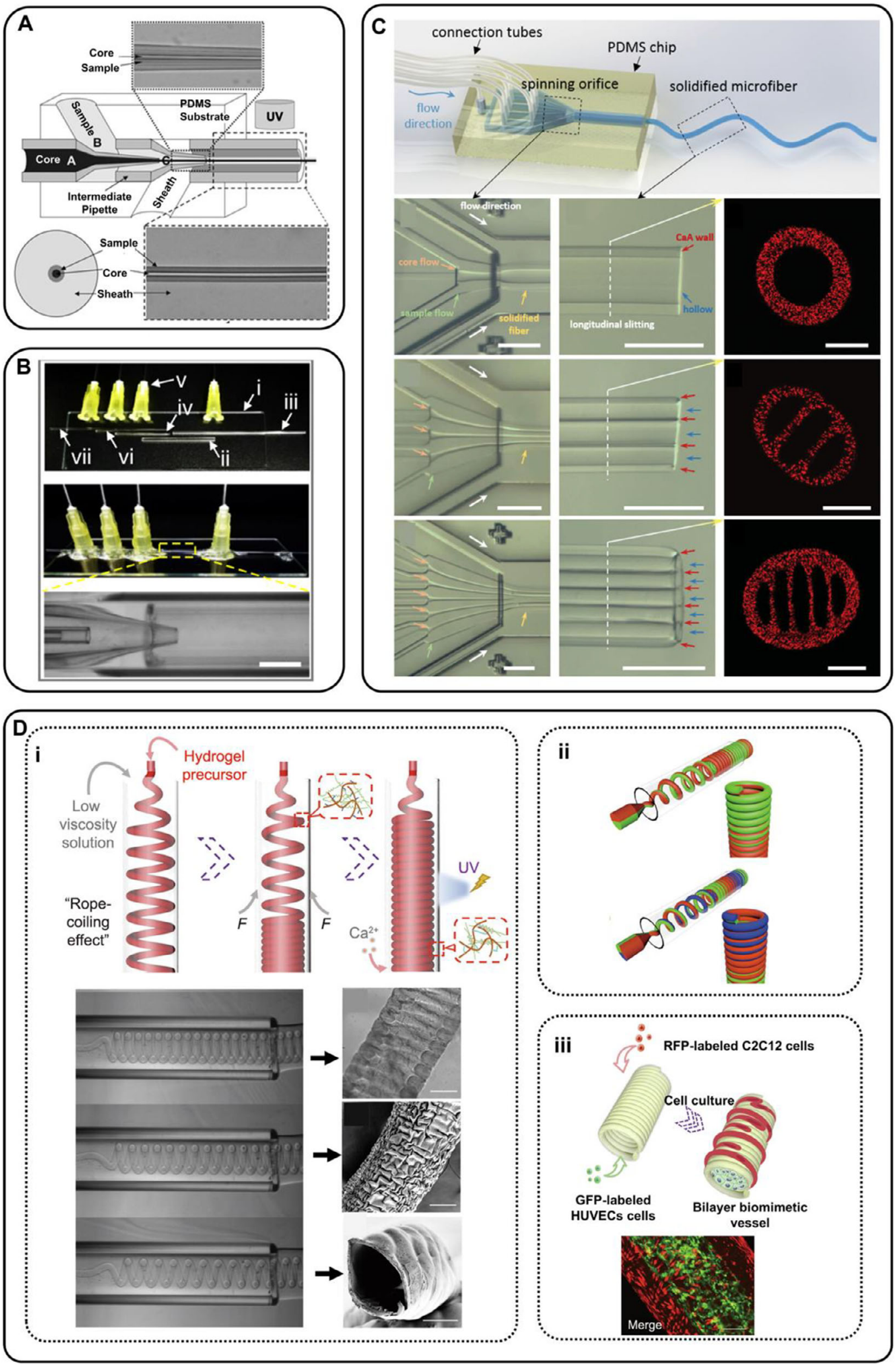

Figure 6. Microfluidic spinning for the fabrication of multilayered hollow tubes. A) Microtube fabrication by using a microfluidic chip. The core fluid and the sample fluid are introduced into inlets "A" and "B", respectively, forming a core–shell flow at position "C". A sheath fluid surrounds the sample fluid for hydrodynamic stability. Reproduced with permission.[99] Copyright 2025, Royal Society of Chemistry. B) Components and detailed views of a nestled capillary microfluidic assembly: i) glass slide; ii) square capillary; iii) collection capillary; iv) original injection capillary; v) needle; vi, vii) spindle

reconstruction. However, further advancements are needed to scale up this technique for larger-diameter tubes. This rope-coiling microfluidic spinning can also be adapted to generate multilayered tubes with microgrooves for blood vessel regeneration, as evidenced by the work of Liu et al. (Figure 6Diii).[110–112] In their study, myoblasts were embedded within the hydrogel, forming the helical outer layer of the construct, while HUVECs were seeded on the inner lumen once myoblasts reached 80% confluency. Both cell lines nicely spread throughout their respective layers with improved alignment of myoblasts in the bioinspired screwed conduits compared to regular tubular constructs.

Microfluidic spinning offers several advantages over PDMS-based systems, including lower cost, improved surface hydrophilicity, stable coaxial flow generation, and precise control of the diameter.[105] This makes it a powerful strategy for scalable fabrication of tubular constructs with high geometric precision. However, the fragility of glass capillaries poses a challenge, making the device synthesis highly time-consuming. Another key challenge is the lack of studies using this technique to produce multilayered constructs with distinct encapsulated cell types. While studies have relied on single-layer cell encapsulation or post-seeding strategies, emerging work demonstrates the feasibility of true multilayered, cell-laden constructs. Continued development of coaxial and multi-stream configurations will be key to achieving reproducible multilayered encapsulation with distinct cell types.

3.5.2. Microfluidic Bioprinting

Recent advancements in microfluidic technology for engineering tubular structures have led to the integration of microfluidic chips with bioprinters. These chips can either be positioned in front of the bioprinter needles[113–116] or serve as the nozzle itself.[117,118] The latter approach is particularly advantageous, as it significantly reduces mechanical stress on cells, which is critical when working with highly sensitive cell lines.[119] A key benefit of microfluidic printheads is their ability to facilitate the immediate processing of generated microfibers. By incorporating multiple inlets and a single outlet, these systems enable the seamless printing of multi-material structures with a single printhead.[116]

Feng et al. used this approach to 3D bioprint large-diameter blood vessels.[116] Their approach involved extruding an alginate bioink containing HUVECs or H9C2 cells through two separate inlets, while a third inlet introduced a Ca solution to crosslink the alginate upon extrusion. This process enabled the deposition of a two-layered bioink in a circular manner, successfully fabricating a structurally relevant large blood vessel. Attalla et al. effectively encapsulated two different cell types, HUVECs and fibroblasts, in alginate bioinks that were extruded as the inner and outer layers, respectively, in a microfluidic printhead, mimicking the architecture of blood vessels.[114] A comparable approach was also used to create a double-layered hollow tube containing HUVECs and osteoblast-like cells.[120] It should be noted that current studies on microfluidic printing are limited to in vitro evaluations, and without supporting in vivo data, the clinical readiness of these approaches cannot yet be assessed.

Recent advances in microfluidic bioprinting have overcome some of the size constraints associated with traditional spinning techniques, enabling the creation of human-scale tubular constructs. This ability to integrate spatial precision with macroscale fabrication represents a key step toward clinical translation. However, scalability remains a critical limitation. While this method excels at fabricating small-diameter tissues, especially vascular structures, it struggles with larger constructs like the urethra, trachea, or esophagus due to the confined dimensions of microfluidic channels. Future developments should focus on scaling up channel architectures and integrating modular printing systems to expand construct size. Additionally, combining microfluidic precision with hybrid bioprinting platforms could enhance both scalability and complexity, paving the way for broader clinical applications.

3.6. Combination of Fabrication Techniques for Engineering Multilayered Tubular Tissues

Recently, researchers have considerably explored the combination of multiple fabrication techniques to overcome the limitations of individual techniques and boost tubular tissue engineering. For example, hybrid methods that combine electrospinning and 3D bioprinting have produced tubular scaffolds with improved structural and biological properties. Fazal et al. introduced a hybrid open-source technology that combines extrusion bioprinting with electrospinning heads.[121] This approach enabled the fabrication of tubular constructs featuring alternating layers of hydrogel and electrospun fibers. By combining these techniques, the system harnesses the personalization and spatial control of 3D bioprinting alongside the biomimetic architecture provided by fibrous scaffolds, offering a versatile strategy for engineering multilayered tubular tissues. Nevertheless, this work currently serves only as a proof-of-concept, as cell-laden hydrogels have not yet been utilized within this system, nor has it been applied to fabricate multilayered cell-laden constructs. Another promising combination is microfluidics with cell electrospraying, which improves cell viability by protecting cells with an organic solvent capsule, as discussed in Section 3.2.[76] However, most of the hybrid strategies to date are adapted for

injection capillaries (scale bar: 500 µm). Reproduced with permission.[102] Copyright 2025, Springer Nature. C) Flow-controlled fabrication of calcium-alginate (CaA) microfibers with varying hollow structures. Flow dynamics in the microfluidic assembly for fibers with one, three, and five hollow channels are shown. Arrows indicate flow directions: white (overall), pink (core), green (sample), yellow (solidified fiber) (scale bar: 500 µm). Corresponding microfibers in an aqueous solution are shown, cut to expose hollow structures. Red arrows mark CaA walls, blue arrows indicate hollow channels (scale bar: 250 µm). Confocal images of longitudinal fiber cross-sections are shown (scale bar: 100 µm). Reproduced with permission.[103] Copyright 2025, John Wiley and Sons. D) Fabrication of bioinspired screwed conduits (BSCs) and biomimetic microvessels. i) Schematic of the coaxial microfluidic setup for continuous BSC fabrication utilizing the rope-coiling effect. Photographs showing flow patterns during BSC formation at varying flow rates (scale bar: 1 mm). Optical microscopy images (scale bar: 300 µm) and scanning electron microscopy (SEM) images (scale bar: 150 µm) of produced BSCs.; ii) construction of multicompartment BSCs; iii) use of BSCs to promote cell differentiation with a confocal image of a cell co-culture in BSCs (scale bar: 200 µm). RFP: red fluorescent protein; GFP: green fluorescent protein. Reproduced with permission.[112] Copyright 2025, Elsevier.

post-fabrication cell seeding and not cell encapsulation during fabrication. For example, electrospinning has been integrated with microfluidics to combine the precise control of fluid handling and perfusion offered by microfluidics with the structural and topographical guidance of aligned nanofibers produced by electrospinning, enabling the fabrication of perfusable chips that promote directed cell alignment and maturation after seeding.[122]

These examples demonstrate how the synergistic integration of some biofabrication techniques, such as extrusion bioprinting, electrospinning, and microfluidics, allows for the creation of advanced tissue constructs with improved mechanical properties, spatial cell organization, and biological performance. These hybrid techniques are crucial for overcoming each technique's particular limitations, such as low mechanical strength in 3D bioprinting or restricted cell placement precision and organic solvent cytotoxicity in electrospinning. The hierarchical, multicellular structures of natural organs may be better mimicked by combining these techniques. However, the next step in advancing hybrid fabrication strategies is the integration of cell encapsulation, enabling these approaches to leverage the biological benefits of controlled cell delivery within complex, multilayered constructs.

3.7. Challenges in the Clinical Translation of Microfabricated Multilayered Tubular Constructs

Each fabrication technique offers unique strengths for engineering multilayered tubular tissues, but also faces distinct limitations that must be addressed for successful clinical translation. Extrusion-based bioprinting excels in versatility and personalization but suffers from shear-induced cell death and limited resolution. Light-based methods like DLP provide excellent precision but are constrained by cytotoxicity concerns and difficulty in achieving true tubular geometries. Electrospinning allows for structural fidelity and scalability, yet integrating viable cells in multilayered configurations remains a challenge. Gel casting is a simple and accessible technique, yet it is time-consuming and lacks the resolution of other methods, requiring future automation and integration with more advanced patterning tools. Self-folding cell sheets represent a mold-free approach for generating tubular constructs, but currently lack precise spatial control and scalability. Microfluidic-based strategies offer fine control over geometry and encapsulation, but are often restricted by fragile device architectures and small-scale throughput, limiting their immediate clinical utility.

While clinical trials have been conducted for cell-seeded tubular grafts, such as vascular,[123] tracheal,[124] or urethral[125] grafts using seeded autologous or allogeneic cells on biodegradable scaffolds, cell-encapsulated multilayered scaffolds have not yet advanced to clinical evaluation. This highlights that the primary translation hurdles are specific to cell-encapsulated fabrication strategies, even though promising in vitro and in vivo results have been obtained for these strategies. Despite encouraging preclinical outcomes, sex as a biological variable is frequently overlooked, limiting the generalizability of the results.[126] Additionally, large animal models, which are critical for bridging the gap to human application, are rarely utilized due to higher complexity and cost.[127] Another major concern for clinical translation is the scale-up of fabrication techniques.[128] While these methods are highly specialized and capable of personalized medicine, the achievable construct dimensions remain small, and production throughput is relatively slow. The high cost of many advanced biomaterials further complicates scalability, making large-scale production challenging. Another key limitation for engineering larger tubular tissue constructs is the thickness of cell-encapsulated constructs, which restricts nutrient and oxygen diffusion, making it difficult to generate fully functional tissues.[129] To address this, the development of effective vascularization strategies within the constructs is essential to enable adequate perfusion throughout the engineered tissue. Furthermore, although cell encapsulation can promote immune engineering by shielding cells within a hydrogel matrix, concerns regarding immunomodulation and immune rejection persist, particularly given the stringent regulatory hurdles associated with advanced therapy medicinal products.[129] Besides regulation, also organization of clinical trials has issues including limited patient availability and communication issues between clinicians and researchers.[130] Another often underappreciated but critical aspect for clinical translation is the sterilization of complex, cell-laden constructs without compromising bioactivity and material integrity.[131] Collectively, these challenges highlight the need for continued development in scalable, reproducible fabrication strategies, integrated vascularization approaches, and advanced immune-engineering techniques to enable the successful clinical translation of cell-encapsulated multilayered tubular tissue constructs.

4. Bioreactors for In Vitro Maturation of Multilayered Tubular Constructs

Bioreactors play a crucial role in tissue engineering by providing a controlled physiological environment that supports cell proliferation, differentiation, and the formation of functional tissues. These systems precisely regulate key parameters such as temperature, nutrient availability, oxygen levels, pH, and waste removal, ensuring optimal conditions for cellular growth.[132] Importantly, bioreactors operate in an automated way, which reduces labor time and minimizes the risk of contamination. Beyond maintaining environmental stability, bioreactors can also replicate the mechanical and biochemical cues that cells experience in vivo. Different bioreactor designs target specific tissue types, with features tailored to support different cellular needs. In this section, we focus on the critical aspects of bioreactor design for tubular tissue engineering, emphasizing the role of perfusion in promoting epithelial cell or EC growth and mechanical stimulation in enhancing SMC development.

4.1. Bioreactor Design for Tubular Tissue Engineering

Each tubular tissue construct has unique structural and functional requirements, necessitating tailored design parameters to replicate its native physiological environment. While all tubular constructs transport fluids, the flow dynamics differ significantly. For example, the urethra experiences intermittent, high-velocity flow, whereas small veins maintain a steady, lower-pressure circulation.[28] Similarly, even within the vascular system, the culture conditions required for large arteries differ from those for small capillaries, highlighting the need for precise bioreactor customization. Despite these variations, certain fundamental design principles apply to all tubular constructs. The core components of any bioreactor are a bioreactor chamber, media reservoirs connected via tubing, pumps to regulate fluid flow, and sensors to monitor physical and metabolic conditions (**Figure** 7A,Bi).[133,134] The tubular construct is housed within the bioreactor chamber, which can be either a commercially available tissue cassette[135] or a custom-built chamber (Figure 7Bii) made from sterilizable polymers to accommodate constructs of varying dimensions.[134,136] Clamps secure the construct in place, while the chamber has two fluid inlets and outlets, one directing flow through the interior of the tubular assembly and the other allowing fluid to circulate around the tube. The construct may be sewn around a silicone tube to increase its structural integrity (Figure 7Biii).[134,137]

Integration of bioreactors with fabrication techniques is an emerging area of interest. In particular, bioprinting approaches that utilize sacrificial inks to define lumens or embed perfusable channels directly within chips are increasingly being coupled with perfusion bioreactors to enable immediate conditioning post-fabrication.[138] Similarly, microfluidic spinning setups offer opportunities for seamless integration with bioreactors by continuously producing hollow fibers under flow.[139] In contrast, most other fabrication strategies such as electrospinning, casting, and cell sheet engineering are not yet directly integrated into bioreactor platforms. With these techniques, constructs are typically fabricated separately and later transferred into dynamic culture systems. Future developments that enable fabrication and conditioning within the same platform, especially for multilayered, cell-laden constructs, could significantly improve structural fidelity, cell viability, and scalability.

4.2. Perfusion and Mechanical Stimulation to Enhance Cellular Growth in Tubular Structures

Epithelial cells, ECs, and SMCs preserve their native phenotype when exposed to physiological conditions that closely resemble their natural environment. Additionally, MSCs can be directed to differentiate into these cell types through the application of specific physical cues.[140,141] In this section, we first review the physical cues that are beneficial for the growth of epithelial cells, ECs, and SMCs. Next, we summarize studies on the use of bioreactors to apply these physical cues for generating functional multilayered tubular tissue constructs.

Since epithelial cells and ECs form the inner layer of tubular tissue constructs, they are primarily subjected to pressure and shear stress from fluid flow through the lumen. This biomechanical environment can be effectively replicated using a bioreactor with controlled fluid perfusion. Wu et al. demonstrated the beneficial effects of flow-induced pressure and shear stress on MSC differentiation into esophageal epithelial cells.[142] In their study, MSCs were cultured on scaffolds under static conditions and in a bioreactor that applied either physiological pressure (50 or 100 mm Hg) or shear stress (0.1 or 1.0 dyne cm^{-2}). Cells exposed to dynamic culture conditions exhibited significantly higher expression of epithelial-specific markers compared to those in static culture, with pressure proving more effective than shear stress. In a follow-up study, the authors implanted an esophageal tissue construct, cultured under the same shear stress conditions (0.1 dyne cm^{-2}), and compared its performance to that of a statically cultured construct and an autologous tissue graft.[143] The bioreactor-cultured scaffold outperformed both the static construct and the control graft.

In addition to epithelial cells, ECs have also been shown to benefit from dynamic culture conditions in a bioreactor.[144] For example, in one study a PCL/collagen electrospun scaffold was seeded with ECs and cultured in a static versus a dynamic environment.[144] Both cultures produced cell-seeded scaffolds with high cell viability that exhibited EC phenotypic characteristics. However, the scaffold cultured in the bioreactor showed a clear alignment of cells in the direction of flow. This alignment improved the homogeneity and organization of the EC layer of the construct, which is expected to provide better barrier function. MSCs could also be directed to differentiate into ECs by exposing them to pulsatile flow that mimics the natural pressure fluctuations found in blood vessels over time.[140]

The primary role of SMCs is to enable the scaffold to contract and stretch, allowing fluid to pass through the tube more effectively. To promote this functionality, the SMC-seeded scaffolds are stretched. To stretch the SMC-containing scaffold in the uniaxial direction, special stretching units can be mounted inside the bioreactor chamber. Perfusion, on the other hand, can be used to generate radial stretching. Luo et al. used a commercial stretching unit from Flexcell International Corporation to stretch a polyglycolic acid scaffold seeded with SMCs.[137] In addition, pulsatile flow through the construct created radial stress on the scaffold. Comparison of mechanically stimulated scaffolds with statically cultured scaffolds showed positive results in terms of increased energy production, increased collagen deposition, and increased cell alignment.

A pulsed-flow perfusion bioreactor is ideal for creating multilayered tubular scaffolds with an inner epithelial or endothelial layer and an outer smooth muscle layer. The regular perfusion creates shear stress for optimal UC growth, while the pulses create radial stretch and release to promote SMC alignment and growth. Recently, a bioreactor capable of independently controlling shear stress and radial expansion through perfusion has been developed, providing a more precise mimic of native physiological conditions.[145] A variety of studies have been performed that utilize perfusion-based reactors to generate multilayered scaffolds.[80,141,146,147]

To mimic the esophagus, a double-layered tubular scaffold was electrospun around a mandrel and seeded with MSCs.[141] The scaffolds were then cultured without perfusion, with perfusion only to generate shear stress, or with pulsatile perfusion to generate mechanical stimulation in addition to shear stress. All groups were compared with statically cultured MSCs in a dish. The results revealed distinct differences in epithelial and SMC-specific markers between the scaffold's two layers. The cells in the inner layer exhibited epithelial-specific markers, whereas the outer layer expressed SMC-specific markers. This underscores the critical role of scaffold design in guiding cell differentiation, as scaffold composition and architecture is the only difference between the two layers. Furthermore, the highest expression of cell markers was observed in cells exposed to pulsatile flow, highlighting

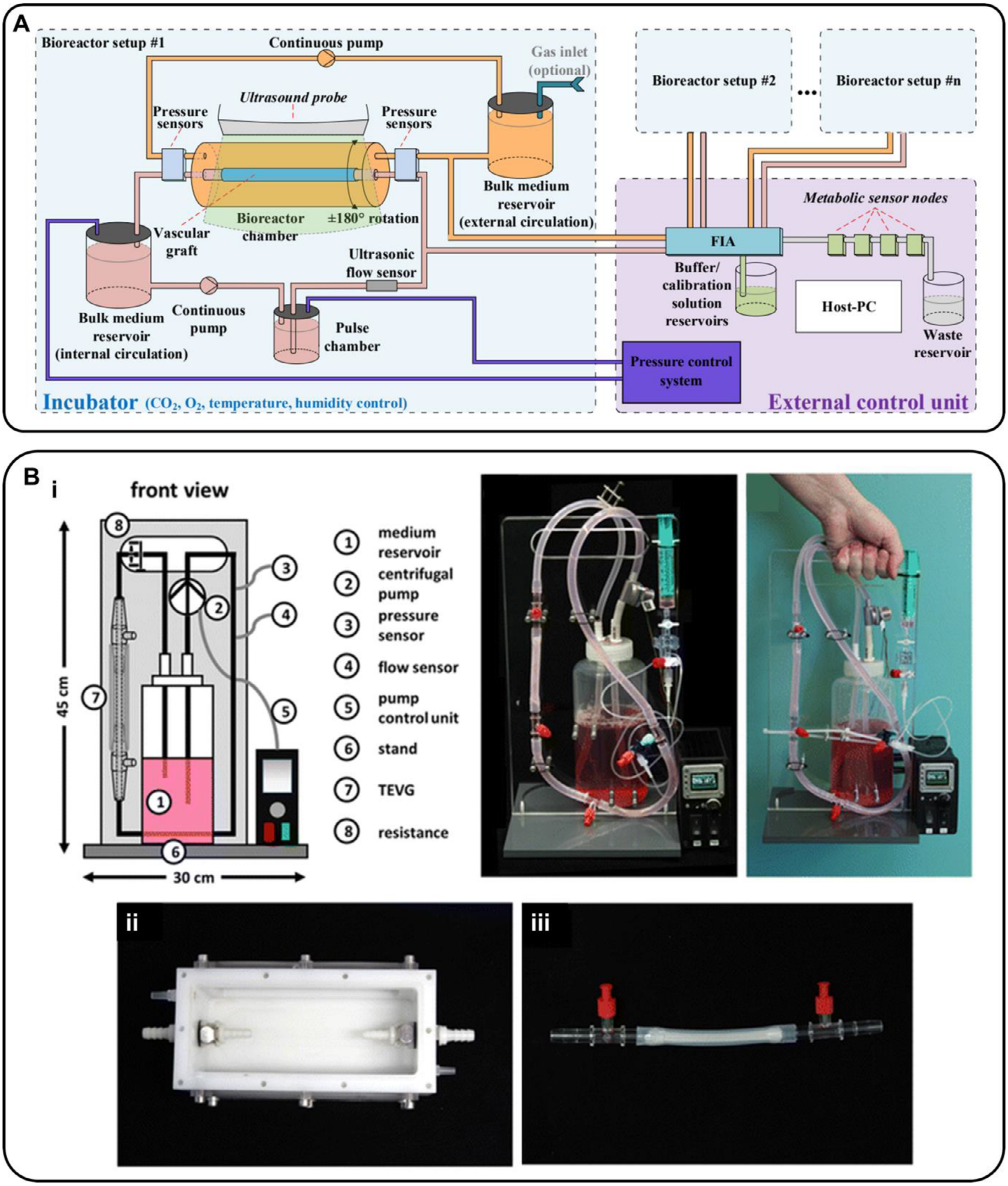

Figure 7. The use of bioreactors for cell maturation in tubular tissue constructs. A) Schematic of a bioreactor system for in vitro cell cultivation. Reproduced with permission.[133] Copyright 2025, Royal Society of Chemistry. B) A bioreactor system for biomimetic blood vessel maturation. i) Schematic and photographs of the bioreactor setup; ii) custom-built bioreactor chamber for tubular tissue constructs; iii) silicone support for tubular tissue construct. Reproduced with permission.[134] Copyright 2018, Springer Nature.

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the combined impact of shear stress and mechanical stimulation on cell growth and differentiation. In a follow-up study, the same scaffold was implanted in a canine model three days after bioreactor cultivation.[147] A fully epithelialized construct with smooth muscle was achieved after 12 months of implantation. In another study, a tubular collagen scaffold seeded with SMCs and UCs was cultured in a bioreactor using a peristaltic pump for fluid delivery to regenerate a urethral tissue construct.[80] The main advantage of the dynamic culture conditions was shown to be the aligned phenotype of the SMCs compared to the random orientation in static culture.

Three-layered tubular scaffolds mimicking blood vessels have also been developed and cultured in bioreactors.[146,148] For example, Baba et al. engineered a three-layered scaffold by mechanically folding cell sheets composed of ECs, SMCs, and fibroblasts into a cohesive hollow tube.[146] Their findings revealed that increasing the flow rate led to a reduction in cell layer thickness, underscoring the sensitivity of cells to shear stress. This highlights the importance of carefully optimizing shear stress parameters to prevent cellular damage. Çelebi-Saltik et al. attempted to culture cell-laden, rather than cell-seeded, multilayered hydrogel scaffolds in a bioreactor for blood vessel regeneration.[148] Using a casting technique around an electrospun support, they created distinct layers embedded with SMCs and fibroblasts. Subsequently, the luminal surface was seeded with HUVECs to complete the three-layered structure. Their engineered vessel demonstrated both histological features and mechanical properties comparable to those of native blood vessels.

Recent studies clearly demonstrate that dynamic culture conditions provided by bioreactors significantly enhance cell differentiation, maturation, and the overall functionality of engineered tubular tissues. Despite these advantages, the integration of cell-laden multilayered tubular scaffolds into bioreactor systems remains limited, largely due to the complexity of coordinating structural integrity, cell viability, and media perfusion in a 3D dynamic setting. Moving forward, future efforts should focus on designing bioreactors specifically tailored to accommodate and support multilayered constructs, including those with varying mechanical properties and spatially organized cell populations.

5. Current Challenges and Future Perspectives

Hollow tubular tissue engineering has emerged as a leading strategy for repairing damaged tubular structures in the human body, including the urethra, vasculature, esophagus, and trachea. A wide range of microfabrication techniques have been explored to replicate the complex, small-scale, and multilayered architecture of these tissues. While most studies to date have relied on post-seeding methods to generate cell-containing multilayered tubes, there is a growing shift toward incorporating cells directly into hydrogels during fabrication. This cell-laden approach offers distinct advantages over post-seeding, such as improved spatial control, enhanced homogeneity, and the ability to localize specific cell types to their native layers. Despite these benefits, the integration of cell-laden hydrogels into microfabricated hollow tubular constructs presents several critical challenges.

The first major challenge is maintaining high cell viability during fabrication. Extrusion-based bioprinting exposes cells to high shear stress, DLP printing involves prolonged light exposure, and cell-embedded electrospinning subjects cells to damaging high voltages. Future efforts should focus on developing more cell-friendly processing conditions by incorporating protective strategies, such as encapsulating cells in microgel particles. Additionally, there is an unmet need to explore novel hydrogel and photoinitiator formulations with improved biocompatibility and reduced cytotoxicity.

A second limitation is the inherent size constraint of most microfabrication methods. Techniques such as bioprinting and microfluidics offer high resolution but often at the expense of overall construct dimensions. To move closer to clinical translation, research should aim to scale up these techniques to produce anatomically relevant structures suitable for large-scale tissue repair. The third challenge lies in hydrogel scaffold design. Each cell type thrives in a distinct microenvironment, requiring hydrogels with tailored mechanical, chemical, and topographical properties. However, these materials must also remain compatible with microfabrication methods, retaining features such as extrudability through fine nozzles or microchannels.

In addition, while there has been substantial progress in microfabrication techniques for creating multilayered tubular scaffolds and in the development of advanced bioreactor systems capable of applying physiologically relevant mechanical cues, these innovations have largely evolved in parallel. The future of tubular tissue engineering lies in the convergence of these technologies by integrating cell-laden hydrogel scaffolds with precisely engineered bioreactors that can independently control parameters such as flow, pressure, and stretch across different scaffold layers. This synergy has the potential to more faithfully replicate in vivo conditions, thereby improving the clinical relevance and scalability of engineered tubular tissues.

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Conflict of Interest

The authors declare no conflict of interest.

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Tess De Maeseneer is a postdoctoral researcher in the Annabi Lab at the University of California, Los Angeles (UCLA). She received both her Master's and Ph.D. degrees in chemical engineering from the Catholic University of Leuven (KUL), Belgium. Her research focuses on the development of advanced biofabrication strategies, particularly 3D bioprinting, for the engineering of complex tissues. She is especially interested in reconstructing challenging structures such as the male urethra and hierarchical vascular networks, with the goal of creating functional, clinically translatable tissue-engineered constructs.

Nasim Annabi is an Associate Professor in the Department of Chemical and Biomolecular Engineering at UCLA. Her team has pioneered engineering innovative multifunctional biomaterials with tunable properties for tissue regeneration. She has published over 170 peer-reviewed articles (H-index: 87, >29 700 citations in July 2025) and holds 17 issued patents. She was named a Senior Member of the National Academy of Inventors due to her remarkable achievements as an academic inventor. Dr. Annabi has received major honors, including the RSC Biomaterials Science Lectureship (2021), SFB Young Investigator Award (2021), AIChE NSEF Award (2020), and Highly Cited Researcher (2022-2024).