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EDITORIAL

Multiscalar circulation system: A key issue in artificial organ manufacturing

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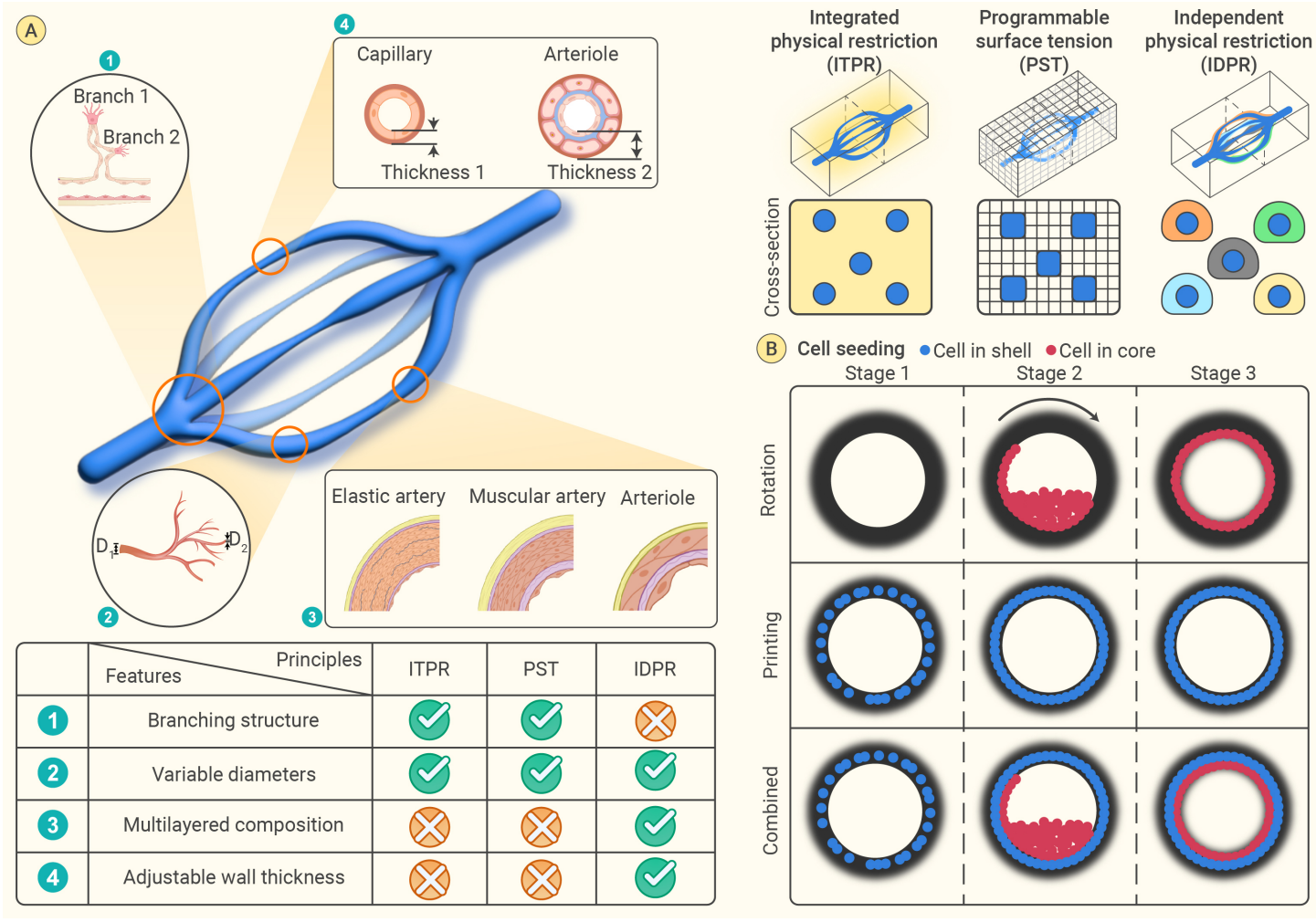
Artificial organ offers a promising solution to the shortage of organ donors for transplantation.[1] The growing interest in organ manufacturing originates from the vision of achieving full-size organ transplants without immune rejection. Fast developments in inorganic functional organs (e.g., heart pumps), and organic miniaturized functional units have been obtained, demonstrating their potential in clinical application. However, current artificial organs still face limitations due to low cell activity or low cell proliferation rate, in which leads to non-expandable structures. To support expandable long-term culture and cell differentiation, incorporating a circulation system within artificial tissue is crucial. This approach ensures sustainable nutrient and waste exchange, and maintains the morphogen gradient. In this article, we introduce fabrication principles, engineering tools, and bioactivation processes involved in creating multiscalar circulation systems for artificial organ manufacturing.



Features \ Principles	ITPR	PST	IDPR
1 Branching structure	✓	✓	✗
2 Variable diameters	✓	✓	✓
3 Multilayered composition	✗	✗	✓
4 Adjustable wall thickness	✗	✗	✓

Figure 1. Fabrication principles for hierarchical vascular network and in-situ cell seeding (A) Fabrication principles for the vascular network with 5 branches. Three common principles are listed: Integrated physical restriction, Programmable surface tension, and Independent physical restriction. And the manufacturing capability of three fabrication principles on four features of blood vessels. "√" means achievable and "X" means not achievable. (B) Different stages of three cell seeding methods. Those are Rotation, Printed, and Combined.

PROPERTIES OF VASCULAR NETWORKS

The human body's circulation systems, primarily composed of blood vessels, have several features that facilitate the transportation of liquids. These features include: (1) a branching structure, (2) variable diameters, (3) a multilayered composition, and (4) adjustable wall thickness. The branching structure is common in circulation systems due to its efficient distribution capabilities. Furthermore, branches help reduce blood pressure and provide redundant pathways for unexpected blockages. Variable diameters, found in

nearly every section of native blood vessels, allow for continuous regulation of the blood flow speed. Sharp changes in diameter often occur at branch junctions, following Murray's law. This tree-like structure enhances the exchange efficiency by increasing microcapillary surface area in deep tissue. In addition to inherent differences in vessel diameter, temporary size changes can be achieved through the contraction of the middle layer, which consists of smooth muscle cells. Through vasoconstriction (narrowing) and vasodilation (widening), blood vessels can adjust their diameter, allowing them to regulate blood flow and pressure in response to various stimuli such as temperature changes, exercise, or stress. For optimal size regulation, the thickness of the three layers - Tunica Intima (inner layer: endothelial cells), Tunica Media (middle layer: smooth muscle cells), and Tunica Externa (outer layer: fibroblasts) - must be precisely designed and fabricated. Mismatched thickness can impair blood flow regulation and lead to systemic disorders.

FABRICATION PRINCIPLES

To construct a perfusable circulation system, several principles have been adopted, including integrated physical restriction (ITPR), programmable surface tension (PST), and independent physical restriction (IDPR) (Figure 1A). Among these, ITPR is the most practical approach. This model consists of two main components: fluidic channels and bulk surrounding physical restriction. Techniques based on this principle often involve remolding a sacrificial vascular network or directly printing physical restrictions using digital light processing (DLP). These methods rely heavily on a casting strategy, which can only create a uniform bulk surrounding tissue, limiting further development. In contrast, PST uses surface tension and capillary force to restrict and guide liquids, respectively.[2] However, the fluidic channels generated with this principle have an open wall, which significantly limits the pumping pressure. To achieve a seamless physical wall, the casting strategy is also employed. Inspired by the concept of multi-cell type tissue construction, IDPR was introduced to create isolated fluidic networks and layered physical restriction. Techniques such as phase separation and coaxial bioprinting are widely used to generate tubular structures. Despite the limitations of existing engineering tools with the IDPR principle, it remains the most feasible approach for fabricating multi-cell type tissues embedded with vascular networks (Figure 1A).

ENGINEERING TOOLS

Several engineering tools have been developed to fabricate complex hierarchical vascular networks, primarily based on the principles of ITPR or IDPR.

ITPR: Initially developed through remolding, this principle uses 3D printing or selective laser sintering (SLS) to construct solid-like filament networks from materials such as carbohydrate glass or resin. These networks serve as patterned 3D vascular structures within engineered tissues. A cell-laden hydrogel pre-gelation solution is cast over these networks, and once crosslinked, the sacrificial filaments are evacuated to create hollow channels.

Recently, a new engineering approach called gallium-based engineered sacrificial capillary pumps for evacuation (ESCAPE) molding has been introduced.[3] This ITPR-based technology extends capability of molding to the capillary level, fully accommodating biological applications. Gallium's suitable liquid-solid transfer temperature makes it an excellent candidate for sacrificial materials of filament networks. Yet, technologies based on casting cannot construct thickness-adjustable multilayer structures for blood vessels, limiting the model's biological functionality.

Another emerging approach is based on constructing physical restrictions directly. By using projection-based stereolithography, DLP can build physical restrictions layer by layer.[4] Moreover, DLP can achieve multi-material and multi-cell type structures by changing the pre-gelation hydrogel solution in the fabrication chamber. While this switching process allows for the fabrication of multilayer blood vessels, it wastes plenty of time during fabrication time-consuming, which is not ideal for biological applications.

IDPR: The original engineering tool based on this principle is microfluidic spinning.[5] Through using a coaxial flow microfluidic device, microfibers with straight or helical inner walls can be generated. However, these hollow microfibers are typically manipulated manually or collected by a roller, which limits their further application.

Inspired by ITPR, phase separation was developed, starting with sacrificial fluidic networks immersed into a polymer solution. Once exposed to an ambient environment, the coated polymer crosslinks during volatilization or through other crosslinking methods. A tubular structure is formed when the sacrificial core material is removed. However, controlling the components and thickness of the coated polymer layer remains challenging, restricting the broader application of this technology.

To realize controllable deposition of those hollow microfibers, 3D printing technology was combined with this coaxial flow device concept. Coaxial printing is a potential method for directly depositing cell-laden tubular structures, using concentric nozzle sets to print a core-shell structure. However, the coaxial nozzle could only generate single-channel tubes without branches. Post-process is required to connect each channel and prevent leakage. Coaxial bioprinting is the only engineering tool capable of rapidly constructing multilayer tubular structures with multi-cell types, but it cannot create seamless branch structures, a key feature of blood vessels.

To date, no engineering tool meets all the requirements for blood vessels. Among these tools, those based on the IDPR principles are closest to realizing the construction of multilayer hierarchical vascular networks.

PROCESS OF BIOACTIVATION

After fabricating vascular networks, it is essential to introduce biological components into the system. Cell seeding is a critical aspect of this process (Figure 1B). Various strategies are commonly employed for seeding different types of cells, such as endothelial cells (ECs) and smooth muscle cells (SMCs).

For the ITPR principle, cells are typically seeded using a rotation strategy. It starts with a cell-free channel where a cell suspension is injected, allowing cells adhere to the inner surface of the vascular networks. This strategy is suitable for ECs, which form a monolayer on the channel's inner surface. However, the rotation strategy is only effective for parallel or isotropic vascular networks. Anisotropic networks require additional rotation directions, resulting in increased time consumption.

Another strategy involves printing, where cells are carried by hydrogel and deposited to simultaneously form vascular network channels and cell-laden shells. However, the presence of cells embedded within the hydrogel makes it challenging to form a monolayer of endothelial cells due to the limitation imposed by the polymer network. To address this, cell-laden sacrificial material is used as the core. Once the sacrificial material is removed, the cells can attach to the inner surface of the channel. However, this method also involves the rotation strategy which may limit its biological application. Consequently, the printing strategy is primarily applicable to SMCs. Therefore, these two strategies are often combined to construct multilayer blood vessels.

PERSPECTIVES

Until now, no engineering tool has been able to rapidly construct hierarchical multilayer blood vessels with adjustable inner and outer diameters. Whether using casting or DLP, tools based on the ITPR principle struggle to build different layers of blood vessels in a bio-friendly timeframe. Besides, the PST principle resembles a pixelated version of the ITPR principle and heavily relies on casting. IDPR principle is the most promising principle that can realize all four features of blood vessels (see PROPERTIES OF VASCULAR NETWORKS). IDPR-based engineering tools (e.g., coaxial bioprinting) have been one step away from the goal of stable bifurcation and rejoining. Once blood vessels are successfully constructed, these engineering tools could also be applied to generate renal tubes, neural tubes, and other tubular structures. This perfusable artificial organ platform will open new possibilities for tissue engineering and fundamental research.

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DECLARATION OF INTERESTS

The authors declare no competing interests.