International Journal of
Science and Engineering Investigations vol. 8, issue 91, August 2019
Received on August 13, 2019 ISSN: 2251-8843

The Fabrication of Vascular Tissue, 3D Printing and Additive Manufacturing

Hind Hamdaoui[1], Zhenying Xu[2], Sikandar Ali Qalati[3], Telezing Kenne Boris Joel[4]
[1,2,4]School of Mechanical Engineering, Jiangsu University, P. R. China
[3]School of Management, Jiangsu University, P. R. China
([1]hamdaoui.hind1990@outlook.com, [2]xuzhenying@ujs.edu.cn, [3]5103180243@stmail.ujs.edu.cn, [4]boris_joel@yahoo.com)

***Abstract*-**Additive manufacturing (AM) comprises a collection of 3D printing approaches supporting the building of multifaceted, biomaterial 3D architecture for vascular tissue engineering and reformative medicine. The capability of 3D printing to design complex materials, types of cells and biomolecules offers a distinctive apparatus to generate tissue fabrication diligently like the arrangement, design and use of biological tissues. Improvements in 3D printing and printable biomimetic stratagems permits the creation of vascularized tissue engineering consisted of several cells entrenched inside appropriate extracellular matrix elements and delivered by useful vasculature. The perfusable and thick vascular tissue fabrication can now be structured, in vitro cultured and printed, proposing an encouraging substitute to customary vascularization schemes. This review offers a brief synopsis of latest 3D printing approaches sightseen to make vascular networks, its tissue constructs, and discusses upcoming aspects concerning the significance of vascularization for clinical application.

***Keywords*-** *Additive Manufacturing, Cell Sheet Engineering, Solvent Casting, Micro Patterning, Electrospinning, Vat Photo-Polymerization, Ink-jet Printing, Laser Assisted Printing*

I. INTRODUCTION

The bodies of human beings are greatly vascularize as it enable healthy working and greater number of multicellular beast to exist. For the continuous functionality of organs and tissues vascular network required which allows the abstraction of left-over materials and the flow of nutrients and oxygen. Consequently, cells are traced in the range of "100 – 200 µm of a capillary", considered as the circulation range of nutrients and oxygen within a tissue, while few exception are there for instances cartilage tissues of cartilage, the lens and cornea part of the eye, and the epithelial layer in skin [1]. Particularly the vascular is structured in vivo as a diverged network of capillaries and small, larger vessel. The establishment of an organized network pertaining to vascular system is operated by two basic procedures of angiogenesis and vasculo-genesis [2]. The process of vasculo-genesis is initiated by somatic powers and essentially befalls during initial embryonic growth to procedure a basic capillary network, nonetheless also happens in adults concerning "tissue revascularization" upon an accident and injury. The formation of "blood vessels" are shaped from "endothelial progenitor cells", however the enlistment of suave muscle cells, "fibroblast" and "pericytes" layers nearby the "endothelial spouts" is necessary for fruition. While, the process of an "angiogenesis" is prompted by hypoxia in the nearby tissue and includes the establishment of fresh vessels from a present network of vascular via the incubation of "endothelial cells" from current vessels. An "angiogenesis" initially prone towards the establishment of capillary setups comprising of EC tubes absent of superfluous wall configurations, which can subsequently advance over arterio-genesis [3]. Medical problems ascend when the vasculature is lengthily bothered by disease/trauma, prominent to a problems of reduction of tissue supply with oxygen and nutrients.

The development of tissue engineering and bio-printing has enabled the establishment of approaches for the repair of organ and tissues, their replacement, and in vitro tissue prototypes, but has upraised the necessity for vascularized tissue concepts. The scope and long-term capability of such tissue constructs are restricted by the configuration, convolution, and functionality of current vascularization. The constructs of tissue in vitro are reliant on on impassive dispersion which limits size of constructs, though, with the assistance of bioreactors mass flow of oxygen, and nutrients via vigorous culture supporting greater and long-term sustainability of tissue and organ constructs [4-5]. Yet, in vivo the quality and the rate of vascular tissue and organ during the development will be restricted which may leads to hypoxia, deficiency of nutrient, and made-up of wastes those having influences on formation of new tissue and cell fate. Consequently, several techniques and approached adopted to improve/engineer vascularization have been suggested such as the "pro-angiogenic drivers, progenitor, de-cellularized matrices, modular assembly, in vitro and in vivo pre-vascularization, synthetic architectures (microfluidic devices and porous scaffolds), novel biomaterials, bio-printing and combinations of all these approaches" [6-7]. Preferably, an "ideal engineered vascular network ought to (i) offer cells with contiguous vessels in close propinquity (100 - 200 mm), (ii) reveal ranked fundamental and practical organization,, where a vascular tree having higher vessels sub categorized into small

vessels and consequently into capillaries well-organized all over the tissue construct, (iii) existing selective sponginess to control the transfer and abstraction of metabolites and nutrients from nearby cells, and (iv) let assimilation with the cloud vasculature in the victim to offer instant perfusion of embedded construct" [8].

The regrowth and capillary of vessels and organ is possible with the aid of an additive manufacturing (AM) in the field of biomedical, it enable the novel ways to regulate and well-organized the installation of biomaterials, tissues, biomolecules and cells [9]. While few of the scholars hold beliefs that one of the best way to produce biomaterial (e.g. Polysaccharides) for biomedical applications is the adoptability of 3D bio-printing technology [10]. Alike [11], used this technology to cellularized human meniscus. In addition, [12], explored the mediation effect of exosome on extracellular matrix and shown that bio-printing greatly controlled and well-organized "exosome-based" microenvironments. Bio-manufacturing is a new encouraging technology and significant field of research, proposing the hope for connecting the gap among organs unavailability and transplantation necessities [13]. The author used this technology to speed up the "bio-fabrication of 3D cellular tissue" concepts impressively, to shape multi-cell insertions of "high density and correctness for vascular system". The previous scholar proved that this technology used for several reasons to improve the functions and applicability of cell laden supports [14], to give shape to several tissue and organs [15], for the components of human [16], vascularized cellular constructs [17], and regenerative medicine [18]. 3D (Three dimensional) also called additive manufacturing. There are several ways to define the term bio-printing according to [19], it is a process, where the "software that provides a computer interface with the printer sends the g-code (or similar) commands to the printer, where a microprocessor translates them into electronic signals that actuate the movements of motors and/or toggling of lights/switches/valves that comprise the physical printing process. This translation is mediated by firmware, a class of software that is stored on the hardware of a device-here the bio-printer".

The present study focus on classification of bio fabrication approaches commonly recycled to generate vasculature and constructs of vascularization as consuming both conservative/additive bio manufacturing approaches. A lasting gestalt of the newest investigation spending both fabrication schemes is delivered. Lastly, defies and future recommendation along with the discussion involved.

II. CONVENTIONAL TECHNIQUES (CT)

This method has been broadly investigated for constructing vascular transplants, blood vessels and for vasculature tissues. A several type of techniques for instance electrospinning, phase separation, solvent casting, micro patterning, cell sheet engineering, and related combination of such techniques have been used [20-21] Despite of having limitation concerning the difficulty and design of "engineered constructs" as well as over the defined setting of cells, biomaterials, and genetic elements in preferred 3D locations, their ease, affordable price and wide variety of procedure enable biomaterials enable them very applied and valuable to advance related combination of bio-fabrication schemes [22-23].

A. Cell Sheet (CS) Engineering

This technique permits the dispassion of a confluent cell stratum by the usage of a temperature receptive "polymer (poly (N-isopropylacrylamide))" implanted on plates of cell culture [24]. CS are isolated deprived of the requirement of enzymatic treatments, conserving intrinsic "extracellular matrix, cell-cell and cell-extracellular matrix anchorage". CS can be transplanted directly to the cloud tissue as solitary sheets or it can be gathered to generate more multifaceted 3D constructs. CS engineering have been utilized by [25], to construct vascularized 3D cardiac tissue by means of numerous CS layers. A vascular cot was established to permit perfusion of the CS construct which indorsed 12-layers. Instead, [26], have utilized CSs to advance blood vessels by means of various ranged mesenchymal stem CSs those are enclosed nearby a transitory supporting mandrel Fig. 1. This construct is permitted to settle afore being perfused by "endothelial progenitor cells" which bestow to the lumen. This association of the mature construct looks like the design of innate blood vessels and displayed vasodilation, nitric release and vasoconstriction, when open to "fluid flow and phenylephrine".

B. Solvent Casting (SC)

In this approach porous organizations are produced by the suspension of polymer in solvent inclosing consistently dispersed salt elements of a particular size. Later "solvent evaporation, the arrangement is absorbed in water to leak out the tricked salt particles, resulting in the creation of a porous structure" which can be advance with the cells of interest" [23]. [27], utilized a SC-co-particulate filtering method to generate a biomimetic complex "chitosan-gelatin hydrogel scaffold with a bi-layered tubular architecture". The inner layer resultants in to a big surface area which enables proliferation and an attachment of fibroblast, while the outer layer delivers preservation and mechanical support. The scaffold shown desired mechanical possessions and sullied "50% in vitro at day 16". Though, the neither angio nor vasculo-genic ability has been assessed but the approach rests an encouraging method to construct vascular structures. The "size of implantable tissue constructs state the vascularization scheme shadowed as small constructs can use dispersion and insinuation from the cloud tissue". Though, bigger constructs cannot trust on vessel ingrowth as necrotic zones usage afore a vascular network is shaped. Hence, in order to deals with this issue, [28], established another forming strategy, called vascular "corrosion casting technique", to totally redo the vascular network inside a kidney Fig. 1. A "polycaprolactone" resolution was utilized into an innate kidney producing a cast copycatting the network of vascular. This functions as a simple, actual and low-cost technique to generate a "sacrificial pattern for the creation of a scaffold for the whole size of a big tissue construct".

C. Micro Patterning (MP) Techniques

There are variety of MP techniques (e.g. Photolithography, micro molding and micro contact) printing. Those have been extensively utilized to govern behavior of cell to biomaterials through the organized demonstration of biological factors by topographical prompts [29-30]. In technique of photolithographic patterning, photo-reactive species are treated with appropriate light sources producing light at definite wavelength via masks to generate designs on substrates. This approach was "utilized to generate 3D connected vascular networks of wide-ranging dimensions among micropatterned gelatine methacryloyl (GelMA) hydrogels of dissimilar degrees of methacrylation" [31]. Micro molding permits generating compound constructions in biomaterials via the "use of master moulds with wanted geometry and topography on silicon wafers". After forming and drying a polymer elucidation on the master mould, "a biomaterial substrate" is achieved with wanted micro patterns. [32], used a "poly dimethylsiloxane pattern invented via a micro patterning procedure to yield aligned 'cords' of encapsulated extracellular among a collagen gel" (Fig.1).

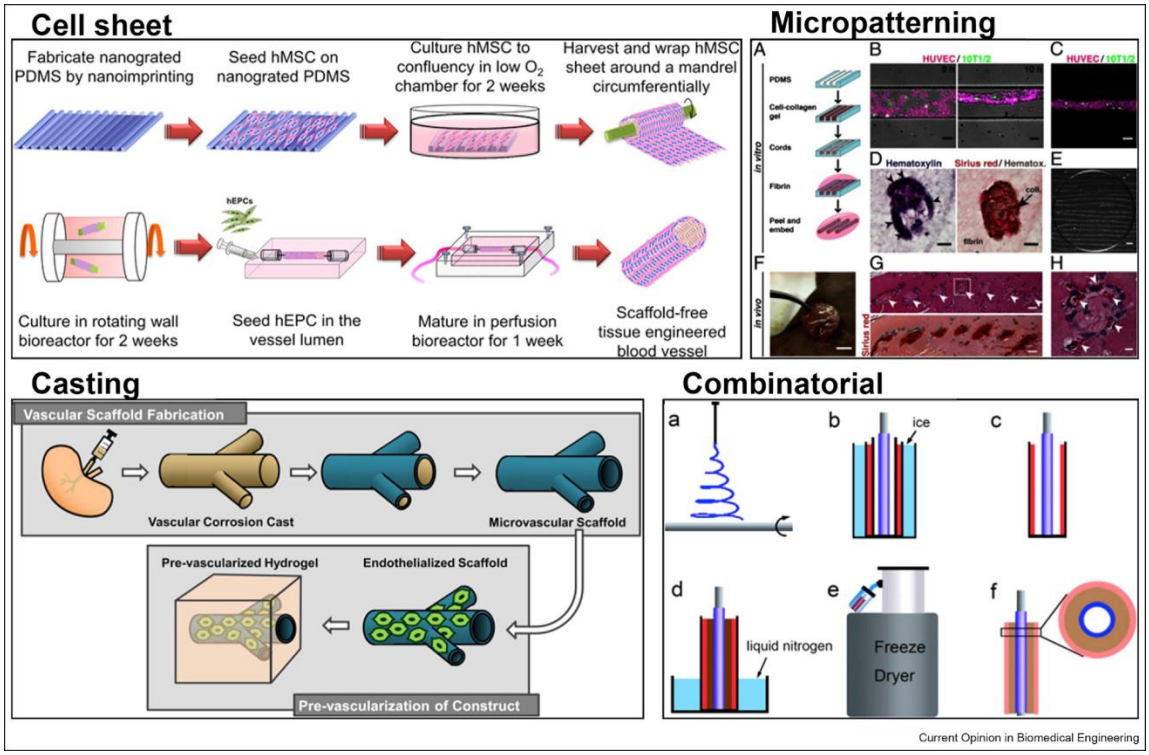

Figure 1. Conventional Bio-fabrication techniques used to produce vascular grafts and perfusable channels

D. Electrospinning

This is the bio-manufacturing method that yields fibers at the Nano and microscale from polymer elucidations by the approaches of an electric field among the collector and the syringe needle [33-34]. Moreover, tailoring functional parameters such as the "needle diameter, flow rate viscosity allows to rheostat the morphology and fiber diameter" [33-34].

Previous scholars have shown considerable interest in the improvement of electrospinning methods for vascular uses [35-36]. [37], proven the construction of multi-layered electro spun tubular configurations using a revolving "mandrel collector, now a common methodology, to create vascular-like structure that could support cell viability". Presently, optimization of physical possessions, fiber morphology, and electrospinning factors are being examined to better moderating cell reaction and prompting particular cell behavior. [38], examined the impact of fiber size on "cell insinuation and scaffold renovation in a vascular insert". On the contrary, smaller sizes lessen "cell infiltration and upheld a pro-inflammatory macrophage phenotype".

E. Combinatorial Techniques

Researchers and experimenters also combined range of techniques to generate novel results for the advancement of fabrications, vascular network. [39], "Fabricated a biomimetic triple-layered vascular scaffold combining electrospinning and thermally induced phase separation" Fig. 1. Additionally [40], discovered the mishmash of CS engineering and electrospinning. This combined technique used an electro spun mixture of PCL and collagen type-I to "generate a tubular scaffold which was wrapped in a smooth muscle cells sheet".

The smooth muscle cell sheet delivered a developed cell layer with pre-existing cell-cell connections and allowed high cell sowing competence of the electro spun scaffolding. Hence, this joined construction procedure allows biomimetic designs and pertinent cells types to be joined into a settled "vascular construct" which has the prospective for instant implantation after "bioreactor culturing".

III. ADDITIVE MANUFACTURING (AM) PPROACHES

3D printing commonly used as AM, has permitted medical experts and academicians to learn and construct tissue fabrication with several and complex design that are not easy to accomplish with CT [17-41]. Additive manufacturing offers higher control, matched with conventional approaches as discussed before, over the architecture and construction procedure resulting in defined "spatial disposition of biomaterials, cells and biomolecules, which closely resemble the designed structure". It allows the uninterrupted unseating of compound channels and tubular constructions, prefiguring of antigenic elements in 3D, and the presence of compound "vascular tissue constructs", letting the growth of dense constructs with inserted vasculature. A range of additive techniques presently utilized alone or in combinations to engineer networks of vascular for tissue construction, comprising "Extrusion, inkjet, vat photo polymerization, and laser-assisted processes".

A. Extrusion Based Processes (EBP)

This process comprises of variety of approaches that extrudes a solution of polymer via a syringe onto a platform, by means of a solenoid, pneumatic, or mechanical, deposition process [42]. EBP can be utilized to construct 3D architecture composed of thermoplastic polymer's, named "fused deposition modelling", else to print either "a cellular hydrogel bio-inks", called extrusion bio-printing [43-44]. EBP are well-known and reasonable due to their changeability, wide-ranging of process able biomaterials and capability to produce 3D tissue constructs.

AM techniques offer the capability to produce "perfusable and interrelated vascular channels", the construction of well-designed "vascular networks" intensely hang on the cell instructive abilities of developed biomaterials. There is extensive appreciation that in vivo cells can vigorously interrelate and modernize the nearby endothelial cells, which in turn diktats and effects cell outcome [45-46]. Apart from printing cells inside indolent hydrogel materials, struggles have been continue to cultivate cell-responsive hydrogels capable to moderate behavior of cell. [47], utilized a cell-responsive bio-ink grounded on "sodium alginate, GelMA, and 4-arm poly (ethylene glycol)-tetra-acrylate and a multi-layered coaxial nozzle extrusion system to directly print perfusable 3D structures". Investigation of alike approach [48], have confirmed the construction of "endothelialised myocardial tissue" structure by employing "a coaxial nozzle to print a micro fibrous scaffold" with extracellular inserted inside a blend bio-ink of alginate and gelatin "methacryloyl" (Fig. 2).

Another approach to produce perfusable channels based on the "printing of sacrificial microfibers inside the hydrogel matrices". Later deposition, sacrificial pattern material is vanished, parting hollow channels which may be perfused and endothelialised. This approach was investigated by [49], "to print useful vascular channel reflecting a perfused open lumen" (Fig. 2). [17] Advanced a bio-printing method proficient of constructing multifaceted tissue constructs with combination of micro channels to improve mass transmission, creation of novel tissue and vascularization (Fig. 2). Constructs were shaped by linking the "extrusion of polymer melt, sacrificial ink and cell-laden bio-inks and tested for their generation of bone, cartilage and skeletal muscle". This scholarship is advancing in proving the construction of large vascularized constructs which is a necessary to be improve inside vascular tissue engineering and also allow clinical rendition of tissue constructs.

B. Inkjet Printing (IP)

This technology submits the small drops of a bio-ink onto a "build platform by the thermal or piezoelectric special effects". In thermal based inkjet, a heating component is employed to prompt the vaporization and on sequent ejection of a small size of bio-ink, whereas "piezoelectric" based inkjet creates usage of "piezoelectric transducers" to stimulate drop creation [50-51]. IJ has been extensively utilized because of matchless features of "high throughput efficiency, resolution and likelihood of similar printing". Though, the prerequisite for low viscous bio-inks to preclude nozzle blockage has restricted its use on the construction of 3D tissue. Other approaches comprising the printing of "endothelial cells with cross linkers or cell-laden bio-inks onto a biomaterial substrate" have been fruitfully applied [52-53].

[54], have validated the "capability to engineer compound vascular architecture that have bifurcations' and existent both overhanging and spanning features" (Fig. 3). The similar approach also applied by [52] via construction of multi-cell varied constructs comprising endothelial cells to prompt vascularization of the construct (Fig. 3). Splicing in vivo revealed construct feasibility and progress but also that sufficient vascularization happened.

C. Vat Photo-polymerization (VP)

This technology also called stereo lithography. VP constructs 3D design via careful photo-originated "curing reaction of a liquid photosensitive material using either laser writing or mask-based writing approach" [55-56]. Initially, a dedicated laser ray is employed to persuade the polymerization of a "fluid photopolymer", whereas in the next a cover is utilized to transmit whole image to a "fluid photopolymer" decreasing the time of printing. These approaches should offer the upmost resolution of print because of the optical centered procedures which permits "up to 100 nm" resolution by means of 2PP (two-photon photo polymerization processes) [57-58]. VP apply a single-photon to recruit photo polymerization whereas 2PP necessitates a material that lets the raptness of 2P, instantaneously, which consequences in a confined reaction therefore permitting application of high resolutions [58].

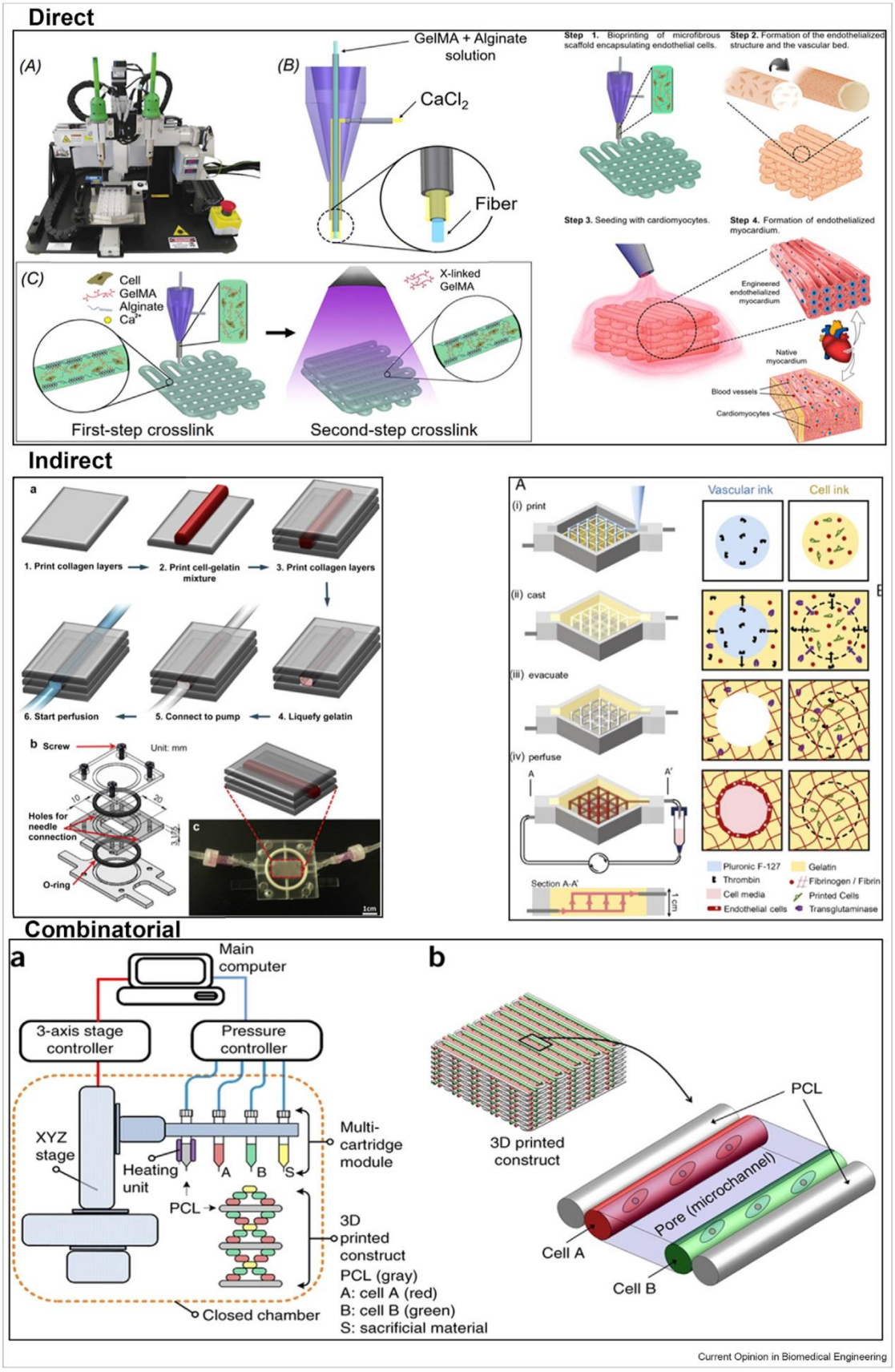

Figure 2. Extrusion based processes to create vascular network in 3D tissue construct via three major approaches

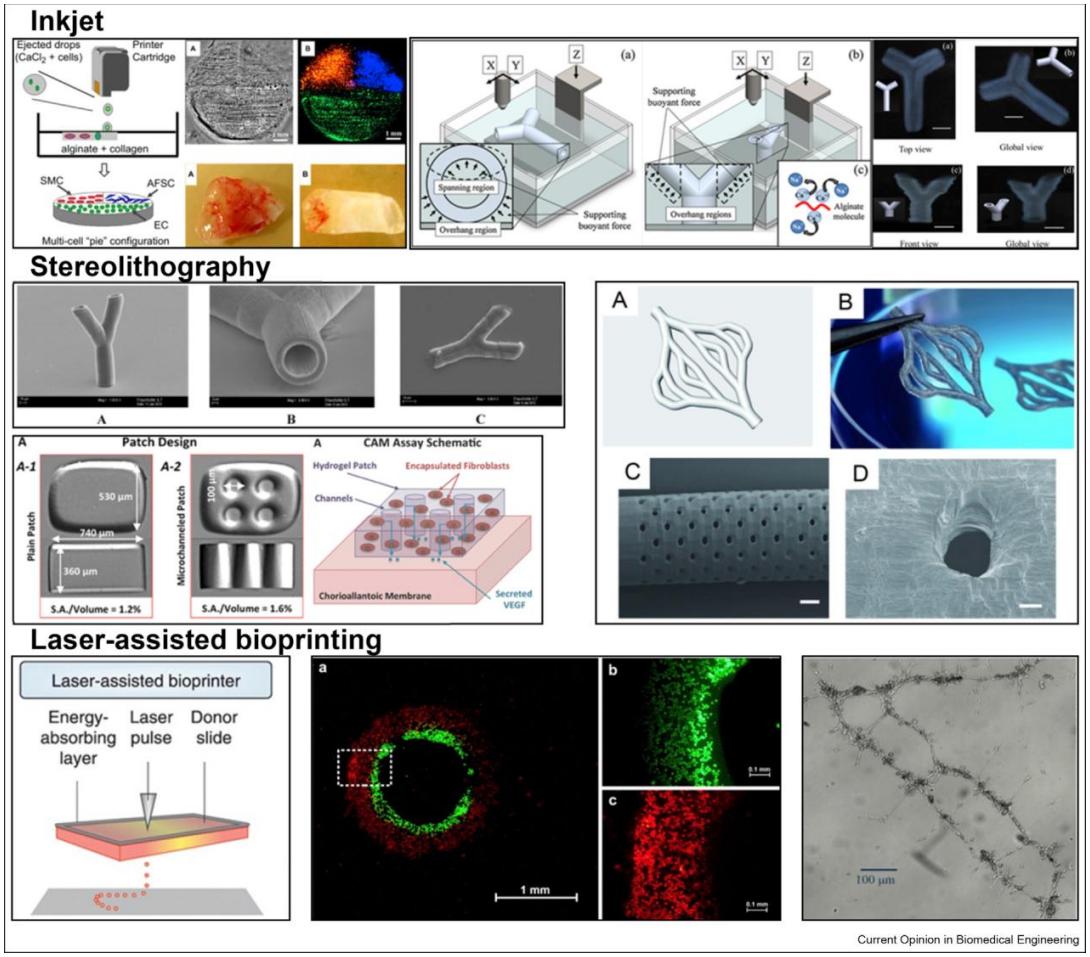

Figure 3. Additive manufacturing techniques and their example used to engineer vascular networks for tissue engineering

[59] Magnificently constructed vessel-like architecture by means of VP and 2PP with the intention of building tubes that satirist the capillaries diameter (Fig. 3). Bifurcated tubular architecture with "18 mm internal diameter and 4 mm thickness of wall were shaped". [60], proven the "utilization of VP to construct 3D tubular design with a photosensitive cytocompatible polyacrylate" (Fig. 3). "Tubular design, containing branching vessels, with 300 mm thickness of wall, 1-2 mm inner diameters, and definite pores throughout the tubes of 100 or 200 mm were constructed".

D. Laser Assisted Bio-printing (LAB)

This technique utilizes the principle of laser induced transmission, formerly established for metallic transmission, to "deposit biological materials onto a substrate" (Fig. 3) [61-62]. Though this technique is not commonly used, a variety of biological materials have been printed by means of this approach [58-63]. [64], employed this technique to build vascular architecture by supervisory the expansion of lumen creation by the direct placing of separable endothelial cells and smooth muscle cell sheets (Fig. 3). Additionally, [65], by means of endothelial cells specified that it is potential to straight-forward the creation and development of "lumen and lumen network using LAB" (Fig. 3). However, the work of above scholars permits expansion of vascular fabrication which conserves a particular form over long time period.

IV. CONCLUSIONS

A variety of approaches have been employed to challenge the problem of vascularization in construct of tissue and reformative medicine, comprising the structure of angiogenic biomaterials and the carriage of endothelial cells and development influences. Such schemes are commonly grounded on vascular stimulation despite of improvement of perfusable vascular systems, which bounds their effectiveness in the vascularization of 3D fabrication of clinically pertinent measurements. Additive manufacturing approaches exemplify an encouraging and operational substitute to openly integrate useful vascular networks with multifaceted association all over the 3D fabrication by the layered deposition of biomolecules, biomaterials, and cells. Whereas, LAB and IP have been mostly examined for printing of cells and expansion factor modeling, EBP approaches are presently used to generate compound "hollow channels" that either be perfused and endothelialised, as long as offering nutrition to the nearby cells. Specifically, additive manufacturing schemes allow the exact

co-printing of endothelial and stromal cells into pre-designed spatial sites to provision and preserve vascular-like architecture, which is exceptionally challenging or difficult when utilizing conventional bio-fabrication approaches. The flexibility of additive manufacturing techniques has also been sightseen for the growth of combinatorial methods with prospective to advance the performance and biomimicry of engineered fabrication.

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Hind Hamdaoui has received her master degree "Mechanical Engineering" from Wuhan University Of Technology, China,. She is currently pursuing PhD in Mechanical Engineering from Jiangsu University P.R. China. Before joining Jiangsu University she has 2 years' work experience as a supervisor and controller of production and sell manager.

Zhenying Xu is Professor as well as PhD Supervisor at Mechanical Department, Jiangsu University. In 2004, she did Ph.D. in Measurement and Control, from Hefei University of Technology, China. She had more than 20 publications in well reputed SSCI, ESCI and international journals. She got an award of Young Back-bone Teacher Training Engineering of Jiangsu University, in 2013.

Sikandar Ali Qalati has received his M.B.A in Marketing from Sukkur, IBA University, Pakistan. He is currently pursuing PhD in management science and engineering from Jiangsu University P.R. China. He has several publications in reputed international journals. Before joining Jiangsu University he has 5 years diverse work experience. He worked with KFC, Pizza Hut, Atlas Honda and Sukkur IBA University. His area of interest are engineering, social media, brand management, marketing and entrepreneurship.

How to Cite this Article:

Hamdaoui, H., Xu, Z., Qalati, S. A. & Joel, T. K. B (2019) The Fabrication of Vascular Tissue, 3D Printing and Additive Manufacturing. International Journal of Science and Engineering Investigations (IJSEI), 8(91), 102-109. http://www.ijsei.com/papers/ijsei-89119-13.pdf