Supplementary

Effects of surface patterning and topography on cellular functions of tissue engineered scaffolds with special reference to 3D bioprinting

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Table S1: Resolution of different bioprinters

Bioprinting process		Minimal print volume	Remarks
Droplet based bioprinting	Acoustic droplet ejection	Avg. droplet dia. 54–183 µm [1], 60-500 µm[2]	***Advantages*** Nozzle free technique Increment in frequency reduces droplet dia. ***Disadvantages*** Frequency of wave negatively impact cell viability
	Inkjet bioprinting	Thermal inkjet bioprinting Orifice dia. ~50 µm Resolution 85 µm Drop volume 85 pL[3,4] Piezoelectric inkjet printhead Orifice dia. 120 µm[5] Electrohydrodynamic jetting Dia. 5µm[6] Electro-assisted bioprinting Dia-100 µm[7] Upward bioprinting[8]	***Advantages*** Ink ejection can be carried out in both continuous and drop by drop mode as per requirement ***Disadvantages*** Application of temperature /pulse voltage/ electric field induces harsh effect on cells Droplet spread out is

		Against gravitational force, offers better printing resolution than downward bioprinting	common occurrence with larger size droplets The interaction with substrate due to gravitational force and sudden deacceleration reduces cell viability
	Micro valve bioprinting	Droplet dia-200–300 µm Printing resolution 300 µm[9]	***Advantages*** Precise cellular positioning Synchronized ejection of hydrogel and cell suspension could be ejected from different nozzles ***Disadvantages*** Droplet dia. > 100 µm is not suitable for patterning, it distorts morphology Cell sedimentation and presence of shear stress Limited cell concentration and cell concentration could be printed due to nozzle clogging
Extrusion based bioprinting	Pneumatic, mechanical and solenoid micro extrusion	Needle diameter varies between 0.25-1.25 mm[10]	***Advantages*** Shear stress during extrusion helps in cellular orientation Introduction of static tensile stress after extrusion (stretching) by modifying deposition mechanism can induce cellular orientation ***Disadvantages*** Shear stress affects cellular functions both short term and long term
Li gh	Laser based bioprinting	Laser Guided Direct Writing Near single cell resolution is possible[11] Laser Induced Forward Transfer Resolution droplet dia. 40-60 µm[12] Laser based stereolithography	***Advantages*** Highly viscous ink with high cell concentration could be printed ***Disadvantages*** Impact of laser energy on cellular functions

		Printing resolution ~ 50μm[13] Two-photon polymerization Printing resolution < 100nm[14]	
op ri	Digital light processing	Resolution of 3–5 μm[15]	***Advantages*** Varying the light intensity in the layer-by-layer method, stiffness gradient could be achieved ***Disadvantages*** Optimization required to use of UV absorbers and photoinitiators for crosslinking to mitigate adverse effects on printed cells

Table S2: Summary of few patterning examples

Sl. No.	Patterning	Printing method	Motive	Ref.
1	Grid of varying fiber diameters and arrangement of fibers to have small and large pores	Electrohydrodynamic printing	Studying structure-induced cell growth for efficient simulation of *in vivo* environment.	[16]
2	Anisotropic patterns having concave and convex interfaces	Microextrusion printing	Fabricating anisotropic networks of type I collage to mimic *in vivo* conditions.	[17]
3	A chessboard of two different types of cells	Laser-assisted bioprinting	Studying the effect of laser-assisted printing on patterning, cell viability differentiating ability and retention of phenotype.	[18]
4	Two-dimensional grid patterns were created by dispensing chitosan or laminin-blended chitosan substrate strands oriented in orthogonal directions.	Dispensing-based rapid prototyping	Perfecting the use of biofunctional pathways in the design of three-dimensional scaffolds for guidance of nerve repair.	[19]
5	Microscaled 3D niches	Two-photon polymerization	Mimicking structural aspects of the native cell/extracellular matrix interaction to highlight the crucial role played by niche 3-D geometry on MSC colonization in culture.	[20]

6	Osteogenic and vasculogenic niches	Extrusion-based bioprinting	Reproduce complex 3D bone architecture.	[21]
7	Broccoli-like nanofibrous particle-scaffolds	Thermally induced phase transition + extrusion-based bioprinting	Reflect natural structure and dimension of collagen fibrils.	[22]
8	Micro/nano surface pores through vigorous agitation of ink with air bubbles and subsequent evaporation of volatile solvent	Extrusion-based bioprinting of polycaprolactone	Promotion of osteogenic differentiation of MSCs	[23]
9	3D scaffolds with micro-patterns (micro-pillar and micro-ridge types) on each layer	Nano-stereolithography	*In vitro* study of the effects of micro-patterns on cellular behaviors of pre-osteoblasts, such as proliferation, adhesion and osteogenic differentiation.	[24]
10	Striped grooved surface structure with many acicular bumps between the grooves.	Cryogenic printing	Efficacy of nutrient deposition/ trapping using modified surface morphology.	[25]
11	Micro-chanelled gelatin scaffolds	Extrusion-based bioprinting	Induction of contractile functionality to cardiomyocytes coupled with helping in orientation, elongation and differentiation of MSCs	[26]
12	Uniaxially aligned surface topography on collagen struts	Extrusion-based bioprinting using polyvinyl alcohol as a sacrificial material and for fibrillation	Higher efficiency of myotube formation noted using myoblasts (C2C12 cell line) with better alignment	[27]
13	Controllably porous half-heart connected with ventricle	Modified inkjet-based bioprinting	Rhythmic contractile function of cardiomyocytes plus periodic beating of entire scaffold	[28]
14	Valentine-shaped heart structure having grooves	Fusion deposition melting utilizing sacrificial nature of PVA	Mimicry of micro-fluid channel-based network in a hollow structure of the human heart	[29]
15	A semi-circular design having two different subpopulations of keratinocytes in a single insert	Inkjet bioprinting	Reproduction of the heterogeneity of the epidermis within an organotypic epidermal model.	[30]
16	Polypyrrole/collagen track	Inkjet bioprinting	Stimulated neurite cell	[31]

	(100 wide, 1.4 μm high)		growth along printed track creating a cell pattern.	
17	Nanostructured ridge/groove-patterns having spacings of 350 nm and height of 500 nm	UV-assisted capillary force lithography	Induction of embryonic stem cells to differentiate into neurons.	[32]
18	Square wells connected by linear channel	Photolithography	Efficient formation of neuron-like networks derived from mouse embryonic stem cells.	[33]
19	Si wafer with linear, circular, and dot micro-patterns	Photolithography	Adult neural stem cell nuclei elongated along the groove axis, and cell extended branches guided by topology on linear and circular patterns	[34]
20	C-shaped rings representing tracheal rings	Extrusion-based bioprinting	Repair of long-segment anterior tracheal defects in a large animal model.	[35]
21	Micropatterns that self-fold into a 3D scaffold.	Four-dimensional (4D) inkjet-printing platform	Human umbilical vein endothelial cells (HUVECs) were embedded in self-folded microtubes to mimic microvessels.	[36]

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