Effects of Macro-/Micro-channels on Vascularization and Immune Response of Tissue Engineering Scaffolds: Literature Review

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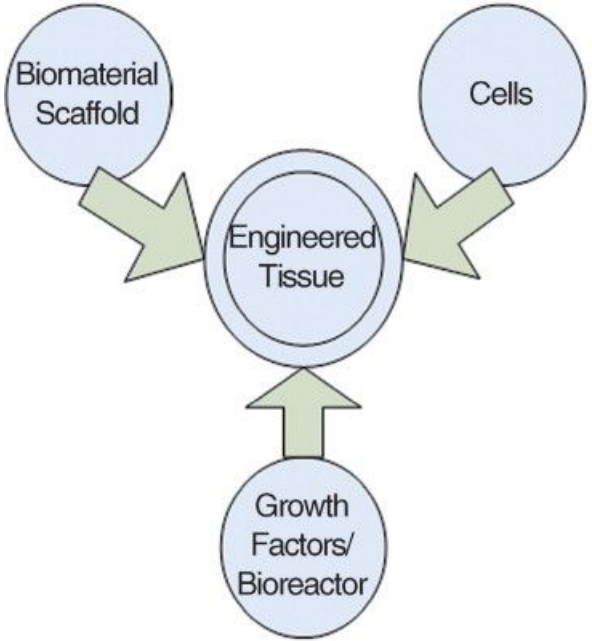
Purpose, Importance and Applications

- Purpose: critically analyze scientific research on effects of macro-/micro-channels on cell recruitment, vascularization, and immune response of tissue engineering scaffolds
- Still a major challenges such as low vascularization, low oxygen and nutrient levels, and immune-induced inflammation
- Recently, research has shown the promise of macro-/micro-channels

Background

- Tissue engineering can be applied to regenerate many different types of tissue, such as bone, liver, heart, skin, and even brain tissue.
- The 3 components of tissue engineering include cells, biomaterial scaffold, and growth factors.
- Scaffolds are extremely vital because they provide an artificial environment for uncommitted stem cells to differentiate, proliferate, and participate in tissue formation.



Background - Cont'd

- Many types of tissues, such as skin, bone, ligament, adipose, and muscle etc., have been developed during in-vitro experimentation; however, there are still limitations
- Recent studies have shown that the incorporation of macro-/micro-channels within scaffolds may address this limitation, allowing the scaffold to better facilitate the survival of cells

Literature Search Methods and Outcomes

- Database: PubMed
- Including only studies published within the last 10 years evaluating the effect of channels on cell response, vascularization and/or immune response
- Two sets of keywords used: “Channel, Vascularization, Tissue Engineering, Scaffold”; “Channel, Vascularization, Tissue Regeneration, Scaffold”
- Exclusions: Reviews, materials research without biological evaluations, simulation or in vitro research models that do not target tissue regeneration applications
- Search outcomes: 117 and 43 articles found by using the above passwords and 12 selected for the current review

Abbreviations

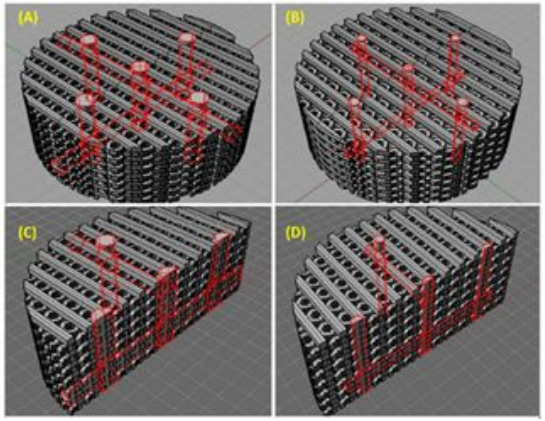
- Mesenchymal stem cells (MSCs)
- Human mesenchymal stem cell (hMSC)
- Human umbilical vein endothelial cell (HUVEC)
- Nano hydroxyapatite (nHA)
- Calcium phosphate cement (CPC)
- Polycaprolactone (PCL)
- Microchanneled 3D printed scaffolds (μCh)
- Neutrophil extracellular traps (NET)
- Vascular endothelial growth factor (VEGF)
- Beta-tricalcium phosphate (β-TCP)
- Bone forming peptide (BFP-1)
- Cardiomyocytes (CM)
- Poly(vinyl alcohol) (PVA)
- Poly (N-isopropylacrylamide) (PNIPAM)

Lead Author, Pub Year	**Scaffold Material**	**Fabrication Method**	**Target Application and Research Model**	**Channel Type**
Holmes, 2016	Polylactic acid (PLA) and nanocrystalline hydroxyapatite (nHA)	3D printing	Bone tissue engineering; In vitro cell culture (hMSC and **HUVEC**)	500 μm and 250 μm diameter microchannels
Yu, 2016	Calcium phosphate cement (CPC)	Dissolution of gelatin fibers of 255 μm and 507 μm in diameter	Bone tissue engineering; In vivo **rat** subcutaneous implantation	Interconnected hollow channels of 500 μm and 250 μm sizes
Won, 2020	Polycaprolactone (PCL)	**3D printing** with camphene and then camphene sublimed	Bone tissue engineering; In vitro cell culture (hMSC and **HUVEC**), in vivo **rat** subcutaneous implantation and in vivo **rat** calvarium defect model	Large pores with microchannels (μCh) 12.9 μm in bulk 21.1 μm on surface
Sheehy, 2014	Agarose hydrogel	Fabricated with a pillared polydimethylsiloxane array structure	Bone tissue engineering; In vivo **mouse** subcutaneous transplantation	Unidirectional longitudinal channels with diameters of 500 μm and a centre-centre spacing of 1 mm
Zhang, 2017	Silicate bioceramic	Coaxial 3D printing with a modified core/shell printer nozzle	Bone tissue engineering; In vitro cell culture (rabbit BMSCs and **HUVEC**) and in vivo **rabbit** radius segmental defect model	Hollow struts with an external diameter of 1 mm and internal diameter of 500 μm
Yu, 2016	Porous β-TCP	β-TCP slurry casted in paraffin-beads filled mold, solidified, dried and sintered	Bone tissue engineering; In vitro cell culture (hBMSC) and in vivo **canine** mandible bony defect model	1 mm diameter hollow channels

Lead Author, Pub Year	Scaffold Material	Fabrication Method	Target Application and Research Model	Channel Type
Fang, 2019	Chitosan and collagen	**3D printed** sacrificial carbohydrate template placed in the mold and dissolved after scaffold preparation	Myocardial tissue regeneration; In vitro cell culture (rat cardiomyocytes, **HUVEC** and C2C12)	The main channel and the branch channel of approximately 744 and 580 μm in diameter
Zieber, 2014	Macroporous alginate scaffolds	Laser piercing technique	Myocardial tissue regeneration; In vitro cell culture (rat cardiac cells and **HUVEC**) and in vivo **mouse** subcutaneous implantation	An array of parallel channels with 200 μm diameter and 400 μm wall-to-wall spacing with shifting between lines of 300 μm
Li, 2016	Gelatin hydrogel	3D printed poly(vinyl alcohol) sacrificial template	Prevasculature and blood perfusion; In vitro cell culture (HUVEC)	2 mm interconnected channels
Lee, 2020	Gelatin hydrogel	N-isopropylacrylamide temperature-dependent water-soluble fibers	Prevasculature and blood perfusion; In vivo **mouse** and **porcine** models of hindlimb ischemia and in vivo **mouse** non-ischemia model	Micro- and macro-channels of 16.37 and 150.46 μm in diameter
Rnjak-Kovacina, 2013	Silk scaffold	Linear wire arrays of either 254 μm or 508 μm diameter in a grid pattern with 1 mm spacing	Large tissue construct engineering; In vitro cell culture (human dermal neonatal fibroblasts) and in vivo **mouse** subcutaneous implantation	254 μm or 508 μm hollow channels
Varoni, 2016	Chitosan scaffold	Aluminum grids with predetermined holes	Neo-vascularization; In vitro cell culture (mouse endothelial cells) and in vivo **mouse** subcutaneous implantation	Regularly-oriented micro-channels (φ 500 μm), which differed for the inter-channel spacing, at 600, 700, or 900 μm

Holmes et al. (2016): Novel 3D bone scaffolds with highly interconnected 3D microvascular mimicking channels

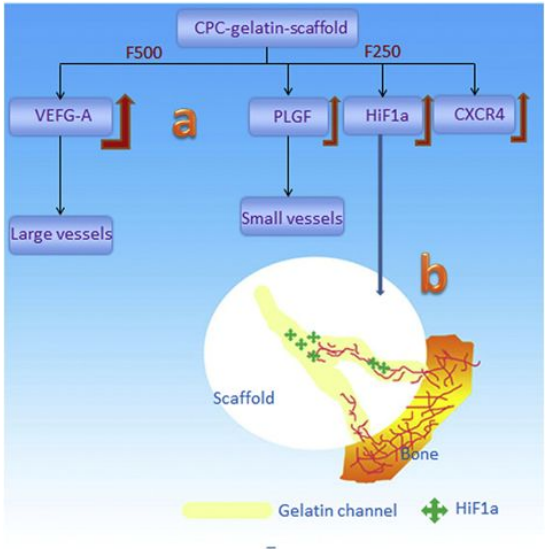
- 3D printed polylactic acid scaffold, channels of 250µm and 500 µm
- Enhanced hMSC adhesion and growth on scaffolds with small microchannels (250 µm) and nHA modification
- Scaffolds with large channels (500 µm) promoted the greatest HUVEC growth
- Powerful construct for further in vitro multiple cell types co-cultured or future in vivo studies



3D CAD modeling of scaffolds with (A), (C) 500 µm microchannels and (B), (D) 250 µm microchannels.

Yu et al. (2016): CPC with interconnected hollow channels formed by dissolution of micro (255 µm) and macro (507 µm) gelatin fibers

- When implanted in vivo, the pre-established vascular networks anastomose with host vessels and accelerate vascular infiltration throughout the whole tissue construct
- Independent variable: 250 µm, 500 µm; different channel sizes induce different vascularization behaviors
- CPC, an extremely promising material, but more experimentation needed



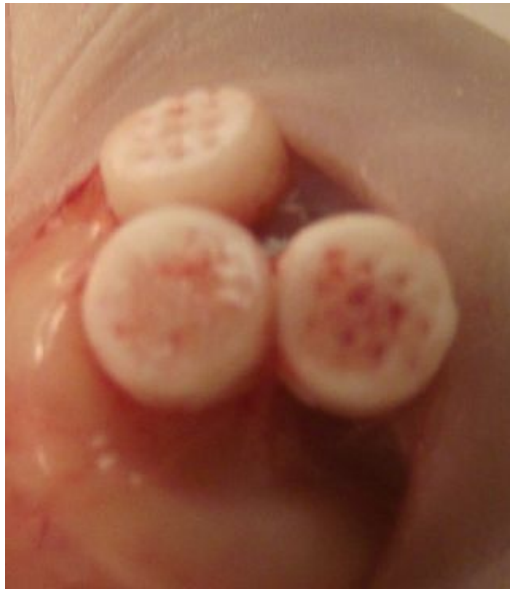
Schematic of the vascularization strategy within channels of CPC scaffold

Won et al. (2020): 3D printed macroporous (a few hundreds μm) PCL scaffolds with microchanneled structures (a few to tens μm)

- NETs present in control group while not present in μCh group.
- Macrophages covered μCh over 7 days polarized toward M2 which contrasted with control where M1 being dominant
- μCh greatly improved vascularization
- The molecular, cellular and tissue reactions to the μCh were coherently favorable for the regeneration process of tissues

Sheehy et al. (2014): Introduction of channels (500 µm) to tissue engineered hypertrophic cartilaginous grafts facilitates vascularization

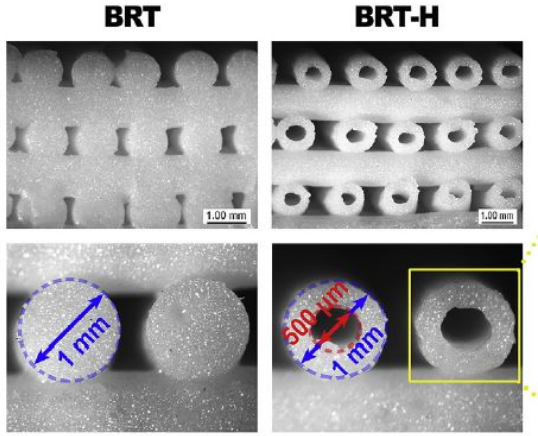
- 6 weeks of cell culturing, then implantation in nude mice
- Upon retrieval from nude mice, channeled group had significantly better results
- The study reinforces the importance of optimizing the architecture of engineered constructs targeting bone tissue regeneration
- The Use of hydrogels as scaffolds for endochondral bone is extremely promising



Retrieval of channelled constructs from the subcutaneous pocket 8 weeks post-implantation

Zhang et al. (2017): 3D printed hollow-pipe-packed silicate bioceramic (BRT-H) scaffolds

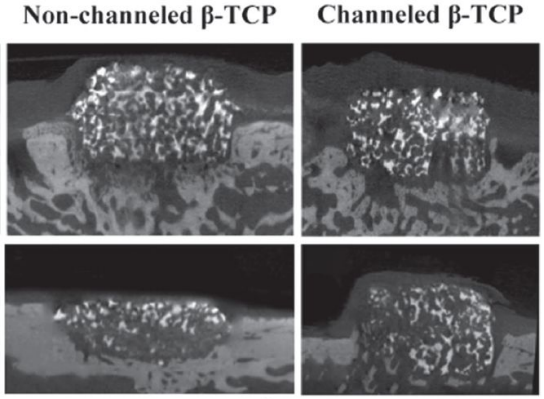
- Implanted in rabbit for 4 weeks, scaffolds with channels contained much greater vascularization
- Hollow pipes in the scaffolds and BRT bioceramic enhanced the angiogenic process
- This is a promising method for large tissue/organ regeneration



The structure of the BRT-H scaffolds with about 500 mm inner channels

Yu et al. (2016) Macroporous β-TCP scaffolds with multiple vertical hollow channels

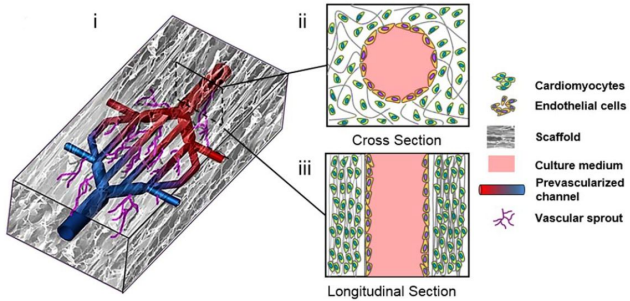
- Scaffolds with BFP-1 implanted in canine mandible bone defects (8mm in diameter and 2mm in depth)
- After 4 and 8 weeks, channeled scaffolds had more vascularization
- Height of tissue was greater in the channeled group
- Future research still necessary



Micro-CT images show that channeled scaffolds can maintain the height of regenerated bone, but scaffolds without channels degraded and lowered the height

Fang et al. (2019): Biomimetic microporous scaffolds with branched channel networks for myocardial tissue engineering

- 7 d of culturing of endothelial cells, they migrated through 10–50 µm micro-holes of branch channel into scaffolds; formed sprouts
- Channels has potential to create healthy myocardial tissue



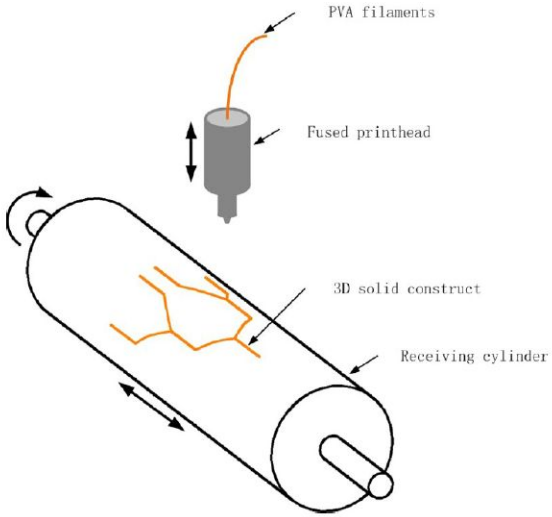
Schematic design of biomimetic scaffold integrated with oriented micro-pores and branched channel networks. Cross section (i) and longitudinal section (ii) of scaffold showed that endothelial cells lining on the lumen surface formed a confluent endothelium while cardiomyocytes aligned directionally along the oriented pores.

Zieber et al. (2014): Microfabrication of channel arrays (200 μm) promotes vessel-like network formation in cardiac cell construct and vascularization

- HUVECs seeded and cultured for three days, then CMs and cardiac fibroblasts added and cultured for another 7 days
- A vessel-like network formed within the cell constructs
- Cells implanted into mice, cells entered channeled scaffolds much more easily than non-channeled
- Greater vascularization in channeled implants

Li et al. (2016): Fabricating tissue engineering scaffolds with a 3D channel (2.2-3 mm) for pre-vasculature networks

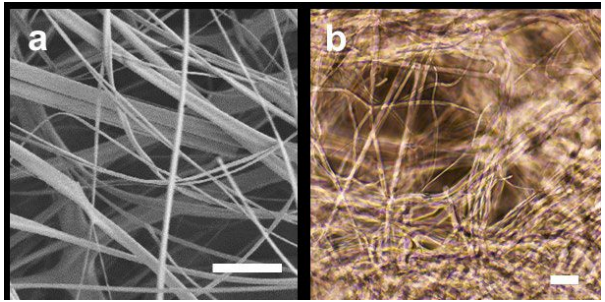
- HUVECs seeded and cultured for 2 days
- Proved their gelatin scaffold was nontoxic and promoted cell growth and vascularization
- No quantitative data
- More future work necessary



Schematic for the printing of the three-dimensional solid sacrificial template.

Lee et al. (2020): Microchannel network hydrogel induced ischemic blood perfusion connection

- Implanted into mouse for 14 days
- This study showed that microchannels were more beneficial than macrochannels
- Significant for regenerative medicine



a. *An SEM image of solvent spun PNIPAM fibres and* ***b.*** *a bright field microscopic image of microchannel network hydrogel. Scale bars = 100 μm*

Lead Author, Pub Year	**Strength**	**Weakness**
Holmes, 2016	Detailed characterizations of materials microstructure and strength; Comparison of two different microchannel sizes	Only in vitro cellular response was evaluated; confounding effect of nHA
Yu, 2016	Comparison of two different microchannel sizes; Researched the effects of cell and growth factor recruitment and vascularization	Subcutaneous implantation model does not directly relate to bony defects
Won, 2020	Used both subcutaneous and calvarium defect models; Researched the effects of cell recruitment and immune response	Only one small size microchannel was evaluated
Sheehy, 2014	MSCs were primed in the hydrogel scaffold for 6 weeks before implantation in vivo (true tissue engineering approach)	Only one size of channel was evaluated; Subcutaneous implantation model does not directly relate to bony defects
Zhang, 2017	Used rabbit radius segmental defects with two different healing periods, i.e., 4 weeks and 12 weeks	Confounding biological effect from inorganic ions release
Yu, 2016	Used large animal model: canine mandible bony defect	Only one size of channel was evaluated; confounding effect of growth factor

Lead Author, Pub Year	Strength	Weakness
Fang, 2019	Used both 3D printed sacrificial template and porogen (NaCl particles) to engineer biomimetic scaffold; Enabled dual cell seeding	Only in vitro cellular response was evaluated
Zieber, 2014	Tricell culture; Multiple implantation time periods, i.e., 4, 6, and 8 weeks	Only one size of channel was evaluated; Confounding effect of growth factor
Li, 2016	Demonstrated a novel strategy toward the engineering of prevasculature thick tissues through the integration of the fused PVA filament deposit (sacrificial material)	Only in vitro cellular response was evaluated
Lee, 2020	Comparison of two different microchannel sizes; Used multiple in vivo models	Random channel structure
Rnjak-Kovacina, 2013	Comparison of two different microchannel sizes	Subcutaneous implantation model does not directly relate to a specific tissue type
Varoni, 2016	Studied multiple time periods, i.e., 3 and 6 weeks	Subcutaneous implantation model does not directly relate to a specific tissue type

Conclusions

- The incorporation of macro-/micro-channels is beneficial for tissue regeneration
- The ideal size of channels may vary depend on the tissue type
- 3D printing and sacrificial template most common methods of scaffold fabrication

Applications

- Tissue engineering can be applied to many tissues/organs
- Today, tissue engineering scaffolds can be used in multiple ways (Mhanna & Hasan, 2017)
- The introduction of macro-/micro-channels can solve the major limitations of tissue engineering
- Channels extremely important for tissue regeneration and are extremely promising

Limitations

- Limited research done on this topic
- No two studies were the same
- Unable to the statistical analysis comparing different studies

Future Studies

- Currently, only proof of principal experimentation and experimentation on small organisms
- Experimentation on larger organism or humans in future
- FDA approve, use during surgery

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