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3D-Bioprinted Constructs that Breathe

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An old but still hot topic in tissue engineering (TE) is the establishment of efficient vascularization networks proving fine, controlled, and long-term distribution of oxygen and nutrients. Combining elegant three-dimensional (3D) fabrication techniques with unconventional living microorganisms, namely photosynthetic species, complex 3D-printed TE constructs are proposed by Maharjan et al. enabling to provide a continuous and on demand oxygen supply to mammalian tissues.

Following the implantation of three-dimensional (3D) bioengineered systems, a series of well-orchestrated and complex biological processes occur aiming towards tissue repair and regeneration.[1] While the *in vitro* delivery of essential biomolecules assuring cell survival can be more easily circumvented, using for example cell culture apparatus such as perfusion bioreactors, most tissue engineered strategies fail upon implantation. *In vivo*, tissue survival is dictated by a 100-200 μm distance from the nearest capillary, thus the implanted biomaterials have to rely on a vascular network to supply oxygen and nutrients. Deprived from that, hypoxic regions are generated, and consequently cell proliferation is significantly jeopardized, as well as other important cellular processes, such as gene expression and matrix deposition. Ultimately, either by the absence of a vascular network, or its delayed establishment, cell death and microtissue necrosis occur, specially at the inner regions of the TE constructs. Considering that situations demanding the intervention of TE approaches are most often those that include large defects, otherwise solved by the innate self-healing of the human body, the establishment of a proper vascular supply is even more critical, and challenging, in such clinical-relevant 3D artificial strategies. Different approaches have been proposed to overcome nutrient perfusion and mass transport limitations of TE constructs, ranging from (1) bioengineered systems functionalized with key binding sites (Figure 1i) to guide and facilitate angiogenesis (e.g. integrins, growth factors);[2] (2) systems based on void-spaced or liquified structures to enhance diffusion and vascular ingrowth – those devices can be produced by sophisticated techniques, such as 3D bioprinting and electrospinning-based approaches, that enable high geometrical precision, and thus playing with bulk features (Figure 1ii-a), such as architecture and porosity,[3] or by using bottom-up strategies (Figure 1ii-b), such as encapsulation strategies in liquefied environments;[4,5] and (3) *in vitro* prevascularized scaffolds (Figure 1iii).[6]

However, the *in vivo* establishment of functional vascular networks still remains a major challenge. While decorated constructs rely on the timely arrival of specific cells that possess the inherent ability to self-organize and achieve this complex stage, thus being limited by a spatiotemporal feature, prefabricated vascular constructs still present limited capacity to anastomose with the host vascular network. Additionally, such TE approaches also present inherent physical barriers that block the ingrowth of host vessels, either resultant from the biomaterial content itself and from the deposited extracellular matrix. Ultimately, besides failing the healing process of the target tissue, the resultant gradients of oxygen that led to the generation of hypoxic regions following the implantation of TE constructs, can also trigger tumor microenvironments by affecting metabolic, inflammatory, and differentiation pathways of endogenous cell subsets.

It is thus easily to understand why vascularization is one of the main challenges that need to be taken to bring tissue engineering into the clinical practice at a large scale. As such, vascularization is an old but still hot topic in the tissue engineering community. More recently, in an attempt to overcome the associated road blocks of poorly vascularized bioengineered systems, a plethora of materials able to release oxygen (Figure 1iv-a) have been proposed.[7] Such oxygen-releasing materials are often composed of solid peroxides (e.g. solid calcium and magnesium peroxides), hydrogen peroxide, sodium percarbonate, and fluorinated compounds. However, the associated burst release of oxygen arises cytotoxic concerns, as well as short-term supply curves. To overcome these limitations, continuous and on demand living suppliers of oxygen have been proposed relying on the co-culture of mammalian cells with unconventional organisms, such as photosynthetic species (Figure 1iv-b), namely microalgae,[8] or bacteria.[9] Different applications have been proposed aiming towards wound healing, cancer therapy, cardiovascular related diseases, and cell transplantation. In this issue of *Matter*, Maharjan et al.[10] take a step

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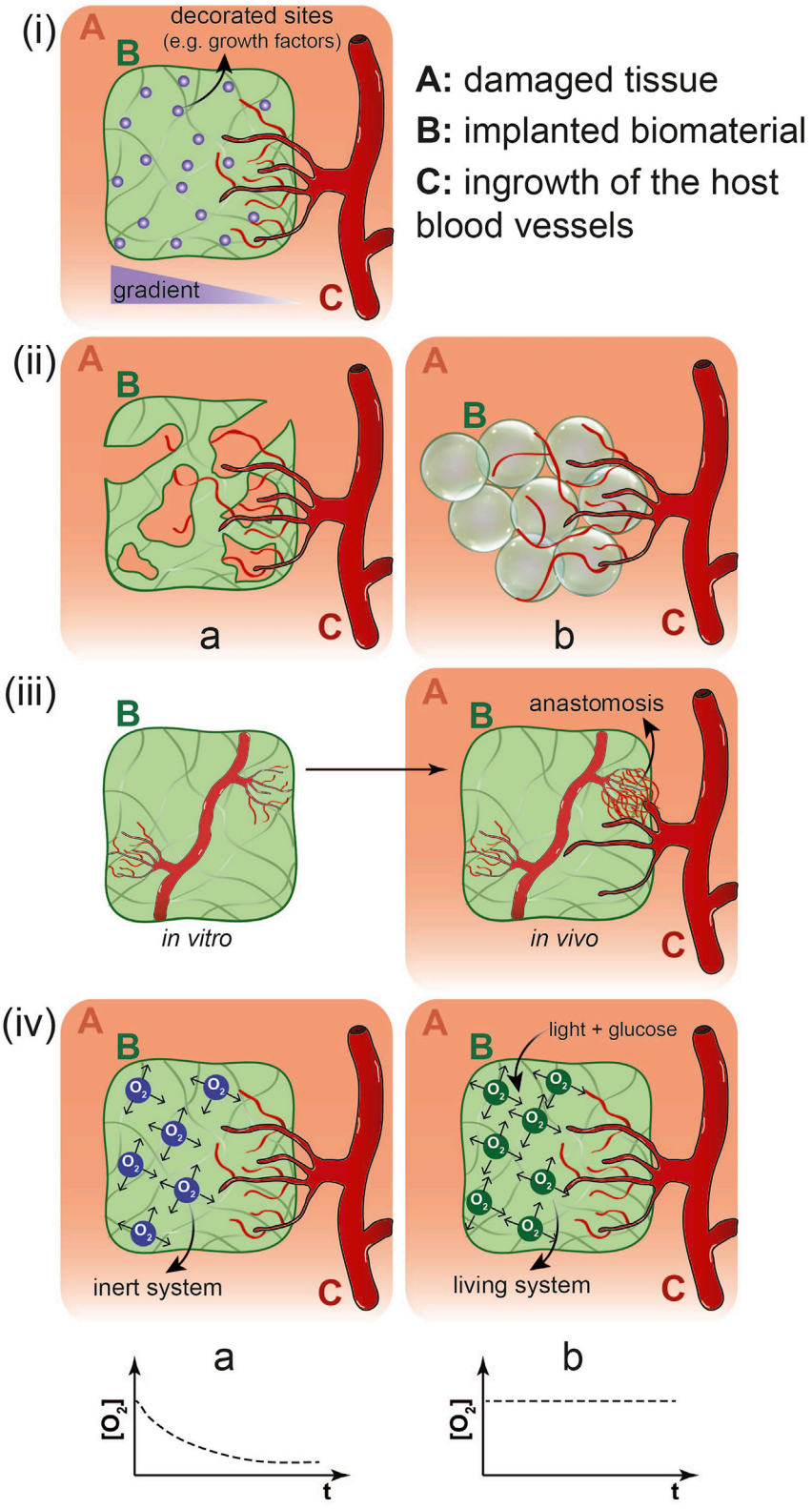

Figure 1. Tissue Engineering Strategies with Enhanced Nutrient Perfusion and Mass Transportation Properties Aiming towards Vascularization

(i) Bioengineered systems decorated with gradients of key biomolecules to guide the ingrowth of the host vessels. (ii) Void-spaced strategies allowing the freely ingrowth of the host vessels, which can be produced by (a) fine-tuning the bulk properties of the scaffold, such as architecture and porosity, or (b) bottom-up assembly of liquified micro-compartments. (iii) Prevascularized strategies prepared *in vitro* aiming rapid anastomosis with the host vasculature upon implantation. (iv) Oxygen releasing systems relying on the presence of (a) inert materials or (b) living organisms, such as photosynthetic species. While the release of oxygen in (a) follows a burst and short-term fashion, in (b) a continuous and on demand supply of oxygen can be achieved.

further in the development of such living oxygen suppliers by embedding bioprinted photosynthetic algae (*C. reinhardtii*) with mammalian cells (HepG2) in a 3D gelatin methacryloyl-based hydrogel matrix to create honeycomb-shaped constructs mimicking the human liver. The bioprinted algae was able to release oxygen upon specific light conditions, and consequently enhanced cell proliferation and production of liver-specific proteins. Remarkably, the authors were also able to produce microchannels taking advantage of the enzymatic degradation of the cellulose-based bioink. Afterwards, vascular networks were created by repopulating the hollow microchannels with endothelial cells (C2C12). These findings further open the door to the prospect of next-generation of vascularization strategies relying on the use of unconventional living components such as algae. We preview that the organelles of such microorganisms may also be explored, such as chloroplasts, since they exhibit similar potential in the continuous and unlimited release of oxygen. We believe that such strategies may contribute to significantly improve the *in vivo* bioperformance of TE constructs or to act as light-responsive drug delivery systems. Detailed

understanding of the living organisms or organelles to be used in combination with biomaterials, its oxygen producing pathways, and the establishment of the adequate balance of the co-culture conditions suitable for the survival of both algae and mammalian cells, are key prerequisites to be considered in the design of such strategies. In order to develop a fully functional living organism-based oxygen release system, it is essential that all the surrounding factors such as type of microalgae species, culture medium, temperature range, and light intensity are mutually accepted by both algae and mammalian cells, which have distinct optimal conditions. We truly believe that photosynthetic microorganisms-based strategies can be harnesses as a sustainable, eco-friendly, cost-effective source of oxygen for human cells, thus bring considerable advantages to the TE field. Of course, the translation of such systems into the clinics will be hindered by important regulatory constrains that should be addressed in the future.

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1. Lee, K., Silva, E.A., and Mooney, D.J. (2011). Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J. R. Soc. Interface *8*, 153–170.

2. Li, S., Nih, L.R., Bachman, H., Fei, P., Li, Y., Nam, E., Dimatteo, R., Carmichael, S.T., Barker, T.H., and Segura, T. (2017). Hydrogels with precisely controlled integrin activation dictate vascular patterning and permeability. Nat. Mater. *16*, 953–961.

3. Kapfer, S.C., Hyde, S.T., Mecke, K., Arns, C.H., and Schröder-Turk, G.E. (2011). Minimal surface scaffold designs for tissue engineering. Biomaterials *32*, 6875–6882.

4. Correia, C.R., Nadine, S., and Mano, J.F. (2020). Cell Encapsulation Systems Toward Modular Tissue Regeneration: From Immunoisolation to Multifunctional Devices. Adv. Funct. Mater. *30*, 1908061.

5. Correia, C.R., Bjørge, I.M., Zeng, J., Matsusaki, M., and Mano, J.F. (2019). Liquefied Microcapsules as Dual-Microcarriers for 3D+ 3D Bottom-Up Tissue Engineering. Adv. Healthc. Mater. *8*, 1901221.

6. Guo, S., Redenski, I., Landau, S., Szklanny, A., Merdler, U., and Levenberg, S. (2020). Prevascularized Scaffolds Bearing Human Dental Pulp Stem Cells for Treating Complete Spinal Cord Injury. Adv. Healthc. Mater. *9*, 2000974.

7. Camci-Unal, G., Alemdar, N., Annabi, N., and Khademhosseini, A. (2013). Oxygen Releasing Biomaterials for Tissue Engineering. Polym. Int. *62*, 843–848.

8. Chávez, M.N., Schenck, T.L., Hopfner, U., Centeno-Cerdas, C., Somlai-Schweiger, I., Schwarz, C., Machens, H.-G., Heikenwalder, M., Bono, M.R., Allende, M.L., et al. (2016). Towards autotrophic tissue engineering: Photosynthetic gene therapy for regeneration. Biomaterials *75*, 25–36.

9. Li, W., Wang, S., Zhong, D., Du, Z., and Zhou, M. (2020). A Bioactive Living Hydrogel: Photosynthetic Bacteria Mediated Hypoxia Elimination and Bacteria-Killing to Promote Infected Wound Healing. Adv. Ther. *2000107*, 1.

10. Maharjan, S., Alva, J., Cámara, C., Rubio, A.G., Hernández, D., Delavaux, C., et al. (2020). Bionic Photosynthetic Oxygenation within 3D-Bioprinted Vascularized Tissues. Matter *4*.

Lithiophilic Covalent-Organic Frameworks Enable Uniform Lithium Deposition

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Construction of lithiophilic sites can effectively facilitate uniform lithium (Li) deposition, which is significantly critical for stable cycling Li metal batteries. Recently, Song et al. report a boroxine covalent-organic framework (COF-1) as an excellent lithiophilic host to achieve very stable Li metal batteries with long lifespan.

To meet the increasing requirements of portable electronics and electric vehicles, it is vital to develop high-energy-density battery systems.[1] Lithium (Li) metal is the most attractive anode for constructing high-energy-density batteries because of its high specific capacity (3860 mAh g^{-1}) and low reduction potential (−3.04 V versus the standard hydrogen electrode).[2] However, the infinite volumetric change of Li metal during electrochemical cycles, leading to un-uniform Li deposition and even Li dendrites, severely impedes the practical applications of Li metal anodes. In the past years, various approaches have been proposed to stabilize the Li deposition,[3,4] of which construction of stable host materials for Li metal is the key to regulate Li cycling behaviors and suppress the growth of Li dendrites.[5–7] To design an appropriate host material, good lithiophilicity, which represents its affinity to Li species, is required.

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