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Spotlight

'Living' Inks for 3D Bioprinting

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A critical aspect in bioprinting is the formulation of bioactive inks. A recent breakthrough in bioink design (Qian *et al. Nano Lett.* 2019; https://doi.org/10.1021/acs.nanolett.9b00066) enables the direct writing of catalytically active microorganisms with unprecedented cell loading, tunable structural properties, and long-term activity. This strategy will create possibilities for bottom-up design and integration of functional living systems.

Bioprinting is a method of constructing engineered biosystems through precise 3D assembly of basic biocomponents, such as cells, growth factors, and supportive biomaterials, into hierarchical structures with rationally designed functionalities. In the past decade, bioprinting has been broadly applied in tissue engineering to regenerate artificial human tissues or organs [1]. In the context of energy and environmental science, microorganisms are also widely exploited as self-sustainable biocatalysts to build systems that are capable of processing complex biochemical reactions and bioenergy transduction with superb efficiency and specificity at low-cost, mild reaction conditions [2,3].

The effective integration of these living components in bioink design, however, remains a major challenge in realizing and enhancing the biologically relevant functions in the printed complex [4]. For example, in extrusion-based 3D printing, the printability of bioink largely relies on its rheological properties. Printability is usually enhanced by formulating with high-volume viscoelastic supporting materials, or fillers, thus compromising the maximum cell load and volumetric productivity in biocatalysis [5]. Qian and colleagues invented a novel approach by directly exploiting freeze-dried cells as both active biocatalysts and fillers, to produce a high-performance bioink with significantly enhanced cell loading density while providing desirable rheological properties for 3D printing (Figure 1A) [6]. As the first bioink prototype, the loading density of living Baker's yeast (*Saccharomyces cerevisiae*) was significantly increased to 750 g/l cell dry weight (orders of magnitudes higher than that in liquid culture). The cells were almost closely packed in the ink. Additionally, by utilizing nanocellulose as an optional secondary filler, the authors of this study achieved comprehensive control of ink rheology (e.g., plateau modulus, a critical parameter characterizing the elasticity of polymeric liquids, can be tuned from 400 to 60 000 Pa) and cell density (0–8.6×10^9 cells/ml) over a wide range for tailored applications.

Low cell viability is another major challenge among extrusion-based bioprinting, attributable to both the printing process (shear stress) and bioink composition (cytotoxicity) [7]. In this regard, the current bioink design demonstrates outstanding biocompatibility, where the encapsulated cells show comparable viability to batch cultured ones with a long life time (up to 4 months). Unlike conventional formulations, the utilization of living cells as the major bioink component minimizes the chemical toxicity from the use of synthetic additives, while also leading to unique shear-thinning properties that could reduce the direct shear stress loading per cell. Additionally, a visible light (405 nm) photoinitiator with less cytotoxicity is used to further improve the biocompatibility. These designs and improvements are expected to provide critical insights about minimally invasive integration and the long-term preservation of living materials through extrusion-based bioprinting.

The high tensile strength and excellent self-supporting properties of the living material enable the fabrication of intricate architectures, from 2D serpentine patterns to 3D hollow cone or circular translating coil, with high resolution (100 μm) and large scale (up to 225 cm^2). From a biocatalytic perspective, this designing freedom provides extensive opportunities to raise efficiency by optimizing the mass transport of nutrients and products within the 3D matrices. For example, by creating a highly porous lattice structure with a substantially expanded liquid–solid interface, the productivity of the printed yeast lattice increases threefold compared with its bulk counterpart, which contains a similar amount of cells but with much lower surface area. This strategy is also fully compatible with existing industrial infrastructure to elevate the biocatalytic volumetric productivity at significantly increased printing dimensions.

To implement the approach toward to a broader scale of engineering applications, more systematic studies will be needed to address the large variation in

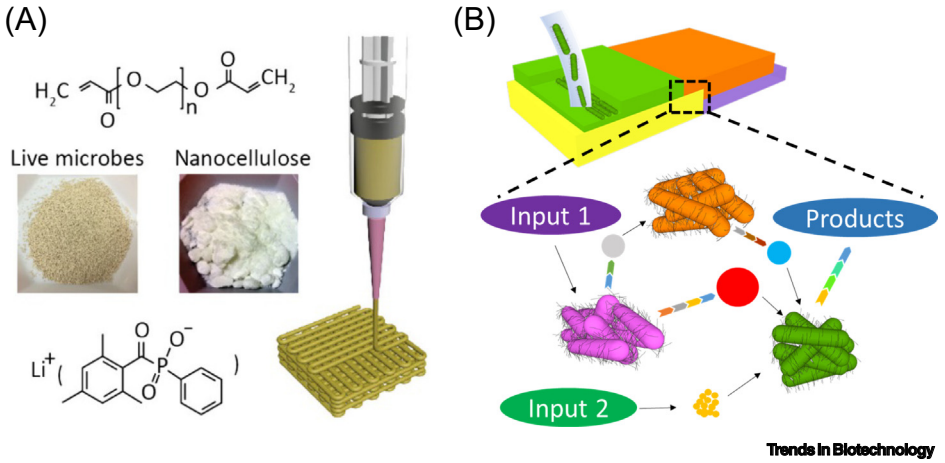

Figure 1. Summary and Prospective of the Living Ink Design. (A) Schematic illustrating the formulation and 3D printing of the bioink. Reproduced, with permission, from [6]. (B) Multidimensional integration of living and synthetic components for programming biological processing and system functionality from the bottom up.

physiochemical properties among different microbes. The size, stiffness, surface chemistry, and cellular interactions of these 'living fillers' are directly associated with the rheological behavior of the bioink and determines its printability. The cellular structure/behavior could also affect the survival rates after freeze drying, which is critical to the success of current strategy. For example, bacterial cells with large flagella production and high motility are known to have low survival rates after freeze drying [8]. These are important factors to consider and optimize when applying the technique to other living systems.

Moving forward, advances in living ink development are expected to assist both fundamental and applied microbiology by constructing and customizing the microbial community from the bottom up (Figure 1B). Particularly when combined with powerful synthetic biology tools, the approach has the potential to create new functionality and improved performance over natural systems by genetically engineering individual living components, spatially modulating their microenvironments and interactions, and programming their ensemble behavior at network levels [9]. These efforts could benefit from continuing research in more sophisticated control of physiochemical properties and multidimensional assembly of bioinks at a range of biologically relevant length scales.

In summary, the advance made by Qian and colleagues represents a new strategy for bioprinting where high-density frozen cells are used as a central component to overcome many intrinsic limitations of traditional bioink formulations. This work could provide important insights into the fundamentals of bioprinting design and process, and unveil its potential toward a range of applications beyond the current paradigm.

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Forum

Safety Assessment of Immune-Mediated Adverse Reactions to Novel Food Proteins

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Current international guidelines for the risk assessment of biotechnology-derived foods date back to 2003. We present new strategies and directions for assessing