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## A definition of bioinks and their distinction from biomaterial inks

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# Biofabrication



## PERSPECTIVE

### A definition of bioinks and their distinction from biomaterial inks

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## Abstract

Biofabrication aims to fabricate biologically functional products through bioprinting or bioassembly (Groll *et al* 2016 *Biofabrication* 8 013001). In biofabrication processes, cells are positioned at defined coordinates in three-dimensional space using automated and computer controlled techniques (Moroni *et al* 2018 *Trends Biotechnol.* 36 384–402), usually with the aid of biomaterials that are either (i) directly processed with the cells as suspensions/dispersions, (ii) deposited simultaneously in a separate printing process, or (iii) used as a transient support material. Materials that are suited for biofabrication are often referred to as bioinks and have become an important area of research within the field. In view of this special issue on bioinks, we aim herein to briefly summarize the historic evolution of this term within the field of biofabrication. Furthermore, we propose a simple but general definition of bioinks, and clarify its distinction from biomaterial inks.

## Introduction

In recent years, the fabrication and characterization of new bioinks gained increasing attention, particularly as the lack of materials suitable for bioprinting was identified as one of the major drawbacks limiting rapid progress in the field [1, 2]. This resulted in the evolution

of new strategies to develop and modify materials to be used as, or in, bioink formulations for bioprinting, which is elegantly reviewed in a number of recent articles [1–9]. However, along with this rising interest, the apparent definition of the term bioink became increasingly divergent. This is particularly obvious when the evolution of the use of the term is reviewed.

## Origin of the term bioink

The term bioink was first used in the context of organ printing in 2003 and was introduced together with the term biopaper [10, 11]. Initially, the concept was to provide, or even print, a biopaper (hydrogel) and then insert living cells or tissue spheroids as the 'bioink' by bioprinting. Thus, the term bioink originally referred to the cellular component that was positioned in three-dimensional (3D) on or within hydrogels. In many of the pioneering studies in the field, cells and cell aggregates were used as the bioink [10–12]. However, even at this stage, some authors were arguing that a practical bioink formulation should be 'structurally and functionally more sophisticated' [10]. Simultaneously, the number of additive manufacturing techniques capable of being used for bioprinting increased over time to include extrusion, droplet deposition such as inkjet and microvalve based techniques, laser forward transfer, and lithography-based techniques, all of which have different physical and rheological requirements for a workable ink.

With the further development of the field of bioprinting and especially the increasing adaptation of direct write extrusion-based printing, appreciation for the understanding of material rheological properties used in the printing process increased. For example, in extrusion-based approaches, the key challenge is that the ink must be dispensed through a narrow nozzle, which is often achieved through exploiting shear-thinning behavior; however, after deposition, the printed 3D object must be stable. Therefore, the material must rapidly increase in viscosity after exiting the nozzle, which is typically supported by post-printing cross-linking. Consequently, the need for a separate biopaper was no longer required, and accordingly, a single unified concept of a bioink being the dispensed material was attained.

## Recent trend for diversified use of the term bioink

Regardless, the term bioink is now ubiquitously used in a growing number of review articles [5–9, 13, 14]. Some of these suggest an extension of the definition towards additively manufactured materials. For example, the term 'fugitive bioink' has been introduced for sacrificial biomaterials that can be processed by an additive manufacturing technology and result in cytocompatible constructs that can be leached or washed away or dissolved to result in pores [15]. Recently, a division of the term bioink into four sub-categories was proposed [16]: support bioinks, fugitive bioinks, structural bioinks and functional bioinks. The suggested discrimination between the different classes in this case was that: (i) *support bioinks* are materials designed to support cell populations during delivery and act as an artificial extracellular matrix as cells

multiply; (ii) *fugitive bioinks* are sacrificial or temporary materials that can be rapidly removed to form internal voids or channels within a printed construct; (iii) *structural bioinks* are used to provide mechanical integrity to printed structures and may also be fugitive but over a relatively long timescale (e.g. thermoplastics such as polycaprolactone), and finally; (iv) functional bioinks provide biochemical, mechanical or electrical cues to influence cellular behavior after a structure is printed. We think that this classification is unnecessarily complicated and, as pointed out in more detail below, derived from the definition of biomaterials, which in our opinion is not reasonable for a definition of bioinks.

Similar to other recent articles, this classification of four classes of bioink has been driven by the role of the constituent materials in the final function of a printed construct, rather than by the fabrication procedure itself. The definition of biomaterials has for a long time been a vital discussion that is still ongoing. This is due to the broad range of biomaterials (including metals, ceramics, polymers and semi-conductors as the most important examples) with strongly deviating properties, and an equally broad application from basic research to clinical translation, covering contact times with the body from seconds to decades. This led to an application and purpose driven definition of the term biomaterial, rather than a material-based definition. A comprehensive and well-accepted article by Williams describes the evolution of biomaterials research and the definitions for terms, such as *biomaterial* and *biocompatible* [17].

It is reasonable to define biomaterials, which comprise a variety of materials that are suitable for a broad range of manufacturing and processing techniques, including spinning, knitting, extruding, machining, chipping and additive manufacturing, from the perspective of their final purpose and utilization. Biofabrication, however, is a more focused field of research, and is characterized by the exploitation of automated procedures to directly create a 3D arrangement of cells, often with the aid of biomaterials. The potential applications of such products are not important for the definition of the bioink. Hence, the 'bio' in the term bioink relates to cells as true biological components of the formulation. This is an important feature, as this biological component drastically limits the processes and most importantly the processing conditions that are suitable, and thus significantly raises the demands on biomaterials and technology.

In our view, and in agreement with the recently updated definition of Biofabrication [18], bioinks can generally be described as a formulation of cells that is suitable to be processed by an automated biofabrication technology. Cell-encapsulating materials are often used, but not necessarily mandatory as an additional bioink component, in line with the pioneering studies mentioned above where in these cases the bioink consisted of only aggregated cells in the form of

cell spheroids or microtissues. Cell-based bioinks are a vital field of research today [19–21] and very promising studies involving functional tissue constructs and translational perspectives have recently evolved [22–24]. Therefore, cells are a mandatory component of a bioink, so that a formulation that includes biologically active components or molecules, but does not contain any cells, would not qualify as a bioink. By way of example, formulations that cannot be considered as *bioinks* include: thermoplasts supplemented with drug molecules, inorganic powders or slurries that release bioactive ions. Furthermore, aqueous formulations of polymers or hydrogel precursors that contain biological factors would be considered *biomaterial inks*, that—by definition—would become *bioinks* following the addition of cells into that formulation.

A relatively new set of technologies applied in Biofabrication are lithography-based technologies, such as stereolithography (SLA), digital light processing (DLP), continuous liquid interface production, or two-photon polymerization, which allow for the fabrication of 3D structures with high spatial resolution [25–30]. These approaches involve spatial patterning of light to photo-crosslink specific regions of a bioink (acting as a resin), usually composed of a low-viscosity photocrosslinkable hydrogel precursor [29, 31]. Thus, bioinks that are suitable for these technologies, so called bioresins, must exhibit characteristics that are compatible for lithographic processes and which differ significantly from the requirements for bioprinting [27]. These difference comprise, for example, rheological properties facilitating layer-by-layer deposition in case of DLP and SLA, and/or supporting high spatial resolution, which is usually a function of reactivity and number of cross-linkable groups per volume of the material. Nonetheless, it is possible to develop systems that can be used for bioprinting and lithography, and in some cases without the need for a photo-absorber [32]. Therefore, in addition to the recently updated definition of Biofabrication and in agreement with our definition of bioinks, bioresins could be considered as a subset of bioinks consisting of a formulation of cells that is suitable to be processed by an automated lithography-based biofabrication technology. Further development and characterization of this potential class of bioinks within this emerging field is required to elaborate on this distinction.

### Bioinks and biomaterial inks

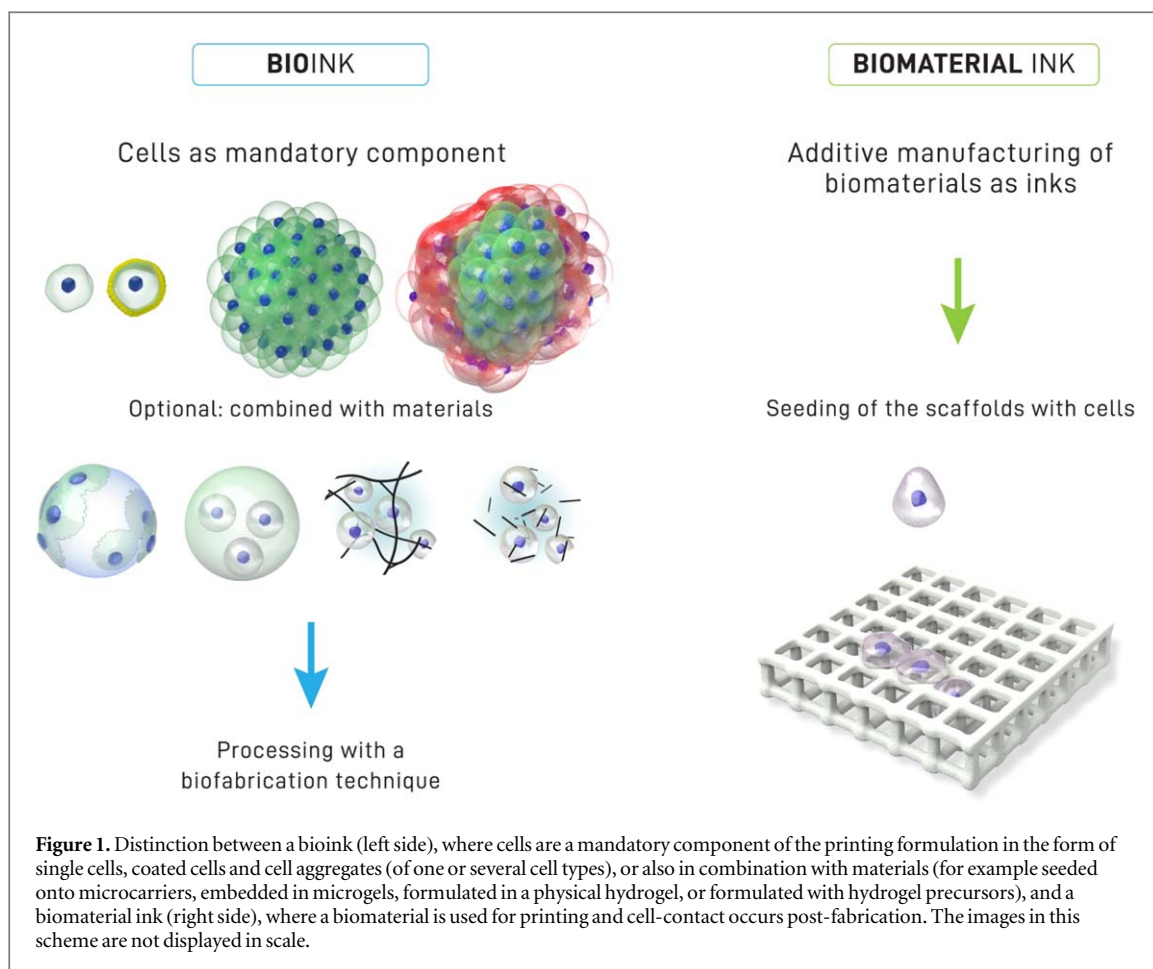
With these definitions in mind, biomaterials that qualify as a bioink must serve as a cell-delivery medium during formulation and processing [33]. It is often stated in literature that hydrogels are the most commonly used bioinks. This is, however, only true for a few studies where physical hydrogels are applied that form a gel before printing. Examples of this include, designed peptide-polymer hybrids [34] and

spider silk protein-based hydrogels [35]. In most studies, materials used for formulation of bioinks are hydrogel precursors that can be cross-linked into hydrogels post-fabrication. A recent intermediate approach is the pre-cross-linking of precursor solutions to a state of higher viscosity, followed by complete cross-linking post-fabrication [36]. This can generally be extended also to cross-linking at the final stage of the extrusion process in the needle immediately before deposition for extrusion-based biofabrication of bioinks [37], or by core-shell approaches at the timepoint when the bioink leaves the nozzle [38]. In addition, it is important to note that bioinks are not restricted to molecular solutions of precursors. Bioinks can also contain microcarriers that may be cell-seeded [39] or nanoparticles that may serve for drug release [40] or to improve rheological and mechanical properties [41]. Also, cell-loaded microgels [42] or microspheres [21] can be used as bioink components to impart additional functionality (figure 1).

For further clarification and distinction, (bio-) materials that can be printed and subsequently seeded with cells after printing, but not directly formulated with cells, thus do not qualify as a bioink. We suggest that these are termed *biomaterial inks*. Such biomaterial inks may be used to produce scaffolds for cell seeding, bioreactors, implants, or they may be used in parallel to bioink-fabrication in hybrid approaches to generate an intrinsic mechanical support within the construct [43, 44]. Accordingly, sacrificial materials that can be printed and dissolved afterwards in a way that does not affect the survival of living cells are not bioinks, but biomaterial inks. This by no means diminishes their importance, but clarifies their terminology for this rapidly growing field. Examples of biomaterial inks are thermoplastic polymers such as polycaprolactone as a biodegradable example, polypropylene as non-degradable example, and polyoxazolines as a non-degradable but thermoresponsive example, biopolymers such as gelatin, inorganic materials such as cements and slurries, and metals, usually in the form of powders, thus covering a broad range of biomaterials, with the additional requirement that the material must be processable by an additive manufacturing or a biofabrication technology. These biomaterial inks may also include lithography-based resins which offer biocompatible substrates with high resolution features to support scaffold fabrication, cell seeding or microfluidic device fabrication, but do not contain cells which distinguishes them from a bioresin.

### Proposal for a definition of bioinks

In summary, we would like to emphasize that, in contrast to the term biomaterials, the term bioink should be defined from a process and technology



point of view. In order to embrace the two main strategies of biofabrication, bioprinting and bioassembly, and the different possible compositions of cells and materials in bioinks discussed above, we propose that bioinks should be defined as ‘a formulation of cells suitable for processing by an automated biofabrication technology that may also contain biologically active components and biomaterials’. Bioinks may include cells in different environments and forms, such as: single cells, cells aggregated in spheroids, cellular rods, cells organized in mini-tissues or organoids, cells coated by a thin layer of material, cells seeded onto microcarriers, or encapsulated in tailored colloidal microenvironments. In addition, bioinks can, but do not have to, contain bioactive molecules such as growth factors, DNA, miRNA, cytokines, exosomes or also biomaterials. This definition of bioinks is independent of the technology used for biofabrication, such as laser forward transfer, microfluidics, assembly, inkjet, dispense plotting, and lithographic techniques. This definition applies to all applications for which biofabrication is performed, including but not limited to, cell printing, tissue or organ printing, fabrication of *in vitro* models, assembly of organ- and body-on-a-chip systems, and the printing of bacteria, algae and plant cells for biotechnological applications.

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