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Biofabrication: Reappraising the definition in an evolving field

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Introduction

Biofabrication is a rapidly growing field of research that continues to mature and is outgrowing its infancy. This is partially due to the expiration of patents covering fused deposition modelling technology [1], which has rapidly made additive manufacturing equipment, commonly known as three-dimensional (3D)-printing, more affordable and widely available. In chorus with the lowered cost of equipment has been the transformation of rapid prototyping into rapid manufacturing [2]. Additive Manufacturing has evolved to be used for the production of high value parts with complex geometries, such as fuel nozzles in airplane engines; and moreover it is used for low number serial production, as demonstrated by the recently FDA approved titanium hip implant components. A similar evolution has occurred in Biofabrication especially for Tissue Engineering (TE) and Regenerative Medicine (RM). The journal Biofabrication was founded in 2009 at the beginning of this transition, which has led to the journal recently further clarifying its scope [3].

This article aims to summarize and assess the history of the term Biofabrication and its different definitions and uses as recorded in the literature to date. More importantly, we also aim to clarify the position of Biofabrication as a research field with a special focus on its relation to and application for TE and RM. Within this context, we propose a refined working definition of Biofabrication, including Bioprinting and 3D assembly as complementary strategies within Biofabrication.

Biofabrication across scientific disciplines

To our knowledge, Biofabrication was first coined in 1994 in relation to the biomineralisation of pearls [4] and later, in 2003, also in association with the deposition of enamel in mammalian teeth [5]. In addition, the US Defence Advanced Research Projects Agency (DARPA) used the definition "*Biofabrication--the use of biological materials and mechanisms for construction*" to describe methods to create high-resolution 3D structures that mimic biological growth mechanisms [6]. In 2004, Biofabrication was used by Payne and co-workers to describe the generation of nanostructured assemblies containing biological materials and/or biocatalysts. In their words, they described a rather broad definition as "*the marriage between biology and microfabrication*" [7]. Nowadays, the term Biofabrication is more broadly used in the context of fabrication of organic/inorganic hybrid materials or generally fabrication of materials [8,9] by living organisms. Within TE and RM, the term Biofabrication emerged with the application of 3D manufacturing strategies incorporating the manipulation and positioning of living cells and/or cell aggregates as will be discussed in more detail in the subsequent section.

The term Biofabrication is thus clearly used across many scientific communities and disciplines to describe different processes and phenomena. There have been attempts to formulate a broad definition of Biofabrication that would embrace its use within the different fields. Luo, for example, stated that "*Regardless of the slight emphasis of the definitions, there are several unique features of Biofabrication: first, the building blocks are cells or biologics; second, the fabrication processes are bio-inspired or bio-friendly; and finally, the products are biological systems, models or devices with transformative properties.*" [10] This definition does not, however, include biological processes such as biomineralisation, because these are not bio-inspired but naturally occurring processes. Hence, because Biofabrication always aims at the fabrication of a product, it could broadly be described as "*a process that results in a defined product with biological function*". This would encompass all the different and novel aspects of Biofabrication, however also several other fields of research and natural processes.

Biofabrication technologies for TE and RM applications

Here we focus on the technology of Biofabrication that uses cells and materials as building blocks, and which is mainly used for TE and RM applications. Our objective is to summarize, specify and classify the rapidly growing and diverging Biofabrication research activities in this field. The use of printing technologies for 3D positioning of cells was first demonstrated in 1988 by Klebe under the term Cytoscribing [11]. Despite the truly pioneering character of this work and possibly because there was a long interval before researchers returned to this concept, this term was not picked up by the subsequent literature. The term "Organ Printing", on the other hand, first appeared in 2003 and was defined as "*a rapid prototyping computer-aided 3D printing technology, based on using layer-by-layer deposition of cells and/or cell aggregates into a 3D gel with sequential maturation of the printed construct into perfused and vascularized living tissue or organs*" [12]. "Organ Printing" is still frequently used, particularly in the popular literature, however its current usage is often broader than this quite narrow original definition.

The historical evolution of the term Biofabrication, when used in the field of TE and RM, has occurred in parallel with the evolution of the term Bioprinting and possibly this has led to a confusion and possible conflation of the two terms, especially in the popular and journalistic media. Bioprinting was, to the best of our knowledge, first used as a term in the title of the "Workshop on Bioprinting, Biopatterning and Bioassembly" held at the University of Manchester, UK, in 2004. In a report on this meeting Mironov, Reis and Derby stated: "*For the purpose of the meeting, Bioprinting was defined as: The use of material transfer processes for patterning and assembling biologically relevant materials--molecules, cells, tissues, and biodegradable biomaterials--with a prescribed organization to accomplish one or more biological functions.*" [13] The term Bioprinting was subsequently used to specifically describe a process where a mechanical fabrication tool is used to

organise or pattern biological entities in 2 or 3 dimensions to build an artificial construct [14]. In 2010 Guillemot *et al.* defined **Bioprinting** as *“the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organization in order to produce bio-engineered structures serving in regenerative medicine, pharmacokinetic and basic cell biology studies”* [15]. It is thus clearly related to the parallel development of Additive Manufacturing that was then emerging as an engineering fabrication tool. Consequently, Biofabrication was first defined by Mironov *et al.* in the inaugural issue of the journal Biofabrication in 2009 as *“the production of complex living and non-living biological products from raw materials such as living cells, molecules, extracellular matrices, and biomaterials”* [16].

It is evident that the terms Bioprinting and Biofabrication are closely related, and since their respective existing definitions overlap, it is extremely difficult to effectively differentiate them. As a consequence, they are often used inconsistently or interchangeably, which underscores the clear need for clarification. As recently pointed out by Hutmacher and colleagues [17, 18], it would be beneficial that a new ASTM/ISO norm is developed, or a new sub-norm is defined under ASTM F2792 in order to unambiguously define the terms such as Biofabrication and Bioprinting. Beyond this more technical approach, and as already suggested by Boland and Mironov [19], we believe that a clarifying positioning of Biofabrication as a research field and its relationship with TE and RM will help to relate the terms and thereby clarify the definitions.

Biofabrication and its relation to TE and RM

Tissue engineering was defined in 1993 as *“an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve biological tissue function or a whole organ”* [20]. The field of TE has grown and expanded since 1993, and its definition has been extended accordingly. In 2007, for example, 12 federal agencies in the US proposed a combined definition of Tissue Science and TE as: *“The use of physical, chemical, biological, and engineering processes to control and direct the aggregate behavior of cells”* [22]. More importantly, the field of RM has been defined as *“the application of tissue science, tissue engineering, and related biological and engineering principles that restore the structure and function of damaged tissues and organs”* [23]. This includes not only *in vivo* but also *in vitro* generation of functional tissue analogues for various purposes such as drug testing, disease models, including cell/tissue/organ-on-a-chip approaches. Within TE and RM, Biofabrication provides a core and vital technology for the above emerging applications. We want to point out that the application of Biofabrication within TE and RM is not restricted to Additive Manufacturing approaches (Figure 1).

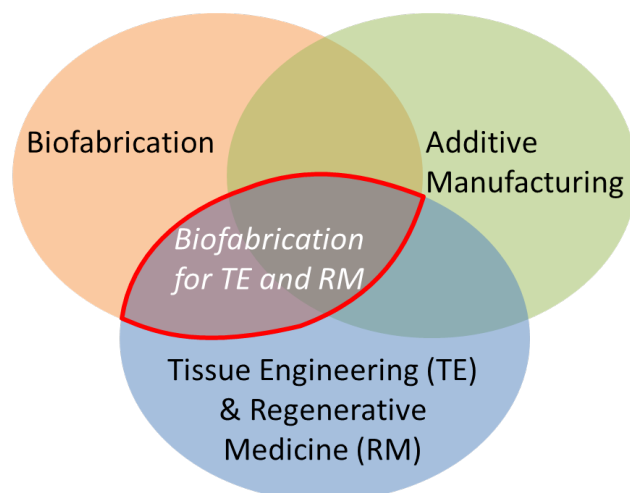


Figure 1: Schematic Venn diagram illustrating the interrelation between Biofabrication, Additive Manufacturing, and the related fields of Tissue Engineering (TE) and Regenerative Medicine (RM). Size of the areas and the overlaps does not represent the scale of the research fields.

In the classical TE approach, cells are seeded onto a prefabricated scaffold typically in conjunction with the delivery of bioactive factors that ensure maintenance of cellular phenotype and appropriate extracellular matrix formation. This is achieved through *in vitro* maturation followed by subsequent implantation with the aim to functionally regenerate tissue (Figure 2). An alternative concept envisions the development of *in vitro* 3D tissue models that exhibit functional features of native tissues for application in an *in vitro* testing or screening system. A more recent approach, in line with the strategy of RM, is so-called *in situ* TE or *in situ* tissue regeneration, which omits *in vitro* cell culture and/or *in vitro* tissue maturation steps. This approach aims instead to design materials and/or exploit the use of chemokines to recruit resident (stem-) cells to the scaffold and modulate the local immune response, inducing regenerative mechanisms *in situ* [21]. It is within this context that Additive Manufacturing has been, and still is, used to generate biomedical implants and scaffolds for seeding with cells in a classical TE approach.

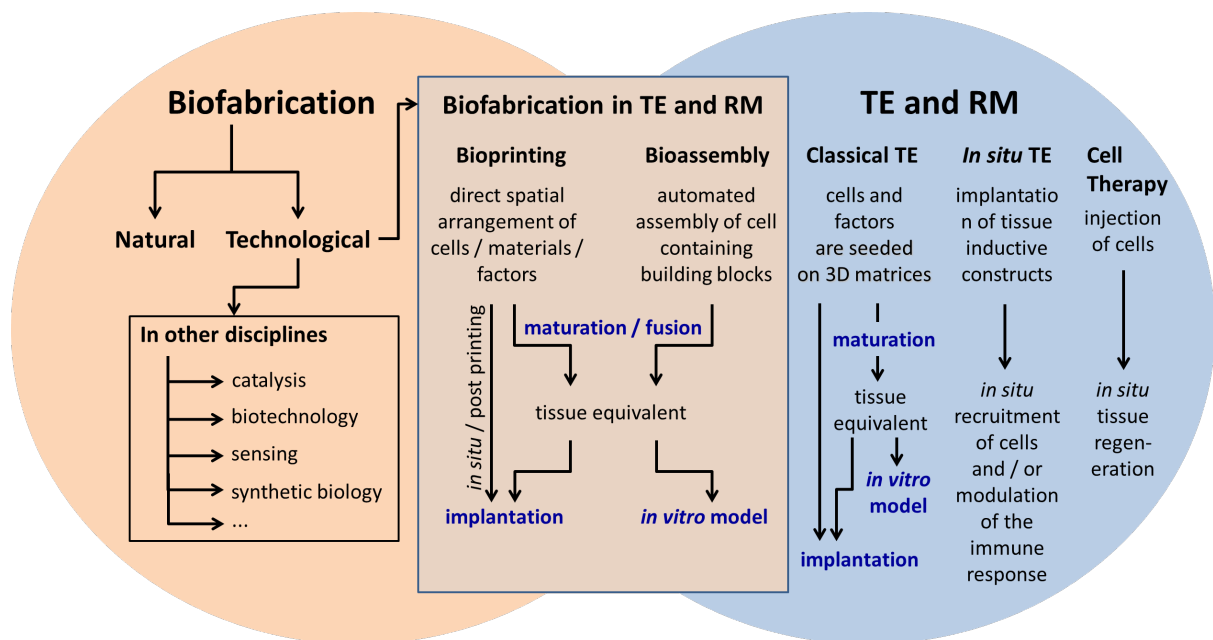


Figure 2: The term Biofabrication is used to describe natural processes such as biomineralization and technological processes in various disciplines such as catalysis, biotechnology, sensing, synthetic biology, and especially TE and RM. For application within TE and RM, Biofabrication can generally be divided into two distinct approaches, Bioprinting and Bioassembly.

From a research strategy perspective, Biofabrication within TE and RM aims at exploiting automated processes, for the most part Additive Manufacturing techniques, to generate cell-biomaterial constructs that, through their spatial arrangement, may mature into functional tissue equivalents. Accordingly, these strategies typically target the development of scaffolds or composite constructs which exhibit tissue mimetic hierarchical features. Alternatively, these constructs are produced with a structural organization that induces or modulates the host response after implantation through paracrine effects. When living single cells, bioactive molecules, biomaterials, or cell-aggregates small enough to be printed are used for fabrication, the mentioned constructs can be achieved by Bioprinting as defined earlier [15], which is one of the two main strategies of Biofabrication.

An alternative strategy for fabricating such constructs is to work with larger pre-formed multicellular fabrication units in the form of cell aggregates, cell fibres, cell sheets or more complex structures, such as organoids or microtissues, comprising cells and their extracellular matrix. These more complex building blocks are formed through cell-driven self-organisation in 3D or lower dimension culture, *i.e.* through a bottom-up approach, often by employing enabling technologies, such as microfabricated molds or microfluidics, followed by tissue fusion and maturation. Another possible

form of these building blocks are hybrid cell-material constructs, also referred to as hybrid tissues, for example cell loaded microgels and microcarrier beads [24-26]. A number of automated assembly techniques are available for Biofabrication using these building blocks, which can be subsumed under the term Bioassembly. Depending on the size, shape and geometry of the cell-containing units, Bioassembly encompasses a number of methods including Additive Manufacturing techniques, but also others, such as textile manufacturing routes like weaving, winding and knitting when cell fibres are used as building blocks [27]. Also, classical Additive Manufacturing of biomaterials can be used to generate templates for Bioassembly. Taken together, **Bioassembly** as the second major strategy of Biofabrication **can be defined as “the fabrication of hierarchical or hybrid cell-material constructs with a prescribed 2D or 3D organization through automated assembly of pre-formed cell containing fabrication units generated via cell-driven self-organisation or the use of bottom-up fabrication technologies, including microfabricated molds or microfluidics.”**

Taking the above into account, it is important to also define which technologies or approaches that cannot not be considered for inclusion in Biofabrication. In our opinion, Biofabrication does not include Additive Manufacturing of simple 3D scaffolds followed by seeding with cells. In order to satisfy our definition, the scaffold must be more than a simple support and instruct, or induce, the cells to develop into a hierarchical tissue mimetic or tissue analogue structure. This may, for example, be achieved through distinctive cell interaction, hierarchical induction of differentiation or functional evolution of the manufactured scaffold.

Thus, we consider the general area of Biofabrication within the context of TE and RM should be distinguished from its use in the area of natural processes, such as biomineralisation and be confined to manufacturing processes as discussed above. Within this technology space, we propose that there are two distinct methodologies that can be distinguished by the length scale of the minimum fabrication unit (pixel or voxel). These are "Bioprinting" according to the definition by Guillemot *et al.* [15] and "Bioassembly". For Bioprinting, the minimum fabrication unit is down to molecular level. In the case of Bioassembly, the minimum fabrication units are pre-formed cell containing building blocks with sizes large enough so that automated assembly can technologically be achieved. Figure 2 shows the interrelation between these terms in a schematic diagram. We note that the aim of Biofabrication is to generate a construct with biological function. Hence, in most cases neither Bioprinting nor Bioassembly fully describe the complete Biofabrication process, which is usually dependent on a maturation phase for the constructed pre-tissue assembly to allow it to develop a continuous and coherent functional structure. This maturation process may occur either *in vitro* with a culture phase – typically adopting the use of bioreactor technology – or conceivably *in vivo* after transplantation.

Taking into account these dynamic and evolving research activities and developments of different technological aspects, we suggest the following revised working definition of **Biofabrication for TE and RM** as **"the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through Bioprinting or Bioassembly and subsequent tissue maturation processes."**

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