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Engineered living bioassemblies for biomedical and functional material applications

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Recent breakthroughs in biofabrication of bioasemblies, consisting of the engineered structures composed of biological or biosynthetic components into a single construct, have found a wide range of practical applications in medicine and engineering. This review presents an overview of how the bottom-up assembly of living entities could drive advances in medicine, by developing tunable biological models and more precise methods for quantifying biological events. Moreover, we delve into advances beyond biomedical applications, where bioassemblies can be manipulated as functional robots and construction materials. Finally, we address the potential challenges and opportunities in the field of engineering living bioassemblies, toward building new design principles for the next generation of bioengineering applications.

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Introduction

A bioassembly is an engineered, predesigned, or self-assembled organization of purely biological (e.g. cells) or composite biosynthetic (e.g. cells plus a synthetic matrix) components into a single or series of fabricated constructs. [1] Biosassemblies have the potential to be used toward growing tissues for organ transplants and as organ-on-a-chip platforms for cancer testing [2–7]. Such progress has been driven by understanding of how biochemical and biophysical cues govern the proliferation, differentiation, and organization of cells into functional living 3D assemblies [8,9]. Moreover, new biofabrication approaches (e.g. 3D printing [10] and external field-driven assembly [11]) have expanded the design possibilities of bioassemblies. In this review, we provide insights into exciting avenues of research in the biofabrication of living bioassemblies from an interdisciplinary approach. We outline and discuss the challenges and opportunities for the use of bioassemblies in biomedical and material applications (Figure 1).

Bioassemblies in leading-edge biomedical applications

Recent scientific developments in biomaterials and cell biology have led to important milestones in biofabrication [12], most notably in the field of tissue engineering and organ-on-chip (OoC) devices.

Regarding OoC systems, these have been adapted to reproduce micro-fluidic and macro-fluidic microphysiological models of entire individual tissues or multiorgan systems (e.g. lung-on-chip) [13], on-chip cancer models [14–16], as well as various commercially available systems. In these platforms, cellular compositional complexity is interfaced with inline sensors and pumping interfaces for liquid flow and gas exchange. OoCs can be used for multiple disease research, for example, to follow viral pathogenesis of SARS-CoV-2 in human airway-mimicking environments [7].

Regarding tissue engineering applications, biofabricated bioassemblies could complement or potentially replace animal-disease models, accelerate biomedical discovery, and enable sufficiently large, highly repeatable, reproducible biological datasets. Moreover, artificial intelligence-enhanced bioengineering approaches may further enable *in silico* biomedical research and clinical studies. For instance, available biological datasets have been used to train machine-learning algorithms to classify cell-shape phenotypes [17,18], which could advance

Figure 1

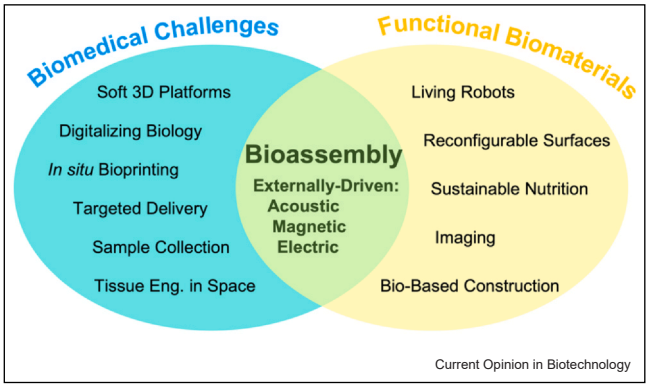


Diagram illustrating various bioassembly applications herein reviewed in two main focus areas: biomedical strategies and functional materials.

reproducibility and automation in bioassembly fabrication [19,20].

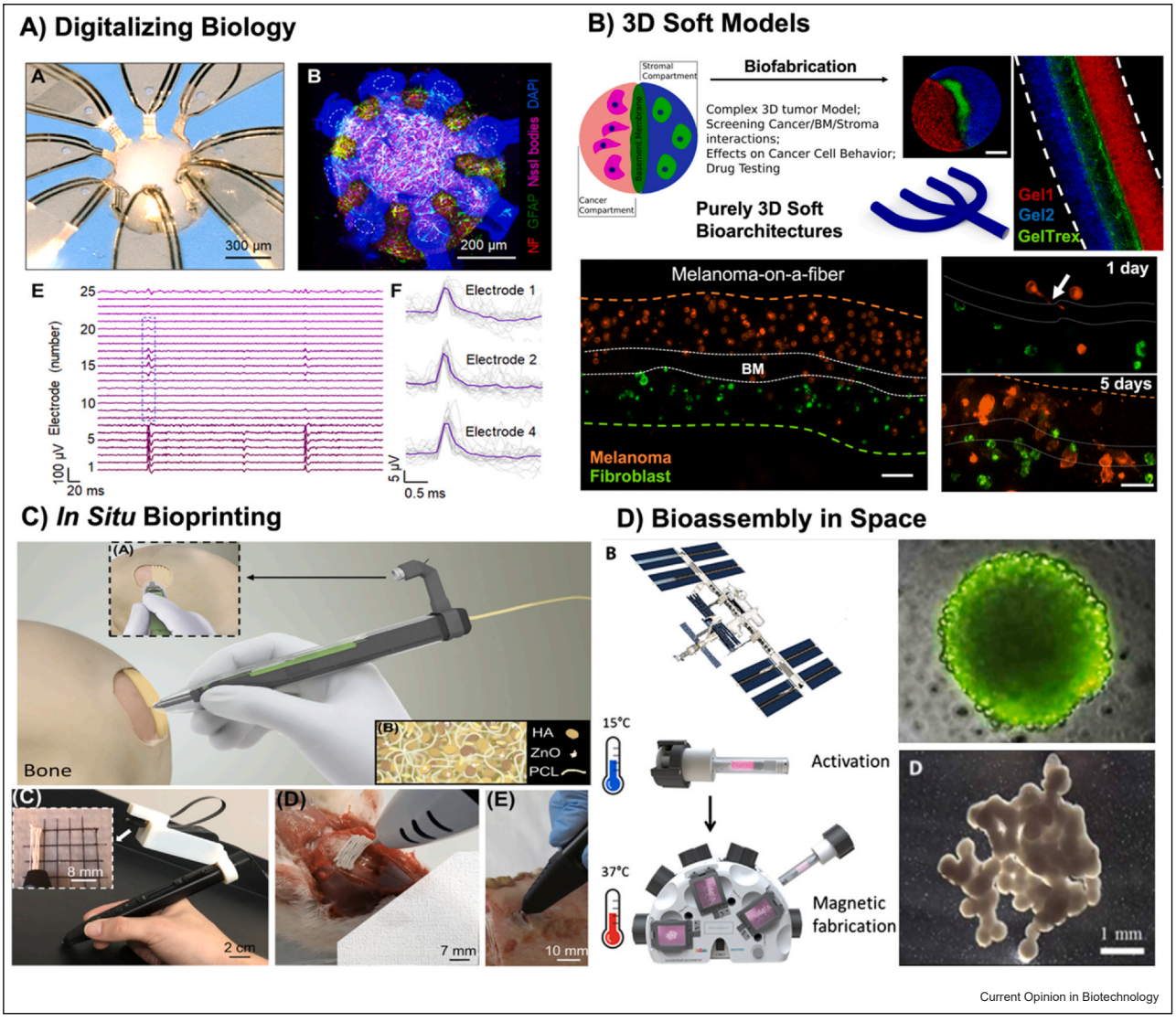
As engineered bioassemblies become larger, more complex, and comprising different types of cells, new challenges arise, particularly in extracting relevant information and data from such living structures and underlying biological events. In this regard, new sensors and analytical methods have been reported, for example, spatially resolved transcriptomics, enabling gene-expression analysis with a spatial resolution. This powerful methodology enables the investigation of intricate biological organization and cell–cell communication within complex engineered tissues and cellular assembloids (assembly of multiple organoids) [21–23]. Additionally, emerging miniaturized 3D tissue platforms allow for the automated digitalization of complex biological phenomena, such as cell proliferation, biomarker expression, and other physiological events (e.g. electrical signals) [24,25]. For example, researchers have recently interfaced neural spheroids and assembloids with soft sensors to obtain direct electrical, optical, chemical, and thermal analyses. Within that platform, the complex neural connections of the bioassemblies, such as synaptic responses and assembloid regrowth, were measured and recorded into quantifiable data (Figure 2a) [26]. In another example, living fiber optics have very recently been explored as a platform capable of supporting and converting complex biological events such as cancer 3D growth and drug susceptibility into directly quantifiable optical data [25].

Further, through the use of autologous cells, these bioassemblies could enable new types of regenerative medicine approaches or bioelectronic hybrid implants for potential organ/tissue replacement or health-monitoring tools. For example, researchers have explored the use of 3D biofabricated constructs as alternatives for synthetic plastic materials, which represent static and stiff materials, unable to be remodeled by living cells and mechanically distant from living tissues [27]. As an alternative to plastic, softer 3D materials can be engineered to imitate the mechanical properties [27], architecture [28], orientation [29], and compositional gradients [30] of living tissues (Figure 2b). Moreover, recent research has focused on studying how the hierarchical assembly of different organoids [31,32] into assembloids leads to multitissue cellular crosstalk [33]. Indeed, assembloids have recently recapitulated important and physiological cell–cell interactions, such as those in multilayered bladder [34], neurovascular [35], and neuromuscular [36] interfaces.

Advances in additive manufacturing have also led to the accurate deposition of biomaterials and cells in situ, potentiating the direct deposition of bioassemblies in living tissues. For instance, bioprinting protocols have been used to sequentially print different bioink layers (from bone to skin) directly on live tissues [37], which may enable direct tissue repair and efficient recovery of hemostasis leveraging the recent developments on tissue-specific 3D bioprinting [38,39]. Further, 3D bioprinters have been miniaturized into smaller handheld devices, such as 3D bioprinting pens, that can deposit multicomponent bioinks in situ, allowing surgeons to manually 'write' tissue scaffolds into defects in muscles [40] or bones [41] (Figure 2c). Moreover, recent studies have reported the use of multinozzle 3D printing to obtain bioassemblies designed and fabricated by voxel (3D version of pixel) units, which enable to integrate multiple materials with different functionalities into a single bioassembly design. [42,43] Such complex 3D bioprinted constructs may be designed to function as soft and deformable biomedical sensors that can be directly printed on living human organs for seamless monitoring of important physiological signals for precision health [44].

Finally, the assembly of living constructs has also expanded into other environments with distinct governing physics, such as outer-space environments with low gravity. It is now clear that altered gravity conditions and environmental pressure have a significant impact on the development of the bioassemblies, for example, endothelial structures [45], tendons [46], and other tissues [47]. Therefore, traditional biofabrication techniques such as 3D bioprinting are likely to fail in an environment where gravity is absent or extremely reduced, as gravitational forces frequently aid in the deposition of materials and cells. Such limitations have driven the search for innovative fabrication approaches that work in gravity-free environments. For instance, the use of externally driven magnetic levitational assembly [48–50] has enabled the hierarchical construction of cartilage spheroids aboard the International Space Station (Figure 2d) [51].

Figure 2

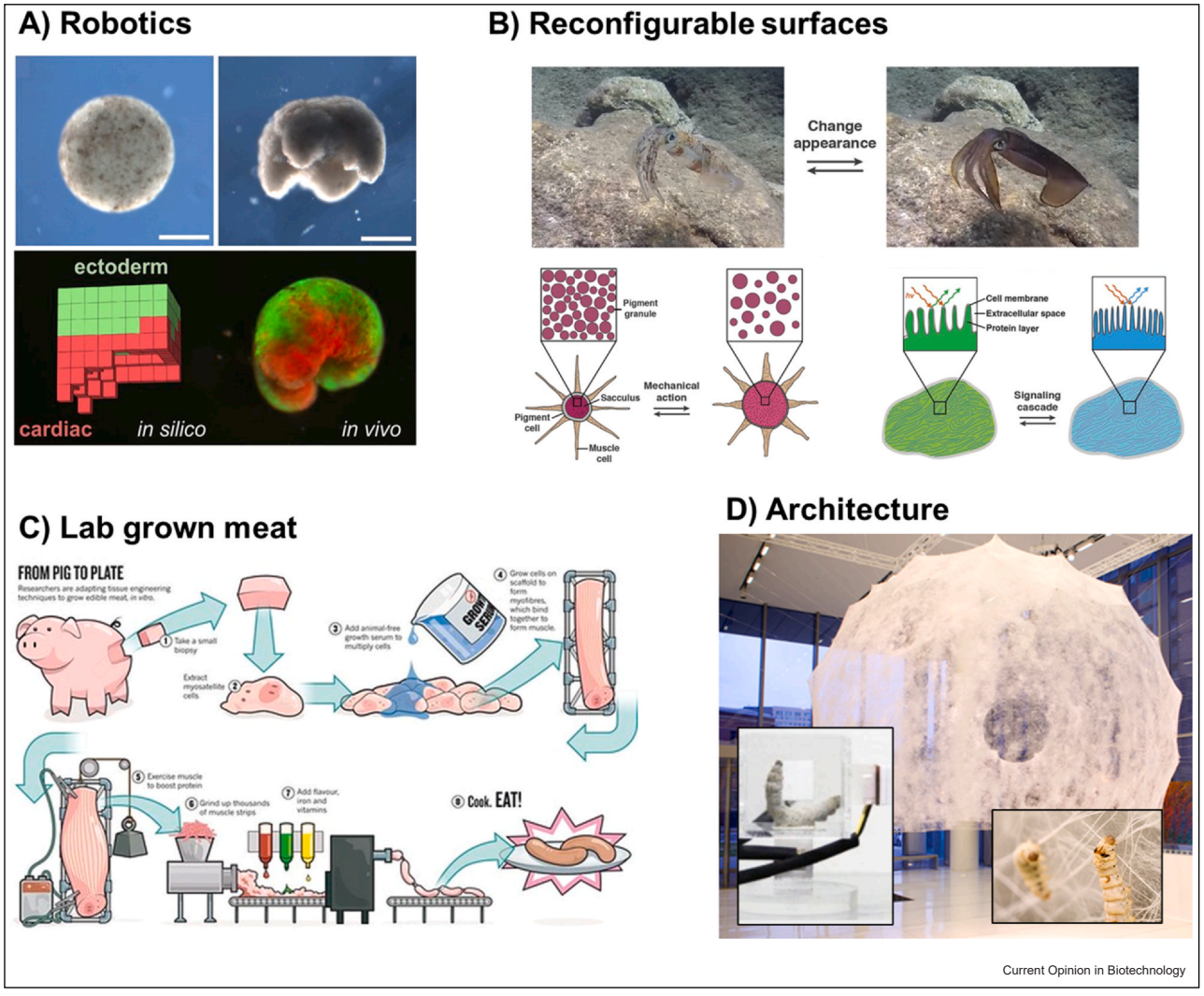


Emerging advances in the engineering and assembly of biological tissues for quantification of biological events, purely 3D soft architectures, portable *in situ* bioprinting and outer space biofabrication.
(a) reprinted from [52], **(b)** adapted from [28], **(c)** adapted from [53], and **(d)** adapted from [51].

Bioassemblies and their use beyond biomedical applications

Bioassemblies could serve as functional materials that perform a specific task or function in response to environmental signals. In this direction, recent biofabrication research has ventured beyond traditional health-related topics as those previously discussed in this review, and demonstrated the potential of using bioassemblies as the engine of functional machines [54]. For example, biorobots with integrated contractile cells (cardiomyocytes and muscle cells) and engineered artificial backbones can convert the contractile motions of the cells into directional locomotion or mechanical actuation [55,56]. For instance, differentiated cardiomyocytes from induced pluripotent stem cells were assembled into a 3D toroid geometry using an acoustic field to create a cardiac robot that is able to move at varying frequencies of beating controlled by the external electical stimulus [57]. More recently, the development of 'xenobots', fabricated by manually shaping *Xenopus laevis* embryos based on designs suggested by an evolutionary algorithm (Figure 3a) [58], has illustrated how machine learning can assist in the design of living systems to construct different engine configurations, for example, for locomotion, mixing, or object manipulation. Moving forward, new robotic designs could explore the use of nontraditional cell models to improve their performance or gain innovative functionality. For instance,

Figure 3



Use of bioassemblies outside material science field: fabrication of fully biological xenobots constructed with the assistance of machine learning. Cephalopods controlled shape and texture change based on mechanical and biochemical signaling. Schematic workflow of lab-grown meat. Bioassembly of the architectural dome by silkworm.
(a) reprinted from [58]. **(b)** reprinted from [67]. **(c)** reprinted from [69]. **(d)** reprinted from [74,75].

the mechanical force acceleration mechanism of the mantis shrimp relies on a mineralized spring structure that releases stored energy (peak acceleration of 65–104 km/s^2) [59,60]. Such spring-based actuation mechanism has not yet been translated into engineered bioassemblies, but it could offer an innovative strategy to construct actuators beyond contractile cells. The fabrication of such spring-type structures would require the coculturing and integrating of soft and rigid biomaterials (e.g. bone and muscle) [61]. Living cells have also been used as templates for biorobots, which provide different functionalities, including biocompatibility and on-site generation of therapeutic targets [62,63]. Further, external stimuli such as acoustic and magnetic fields have also been to assemble a large groups of biorobots [64] and nonbiological robotic systems [65].

Another emerging area of research consists of developing shape-morphing biomaterials into functional devices. Nature has multiple examples of organisms that can change shape or texture on demand [66]. For instance, cephalopods (cuttlefish, octopus) can change their appearance to mimic other species and camouflage into their surroundings. This behavior is possible due to a combination of tissues and cells that change their size, as light interacts with pigmented cells that alter the way light reflects from the animal skin [67] (Figure 3b). The integration of shape-shifting

bioassemblies with electronic systems could entail reconfigurable surfaces [68].

Bioassemblies could also be used as lab-grown food, which promises a potential sustainable alternative strategy to factory farming (Figure 3c) [69]. For instance, scientists have explored engineered plants as ingredients for meat substitutes [70]. On the other hand, cells have also been assembled into larger tissue constructs to produce engineered meat with comparable characteristics to animal-derived protein sources [71,72]. Nutrient-dense food that can be grown efficiently regardless of climate might help combat hunger and reduce pollution caused by animal farming.

The development of engineered plant-based bioassemblies could also enable creating alternatives for buildings and furniture-construction materials. For instance, the ability to fabricate bioassemblies that mimick wood, could potentially help reduce deforestation and loss of habitats [73]. Further, living organisms could also be guided for generating other biological materials on-site, serving as an alternative for construction materials. For instance, silkworms were directed to construct a full, meter-scale silk biodome, illustrating that biomaterials have a potential in constructing architectural features and buildings (Figure 3d) [74,75]. Textiles incorporating living cells have also gained increasing attention in the fashion industry. These textiles boost physiological functions by incorporating living cells into cellulose-based materials. For instance, by using textiles as a substrate, unsynthetic living algae can act as CO_2 capture and storage entities for carbon fixation [76]. Graphene-based electrodes with an integrated 3D honeycomb structure can be fabricated by using bioassembly to meet the growing demands for flexible and mechanically robust materials for energy-storage systems [77].

Outlook

In summary, we have reviewed the advances and potential trends in the engineered assembly of living systems. We have provided insights into possible interdisciplinary developments of biofabrication from a bioengineering and materials perspective, including their use as disease models, robots, and construction materials. Nevertheless, there are various challenges ahead. For instance, the diversity of fabrication technologies will require the development of universal standardized characterization and reporting protocols to compare and evaluate the performance and viability of bioassembled constructs. Moreover, with the availability of a wide range of diverse biological building blocks (bacteria, cells, and biological matrices), a new challenge arises for endless possibilities of combinations and designs; thus, with the use of machine-learning approaches, one could potentially predict and suggest the desired combination of building blocks to achieve particular functionalities or material properties. Moreover, the integration of living bioassemblies with electronic systems could obtain relevant information and drive the digitalization of biology.

The development of more sophisticated bioassemblies could enable better modeling of physiological phenomena ranging from tissue development to investigating diseases and their early detection, progression and treatment such as cancer and infectious diseases, enhancing in vitro testing of pharmacological compounds, and potentially patient-specific, personalized therapeutics. Further, the fabrication of living tissues using human cells and engineered matrices could also accelerate the translation of innovative biotechnology advances from the lab bench to the clinical settings, complementing and potentially significantly reducing the need for animal testing. Nevertheless, use-cases for biomedical application-related bioassembly could potentially be governed by complex regulatory frameworks and extensive market regulation, thus can be strategically important for their translational potential. Identifying commercially viable applications outside of biomedical applications could help accelerate the translation of bioassemblies into everyday technologies. Biological building blocks (cells, bacteria, and biological matrices) that are compatible, simple and programmable culture conditions would potentially enable rapid adoption, as the use of specialized strains may present scale-up limitations.

Finally, it is important to consider the bioethical angles of expanding tissue engineering to create engineered organisms. The potential broad adoption of these bioassemblies in environmental and medical applications could result in wide environmental or health impact. As technologies and tools advance, future trends in bioassembly with frameworks and developmental tools becoming widely available, the use of bioassemblies could have a profound impact on our everyday lives and at the clinic, ranging from extended life expectancy to healthier sustainable foods, and environmentally sustainable materials.

Conflict of interest statement

Prof. Utkan Demirci (UD) is a founder of and has an equity interest in (i) DxNow Inc., a company that is developing microfluidic IVF tools and imaging technologies, (ii) Koek Biotech, a company that is developing microfluidic technologies for clinical solutions, (iii) Levitas Inc., a company focusing on developing microfluidic sorters using magnetic levitation, (iv) Hillel Inc., a company bringing microfluidic cellphone tools to home settings, and (v) Mercury Biosciences, a company

developing vesicle-isolation technologies. UD's interests were viewed and managed in accordance with the conflict-of-interest policies.

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