Chapter 6 Rapid prototyping of soft bioactuators

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6.1 Background: Bioinspiration in tissue engineering and robotic actuators

The driving principle behind man-made robots is force actuation leading to a form of directed movement or locomotion. Natural systems can motivate the design and development of robots that replicate or enhance many basic locomotive strategies—such as climbing, crawling,[1] walking,[2] jumping,[3,4] or swimming[5–9]—with novel solutions. Biological soft robotics derives inspiration and design principles from organic systems to facilitate engineering approaches to challenges that have historically plagued conventional robotic actuators. Traditional hard skeletons (made of high stiffness metals or plastics) and electromagnetic actuators can

result in rigid bioactuators that exhibit few degrees of freedom (DOF), low complexity, difficulty in grasping actions, and aggressive collisions with living tissues. Moreover, they rarely present multifunctionality, versatility, or adaptability.[10]

Conversely, soft bioactuators (typically composed of gels, polymers, and fluids, sometimes with the addition of biological materials) must not only be functional in a research laboratory but also effective in situations where they may be called on to move over unstable terrain while carrying heavy loads of sensors, imagers, or samplers. These devices would also ideally be capable of untethered as well as directional locomotion, elastic deformation or stiffness modulation, efficient energy storage, and robust motion control, to be both effective and useful. Finally, these continuum robots must be environmentally safe and sufficiently low cost such that they can be abandoned if damaged or polluted.[11] Soft biorobotic manipulators with high power-to-weight ratios generally have more DOF and are more compliant than their rigid counterparts, and can manipulate fragile and unknown objects via a simple control algorithm. The lightweight and flexible polymers, hydrogels, and elastomers used to form soft robots have lower stiffness (moduli of 10^4–10^9 Pa) that corresponds to properties of biological matter with which they might interact (Figure 6.1a). Due to recent manufacturing advancements, they can be rapidly produced with high spatial control and a range of properties in three dimensions.[3,12–15]

Beyond structures, soft biorobotic systems require an actuating source and fuel supply. It therefore follows that living biological materials (or relevant mimics thereof) would inspire and comprise a large portion of demonstrated bioactuators.[15,16] Beyond simple biomimicry, the field of *biodesign* incorporates living organisms into artificial or manmade systems.[17] The addition of living biological actuator sources (e.g., muscle tissues) can increase the efficiency and responsiveness of soft actuators, as many of these living components have evolved with efficient standard processes for force production, energy consumption, or net movement.[18–21] The world of biology is full of intricate systems designed to solve extremely complex locomotive and manipulative tasks with high efficiency at a wide variety of scales. Depending on the ecosystem, there are numerous methods of potential locomotion among diverse structures and species, including both plants and animals.[22] Considering the breadth of methods of propulsion, it is perhaps unsurprising that there are also many ways for these robots to generate a range of locomotive forces, from molecular (e.g., motor proteins; 10^{-9} m) to cellular (e.g., individual microorganisms or cells; 10^{-6} m) to tissue (e.g., muscles or cell clusters; 10^{-3} m) length scales.[21] In addition to locomotors, there are also biorobots that imitate peristalsis to act as pumps or valves, transport cargo, actuate a joint, sense a signal, or perform as microgrippers, rotors, mixers, or manipulators to achieve other tasks.[23–26] It is apparent that the development of soft bioactuators necessitates the intersection and integration of advancements in diverse fields such as nanotechnology, tissue engineering (TE), and developmental biology.[27] In this chapter, we discuss the use of rapid prototyping technologies to achieve that end.

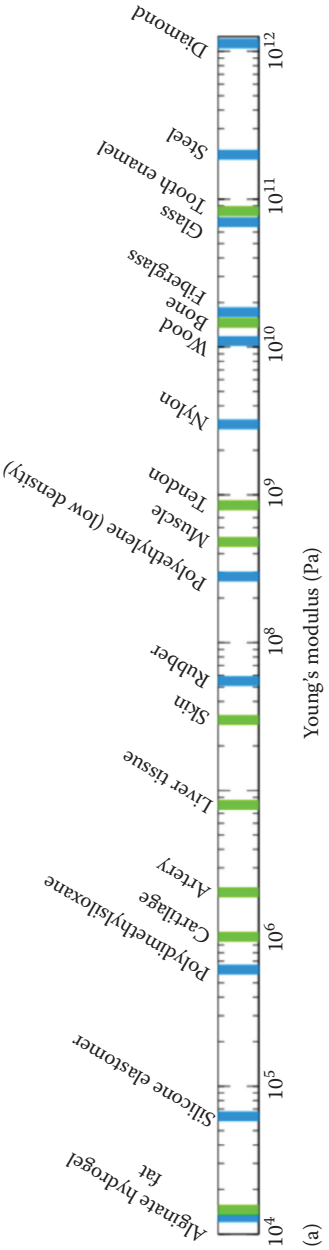

Figure 6.1 Rapid prototyping methods. (a) Range of tensile elastic moduli of biological and synthetic materials. Soft bioactuators mimic the material properties of biological tissues. (Reprinted by permission from Macmillan Publishers Ltd. *Nature*, Rus, D. and Tolley, M.T., 2015, copyright 2015.) (*Continued*)

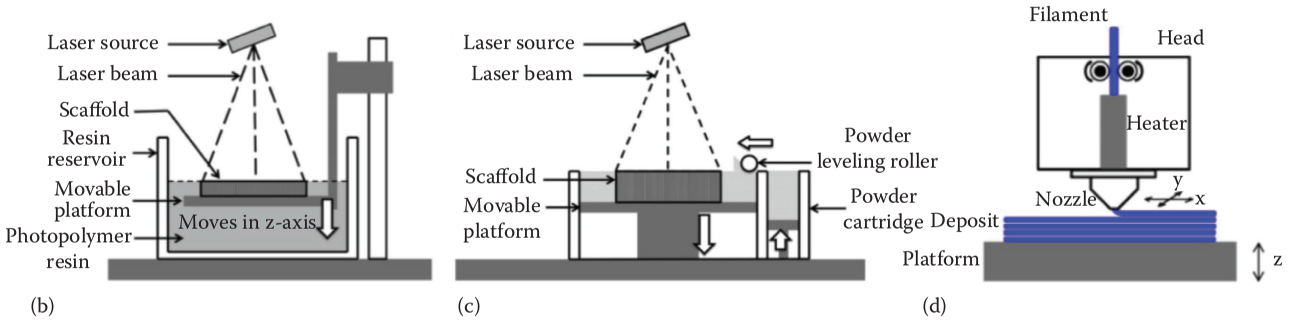

Figure 6.1 (Continued) Rapid prototyping methods. (b) Stereolithography (SL) is a maskless solid free-form method that requires a photosensitive resin and a UV light source. The apparatus (SLA) contains a platform that moves vertically. This technique traced the laser or projected image to photopolymerize a liquid resin in a layer-by-layer fashion. (Reprinted from Wu, G.H. and Hsu, S.H., *J. Med. Biol. Eng.*, 35, 285–292, 2015. With permission.) (c) Selective laser sintering (SLS) is the laser-based fusing of thermoplastics or metal powders to create a desired 3D structure. When a laser hits the first layer of powder on the stage, the particles fuse. A new powdered layer is deposited on the first layer and exposed to the laser again. After the fabrication is complete, the unsintered powder is removed. (Reprinted from Wu, G.H. and Hsu, S.H., *J. Med. Biol. Eng.*, 35, 285–292, 2015. With permission.) (d) Fused deposition modeling (FDM) is a solid-based system that utilizes extrusion. The nozzles (which can dispense different materials) extrude a filament-shaped material on the stage to pattern the first layer; then, the platform lowers and the next layer subsequently forms on the first. As the nozzle dispenses the material at high temperature, all the layers bind well without additional treatment. (Reprinted from *J. Mater. Process Technol.*, 209, Ahn, D. et al., Representation of surface roughness in fused deposition modeling, 5593–5600, Copyright 2009, with permission from Elsevier.) (*Continued*)

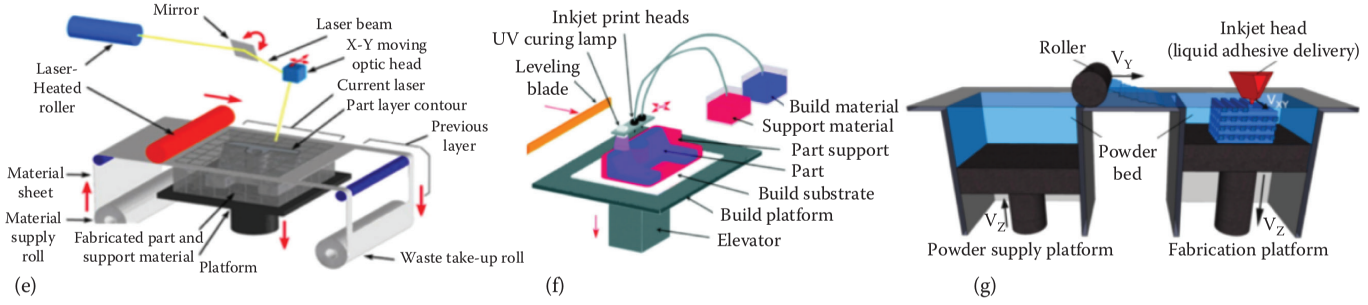

Figure 6.1 (Continued) Rapid prototyping methods. (e) With laminated object manufacturing (LOM), a single layered material, such as paper, plastic, or metal, is adhered to the platform. Then, a laser beam sketches the outline by cutting and removing the excessive material. The platform moves down by thickness of a single layer and another sheet of material is adhered on the first layer. The process is repeated until the final layer is patterned. LOM is economical and fast but not effective for fabricating geometrically complex designs. (Reprinted from *J. Mater. Process Technol.*, 212, Ahn, D. et al., Quantification of surface roughness of parts processed by laminated object manufacturing, 339–346, Copyright 2012, with permission from Elsevier.) (f) MultiJet Modeling (MJM) combines different RP techniques. A base thermoplastic polymer material is dispensed from an array of nozzles moving in the X–Y plane. The array draws the desired shape with the polymer on the platform, which then lowers relative to the array before the next layer is formed. (Bhattacharjee, N. et al., *Lab Chip*, 16, 1720–1742, 2016. Reproduced by permission of The Royal Society of Chemistry.) (g) Three-dimensional printing (3DP) can employ an inkjet type processing method. There are two adjacent chambers in this system. A feed roller on top of the material chamber spreads a powdered plaster material evenly on a platform moving vertically in the build chamber. A cartilage hanging above the build chamber moves over the surface to dispense a binding material. The build chamber moves down by one layer thickness, the process is repeated, and excess material is removed. Compared to other RP technologies, 3DP is fast and starting material is inexpensive. (Reprinted from *Biomaterials*, 33, Billiet, T. et al., A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering, 6020–6041, Copyright 2012, with permission from Elsevier.)

6.2 Rapid prototyping techniques and applications

The malleable nature, micro- to macro-scales, and potential for intricate composition and structure of bioactuators suggest that researchers will require a different manufacturing approach than can generally be employed for rigid robots. Rapid prototyping (RP) refers to a group of techniques that collect digital information to robotically fabricate physical 3D polymers, metals, and ceramic solids.[28–30] These 3D structures are dictated by a computer-aided design (CAD) model of the desired part, which can be built from scratch or derived from medical images to print patient-specific structures. Although various techniques exist (Figure 6.1b–g), all methods of RP adapt a similar fundamental approach: a CAD model is converted into a standard tessellated language (STL) file; a computer program then receives the information and slices the 3D model; and finally, these cross sections are sequentially layered using additive manufacturing technologies that employ extrusion, melting, jetting, or photopolymerization to create a final structure. All RP techniques have short fabrication times, low costs, minimal postprocessing and waste, and variable material choices and properties, with resolutions that extend from micron to centimeter scale.[18,31–33]

CAD not only serves as the input for an important method by which soft robotic actuators or patient-specific scaffolds can be fabricated but also provides a substrate for virtual testing and development. Due to both the increasing ubiquity of (and immense improvements in) computational power, simulation tools can now be used to calculate kinematic, dynamic, and finite-element analysis-based responses of a prototype and visualize the results in an interactive, 3D virtual environment. The ability to model prototypes realistically and accurately while validating preliminary prototype results has become integral in nearly all facets of engineering design, including bioinspired robotics.[34] Finally, CAD libraries can also be utilized for input on material selection and design optimization.[35]

RP allows for the fabrication of complex and multilayered 3D structures and geometries that cannot be achieved using conventional multistep processes such as mask-based soft lithography and was therefore introduced to TE to overcome limitations of conventional fabrication techniques.[24,36,37] For example, some RP techniques could allow for printing of spatially controlled growth factors or the use of multiple nozzles loaded with different biomaterials to create more advanced tissue structures composed of diverse matrix components.[38–40] Most important, stereolithography (SL) enables the construction of micro- or mesoscale tissue structures with desired shapes and physical properties,[41] and provides the user with a wide range of synthetic and natural material options (e.g., hydrogels that can encapsulate living cells[42,43]) that offer greater versatility and compatibility than PDMS or metallic structures. Furthermore, through the specification of light intensity, irradiation time, and chemical makeup of the liquid resin, the mechanical properties of a printed part can be precisely regulated. The ability to fine-tune

these features, including strength and porosity, can have implications for cells that are sensitive to the stiffness, topography, and geometry of their microenvironment.[42,44–47] Moreover, CAD allows for the production of advanced designs that closely resemble the physical morphology, orientation, or finer details of native tissue; interconnected pores, complex surface topography, and internal structures can be easily reconstructed into rapidly manufactured scaffolds.[48–50]

Due to increasing automation speeds as well as high throughput and iterative capabilities that allow for design optimization, rapid prototyping technologies are especially useful for the production of soft bioactuators in an inexpensive and mass-producible manner.[10,30,51] These techniques can be utilized to construct temporary or sacrificial shape-specific molds, or to print the bodies of flexible bioactuating devices themselves.[19] In the case of the latter, high-yield RP allows for manufacturing of complex structures in a single-step process, with the possibility of shape variations or heterogeneous properties.[18]

Each RP technique requires different materials in a specific form. The selected material thus needs to be compatible with the fabrication method as well as the intended application. In addition, the specific architecture of the scaffold depends on the type of RP technique. For example, selective laser sintering (SLS) of powders is not suitable for building porous structures or smooth surfaces; on the other hand, extrusion-based fused deposition modeling (FDM) produces thermoplastic parts with smooth surfaces that need further modification to ensure cell adhesion. Soft actuators necessitate a flexible biodegradable or biocompatible polymer.[18,40,51] Therefore, it is important to consider material properties and the design of the scaffold, whether the user desires to successfully regenerate a tissue or build a functional bioactuator. The user's choice of RP fabrication technique, materials, and biological actuating source (if applicable), should be entirely context- and application-dependent.

6.3 Nonliving bioactuators

In many cases, synthetic RP devices have been developed to mimic the agonist–antagonist style of actuation that characterizes living tissues (Figure 6.2a–d). While they do exhibit many favorable characteristics (such as greater DOF and flexibility) compared to rigid actuators and are not subject to the sensitivity or strict environmental conditions necessitated by metabolically active cells, nonliving soft bioactuators can be at a loss when compared to the volumetric efficiency of native muscle, or the controllability of rigid actuators. Moreover, these systems often require an external power source, which adds extraneous weight that further increases energy requirements. This additional hardware, however, can contribute to significantly improved power density for synthetic actuators. The movement of nonliving soft devices can be controlled with fluidic elastomer actuators (FEAs),

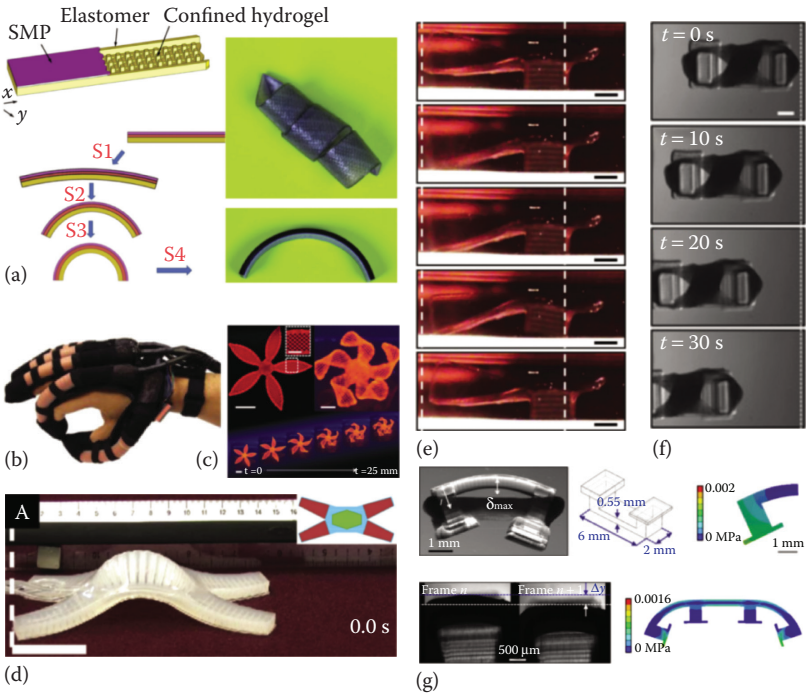

Figure 6.2 Soft bioactuators. Examples of soft bioactuators fabricated with rapid prototyping methods are demonstrated in both nonliving (a–d) and living (e–g) systems. (a) The combination of hydrogels and SMPs allowed for a two-way actuator that could reversibly bend or coil. (Adapted from Mao, Y. et al., *Sci. Rep.*, 6, 24761, 2016. With permission.) (b) A hydraulic-powered soft robotic glove fabricated from a 3D-printed mold was able to demonstrate precise grasping. (Reprinted from *Rob. Auton. Syst.*, 73, Polygerinos, P. et al., Soft robotic glove for combined assistance and at-home rehabilitation, 135–143, Copyright 2015, with permission from Elsevier.) (c) The complex morphological transformation of a flower structure was made possible with 4D bioprinting. (Reprinted by permission from Macmillan Publishers Ltd. *Nat. Mater.*, Gladman, A.S. et al., 2016, copyright 2016.) (d) An elastomeric crawling soft robot was powered by pneumatic pressure. (Reprinted from Shepherd, R.F. et al., *Proc. Natl. Acad. Sci. USA*, 108, 20400–20403, 2011. With permission.) (e) A 3D-printed biohybrid cantilever was powered by the spontaneous contraction of cardiac muscle cells. (Reprinted by permission from Macmillan Publishers Ltd. *Sci. Rep.*, Chan, V. et al., 2012, copyright 2012.) (f) An electrically powered biobot was fabricated from a hydrogel skeleton made with a stereolithographic 3D printer and a combination of skeletal muscle cells and ECM proteins. (Reprinted from Cvetkovic, C. et al., *Proc. Natl. Acad. Sci. USA*, 111, 10125–10130, 2014. With permission.) (g) An optically powered and exercised skeletal muscle biobot demonstrated directionality and control. (Reprinted from Raman, R. et al., *Nat. Protoc.*, 12, 519–533, 2017. With permission.)

(which utilize pneumatic or hydraulic pressure), variable-length tendons (such as tension cables or shape-memory materials), or electroactive polymers (EAPs), (which can be ionic or electronic).[18,52] Considering the advantages, it is not surprising that RP fabrication methods also permit low production costs and printing times for soft bioactuators.[19,53] 3D-printed soft actuator materials, utilized stimuli, speeds, and pros and cons are reviewed in References.[1,51,52]

6.3.1 Fluidic elastomer actuators

FEAs are a frequently utilized actuation method that relies on pressurized fluid or compressed air for controlled structural deformation. Design or geometric asymmetry can allow for net movement on fluid inflation. Pneumatic systems (such as McKibben actuators[54] or artificial muscles) generally consist of both extensible and inextensible but flexible layers, broken up into a series of internal chambers or channels. On pressurization, the more inflexible shell constrains the material to increase its diameter, shorten, and exhibit greater rigidity or stiffness—that is, to contract like muscle.[52,55] These versatile soft robots can be modeled after worms,[56] octopuses,[57] flat muscles,[58] and novel multilimbed organisms.[59] Recent advances have resulted in the development of soft, miniaturized pneumatic hardware that allows the robots to behave somewhat autonomously, such as crawling through tightly confined spaces—which would be impossible to navigate with rigid or tethered robots.[60,61] Pneumatic actuators are lightweight, robust, and easily controlled; moreover, they can operate in wider temperature ranges than muscle *in vivo*.[62] However, some pneumatic systems still lack robustness, reliability, and overall control.[63]

Yang et al. created a variable stiffness robotic finger that exhibited a change in elastic modulus of the 3D-printed shape memory polymer (SMP) (Section 6.3.2) skeleton with temperature. Heating of selective regions within the pneumatic actuator caused bending of the substrate and could be modified to achieve gripping or grasping.[64] Bartlett et al. 3D printed a multimaterial robot that exhibited a wide stiffness gradient within its body. The jumping robot was powered by both a combustion reaction within the body's chamber as well as inflation of its pneumatic legs.[4] Recently, Wang et al. directly 3D printed an air-driven soft robot, with integrated curvature sensors, capable of gripping. It has been hypothesized that the use of soft grippers could increase safety and decrease scar formation in surgical applications.[55,65]

Some work has moved away from the use of air as its medium and instead incorporates denser fluids in the creation of hydraulic-powered soft robots.[66] MacCurdy et al. used a five-head printer to fabricate a bellows actuator of solid polymers and liquid material simultaneously. The legs were actuated by pumping fluid throughout the bellows of the hexapod robot's body.[67] Using propulsion principles seen in the octopus, Fischer et al. created a hydraulic underwater actuator by using

FDM to fabricate a flexible thermoplastic material.[68] Hydraulic power increases the ceiling frequency of actuation and provides higher forces and durations of actuation than pneumatic pressure. However, pneumatic actuation is more environmentally benign and exhibits less weight.[19,69]

6.3.2 Variable-length tendon actuators and *smart* materials

Variable-length tendon actuators that conform or morph their properties can take the form of shape memory alloy (SMA) actuators and lightweight metals with highly tunable mechanical properties depending on the specific alloy used, or tension cables, which require an external conventional motor. Advantages of SMAs include minimal weight and bidirectional scaling. As shown in tentacular soft robots,[60] application of a thermoelectric stimulus to composite SMAs can result in directed movement with high force and large DOF during a temperature-induced phase transformation. However, the phase transition is relatively slow and lacking in high precision, and researchers lack a targeted method for heating the wires.[55,70]

The materials used in soft robotics must contain an additional level of complexity that allows them to be stable along a wide range of environmental conditions but undergo drastic conformational changes on variation of this environmental stimulus around a given critical point.[71] A common category of bioactuator utilizes rapidly prototyped or printed *smart* materials that can physically react to dynamic stimuli. Though response time and control mechanisms vary by stimulus, these materials can controllably and reversibly respond to changes in pH, light, pressure, moisture, temperature, ionic gradient, and electric or magnetic fields by altering one or more physical properties, such as contraction or expansion of shape—much like an organism might do when subjected to varying ecological conditions.[71–75]

Combining smart materials with rapid prototyping can have interesting outcomes for soft biorobotics. For example, a composite biomimetic actuating system could contain SMAs embedded in soft 3D-printed materials for greater control. These smart materials can provide actuation power and structural support, lending soft bioactuators' increased flexibility and dexterity. Walters et al. prototyped a tentacle-like elastomer fabricated by 3D printing and actuated by inserted SMAs.[76] Gui et al. manufactured a tripedal soft robot modeled after a spider. Forward locomotion was powered by an SMA *muscle* (a metal fiber) directly printed into a soft 3D photopolymer adhesive structure.[11] Drawing inspiration from the deformation of crawling and climbing insects, Umedachi et al. designed electrically powered, SMA- or electric tendon-actuated *softworms*. These multi-limbed actuators contained a rubber body fabricated from multimaterial 3D printing. SMAs provided structural support and actuation; variable friction control allowed net forward crawling, as well as control of speed and steering.[1,77,78] The combination of 3D

fabrication with controllable spatiotemporal properties is sometimes referred to as *four-dimensional (4D) printing*.[70,72]

SMPs can recover their original conformation from a temporary stimulus-induced change in shape. Compared to SMAs, these softer materials are cheaper and have a larger range of tunable properties (mechanical, thermal, or optical), and are more similar to native muscle.[72,79,80] Most important, they do not require the extensive current supply or activation heat of SMAs.[18] Bodaghi et al. printed SMPs into various arrangements of flexible beams in planar and tubular arrangements. A stress anisotropy resulted in expansion and shrinking of the polymer bioactuator on thermomechanical stimulus.[81] Mao et al. designed a 3D-printed arrangement of two different materials. Two-way actuation was achieved as the system could reversibly switch between twofold stable configurations in response to temperature (SMPs) or water absorption (hydrogel) stimuli.[82] Wu et al. controlled the bending, folding, and opening of 2D substrates by adjusting the SMP fiber-volume fraction within a 3D-printed composite to mimic insect, helix, and hook designs.[83]

Hydrogels are capable of swelling on water absorption and can be considered *smart* materials for 4D printing. Bakarich et al. developed a thermally stimulated actuator with large strain by printing an ionic covalent entanglement hydrogel (PNIPAAm) with high toughness. The transition of the material at a critical temperature caused a decrease in water content and change in volume.[74] Sydney Gladman et al. printed a patterned hydrogel composite out of a soft polyacrylamide matrix with embedded stiff cellulose fibrils. The multimaterial system mimicked a plant cell wall composition and produced a controlled curvature due to anisotropic swelling when immersed in water.[72] Zolfagharian et al. demonstrated a photothermal-responsive bioactuator, additively manufactured from an extrusion-based chitosan hydrogel, with remote control over folding.[84] Zhu et al. developed an optical 3D-printing technology to fabricate an artificial PEG–hydrogel microfish with magnetic guidance.[85]

6.3.3 Electroactive polymer actuators

When subjected to an electric field stimulus, EAPs are capable of changes in overall shape, resulting in strain and therefore actuation. These materials have been utilized for a range of applications, including the development of electrically active soft bioactuators.[55,86] Asaka et al. presented a thorough review of EAP materials and biomedical applications in *Current Status of Applications and Markets of Soft Actuators*.[87] Dielectric elastomer actuators (DEAs) are capable of thickness contraction and area expansion under high voltage. They can demonstrate high strain (200%), elasticity, efficiency, and energy density. Rossiter et al. fabricated a DEA using a combination of 3D-printing techniques with soft and rigid materials. A simple antagonistic structure composed of two membranes was proposed as a prototype for soft robotics.[88] Recently, Cai et al. developed an

acrylic DEA to robotically mimic native facial muscles using both FDM for the frame and a multimaterial 3D printer for the dielectric film,[89] and Nguyen et al. fabricated a scalable DEA hexapod robot with controllable directional locomotion, rotation, and turning.[90]

Ionic polymer-metal composites (IPMCs) require low actuating voltages to change shape or bend and are therefore promising for soft robotics. They must operate in wet conditions (amenable to swimming bioactuators) and thus require some protection in air.[14,19,52,76] While these compliant materials have been developed as intelligent artificial muscles, only few groups have constructed soft EAP bioactuators with rapid prototyping technologies.[86] Carrico et al. demonstrated a novel method for printing soft IMPC structures using fused filament additive manufacturing. A polymer was printed in a layer-by-layer fashion and rendered electroactive via subsequent chemical functionalization.[91]

6.3.4 3D-printed molds for fabrication of soft bioactuators

In addition to the printing of entire bioactuators themselves, RP has been used to manufacture both sacrificial and permanent molds[60,63,92] in which to shape bioactuators from rubber, PDMS, or other soft materials (sometimes dubbed *semi-printing*). For example, Ahn et al. created a smart material actuator, capable of bending and twisting, embedded in a soft matrix that was cast with a 3D-printed mold.[93] Low et al. formed silicone-based soft pneumatic grippers,[55] and Martinez et al. demonstrated a micropneumatic tentacle that could grasp and manipulate complex objects,[57] by casting soft materials into custom-printed 3D molds. An IMPC-embedded tube (cast into a 3D-printed mold) with multi-DOF capability was developed by Liu et al. to aid in minimally invasive surgical procedures.[94]

Regarding actuators whose entire structures can achieve net locomotion, Jin et al. fabricated a soft robot capable of swimming, gripping, and crawling, and whose body was integrated with SMA wires and molded using 3D-printed parts.[70] Lin et al. used 3D-printed plastic molds to create a soft, rolling, coiled SMA-actuated *GoQBot*.[95] Mosadegh et al. molded a *pneu-net* (pneumatic network) soft robot whose body was actuated by air inflation.[56] Most recently, Yuk et al. published a hydraulic, polyacrylamide–alginate hydrogel actuator molded from 3D-printed solids,[96] and Wehner et al. cured an elastomer containing an embedded controller in a 3D-printed mold to fabricate a multimaterial pneumatic soft *octobot* with eight arms.[97]

However, it should be noted that elastomeric bioactuators printed in whole, as compared to those cast in a 3D-printed mold, can boast easier and shorter fabrication without the subsequent need for postprocessing or assembly.[76] Morrow et al. modified a FDM printer to directly fabricate a silicone pneumatic actuator; comparison to an identical structure made from a molding process demonstrated no tradeoff in force.[98]

6.4 Living bioactuators

The use of biological materials (including DNA, motor proteins, myosin–actin complexes, bacteria, algae, single cells or clusters, and natural or engineered tissues—either independently or collectively) as the primary actuators of locomotive force is still an extremely young field, but has resulted in some interesting possibilities (References[21,99] for a review). The basic requirements of an ideal living biological actuation source include the ability to generate a controllable or repeatable force, operate under a range of environments, and be easily maintained.[24] Evolution has produced optimal living actuators that can operate for long term at physiological conditions (favorable for biomedical applications), wirelessly convert chemical energy (from glucose or fats, for example, which can boast energy densities up to 100 times that of a battery[10]) to mechanical work more efficiently than nonliving power sources, produce nontoxic and biodegradable by-products from fuel conversion, and be stimulated electro- or pharmacomechanically—thus eliminating the need for an external energy source. In addition, they are proficient at self-assembly and replication, protein synthesis, rapid adaptation (responses as short as tens of milliseconds), and are highly sensitivity to environmental conditions. Understandably, they are also biodegradable and biocompatible, and can dynamically interact with other living or nonliving components.[15,53,99]

In general, when exploiting the innate contractility of cells or tissue to power a bioactuator, it is critical to consider the stimuli (mechanical, electrical, and biochemical) necessary for differentiation, development, or maintenance.[46,53] RP techniques can assist in providing a suitable scaffold or environment in which appropriate cues can be tuned or added (Figure 6.2e–g). For example, a stereolithography apparatus (SLA) can print hydrogels with tissue-like stiffness values that are mechanically similar to cells' extracellular environment *in vivo*. The elastic modulus of the extracellular matrix (ECM) not only affects viability and proliferation, but also dictates the differentiation bias of cultured stem cells[100]; for this reason, SL has been used to fabricate a variety of matrices that can realistically simulate cellular microenvironments and aid in an engineered tissue development.[42]

Though engineered molecular[21,24,73] and bacterial[101] bioactuators are capable of cargo transport and fluidic pumping and can thrive in a range of environmental temperatures or pH, few have been incorporated with RP techniques.[99] The combination of many cells can allow for a collective output that is greater than the sum of its parts. Moreover, complexity (and thus functionality) increases when progressing from single cells to cell clusters (2D sheets or 3D arrangements) to tissues and systems.[24,27] Therefore, in this section we will focus only on living eukaryotic bioactuators at the multicellular and tissue scale, comprising a synthetic mechanical scaffold and one or more actuating biological components.

Scalable molecular motors and contraction machinery that comprise muscular sarcomeres in particular can generate active contraction in multiple forms and size scales, be hierarchically combined in series or in parallel, and has evolved over millions of years with extremely high plasticity and volumetric efficiency.[21,24,35] Functional bioactuators have been devised using whole explanted tissues,[8,102,103] cells differentiated within a scaffold or gel,[104] or self-organized engineered tissue.[105] It is worth noting that although smooth muscle[106] is capable of force production, its relatively slow contraction has prohibited its employment in biorobots that require rapid actuation.[99]

6.4.1 Cardiac muscle

Cardiomyocytes (cardiac muscle cells) provide an excellent source for bioactuation due to their intrinsic, synchronous contraction; thus, external stimulation is unnecessary. The cells can form a syncytium through gap junctions and cell–cell adhesions, and produce spontaneous contractions.[21,27] The original developments in cardiac-based bioactuators included locomotive machines such as walking microrobots,[5,107,108] on-chip pumps,[109] and swimming robots[110] and *jellyfish*.[9] However, most were constructed on silicon or PDMS substrates that did not mimic the native cellular microenvironment nor allow for dynamic adaptation; few have been coupled to substrates fabricated from RP methods.

To demonstrate the ability of cardiac cells to induce the locomotion of a material with an elastic modulus similar to that of the native myocardium, Chan et al. developed a biological robot (dubbed *biobot*) from polyethylene glycol (PEG) hydrogel.[41,111] A modified SLA was utilized to fabricate a microcantilever, the surface of which was functionalized with collagen to adhere a culture of primary neonatal rat cardiomyocytes. To transform this hybrid structure into a bioactuator capable of directional locomotion, a net asymmetry of actuation was introduced in the cantilever design. Furthermore, the thickness of the cantilever was optimized to control the curvature of the actuating leg and maximize the locomotive speed. The occurrence of a *power stroke* induced by rhythmic spontaneous cardiac sheet contraction drove the actuating leg to bend downward, increasing the friction and causing the biobot to propel forward. With a maximum velocity of 236 μm/s, the cardiac biohybrid actuator demonstrated efficient mechanisms of autonomous locomotion and a novel approach to spatially control biochemical and physical cues during fabrication.

6.4.2 Skeletal muscle

The behavioral complexity and degree of external control that can be imposed on cardiac muscle are limited by its intrinsic spontaneous contractility. The ability to regulate an actuator through the modulation of an applied stimulus not only

allows for precise control over its motion but also opens up avenues for greater functionality. Skeletal muscle is the primary generator of animal locomotion, with a dense structure comprising a modular hierarchy with an arrangement of motor units that can be recruited individually or in summation. It exhibits a greater force-to-weight ratio in comparison to many rigid mechanical actuators.[21,24,27,99] A high degree of spatial and temporary control over actuation, even of single fibers, is possible via external sources such as electrical,[2] optical,[112,113] or neural[114,115] stimulation.

A skeletal muscle-powered biobot developed by Cvetkovic et al. mimicked the mammalian musculoskeletal system, wherein muscle contraction drives the articulation of bones across flexible joints.[2,116] A 3D-printed skeleton (comprised of a flexible beam connected to two stiff pillars) was fabricated using a SLA and subsequently anchored to an engineered muscle strip containing differentiating C2C12 myoblasts. The 3D muscle strip contained natural ECM hydrogels (fibrin and Matrigel™) that supported the densely embedded cells. To induce the locomotion-driving contraction of the muscle strip, the biobot was positioned within an electric field and subjected to a pulse stimulation of 1–4 Hz, resulting in a global response of the excitable cells. The introduction of deliberate asymmetry in the pillars (achievable with a slight modification in the rapid prototyping technique) allowed the biobot to move in a unidirectional trajectory along a surface in a fluid with a maximum locomotion of ~150 μm/s.

Although the muscle strip successfully induced net locomotion, the muscle was permanently tethered to the skeleton, preventing the adaptation of the muscle to other skeleton structures.[2] A second iteration by Raman et al. was devised with a muscle ring structure, formed in a separate 3D-printed mold before being transferred to the skeleton.[113] Muscle rings exhibited higher myofiber alignment and increased viability.[116] The C2C12s were also genetically modified to express Channel rhodopsin (ChR2), a membrane protein that causes muscle contraction under the presence of blue light.[117] This allowed for the control of locomotion through an optical stimulus, which could be positioned on localized regions of the biobot, thus enabling the development of a symmetrical yet bidirectional bioactuator whose direction of locomotion was determined by which ring the light stimulated. Similarly, a single device could also be forced to rotate by stimulating only one half of a single muscle ring. To maximize force production, the biobots underwent an *exercise regimen* of daily optical stimulation throughout muscle differentiation. Exercise was shown to improve myotube formation, leading to an increased tension and locomotive speed up to 310 μm/s.

6.4.3 Control mechanisms for living bioactuators

Cell- and tissue-based bioactuators can be controlled in a variety of manners, both internally regulated and externally applied. Internal, cell-based control

utilizes intrinsic sensory pathways or mechanisms within the biological material. External, noncell-based control involves remote operation or local environmental stimuli—whether chemical, magnetic, electrical, optical, or a combination thereof.[24] Cardiomyocytes, for example, can be controlled with temperature variance, and skeletal muscle cells can be activated via electrical fields, optogenetics, or a neuronal network (requiring acetylcholine release from an innervating motor neuron). Optical control is negligibly invasive, irreversible, and can provide precise spatiotemporal control. It can also be used as an on/off toggle switch to quickly modulate contraction, pacing, or net actuation.[21,118,119]

The ability to noninvasively control living bioactuators with such specificity sets the stage for the development of future biological machines for a variety of applications. However, care must be taken to ensure that stimuli are applied within ranges that are acceptable or minimally invasive for living biological material, especially when dealing with exposure to ultraviolet (UV) light or electromagnetic fields, changes in pH and temperature, media electrolysis, and toxic waste by-products.[24]

6.5 Applications

Rapid prototyping technologies, which continue to advance in efficiency, resolution, and biocompatible material selection,[72,120] provide a controlled, economical, and potentially high-throughput solution to the production of responsive bioactuators for myriad applications. The extreme diversity of fabrication approaches, material composition, and functionalities suggest that soft bioactuators can be used in a variety of manners and systems. They boast many useful capabilities, including shape deformation, conformation, and sensitivity to their surroundings, movement in unstructured environments, and manipulation of delicate objects.[57] Their scalability also enables operation in environments where movement of their larger counterparts would be impractical or impossible. Furthermore, these devices are lighter, undergo more continuous and natural deformation with simple control inputs, and are more easily mass produced than their motor-driven counterparts.[121]

It is expected that custom-printed 3D robots and actuator structures will appear in application areas as diverse as devices for human–computer interaction, chemical and environmental remediation, or surgical tools for training.[88] For example, Alblalaihid et al. used a projection microstereolithography system to 3D-print polymer components on top of which thin metals could be coated to create a microscale gripper. The gripper was thermoelectrically activated and could be used for surgical manipulation.[122] Also, applications are being explored with fluid-powered actuators demonstrating human potential in macroscale self-healing medical implants or wearable orthotics.[18,123] Park et al. designed an artificial

soft biorobotic pneumatic muscle actuator attached to a 3D-printed leg model that could be worn over the knee,[124] and Doncieux et al. developed a bioinspired *Gummi Arm* fabricated of 3D-printed plastic structures connected by agonist–antagonist joints that mimicked soft tendons.[125]

Bioactuators with or without cells might be designed for drug screening or delivery,[126] bioreactors or lab-on-a-chip devices, vascular pumps and monitors, or adaptive prosthetics.[24,27,53] Some aspects of this technology have been proposed for use as part of drug delivery systems where a drug could detect body-site specific temperatures during local infection or low-pH tumor environments, and intelligently self-locate and self-release pharmaceuticals.[71] Independently or in large numbers, these actuators might also be programmed to form mobile and robust sensor and communication networks, allowing them to work in rubble fields, utility conduits, or the ocean floor to assist work in multiple industries.[127,128] Terrestrial actuators that could navigate dense environments and change shape, color, or surface temperature for camouflage would fit in well with outdoor research or military operations.[70]

Both living and nonliving bioactuators must be designed such that the form matches the intended function.[46,99,129] When building with muscle, for example, the devices might need to closely match the performance of their *in vivo* equivalents, especially if their intended use is to mimic a native tissue for a drug testing or regenerative application, or to provide a novel platform for understanding fundamental biological phenomena. However, when physiological relevance is less important to the end objective, replication of natural performance might be overlooked in favor of maximal efficiency, contractility, or power output. For example, primary cardiomyocytes might be overlooked in favor of a skeletal muscle cell line if extensive scaling-up or wider contractile ranges were necessary to the functionality of the bioactuator, but favored in certain temperatures or situations requiring cellular synchrony.

6.6 Limitations and future directions

Though much progress has been made, significant fundamental challenges still remain. Soft bioactuators must be manufactured in a manner that preserves the mechanical integrity of their structure and allows shock absorption, deformation, flexibility, and minimal damage. A major challenge is attempting to increase force output from elastic or synthetic systems without compromising the biomimetic properties that promote integration with living materials or the complexity of design and function, which characterizes the natural world.[15,18,19,40,120] When integrating living biomaterials, researchers must consider scenarios, which might enhance or hinder force production—for example, cellular viability, (self-)adhesion, organization, alignment, directionality, and overall struture, which all contribute

significantly to function. Moreover, cells must be maintained in cell culture media at highly regulated conditions ensuring nutrient and oxygen delivery, with the application of appropriate external cues for guidance of tissue development and mechanical performance. They are subject to a variety of failure modes, including mechanical (within the tissue or at the interface), metabolic, fatigue, damage or injury, and necrosis.[24,53] Finally, it can also be difficult to model the active and passive response of a cellular or living system in uncertain environmental conditions. Researchers still lack a deep understanding of how fundamental processes of cells (such as local interactions) function globally across length scales.[27]

Thus far, 3D-printed actuators have been assembled mostly with singular modalities. However, just as traditional robots contain multiple systems for various modalities (actuation, perception, computation, power, etc.), future designs of soft bioactuators should integrate multiple components and functionalities—including sensing[121,130] and processing of information—as well as multiple cell types or materials to achieve more complex, precise, and useful actuation.[52,99] In the future, it will be necessary to implement feedback systems and control mechanisms that help to extend the lifetime, precision, repeatability, and outputs of bioactuators, while also enhancing their operation outside encapsulated or restricted environmental conditions.[24,35,131,132] With living bioactuators, coculture systems can provide a synergistic support system to enhance the overall performance. To meet metabolic demands of living cells on larger size (>0.5 mm) or time scales, a vascular component will be necessary for consistent nutrient delivery. Indeed, the lack of perfusive blood vessels within regenerated tissues has historically plagued developments in the TE field. Guo, Miller, and Kolesky all have demonstrated various 3D-bioprinting methods that could be used in microvascular network formation.[133–135] In addition, innervation of muscle fibers with motor neurons can aid in the preservation of skeletal muscle phenotype, while allowing for better control, directed motion, or more complex functional outputs.[46,53]

With regards to fabrication, some limitations exist within the realm of technological advancements that might permit the construction of specific structures applicable to bioactuators. In the future, researchers will need to consider how to develop materials and scaffolds that can support cell and tissue outputs with maximal efficiency or power. This may include specific nano- or microscale geometries, shorter print times for large structures, higher resolution, greater range of material properties, improvement of surface adhesion techniques, and attachment of ECM.[29,35] Moreover, there exists a demand for multimaterial rapid prototyping advancements that could combine properties of multiple materials in one product.[11,36,51] Soft bioactuators might integrate new techniques in 3D printing of layered fabrics,[136,137] SMPs for flexible electronics,[80] and direct printing of electronic fluidic components[138] for onboard automation. From any perspective, these advancements represent a rapidly growing field with the potential to significantly benefit human life.

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