3D Printed Bionic Ears

Manu S. Mannoor,‡ Ziwen Jiang,‡ Teena James,‡ Yong Lin Kong,‡ Karen A. Malatesta,‡ Winston O. Soboyejo,† Naveen Verma,§ David H. Gracias,‡ and Michael C. McAlpine*,‡

†Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, New Jersey 08544, United States
‡Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland 21218, United States
§Department of Electrical Engineering, Princeton University, Princeton, New Jersey 08544, United States

Supporting Information

ABSTRACT: The ability to three-dimensionally interweave biological tissue with functional electronics could enable the creation of bionic organs possessing enhanced functionalities over their human counterparts. Conventional electronic devices are inherently two-dimensional, preventing seamless multidimensional integration with synthetic biology, as the processes and materials are very different. Here, we present a novel strategy for overcoming these difficulties via additive manufacturing of biological cells with structural and nanoparticle derived electronic elements. As a proof of concept, we generated a bionic ear via 3D printing of a cell-seeded hydrogel matrix in the anatomic geometry of a human ear, along with an intertwined conducting polymer consisting of infused silver nanoparticles. This allowed for in vitro culturing of cartilage tissue around an inductive coil antenna in the ear, which subsequently enables readout of inductively-coupled signals from cochlea-shaped electrodes. The printed ear exhibits enhanced auditory sensing for radio frequency reception, and complementary left and right ears can listen to stereo audio music. Overall, our approach suggests a means to intricately merge biologic and nanoelectronic functionalities via 3D printing.

KEYWORDS: Cybernetics, tissue engineering, bioelectronics, cyborg organs, electronic implants, additive manufacturing

The design and implementation of bionic organs and devices that enhance human capabilities, known as cybernetics, has been an area of increasing scientific interest.1,2 This field has the potential to generate customized replacement parts for the human body, or even create organs containing capabilities beyond what human biology ordinarily provides. In particular, the development of approaches for the direct multidimensional integration of functional electronic components with biological tissue and organs could have tremendous impact in regenerative medicine, prosthetics, and human-machine interfaces.3,4 Recently, several reports have described the coupling of electronics and tissues using flexible and/or stretchable planar devices and sensors that conform to tissue surfaces, enabling applications such as biochemical sensing and probing of electrical activities on surfaces of the heart,3 lungs,6 brain,5 skin,8 and teeth.9 However, attaining seamless three dimensionally (3D) entwined electronic components with biological tissues and organs is significantly more challenging.4

Tissue engineering is guided by the principle that a variety of cell types can be coaxied into synthesizing new tissue if they are seeded onto an appropriate three-dimensional hydrogel scaffold within an accordant growth environment.10−15 Following in vivo or in vitro culture, tissue structures form which possess the morphology of the original scaffold.16 A major challenge in traditional tissue engineering approaches is the generation of cell-seeded implants with structures that mimic native tissue, both in anatomic geometries and intratissue cellular distributions.17 Techniques such as seeding cells into nonadhesive molds or self-folding scaffolds have been used to fabricate three-dimensional tissue constructs with complex 3D geometries.18,19 Yet, existing techniques are still incapable of easily creating organ or tissue parts with the required spatial heterogeneities and accurate anatomical geometries to meet the shortage of donor organs for transplantation.20−22 For instance, total external ear reconstruction with autogenous cartilage with the goal of recreating an ear that is similar in appearance to the contralateral auricle remains one of the most difficult problems in the field of plastic and reconstructive surgery.23

Additive manufacturing techniques such as 3D printing offer a potential solution via the ability to rapidly create computer-aided design (CAD) models by slicing them into layers and building the layers upward using biological cells as inks in the precise anatomic geometries of human organs.24−27 Variations of 3D printing have been used as methods of solid freeform fabrication, although its use has mainly been limited to the creation of passive mechanical parts.24,28 Extrusion-based 3D printing has been used to engineer hard tissue scaffolds such as
knee menisci and intervertebral discs complete with encapsulated cells. This technique offers the ability to create spatially heterogeneous multimaterial structures by utilizing deposition tools that can extrude a wide range of materials. Further, nanoscale functional building blocks enable versatile bottom-up assembly of macroscale components possessing tunable functionalities. This could allow for the simultaneous printing of nanoelectronic materials and biological cells to yield three dimensionally integrated cyborg tissues and organs exhibiting unique capabilities.

Here we introduce a conceptually new approach that addresses the aforementioned challenges by fully interweaving functional electronic components with biological tissue via 3D printing of nanoelectronic materials and viable cell-seeded hydrogels in the precise anatomic geometries of human organs. Since electronic circuitry is at the core of sensory and information processing devices, in vitro culturing of the printed hybrid architecture enables the growth of “cyborg organs” exhibiting enhanced functionalities over human biology. Our approach offers the ability to define and create spatially heterogeneous constructs by extruding a wide range of materials in a layer-by-layer process until the final stereolithographic geometry is complete. This concept of 3D printing living cells together with electronic components and growing them into functional organs represents a new direction in merging electronics with biological systems. Indeed, such cyborg organs are distinct from either engineered tissue or organs.

As a proof of concept of this approach, we evaluated the ability of 3D printing to create a viable ear auricle that also contains electronics that enable alternative capabilities to human hearing. Human organs comprising predominantly of cartilaginous tissue, such as the ear auricle, represent suitable prototype candidates to investigate the feasibility of our approach. This is due to (1) the inherent complexity in the ear’s anatomical geometry, which renders it difficult to bioengineer via traditional tissue engineering approaches as well as (2) the simplicity in its cartilage tissue level structure due to the lack of vasculature. Additionally, bottom-up assembly of nanoelectronic matrices provides the ability to hierarchically generate functional macroscale electronic components. Specifically, we demonstrate 3D printing of a chondrocyte-seeded alginate hydrogel matrix with an electrically conductive silver nanoparticle (AgNP) infused inductive coil antenna, connecting to cochlea-shaped electrodes supported on silicone. Taken together, the result is three-dimensional integration of functional electronic components within the complex and precise anatomic geometry of a human ear (Figure 1).

The following steps are involved in the process. First, a CAD drawing of the bionic ear (Figure 1A) is used to prescribe the anatomic geometry and the spatial heterogeneity of the various functional materials. As described above, three materials comprise the three functional constituents (structural, biological, and electronic) of the bionic ear. These materials are fed into a syringe extrusion based Fab@Home 3D printer (The NextFab Store, Albuquerque, NM) (Figure 1B). The printed bioelectronic hybrid ear construct is then cultured in vitro to enable cartilage tissue growth to form a cyborg ear with the capability of sensing electromagnetic signals in the radio frequency (RF) range by means of an inductive coil acting as a receiving antenna (Figure 1C).

To demonstrate our approach, we printed the bionic ear construct as follows. For the scaffold, we preseeded an alginate hydrogel matrix with viable chondrocytes at a density of ~60 million cells/mL (see Supporting Information). Alginate matrix is three dimensionally stable in culture, nontoxic, preseeding, and extrusion compatible, and a suitable cell delivery vehicle because cross-linking can be initiated prior to deposition. Chondrocytes used for the printing were isolated from the articular cartilage of one month old calves (Astarte Biologics, Redmond, WA). A CAD drawing of a human ear auricle in stereolithography format (STL) with an integrated circular coil antenna connected to cochlea-shaped electrodes was used to define the print paths by slicing the model into layers of contour and raster fill paths. Cross-linking was initiated in the alginate hydrogel matrix preseeded with viable chondrocytes, which was then 3D printed along with conducting (AgNP-infused) and nonconducting silicone solutions (Supporting Information Movie 1). Together, this method produced the biological, electronic, and structural components of the bionic organ in a single process.
Figure 2A shows the 3D printed bionic ear immediately after printing. Notably, it is found to faithfully reproduce the CAD drawing, in the precise spatiality for each material as dictated by the design. The printed ear construct was immersed in chondrocyte culture media containing 10 or 20% fetal bovine serum (FBS), which was refreshed every 1−2 days (see Supporting Information). The hybrid ear showed good structural integrity and shape retention under culture (Figure 2B). Over time, the construct gradually became more opaque; this was most apparent after four weeks of culture and is grossly consistent with developing an extracellular matrix (ECM). The gross morphology of the bionic ear after 10 weeks of in vitro culture is shown in the Supporting Information.

Viability was tested immediately before and during the various stages of the printing process. Initial viability of cells was determined after culturing using a Trypan blue cell exclusion assay (Corning Cellgrow, Mediatech, VA) and was found to be 96.4 ± 1.7% (Figure 2C) (see Supporting Information). The printed cell-seeded alginate ear was also tested with a LIVE/DEAD Viability Assay (Molecular Probes, Eugene, OR) and exhibited a cell viability of 91.3 ± 3.9% with homogeneous chondrocyte distribution. This result suggests that the printing process, including cell encapsulation and deposition, does not appreciably impact chondrocyte viability.

Notably, this approach of printing a preseeded hydrogel matrix eliminates the major problems associated with seeding depth limitations and nonuniform seeding in traditional
methods for seeding premolded 3D scaffolds. Seeding chondrocytes into a bioabsorbable alginate matrix and shaping it via 3D printing localizes the cells to a desired geometry, allowing for new ECM production in defined locations when cultured in nutritive media. As tissue develops, the polymer scaffold is reabsorbed (Figure 2D), so that the new tissue retains the shape of the polymer in which the cells were seeded. The biodegradable scaffolding provides each cell with better access to nutrients and more efficient waste removal.

Next, histologic evaluation was used to compare the morphology of chondrocytes in the neocartilage of the bionic ear to that of the native cartilaginous tissue. Hematoxylin and eosin (H&E) staining revealed uniform distribution of the chondrocytes in the constructs (Figure 2E) (see Supporting Information). Histology of the ear tissue with Safranin O staining indicated relatively uniform accumulation of proteoglycans in the cultured ear tissue (Figure 2F). These biochemical data are consistent with the development of new cartilage. Finally, fluorescent measurements were used to ascertain the viability of the 3D printed bionic ear tissue after 10 weeks of in vitro growth culture using fluorescein diacetate (FDA) and propidium iodide (PI) stains. Figure 2G,H shows the tissue covering the coil antenna and the internal tissue that is in contact with the electrode that runs perpendicular through the tissue, respectively. In both cases, the grown cartilage exhibited excellent morphology and tissue level viability. Notably, this approach of culturing tissue in the presence of abiotic electronic materials could minimize the immune response of the grown tissue.

We then characterized the mechanical properties of the cartilage at various stages of growth, as ECM development correlates strongly with the developing tissue’s mechanical properties. First, extensive biochemical and histologic characterizations were performed. Samples were removed from cultures containing 10 and 20% FBS at 2, 4, 6, 8, and 10 weeks and frozen to measure DNA content of the neocartilage and for biochemical evaluation of the ECM (see Supporting Information). ECM accumulation in the constructs was evaluated by quantifying the amount of two important components of ECM: (1) hydroxyproline (HYP) as a marker of collagen content and (2) sulfated glycosaminoglycan (GAG) as a marker of proteoglycans. By week 10, the HYP content increased to $1.2 \pm 0.1$ and $1.4 \pm 0.2 \mu g/mg$ for cultures containing 10 and 20% FBS, respectively (Figure 3A). The corresponding values of GAG content for week 10 were $10.6 \pm 0.6$ and $12.2 \pm 1.0 \mu g/mg$ (Figure 3B). This increase in GAG and HYP content indicates that chondrocytes are alive and metabolically active in culture.

Next, tensile properties were analyzed by testing 3D printed chondrocyte-alginate dogbone samples at various points in culture in which the dogbones contained the same cell densities and identical culturing conditions as the ear (see Supporting Information). Evaluation of the mechanical properties indicated that the Young’s modulus of the dogbones increased with time from 14.16 to 111.46 kPa at week 10 (Figure 3C). Dogbones of a lower chondrocyte density of 20 million cells/mL were also tested under similar conditions to understand the effect of the initial chondrocyte density in the mechanical properties of the grown tissue. These were found to possess a lower Young’s modulus of 73.26 kPa at week 10. Next, the hardness of the grown cartilaginous tissue of the 3D printed auricle was characterized using nanoindentation measurements. The indentations were performed at the various anatomic sites of the auricle (Figure 3D). As shown in Table 1, these hardness values were found to be relatively uniform, ranging from 38.50 to 46.80 kPa, confirming the structural integrity of the printed ear.

To demonstrate the enhanced functionalities of the 3D printed bionic ear, we performed a series of electrical characterizations. First, the resistivity of the coil antenna was measured using four point probe measurements and found to be dependent on the volumetric flow rate used for printing the
conducting AgNP-infused silicone (see Supporting Information). At the optimum flow rate, the resistivity of the printed coil was found to be $1.31 \times 10^{-6} \, \Omega \cdot m$, which is only 2 orders of magnitude higher than pure silver ($1.59 \times 10^{-8} \, \Omega \cdot m$). Next, we performed wireless radio frequency reception experiments. To demonstrate the ability of the bionic ear to receive signals beyond normal audible signal frequencies (in humans, 20 Hz to 20 kHz), we formed external connections to the cochlea-shaped electrodes stemming from the inductive coil of the bionic ear (Figure 4A). The ear was then exposed to sine waves of frequencies ranging from 1 MHz to 5 GHz. The S21 (forward transmission coefficient) parameter of the coil antenna was analyzed using a network analyzer and was found to transmit signals across this extended frequency spectrum (Figure 4B).

Most importantly, as a demonstrative example of the versatility in modifying the final organ by modifying the CAD design, we printed a complementary left ear by simply reflecting the original model (see Supporting Information). Left and right channels of stereophonic audio were exposed to the left and right bionic ear via transmitting magnetic loop antennas with ferrite cores (Figure 4C). The signals received by the bionic ears were collected from the signal output of the dual cochlea-shaped electrodes and fed into a digital oscilloscope and played back by a loud speaker for auditory and visual monitoring. Excerpts of the transmitted and received signals of duration 1 ms for both the right and left bionic ears are shown in Figure 4D and are found to exhibit excellent reproduction of the audio signal. Significantly, the played back music (Beethoven’s “Für Elise”) from the signal received by the bionic ears possessed good sound quality (Supporting Information Movie 2).

In summary, designer cyborg ears were fabricated that are capable of receiving electromagnetic signals over an expansive frequency range from hertz to gigahertz. Our strategy represents a proof of principle of intertwining the versatility of additive manufacturing techniques with nanoparticle assembly and tissue engineering concepts. The result is the generation of bona fide bionic organs in both form and function, as validated by tissue engineering benchmarks and electrical measurements. Such hybrids are distinct from either engineered tissue or planar/ flexible electronics and offer a unique way of attaining a seamless integration of electronics with tissues to generate “off-the-shelf” cyborg organs. Finally, the use of 3D printing with other classes of nanoscale functional building blocks, including semiconductor, magnetic, plasmonic, and ferroelectric nanoparticles, could expand the opportunities for engineering bionic tissues and organs.

■ ACKNOWLEDGMENTS

We thank Kelylee Cung, Yao-Wen Yeh, and Dr. Ismaiel Yakub for valuable discussions and technical assistance. The 3D CAD model of the ear was downloaded from thingiverse.com and was also used to render the ear images in the manuscript (Figures 1a, 1c, 4c, and the TOC image). The Creative Commons License is available at http://creativecommons.org/licenses/by-nc/3.0/. A student version of the Autodesk 3ds Max software package was used to modify and render the 3D images. Beethoven’s Für Elise music was obtained from the online collection created by Jason Shaw with permission through audionautix.com. Released under Creative Commons License 3.0. Downloadable release form is available at http://www.audionautix.com/Saved/CCrelease.jpg. M.C.M. acknowledges support of this work by the Defense Advanced Research Projects Agency (No. D12AP00245) and the Air Force Office of Scientific Research (No. FA9550-12-1-0367). D.H.G. acknowledges support from the NIH Director’s New Innovator program (DP2-OD004346-01). This material is based upon work supported by the Grand Challenges Program at Princeton University.

■ REFERENCES

(1) Lavine, M.; Roberts, L.; Smith, O. Science 2002, 295, 995.
(2) Craelius, W. Science 2002, 295, 1018–21.
(3) Green, D. W. Biomed. Mater. 2008, 3, 034010.
(4) Tian, B.; Liu, J.; Dvir, T.; Jin, L.; Tsui, J. H.; Qing, Q.; Suo, Z.; Langer, R.; Kohane, D. S.; Lieber, C. M. Nat. Mater. 2012, 11, 986–94.
(5) Timko, B. P.; Cohen-Karni, T.; Yu, G.; Qing, Q.; Tian, B.; Lieber, C. M. Nano Lett. 2009, 9, 914–8.
(6) Nguyen, T. D.; Deshmukh, N.; Nagaraj, J. M.; Kramer, T.; Purohit, P. K.; Berry, M. J.; McAlpine, M. C. Nat. Nanotechnol. 2012, 7, 878–93.
(7) Viventi, J.; Kim, D. H.; Vigeland, L.; Frechette, E. S.; Blanco, J. A.; Kim, Y. S.; Arriv, A. E.; Tiruvadi, V. R.; Hwang, S. W.; Vanleeer, A. C.; Wulsin, D. F.; Davis, K.; Gelber, C. E.; Palmer, L.; Van der Spiegel, J.; Wu, J.; Xiao, J.; Huang, Y.; Contreras, D.; Rogers, J. A.; Litt, B. Nat. Neurosci. 2011, 14, 1599–605.
(8) Kim, D. H.; Lu, N.; Ma, R.; Kim, Y. S.; Kim, R. H.; Wang, S.; Wu, J.; Won, S. M.; Tao, H.; Islam, A.; Yu, K. J.; Kim, T. I.; Chowdhury, R.; Ying, M.; Xu, L.; Li, M.; Chung, H. J.; Keum, H. Z.; McCormick, M.; Liu, P.; Zhang, Y. W.; Omenetto, F. G.; Huang, Y.; Coleman, T.; Rogers, J. A. Science 2011, 333, 838–43.
(9) Mannoor, M. S.; Tao, H.; Clayton, J. D.; Sengupta, A.; Kaplan, D. L.; Naik, R. R.; Verma, N.; Omenetto, F. G.; McAlpine, M. C. Nat. Commun. 2012, 3, 763.
(10) Pampaloni, F.; Reynaud, E. G.; Stelzer, E. H. Nat. Rev. Mol. Cell Biol. 2007, 8, 839–45.
(11) Langer, R.; Vacanti, J. P. Science 1993, 260, 920–6.
(12) Langer, R.; Vacanti, J. P. Science 1993, 260, 130–3.
(13) Jayawarna, V.; Ali, M.; Jowitt, T. A.; Miller, A. F.; Saiani, A.; Gough, J. E.; Ulijn, R. V. Adv. Mater. 2006, 18, 611–614.
(14) Lee, M. Y.; Kumar, R. A.; Sukumar, S. M.; Hogg, M. G.; Clark, D. S.; Dordick, J. S. Proc. Natl. Acad. Sci. USA 2008, 105, 59–63.
(15) Mapili, G.; Lu, Y.; Chen, S.; Roy, K. J. Biomed. Mater. Res., Part B 2005, 75, 414–24.
(16) Marler, J. J.; Upton, J.; Langer, R.; Vacanti, J. P. Adv. Drug Delivery. Rev. 1998, 33, 165–182.
(17) Shieh, S. J.; Terada, S.; Vacanti, J. P. Biomaterials 2004, 25, 1545–57.
(18) Napolitano, A. P.; Dean, D. M.; Man, A. J.; Youssef, J.; Ho, D. N.; Rago, A. P.; Lech, M. P.; Morgan, J. R. BioTechniques 2007, 43 (494), 496–500.

■ ASSOCIATED CONTENT

1 Supporting Information

Additional experimental details with materials, methods, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone number: (609) 542-0257. E-mail: mcm@princeton.edu.

Notes

The authors declare no competing financial interest.
(19) Jamal, M.; Kadam, S. S.; Xiao, R.; Jivan, F.; Onn, T. M.; Fernandes, R.; Nguyen, T. D.; Gracias, D. H. Adv. Healthcare Mater. 2013, DOI: 10.1002/adhm.201200458.
(20) Chang, S. C.; Tobias, G.; Roy, A. K.; Vacanti, C. A.; Bonassar, L. J. Plast. Reconstr. Surg. 2003, 112, 793–9.
(21) Khademhosseini, A.; Langer, R.; Borenstein, J.; Vacanti, J. P. Proc. Natl. Acad. Sci. U.S.A. 2006, 103, 2480–7.
(22) Atala, A. Curr. Opin. Biotechnol. 2009, 20, 575–92.
(23) Cao, Y.; Vacanti, J. P.; Paige, K. T.; Upton, J.; Vacanti, C. A. Plast. Reconstr. Surg. 1997, 100, 297–302 discussion 303–4.
(24) Symes, M. D.; Kitson, P. J.; Yan, J.; Rich mond, C. J.; Cooper, G. J.; Bowman, R. W.; Vilbrandt, T.; Cronin, L. Nature Chem. 2012, 4, 349–54.
(25) Jones, N. Nature 2012, 487, 22–3.
(26) Reiffel, A. J.; Kafka, C.; Hernandez, K. A.; Popa, S.; Perez, J. L.; Zhou, S.; Pramanik, S.; Brown, B. N.; Ryu, W. S.; Bonassar, L. J.; Spector, J. A. PLoS One 2013, 8, e56506.
(27) Villar, G.; Graham, A. D.; Bayley, H. Science 2013, 340, 48–52.
(28) Yeong, W. Y.; Chua, C. K.; Leong, K. F.; Chandrasekaran, M. Trends Biotechnol. 2004, 22, 643–52.
(29) Cohen, D. L.; Malone, E.; Lipson, H.; Bonassar, L. J. Tissue Eng. 2006, 12, 1325–35.
(30) Khalil, S.; Nam, J.; Sun, W. Rapid Prototyping J. 2005, 11, 9–17.
(31) Xu, T.; Binder, K. W.; Albanna, M. Z.; Dice, D.; Zhao, W.; Yoo, J. J.; Atala, A. Biofabrication 2013, 5, 015001.
(32) Malone, E.; Berry, M.; Lipson, H. Rapid Prototyping J. 2008, 14, 128–140.
(33) Ahn, B. Y.; Duoss, E. B.; Motara, M. J.; Guo, X.; Park, S. I.; Xiong, Y.; Yoon, J.; Nuzzo, R. G.; Rogers, J. A.; Lewis, J. A. Science 2009, 323, 1590–3.
(34) Wu, W.; DeConinck, A.; Lewis, J. A. Adv. Mater. 2011, 23, H178–83.
(35) Someya, T.; Sekitani, T.; Iba, S.; Kato, Y.; Kawaguchi, H.; Sakurai, T. Proc. Natl. Acad. Sci. U.S.A. 2004, 101, 9966–70.
(36) Bichara, D. A.; O’Sullivan, N. A.; Pomerantseva, I.; Zhao, X.; Sundback, C. A.; Vacanti, J. P.; Randolph, M. A. Tissue Eng., Part B 2012, 18, 51–61.
(37) Marinissen, W. J. C. M.; van Osch, G. J. V. M.; Aigner, J.; van der Veen, S. W.; Hollander, A. P.; Verwoerd-Verhoef, H. L.; Verhaar, J. A. N. Biomaterials 2002, 23, 1511–1517.
(38) Dobratz, E. J.; Kim, S. W.; Voglewede, A.; Park, S. S. Arch. Facial Plast. Surg. 2009, 11, 40–47.
(39) Kelly, D. J.; Crawford, A.; Dickinson, S. C.; Sims, T. J.; Mundy, J.; Hollander, A. P.; Prendergast, P. J.; Hatton, P. V. J. Mater. Sci. Mater. Med. 2007, 18, 273–81.
(40) Li, C.; Pruitt, L. A.; King, K. B. J. Biomed. Mater. Res. A 2006, 78, 729–38.