Metallic Nanoislands on Graphene for Biomechanical Sensing
Julian Ramírez, Beril Polat, and Darren J. Lipomi*

ABSTRACT: This minireview describes a nanomaterial-based multimodal sensor for performing biomechanical measurements. The sensor consists of ultrathin metallic films on single-layer graphene. This composite material exhibits physical properties that neither material possesses alone. For example, the metal, deposited by evaporation at low (<10 nm) nominal thicknesses, renders the film highly sensitive to mechanical stimuli, which can be detected using electrical (i.e., resistance) and optical (i.e., plasmonic) modalities. The electrical modality, in particular, is capable of resolving deformations as small as 0.0001% engineering strain, or 1 ppm. The electrical and optical responses of the composite films can be tailored by controlling the morphology of the metallic film. This morphology (granular or island-like when deposited onto the graphene) can be tuned using the conditions of deposition, the identity of the substrate beneath the graphene, or even the replacement of the graphene for hexagonal boron nitride (hBN). This material responds to forces produced by a range of physiological structures, from the contractions of heart muscle cells, to the beating of the heart through the skin, to stretching of the skin due to the expansion of the lungs and movement of limbs. Here, we provide an update on recent applications of this material in fields ranging from cardiovascular medicine (by measuring the contractions of 2D monolayers of cardiomyocytes), regenerative medicine (optical measurements of the forces produced by myoblasts), speech pathology and physical therapy (measuring swallowing function in head and neck cancer survivors), lab-on-a-chip devices (using deformation of sidewalls of microfluidic channels to detect transiting objects), and sleep medicine (measuring pulse and respiration with a wearable, unobtrusive device). We also discuss the mechanisms by which these films detect strain.

INTRODUCTION
Mechanical forces are ubiquitous in human physiology, and thus, the detection of biomechanical deformation is critical in research and the clinic. To address this need, many groups have developed strain gauges composed of a variety of materials and which operate by a range of mechanisms.1 In particular, nanomaterial-based strain sensors have become a topic of interest due to their superior sensitivity when compared to conventional strain gauges based on bulk metals and alloys. For example, bulk metallic traces can be used to sense tensile strain through detection of changes in electrical resistance due to constriction of the cross-sectional dimensions upon stretching. In contrast, the sensitivity of nanomaterial-based strain gauges arises from piezoresistive effects, in which mechanical deformation actually produces a change in the intrinsic resistivity of the material. Along with the high sensitivity of nanomaterial-enabled strain gauges, their amenability to various substrates (from rigid to elastomeric) expands the possible utility of these materials for in vitro and wearable applications.3 Recent progress in the field of highly sensitive strain gauges has been driven by the development of thin metallic films containing fine cracks. In these systems, the response to mechanical strain is mediated by the propagation, opening, and closing of these cracks upon mechanical deformation.4,5 The high sensitivity and mechanical stability offered by this class of materials have
enabled the development of devices for applications ranging from wearable sensors to human-machine interfaces, exemplified in Figure 1. For example (Figure 1a), Kwon and coworkers developed a device capable of estimating energy expenditure by measuring the bending of the knee during walking and running.6 In Figure 1b, a piezoresistive tactile sensor device was developed by Moon et al., where the device contained two sensors made from metallized nanofibril networks.7 Kim and collaborators recently paired a cracked metal film strain sensor with a deep neural network to decode the complex motion of five finger motions in real-time (Figure 1c).8

Over the last five years, our research group has been exploring a type of ultrasensitive strain gauge for measuring biomechanical deformation originating from both cells and physiological processes. These strain gauges are based on metallic nanoparticles supported by single-layer graphene. When combined, these composite materials possess properties found in neither bulk metal films nor single-layer graphene. For example, when used as piezoresistive strain gauges, a limit of detection of 0.0001% strain (resolution of 1 ppm or 1 microstrain) is possible. Applications for this material range from mechanical sensing of human biosignals (e.g., swallowing activity, respiration, and cardiac pulse rate) and elastohydrodynamic deformation in microfluidic channels, to optical monitoring of mechanical contractions of myoblast cells. In our experiments, we have used gold, silver, and palladium as the metallic film on graphene. Gold is unreactive and biocompatible and thus a useful metal to use when detecting mechanical activity piezoresistively. Palladium and silver, however, are cytotoxic but possess ultrasensitive piezoresistive and “piezoplasmonic” sensitivity, respectively. By exploiting the biocompatibility of graphene and the use of encapsulation, these materials are capable of measuring biomechanical activity with a better response than gold while mitigating possible toxicity. A summary of the biomechanical forces measured using this material is shown in Figure 2.

■ INITIAL FINDINGS: METALLIC FILMS ON SINGLE-LAYER GRAPHENE

In a serendipitous discovery during an unrelated project, we found that it was possible to control the morphologies of the...
metallic films evaporated onto graphene by changing the identity of the substrate supporting the graphene. This difference in morphologies depended on the lattice mismatch between the evaporated metal and the graphene, along with differences in surface energy between the substrate supporting the graphene and the evaporated metal. With the ability to generate granular metallic films with very small spacings between adjacent particles, we reasoned that the composite films might behave as ultrasensitive strain gauges. Some of these strain gauges, as described by others, operate by modulation of the tunneling current between adjacent particles by mechanical strain. While we would later determine that tunneling is not likely to play a dominant role in the strain response in the films that we were making, we nevertheless found that the change in electrical resistance of strain gauges made from these films exhibited extraordinarily high sensitivity and resolution.

The sensitivity of a strain gauge is quantified by the gauge factor, defined as the ratio of the normalized change in electrical resistance to the applied strain. Commercial strain gauges that detect strain through geometrical changes in the metallic film have gauge factors of ~2 for modest strains (~1–5%). For comparison, we found that graphene/metal films had gauge factors over 10 in the ultralow strain regime (~0.001%) and could thus enable detection of minute biomedical signals. For example, gold islands on single-layer graphene were capable of detecting the contractions of rat cardiomyocytes by a change in the electrical resistance of the gold-graphene composite. The sensor had a response time of 0.8 ± 0.2 ms. Critically, because gold is a noble metal that is not cytotoxic, we saw no adverse effects in the cells due to exposure to the metal. In the first on-body application, it was possible to measure the pulse waveform of the radial artery on the surface of the wrist of a human subject. For palladium on graphene, the smallest detectable strain, i.e., resolution starting in an unstrained state, was at the time measured to be 0.001% (later found to be an order of magnitude lower than this). Moreover, the sensors exhibited a useful degree of dynamic range. When placed on a silicone elastomer, these sensors could be cyclically stretched up to 9% (the largest strain tested before the supporting substrate ruptured) while returning to the baseline resistance at mechanical equilibrium.

Near-Zero Temperature Coefficient of Resistance. One disadvantage of strain sensors that use a change in electrical resistance as the output is that the resistance tends to have an unwanted dependence on temperature. In conventional strain gauges, the problem of nonzero temperature coefficient of resistance (TCR) of pure metals is circumvented by the use of alloys such as constantan, an alloy of copper and nickel, whose resistance exhibits only a small dependence on temperature. We were thus interested in how we might tune the composition of the metal–graphene composite films to achieve a similar effect. In their pristine form, single-layer graphene has a negative TCR, while bulk metal has a positive TCR, and hence, the rates of change in resistance with respect to increasing temperature are negative and positive for graphene and metal, respectively. We found that the addition of a small amount of metal to graphene would change the TCR of the composite film to near zero. This was due to the fact that the graphene provides a high resistance to the current flow, while the metal provides a low resistance. As the temperature increases, the resistance of the graphene increases less than the resistance of the metal, resulting in a near-zero TCR for the composite film.

Figure 3. Piezoresistive biomechanical sensing with wearable devices comprising graphene/metal composites. Graphene/palladium (Gr/Pd) devices have been used for (a) the piezoresistive detection of swallowing activity in head and neck cancer patients for the monitoring of the onset of swallowing dysfunction due to radiation. The swallowing data acquired by the sensor were used to (b) develop machine learning algorithms designed to (c) distinguish swallows for the same food type between a healthy human (bottom plot, blue) and a dysphagic patient (top plot). Reproduced (adapted) with permission from ref 9. Copyright (2018) American Chemical Society. By combining (d) Gr/Pd with PEDOT:PSS, stretchable devices could be used for the simultaneous measurement of human pulse pressure and respiration waveforms by placing the device on the torso of a human participant (e). (f) The deformation in the structure was modeled using finite element analysis. Reproduced (adapted) with permission from ref 19. Copyright (2019) American Chemical Society.

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temperature have the opposite sign. By measuring the TCR of the films as a function of the nominal thickness of metal, we could generate films that were insensitive to temperature but not to strain. Analysis of the morphology of the metallic film as a function of increasing nominal thickness (and surface coverage) led to a simple model to correlate the thermo resistive behavior of the composite material as a function of surface coverage. The model shows that the TCR of the composite becomes increasingly positive with increasing coverage of the metal. Thus, the resistance of the composite material can be tuned to have a near-zero dependence on temperature because the positive TCR of the metal counterbalances the negative TCR of graphene. As a proof of concept, wearable sensors with near-zero TCR were fabricated to demonstrate the stability of the electrical signal when detecting the pulse waveform on the wrist, while suppressing variations in surface temperature produced by simulated sunlight.

DEVELOPMENTS IN WEARABLE APPLICATIONS

Wearable Sensor for Monitoring Swallowing Activity. We then sought to develop devices capable of exploiting the exceptional piezoresistance of this material in real-world scenarios. The monitoring of swallowing activity is of particular interest in populations who suffer from head and neck cancer. While this condition is curable with radiation therapy, up to 40% of patients develop radiation-associated fibrosis of the swallowing muscles and subsequent dysphagia, which severely reduces swallowing function. The current gold-standard method to monitor the onset of dysphagia is videofluoroscopy, where an X-ray video of a patient is obtained during the swallowing of a barium-containing paste. While videofluoroscopy provides a complete assessment of a patient’s swallowing function, this exam requires visits to the clinic. Because of the episodic nature of this exam, fibrosis is often not detected until it is too late, and thus, there is a need for continuous monitoring, possibly using an at-home device.

To address this need, we developed a flexible device comprising strain-sensitive palladium nanoislands on single-layer graphene to be placed on the skin underneath the chin to detect mechanical deformation due to swallowing (Figure 3a). To complement the strain measurements, surface electro myography (sEMG) measurements of the muscle activity were obtained using conventional metallic electrodes. The utility of our device was tested by measuring the swallowing activity in a cohort of 14 head and neck cancer patients, 7 of whom exhibited signs of dysphagia, and 7 of whom had normal swallowing function. Assisted by a machine-learning algorithm, measurements of strain could be used to detect differences in swallowing activity based on the consistency of the swallowed food (i.e., water, yogurt, cracker). The algorithm was also able to distinguish dysphagic from nondysphagic swallowing (Figure 3b and 3c). The algorithm was trained by asking the user or patient to swallow the same bolus several times (red curves) to develop the classifier models for each signal (blue curve). After developing the models, a new swallowing signal (green curve) was then placed into a category. The accuracy of the classifier models was tested through cross-validation techniques. An efficient classification model based on the L1 distance of the per-class average (i.e., the distance between the model signal and the new signal at every time point) was found to be the most accurate model. In a separate experiment (not shown), visual comparison of the strain data with sEMG data made it possible to distinguish swallowing from motion artifacts (e.g., head turning and coughing). Comparison of the strain data with sEMG data made it possible to distinguish swallowing from motion artifacts (e.g., head turning and coughing). For one patient, the data from the sensor were compared to the results obtained from a videofluoroscopy examination. It was thus possible to attribute changes in electrical resistance with time to different phases in the swallowing process. This study thus represents an example of a nanomaterial-enabled device in a patient cohort study, and further establishes wearable devices as potential complements to clinically accepted practices in patient care.

Wearable Sensor for Monitoring Interpolated Biomechanical Signals. A constant challenge in the development of strain gauges is the ability to combine sensitivity and dynamic range. This challenge is especially salient for strain gauges based on graphene, which fractures at only a few percent strain, whereas skin is significantly more stretchable (i.e., up to 50%). To develop stretchable sensors capable of withstanding greater strains while retaining piezoresistive sensitivity, we combined the graphene/metal film with a formulation of plasticized PEDOT:PSS by depositing the conductive polymer directly on top of the nanomaterial composite, seen in Figure 3d. The role of the conductive polymer was to introduce an alternative pathway for electrical conduction upon cracking of the graphene film. Hence, the electrons would travel through the graphene/metal film at low strains (~0.001%), as it is the path of lowest electrical resistance, but would travel through a contiguous path comprising all three materials at larger strains. We found that this strategy was largely successful, as our “structured composite” consisting of graphene/metal/PEDOT:PSS provided high resolution at low strains (strains of 0.001% produced a change in resistance well above the noise) while the highly plasticized nature of the PEDOT:PSS formulation allowed for the retention of conductivity up to 86% strain. To develop a wearable device using this material, we encapsulated the graphene/metal film between two PDMS layers. The encapsulation of the film between elastomeric layers resulted in a reduction of the piezoresistive sensitivity but an increase in the working range of the material. The piezoresistive response of the material demonstrated some hysteresis at strains above 10% but was able to withstand up to 250 cycles (the maximum number of cycles tested). Moreover, experiments in which the material was cyclically stretched suggested that the conductive pathways involving the graphene reformed upon return to mechanical equilibrium (i.e., physical contact after the material fractures). The utility of the improved material was demonstrated by attaching a stretchable device, comprising graphene/metal/PEDOT:PSS, on the torso of a human subject simulating sleep and detecting interpolated signals stemming from respiration and pulse, as seen in Figure 3e and Figure 3f. This proof-of-concept experiment, using the diagnosis of sleep apnea using polysomnography as inspiration, highlights the potential of wearable nanomaterial devices to reduce the complexity and costs of devices for distributed healthcare.

ASSAYING CELLULAR BIOMECHANICS

Optical Measurements of Myocyte Activity. All cells produce mechanical forces; in the case of myocytes, mechanical activity is central to their function. Assays of mechanical activity of cells in vitro has thus stimulated the development of sensing platforms. In particular, platforms amenable to high-throughput measurements are desirable to determine the effect of environmental stresses (e.g., in evaluating the cardiotoxicity of
drug candidates.\textsuperscript{23} Sensors based on metallic nanoparticles supported by graphene have significant potential for use in this field, as they are both piezoresistive and plasmonically active. In particular, the morphology of island-like metallic films on graphene enables the detection of mechanical activity using an optical modality.\textsuperscript{24} These composite materials are thus in principle capable of detecting mechanical activity through more than one sensing modality. For example, by reacting a film of silver nanoislands on graphene with a solution of benzene thiol, it was possible to use the resulting self-assembled monolayer of benzene thiolate as a reporter. That is, we reasoned that the small gaps between adjacent nanoislands would produce an intense electric field, which would substantially amplify the characteristic surface-enhanced Raman scattering (SERS) spectrum of benzene thiolate.\textsuperscript{10} A schematic diagram illustrating our hypothesis can be seen in Figure 4a. Given that the spacing between nanoislands could be modulated by strain, we hypothesized that the composite film would be an effective optical strain sensor. That is because the intensity of the electric field between adjacent nanoparticles is nonlinearly dependent on the spacing between particles, and the SERS intensity is nonlinearly dependent on the intensity of the electric field, the compounded nonlinear effects would lead to exceptional sensitivity to strain. Moreover, the electrical conductivity of the graphene would permit electrical stimulation of or measurement from structures (i.e., cells) with which it was in contact.

To investigate the possibility of simultaneous electrical stimulation with optical detection, we deposited a 2D monolayer of myoblast cells on a nanoisland/graphene film (Figure 4b). Although silver has the potential for cytotoxicity, the biocompatibility of the composite was assured by placing the silver on the bottom, with the cells adhered to the graphene. We applied a pulsed voltage to the cells through the graphene/silver film, which caused them to contract and thus pull the silver nanoislands apart around the periphery of the cells. Increased separation between individual silver nanoislands led to a decrease in plasmonic coupling and hence to a decreased signal, as seen in Figure 4c. This plasmonic behavior was not observed in electrically pulsed silver nanoisland substrates without cells. This control experiment confirmed that the effect was not based on an inherent piezoelectric effect in metal nanoislands that causes them to contract. The complementary modalities of electrical stimulation and optical sensing for cellular media could be useful in monitoring actutable cells, especially in a future high-throughput format.

**Detecting Particles and Cells in Microfluidic Channels by Deflection of the Sidewalls.** The high sensitivity of the graphene–metal composite films led us to investigate whether it would be possible to perform mechanically-based analyses of nonadherent cells as they flowed through microfluidic channels. That is, our calculations suggested that the transit of particles through microchannels in elastomeric slabs would deform the sidewalls, even if the diameter of the particle was smaller than the diameter of the channel. At the very least, the technique could permit the enumeration of flowing objects. Such a capability is useful for blood-based detection of cells of different sizes and possibly also for rapid screening of mechanical properties, e.g., circulating tumor cells. With this motivation, we embedded our graphene-based sensors in the sidewalls of microfluidic channels (Figure 4d).\textsuperscript{11} In our initial experiments, our device was able to detect the transit of bubbles, solid particles, and individual human-derived mesenchymal stem cells through a microfluidic channel by measuring the changes in electrical resistance that corresponded to the deflection of the sidewalls of the channel (Figure 4e and 4f). We note that lag due to the viscoelasticity of the channel walls could decrease the maximum frequency with which transiting particles could be counted. While at present, the device is only able to differentiate objects based on size, it might be possible to use theory to reconstruct the stiffness of the particles (or cells) as they transit based on the time-dependent deformation profile of the sidewalls.

**Understanding the Mechanism of Strain Detection.** We then sought to understand the piezoresistive mechanism(s) of these films. Our analysis was confounded at the outset by the fact that both metallic films and graphene exhibit a change in resistance under strain when used alone. For bulk metallic foils, the change in resistance is geometrical in origin, while piezoresistance in disconnected metallic particles has been shown to arise from tunneling in systems where the spacing between particles is especially small.\textsuperscript{25} Theoretical calculations on the electronic properties of single-layer graphene indicate that the increase in electrical resistance due to mechanical strain is due to scattering effects, stemming from random strain fluctuations in the film.\textsuperscript{26} Other simulation-based studies have also observed an opening in the band gap of graphene due to the break in sublattice symmetry when C–C bonds are elongated.\textsuperscript{27} Such calculations, however, assume pristine, single-crystal, and defect-free graphene, which is difficult to obtain on scales larger than hundreds of micrometers.\textsuperscript{28} Throughout our studies with...
graphene/metal films, we determined that the piezoresistive effect of single-layer graphene is amplified through the addition of metal adatoms on its surface.

The central feature of our investigation was the replacement of graphene with hexagonal boron nitride (hBN). A visual schematic of our approach can be seen in Figure 5. While graphene is a conductor and exhibits piezoresistance, hBN is an insulator and has no electrical response to strain. Thus, it would be possible to separate contributions of metal to the piezoresistance of the composite film from those of the graphene. We first compared the morphologies of these metal films on single-layer graphene and hexagonal boron nitride to gain insight into the influence of the 2D substrates on the morphology of the metal film being deposited. Through analysis of scanning and transmission electron microscopy (SEM and TEM) images, we were able to calculate the fractional coverage, extent of connectivity, and percolation thresholds as a function of nominal thickness of the metallic film on each of the 2D substrates.

After analyzing the morphology of the granular metallic films (Figure 5a), we measured the piezoresistive response of these films at ultralow strains for the different 2D substrates (Figure 5c). For the regime of small strains tested, our experiments demonstrated that films on hBN in which the metal was unpercolated produced open circuits and thus did not produce a response to strain. Thus, physical continuity of the metallic film on hBN was required. Films comprising metal on graphene, on the other hand, were capable of resolving strains ≥0.0001%, regardless of the degree of percolation in the metal film. While films in which the metal was unpercolated were both conductive and piezoresistive, we were not able to resolve these small mechanical strains with pristine single-layer graphene. While the reason for this transition is not clear, several changes in the graphene occur at the initial stages of metallization and could possibly affect the evolution in piezoresistive response with strain. As palladium is deposited onto graphene, the metal adatoms form palladium carbide bonds and etch pristine graphene, which degrades the conductivity of the film in the process. Additionally, the effect of mechanical deformation on the electronic structure of graphene is not clear. However, the addition of metal islands to graphene could produce an inhomogeneous strain field in the composite film, whose effect on the electromechanical behavior would be difficult to predict.

After characterizing the strain response, we measured the response of the films to temperature, as piezoresistive films that operate under a tunneling mechanism should exhibit a negative TCR. Instead, we observed a positive TCR in all the metal films on hBN (at least those that exhibited any conductivity), while the TCR of graphene/metal systems depended heavily on the degree of percolation of the metal. The reason for this dependence is that metals have a positive TCR and graphene has a negative one, and the TCR of the composite is thus determined by the relative amounts of the two materials. The results of these experiments point to the absence of tunneling as a dominant mechanism of either conductivity or piezoresistance in the films.

Our work in elucidating the mechanism of piezoresistance, however, does not explicitly consider electronic interactions between the metal and the 2D substrates or quantization of charge transport in the metallic films due to their extremely small thicknesses. Even with these recent advances, further work must be done to gain better insight into the fundamentals behind the optical, electronic, and mechanical properties that result from interactions between metal films and 2D substrates.

Figure 5. Elucidating mechanism of piezoresistive strain detection. (a) Transmission electron micrographs of disconnected and percolated films of palladium on single-layer graphene, with nominal thicknesses of 2 and 8 nm, respectively. (b) Schematic diagrams showing the electron path across the films of graphene/metal and hBN/metal films. (c) Cantilever apparatus for measuring the piezoresistive response of graphene/metal and hBN/metal films as they undergo step bending strains of 1 ppm (0.0001%). The magnitude of sensitivity is determined by calculating the gauge factor of the films at a chosen bending strain. Reproduced from ref 30.
traditional methods using first-principles mechanical and electronic simulations of graphene/metal composites could provide deeper fundamental understanding as to which physiochemical interactions enable the sensing capabilities of these materials.

■ CONCLUSIONS

This minireview summarized our recent work in developing strain gauges based on metallic nanoislands supported by single-layer graphene. The sensitivity and resolution of these devices have permitted us to explore applications in biomechanics and mechanobiology. We have shown that these films, which exhibit piezoresistive effects to strains as low as 0.0001%, are among the most sensitive and highest-resolution strain gauges reported. Moreover, in concert with stretchable conductive polymers, layered composites can be shown to combine high dynamic range to the already high sensitivity and resolution. In the context of biomechanical sensors, we have shown that machine learning has the potential to transform the raw output into actionable data. Our work to elucidate the mechanism of piezoresistance in the composite materials suggests that neither the graphene nor the metal alone is responsible for the performance of the devices, and that both materials are required. We believe that the development of a deeper understanding of the operational mechanisms of these interesting composite materials will lead to the development of more effective nanomaterial-enabled strain gauges in the future.

■ AUTHOR INFORMATION

Corresponding Author
Darren J. Lipomi — Department of NanoEngineering, University of California, San Diego, La Jolla, California 92093-0448, United States; orcid.org/0000-0002-5808-7765; Email: dlipomi@eng.ucsd.edu

Authors
Julian Ramirez — Department of NanoEngineering, University of California, San Diego, La Jolla, California 92093-0448, United States
Beril Polat — Department of NanoEngineering, University of California, San Diego, La Jolla, California 92093-0448, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c01967

Notes
The authors declare no competing financial interest.

Biographies

Julian Ramirez received his B.S. and his M.S. from UC San Diego in chemical engineering in 2015 and 2017, respectively. He received his Ph.D. degree in chemical engineering in 2020 from UC San Diego in the laboratory of Prof. Darren Lipomi. His main research interests are printed electronics, hybrid micro/nanofabrication, nanomaterial-based chemical and physical sensors, and mechanical reliability of micro/nano devices. He is a recipient of the National Science Foundation Graduate Research Fellowship.

Beril Polat is a Ph.D. student in the Chemical Engineering program in the Department of NanoEngineering at UC San Diego. She earned her Bachelor’s/Master’s degrees in Chemical and Biomolecular Engineering from Johns Hopkins University in 2016. Her current research focuses on the applications of metallic nanoislands on graphene films as wearable strain sensors for healthcare monitoring.

Darren J. Lipomi is a professor in the Department of NanoEngineering as well as programs in Chemical Engineering and Materials Science at UC San Diego. He earned his Ph.D. from Harvard University in 2010 in the laboratory of Prof. George M. Whitesides and was a postdoctoral fellow at Stanford University in the laboratory of Prof. Zhenan Bao from 2010 to 2012. His current research focuses on the mechanical properties of polymeric and nanostructured thin films for applications in energy and healthcare. He is a recipient of the NIH Director’s New Innovator Award and the Presidential Early Career Award for Scientists and Engineers.

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