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ABSTRACT

Acclaimed as one of the biggest scientific breakthroughs, the technology of CRISPR has brought significant improvement in the biotechnological spectrum—from editing genetic defects in diseases for gene therapy to modifying organisms for the production of biofuels. Since its inception, the CRISPR-Cas9 system has become easier and more versatile to use. Many variants have been found, giving the CRISPR toolkit a great range that includes the activation and repression of genes aside from the previously known knockout and knockin of genes. Here, in this Perspective, we describe efforts on automating the gene-editing workflow, with particular emphasis given on the use of microfluidic technology. We discuss how automation can address the limitations of gene-editing and how the marriage between microfluidics and gene-editing will expand the application space of CRISPR.

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INTRODUCTION

Genome engineering is traditionally performed by the delivery of exogenous genes into the cells with the hope that the cell will integrate the genes into its genome. With recent advances in gene-editing technologies, genome engineering is being revolutionized by the powerful CRISPR-Cas9 system replacing the first gene-editing tools such as zinc finger nucleases[1–4] and transcription activator-like effectors (TALENs).[5–8] This RNA-guided Cas9 system is derived from the adaptive immune system of bacteria and is now being used in almost all laboratories to genetically modify the genome in mammalian cells. CRISPR-Cas9 is short for clustered regularly interspaced short palindromic repeats, and CRISPR-associated protein 9 enables a site-specific double-stranded break at the target site that is followed by two natural DNA repair mechanisms of the cell: (1) non-homologous end joining (NHEJ) or via (2) homology directed repair (HDR) when a template DNA fragment is available. Both pathways allow for the cell to repair the breaks that can lead to permanent deletions, modifications, or insertions. Although NHEJ repair pathways are more efficient (and hence more frequent) in the cell, there are several new mechanisms that can improve HDR efficiency.[9–11] Regardless of the efficiency, the multi-functional capabilities of CRISPR are enabling scientists to perform unimaginable experiments that would have been impossible a few years ago—e.g., engineering living organisms with broad applications in the bio-industrial space ranging from treating inherited diseases to solving the bioenergy crisis by modifying agricultural gene crops for accelerated crop improvement—CRISPR has reshaped the ability to edit DNA in living cells.[12–24]

Since the discovery of Cas9, gene-editing using CRISPR is rapidly becoming more versatile and easier to use.[25–29] Recently, there are many other endonucleases orthologs (Cas12 and Cas13a) that have been discovered for a variety of applications such as class II Cas12a and Cas13 and class I cascade. Type V Cas12a contrasts from Cas9 by creating staggered cuts with a 5′ overhang at the DNA target site, thereby allowing for integration of orientation specific DNA sequences, which promotes HDR instead of NHEJ.[30] The other recently discovered type VI endonuclease is Cas13a that cleaves ssRNA.[31] As a result, Cas13a has been programmed to cleave target areas on mRNA in bacterial and eukaryotic cells.[32] Finally, type I cascade is a complex of multiple proteins that targets DNA and recruits the endonuclease Cas3. Cas3 creates single-stranded nicks as well as degrades the target DNA through its additional exonuclease function. Due to its commonality in nature,

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researchers are still looking into harnessing the exonuclease activity of Cas3 for novel applications with mammalian cells.[33]

Primarily known for its purpose to knockout or knockin genes, CRISPR is being redefined for genome regulation functions like CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa).[31,34,35] Targeted gene silencing or activation is achieved by using a nuclease-deficient Cas9 (or dCas9) to prevent double-stranded breaks while accurately binding to the target DNA with a synthetic guide RNA (sgRNA) and helping to recruit transcriptional factors for the desired regulation.[35] Aside from its functions, CRISPR is affecting the whole biotechnological spectrum, leading industrial and academic genetic efforts to discover genetic defects in diseases to create new therapeutics[36–38] to modifying organisms or cellular pathways to fulfill the biofuel's promise.[39–41] Despite the oft-hyped popularity, the steps to perform an effective gene edit for these projects are very time consuming, manually intensive, and laborious. The physical workflow of gene-editing consists of four steps: (1) to design guides that will target the gene of interest, (2) to deliver guides and the endonuclease to the cell, (3) to select single-cell clones, and (4) to expand a CRISPR-modified cell line—all of which can take at least two months to perform (without taking into account the attempts needed for a successful edit).[42–44] The manual handling of these four steps are extremely inefficient due to the constant pipetting and the transferring of cells and reagents to multiple platforms. As a consequence, the inefficiency leads to inconsistencies in the editing and in obtaining single-cell clones through a dilution series. These limitations have halted the scalability of this technology and scaled-back the engineering of custom cell lines. One solution that can provide a "hands-off" workflow and the scalability for gene-editing is microfluidics. This technology is modular, enabling easy integration with automation capabilities for high-throughput and single-cell selection studies.[39,45–47] The low-volume and inexpensive "lab-on-a-chip" can culminate different operations (sensing, detecting, sorting, etc.) on one or several devices to provide exceptional opportunity in achieving a fully, self-driving gene-editing system.

The combination of automation, gene-editing, and microfluidics are mainly focused on improving the efficiency of delivering the gene-editing machinery into the cells,[43,48–51] but there is lack of emphasis on the rest of the gene-editing workflow, namely, processes like validation, isolation, and expansion of a single viable edited clone since there is a high-level of control required for these steps. This is particularly true for larger scale experiments and, therefore, the need for automation becomes more prominent as can be seen by collaboration between industries (e.g., Sphere Fluidics, Tecan) and biofoundries worldwide[24,52] to automate these complex gene-editing workflows. In this Perspective, we will describe current automation approaches toward implementing a "hands-off" workflow with a focus on current microfluidic technologies that are used to miniaturize and to automate the gene-editing workflow (see Table I for a summary of microfluidic devices used for the gene-editing workflow). We will describe the future of automation and gene-editing and how the field is moving toward a next-generation gene-editing platform with the use of microfluidics. Finally, we will give future perspectives on how other fields (machine learning, diagnostics, and optogenetics) will be affected by automating the gene-editing workflow.

STEP 1: DESIGN OF SYNTHETIC GUIDE RNA (sgRNA)

The design of custom synthetic guide RNAs has already become quick work for researchers—using computational tools developed by large--scale companies like Synthego, Benchling, and IDT Technologies. These companies use empirical data based on previous large-scale studies done with genome-wide libraries for genetic screens. These studies helped in understanding the reasoning (and prediction) to Cas9 off-target activity in accordance with the sgRNA sequence and experimental conditions.[53–56] Thus, the computational tools are created to design guides with the highest predictive on-target activity and with the lowest off-target effects, allowing the researcher to pre-select sgRNAs that are deemed to be most effective for their editing experiments. The design is a critical step as less effective sgRNAs can lead to undesired outcomes such as off-target mutagenesis,[56] especially in research with cancer cell lines that have an aneuploidy nature.[57] As shown in the study by Yuen *et al.*, different sgRNAs with different efficiency ranges were used against a target with known variant copy number. They show that the knockout efficiency is independent to target gene copy number; however, less effective sgRNAs are more sensitive when they are used against a target that is high copy. This is due to the disposition of higher copy targets to allow partially knocked out cells to increase in amount while diminishing the portion of completely knocked out cells.[58] Alongside with the guide design, some companies such as Synthego developed their own kits for applying the gene-editing system without the need for researchers to focus on the logistics of an experiment but rather on the experiments' outcome.[59] Together, these tools provide a more streamlined approach for researchers to construct their gene target strategy.

STEP 2: DELIVERY OF Cas9 AND sgRNAs

Given the capability of microfluidics to generate multiple droplets at a very small scale (pl to nl range) and to have the ability to manipulate the cells at a single level, this technology has been exploited for the delivery of gene-editing machinery into cells (Fig. 1). Microfluidics provide a microscale environment that allows for many advantages such as lower reagent volumes, mass number of culture chambers for high-throughput studies, and an increased spatiotemporal control of target cells. The study by Sinha *et al.* is one such work that focused on automating gene-editing using digital microfluidics by knocking out a stably expressing reporter gene in a small lung carcinoma cell line as a proof-of-principle and then targeted an oncogene in the same cell line using an image-based analysis technique to analyze the edited cells.[60] Another group, Sharei *et al.*, have designed a device with microchannels causing deformation of cells passing through which allowed for transient membrane disruptions for the diffusive intracellular uptake of exogenous material.[48] Their work, termed as "cell squeezing," has been further explored with the engineering of primary cells to show that this operation of cell squeezing delivery compared to electroporation retained more similar gene expression profiles to untreated cells, therefore, showing that mechanical stress preserved cell functionality.[49] The group later established a company, Cell Squeeze® (SQZBiotech®),[61] to expand their delivery approach on cell-based therapies.

A similar work was published by Han *et al.* that involved cell mechanical deformation and applying stress on the cell membrane

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TABLE I. Microfluidic studies involved in or potentially used for gene-editing.

	Reference	Type of device	Purpose	Strength	Application
Delivery of Cas9 and sgRNAs	Han *et al.*[62]	Channel microfluidics	Plasmid and exogenous material delivery into the cells	High cell viability and high delivery efficiency of various macromolecules into different cell types	Shown for "hard-to-transfect" lymphoma cells and embryonic stem cells
	Sinha *et al.*[60]	Digital microfluidics	Automating gene-editing using digital microfluidics	Fully automated pipeline of CRISPR knockout on chip (i.e., culture, edit, image-based analysis)	Knocking out a stably expressing reporter and a RAF1 oncogene in a small lung carcinoma cell line
	Li *et al.*[66]	Droplet-based microfluidics	A lipoplex (cationic lipid-nucleic acid complex)-mediated single-cell transfection in a droplet-based microfluidic	Significant increase of gene delivery efficiency via single-cell transfection going from ~5% to ~50% in K562, THP-1, and Jurkat cells	Significant improvement in transfection efficiency for three suspension cell lines, i.e., K562, THP-1, and Jurkat
Validation and expansion of the gene-edited cells	Mocciaro *et al.*[42]	Optical electrowetting	Light-activated cell identification and sorting system	3500 "NanoPens" for selecting and expansion of mammalian cells	Single-cell manipulation, clonal expansion, and phenotypic analysis for primary T-cells in nanoliter volumes
	Samlali *et al.*[74]	Hybrid microfluidics (i.e., combination of droplet and digital microfluidics)	Selection, isolation, and expansion of successfully edited mammalian clones using a hybrid microfluidic system	Individual addressability of single cells; *in situ* encapsulation of single cells in droplets for direct expansion	On-demand selection, isolation, and expansion of gene-edited small lung carcinoma cells
Potential for gene-editing	Sharei *et al.*[48]	Channel microfluidics	Cell squeezing for the diffusive intracellular uptake of exogenous material	Non-traditional means of transfection—i.e., cell squeezing—minimally invasive	For the delivery of transcription factors toward cell-based therapeutics
	Madison *et al.*[67]	Digital microfluidics	Electroporation on a digital microfluidic platform	High on-chip transformation efficiency ($8.6 \pm 1.0 \times 10^8$ cfu μg^{-1})	Electroporation for bacterial transformation
	SEED Biosciences[73]	Robotics	Traceable single-cell isolation and detecting single cells using impedance profiling	An *in situ* based method for detecting the presence of single cells without fluorescence labeling	A pipetting robot for single-cell isolation and detection
	Hur *et al.*[65]	Channel microfluidics	Versatile intercellular delivery platform for hard-to-transfect primary stem and immune cells	High delivery efficiency (up to 98%), user-friendly (single step operation), and high scalability (1×10^6 cells/min)	Universal intracellular transfection platform for highly efficient transport of different biomolecules into hard-to-transfect primary cells

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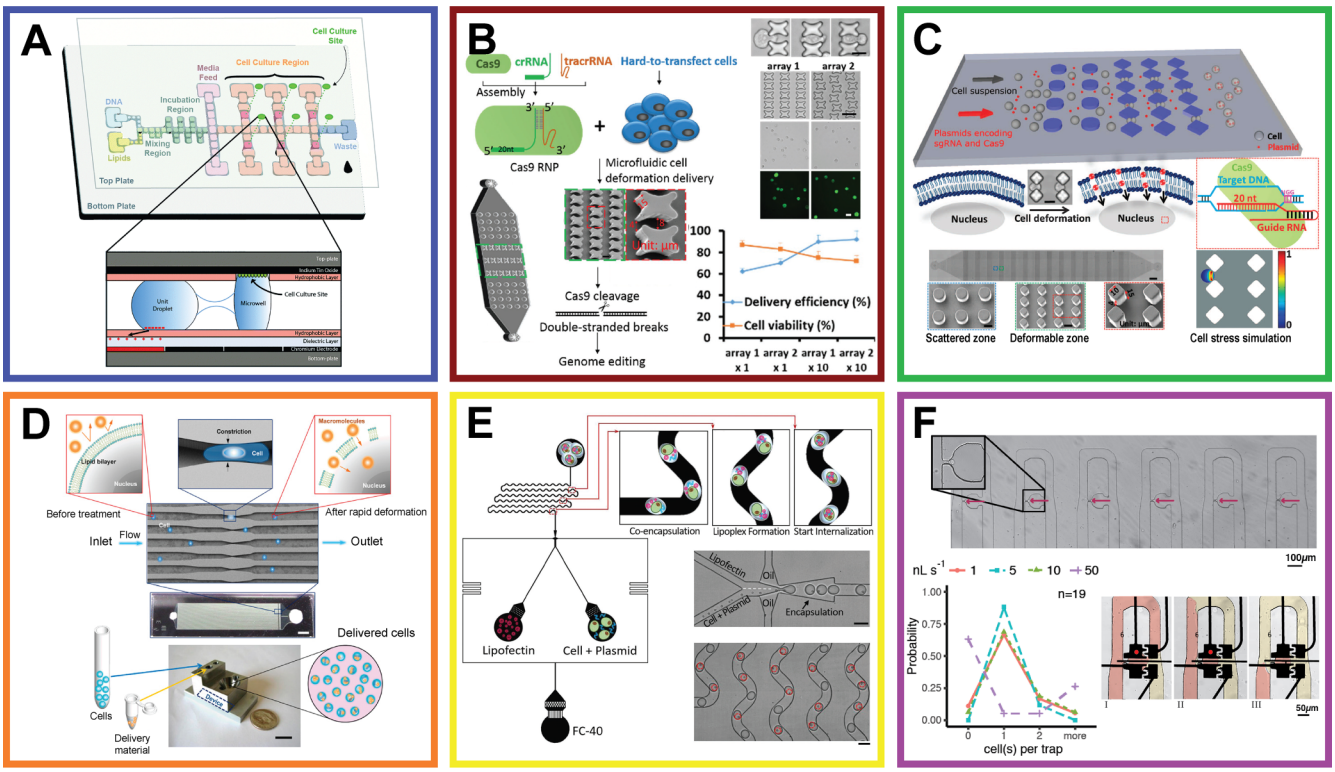

FIG. 1. Microfluidic devices used to automate gene-editing workflows. (a) A schematic of a digital microfluidic device showing the device's layout used for cell culturing, lipo-mediated transfection, gene-editing, and analysis at the culture sites. Side view schematic of a two-plate device with adherent cells on the top-plate. Workflow of the automated gene-editing pipeline executed on a digital microfluidic platform. Reproduced with permission from Sinha *et al*., Lab Chip **18**(15), 2300–2312 (2018). Copyright 2018 Royal Society of Chemistry. (b) Microfluidic device to induce mechanical deformation to hard-to-transfect cells as a delivery method for Cas9-sgRNA ribonucleoprotein complexes. Scanning electron microscopy of deformable zones with different array formatting. Graph with delivery efficiency and cell viability shown for the two array formats. Reproduced with permission from Han *et al*., Adv. Biosyst. **1**(1–2), 1600007 (2017). Copyright 2017 from John Wiley & Sons. Inc. (c) Delivery mechanism where a cell suspension is flowed through the device with deformable zones for microconstriction of the cells. Plasmids encoding sgRNAs and Cas9 protein are delivered into the cell after passing through the microconstriction area. Reproduced with permission from Han *et al*., Sci. Adv. **1**(7), e1500454 (2015). Copyright 2015 American Association for the Advancement of Science. (d) Channel microfluidic device containing constrictive channels, which generates transient membrane pores as a cell passes by, allowing for cytosolic delivery by diffusion. Reproduced with permission from DiTommaso *et al*., Proc. Natl. Acad. Sci. U.S.A. **115**(46), E10907–E10914 (2018), Copyright 2018 National Academy of Sciences. (e) Schematic image of a droplet microfluidics-based single-cell lipofection platform. Single cells are coencapsulated with plasmids (encoding the Cas9 protein and sgRNA) and lipofectin. Reproduced with permission from Li *et al*., Small **14**(40), e1802055 (2018), Copyright 2018 John Wiley & Sons Inc. (f) Integrated hybrid microfluidic device consisting of a digital microfluidic layer and a channel layer. The channel layer contains a trapping area to encapsulate gene-edited single cells *in-situ* with high efficiency (∼88%). Applied potentials to the electrodes (shown by the red dot) provide the capability to release or to keep the droplets containing the single cell. Reproduced with permission from Samlali *et al*., Small **16**(34), 2070190 (2020), Copyright 2020 John Wiley & Sons Inc.

via squeezing through diamond shaped microstructures on the chip to generate transient membrane pores for plasmid and exogenous material delivery into the cells. While maintaining high cell viability, they reported high delivery efficiency of various macromolecules into different cell types, including hard-to-transfect lymphoma cells and embryonic stem cells.[62,63] Later, the same group enhanced the delivery efficiency of the chip by modifying the constriction shape to a curved tunnel and extending the cell deformation time for Cas9 ribonucleoprotein (RNP) delivery.[64] Analogous to the cell mechanical deformation approach, Hur *et al.*[65] very recently developed a device that focused on getting the optimal mode of cell deformation to maximize intracellular delivery. Although not used for gene-editing, they designed a T-junction microchannel with a cavity that would allow elongating cells hydrodynamically with the help of inertial effects, bypassing irregular cell deformation. After hitting the cavity, the cells are extensively elongated due to the local downstream flow that stretches the cell from two sides in the T-junction channel, permitting the cells to stretch uniformly. Thus, these cell perturbations allow for very efficient delivery of various biomolecules (DNA, mRNA, siRNA), nanomaterials (quantum dots, large nanoparticles), and potentially gene-editing materials (Cas9, sgRNA) into hard-to-transfect primary and immune cells.

Li *et al.* presented a lipoplex (cationic lipid-nucleic acid complex)-mediated single-cell transfection method for hard-to-transfect suspension cells with droplet microfluidics. Chaotic advection via serpentine

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channels and co-encapsulation of single cells with plasmids and lipofectamine were described to induce transfection via endocytosis. They reported significant improvement in transfection efficiency for three suspension cell lines, i.e., K562, THP-1, and Jurkat, and efficiently targeted and knocked out the TP53BP1 gene for K562 cells via the CRISPR-Cas9.[66] Electroporation on a digital microfluidic platform is another approach taken by Madison *et al.*[67] for non-mammalian-based transformation (and potentially for gene-editing). They reported an on-chip transformation efficiency of $8.6 \pm 1.0 \times 10^8$ cfu μg^{-1}; however, the cell survival rate was shown to be very low ($1.5 \pm 0.3\%$) due to the excessive electropore accumulation, joule heating, and water electrolysis.[67]

The customary role of microfluidics in gene-editing is to use it for the delivery of CRISPR-Cas9 and exogenous material—an essential step toward automating gene-editing. However, many challenges still exist in this area, especially in terms of understanding the mechanisms of transfection since the commonly used transfection methods (lipofectamine and electroporation) have their own unique concerns.[68] There needs to be further exploration on the changes in gene expression that are caused by transfection[49] or integration of other types of technologies[68,69] such that a full assessment is performed to determine the causes of lower efficiency and viability of cells (especially hard-to-edit cell lines) to be able to use microfluidics for promising clinical applications downstream.[70]

STEP 3 AND 4: VALIDATION AND EXPANSION OF THE GENE-EDITED CELLS

The selection, validation, isolation, and expansion of edited cells remain a tedious task for researchers endeavoring into genome engineering projects. The approach of antibiotic selection or the use of a fluorescence marker for sorting with FACS is typically applied for selection of gene-edited cells. Detection of validated cells is crucial to verify if the desired edit is introduced to the gene of interest. Generally, this step can involve different strategies, from performing phenotypic (e.g., fluorescence microscopy) and genotypic assays (e.g., mismatch detection) to genomic sequencing to confirm for successful deletions or insertions or even disease modeling (using "organ-on-chip"[71]) to understand therapeutic efficacy of their targeted edits. Next, isolation and expansion steps require the use of a limited dilution technique or the operation of FACS to isolate single cells, which require the maintenance of many clones to ensure viable cellular expansions. Finally, genotyping of cell populations or edited clones requires Sanger or Next-Generation sequencing to relate the genotypical edits to phenotype.[72]

Thus, as detailed, these validation steps cannot easily be streamlined into one automated framework as the exact approach needed to validate the genetic modification will depend on the researcher's application. Nonetheless, many academic studies and companies are currently exploring different methodologies to simplify parts of the process through automation. In particular, SEED Biosciences, a Swiss start-up company, has built a pipetting robot, DispenCell, capable of detecting single cells using impedance profiling and uses a low-pressure pump to dispense single cells into a 96-well plate or a 384-well plate for traceable single-cell isolation.[73] To mitigate robotic costs and challenges of single-cell isolation, a recent study by Samlali *et al.*[74] aims to select for successfully edited mammalian clones, isolate, and expand them using an integrated droplet and digital-based microfluidic system. Their droplet-based microfluidic system allows for ordered manipulation of single cells and provides the controllability due to the digital microfluidics component of their device. Hence, using electrode actuation, droplet-containing single cells are placed into trapping chambers and can be released on demand for further maintenance and expansion. Aside from electrode-based actuation, Mocciaro *et al.* introduced a light-activated cell identification and sorting system that is based on a platform that has 3500 nanopens, which are traps for each edited clone. This device enables single-cell manipulation, clonal expansion, and phenotypic analysis in nanoliter volumes—a major step forward in terms of throughput and isolation.[42]

STEP N: TOWARD A NEXT-GENERATION STANDARDIZED GENOME ENGINEERING PLATFORM

Given the tremendous utility of automation for CRISPR-Cas9 gene-editing, a fully automated system that combines the four key procedural steps for gene-editing will offer the opportunity to reshape how we currently design, build, test, and learn gene-editing for different cell lines. The goals for a next-generation automation gene-editing platform are to be able to obtain high efficiency, to increase throughput, and to execute parallel experiments.

Introducing a large number of edits is intrinsically limited to manual and to low throughput gene-editing protocols. Large-scale genomic changes are either impossible or confined to single base edits, which translate to single desired knockouts (and possible off-target effects). Droplet-based microfluidic platforms are excellent for high-throughput experiments (e.g., screening[46,75,76] and gene profiling[77,78]) and are perfectly suited to be used for "pooled" gene-editing experiments[29] that will be integrated with Drop-seq[79] (or its derivatives[80,81]) platforms to validate the edits at a single-cell level. The bridge between these platforms can expedite the validation of a single selected clone. Another major concern when moving toward a next-generation automated genome engineering platform is the efficiency of knockouts and transfection. The number of cells containing the target edits drastically decreases at higher throughput.[82] Trying to optimize the design of synthetic guide RNAs,[55] concentrations of nucleases, and DNA constructs[61] to improve transfection efficiency are also tedious and are not standardized approaches to evaluate gene-editing.

These major challenges have been a driving force for industrial companies focused on gene-editing and synthetic biology to invest in collaborations and expand the field further through automation. Oxford Genetics, Sphere Fluidics, Twist Biosciences, and the University of Edinburgh have recently started a collaboration to develop an automation platform to perform gene-editing workflows. Their belief is that automating gene-editing workflows not only reduces the process time but also remarkably helps in generating reproducible and reliable data and enables scaling up the process. Other companies (e.g., Ginkgo, Zymergen, Inscripta, Synthego, Amyris, and Genomatica) and biofoundries all around the world[52] (e.g., Edinburgh Genome Foundry,[83] Illinois Biological Foundry for Advanced Biomanufacturing,[84,85] London DNA Foundry,[71] Singapore SynCTI Foundry,[65] U.S. DOE Agile BioFoundry,[86] and Concordia Genome Foundry[87]) have also invested on these approaches with the

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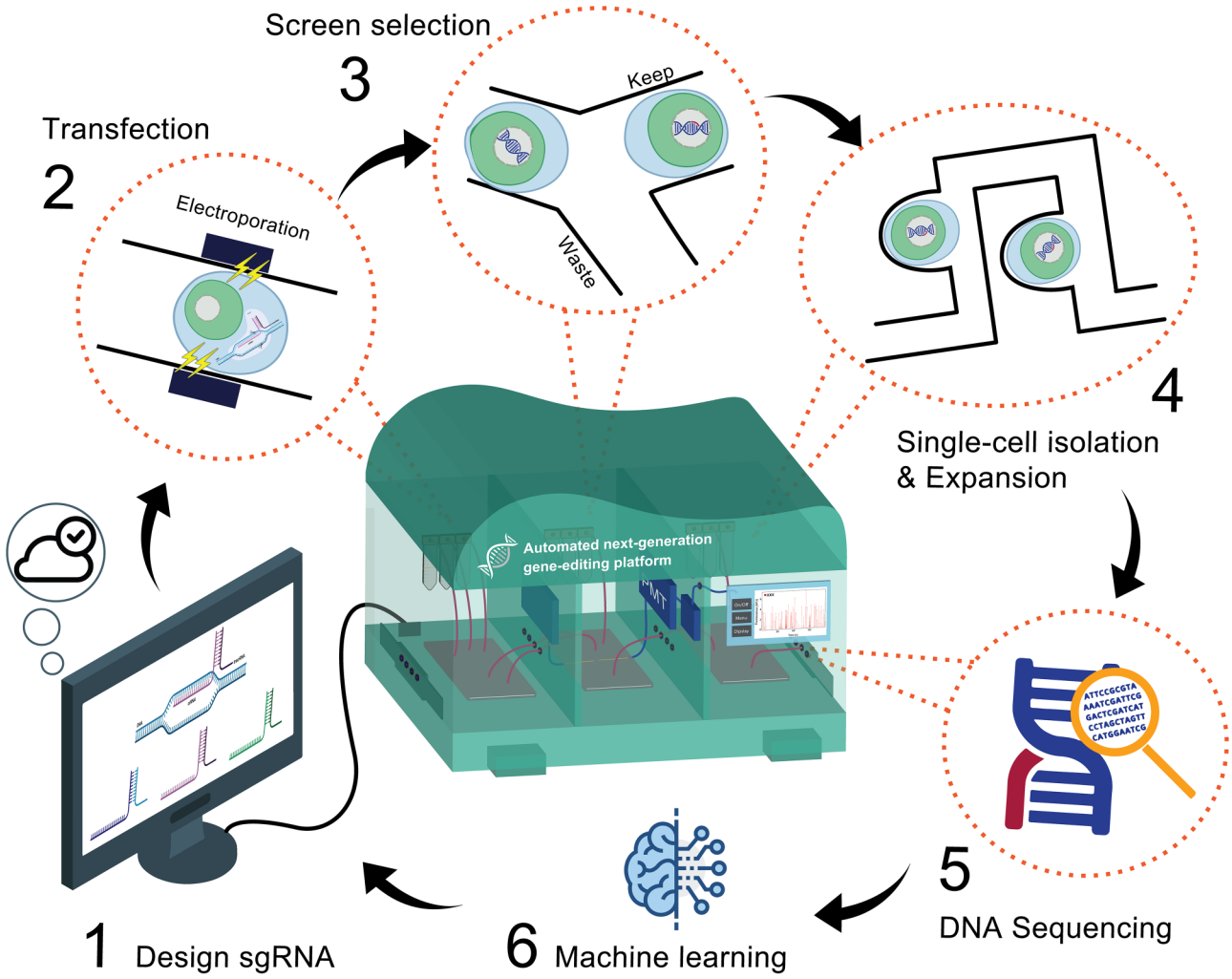

FIG. 2. Schematic of a visionary automated next-generation gene-editing platform that is combined with machine learning for an easy and hands-free workflow of CRISPR editing. The platform features: (1) cloud-based data for CRISPR design strategies and modular microfluidic devices that performs each step involved in the CRISPR pipeline, (2) CRISPR machinery for delivery, (3) screen selection, (4) single-cell isolation and expansion; (5) Sequencing is integrated for a rapid turnaround of results and (6) machine learning algorithms are incorporated to design and analyze the processes, which can inform future decisions in design.

goal to establish platforms enabling high-throughput assays and automating and standardizing the gene-editing protocol.

In our opinion, a promising advancement will be to develop a fully automated machine learning platform that is capable of running the whole pipeline of CRISPR gene-editing based on a standard data set and proper experimental designs (see our vision in Fig. 2). The ideal platform will possess cloud-based data for the design of CRISPR strategies (knockin, knockout, activation, and repression) integrated with a system that assembles modular device pieces for each step of the pipeline as described above (steps 1–4) and the capacity for a streamlined with feedback input/output performance. By doing so, the modularity will allow for the pipeline to be tailored to the researcher's application all while making the CRISPR workflow simple by allowing the researcher to input all the necessary components into the platform, which will produce an output of the edited and expanded cell line. For example, based on the researcher's desired method of delivery, there will be a choice between physical (i.e., electroporation) and chemical transfections (lipofection, calcium phosphate transfection, etc.) or viral transduction methods with the option to deliver CRISPR machinery as ribonucleoproteins or plasmids. Thus, this range of choices will permit for transient or stable expression of CRISPR components within easy to hard-to-transfect cell lines. Moreover, this next-generation automated platform is not limited to only to mammalian cells but is used with other organisms such as prokaryotic cells thanks to the low cost of fabrication for each modular microfluidic device. In this case, equipped with UV sterilization, cross-contamination can be completely avoided. For

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screening and selection, due to the modularity of the microfluidic devices, the incorporation of optical fibers will serve as a way for sorting cells like FACS or impedance sensing can be done to select for healthy cells vs dead cells when selecting with an antibiotic resistance. Finally, having integrated sequencing in the platform will produce results within a quick time frame for researchers to assess their data. The whole pipeline will be based on standard data set and experimental designs, and machine learning will continuously update the analytical model allowing researchers to observe in real-time if each step was successfully completed for a conclusive edit. Thus, this platform will provide an easy and hands-free workflow of CRISPR gene-editing for any researcher to use, giving everyone an opportunity to engineer new cells to serve the greater good without having to go through the struggles of optimizing each step for successful gene-editing.

FUTURE PERSPECTIVE

Looking forward, given the versatility and ease of use afforded by automation for gene-editing, we envision several routes for expanding CRISPR and its applications.

Machine learning and AI

There are a growing number of innovations combining machine learning with gene-editing techniques. For instance, using machine learning algorithm and big data analysis shows that the DNA repair process is, in fact, not random even if there is no template inserted into the cells after Cas9 DNA cut. Shen *et al.*[88] have analyzed a library of 2000 Cas9 guide RNAs paired with DNA target sites to develop a machine learning algorithm called inDelphi to predict repairs made to DNA snipped with Cas9. Using this machine learning algorithm, they report that 5%–11% of the guide RNAs used induced a single, predictable repair genotype in the human genome in more than 50% of editing products. In another work,[89] the FORECasT model (favored outcomes of repair events at Cas9 targets) shows similar results (based on a library 41 630 guide RNAs), which revealed that the majority of repairs are based on specific sequences that exist at the Cas9-cut site. Using those specified sequences on a Cas9-cut site, the algorithm automatically predicts the result of Cas9 gene-editing.

Similarly, there is immense potential in pairing high-throughput automated microfluidics and machine learning.[90–94] The idea is to use the microfluidic systems to not only automate the fluidics but to be able to derive a training data set that can be used by machine learning algorithms to predict answers that will rapidly help optimize the experiment. For example, in relation to gene-editing, there is potential on using microfluidics to perform the actual editing of the cells (i.e., delivery and knockout) under many different conditions. These samples can then be characterized across numerous parameters (e.g., type of delivery construct, and concentrations) to identify the most predictive parameters through machine learning techniques[95,96] that will increase expression efficiency and/or cell viability. The fast capabilities of machine learning can derive quick results and narrow the search space thereby reducing the number of trial-and-error experimental runs. Hence, insights from these techniques can derive a set of criteria that will enable efficient editing for future biological applications.

Diagnostics

In this time of COVID-19, next-generation diagnostics that apply CRISPR machinery would greatly aid in early disease detection and intervention. With respect to public health safety, these rapid diagnostic tests would reduce the spread of the disease in resource-poor settings and medical centers. For systematic and exact identification of infectious diseases, point-of-care tools should be precise, low in cost, and portable to use such that a laymen user can operate it to obtain a quick readout.[97]

A research group from the University of Freiburg has built a point-of-care biosensor microfluidic device to identify brain tumor microRNA markers (miR-19b and miR-20a) with the use of CRISPR-Cas13a. By combining Cas13a's dual RNase ability[98] of cleavage upon recognition of target RNA and cleavage of reporter RNA, the group has been able to detect the miRNAs without the need of nucleic acid amplification. The chip design consists of an immobilization area where samples are loaded and an electrochemical measurement cell, which assessed an amperometric signal that is inversely proportional to the concentration of target miRNA in samples. Within a total processing time of 4 h, their readout time is achieved within 9 min with a working volume of 0.6 μl (at a detection limit of 10 pM). The cost of device fabrication is less than 2 euros (~2.34 USD), thus making this biosensor an ideal alternative care for places with limited resources.[99]

A recent collaboration work between Binx Health Ltd. and Sherlock Biosciences have come together for the first CRISPR-based point-of-care test for COVID-19.[100] The SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) detection system was first reported by Gootenberg *et al.*, which is an *in vitro* nucleic acid detection platform that utilizes Cas13a for cleavage of a reporter RNA, producing a fluorescent signal for real-time detection of a target.[101] In the context of the specific SARS-CoV-2, the Sherlock™ system uses the described method to program the CRISPR machinery to uncover the presence of the coronavirus' genetic signature in samples collected from patients. The Binx *io* molecular platform involves a desktop instrument that can process a single-use cartridge with a capacity to multiplex up to 24 targets. Once the cartridge is loaded, the procedure is completely automated and produces a binary readout on screen. As Binx Health has the first platform to have FDA clearance for chlamydia and gonorrhea testing, its promising test for COVID-19 using the SHERLOCK detection system would require only an hour to yield results.[102]

In very recent work, Ramachandran *et al.*[103] have developed a microfluidic chip combined with on-chip electric field control for the rapid detection of SARS-CoV-2. Their device has the capability to carry out automatic nucleic acid extraction from raw nasopharyngeal swab samples and using electric fields to control CRISPR-Cas12 enzymatic activity when target nucleic acids are recognized. To extract nucleic acids from the samples, they use an electrokinetic microfluidic technique called isotachophoresis (ITP), which consists of a two-buffer system: a high-mobility leading electrolyte (LE) and a low-mobility trailing electrolyte (TE) buffer. By applying an electric field, the sample ions are sandwiched in between a LE-to-TE interface in a microchannel. This permits for the preconcentration, purification, mixing, and acceleration of reactions between reagents and samples. Thus, after nucleic acid extraction, using this technique,

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they continue to co-focus CRISPR-Cas12/gRNA, fluorescent reporters, and target nucleic acids together in an enzymatic assay, which grants a fluorescent readout after 30 min.

Optogenetics

Another major improvement done to CRISPR technology is the use of optogenetics for the precise control of CRISPR.[104–106] One of CRISPR's long-term challenge of off-target effects has been answered through this new application of a developed technique. By using modified light-sensitive RNA, Liu *et al.*[105] have been able to control Cas9 nuclease activation when stimulated with light that is non-phototoxic to cells. Furthermore, through the modification of the RNA, they have formed caged RNA (cgRNA), which as the name suggests forms a cage when hybridized with transactivating CRISPR RNA (tracrRNA). Thus, due to the steric hindrance produced by the cage formation, which blocks nuclease activation and full DNA unwinding, the Cas9-cgRNA complex cannot cleave the target DNA but can still bind to it. By stimulating the cells at specific wavelengths, the caging groups are withdrawn and the activated bound complex can then quickly cleave the target DNA. This whole process takes a few seconds to occur as opposed to hours for the normal current approaches; hence, the researchers have referred their method as very fast CRISPR (vfCRISPR).[105]

CONCLUSION

With the advent of this developed method, researchers gain access to a more precise spatiotemporal resolution for on-demand activation of the gene-editing machinery. An interesting inquiry to investigate further is to combine vfCRISPR and the RNA-targeting Cas13a as this will prove to be a powerful combination to apply in diagnostics. Additionally, microfabrication of devices with optogenetic technologies has been previously done.[107,108] Thus, with the sorting addressability of high-throughput production of droplets, automating the specific emission of wavelengths from optical fibers to edited target cells would grant a powerful platform for the detection of end result readouts. We believe that with the suggested next-generation automated gene-editing platform from this Perspective, the exploration of this idea would be rendered faster to achieve and easier to optimize. There are endless possibilities of studies to dive into with the platform: from looking into kinetics of CRISPR machinery at the single-cell level to novel rapid point-of-care diagnostics.

AUTHORS' CONTRIBUTIONS

F.A. and A.B.V.Q. are co-first authors.

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DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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