METHODS AND SYSTEMS FOR MICROFLUIDIC SCREENING

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/913,624, filed October 10, 2019, and U.S. Provisional Application No. 62/954,348, filed December 27, 2019, both of which are incorporated by reference herein in its entirety.

BACKGROUND

[0002] Drug development often requires a significant amount of testing and analysis to determine how specific chemical substances impact cellular and other biological components. As such, devices and specific methodologies that focus on correlating a relationship between specific chemical substances and biological components is an integral component for a drug developer, such as pharmaceutical companies.

SUMMARY

[0002a] In an aspect of the present disclosure, there is provided a method comprising:

(a) providing a plurality of encapsulations comprising:
  (i) a scaffold comprising an effector bound thereto via a cleavable linker, wherein the effector is releasable from the scaffold via cleavage of the cleavable linker;
  (ii) a nucleic acid encoding corresponding to and identifying the effector; and
  (iii) a cell;

(b) releasing the effector from the scaffold via cleavage of the cleavable linker, thereby generating a released effector ;

(c) incubating the released effector with the cell, thereby allowing the released effector to interact with the cell;

(d) detecting a signal from an encapsulation of the plurality of encapsulations, wherein the signal measures an activity or characteristic of the cell; and,

(e) based on the signal detected in (d), performing at least one of: sorting the effector, sorting the encapsulation, or adding a barcode to the nucleic acid encoding.

[0003] Provided herein are systems and methods for performing high-throughput assays using microfluidic systems and encoded effectors. The systems and methods described herein can be used to perform nearly any assay in a high-throughput manner and provide detailed information about the effect of various effector molecules on biological systems. The systems and methods

provided herein utilize encoded effectors, which allow a user to readily ascertain which of the effectors has an effect on a biological sample.

[0004] Other systems have various drawbacks, including an inability to customize the addition of reagents at concentrations of interest at different unit operations during a screen. The systems and methods described herein address these drawbacks. For example, methods and systems described herein allow for the introduction of reagents at specified concentrations at different steps in a screening procedure. In some instances, adding reagents at defined concentrations allows uniform doses of effectors to be administered across a library being screened. This may allow for decreased false positives in a screen because low potency but highly-loaded effectors may be dosed against samples at a uniform concentration across a library screen. In some instances, the customizable additions of reagents allow for facile deconvolution of screening hits without a step of physical sorting of effectors that elicit a positive or negative response in the screen.

[0005] In another example are methods of monitoring biological samples in a microfluidic based screen without utilizing light (e.g. fluorescence) emitted from a sample. These methods may allow

for more detailed information about a sample being analyzed than is available by other methods. Further provided herein are methods and systems for incorporating genetic or cellular information from a sample into the encoded effectors. This incorporation step can allow for an improved analysis of the response of a cell or other biological sample contacted with an effector than is available by other methods. In another example, information encoded in a sample, such as a DNA barcode, is incorporated from the sample into the encoding to allow determination of synergistic benefits of multiple effectors. This can be used for conducting a small-molecule fragment-based screen to generate compound leads.

[0006] The methods provided herein provide advantages over existing DNA encoded libraries being used for drug screening. In some examples, the methods enclosed herein are functional "activity-based" assays, not just "affinity-based" assays: they allows the screening of functional assays. In some examples, the methods herein are not limited to testing if a candidate drug binds to a disease target. In contrast, the methods herein may be capable of testing whether the candidate drug functions against that disease target. Such functions may comprise inhibition, disruption of protein-protein interactions, or activating an enzyme or allosteric pocket.

[0007] In some instances, the methods provided herein can screen in complex environments such as cell lysates, cells, or other multi-component mixtures in a single assay. In some examples, the functional activity test is orthogonal to all other components in a mixture and is specifically testing for functional activity of a target of interest. The screening modalities provided herein are diverse. Such modalities can screen for potency, selectivity, toxicity, liabilities, or other key metrics critical for drug discovery campaigns. The methods provided herein may allow for speed and diversity at 1000 times lower operational cost than other methods. In some instances, the speed, low reagent needs, and exceptional validation rates allow fast, iterative screening of potentially an unlimited set of chemically diverse compounds. The flexibility and speed allow for testing or screening of compounds in many different assays or formats for a single target, allowing multiple sampling of conditions, easy "restarts", fast "hit to lead" starts, and "immediate" validation of library designs.

[0008] In some instances, the methods provided herein do not require high sequencing depth, thus reducing costs for analysis. Additionally, the methods disclosed herein may allow for the quantification of yields of each chemistry step, allowing normalized dose-response curves and possibly quantitative analysis.

[0009] In some instances, the methods provided herein enable the use of DNA damaging chemistries that require organic solvents, or conditions that would otherwise be DNA damaging in the synthesis of encoded beads. For example, some chemistries needed to construct small-

molecules may degrade or cause DNA to become non-amplifiable and thus the DNA barcode information can no longer be read. In some instances, this challenge is overcome by providing DNA encodings bound to scaffolds at high levels. In some examples, the scaffolds comprise 10 million or more encodings bound to a scaffold. Additionally, in some examples, as few as 10 encodings are required to be present in order to detect a positive hit.

[0010] Provided herein are methods for cell phenotypic screening. Cells directly within droplets can be tested and probed for a variety of different phenotypes. For example, an entire library can be screened for toxicity against a particular cell type, or an entire library can be screened for its ability to affect a particular disease target in its native cell context, or an entire library can be screened for its ability to affect a panel of targets (transcriptome, protein panel, etc.). This is allowed because a small molecule can be liberated off of the bead where it can then penetrate intracellularly a cell (or affect an extracellular target) and affect a particular disease target

[0011] Further provided herein are methods for normalizing the results of screens of encoded effectors. Other methods of ascertaining the results from a screen suffer from high rates of false negative results, where an effector displays potency against a target sample, but due to damage to the encoding during the screen or low abundance of the encoding during the synthesis of the encoded effector, the "hit" is missed in the subsequent analysis. Provided herein are methods for normalizing the amount of encodings present after a screen has been performed in order to minimize false negative results due to low abundance of encodings of potent effectors.

[0012] Also provided herein are devices for performing the methods provided herein. In some instances, the method provided herein are performed on microfluidic devices or in microfluidic channels.

[0013] Further provided herein are devices useful for the performance of high-throughput screen using encoded libraries. These devices can allow for fixing a target sample, in some instances a single cell, in a fixed location in space with a single encoded effector. Such devices can allow for screening single compounds against cells to determine desired effects without the need to create *in situ* encapsulations separating each individual sample/effector combination.

[0014] Disclosed herein, in some examples is a method for screening an encoded effector, the method comprising: a) providing at least one cell and a scaffold in an encapsulation, wherein the scaffold comprises an encoded effector bound to the scaffold by a photocleavable linker and a nucleic acid encoding the effector; b) cleaving the photocleavable linker to release the encoded effector from the scaffold; and c) detecting a signal from the droplet, wherein the signal results from an interaction between the encoded effector and the at least one cell. In some examples, cleaving the photocleavable linker releases a pre-determined amount of the

encoded effector into the droplet. In some examples, the photocleavable linker is cleaved using electromagnetic radiation. In some examples, cleaving the photocleavable linker comprises exposing the encapsulation to a light from a light source. In some examples, the light intensity of the light is from about 0.01 J/cm^2 to about 200 J/cm^2. In some examples, the method further comprising the step of lysing the one or more cells. In some examples, the method further comprising providing an activating reagent to activate the photocleavable linker, so as to enable the photocleavable linker to be cleaved from the encoded effector.

[0015] Disclosed herein, in some examples, is a system for screening an encoded effector, the system comprising: a) one or more cells; b) a scaffold, wherein an encoded effector is bound to the scaffold by a cleavable linker, wherein a nucleic acid encoding the effector is bound to the scaffold; and c) a microfluidic device configured to: i) receive the one or more cells and scaffold; ii) encapsulate the one or more cells and scaffold within an encapsulation; iii) cleave the cleavable linker from the encoded effector to release a predetermined amount of the encoded effector within the encapsulation; iv) incubate the encoded effector with the one or more cells for a period of time; v) detect a signal from the encapsulation, wherein the signal results from an interaction between the encoded effector and one or more cells; and vi) sort the encapsulation based on the detection of the signal. In some examples, the cleavable linker is a photocleavable linker. In some examples, the microfluidic device further comprises a first collection tube and second collection tube for sorting the encapsulation, wherein the encapsulation is placed in 1) the first collection tube if the signal is at or above a predetermined threshold or 2) the second collection tube if the signal is below a predetermined threshold. In some examples, the system further comprising a waveform pulse generator to move the encapsulation to the first or second collection tube by an electrical field gradient, by sound, by a diaphragm, by modifying geometry of the microfluidic channel, or by changing the pressure of a microfluidic channel of the microfluidic device. In some examples, the signal is detected based on detecting morphological changes in the one or more cells measured by recording a series of images of the droplet or detecting fluorescence emitted by a molecular beacon or probe. In some examples, the period of time is controlled by residence time as the encapsulation travels through a microfluidic channel of the microfluidic device.

[0016] Disclosed herein, is a method for amplifying a primer to maximize cellular nucleic acid capture comprising: a) providing an encapsulation comprising a nucleic acid encoded scaffold with one or more cells, an amplification mix, and a nicking enzyme, wherein a nucleic acid encoding is bound to the nucleic acid encoded scaffold; b) lysing the one or more cells to release one or more cellular nucleic acids; c) nicking the nucleic acid encoding with the nicking enzyme, thereby creating an encoded nucleic acid primer; d) amplifying the encoded nucleic

acid primer via the nicking site and amplification mix; and e) labeling a released cellular nucleic acid with the encoded nucleic acid primer. In some examples, the specific site comprises a specific nucleotide sequence. In some examples, amplifying the encoded nucleic acid primer comprises 1) creating a copy of the nucleic acid encoding that extends from the nicking site, and 2) nicking the nucleic acid encoding copy to create another encoded nucleic acid primer. In some examples, amplifying the encoded nucleic acid primer comprises simultaneously 1) creating a copy of the nucleic acid encoding that extends from the nicking site, and 2) displacing the nucleic acid encoding copy to create another encoded nucleic acid primer. In some examples, the amplification mix comprises an amplification enzyme, such that the amplification enzyme enables for a copy of the nucleic acid encoding to be simultaneously created and displaced. In some examples, the amplification enzyme comprises a polymerase. In some examples, each nucleic acid encoding comprises a capture site that prescribes a target cellular coding or a target cellular nucleic acid to label a released cellular nucleic acid.

INCORPORATION BY REFERENCE

[0017] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0019] **FIG. 1** provides a depiction of a nucleic acid encoded effector bound to a bead along with the nucleic acid encoding.

[0020] **FIG. 2A** shows an exemplary workflow of a screen using a nucleic acid encoded effector bound to a bead.

[0021] **FIG. 2B** shows an exemplary workflow of a screen using a microfluidic device.

[0022] **FIG. 2C** shows an exemplary workflow for an encapsulation assay screen.

[0023] **FIG. 2D** shows an exemplary workflow for an encapsulation assay screen using pico-injection.

[0024] **FIG. 3** illustrates an exemplary method for amplifying a primer to maximize cellular nucleic acid capture.

CLAIMS

1. A method comprising:

(a) providing a plurality of encapsulations comprising:

(i) a scaffold comprising an effector bound thereto via a cleavable linker, wherein the effector is releasable from the scaffold via cleavage of the cleavable linker;

(ii) a nucleic acid encoding corresponding to and identifying the effector; and

(iii) a cell;

(b) releasing the effector from the scaffold via cleavage of the cleavable linker, thereby generating a released effector ;

(c) incubating the released effector with the cell, thereby allowing the released effector to interact with the cell;

(d) detecting a signal from an encapsulation of the plurality of encapsulations, wherein the signal measures an activity or characteristic of the cell; and,

(e) based on the signal detected in (d), performing at least one of: sorting the effector, sorting the encapsulation, or adding a barcode to the nucleic acid encoding.

2. The method of claim 1, wherein the effector and the nucleic acid encoding are both covalently bound to the scaffold, and wherein the effector and the nucleic acid encoding form an effector/encoding pair linked in space on the scaffold.

3. The method of claim 1 or 2, wherein the effector comprises an effector subunit, the nucleic acid encoding comprises a nucleic acid encoding subunit, and the nucleic acid encoding subunit corresponds with the effector subunit.

4. The method of any one of claims 1-3, wherein the scaffold is a bead, and the nucleic acid encoding further comprises a bead barcode corresponding to and identifying the bead.

5. The method of any one of claims 1-4, wherein the cleavable linker is cleavable by electromagnetic radiation.

6. The method of any one of claims 1-5, wherein the releasing of (b) releases a predetermined amount or dose of the released effector in the encapsulation.

7. The method of any one of claims 1-6, wherein the encapsulation comprises a droplet, a macrowell, a microwell, a picowell, or a microfluidic confinement.

8. The method of any one of claims 1-7, wherein the scaffold is selected from the group consisting of: a solid support, a bead, a fiber, a nanofibrous scaffold, a molecular cage, a dendrimer, and a multi-valent molecular assembly.

9. The method of any one of claims 1-8, wherein the scaffold is a bead with a diameter of at least 1 micrometer (μm).

10. The method of any one of claims 1-9, wherein the signal comprises electromagnetic radiation, thermal radiation or any combination thereof.

11. The method of any one of claims 1-10, further comprising measuring a property of the encoding to determine the structure of the effector.

12. The method of claim 11, wherein measuring a property of the nucleic encoding comprises sequencing.

13. The method of any one of claims 1-12, wherein the method comprises selectively adding the barcode to the encoding based on the signal, wherein the barcode comprises a primer sequence to allow for amplification of the encoding upon the addition of the barcode thereto.

14. The method of any one of claims 1-13, wherein (e) further comprises identifying a subset of the encapsulations as containing hits based on a threshold set for the signal detected in (d).

15. The method of claim 14, wherein the identifying comprises defining a threshold for the signal using a computer or algorithm.

16. The method of claim 14 or 15, wherein the method comprises sorting the hits, and wherein sorting comprises physically separating the hits in space.

17. The method of any one of claims 1-16, wherein sorting comprises by a waveform pulse generator, electrical field gradient, sound, a diaphragm, a valve, electrophoresis, electro-

osmosis, modifying geometry of the microfluidic channel, or changing the pressure of the microfluidic channel.

18. The method of any one of claims 1-17, wherein the signal is measured by performing an activity-based assay indicative of an activity of a target in presence of the interaction, and sorting is activity-based sorting.

19. The method of any one of claims 1-18, further comprising transferring one or more cellular nucleic acids from the cell to the nucleic acid encoding.

20. The method of claim 19, further comprising quantifying the cellular nucleic acids by sequencing the nucleic acid encoding after the cellular nucleic acids have been transferred.

21. The method of claim 19 or 20, wherein the cellular nucleic acids transferred to the nucleic acid encoding comprise a complementary sequence to a sequence on the nucleic acid encoding, and wherein the transferring comprises at least one of: annealing, ligating, chemically cross-linking, hybridization, amplifying the cellular nucleic acids on the nucleic acid encoding, or extending the nucleic acid encoding.