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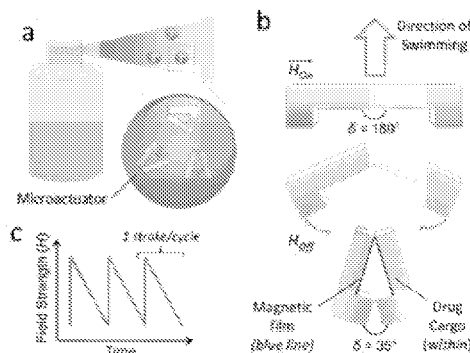
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(54) Title: MICROSCALE ROBOTS FOR DRUG DELIVERY THROUGH VISCOUS FLUIDS

Figure 1



(57) Abstract: The present invention is related to the field of microrobotics. In particular, microscale actuators are contemplated that are L-shaped microparticle dimers and configured to self-propel through non-Newtonian viscous fluids in a time-asymmetric cyclic magnetic field, thereby providing an improved therapeutic agent delivery platform. The microscale robots can be used to treat any condition associated with non-Newtonian fluids such as pulmonary conditions including, but not limited to chronic obstructive pulmonary disease, cystic fibrosis and/or viral infections.



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Microscale Robots For Drug Delivery Through Viscous Fluids

Field Of The Invention

The present invention is related to the field of microrobotics. In particular, microscale robots are contemplated that propel themselves through non-Newtonian viscous fluid barriers. This capability provides therapeutic agent delivery to previously inaccessible tissues. For example, the microscale robots can be used to treat pulmonary conditions including, but not limited to chronic obstructive pulmonary disease, cystic fibrosis and/or viral infections by delivering therapeutic agents at close proximity to the diseased tissues.

Background Of The Invention

Over the past decade, there has been much progress in the fields of microscale robotics and active particles. Such active devices are capable of self-propulsion and represent a promising platform for the design of delivery vehicles that navigate through biological barriers. Ghosh et al., “Active matter therapeutics” Nano Today 31:100836 (2020); and Li et al., “Micro/nanorobots for biomedicine: Delivery, surgery, sensing, and detoxification” Sci. Robot. 2:eaam6431 (2017).

What is needed in the art are microscale actuators that penetrate non-Newtonian fluids such as biological mucus. Such devices encapsulate and deliver therapeutic agents, including but not limited to drugs, nucleic acids, and other therapeutics.

Summary Of The Invention

The present invention is related to the field of microrobotics. In particular, microscale robots are contemplated that propel themselves through non-Newtonian viscous fluid barriers. This capability provides therapeutic agent delivery to previously inaccessible tissues. For example, the microscale robots can be used to treat pulmonary conditions including, but not limited to chronic obstructive pulmonary disease, cystic fibrosis and/or viral infections by delivering therapeutic agents at close proximity to the diseased tissues.

In one embodiment, the present invention contemplates an L-shaped microparticle dimer and a dipole-dipole hinge, wherein each L-shaped microparticle comprises a first arm comprising a dipole and a second arm that is shorter than the first arm. In one embodiment, the dipole-

dipole hinge links the first arm of the each L-shaped microparticle. In one embodiment, the second arm of each L-shaped microparticle is oriented in the same direction. In one embodiment, the side surface of the first arm comprises a magnetic metal patch. In one embodiment, the magnetic patch is a ferromagnetic metal patch. In one embodiment, the first arm has a terminus, the terminus comprising the dipole. In one embodiment, the plurality of ferromagnetic metal patches comprises a metal including, but not limited to, iron, cobalt, nickel and other ferromagnetic alloys. In one embodiment, the L-shaped microparticle dimer encapsulates a therapeutic drug. In one embodiment, the encapsulated therapeutic drug is releasable. In one embodiment, the surface of the L-shaped microparticle is coated with a therapeutic drug.

In one embodiment, the present invention contemplates a method, comprising: a) providing; i) a first L-shaped microparticle comprising a first arm and a second arm that is shorter than the first arm; ii) a second L-shaped microparticle comprising a third arm and a fourth arm that is shorter than the third arm; iii) a magnetic metal; and iv) a magnetic field; b) coating a side surface of the first and third arms of the first and second L-shaped microparticles with the magnetic metal to create a plurality of magnetic metal patches; c) exposing the first and second L-shaped microparticles to the magnetic field to induce a dipole in each of the first and third arms; d) linking the first and second L-shaped microparticles with the dipoles to create an L-shaped microparticle dimer comprising a dipole-dipole hinge, such that the second and fourth arms are oriented orthogonal to the direction of the magnetic field. In one embodiment, the coating further comprises a ferrofluid. In one embodiment, the plurality of magnetic metal patches are ferromagnetic metal patches. In one embodiment, the coating comprises photolithography. In one embodiment, the photolithography is patterned. In one embodiment, the coating comprises GLAD. In one embodiment, the coating comprises resist lift-off. In one embodiment, the linking comprises self-limiting assembly. In one embodiment, the ferromagnetic metal patch comprises a metal including, but not limited to, iron, cobalt, nickel and other ferromagnetic alloys. In one embodiment, the first and second L-shaped microparticles encapsulate a therapeutic drug. In one embodiment, the encapsulated therapeutic drug is releasable. In one embodiment, the surface of the L-shaped microparticle is coated with a therapeutic drug.

In one embodiment, the present invention contemplates a method, comprising: a) providing; i) a microscale actuator comprising an L-shaped microparticle dimer and a therapeutic compound; ii) a patient comprising at least one symptom of a disorder or disease comprising a non-Newtonian fluid; and iii) a cyclic magnetic field; b) administering the
5 microscale actuator to the patient; and c) exposing the patient to the cyclic magnetic field wherein the at least one symptom of the disorder or disease is reduced. In one embodiment, the microscale actuator self-propels through the non-Newtonian fluid. In one embodiment, the self-propulsion comprises a time-asymmetric motion of the L-shaped microparticle dimer induced by the cyclic magnetic field. In one embodiment, the non-Newtonian fluid comprises a thick
10 mucus. In one embodiment, the non-Newtonian fluid comprises blood. In one embodiment, the non-Newtonian fluid comprises vitreous humor. In one embodiment, the disease or disorder comprises a pulmonary disorder or disease. In one embodiment, the pulmonary disorder or disease includes, but is not limited to cystic fibrosis, chronic obstructive pulmonary disorder and/or chronic bronchitis. In one embodiment, the disease or disorder comprises a
15 gastrointestinal disease or disorder. In one embodiment, the gastrointestinal disease or disorder is a digestive disease or disorder. In one embodiment, the gastrointestinal disease or disorder is structural. In one embodiment, the exposing further comprises releasing the therapeutic compound from the microscale actuator. In one embodiment, the therapeutic compound is a drug. In one embodiment, the drug is a small organic molecule. In one embodiment, the drug is
20 salbutamol. In one embodiment, the drug is a steroid. In one embodiment, the drug is an antibacterial drug. In one embodiment, the therapeutic compound is a nucleic acid. In one embodiment, the nucleic acid is antisense. In one embodiment, the antisense includes, but is not limited to, siRNA, shRNA and/or miRNA. In one embodiment, the therapeutic compound is a polypeptide. In one embodiment, the polypeptide is an antibody. In one embodiment, the
25 antibody is a monoclonal antibody. In one embodiment, the L-shaped microparticle dimer comprises a plurality of magnetic metal patches and a dipole-dipole hinge, wherein each L-shaped microparticle comprises a first arm comprising a dipole and a second arm that is shorter than the first arm. In one embodiment, the dipole hinge comprises a dipole-dipole interaction. In one embodiment, the polypeptide is a cytokine. In one embodiment, the therapeutic drug is
30 encapsulated within said microscale actuator. In one embodiment, the therapeutic drug is coated

on the surface of said microscale actuator. In one embodiment, the therapeutic drug is releaseable,

Definitions

5 To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as “a”, “an” and “the” are not intended to refer to only a singular entity but also plural entities and also includes the general class of which a specific example may be used for illustration. The terminology herein is used to describe
10 specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

The term “about” or “approximately” as used herein, in the context of any of any assay measurements refers to +/- 5% of a given measurement.

15 The term “microscale actuator” as used herein, refers to a microparticle assembly having a geometric design including, but not limited to a dimer, a chain, or a colloid, having sufficient flexibility to undergo reciprocal motion thereby providing self-propulsion.

The term “L-shaped microparticle” as used herein, refers to a microparticle containing an approximate 90° angle, wherein one side extends into an arm that is equal to or longer than the other arm.

20 The term “dimer” as used herein, refers to a composition comprising a linkage between two identical components. For example, an L-shaped microparticle dimer is composed of a linkage between two L-shaped microparticles held together with a dipole-dipole interaction.

The term “non-Newtonian” as used herein, refers to a material that does not follow Newton's law of viscosity, which states that viscosity is independent of applied shear stress. In
25 non-Newtonian fluids, viscosity can change when under force to either more liquid-like or more solid-like. Ketchup, for example, becomes runnier when shaken and is thus a non-Newtonian fluid. Many salt solutions and molten polymers are non-Newtonian fluids, as are many commonly found substances such as custard, toothpaste, starch suspensions, corn starch, paint, blood, melted butter, and shampoo. Biological examples of non-Newtonian fluids include, but
30 not limited to biological mucus, blood, vitreous humor, synthetic mucus, weak hydrogels, and/or xanthan gum.

The term “time-asymmetric actuation” as used herein, refers to a time duration difference when comparing the opening and closing time of the two respective arms of a microscale actuator. In particular, a microscale device may exhibit a slow open/fast close actuation or a fast open/slow close actuation. For example, i) a slow opening is defined as one that is driven by 0 to 15 mT over 5 seconds; ii) a fast closing is defined as one that is driven by 15 to 0 mT over 1 second; iii) a fast opening is defined as a one that is driven by 0 to 15 mT over 1 second; and iv) a slow closing is defined as one that is driven by 15 to 0 mT over 5 seconds.

The term “suspected of having”, as used herein, refers a medical condition or set of medical conditions (e.g., preliminary symptoms) exhibited by a patient that is insufficient to provide a differential diagnosis. Nonetheless, the exhibited condition(s) would justify further testing (e.g., autoantibody testing) to obtain further information on which to base a diagnosis.

The term “effective amount” as used herein, refers to a particular amount of a pharmaceutical composition comprising a therapeutic agent that achieves a clinically beneficial result (i.e., for example, a reduction of symptoms). Toxicity and therapeutic efficacy of such compositions can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The term “symptom”, as used herein, refers to any subjective or objective evidence of disease or physical disturbance observed by the patient. For example, subjective evidence is usually based upon patient self-reporting and may include, but is not limited to, pain, headache, visual disturbances, nausea and/or vomiting. Alternatively, objective evidence is usually a result of medical testing including, but not limited to, body temperature, complete blood count, lipid panels, thyroid panels, blood pressure, heart rate, electrocardiogram, tissue and/or body imaging scans.

The term “disease” or “medical condition”, as used herein, refers to any impairment of the normal state of the living animal or plant body or one of its parts that interrupts or modifies the performance of the vital functions. Typically manifested by distinguishing signs and symptoms, it is usually a response to: i) environmental factors (such as malnutrition, industrial hazards, or climate); ii) specific infective agents (such as worms, bacteria, or viruses); iii) inherent defects of the organism (such as genetic anomalies); and/or iv) combinations of these factors.

The terms "reduce," "inhibit," "diminish," "suppress," "decrease," “prevent” and grammatical equivalents (including “lower,” “smaller,” etc.) when in reference to the expression of any symptom in an untreated subject relative to a treated subject, mean that the quantity and/or magnitude of the symptoms in the treated subject is lower than in the untreated subject by any amount that is recognized as clinically relevant by any medically trained personnel. In one embodiment, the quantity and/or magnitude of the symptoms in the treated subject is at least 10% lower than, at least 25% lower than, at least 50% lower than, at least 75% lower than, and/or at least 90% lower than the quantity and/or magnitude of the symptoms in the untreated subject.

The term “fibrosis” as used herein, refers to any medical condition marked by increase of interstitial fibrous tissue. For example, “pulmonary fibrosis” is characterized by a scarring or thickening of the lungs.

The term “inhibitory compound” as used herein, refers to any compound capable of interacting with (i.e., for example, attaching, binding etc.) to a binding partner under conditions such that the binding partner becomes unresponsive to its natural ligands. Inhibitory compounds may include, but are not limited to, small organic molecules, antibodies, and proteins/peptides.

The term “pulmonary injury” as used herein, refers to any effect on pulmonary tissue that impairs its functional or structural integrity. For example, injury may be a result of, but not limited to, inhalation of toxins, surgical procedures, or accident.

The term “therapeutic agent”, "drug" or "compound" as used herein, refers to any pharmacologically active substance capable of being administered which achieves a desired effect. Drugs or compounds can be synthetic or naturally occurring, non-peptide macromolecules, proteins or peptides, oligonucleotides or nucleotides, polysaccharides or sugars.

The term "administered" or "administering", as used herein, refers to any method of providing a composition to a patient such that the composition has its intended effect on the patient. An exemplary method of administering is by a direct mechanism such as, local tissue administration (*i.e.*, for example, extravascular placement), oral ingestion, transdermal patch, topical, inhalation, suppository *etc.*

The term "patient" or "subject", as used herein, is a human or animal and need not be hospitalized. For example, out-patients or persons in nursing homes are "patients." A patient may comprise any age of a human or non-human animal and therefore includes both adult and juveniles (*i.e.*, children). It is not intended that the term "patient" connote a need for medical treatment, therefore, a patient may voluntarily or involuntarily be part of experimentation whether clinical or in support of basic science studies.

The term "pharmaceutically" or "pharmacologically acceptable", as used herein, refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human.

The term, "pharmaceutically acceptable carrier", as used herein, includes any and all solvents, or a dispersion medium including, but not limited to, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils, coatings, isotonic and absorption delaying agents, liposomes, commercially available cleansers, polymeric micro- and nanoparticles, and the like. Supplementary bioactive ingredients also can be incorporated into such carriers.

The term "biocompatible", as used herein, refers to any material that does not elicit a substantial detrimental response in the host. There is always concern, when a foreign object is introduced into a living body, that the object will induce an immune reaction, such as an inflammatory response that will have negative effects on the host. In the context of this invention, biocompatibility is evaluated according to the application for which it was designed: for example; a bandage is regarded a biocompatible with the skin, whereas an implanted medical device is regarded as biocompatible with the internal tissues of the body. Preferably, biocompatible materials include, but are not limited to, biodegradable and biostable materials.

The term "biodegradable" as used herein, refers to any material that can be acted upon biochemically by living cells or organisms, or processes thereof, including water, and broken down into lower molecular weight products such that the molecular structure has been altered.

The term "bioerodible" as used herein, refers to any material that is mechanically worn away from a surface to which it is attached without generating any long term inflammatory effects such that the molecular structure has not been altered. In one sense, bioerosion represents the final stages of "biodegradation" wherein stable low molecular weight products undergo a final dissolution.

The term "bioresorbable" as used herein, refers to any material that is assimilated into or across bodily tissues. The bioresorption process may utilize both biodegradation and/or bioerosion.

The term "biostable" as used herein, refers to any material that remains within a physiological environment for an intended duration resulting in a medically beneficial effect.

The term "small organic molecule" as used herein, refers to any molecule of a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e.g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size from approximately 10 Da up to about 5000 Da, more preferably up to 2000 Da, and most preferably up to about 1000 Da.

The terms "amino acid sequence" and "polypeptide sequence" as used herein, are interchangeable and to refer to a sequence of amino acids.

The term "portion" when used in reference to a nucleotide sequence refers to fragments of that nucleotide sequence. The fragments may range in size from 5 nucleotide residues to the entire nucleotide sequence minus one nucleic acid residue. When used in reference to an amino acid sequence "portion" refers to fragments of that amino acid sequence. The fragment may range in size from 2 amino acid residues to the entire amino acid sequence minus one amino acid residue.

"Nucleic acid sequence" and "nucleotide sequence" as used herein refer to an oligonucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

The term "an isolated nucleic acid", as used herein, refers to any nucleic acid molecule that has been removed from its natural state (e.g., removed from a cell and is, in a preferred embodiment, free of other genomic nucleic acids).

As used herein, the term "antisense" is used in reference to RNA sequences which are complementary to a specific RNA sequence (e.g., mRNA). Antisense RNA may be produced by any method, including synthesis by splicing the gene(s) of interest in a reverse orientation to a viral promoter which permits the synthesis of a coding strand. Once introduced into a cell, this transcribed strand combines with natural mRNA produced by the cell to form duplexes. These duplexes then block either the further transcription of the mRNA or its translation. In this manner, mutant phenotypes may be generated. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. The designation (-) (i.e., "negative") is sometimes used in reference to the antisense strand, with the designation (+) sometimes used in reference to the sense (i.e., "positive") strand.

As used herein, the terms "siRNA" refers to either small interfering RNA, short interfering RNA, or silencing RNA. Generally, siRNA comprises a class of double-stranded RNA molecules, approximately 20-25 nucleotides in length. Most notably, siRNA is involved in RNA interference (RNAi) pathways and/or RNAi-related pathways. wherein the compounds interfere with gene expression.

As used herein, the term "shRNA" refers to any small hairpin RNA or short hairpin RNA. Although it is not necessary to understand the mechanism of an invention, it is believed that any sequence of RNA that makes a tight hairpin turn can be used to silence gene expression via RNA interference. Typically, shRNA uses a vector stably introduced into a cell genome and is constitutively expressed by a compatible promoter. The shRNA hairpin structure may also be cleaved into siRNA, which may then become bound to the RNA-induced silencing complex (RISC). This complex binds to and cleaves mRNAs which match the siRNA that is bound to it.

As used herein, the term "microRNA", "miRNA", or "µRNA" refers to any single-stranded RNA molecules of approximately 21-23 nucleotides in length, which regulate gene expression. miRNAs may be encoded by genes from whose DNA they are transcribed but miRNAs are not translated into protein (i.e. they are non-coding RNAs). Each primary transcript (a pri-miRNA) is processed into a short stem-loop structure called a pre-miRNA and finally into a functional miRNA. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to down-regulate gene expression.

The term "sample" or "biopsy" as used herein is used in its broadest sense and includes environmental and biological samples. Environmental samples include material from the environment such as soil and water. Biological samples may be animal, including, human, fluid (e.g., blood, plasma and serum), solid (e.g., stool), tissue, liquid foods (e.g., milk), and solid
5 foods (e.g., vegetables). For example, a pulmonary sample may be collected by bronchoalveolar lavage (BAL) which comprises fluid and cells derived from lung tissues. A biological sample may comprise a cell, tissue extract, body fluid, chromosomes or extrachromosomal elements isolated from a cell, genomic DNA (in solution or bound to a solid support such as for Southern blot analysis), RNA (in solution or bound to a solid support such as for Northern blot analysis),
10 cDNA (in solution or bound to a solid support) and the like.

The term "biologically active" refers to any molecule having structural, regulatory or biochemical functions. For example, biological activity may be determined, for example, by restoration of wild-type growth in cells lacking protein activity. Cells lacking protein activity may be produced by many methods (i.e., for example, point mutation and frame-shift mutation).
15 Complementation is achieved by transfecting cells which lack protein activity with an expression vector which expresses the protein, a derivative thereof, or a portion thereof.

The term "immunologically active" defines the capability of a natural, recombinant or synthetic peptide, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and/or to bind with specific antibodies.

20 The term "antibody" refers to immunoglobulin evoked in animals by an immunogen (antigen). It is desired that the antibody demonstrates specificity to epitopes contained in the immunogen. The term "polyclonal antibody" refers to immunoglobulin produced from more than a single clone of plasma cells; in contrast "monoclonal antibody" refers to immunoglobulin produced from a single clone of plasma cells.

25 As used herein, the term "coding region" when used in reference to a structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of a mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3' side by one of the three triplets which specify stop codons (i.e., TAA, TAG, TGA).

As used herein, the term "structural gene" refers to a DNA sequence coding for RNA or a protein. In contrast, "regulatory genes" are structural genes which encode products which control the expression of other genes (e.g., transcription factors).

As used herein, the term "gene" means the deoxyribonucleotide sequences comprising the coding region of a structural gene and including sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb on either end such that the gene corresponds to the length of the full-length mRNA. The sequences which are located 5' of the coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene which are transcribed into heterogeneous nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences which are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region may contain regulatory sequences such as promoters and enhancers which control or influence the transcription of the gene. The 3' flanking region may contain sequences which direct the termination of transcription, posttranscriptional cleavage and polyadenylation.

The terms "binding component", "molecule of interest", "agent of interest", "ligand" or "receptor" as used herein may be any of a large number of different molecules, biological cells or aggregates, and the terms are used interchangeably. Each binding component may be immobilized on a solid substrate and binds to an analyte being detected. Proteins, polypeptides, peptides, nucleic acids (nucleotides, oligonucleotides and polynucleotides), antibodies, ligands, saccharides, polysaccharides, microorganisms such as bacteria, fungi and viruses, receptors, antibiotics, test compounds (particularly those produced by combinatorial chemistry), plant and

animal cells, organs or fractions of each and other biological entities may each be a binding component.. Each, in turn, also may be considered as analytes if same bind to a binding component on a chip.

The term "macromolecule" as used herein, refers to any molecule of interest having a
5 high molecular weight, including synthetic and biological polymers. For example, some biopolymers having a high molecular weight would be comprised of greater than 100 amino acids, nucleotides or sugar molecules long.

The term "bind" as used herein, includes any physical attachment or close association, which may be permanent or temporary. Generally, an interaction of hydrogen bonding,
10 hydrophobic forces, van der Waals forces, covalent and ionic bonding etc., facilitates physical attachment between the molecule of interest and the analyte being measuring. The "binding" interaction may be brief as in the situation where binding causes a chemical reaction to occur. That is typical when the binding component is an enzyme and the analyte is a substrate for the enzyme. Reactions resulting from contact between the binding agent and the analyte are also
15 within the definition of binding for the purposes of the present invention.

The term "binding site" as used herein, refers to any molecular arrangement having a specific tertiary and/or quaternary structure that undergoes a physical attachment or close association with a binding component. For example, the molecular arrangement may comprise a sequence of amino acids. Alternatively, the molecular arrangement may comprise a sequence a
20 nucleic acids. Furthermore, the molecular arrangement may comprise a lipid bilayer or other biological material.

Brief Description Of The Figures

Figure 1 presents schematic illustrations of:

- 25 Figure 1A: Nebulized droplets containing prefabricated microactuators.
 Figure 1B: Magnetic reconfiguration of a microactuator that generates propulsive force.
 Figure 1C: One embodiment of a cyclic magnetic fluctuation that results in microactuator propulsion due to repetitive time-asymmetric reconfiguration
30 cycles.

Figure 2 presents illustrative prototype microscale actuators fabricated from patchy microcubes. Scale bar = 10 μm .

Figure 2A: Polymeric microcubes with magnetic patches.

Figure 2B: Particle assembly in a uniform magnetic field.

5 Figure 2C: Reversible folding by toggling the magnetic field on and off.

Figure 3 presents exemplary data showing microscale actuator reconfiguration-driven self-viscophoresis.

Figure 3A: Time-symmetric strokes.

Figure 3B: Rapid opening and slow closing.

10 Figure 3C: Slow opening and rapid closing.

Figure 3D: Locomotion time course data of microactuator dynamics in different magnetic fields through a solution containing Xanthan gum.

Figure 4 presents an illustrative micrograph of poly(methyl methacrylate) (PMMA) microparticles.

15 Figure 4A: Chemical structure of a type of PMMA.

Figure 4B: A fabricated L-shaped microparticle made from PMMA.

Figure 5 presents representative photomicrographs and an associated schematic representation of an actuating chain of microparticles (e.g., on/off).

20 Figure 6 presents exemplary data of a cumulative release assay of encapsulated salbutamol from SU-8 microparticles.

Figure 7 presents one embodiment of a fabrication method for dimeric magnetic actuators made from L-shaped microparticles.

Figure 7A: A representative step-by-step photolithography method.

25 Step 1) A diluted photoresist SU-8 3010 (blue) is spin-coated on a silicon wafer (silver) to a film thickness of 3.5 μm .

Step 2) The photoresist is patterned using a maskless aligner and developed to fabricate an array of L-shaped particles.

30 Step 3) The photoresist AZ nLOF 2070 or 9260 (red) is spin-coated on top of the particle layer and patterned using alignment photolithography. The film thickness of this lift-off layer is 7-10 μm .

Step 4) The AZ pattern is developed to reveal an array of holes, which exposes the inner side of the long arm of the L-shaped particles.

Step 5) 10 nm of chromium followed by 100 nm of cobalt is evaporated at an angle (not shown). All metal evaporation is performed using an electron-beam evaporator. The metal-coated AZ layer is removed in AZ Kwik Strip, leaving side-coated SU-8 structures. The metal layer is shown in gold for easy visualization.

Figure 7B: Schematic illustration of L-shaped microparticles during magnetic assembly. Particles are mechanically released and suspended in solution (top). A uniform magnetic field is applied, and particle dipoles form in alignment with the direction of the applied field (middle). Dipole-dipole interactions drive particles to assemble into dimers. Turning the applied field off causes dipole-dipole interactions to dominate that close the assemblies. The last two steps are reversible, leading to fully switchable opening and closing motions.

Figure 8 presents a representative system for assembling and operating the time-asymmetric microscale actuators.

Figure 8A: An assembly cell containing magnetic microparticles between a collinear pair of electromagnetic coils. The electromagnetic coils are connected in parallel to a function generator that outputs time-varying signals.

Figure 8B: A graphical representation of slow opening and fast closing actuation.

Figure 8C: A graphical representation of fast opening and slow closing actuation.

Figure 9 presents one embodiment of a fabrication assembly process of L-shaped microparticles into microscale actuators.

Figure 9A: Metal-coated L-shaped microparticles after lift-off of AZ 9260. Metal coatings on the tops of the L-shaped microparticles are clearly visible.

Figure 9B: L-shaped microparticles released into solution.

Figure 9C: L-shaped microparticle configurations after magnetic field-induced assembly.

(i) Particles in the staggered configuration, where actuation is prevented.

(ii) Particles assembled into an actuator configuration, where actuation occurs in the presence of a cyclic magnetic field.

Figure 10 presents exemplary data showing microscale actuator response to cyclic magnetic fields and drug release.

Figure 10A: An open microscale actuator aligned with an externally applied magnetic field.

5 Figure 10B: A closed microscale actuator in the absence of an externally applied magnetic field.

Figure 10C: Cumulative release profile of encapsulated salbutamol from SU-8 microparticles.

10 Figure 11 presents representative illustrations of conventional helical microrobots and time-asymmetric microscale actuators. Helical robots (left) travel along a single direction in response to a rotating magnetic field. Time-asymmetric microscale actuators (right) self-propel along any direction, including perpendicular to the applied magnetic field vector.

Detailed Description Of The Invention

15 The present invention is related to the field of microrobotics. In particular, microscale robots are contemplated that propel themselves through non-Newtonian viscous fluid barriers. This capability provides therapeutic agent delivery to previously inaccessible tissues. For example, the microscale robots can be used to treat pulmonary conditions including, but not limited to chronic obstructive pulmonary disease, cystic fibrosis and/or viral infections by
20 delivering therapeutic agents at close proximity to the diseased tissues.

In one embodiment, the present invention contemplates a method for fabricating dimeric microrobots (e.g., microscale actuators) comprising two L-shaped microparticles that self-propel in a cyclic magnetic field. Standard alignment photolithography is combined with directed microparticle assembly in magnetic fields. Once fabricated and assembled, these microrobots
25 can perform reciprocal strokes in time-varying magnetic fields at low magnetic field strengths. In one embodiment, the method exhibits a self-limited assembly. This is an improvement over existing fabrication methods by eliminating formation of obstructive microparticle clusters containing inoperative assemblies. This improved method allows for a bottom-up fabrication of microscale actuators with well-defined dimensions and properties.

30 Because the presently disclosed technology combines several commonly used microfabrication methods (photolithography, GLAD, and metal lift-off), it is amenable to scale-

up. Scalability and reconfigurability offer many advantages over existing designs of microrobots currently being investigated. Sitti et al., “Pros and Cons: Magnetic versus Optical Microrobots” *Adv. Mater.* 32:1906766 (2020). In addition to practical applications, the self-terminating behavior of the microparticles described herein can be applied to fundamental studies of
5 magnetic colloidal assembly. Klinkova et al., “Colloidal analogs of molecular chain stoppers” *Proc. Natl. Acad. Sci. U.S.A.* 110:18775–18779 (2013).

In one embodiment, the present invention contemplates reconfigurable microscale actuators (“microactuators”) that can be inhaled and externally operated to mechanically disrupt mucosal films and enhance drug penetration in the airways. Preliminary data showed that such
10 actuators are made from a directed assembly of cubic microparticles fabricated from monolithic techniques into long chains and/or clusters. Shields IV et al., “Field-directed assembly of patchy anisotropic microparticles with defined shape” *Soft Matter* 9:9219 (2013); and Han et al., “Sequence-encoded colloidal origami and microbot assemblies from patchy magnetic cubes” *Sci. Adv.* 3:e1701108 (2017).

15 There is a long felt need to enhance drug penetration through mucosal barriers deep within the lungs to treat airway diseases such as chronic obstructive pulmonary disease (COPD), cystic fibrosis and asthma. Teno et al., “Dying Trajectory in the Last Year of Life: Does Cancer Trajectory Fit Other Diseases?” *Journal of Palliative Medicine.* 4(4):457–464 (2001). Of these, COPD is the fourth leading cause of death in the U.S. Pires et al., “Pharmacological treatment of
20 COPD – New evidence” *Pulmonology* 25(2):90–96 (2019). Chronic bronchitis (CB) is a subtype of COPD and is associated with an increased risk of respiratory infection, lung function decline, and elevated all-cause mortality. Kim et al. “The chronic bronchitis phenotype in chronic obstructive pulmonary disease: features and implications” *Current Opinion in Pulmonary Medicine* 21(2):133–141 (2015); and Kim et al., “Chronic Bronchitis and Chronic Obstructive
25 Pulmonary Disease” *Am J Respir Crit Care Med.* 187(3):228–237 (2013). These diseases are characterized by mucus buildup, which leads to bacterial colonization, airway inflammation, tissue destruction, and ultimately respiratory failure. Fernández-Blanco et al., “Attached stratified mucus separates bacteria from the epithelial cells in COPD lungs” *JCI Insight.* 6;3(17):e120994 (2018). Common drugs, especially bronchodilators, exhibit poor penetration
30 through mucosal biofilms and are of limited effectiveness in advanced cases of COPD. Paranjpe et al., “Nanoparticle-Mediated Pulmonary Drug Delivery: A Review” *IJMS* 8;15(4):5852–5873

(2014); and Tyrrell et al., “Gaining the Upper Hand on Pulmonary Drug Delivery”. J Pharmacovigil. 1;2(1):118 (2014).

I. Conventional Microrobots

5 Over the past decade, numerous advances in microscale robotics have generated new applications across science, engineering, manufacturing, and medicine. Some of the most promising examples include those for drug delivery, minimally invasive surgery, environmental remediation, and microscale manufacturing. Ghosh et al., “Active matter therapeutics” Nano Today 31:100836 (2020); Jin et al., “Untethered Single Cell Grippers for Active Biopsy” Nano
10 Lett. acs.nanolett.0c01729 (2020); Jurado-Sánchez et al., “Micromotors for environmental applications: a review” Environ. Sci.: Nano 5:1530–1544 (2018); and Chung et al., “Three-dimensional heterogeneous assembly of coded microgels using an untethered mobile microgripper” Lab Chip 15, 1667–1676 (2015).

A method and system has been reported for propelling and controlling displacement of a
15 microrobot in a space having a wall. This system includes the steps of: fabricating the microrobot with a body containing a magnetic field-of-force responsive material, wherein, in response to a magnetic field of force, a force is applied to the material in a direction determined by the magnetic field of force; positioning the microrobot in the space for displacement in that space; and generating the magnetic field of force with a predetermined gradient and applying the
20 magnetic field of force to the microrobot propelling the microrobot through the space in a direction of a field of force. Then, a sequence of field generating steps are executed, wherein each step includes calculating the direction, amplitude and spatial variation of the net field of force to control displacement of the microrobot in the space and against the wall from one equilibrium point to another. Chautems et al., “Method And System For Propelling And
25 Controlling Displacement Of A Microbot In A Space Having A Wall” United States Patent Application Publication Number 2020/0193586 (herein incorporated by reference).

A method has been reported for simultaneously calibrating magnetic actuation and sensing systems for a workspace, wherein the actuation system comprises a plurality of magnetic actuators and the sensing system comprises a plurality of magnetic sensors, wherein all the
30 measured data is fed into a calibration model, wherein the calibration model is based on a sensor measurement model and a magnetic actuation model, and wherein a solution of the model

parameters is found via a numerical solver order to calibrate both the actuation and sensing systems at the same time. Sitti et al., “Simultaneous Calibration Method For Magnetic Localization And Actuation Systems” United States Patent Application Publication Number 2020/0196844 (herein incorporated by reference).

5 Within the drug delivery field, conventional microrobots have been unable to address long-term challenges such as navigation through obstructive biological barriers and targeted delivery to inaccessible sites in the body. These capabilities would offer the potential to improve the therapeutic efficacy and safety of drugs to treat a variety of diseases. However, despite this promise, issues related to fabrication, actuation, and steerability of microdevices continue to
10 limit their ability to improve *in vivo* therapeutics. Shields et al., “The Evolution of Active Particles: Toward Externally Powered Self-Propelling and Self-Reconfiguring Particle Systems” Chem 3:539–559 (2017).

 Microrobots are microscale devices that exhibit autonomous behaviors, typically untethered from power sources. Sitti, M., “Mobile microrobotics” MIT Press (2017).

15 Microrobots are typically fabricated monolithically using techniques such as photolithography, two-photon lithography, and microcontact printing. During fabrication, catalysts and other metals are often included to asymmetrically dissipate energy to generate motion.

 Once fabricated, microrobots can be made active or self-propelling by introducing chemical fuels or external energy sources, including acoustics, light, or electromagnetic fields.
20 Aghakhani et al., “Acoustically powered surface-slipping mobile microrobots” Proc Natl Acad Sci USA 117:3469–3477 (2020); Palagi et al., “Structured light enables biomimetic swimming and versatile locomotion of photoresponsive soft microrobots” Nature Materials 15:647–653 (2016); and Han et al., “Engineering of Self-Propelling Microbots and Microdevices Powered by Magnetic and Electric Fields” Adv. Funct. Mater. 28:1705953 (2018). Of these energy sources,
25 magnetic fields offer many advantages, including high spatiotemporal resolution and compatibility with biological organisms. Sitti et al., “Pros and Cons: Magnetic versus Optical Microrobots” Adv. Mater. 32, 1906766 (2020).

 Recent work with magnetic microrobotics has focused mostly on paramagnetic helices driven by rotating magnetic fields. Such helices are often fabricated using two-photon
30 lithography of photoresists containing magnetic nanoparticles. Ceylan et al., “3D-Printed Biodegradable Microswimmer for Theranostic Cargo Delivery and Release” ACS Nano

13:3353–3362 (2019). Other examples of magnetic microrobots include Janus particles and microwheels that roll on biological surfaces. Yang et al., “Microwheels on microroads: Enhanced translation on topographic surfaces” *Sci. Robot.* 4, eaaw9525 (2019); and Bozuyuket al., “High-Performance Magnetic FePt (L10) Surface Microrollers Towards Medical Imaging-Guided Endovascular Delivery Applications” *Adv Funct Materials* 32:2109741 (2022). The navigation of such microdevices is highly dependent on surface topology, typically tissue surfaces of the circulatory or pulmonary systems, and the direction of the applied magnetic field. Thus, there remains a need to develop microrobots whose fabrication is scalable and whose direction of motion is decoupled from the direction of the applied field.

10 It has been previously reported that a chain of polymeric microcubes made with magnetic films on one side can be assembled in a uniform magnetic field. See, Figure 2. These chains of microcubes close due to dipole-dipole interactions between the assembled films on adjacent cubes and reopen due to strong dipole-field interactions. The propulsion of these microcube chains were studied at low Reynolds numbers, where viscous forces dominate. In Newtonian fluids, time-reversible motions do not lead to displacements. However, mucus is a non-Newtonian fluid and these microcube chains can efficiently swim through non-Newtonian fluids by folding with time-asymmetric strokes. See, Figure 3. Han et al., “Sequence-encoded colloidal origami and microbot assemblies from patchy magnetic cubes” *Sci. Adv.* 3:e1701108 (2017); and Han et al., “Active Reversible Swimming of Magnetically Assembled
20 “Microscallop” in Non-Newtonian Fluids” *Langmuir* acs.langmuir.9b03698 (2020) doi:10.1021/acs.langmuir.9b03698.

In Newtonian fluids, time-reversible motions do not lead to net displacements. However, most biological fluids are non-Newtonian: blood, saliva, and mucus. Propulsion in non-Newtonian fluids must overcome viscous forces, which means that reconfigurations must be time asymmetric (e.g., open faster than close). Microswimmers that use flexible elements are inspired by the motion of spermatozoa. Palagi et al., “Bioinspired microrobots” *Nat Rev Mater.* 3(6):113–124 (2018). In contrast, the presently disclosed microactuators take advantage of the non-Newtonian rheology of mucus to enable transport. When placed in shear-thinning fluids, the local viscosity gradient resulting from non-uniform shear stresses exerted by time-asymmetric strokes of the microactuators generate propulsive thrust through self-viscophoresis. Thus, the

microscale actuators described herein circumvent the need for flexible elements, which simplifies fabrication procedures needed for scale-up.

II. Time-Asymmetric Microscale Actuators - Overview

5 In one embodiment, the present invention contemplates L-shaped microparticle dimer actuators that facilitate active matter therapeutics by improving drug delivery to mucosal cells (e.g., bronchial epithelial cells) by transducing energy from cyclic magnetic fields into directed motion through non-Newtonian fluids.

10 In contrast to top-down fabrication of conventional microrobots, the present invention contemplates microscale actuators using a bottom-up assembly of magnetic particles as a method to scale-up production. For example, magnetic microparticles can be assembled into various colloidal assemblies including but not limited to, dimers, chains, clusters, and other geometries depending on the shape of the magnetic particles and the properties of the magnetic field applied during fabrication. Colloidal assemblies made of cubic magnetic microparticles adopt many
15 chain configurations in time-varying magnetic fields. Geometry, magnetic properties, and field conditions all play deterministic roles in the configuration space of these cubic particle chains. These properties make colloidal assemblies a potential platform to fabricate the presently disclosed reconfigurable microrobotic structures comprising L-shaped microparticle dimers.

20 In particular, ferromagnetic materials such as iron, cobalt, nickel or other ferromagnetic alloys are preferred materials as they retain a magnetic dipole upon removal of an external magnetic field. This remanent magnetization allows particles to remain assembled upon removal of the field. It has been reported that assemblies of magnetic microcubes folded in on themselves in response to a cyclic magnetic field, and that the folding behavior was dependent on the geometric sequence of cubes within the assembly.

25 Further, it was observed that folding behavior of microcube chains resulted in a self-propulsion through time-asymmetric cyclic folding motions in non-Newtonian fluids. Han et al., “Sequence-encoded colloidal origami and microbot assemblies from patchy magnetic cubes Sci. Adv. 3, e1701108 (2017); and Han et al., “Active Reversible Swimming of Magnetically Assembled “Microscallop” in Non-Newtonian Fluids”. Langmuir acs.langmuir.9b03698 (2020).
30 This observation was in contrast to Purcell’s scallop theorem proposing that reciprocal motions (such as the opening and closing of a scallop) at low Reynold’s numbers inhibits self-propulsion

in Newtonian fluids. Purcell E. M., “Life at low Reynolds number” American Journal of Physics 45, 3–11 (1977).

In one embodiment, the present invention contemplates a microscale actuator comprising an assembly of a plurality of magnetically engineered L-shaped microparticle dimers. In one
5 embodiment, the assembly is self-limited. In one embodiment, the self-limited assembly comprises a geometric design, such as a dimer. In one embodiment, the dimer reliably opens and closes as a response to a cyclic magnetic field. In one embodiment, the response is time-asymmetric wherein the relative actuation velocities of the opening and closing are different. In one embodiment, the microscale actuator comprises an anisotropic shape. In one embodiment,
10 the surface of the microscale actuator comprises a plurality of selectively placed ferromagnetic patches. In one embodiment, the microscale actuator is a dimeric microscale actuator. In one embodiment, the dimeric microscale actuator consists of two particles.

Although it is not necessary to understand the mechanism of an invention, it is believed that the geometric design of the presently disclosed microscale actuators results from a
15 controlled assembly of specifically oriented microparticles. It is also believed that the microparticle substrate is capable of drug encapsulation or surface attachment and subsequent drug release to provide various biomedical applications. It is also believed that these presently disclosed microscale actuators self-propel through non-Newtonian fluids, such as mucus.

Particles conventionally used as drug carriers are typically passive, relying on diffusion
20 to navigate through biological barriers. Alternatively, self-propelled or active particles transduce energy from their environment into directed motion. Active particles can be energized by light or electric, magnetic, and acoustic fields to overcome Brownian-limited diffusion. Shields et al., “The Evolution of Active Particles: Toward Externally Powered Self-Propelling and Self-Reconfiguring Particle Systems” Chem. 3(4):539–559 (2017); and Ghosh et al., “Active matter
25 therapeutics Nano Today 31:100836 (2020).

These enhanced transport characteristics make active particles a useful platform for drug delivery. The most promising active particles for biomedical applications are those powered by magnetic fields, ultrasound, and light. The advantages of magnetic fields include deep tissue penetration, specificity to not interact with biological tissues, and high spatiotemporal resolution.
30 Sitti et al., “Pros and Cons: Magnetic versus Optical Microrobots” Adv Mater. 13;1906766 (2020).

Reconfiguring (e.g., actuating) particle assemblies facilitate biological applications that involve advanced self-propulsive motions through complex environments. In contrast, particle-based drug delivery systems developed so far, most are geometrically simple (e.g., spheres, cylinders, helices) and are incapable of reconfiguration. Li et al., “Micro/nanorobots for
5 biomedicine: Delivery, surgery, sensing, and detoxification” *Sci Robot.* 1;2(4):eaam6431 (2017). Some particles with more complex shapes that can change shape have been fabricated from elastomeric materials; however, this method of actuation is generally limited to materials that are not typically biocompatible (e.g., liquid-crystal elastomers and thus not amenable to *in vivo* deployment. While there has been some progress in making biodegradable microrobots, their
10 structures are generally not amenable to scalable production. Evans et al., “Active Shape-Morphing Elastomeric Colloids in Short-Pitch Cholesteric Liquid Crystals” *Phys Rev Lett.* 30;110(18):187802 (2013); and Ceylan et al., “3D-Printed Biodegradable Microswimmer for Theranostic Cargo Delivery and Release” *ACS Nano.* 26;13(3):3353–3362 (2019).

In one embodiment, the present invention contemplates method comprising fabricating
15 biocompatible microactuators by self-assembly of microparticles. Although it is not necessary to understand the mechanism of an invention, it is believed that the present method combines both top-down and bottom-up techniques to create advanced microstructures capable of controlled reconfiguration and transport.

In one embodiment, the present invention contemplates a fabrication and/or use of
20 reconfigurable, self-propulsive, microscale actuators. For example, such microscale actuators can be inhaled and externally operated to enhance drug penetration through mucosal biofilms and potentially other biological barriers. In one embodiment, the microscale actuators are made from an assembly of microscale particle building blocks (e.g., microparticles) fabricated from monolithic techniques (e.g., photolithography or microcontact printing). These microparticles
25 are then coated with magnetic films in well-defined regions along their surfaces.

Microscale actuators are then created by an assembly of coated microparticles using a magnetic field. Although it is not necessary to understand the mechanism of an invention, it is believed that microscale actuators close due to dipole-dipole interactions between the magnetic films on the particles. Conversely, microscale actuators open on demand due to strong dipole-
30 field interactions through the reapplication of a magnetic field.

Advantages of microscale actuators include, but are not limited to: (i) creation with biocompatible materials (e.g., poly(methyl methacrylate), PMMA, or poly(lactic-co-glycolic acid), PLGA), (ii) encapsulation of a range of drugs, such as bronchodilators and antibiotic drugs, and (iii) mucosal dispersing agent coatings to improve penetration through mucosal
5 biofilms. Consequently, microscale actuators may self-propel through viscous, non-Newtonian fluids, driven by magnetically induced asymmetric actuations. Mucus, a characteristic non-Newtonian fluid, is a medium through which time-asymmetric self-propelled microscale actuators can traverse.

In one embodiment, microscale actuators can be dispersed using nebulizers or dry
10 powder inhalers and powered remotely. Nebulizers and dry powder inhalers can be used to disperse microscale actuators. Nebulization may use an electrically powered pump to deliver air to a suspension that creates a fine aerosol which can be inhaled by the patient. Commercial nebulizers are usually comprised of a base, a compressed air chamber, and a container for holding the suspended medicine. The carrier fluid is a sodium chloride solution through which
15 pressurized air is directed to create a mist. Nebulization may be used to disperse microactuators across multiple artificial mucosal films (e.g., a 10 cm x 10 cm surface, each) at fixed separation distances (e.g., ~ 50 cm). Advantages of nebulized microscale actuators include, but are not limited to: (i) the mist is generally easier to breathe; and (ii) COPD patients are more familiar with a nebulizer's use and function, as it is commonly used to deliver salbutamol. A dry powder
20 inhaler can also be used to disperse microactuators over such 10 cm x 10 cm artificial mucosal films. To disperse the powder, actuators can be lyophilized with an excipient (i.e., lactose monohydrate), to enhance delivery. Rahimpour et al., "Lactose Engineering for Better Performance in Dry Powder Inhalers" Adv Pharm Bull. 2(2):183–187 (2012). Fluorescence imaging can be used to study the distribution of particles on surfaces using both methods and
25 quantitatively compared via root mean square (RMS) spatial deviations.

Microscale actuator technology can be used for transport through mucus or in the bloodstream. In one embodiment, the microscale actuators range in size from between 5-15 micrometers across. In one embodiment, the microscale actuators are less than 5 micrometers.

III. Time-Asymmetric Self-Propelled Microscale Actuators - Fabrication

Previous results showing that magnetic microcubes assemble into long chain-like structures were replicated. Han et al., “Active Reversible Swimming of Magnetically Assembled “Microscallop” in Non-Newtonian Fluids” *Langmuir* 36:7148–7154 (2020); and Han et al., “Sequence-encoded colloidal origami and microbot assemblies from patchy magnetic cubes” *Sci. Adv.* 3:e1701108 (2017). Contact photolithography was used and metal deposition to coat the top sides of cubes with 10 nm chromium (Cr) and 100 nm cobalt (Co) layers. Chain assembly was accomplished using permanent neodymium magnets, which allowed for manual control of actuator position and mechanics. Actuation was achieved with staggered sequences of the magnetic microcubes. See, Figure 5.

In one embodiment, the present invention contemplates a method for manufacturing microscale actuators by L-shaped microparticle self-assembly. In one embodiment, the L-shaped microparticles are generated by alignment photolithography and GLAD. In one embodiment, metals (e.g., cobalt) are evaporated onto the microparticles to form a magnetic film. In one embodiment, the self-assembly of the metal-coated microparticles into microscale actuators are guided by magnetic fields. In one embodiment, the microscale actuators are made from biocompatible materials. In one embodiment, the microscale actuators encapsulate drugs including, but not limited to, bronchodilators. In one embodiment, the microscale actuators are coated with mucosal dispersing agents. In one embodiment, the mucosal dispersing agent is dithiothreitol. Although it is not necessary to understand the mechanism of an invention, it is believed that mucosal dispersing agents reduce mucosal disulfide bonds to permit penetration of the microscale actuator through a thick non-Newtonian fluid, such as mucus. Recent Advances in the Pathophysiology of COPD. (Birkhäuser Basel, 2004). doi:10.1007/978-3-0348-7939-2.

In one embodiment, the present invention contemplates methods of fabricating microscale actuator dimers comprising a plurality of shapes and sizes. In one embodiment, the plurality of shapes and sizes improve self-propulsion as compared to conventional microscale actuators. In one embodiment, the improved self-propulsion comprises improved speed, improved maneuverability, improved navigation, and improved fluid displacement.

A. Photolithography

A physical or virtual photomask can be designed to determine the effect of microactuator size on propulsion. Physical masks are used in contact photolithography, and virtual masks are

used in maskless photolithography. The photomask can create particles of different sizes ranging from 2 to 7 μm , producing actuator assemblies of between 4 to ~ 15 μm . Particles can be fabricated from PMMA or SU-8, which are biocompatible and amenable to photopatterning. Carbaugh et al., “Photolithography with polymethyl methacrylate (PMMA)” *Semicond Sci Technol.* 1;31(2):025010 (2016).

Microactuators can be made using microcontact printing, which entails soft lithography to make microparticles using a rubber stamp. This method allows the microactuators to be fabricated in a more scalable format from biodegradable materials (e.g., PLGA) instead of photoresists, thereby imparting better control over the release of drugs. Encapsulation and release studies with therapeutic agents may also be achieved from this fabrication method. For example, one would expect the release of $>50\%$ of an encapsulated therapeutic agent within 24 hours.

Soft lithography is a method for replicating particle structures using elastomeric stamps, which are usually made from polydimethylsiloxane (PDMS). Shields et al., “Cellular backpacks for macrophage immunotherapy” *Sci Adv.* 1;6(18):eaaz6579 (2020). Soft lithography offers several advantages over contact photolithography, enabling (i) scale-up for mass production, (ii) access to a greater selection of materials, and (iii) ease of fabrication. Qin et al., “Soft lithography for micro- and nanoscale patterning” *Nat Protoc.* 5(3):491–502 (2010).

In one embodiment, the present invention contemplates a microcontact printing method of making microparticles. Microcontact printing is a form of soft lithography in which the stamp is used to deposit material onto a substrate. Microcontact printing can be used to fabricate biodegradable particles made of PLGA and has a successful history in pulmonary drug delivery. Wang et al., “Facile functionalization and assembly of live cells with microcontact-printed polymeric biomaterials” *Acta Biomaterialia* 11:80–87 (2015); Han et al., “Bioerodable PLGA-Based Microparticles for Producing Sustained-Release Drug Formulations and Strategies for Improving Drug Loading” *Front Pharmacol.* 7 (2016); and Sun et al., “Magnetically Powered Biodegradable Microswimmers” *Micromachines* 13;11(4):404 (2020).

B. Magnetic Patches

In one embodiment, the present invention contemplates layering magnetic patches onto well-defined regions inside of the long arm of an L-shaped microparticle using techniques including alignment photolithography and glancing angle deposition (GLAD). Pawar et al.,

“Multifunctional Patchy Particles by Glancing Angle Deposition” *Langmuir* 18;25(16):9057–9063 (2009). Each magnetic patch comprises 10 nm chromium as an adhesive and 100 nm cobalt for magnetic susceptibility. Once the metals are deposited, the second (sacrificial) layer of photoresist can be removed using a solvent wash. These fabricated L-shaped microparticles can then be assembled into microactuators under a directed electromagnetic field. Shields et al., “Engineering of Self-Propelling Microbots and Microdevices Powered by Magnetic and Electric Fields” *Advanced Functional Materials* 28(25):1705953 (2018).

L-shape microparticles can be suspended in low concentrations of ferrofluid (e.g., 0.6–1.2 vol.%), wherein only two particles can click together with aligned patches due to steric hindrance from the short arm of the L-shape. This is a distinct difference from the microcube chains as reported by Han et al. (*supra*). The diamagnetic regions generated by the ferrofluid repels the magnetic field from the electromagnets and nanoparticles, driving their assembly to attract the diamagnetic regions (e.g., dipoles) of the engineered L-shaped microparticles. Once assembled, the microscale actuators are resuspended in physiological buffer and the percentage of assemblies that formed the intended structure as a function of ferrofluid concentration can be determined.

When particles blocked with AZ 9260 were removed using mechanical forces, small amounts of cobalt were sometimes released on the silicon wafer along with the particles during extraction. These cobalt flakes were visible in solution and, in some cases, were attracted to the magnetic patches of the particles. The resultant assembly of microscale actuators using this resist lift-off technique was successful on approximately 90% of a 2-inch wafer. See, Figure 9A and 9B. A dedicated lift-off resist such as the AZ nLOF series may lessen the occurrence of this anomaly. Kim et al., “Review of polymer MEMS micromachining” *J. Micromech. Microeng.* 26:013001 (2016).

In one embodiment, the present invention contemplates a method for creating a plurality of microscale actuator configurations comprising assembling L-shaped microparticles in a ferrofluid. The fabrication of L-shaped actuators with precisely defined magnetic patches can be accomplished using magnetically directed assembly in ferrofluids. Although it is not necessary to understand the mechanism of an invention, it is believed that ferrofluids alter a local magnetic field around an L-shaped microparticle, thereby allowing for L-shaped microparticles to assemble side-to-side as opposed to staggering, as has previously been shown with assembly of

cubic microparticles into long chains and/or clusters. Shields IV et al., “Field-directed assembly of patchy anisotropic microparticles with defined shape” *Soft Matter* 9:9219 (2013).

L-shaped microparticles were fabricated and GLAD was used to selectively coat ferromagnetic patches on their surfaces. In one embodiment, ferromagnetic patches are deposited along one side surface of the long arm of the microparticle. In one embodiment, the selective coating comprises adding a blocking layer (e.g., AZ 9260). After the microparticles were extracted from the substrate, these ferromagnetic patches were then magnetized in a uniform magnetic field such that the magnetic dipoles aligned with the field, resulting in the assembly of a microscale actuator with neighboring dipoles.

Ferromagnetic patch placement was evaluated for obtaining the correct assembly of the L-shaped microparticles into microscale actuator dimers. For example, magnetic patches were placed on a top surface of an L-shaped microparticle as opposed to a side surface as described above. Top surface magnetic patch placement resulted in an assembly of particle chains aligned along the long arms of the L-shaped microparticles. Despite this, a very small percentage of particles assembled in the preferred configuration in dilute solutions (estimated to be <1% of assemblies). Thus, the placement of magnetic metal along the side surface of the microparticles was deemed a preferred location.

C. Magnetic Field Alignment – Differential Orientation Assembly

Although it is not necessary to understand the mechanism of an invention, it is believed that the L-shaped microparticle dimer geometric design terminates assembly without further chain growth in the absence of excess cobalt. In particular, dimeric microparticle assemblies can be controlled by a magnetic field alignment.

For example, alignment of L-shaped microparticles into dimers with a magnetic field permits the microparticles to face one of two different directions: short arm pointing up and short arm pointing down. It is preferred that the short arm alignment be positioned orthogonal to the direction of the magnetic field. See, Figure 7B. Because both alignments minimize the dipolar interaction energy of the L-shaped microparticles, each configuration leads to a different assembly type. The magnetic-field-induced L-shaped microparticle assembly into microscale actuator is limited to a dimer because steric hindrance provided by the short arm prevents additional L-shaped microparticles from interacting with their magnetic patches. The L-shaped

particle effectively acts stop the assembly as a dimer, analogous to a termination reaction in polymer chemistry.

Although it is not necessary to understand the mechanism of an invention, it is believed that a microscale actuator dimer in which both L-shaped microparticles are oriented with the arms in the same direction is a preferred orientation for proper actuation in a cyclic magnetic field. See, Figure 9C(ii). The alternative assembly type creates a microscale actuator dimer in which L-shaped particles are pointing in opposite directions. See, Figure 9C(i). This configuration prevents actuation due to a slight overlap in dipoles. Han et al., "Sequence-encoded colloidal origami and microbot assemblies from patchy magnetic cubes" Sci. Adv. 3:e1701108 (2017).

Due to the thicknesses and alignment resolution of photoresist layers during fabrication, it is likely unavoidable that some metal will be evaporated from the top surface of the particles in addition to side wall surfaces. Therefore, the alignment geometry and angle of evaporation must be calculated such that the side wall ferromagnetic patches are thicker than the top surface. To ensure this, a thickness limit of the top surface cobalt (Co) patches was set to 20 nm. The angle of evaporation was also set such that the top surface metal patch-to-side surface metal patch ratio was 20:80. Preliminary data demonstrated that these conditions were sufficient to achieve the desired reconfigurations. If the metal films on top of the L-shaped microparticles are too thick, then actuation is minimal.

The fabricated microscale actuators were then subjected to cyclic magnetic fields using a magnetic coil setup as shown above. See, Figure 8. Alternatively, actuation of these devices may also be accomplished manually using neodymium magnets, whereby cyclic magnetic fields were produced by repeatedly moving the magnets close to and subsequently away from the assembly chamber. For example, actuator opening occurs in the presence of a magnetic field and actuator closing occurs in the absence of a magnetic field. See, Figures 10A and 10B, respectively. In one embodiment, the microscale actuators comprise a single hinge comprising dipole attraction between a magnetic metal coating on the inner side of each long arm.

IV. Methods Of Using Time-Asymmetric Microscale Actuators

In one embodiment, the present invention contemplates a method for using a microscale actuator that is aligned in parallel to a magnetic field vector and actuated perpendicular to the magnetic field vector. Although it is not necessary to understand the mechanism of an invention, it is believed that the presently disclosed microscale actuators respond to a magnetic field only in terms of orientation and not direction. In one embodiment, the presently disclosed microscale actuators self-propel in any direction (e.g., 360°) around a magnetic field vector. Such a capability is a major advantage and permits traversal of a large volume that is unattainable by conventional microrobots. See, Figure 11.

Conventional microrobots rely on magnetic helices to break symmetry; however, these devices are only capable of traveling along the vector defined by the magnetic field, which greatly limits their coverage. Yasa et al., “Elucidating the interaction dynamics between microswimmer body and immune system for medical microrobots” *Sci. Robot.* 5:eaaz3867 (2020).

In one embodiment, the present invention contemplates a time-asymmetric microscale actuator comprising an L-shaped microparticle dimer that generates a large volume displacement during self-propulsion. Consequently, the presently disclosed self-propelled time-asymmetric microscale actuators are useful for pulmonary drug delivery given the physiology and volume of human lungs. In one embodiment, the presently disclosed microscale actuators self-propel through non-Newtonian fluids including, but not limited to, blood, vitreous humor, and mucus unlike conventional microrobots. Wu et al., “A swarm of slippery micropropellers penetrates the vitreous body of the eye” *Sci. Adv.* 4:eaat4388 (2018).

In one embodiment, the presently disclosed self-propelled time-asymmetric microscale actuators are delivery platforms for small molecule drugs, biologics, antibodies, aptamers, antigens, proteins, cytokines, mRNA, CRISPR-Cas9 components to previously inaccessible regions of the body.

It has been reported that microcube chains self-propel through viscous, non-Newtonian fluids when driven by asymmetric strokes. In operation, the microcube chains reconfigure due to dipole-dipole interactions between the magnetic films on the particles and controllably reopen due to strong dipole-field interactions. Han et al., “Active Reversible Swimming of Magnetically Assembled “Microscallop” in Non-Newtonian Fluids” *Langmuir* *acs.langmuir.9b03698* (2020).

In one embodiment, the present invention contemplates a method for using a microscale actuator comprising an L-shaped microparticle dimer that self-propels through mucus thereby enhancing drug and/or genetic material transport. Mucus, a characteristic non-Newtonian fluid, is an ideal medium through which the microactuators can penetrate. In one embodiment, the method
5 further comprises dispersing the microactuators onto mucous. In one embodiment, the dispersing comprises a nebulizer. In one embodiment, the method further comprises penetrating the microactuators through the mucous driven by magnetic non-reciprocal strokes.

Self-propelled or active particles (such as microactuators) transduce energy from their environment (e.g., from magnetic fields, ultrasound, light) into directed motion. Shields et al.,
10 “The Evolution of Active Particles: Toward Externally Powered Self-Propelling and Self-Reconfiguring Particle Systems” *Chem* 3:539–559 (2017). The advantages of using magnetic fields include, but are not limited to, deep tissue penetration, specificity to not interact with biological tissues, and high spatiotemporal resolution. Sitti et al., “Pros and Cons: Magnetic versus Optical Microrobots” *Adv. Mater.* 1906766 (2020). For example, when delivery to the
15 lungs is desired, deep penetration is needed and magnetic fields represent a useful energy source.

In one embodiment, the present invention contemplates a method comprising a time-asymmetric microscale actuator comprising an L-shaped microparticle dimer that self-propels with reciprocal, time-asymmetric (e.g., fast opening and slow closing) motions to generate propulsion when submerged in non-Newtonian fluids. In one embodiment, local viscosity
20 gradients are formed around the microscale actuators. Although it is not necessary to understand the mechanism of an invention, it is believed that such properties provide control of self-assembled time-asymmetric microscale actuators disclosed herein that provide effective clinical benefit.

Because many biological fluids (e.g., mucus, blood, and vitreous humor) are non-
25 Newtonian, such time-asymmetric microscale actuators represent improved therapeutic agent delivery vehicles. Those in the art have not pursued controlling the length, conformation, and configurations of magnetic microstructures assembled into chains (e.g., microcubes).

Although it is not necessary to understand the mechanism of an invention, it is believed that the presently disclosed microscale actuators enable a scalable production of such
30 biocompatible microcarriers. For example, standard lithographic tools may be used for fabricating the microparticles and bottom-up assembly methods increase translational readiness.

Further, the mechanics of the actuators enable fluid displacements that disrupt thick mucus, reach specific target cells (e.g., epithelial cells) and release therapeutic payloads deep within pulmonary airways. For example, the mechanism of self-propulsion has been reported to increase mucus displacement and reduce the burden of continued exposure to bronchodilators. Oehme et al., “Agonist-induced β 2-adrenoceptor desensitization and downregulation enhance pro-inflammatory cytokine release in human bronchial epithelial cells” *Pulmonary Pharmacology & Therapeutics* 30:110–120 (2015). Furthermore, magnetic field orientation and the direction of actuator propulsion are decoupled.

Current active particles used for drug delivery are usually driven by rotating magnetic fields, resulting in thrust perpendicular to the field direction. Palagi et al., “Bioinspired microrobots” *Nat. Rev. Mater.* 3:113–124 (2018). Thus, these particles *only* move along a vector defined by the magnetic field. In contrast, the presently disclosed microscale actuators can undergo locomotion any direction around the axis of the magnetic field, and thereby have a 360° range of motion that can be accessed simultaneously to drastically enhance dispersion in the complex geometry of the lungs.

V. Therapeutic Agent Release From L-Shaped Microparticles

Real time reconfiguration of microparticle-based microactuator assemblies facilitate biological applications that involve advanced motions through complex environments. Of the particle-based drug delivery systems developed so far, most are incapable of reconfiguration. Some particles can change shape; however, these particles are generally made from materials that are not biocompatible (e.g., liquid crystal elastomers). Evans et al., “Active Shape-Morphing Elastomeric Colloids in Short-Pitch Cholesteric Liquid Crystals” *Phys. Rev. Lett.* 110:187802 (2013). While there are some examples of biodegradable actuators, their structures are minimally amenable to scalable production. Ceylan et al., “3D-Printed Biodegradable Microswimmer for Theranostic Cargo Delivery and Release” *ACS Nano* 13:3353–3362 (2019).

In one embodiment, the present invention contemplates an assembly of L-shaped microparticle dimers comprising biocompatible materials that enable a scalable fabrication of microactuators configured to perform advanced motions to successfully travel and deliver therapeutic compounds at a desired target site. In one embodiment, these L-shaped microparticle dimers are microscale actuators created by a bottom-up assembly of prefabricated building

blocks. In one embodiment, the microscale actuator is repeatedly reconfigured (e.g., actuated) to induce propulsion by a mechanical disruption of a non-Newtonian medium, such as mucus. In one embodiment, magnetic reconfiguration permits propulsion in directions that are fundamentally decoupled from the directionality of the applied magnetic field.

5 The data presented herein demonstrates the capacity of microscale actuators to encapsulate and release therapeutic agents. For example, therapeutic agents were dissolved in a SU-8 photoresist and encapsulated into microscale actuators during fabrication.

 Salbutamol is a known bronchodilator that is a treatment for chronic obstructive pulmonary disease (COPD). Haghi et al., “Mono- and Cocultures of Bronchial and Alveolar
10 Epithelial Cells Respond Differently to Proinflammatory Stimuli and Their Modulation by Salbutamol and Budesonide” *Mol. Pharmaceutics* 12:2625–2632 (2015). Because SU-8 is hydrophobic, hydrophilic drugs such as salbutamol require the use of cosolvents to facilitate their homogeneous incorporation. Consequently, salbutamol release was studied from individual SU-
8 L-shaped microparticles without metal coatings.

15 L-shaped microparticle concentrations of $10^6/\text{mL}$ were used in all drug release experiments as an approximation of clinical relevance and release profiles were generated in a buffer of just physiological buffered saline (PBS) or 5 vol.% dimethyl sulfoxide (DMSO) in PBS. See, Figure 10C.

 The PBS-only release profiles reveal a characteristic burst release from the polymer
20 network over a period of 32 hours, and the release significantly exceeds a cell activation threshold of $10\text{ }\mu\text{M}$. However, release in a buffer containing 5 vol.% DMSO in PBS was more dramatic. This data suggests that microscale actuator devices encapsulating a therapeutic agent can serve as a drug delivery vehicle.

 Because the microscale actuator fabrication process is independent of any specific drug,
25 other small molecule drugs could be encapsulated within the L-shaped particles. Higham et al., “Effects of corticosteroids on COPD lung macrophage phenotype and function” *Clinical Science* 134:751–763 (2020). Additionally, the surfaces of the L-shaped microparticles or microscale actuators can be modified to facilitate facile loading of biological drugs (e.g., proteins, nucleic acids) or made from other polymers to tune drug release kinetics. Garcia et al., “Microfabricated
30 Engineered Particle Systems for Respiratory Drug Delivery and Other Pharmaceutical Applications” *Journal of Drug Delivery* 2012, 1–10 (2012); and Pulivendala et al., “Inhalation of

sustained release microparticles for the targeted treatment of respiratory diseases” *Drug Deliv. and Transl. Res.* 10:339–353 (2020).

It has been shown that the bronchodilator, salbutamol, can be encapsulated and released from SU-8 microparticles. For example, salbutamol at a concentration of 1 mg/mL was mixed
5 into the SU-8 photoresist, and contact photolithography was used to fabricate L-shaped microparticles. These particles were suspended in phosphate buffered saline (PBS and PBS containing 5% DMSO. Salbutamol release profiles are shown in each solvent and were determined to be sufficient for cell activation. See, Figure 6.

10 VI. Therapeutic Applications

In one embodiment, the present invention contemplates a method comprising delivering a microactuator encapsulating a therapeutic agent for treatment of a pulmonary disease or disorder. In one embodiment, the pulmonary disease or disorder includes, but is not limited to, cystic fibrosis, chronic obstructive pulmonary disease and/or chronic bronchitis.

15 A. Cystic Fibrosis

Cystic fibrosis is a chronic, progressive, and often fatal genetic disorder of the mucus glands, affecting over 30,000 Americans. The pathophysiology of cystic fibrosis is derived from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, leading to mucus buildup, bacterial infection, and respiratory failure. Mucosal barriers impede the delivery
20 of inhalable genes and drugs to treat symptoms and the underlying disease. Boeck K. D., “Cystic fibrosis in the year 2020: A disease with a new face” *Acta Paediatr.* 109:893–899 (2020); Martin et al., “Causes of death in French cystic fibrosis patients: The need for improvement in transplantation referral strategies!” *J. Cyst. Fibros.* 15:204–212 (2016); and Duncan et al., “The Mucus Barrier to Inhaled Gene Therapy” *Mol. Ther.* 24:2043–2053 (2016).

25 Cystic fibrosis (CF) is a genetic disorder causing chronic illness and eventually respiratory failure. Most patients with cystic fibrosis display symptoms soon after birth, with a variety of presentations that include respiratory infections and poor weight gain. The genetic basis of this disease has been linked to more than 2,000 mutations of CF transmembrane conductance regulator (*CFTR* gene. Anonymous “Cystic Fibrosis Mutation Database’
30 genet.sickkids.on.ca/. The CFTR protein is responsible for regulating the transport of chloride and sodium ions across epithelial cell membranes, and CF mutations lead to dysregulated ion

transport, causing mucus buildup, chronic infections, and the need for lifelong treatment regimes. Brown et al., “Keep them breathing: Cystic fibrosis pathophysiology, diagnosis, and treatment” J. Am. Acad. PAs 30: 23–27 (2017).

Importantly, mucus overproduction interferes with mucociliary clearance, which
5 advances disease progression and limits delivery of therapeutics to the lung epithelium. Because CF is a monogenic disorder, there is a need to improve the delivery of therapeutic agents to bronchial epithelial cells to compensate for the physiological imbalance caused by mutations in the CFTR gene. For example, pulmonary delivery of therapeutic nucleic acids is greatly limited by the physical presence of mucosal and biofilm buildup.

10 Therapeutics delivered via inhalation for CF currently include mucolytics and antibiotics. However, treatments for pulmonary delivery are limited because mucosal burdens pose a significant barrier to transport into the pulmonary tract. As a result, oral drugs (e.g., lumacaftor) have gained attention in recent years for managing the chronic effects of CF due to its ease of administration and ability to reduce the need for antibiotics in less advanced cases. Connett, G.,
15 “Lumacaftor-ivacaftor in the treatment of cystic fibrosis: design, development and place in therapy” Drug Des. Devel. Ther. 13:2405–2412 (2019). Despite this, oral administration of therapeutic agents is unlikely to fully replace inhalation administration for advanced cases of CF. Furthermore, gene and/or nucleic acid delivery to diseased epithelial cells requires that therapeutics be delivered directly to the target cells comprising the lung epithelium.

20 Therefore, there remains a need to efficiently deliver drugs and nucleic acids through mucosal biofilms for patients with CF. Several drug delivery systems have been developed to deliver micro- and nanoparticles for controlled release of drugs. However, particles used as drug carriers are typically passive and rely on diffusion to penetrate biological barriers. Ungaro et al., “Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises:
25 PLGA carriers for inhalation”. J. Pharm. Pharmacol. 64:1217–1235 (2012).

B. Chronic Obstructive Pulmonary Disease And Chronic Bronchitis

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the US with >150,000 deaths annually. Croft JB., “Urban-Rural County and State Differences in Chronic Obstructive Pulmonary Disease — United States”, Morb Mortal Wkly Rep. (2018).

30 Chronic bronchitis (CB) and emphysema are the phenotypes of COPD, which are understood as two ends of a disease spectrum, with most patients displaying symptoms of both conditions. CB

is more common, with around 9 million new diagnoses last year. CB is diagnosed by coughing and sputum production for at least 3 months per year for 2 consecutive years. Outcomes include reduced lung function, increased airflow obstruction in smokers, lower respiratory tract infection, exacerbation of COPD symptoms, lower health-related quality of life, and worsened all-cause mortality. Treatment options for CB are limited because this condition poses significant barriers to drug transport. Kim et al., “The chronic bronchitis phenotype in chronic obstructive pulmonary disease: features and implications” *Current Opinion in Pulmonary Medicine* 21(2):133–141 (2015); and Kim et al., “Chronic Bronchitis and Chronic Obstructive Pulmonary Disease” *Am J Respir Crit Care Med*. 187(3):228–237 (2013).

The proper function of mucus in the lungs is to form a protective layer that clears pathogens and particulate matter. In COPD, excessive mucus accumulates in the lungs, and inflammation, oxidative stress, infections, and particulate exposure all contribute to its pathogenesis. Mucus overproduction interferes with mucociliary clearance, compounding its progression. COPD is characterized by increased inflammation of the inner lining of the airways, increased mucin production by epithelial cells, and ionic imbalances that dry out the mucosal environment. Viscoelasticity of mucus increases when it begins to dry, and mechanical properties limit therapeutic efficacy by reducing drug diffusion. In the case of CB, prolonged exposure to proinflammatory factors and particles, like those found in tobacco smoke, cause mucus metaplasia, which limits drug transport to lung epithelial cells. Tesfaigzi Y., “Regulation of Mucous Cell Metaplasia in Bronchial Asthma” *CMM* 1;8(5):408–415 (2008).

CB treatments are few in number, and there remains a significant need to improve delivery to the bronchial epithelium to reduce mucus production and increase ciliary transport. Pulivendala et al., “Inhalation of sustained release microparticles for the targeted treatment of respiratory diseases” *Drug Deliv and Transl Res*. 10(2):339–353 (2020). Treatments for CB include smoking cessation, physical measures, short- and long-acting β 2 adrenergic receptor agonists (e.g., SABAs and LABAs), mucolytics, glucocorticoids, phosphodiesterase-4 inhibitors, and antioxidants. Unlike other pulmonary diseases, anticholinergics and antibiotics are not recommended for CB. Bronchodilators (e.g., for delivering salbutamol) are a common treatment for CB. Croft JB., “Urban-Rural County and State Differences in Chronic Obstructive Pulmonary Disease — United States” *Morb Mortal Wkly Rep*. 2018;67; [cdc.gov/mmwr/volumes/67/wr/mm6707a1.htm](https://www.cdc.gov/mmwr/volumes/67/wr/mm6707a1.htm). There are many drug delivery systems for

bronchodilators that have been used to deliver micro- and nanoparticles for controlled release of drugs. Ungaro et al., “Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises: PLGA carriers for inhalation” *Journal of Pharmacy and Pharmacology* 64(9):1217–1235 (2012). However, propelled transport of these particles through mucosal films
5 remains a major challenge.

C. Cellular Responses

In one embodiment, the present invention contemplates a method comprising administering a microscale actuator encapsulating a therapeutic compound and releasing the therapeutic compound at a biological target site such that the therapeutic compound elicits a
10 cellular response. For example, microactuators that release salbutamol within a pulmonary tract would be expected to potentiate an increase in interleukin (IL)-6/8 secretion and induce significant changes in the expression of intracellular cyclic adenosine monophosphate (cAMP) and $\beta 2$ adrenergic receptor ($\beta 2$ -AR). This cellular response to salbutamol can be demonstrated in 16HBE14o- cell cultures by comparing salbutamol-negative controls to salbutamol alone.

15 Drug release from conventional PLGA microparticles involves heterogeneous mechanisms and is diffusion-driven. Han et al., “Bioerodable PLGA-Based Microparticles for Producing Sustained-Release Drug Formulations and Strategies for Improving Drug Loading” *Front Pharmacol.* (2016). Salbutamol is a short-acting $\beta 2$ -AR agonist. $\beta 2$ -AR receptors are found throughout the respiratory system, including epithelial and airway smooth muscle cells.
20 The cellular responses to salbutamol are well-understood, providing a clear avenue to test their efficacy. The 16HBE14o- human bronchial epithelial cell line is widely used to model barrier function of the airway epithelium and to study respiratory ion transport. This cell line has been shown to exclusively express $\beta 2$ -AR subtypes and secrete IL-6 and IL-8 in response to salbutamol. Forbes et al., “The human bronchial epithelial cell line 16HBE14o- as a model
25 system of the airways for studying drug transport” *International Journal of Pharmaceutics* 257(1–2):161–167 (2003). Molecular expression and cytokine secretion has been investigated using 2D 16HBE14o cell cultures. Cellular responses to treatment with PLGA microactuators loaded with salbutamol can be ascertained after penetration through mucosal barriers ranging in thickness from 10 to 50 μm .

30 Bronchodilators, such as salbutamol, have been reported to generate pro-inflammatory responses after prolonged use, causing an increase in the production of pro-inflammatory

cytokines mediated by cAMP. At certain concentrations (i.e., $\geq 10 \mu\text{M}$), $\beta 2$ agonism by salbutamol triggers an initial increase in cAMP, followed by significant decrease by 24 hours. Changes in intracellular cAMP expression can be detected using a cAMP ELISA. Salbutamol-treated cells may be collected at different time points (i.e., 1, 6, and 24 hours) and lysed using a
5 lysis buffer kit and stored for later analysis at -80°C . To correlate cAMP concentration with cytokine secretion, samples can be collected from the same set of plates for both purposes. Cells without mucus may be incubated with different doses of free salbutamol as positive controls.

IL-6 and IL-8 are mediators of an innate immune response which is increased in response to $\beta 2$ -AR agonism by salbutamol. Each cytokine can be measured after 24 hours following
10 introduction of microactuators. To collect samples, media from the bottom of the transwell plates may be taken and stored at -80°C until analysis by ELISA. Cells without mucus can be incubated with different doses of free salbutamol as positive controls. Negative controls can include salbutamol alone (no actuators) and no salbutamol.

To determine the molecular expression of $\beta 2$ -AR, cells can be cultured with the drug-
15 loaded microactuators after penetrating a mucosal film. Subsequently, the cells are extracted and stained with fluorescent anti- $\beta 2$ -AR antibodies to measure surface expression. Unlike cAMP, $\beta 2$ -AR only decreases with time in response to $\beta 2$ agonism; thus, $\beta 2$ -AR expression is measured after 24 hours. As before, expression of $\beta 2$ -AR can be studied as a function of drug concentration and time against the same controls (positive and negative) as before.

20 Treatment with microactuators encapsulated with salbutamol is expected to produce potent changes in biomarkers of salbutamol activity in bronchial epithelial cell cultures. This effect may follow a brief time delay due to: (i) the transport of actuators through the mucosal films on the apical side of the cell culture; and (ii) the time needed for expression levels to significantly change in response to salbutamol. Biomarker levels can also be validated after 48
25 hours.

Alternatively, the microscale actuators can be validated using BEAS2B cells, which have been reported to demonstrate β -agonism effects. Yan et al., "Analysis of the Indacaterol-Regulated Transcriptome in Human Airway Epithelial Cells Implicates Gene Expression Changes in the Adverse and Therapeutic Effects of $\beta 2$ -Adrenoceptor Agonists" J Pharmacol Exp
30 Ther. 1;366(1):220–236 (2018). This cell line does not form polarized epithelia, which complements data collected with the 16HBE14o cell line. qPCR may be used in parallel with

ELISA to confirm the expression of genes related to β -agonism (e.g., *AREG*, *BDNF*, *CCL20*, *CXCL2*, *EDNI*, *IL6*, *IL15*, and *IL20*). Uhlig et al., “The effects of salbutamol on epithelial ion channels depend on the etiology of acute respiratory distress syndrome but not the route of administration” *Respir Res.* 15(1):56 (2014).

5 **D. Digestive Diseases Or Disorders**

In one embodiment, the present invention contemplates a method of treating a gastrointestinal disease or disorder comprising a non-Newtonian fluid with a microscale actuator as described herein. In one embodiment, the non-Newtonian fluid includes, but is not limited to, mucus, pus, blood and/or digestive fluid.

10 Gastroesophageal reflux disease (GERD), diarrhea and colorectal cancer are examples of gastrointestinal diseases. When examined, some diseases show nothing wrong with the GI tract, but there are still symptoms. Other diseases have symptoms, and there are also visible irregularities in the GI tract. Most gastrointestinal diseases can be prevented and/or treated.

Gastrointestinal diseases affect the gastrointestinal (GI) tract from the mouth to the anus.

15 There are two types: functional and structural. Some examples include nausea/vomiting, food poisoning, lactose intolerance and diarrhea.

Functional diseases are those in which the GI tract looks normal when examined, but doesn't move properly. They are the most common problems affecting the GI tract (including the colon and rectum). Constipation, irritable bowel syndrome (IBS), nausea, food poisoning, gas,

20 bloating, GERD and diarrhea are common examples.

Many factors may upset your GI tract and its motility (ability to keep moving), including:

Eating a diet low in fiber.

Not getting enough exercise.

Traveling or other changes in routine.

25 Eating large amounts of dairy products.

Stress.

Resisting the urge to have a bowel movement, possibly because of hemorrhoids.

Overusing anti-diarrheal medications that, over time, weaken the bowel muscle movements called motility.

30

Taking antacid medicines containing calcium or aluminum.

Taking certain medicines (especially antidepressants, iron pills and strong pain medicines such as narcotics).

Pregnancy.

5 Structural gastrointestinal diseases are those where your bowel looks abnormal upon examination and also doesn't work properly. Sometimes, the structural abnormality needs to be removed surgically. Common examples of structural GI diseases include strictures, stenosis, hemorrhoids, diverticular disease, colon polyps, colon cancer and inflammatory bowel disease.

1. Constipation

10 Constipation, which is a functional problem, makes it hard for you to have a bowel movement (or pass stools), the stools are infrequent (less than three times a week), or incomplete. Constipation is usually caused by inadequate "roughage" or fiber in your diet, or a disruption of your regular routine or diet. Constipation causes you to strain during a bowel movement. It may cause small, hard stools and sometimes anal problems such as fissures and hemorrhoids. Constipation is rarely the sign that you have a more serious medical condition.

15 2. Irritable bowel syndrome (IBS)

Irritable bowel syndrome (also called spastic colon, irritable colon, IBS, or nervous stomach) is a functional condition where your colon muscle contracts more or less often than "normal." Certain foods, medicines and emotional stress are some factors that can trigger IBS. Symptoms of IBS include:

- 20 Abdominal pain and cramps.
Excess gas.
Bloating.
Change in bowel habits such as harder, looser, or more urgent stools than normal.
Alternating constipation and diarrhea.

25 3. Hemorrhoids

Hemorrhoids are dilated veins in the anal canal, structural disease. They're swollen blood vessels that line your anal opening. They are caused by chronic excess pressure from straining during a bowel movement, persistent diarrhea, or pregnancy. There are two types of hemorrhoids: internal and external. Internal hemorrhoids are blood vessels on the inside of your
30 anal opening. When they fall down into the anus as a result of straining, they become irritated and start to bleed. Ultimately, internal hemorrhoids can fall down enough to prolapse (sink or

stick) out of the anus. External hemorrhoids are veins that lie just under the skin on the outside of the anus. Sometimes, after straining, the external hemorrhoidal veins burst and a blood clots form under the skin. This very painful condition is called a “pile.”

4. Anal fissures

5 Anal fissures are also a structural disease. They are splits or cracks in the lining of your anal opening. The most common cause of an anal fissure is the passage of very hard or watery stools. The crack in the anal lining exposes the underlying muscles that control the passage of stool through the anus and out of the body. An anal fissure is one of the most painful problems because the exposed muscles become irritated from exposure to stool or air, and leads to intense
10 burning pain, bleeding, or spasm after bowel movements. Initial treatment for anal fissures includes pain medicine, dietary fiber to reduce the occurrence of large, bulky stools and sitz baths (sitting in a few inches of warm water). If these treatments don't relieve your pain, surgery might be needed to repair the sphincter muscle.

5. Perianal abscesses

15 Perianal abscesses, also a structural disease, can occur when the tiny anal glands that open on the inside of your anus become blocked, and the bacteria always present in these glands causes an infection. When pus develops, an abscess forms. Treatment includes draining the abscess, usually under local anesthesia in the healthcare provider's office.

6. Anal fistula

20 An anal fistula – again, a structural disease – often follows drainage of an abscess and is an abnormal tube-like passageway from the anal canal to a hole in the skin near the opening of your anus. Body wastes traveling through your anal canal are diverted through this tiny channel and out through the skin, causing itching and irritation. Fistulas also cause drainage, pain and bleeding. They rarely heal by themselves and usually need surgery to drain the abscess and
25 "close off" the fistula.

7. Perianal infections

 Sometimes the skin glands near your anus become infected and need to be drained, like in this structural disease. Just behind the anus, abscesses can form that contain a small tuft of hair at the back of the pelvis (called a pilonidal cyst). Sexually transmitted diseases that can affect the
30 anus include anal warts, herpes, AIDS, chlamydia and gonorrhea.

8. Diverticular disease

The structural disease diverticulosis is the presence of small outpouchings (diverticula) in the muscular wall of your large intestine that form in weakened areas of the bowel. They usually occur in the sigmoid colon, the high-pressure area of the lower large intestine.

Diverticular disease is very common and occurs in 10% of people over age 40 and in 5 50% of people over age 60 in Western cultures. It is often caused by too little roughage (fiber) in the diet. Diverticulosis can sometimes develop/progress into diverticulitis

Complications of diverticular disease happen in about 10% of people with outpouchings. They include infection or inflammation (diverticulitis), bleeding and obstruction. Treatment of diverticulitis includes treating the constipation and sometimes antibiotics if really severe. 10 Surgery is needed as last resort in those who have significant complications to remove the involved diseased segment of the colon.

9. Colon polyps and cancer

Each year, 130,000 Americans are diagnosed with colorectal cancer, the second most common form of cancer in the United States. Fortunately, with advances in early detection and 15 treatment, colorectal cancer is one of the most curable forms of the disease. By using a variety of screening tests, it is possible to prevent, detect and treat the disease long before symptoms appear. Almost all colorectal cancers begin as polyps, benign (non-cancerous) growths in the tissues lining your colon and rectum. Cancer develops when these polyps grow and abnormal cells develop and start to invade surrounding tissue. Removal of polyps can prevent the 20 development of colorectal cancer. Almost all precancerous polyps can be removed painlessly using a flexible lighted tube called a colonoscope. If not caught in the early stages, colorectal cancer can spread throughout the body. More advanced cancer requires more complicated surgical techniques.

Most early forms of colorectal cancer do not cause symptoms, which makes screening 25 especially important. When symptoms do occur, the cancer might already be quite advanced. Symptoms include blood on or mixed in with the stool, a change in normal bowel habits, narrowing of the stool, abdominal pain, weight loss, or constant tiredness.

10. Colitis

There are several types of colitis, which are conditions that cause an inflammation of the 30 bowel. These include:

Infectious colitis.

Ulcerative colitis (cause unknown).

Crohn's disease (cause unknown).

Ischemic colitis (caused by not enough blood going to the colon).

Radiation colitis (after radiotherapy).

- 5 Colitis causes diarrhea, rectal bleeding, abdominal cramps and urgency (frequent and immediate need to empty the bowels). Treatment depends on the diagnosis, which is made by colonoscopy and biopsy.

Experimental

10

Example I

Finite Volume Simulations

- Various particle designs were simulated using the computational fluid dynamics (CFD) software ANSYS. Previous results were verified, and new particle shapes were designed to
15 establish relationships between geometry and transport behavior. It was found that particles with greater “sweep angles” showed faster propulsion. Sweep angle in this context refers to the angle through which one side of the actuator moves during a single stroke. Photomasks were designed including four different particle shapes so that experimental and computational findings could be compared.

20

Example II

Microactuator Fabrication And Device Design

- Particles of various sizes and geometries were made by photolithography with poly(methyl methacrylate) (PMMA) and SU-8. It has been reported that different sizes and
25 geometries of particles be used to target different regions within the lungs. For example, an actuator size range of 4–15 μm prevents aggregation and controls deposition deep within the lungs. Ruge et al., “Pulmonary drug delivery: from generating aerosols to overcoming biological barriers—therapeutic possibilities and technological challenges” *Lancet Respir. Med.* 1:402–413 (2013); and Pulivendala et al., “Inhalation of sustained release microparticles for the targeted
30 treatment of respiratory diseases” *Drug Deliv. Transl. Res.* 10:339–353 (2020).

PMMA was synthesized and made into a photoresist material following established methods. Carbaugh et al., “Photolithography with polymethyl methacrylate (PMMA)” *Semicond. Sci. Technol.* 31:025010 (2016); and Christensen et al., “Gelation of Copolymers with Pendent Benzophenone Photo-Cross-Linkers” *Macromolecules* 45:5237–5246 (2012).

Automated alignment with maskless photolithography and GLAD can be performed to add magnetic patches onto well-defined regions of the particles (Fig. 1). Pawar et al., “Multifunctional Patchy Particles by Glancing Angle Deposition” *Langmuir* 25:9057–9063 (2009). Each metal patch consists of 10 nm chromium and 100 nm cobalt for magnetic susceptibility. Once the metal is deposited, the second (sacrificial) layer of photoresist can be removed. Particles can be suspended in low concentrations of ferrofluid (i.e., 0.6–1.2 vol.%) for directed assembly. Shields et al., “Supercolloidal Spinners: Complex Active Particles for Electrically Powered and Switchable Rotation” *Adv. Funct. Mater.* 28:1803465 (2018).

Alternatively, particle orientation control during assembly can be managed with magnetic templating methods to orient particles on microparticle surfaces which then drive their assembly into actuators. Yellen et al., “Programmable Assembly of Colloidal Particles Using Magnetic Microwell Templates” *Langmuir* 20:2553–2559 (2004).

The fabricated microscale actuator device comprises a drug formulation and a dispersing instrument. Different types of microscale actuator devices include, but are not limited to, a pressurized metered dose inhaler, a dry powder inhaler, and a nebulizer.

Example III

Actuator Transport Through Mucosal Films

Mucosal films 10 to 50 μm thick can be made from synthetic materials following earlier methods. Hamed et al., “Synthetic tracheal mucus with native rheological and surface tension properties: Synthetic Tracheal Mucus with Native Properties” *J Biomed Mater Res.*

102(6):1788–1798 (2014). Microactuators can be fabricated from PMMA, and sizes can be varied from 4–15 μm , which is within previously tested ranges for nebulization and inhalation.

Garcia et al., “Microfabricated Engineered Particle Systems for Respiratory Drug Delivery and Other Pharmaceutical Applications” *Journal of Drug Delivery* 2012:1–10 (2012). Dimensions

can be tuned to target the peripheral and/or central regions of the respiratory system. Ruge et al., “Pulmonary drug delivery: from generating aerosols to overcoming biological barriers—

therapeutic possibilities and technological challenges” *The Lancet Respiratory Medicine* 1(5):402–413 (2013); and Tsuda et al., “Particle Transport and Deposition: Basic Physics of Particle Kinetics” In: Terjung R, editor. *Comprehensive Physiology*. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2013. p. 1437–1471. Penetration through artificial mucosal films can be tested, and magnetic field parameters can be optimized, including field strength, frequency, on/off-rates, and duration. Penetration can be assessed at different time points. Optimized actuators can be validated using sputum samples from patients with COPD.

A variety of mucus models can be used to test drug transport, including intact tissues, *in vitro* cell cultures, reconstituted mucin samples, and patient samples. In the present example, synthetic mucus was chosen, as its rheology can be tuned to match the high variability of patient-derived mucus samples. Lock et al., “Mucus models to evaluate the diffusion of drugs and particles” *Adv. Drug Deliv. Rev.* 124:34–49 (2018).

Synthetic mucus was prepared following earlier methods from commercially available proteins, lipids, glycoproteins, ions, and water. Viscoelasticity of mucus was measured using a parallel plate rheometer to tune compositions to match the range of patient samples with CF. Hamed et al., “Synthetic tracheal mucus with native rheological and surface tension properties: Synthetic Tracheal Mucus with Native Properties” *J. Biomed. Mater. Res. A* 102:1788–1798 (2014). After dispersing particles onto mucosal films, multiparticle tracking (MPT) can be conducted using time-lapse microscopy and image-processing scripts. The performance of the microactuators can be energized by different cyclic magnetic fields (i.e., 70 Gauss field strengths) and suspended in different fluid compositions mimicking native mucus samples. Particles without magnetic films can be used as controls. Actuators are then validated in sputum from patients diagnosed with COPD.

Nebulization uses an electrically powered pump to deliver air to a suspension and creates a fine aerosol that can be inhaled by the patient. Nebulization can be used to disperse actuators across artificial mucosal films over a 10 cm x 10 cm surface at a fixed separation distance (e.g., 50 cm). Nebulization was chosen because CF patients commonly use this method to inhale mucolytics. Henke et al., “Mucolytics in cystic fibrosis” *Paediatr. Respir. Rev.* 8:24–29 (2007).

Fluorescence imaging and multiparticle tracking (MPT) can be used to study the distribution of particles on surfaces using both methods and quantitatively compared via root mean square (RMS) spatial deviations.

Subsequently, particles can be coated with dithiothreitol, a disulfide reducing agent, to improve mucus penetration. Seo et al., “Using UV-absorbance of intrinsic dithiothreitol (DTT) during RP-HPLC as a measure of experimental redox potential *in vitro*” *Anal. Bioanal. Chem.* 405, 6379–6384 (2013). Two coating strategies can be tested. First, due to its negative charge, dithiothreitol can be added to the surfaces of the particles using electrostatic complexation with the cationic poly(allylamine) hydrochloride. Shields et al., “Cellular backpacks for macrophage immunotherapy” *Sci. Adv.* 6:eaaz6579 (2020). Second, particles can be modified with a metal phenolic network coating to display free galloyl groups. Available galloyl groups can then bind to dithiothreitol prior to washing and dispersion. UV-Vis can be used to confirm the presence of dithiothreitol from both methods after solvent-mediated extraction into purified solution (peak absorbance at 280 nm). Zhao et al., “Engineering of Living Cells with Polyphenol-Functionalized Biologically Active Nanocomplexes” *Adv. Mater.* n/a, 2003492.

Viscoelasticity of mucus was measured using a parallel plate rheometer. After dispersing particles onto mucosal films, multiparticle tracking can be conducted using time-lapse microscopy and image-processing scripts.

Example IV

Magnetic Programming Of In Vitro Drug Release In Cell Culture

Microscale actuators for *in vitro* salbutamol release can be programmed by diffusion and magnetic hyperthermia.

It is expected that *in vivo* salbutamol release onto 16HBE14o- cell cultures will potentiate an increase in IL-6 and IL-8 secretion and induce significant changes in the expression of cAMP and β -AR as compared to salbutamol-negative controls. While most inhalable drugs for CF therapy are mucolytics and antibiotics, these drugs do not target the epithelium. Thus, bronchodilator delivery should be predictive of an ability to deliver drugs and genetic material to epithelial cells. Salbutamol is a short-acting β -AR agonist bronchodilator. Cellular responses to salbutamol are well-known, providing a clear route to test their efficacy. Uhlig et al., “The effects of salbutamol on epithelial ion channels depend on the etiology of acute respiratory distress syndrome but not the route of administration” *Respir. Res.* 15:56 (2014); Haghi et al., “Mono- and Cocultures of Bronchial and Alveolar Epithelial Cells Respond Differently to Proinflammatory Stimuli and Their Modulation by Salbutamol and Budesonide” *Mol. Pharm.*

12:2625–2632 (2015); and Oehme et al., “Agonist-induced β 2-adrenoceptor desensitization and downregulation enhance pro-inflammatory cytokine release in human bronchial epithelial cells” *Pulmonary Pharmacology & Therapeutics* 30:110–20 (2015).

Salbutamol can be encapsulated into particles at different concentrations using
5 established techniques. Release may be studied by diffusion and magnetic hypothermia using a UV-Vis-based cumulative release assay (peak absorbance at 280 nm). Shields et al., “Encapsulation and controlled release of retinol from silicone particles for topical delivery” *J. Control. Release Off. J. Control. Release Soc.* 278:37–48 (2018).

Varying magnetic field strengths (1–20 mT), frequencies (100–500 kHz) and durations
10 (10–600 sec) can be used to induce release compared to no magnetic stimulation, following established conditions. Rivera-Rodriguez et al., “Magnetic nanoparticle hyperthermia potentiates paclitaxel activity in sensitive and resistant breast cancer cells” *Int. J. Nanomedicine* 13:4771–4779 (2018); and Torres-Lugo et al., “Thermal potentiation of chemotherapy by magnetic nanoparticles” *Nanomed.* 8:1689–1707 (2013).

15 It has been reported that epithelial cell responses occur at ~ 10 μ M salbutamol. Unwalla et al., “Albuterol Modulates Its Own Transepithelial Flux via Changes in Paracellular Permeability” *Am. J. Respir. Cell Mol. Biol.* 46:551–558 (2012). Salbutamol release onto 16HBE14o- human bronchial epithelial cells cultured in transwell plates can be performed to study respiratory ion transport. This cell line exclusively expresses β 2-AR subtypes and secretes
20 IL-6 and IL-8 in response to salbutamol. Forbes et al., “The human bronchial epithelial cell line 16HBE14o- as a model system of the airways for studying drug transport” *Int. J. Pharm.* 257:161–167 (2003).

Actuators can be deposited on top of cell cultures prior to depositing mucus for studying drug release. Cells without mucus can be incubated with free salbutamol as positive controls.
25 Negative controls can include actuators without salbutamol and no exogenous salbutamol or actuators.

Example V

Therapeutic Agent Encapsulation

30 Therapeutic agents were added to the fabrication process in accordance with Examples II or VIII for their encapsulation into time-asymmetric microscale actuators. For example, the

therapeutic agents were incorporated into the polymer regions of the microscale actuators by dissolving the drug directly into the PLGA solution prior to spin coating on the polydimethylsiloxane (PDMS) stamp at different loadings (e.g., 1, 2, 4, and 8 wt.% in solids).

20 mg of the bronchodilator salbutamol was dissolved in 20 mL SU-8 photoresist (SU-8 TF 6002). 20 μ L of acetone was used as a cosolvent to improve solubility. Photolithography was performed using a photomask and mask aligner (Karl Suss MJB3). 3-inch wafers were used in fabrication. After fabrication, particles were extracted using a rubber policeman and suspended in one of two solvents: PBS or PBS containing 5 vol.% DMSO. Particle concentrations were fixed at 10^6 /mL.

Drug release in cultured bronchial epithelial cells is achieved by culturing cells on transwell plates and quantified using ELISA for all biomarkers, including cAMP, and interleukins-6 and -8. Oehme et al., “Agonist-induced β 2-adrenoceptor desensitization and downregulation enhance pro-inflammatory cytokine release in human bronchial epithelial cells” *Pulm. Pharmacol. Ther.* 30:110–120 (2015). Drugs as well as nucleic acids are delivered from microscale actuators.

Example VI

Cell Transfection

Transfecting epithelial cells with mRNA encoding for the CFTR protein has been shown to treat several phenotypes of CF. Harvey et al., “Airway epithelial CFTR mRNA expression in cystic fibrosis patients after repetitive administration of a recombinant adenovirus”. *J. Clin. Invest.* 104:1245–1255 (1999). Incorporation of *CFTR* mRNA onto the surfaces of the microscale actuators using electrostatic assembly can be achieved with polyallylamine hydrochloride. Wu et al., “Electrostatic Nucleic Acid Nanoassembly Enables Hybridization Chain Reaction in Living Cells for Ultrasensitive mRNA Imaging” *J. Am. Chem. Soc.* 137:6829–6836 (2015). It is expected that this system could prove to be a drug and nucleic acid delivery system for treating pulmonary conditions including, but not limited to, cystic fibrosis.

Example VII Microcontact Printing

Microcontact printing can be used to make actuators from PLGA following a reported method. Shields et al., “Cellular backpacks for macrophage immunotherapy” Sci Adv. 1;6(18):eaaz6579 (2020). Photolithography can be used to pattern SPR 220 on a silicon wafer using a photomask. Photopatterned wafers can be passivated with trichloro(1H,1H,2H,2H-perfluorooctyl) silane as a non-stick material. PDMS (10:1 base to crosslinker) can be poured over the wafers, which can then be degassed in a vacuum desiccator and cured. Cured stamps can then be separated from the patterned substrate. PLGA microparticles can be formed by spin-coating an ink (i.e., a solution of PLGA in acetone) on the PDMS stamp, which can then be pressed on a surface of polyvinyl alcohol (PVA) above a heated water bath. Next, the particles are coated with a sacrificial layer to guide the placement of metallic patches via GLAD. Chromium and cobalt can be deposited, as described above. Finally, the sacrificial layer can be removed using a gentle solvent wash, leaving behind the finished the biodegradable particles for assembly and storage.

Example VIII Time-Asymmetric Microscale Actuator Fabrication

L-shaped, microparticles were fabricated from an SU-8 photoresist (SU-8 3010, Kayaku Advanced Materials) using maskless photolithography (μ MLA, Heidelberg). The SU-8 resist was diluted with cyclopentanone and spin coated to achieve a film thickness of 3.5 μ m, as measured by stylus profilometry. SU-8 was spin-coated at 2000 rpm for 30 seconds, soft-baked for 2 minutes at 95°C, and exposed to specific patterns using maskless photolithography. The SU-8 particles were revealed after a post-exposure bake of 1 minute at 65°C and 2 minutes at 95°C followed by development in propylene glycol methyl ether acetate (PGMEA) for 1 minute.

In addition to the array of L-shaped particles, eight alignment markers were fabricated for alignment lithography. The array of microparticles was coated with a second photoresist layer (AZ 9260, Integrated Micromaterials) to serve as a blocking layer on top of the SU-8. See, Figure 7A: Steps 3 and 4. For the lift-off steps to be successful, it is ideal that dedicated lift-off resists are used.

A positive-tone AZ 9260 was used to fabricate holes around the L-shaped particles. AZ 9260 was spin-coated at 2000 rpm for 60 seconds to a thickness of 10 μm , baked at 110°C for 3 minutes, and patterned using maskless photolithography. An array of holes was patterned on top of the L-shaped particles to reveal the inside of the long arm. This resist can be removed in
5 common solvents such as dimethyl sulfoxide (DMSO), acetone, and N-methyl-2-pyrrolidone (NMP).

However, stripping of this resist leaves small sections of the wafer still coated with AZ 9260 and metal. Thus, subsequent release of particles cannot easily be accomplished without excess cobalt releasing into solution. This negatively affects the magnetic assembly of the
10 particles by the formation of large contaminants.

All microscopy images shown herein come from particle fabrication using AZ 9260. Glancing angle deposition (GLAD) was performed using an e-beam evaporator (Edwards Auto 306 Cryo). 10 nm of chromium and 100 nm of cobalt were evaporated onto the substrate held at an angle of 75° from the plane normal to the vapor direction. This angle was necessary to guide
15 the deposition of metal to the interior edge of the particles. See, Figure 7A (bottom).

Cobalt was evaporated from a tungsten crucible to prevent alloying, as tungsten has a higher melting point. Kannan et al., “Ultrahigh supercapacitance in cobalt oxide nanorod film grown by oblique angle deposition technique” *Current Applied Physics* 18:1399–1402 (2018). After metal deposition, the AZ layer can be removed using AZ Kwik Strip according to
20 recommendations provided by the manufacturer. Washing with Kwik Strip was performed using two baths of solution with gentle agitation. Briefly, two beakers containing Kwik Strip were heated to 60°C. The sample was placed in the first beaker for 30 minutes. Then the sample was placed in the second beaker for an additional 30 minutes to remove any remaining photoresist.

Particles were removed by submerging the silicon wafer in a solution of NMP for
25 approximately one hour. NMP swells the SU-8 polymer network, making particles susceptible to detachment from the silicon. Kim et al., “Review of polymer MEMS micromachining” *J. Micromech. Microeng.* 26, 013001 (2016). Particles could then be mechanically removed using a razor blade and were resuspended in a 0.1% solution of Tween 20 at a concentration of 10^6 particles/mL. Resuspension was carried out by centrifugation at 5000xg for 5 minutes, aspiration
30 of solvent, addition of fresh solvent, and sonication for an additional 10 minutes. Prior to

experiments, the particles solution was filtered through a cell strainer to remove larger strands of cobalt metal.

A dedicated lift-off resist (e.g., AZ nLOF 2070) may improve fabrication procedures by eliminating the formation of debris and thus the need for a separation step using a cell strainer and the formation of large contaminants after extracting particles from silicon. AZ nLOF 2070 is a lift-off photoresist used industrially in applications requiring metal deposition.

The sidewall of the photoresist should have a negative profile, preventing the deposition of continuous metal films. Although this is difficult to achieve with AZ 9260, negative profiles are easily achievable with AZ nLOF resists so that complete stripping using solvent washes is trivial. The profile of AZ nLOF resists can be tuned by different process conditions to allow for easier lift-off. A layer of AZ nLOF 2070 exposes only the vertical sidewall of the SU-8 structure, allowing for metal deposition.

Example IX

Microscale Actuator Testing System

A 15 μ L solution suspension of microscale actuators was sandwiched between two glass coverslips. See, Figure 8A. Vacuum grease was applied around the edges of the smaller (22 mm x 22 mm) coverslip to create a sealed chamber and prevent the evaporation of water.

The microscale actuators were magnetized by a uniform magnetic field generated by a collinear pair of electromagnetic coils. The coils rested on a 3D-printed microscope stage on an inverted fluorescence microscope. The coils were wound by hand and produced field strengths up to 15 mT at a current of 5 A, which is sufficient to magnetize the metallic films on the particles, estimated to be 8 nm chromium and 80 nm cobalt after GLAD. The electromagnetic coils were then connected to a function generator (33120A, Agilent Technologies) so that arbitrary, time-varying magnetic fields could be generated. Two different signal outputs were programmed: 1) slow increase and rapid decrease in the applied field strength and 2) rapid decrease and slow increase in the applied field strength. See, Figure 8B and 8C, respectively. Videos of magnetic assembly and actuation were taken using a CCD camera connected to the microscope at 15 frames/s.

Particles were sometimes made fluorescent through the incorporation of fluorophores (e.g., Nile red) by dissolving the fluorophores directly into the photoresist (1 mg fluorophore into

10 mL photoresist). Fluorescence microscopy could then be used to observe particle trajectories in fluids in which visibility is limited. For example, solutions of mucin proteins used to simulate the rheology of mucus introduce high scattering that makes it difficult to resolve individual particles.

5 Therapeutic release may also be investigated following microscale actuator propulsion through fluids of variable thickness and viscosity using fluorescence imaging and MPT, as described above.

Example X

10 Therapeutic Agent Release From Time-Asymmetric Microscale Actuators

Microscale actuators encapsulating a therapeutic agent were fabricated in accordance with Example V. After fabrication, microactuators were mixed with artificial mucus samples and stored at physiological temperatures. Aliquots were taken and particles extracted from the mucus.

15 Drug release experiments were carried out as follows: 1) vials containing 1 mL of solution were centrifuged at 2000xg for 5 min, 2) solvents were aspirated from each vial, and 3) particles were resuspended in fresh solvent.

Salbutamol concentration was measured by LCMS (characteristic absorbance peak is at 276 nm) (43) at different time points (e.g., 1, 6, 12, 24, 48, and 72 hours). If components from
20 the artificial mucus interfere with spectroscopic readings, collected particles may be dissolved with acetone, and total amount of drug released determined by subtracting the initial drug amount from the amount of drug retained in the microactuators.

This data was used to form cumulative release curves. To tune salbutamol release rates, drug release can be investigated from microactuators formed from PLGA of increasing
25 molecular weight (e.g., 10, 25, 40, and 65 kDa). Hines et al., "Poly (lactic-co-glycolic acid) controlled release systems: experimental and modeling insights" Crit Rev Ther Drug Carrier Syst. 30(3):257–276 (2013).

Example XI

Computational Fluid Dynamics Simulations And Microparticle Design

Numerical simulations were done to establish relationships between particle shape and transport properties. For each design, actuators underwent time-asymmetric strokes in a shear-thinning fluid which identified a significant dependence of transport on particle size and sweep angle. Variations in the sweep angle led to significant changes in propulsion. These findings led to a design of new particle shapes for use in contact or maskless photolithography.

Photolithography has been used to fabricate $\sim 10^7$ particles per wafer. See, Figure 4B. SU-8 was used in fabrication following the manufacturer's protocol. SU-8 and PMMA were used in fabrication to a resolution down to 1.5 μm .

Example XII

Poly(methyl methacrylate) (PMMA) Synthesis And Characterization

PMMA copolymers containing benzophenone groups were synthesized and used to formulate a new photoresist material. See, Figure 4A. This material was characterized using NMR, which showed 2.5% benzophenone groups within the polymer chain. PMMA was selected as the material of interest because of its successful history in drug delivery. Bettencourt et al., "Poly(methyl methacrylate) particulate carriers in drug delivery" J. Microencapsul. 29, 353–367 (2012).

PMMA copolymers were dissolved in toluene at various concentrations, and relationships between PMMA concentration and film thickness were established. PMMA film thickness was measured using a Dektak stylus profilometer. PMMA fabrication was performed without soft or post-exposure bakes, and exposure energies in the range of 3.3–3.5 J/cm² were successful. See, Figure 4B.

Example XIII

Synthetic Mucus

Synthetic mucus that mimics the rheological properties of native mucus was made and characterized by rheology. This was accomplished by crosslinking porcine mucin type III proteins with glutaraldehyde in a buffer solution similar to that lining human airways. Small volumes of the protein gel were subject to oscillatory rheology. The data give evidence of an

extremely weak gel with shear-thinning properties, enabling the study of actuator self-propulsion in a biomimetic material system. The storage and loss moduli were on the order of 3–4 Pa, which is in agreement with literature values for synthetic mucus materials.

5

10

Claims

We claim:

- 5 1. An L-shaped microparticle dimer and a dipole-dipole hinge, wherein each L-shaped microparticle comprises a first arm comprising a dipole and a second arm that is shorter than or equal to the first arm.
2. The dimer of Claim 1, wherein said dipole-dipole hinge links said first arm of each of
10 said L-shaped microparticles.
3. The dimer of Claim 1, wherein said second arm of said each L-shaped microparticle is oriented in the same direction.
- 15 4. The dimer of Claim 1, wherein a side surface of said first arm comprises a magnetic metal patche.
5. The dimer of Claim 4, wherein said plurality of magnetic patches are ferromagnetic metal patches.
20
6. The dimer of Claim 1, wherein said first arm has a terminus, said terminus comprising a dipole.
7. The dimer of Claim 5, wherein said plurality of ferromagnetic metal patches comprise a
25 metal selected from the group consisting of iron, cobalt and nickel.
8. The dimer of Claim 1, wherein said L-shaped microparticle dimer encapsulates a therapeutic drug.
- 30 9. The dimer of Claim 8, wherein said encapsulated therapeutic drug is releasable.

10. A method, comprising:
- a) providing;
 - i) a first L-shaped microparticle comprising a first arm and a second arm that is shorter than or equal to said first arm;
 - ii) a second L-shaped microparticle comprising a third arm and a fourth arm that is shorter than or equal to said third arm;
 - iii) a magnetic metal; and
 - iv) a magnetic field;
 - b) coating a side surface of said first and third arms of said first and second L-shaped microparticles with said magnetic metal to create a plurality of magnetic metal patches;
 - c) exposing said first and second L-shaped microparticles to said magnetic field to induce a dipole in each of said first and third arms;
 - d) linking said first and second L-shaped microparticles with said dipoles to create an L-shaped microparticle dimer comprising a dipole-dipole hinge, such that said second and fourth arms are oriented orthogonal to the direction of said magnetic field.
11. The method of Claim 10, wherein said coating further comprises a ferrofluid.
12. The method of Claim 10, wherein said plurality of magnetic metal patches are ferromagnetic metal patches.
13. The method of Claim 10, wherein said coating comprises photolithography.
14. The method of Claim 10, wherein said coating comprises glancing angle deposition.
15. The method of Claim 10, wherein said coating comprises resist lift-off.
16. The method of Claim 10, wherein said linking comprises self-limiting assembly.

17. The method of Claim 12, wherein said ferromagnetic metal patche comprises a metal selected from the group consisting of iron, cobalt and nickel.
- 5 18. The method of Claim 10, wherein said first and second L-shaped microparticles encapsulate a therapeutic drug.
19. The method of Claim 18, wherein said encapsulated therapeutic drug is releasable.
- 10 20. The method of Claim 10, wherein said first and second L-shaped microparticles are coated with a therapeutic drug.
21. The method of Claim 20, wherein said coated therapeutic drug is releaseable
- 15 22. A method, comprising:
- a) providing;
 - i) a microscale actuator comprising an L-shaped microparticle dimer and a therapeutic compound;
 - ii) a patient comprising at least one symptom of a disorder or disease comprising a non-Newtonian fluid; and
 - 20 iii) a cyclic magnetic field;
 - b) administering said microscale actuator to said patient; and
 - c) exposing said patient to said cyclic magnetic field wherein said at least one symptom of said disorder or disease is reduced.
- 25
23. The method of Claim 22, wherein said microscale actuator self-propels through said non-Newtonian fluid.
24. The method of Claim 23, wherein said self-propulsion comprises a time-asymmetric motion of said L-shaped microparticle dimer induced by said cyclic magnetic field.
- 30

25. The method of Claim 22, wherein said non-Newtonian fluid comprises mucus.
26. The method of Claim 22, wherein said non-Newtonian fluid comprises blood.
- 5 27. The method of Claim 22, wherein said non-Newtonian fluid comprises vitreous humor.
28. The method of Claim 22, wherein said disease or disorder comprises a pulmonary disorder or disease or a digestive disorder or disease.
- 10 29. The method of Claim 28, wherein said pulmonary disorder or disease is selected from the group consisting of cystic fibrosis, chronic obstructive pulmonary disorder and chronic bronchitis.
30. The method of Claim 22, wherein said exposing further comprises releasing said
15 therapeutic compound from said microscale actuator.
31. The method of Claim 22, wherein said therapeutic compound is a drug.
32. The method of Claim 31, wherein said drug is a small organic molecule.
- 20 33. The method of Claim 31, wherein said drug is a bronchodilator.
34. The method of Claim 31, wherein said drug is a steroid.
- 25 35. The method of Claim 31, wherein said drug is an antibacterial drug.
36. The method of Claim 22, wherein said therapeutic compound is a nucleic acid.
37. The method of Claim 36, wherein said nucleic acid is an antisense.
- 30

38. The method of Claim 37, wherein said antisense is selected from the group consisting of siRNA, shRNA and miRNA.
39. The method of Claim 22, wherein said therapeutic compound is a polypeptide.
- 5 40. The method of Claim 39, wherein said polypeptide is an antibody.
41. The method of Claim 40, wherein said antibody is a monoclonal antibody.
- 10 42. The method of Claim 22, wherein said L-shaped microparticle dimer comprises a plurality of magnetic metal patches and a dipole-dipole hinge, wherein each L-shaped microparticle comprises a first arm comprising a dipole and a second arm that is shorter than or equal to said first arm.
- 15 43. The method of Claim 42, wherein said hinge comprises a dipole-dipole interaction.
44. The method of Claim 37, wherein said polypeptide is a cytokine.
- 20 45. The method of Claim 22, wherein said therapeutic drug is encapsulated within said microscale actuator.
46. The method of Claim 22, wherein said therapeutic drug is coated on the surface of said microscale actuator.
- 25 47. The method of Claim 22, wherein said therapeutic drug is releaseable,

Figure 1

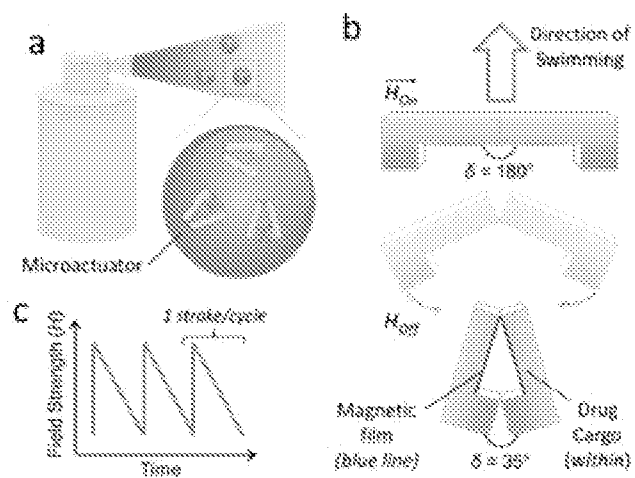


Figure 2

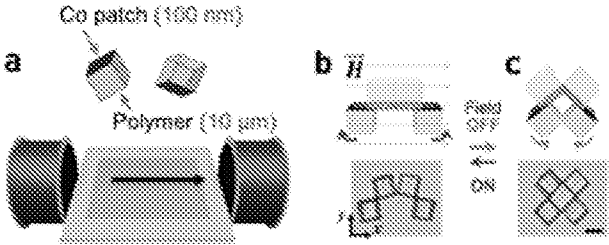


Figure 3

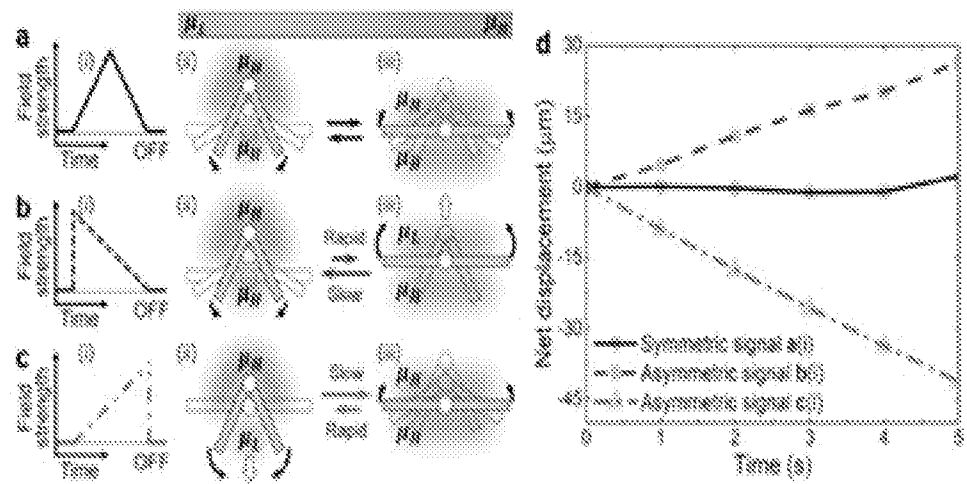


Figure 4

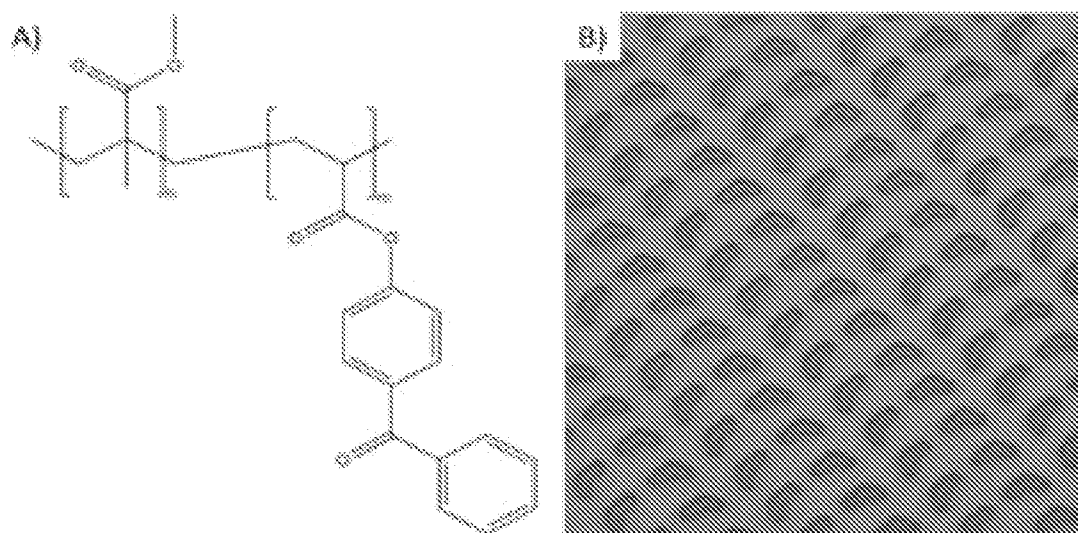


Figure 5

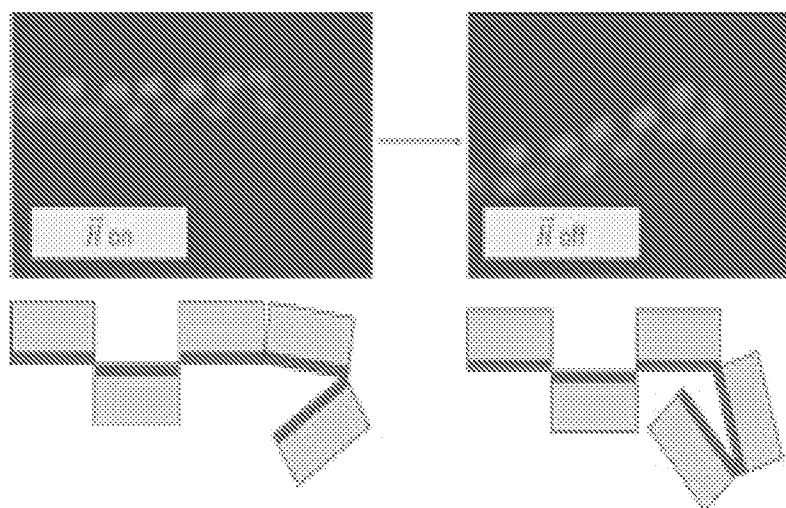


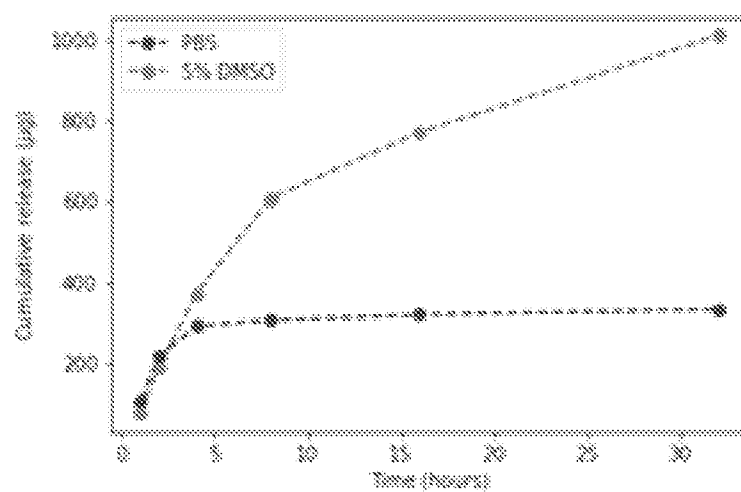
Figure 6

Figure 7

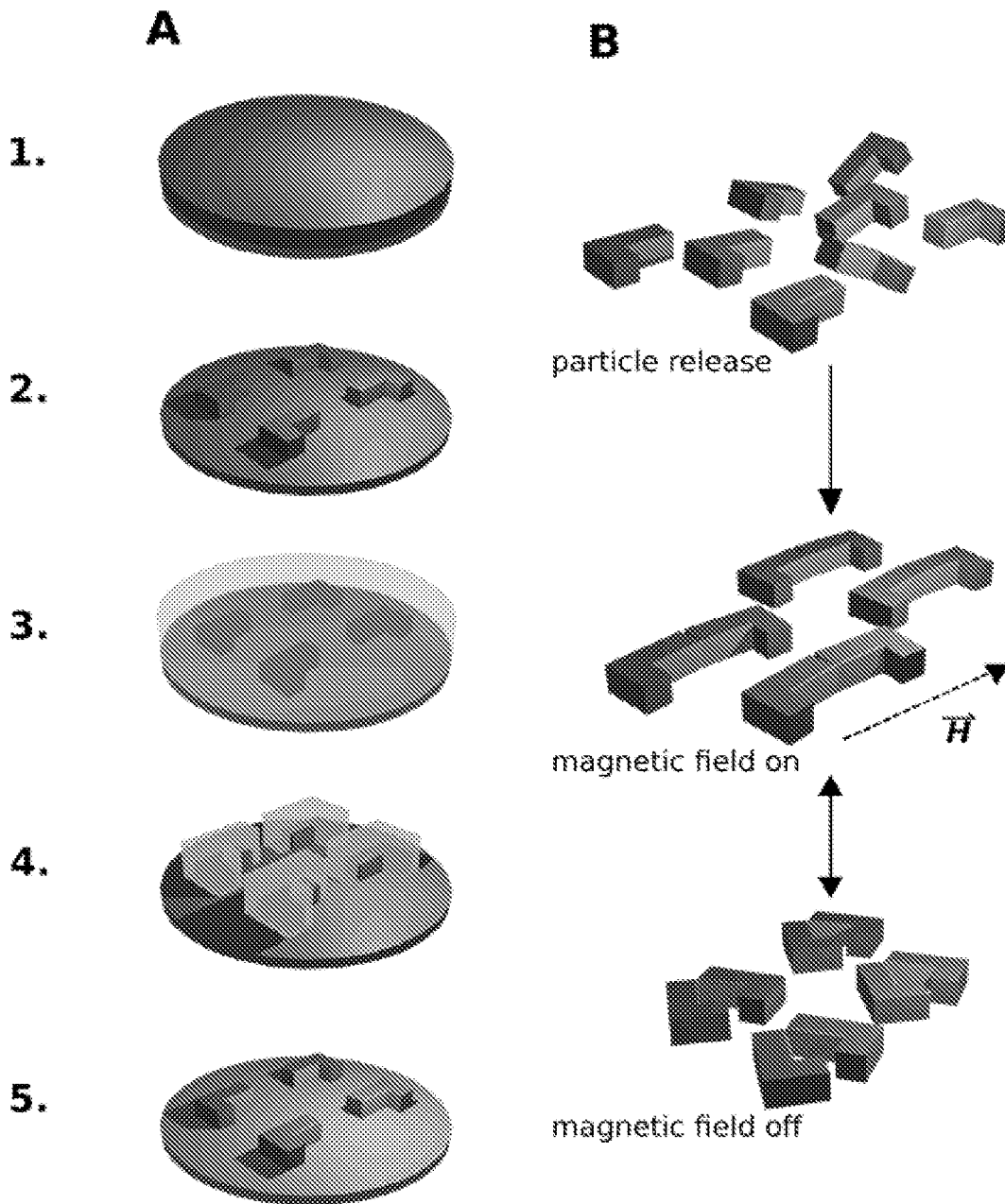


Figure 8

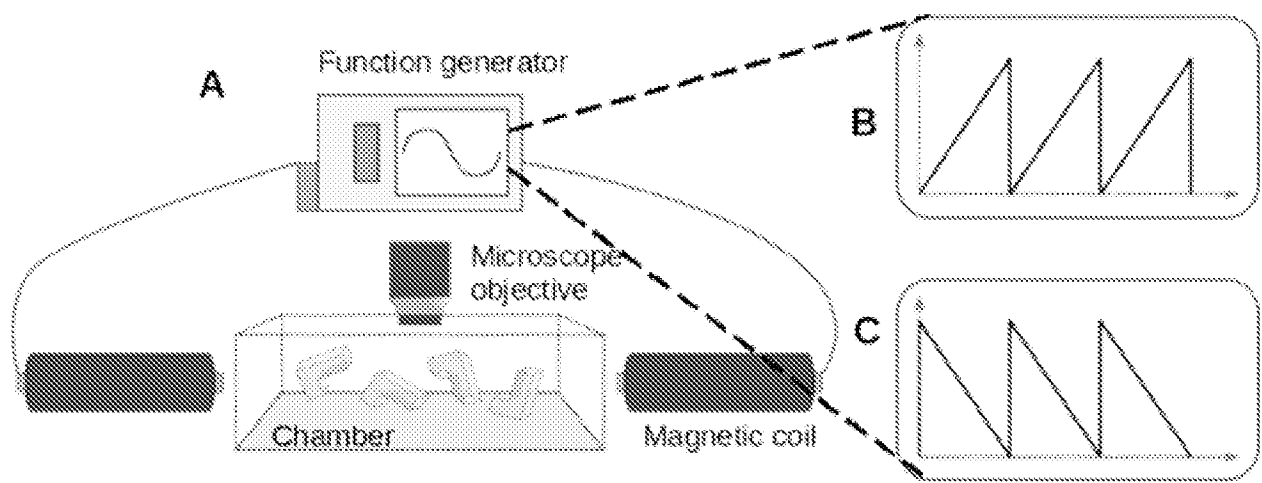


Figure 9

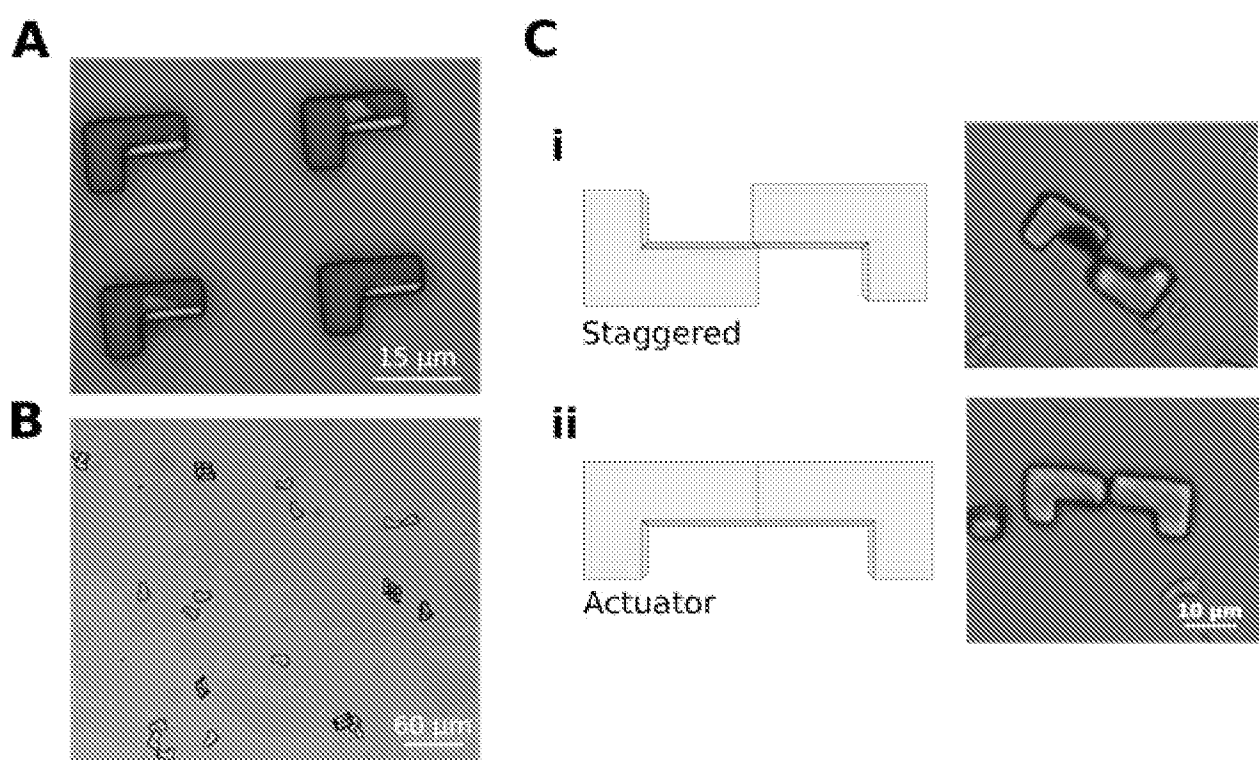


Figure 10

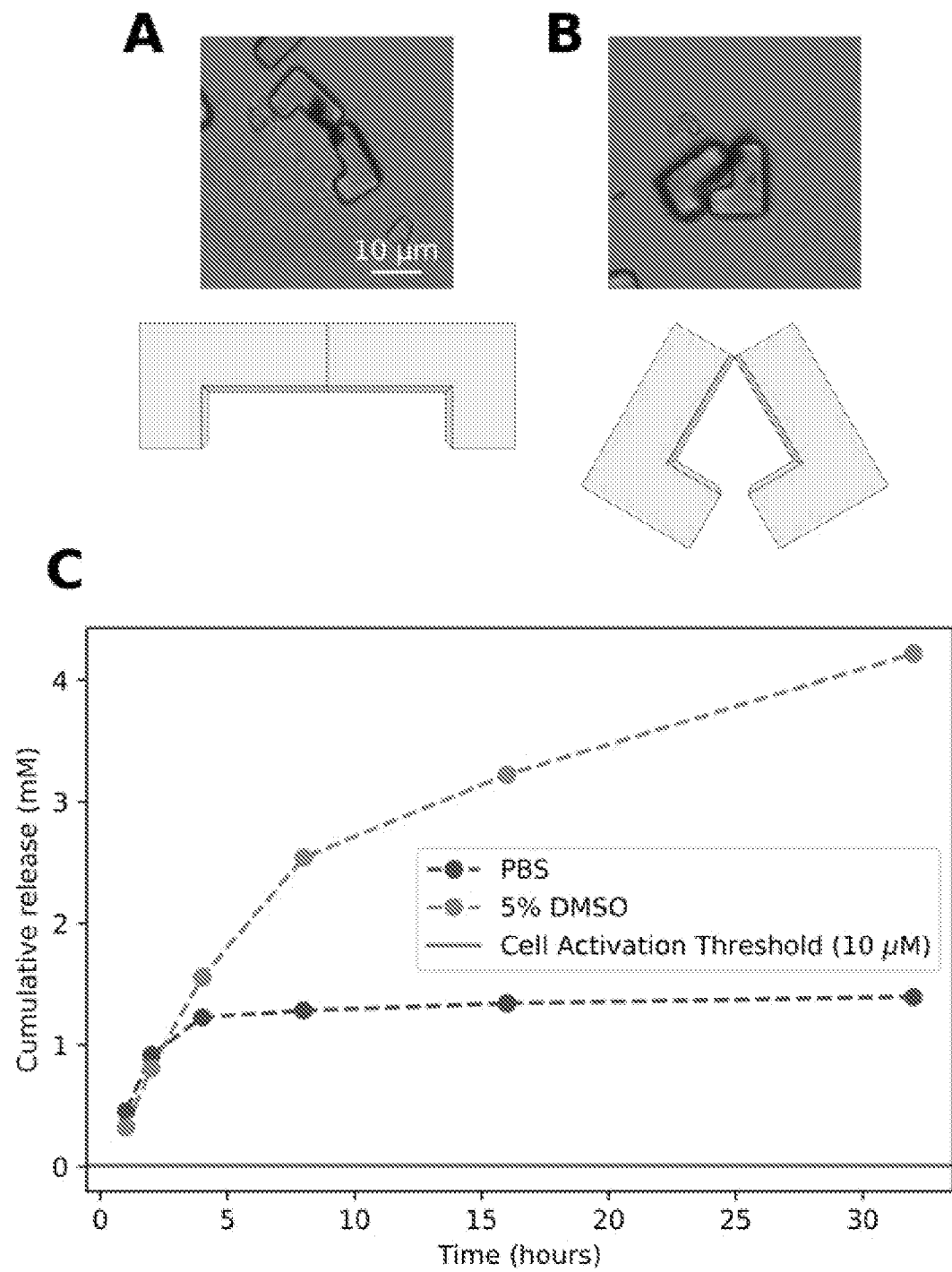
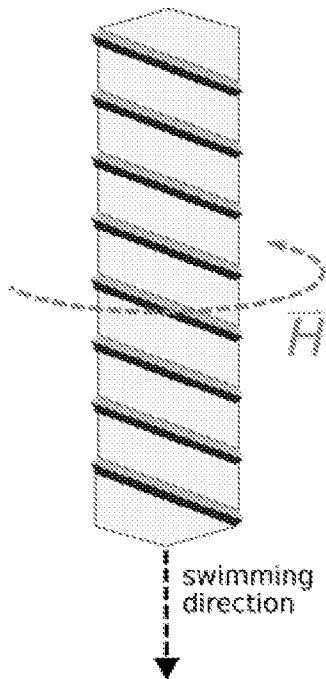


Figure 11

Helical microrobot**Microactuators**