[0006] Nucleic acids like DNA and RNA are of increasing interest for various therapeutic treatments. Various reports describe approaches for administration of nucleic acids. See, e.g., US10485884, herein incorporated by reference for all purposes. One approach is leveraging the ability of cationic liposomes (which induce DNA/RNA condensations) to facilitate cellular uptake of DNA or RNA into specific cells. The cationic liposomes usually consist of a cationic lipid, like DOTMA and/or DOTAP, and one or more helper lipids, like DOPE. So-called 'lipoplexes' can be formed from the cationic (positively charged) liposomes and the anionic (negatively charged) nucleic acid. Lipoplexes may form spontaneously by mixing the nucleic acid with the liposomes, driven by electrostatic interactions between the positively charged liposomes and the negatively charged nucleic acid. Accordingly, methods and systems for producing, mixing, transferring and/or manufacturing pharmaceutical compositions and formulations comprising RNA and lipid (e.g., liposomes) are required.

SUMMARY

[0007] Provided herein are peristaltic pump systems that include a dampener for reducing the pulsations or oscillations of the flowrate from the peristaltic pumps in the system. In some examples, these systems are useful to produce, mix, transfer and/or manufacture pharmaceutical compositions and formulations, including, e.g., compositions and formulations comprising RNA and lipids, including lipoplexes or liposomes. As explained above, the flowrate from a peristaltic pump can pulse or oscillate over time due to the nature of peristaltic pumps. Thus, peristaltic pumps may not be suitable for certain uses where a smooth or consistent flow rate is required. An example of such a use can be when the peristaltic pumps are used to displace pharmaceutical compositions. These pharmaceutical compositions can include delicate and expensive components. In addition, the amount of a given component in the pharmaceutical composition can be critical to whether that pharmaceutical composition will be effective and safe for its intended use. For example, pharmaceutical compositions and formulations comprising RNA and lipids (such as lipoplexes or liposomes) are sensitive to: (1) the dynamic ratio of nucleic acids and lipids/liposomes when they are formed upon mixing and; (2) the average flow rate used during mixing. If the dynamic flow rate of the nucleic acids varies dynamically during operation, the ratio of nucleic acids to lipids/liposomes will vary throughout the mixing operation resulting in greater heterogeneity in the quality attributes of the resulting lipoplexes (size, polydispersity index, surface charge, etc.). Syringe pumps can be used to mix nucleic acids and liposomes/lipids (including, e.g., RNA and lipids) to form lipoplexes useful in manufacturing RNA vaccines (see, e.g., Oberli M.A et al. Nano Lett. 2017,

17, 1326−1335, or Kauffman, K. J. et al. Nano Lett. 2015, 15, 7300−7306; see also WO2019077053). Syringe pumps generate flow that has relatively low pulsation and the mixing ratio of two or more solutions can be well-controlled. However, normal syringes do not offer a closed, aseptic boundary outside of a Grade A cleanroom environment because the inside of the syringe barrel is exposed to the ambient environment before the syringe plunger is pulled to load fluid into the syringe barrel. This is particularly critical for systems in which the liposomes and/or lipoplex are too big to pass through a sterilizing grade filter and cannot be terminally sterilized without significant degradation. Peristaltic pumps, by contrast, can be used with fully closed fluid paths that do not require operation in a Grade A cleanroom environment to maintain aseptic processing and sterility assurance. This is a large advantage, because Grade A cleanroom environments are costly to maintain and require time-consuming environmental monitoring controls. Accordingly, Applicants have discovered methods and systems using peristaltic pumps that includes a dampener to drastically reduce the pulsations or oscillations from peristaltic pumps useful to produce, mix, transfer, and/or manufacture pharmaceutical compositions and formulations, including, e.g., compositions and formulations comprising RNA and lipids, including lipoplexes or liposomes, e.g., RNA vaccines. Although the dampeners disclosed herein are discussed in combination with peristaltic pumps, the pump system does not necessarily have to be a peristaltic pump system, as dampeners could be combined with any pumping system that generates pulses as part of its mechanism of action (including, e.g., membrane, piston, etc.). For example, a syringe pump system can use the dampeners disclosed herein. In some examples, these methods and systems using peristaltic pumps that includes a dampener are suitable to ensure smooth or consistent flow rate to produce, mix, transfer and/or manufacture pharmaceutical compositions comprising RNA and lipids (including lipoplexes or liposomes), including, e.g., RNA vaccines.

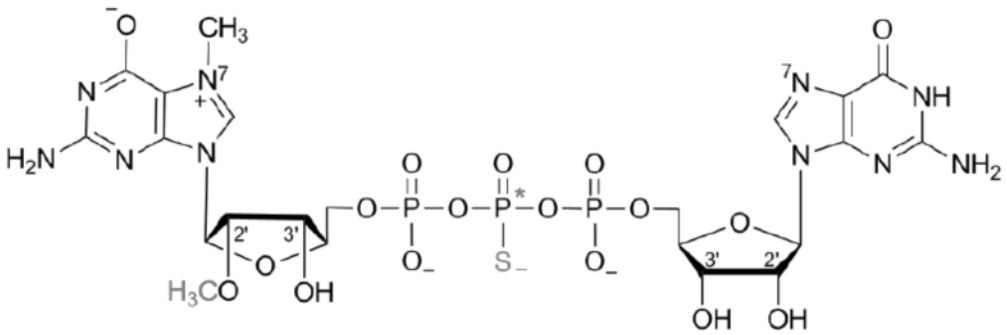
[0008] In one aspect, the present disclosure refers to a tubing kit for forming a mixture comprising: a first portion of tubing configured to be fluidly connected to a container containing a first composition; a second portion of tubing configured to be fluidly connected to a container containing a second composition; a dampener fluidly connected to the first portion of tubing and fluidly connected to the second portion of tubing; a mixer for mixing the first composition from the first portion of tubing and the second composition from the second portion of tubing; a mixture container for collecting the mixed first composition and second composition from the mixer, wherein the first portion of tubing is configured to be connected to at least one peristaltic pump head for pumping the first composition from the container containing the first composition to the mixture container, and the second portion of tubing is configured to be connected to at least one peristaltic pump head for pumping the second composition from the container containing the second composition to the mixture container. In some examples, a tubing kit for forming a mixture includes: a first portion of tubing configured to be fluidly connected to a container containing a first composition; a second portion of tubing configured to be fluidly connected to a container containing a second composition; a dampener fluidly connected to the first portion of tubing and fluidly connected to the second portion of tubing; a mixer for mixing the first composition from the first portion of tubing and the second composition from the second portion of tubing; a mixture container for collecting the mixed first composition and second composition from the mixer, wherein the first portion of tubing is configured to be connected to at least one peristaltic pump head for pumping the first composition from the container containing the first

composition to the mixture container, and the second portion of tubing is configured to be connected to at least one peristaltic pump head for pumping the second composition from the container containing the second composition to the mixture container. In some examples, the dampener comprises an enclosed volume of fluid. In some examples, the fluid is air. In some examples, the dampener is a tubing dampener. In some examples, the dampener comprises a flexible membrane. In some examples, the tubing kit includes a first tee connector that fluidly connects the dampener, the first portion of tubing, and a first mixer input portion of tubing, wherein the first mixer input portion of tubing fluidly connects to the mixer. In some examples, the tubing kit includes a second tee connector that fluidly connects the dampener, the second portion of tubing, and a second mixer input portion of tubing, wherein the second mixer input portion of tubing fluidly connects to the mixer. In some examples, the first portion of tubing comprises a first segment of tubing and a second segment of tubing, wherein the first segment of tubing and the second segment of tubing are fluidly connected in parallel. In some examples, the first segment of tubing is configured to be connected to a first peristaltic pump head, and the second segment of tubing is configured to be connected to a second peristaltic pump head. In some examples, the second portion of tubing comprises a third segment of tubing and a fourth segment of tubing, wherein the third portion of tubing and the fourth portion of tubing are fluidly connected in parallel. In some examples, the third segment of tubing is configured to be connected to a third peristaltic pump head, and the fourth segment of tubing is configured to be connected to a fourth peristaltic pump head. In some examples, the mixer comprises an input fluidly connected to the first portion of tubing, an input fluidly connected to the second portion of tubing, and output fluidly connected to the mixture container. In some examples, the mixer comprises a Y-connector, a helical mixer, or a static mixer. In some examples, the tubing kit includes a first dampener connector that fluidly connects the first portion of tubing to the dampener and to the mixer and a second dampener connector that fluidly connects the second portion of the tubing to the dampener and to the mixer. In some examples, the mixture container is a bag, vessel, or bottle.

[0009] In one aspect, the present disclosure refers to a system for forming a pharmaceutical composition or mixture of pharmaceutical compositions, the system comprising: a first container containing a first pharmaceutical composition; a second container containing a second pharmaceutical composition; a first portion of tubing fluidly connected to the first container; a second portion of tubing fluidly connected to the second container; a dampener fluidly connected to the first portion of tubing and fluidly connected to the second portion of tubing; a mixer for mixing the first pharmaceutical composition from the first portion of tubing and the second pharmaceutical composition from the second portion of tubing; and a mixture container for collecting the mixed first pharmaceutical composition and second pharmaceutical composition from the mixer. In some examples, a system for forming a pharmaceutical composition or mixture of pharmaceutical compositions, the system includes: a first container containing a first pharmaceutical composition; a second container containing a second pharmaceutical composition; a first portion of tubing fluidly connected to the first container; a second portion of tubing fluidly connected to the second container; a dampener fluidly connected to the first

portion of tubing and fluidly connected to the second portion of tubing; a mixer for mixing the first pharmaceutical composition from the first portion of tubing and the second pharmaceutical composition from the second portion of tubing; and a mixture container for collecting the mixed first pharmaceutical composition and second pharmaceutical composition from the mixer. In some examples, the system includes at least one peristaltic pump head connected to the first portion of tubing for pumping the first composition from the container containing the first composition to the mixture container, and at least one peristaltic pump connected to the second portion of tubing for pumping the second composition from the container containing the first composition to the mixture container. In some examples, the first composition or second composition comprises a nucleic acid, one or more lipids, one or more proteins, or a buffer. In some examples, the first composition comprises a nucleic acid and the second composition comprises one or more lipids. In some examples, the first composition comprises RNA and the second composition comprises one or more lipids. In some examples, the RNA comprises one or more polynucleotides encoding 10-20 neoepitopes resulting from cancer-specific somatic mutations present in a tumor specimen. In some examples, the RNA is formulated in a lipoplex nanoparticle or liposome. In some examples, the lipoplex nanoparticle or liposome comprises one or more lipids that form a multilamellar structure that encapsulates the RNA. In some examples, the one or more lipids comprises at least one cationic lipid and at least one helper lipid. In some examples, the one or more lipids comprises (R)-N,N,N–trimethyl–2,3–dioleyloxy–1–propanaminium chloride (DOTMA) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). In some examples, at physiological pH the overall charge ratio of positive charges to negative charges of the liposome is 1.3:2 (0.65). In some examples, the RNA comprises an RNA molecule comprising, in the 5'→3' direction: (1) a 5' cap; (2) a 5' untranslated region (UTR); (3) a polynucleotide sequence encoding a secretory signal peptide; (4) a polynucleotide sequence encoding the one or more neoepitopes resulting from cancer-specific somatic mutations present in a tumor specimen; (5) a polynucleotide sequence encoding at least a portion of a transmembrane and cytoplasmic domain of a major histocompatibility complex (MHC) molecule; (6) a 3' UTR comprising: (a) a 3' untranslated region of an Amino-Terminal Enhancer of Split (AES) mRNA or a fragment thereof; and (b) non-coding RNA of a mitochondrially encoded 12S RNA or a fragment thereof; and (7) a poly(A) sequence. In some examples, the RNA molecule further comprises a polynucleotide sequence

encoding an amino acid linker; wherein the polynucleotide sequences encoding the amino acid linker and a first of the one or more neoepitopes form a first linker-neoepitope module; and wherein the polynucleotide sequences forming the first linker-neoepitope module are between the polynucleotide sequence encoding the secretory signal peptide and the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule in the 5'→3' direction. In some examples, the amino acid linker comprises the sequence GGSGGGGSGG (SEQ ID NO:21). In some examples, the polynucleotide sequence encoding the amino acid linker comprises the sequence GGCGGCUCUGGAGGAGGCGGCUCCGGAGGC (SEQ ID NO:19). In some examples, the RNA molecule further comprises, in the 5'→3' direction: at least a second linker-epitope module, wherein the at least second linker-epitope module comprises a polynucleotide sequence encoding an amino acid linker and a polynucleotide sequence encoding a neoepitope; wherein the polynucleotide sequences forming the second linker-neoepitope module are between the polynucleotide sequence encoding the neoepitope of the first linker-neoepitope module and the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule in the 5'→3' direction; and wherein the neoepitope of the first linker-epitope module is different from the neoepitope of the second linker-epitope module. In some examples, the RNA molecule comprises 5 linker-epitope modules, and wherein the 5 linker-epitope modules each encode a different neoepitope. In some examples, the RNA molecule comprises 10 linker-epitope modules, and wherein the 10 linker-epitope modules each encode a different neoepitope. In some examples, the RNA molecule comprises 20 linker-epitope modules, and wherein the 20 linker-epitope modules each encode a different neoepitope. In some examples, the RNA molecule further comprises a second polynucleotide sequence encoding an amino acid linker, wherein the second polynucleotide sequence encoding the amino acid linker is between the polynucleotide sequence encoding the neoepitope that is most distal in the 3' direction and the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule. In some examples, the 5' cap comprises a D1 diastereoisomer of the structure:



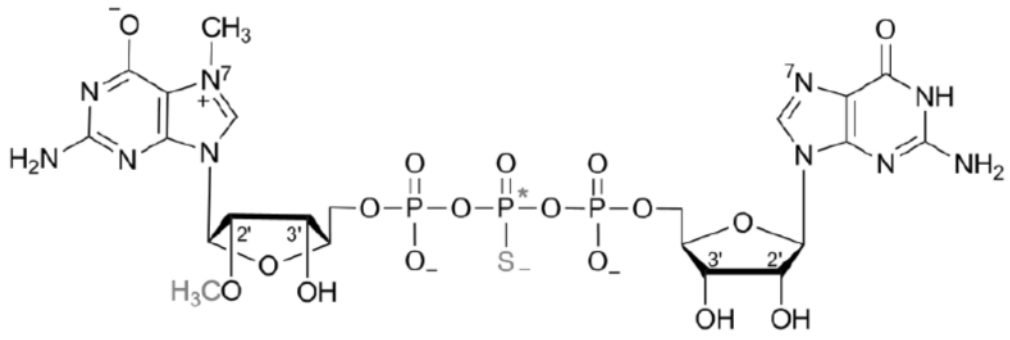
. In some examples, the 5’ UTR comprises the sequence UUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACC (SEQ ID NO:5). In some examples, the 5’ UTR comprises the sequence GGCGAACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACC (SEQ ID NO:3). In some examples, the secretory signal peptide comprises the amino acid sequence MRVMAPRTLILLLSGALALTETWAGS (SEQ ID NO:9). In some examples, the polynucleotide sequence encoding the secretory signal peptide comprises the sequence AUGAGAGUGAUGGCCCCCAGAACCCUGAUCCUGCUGCUGUCUGGCGCCCUGGC CCUGACAGAGACAUGGGCCGGAAGC (SEQ ID NO:7). In some examples, the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule comprises the amino acid sequence IVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSDVSLTA (SEQ ID NO:12). In some examples, the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule comprises the sequence AUCGUGGGAAUUGUGGCAGGACUGGCAGUGCUGGCCGUGGUGGUGAUCGGAG CCGUGGUGGCUACCGUGAUGUGCAGACGGAAGUCCAGCGGAGGCAAGGGCGGC AGCUACAGCCAGGCCGCCAGCUCUGAUAGCGCCCAGGGCAGCGACGUGUCACU GACAGCC (SEQ ID NO:10). In some examples, the 3’ untranslated region of the AES mRNA comprises the sequence CUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCUUUCCCGUCCUGGGUACCCC GAGUCUCCCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGCCCCACU CACCACCUCUGCUAGUUCCAGACACCUCC (SEQ ID NO:15). In some examples, the non-coding RNA of the mitochondrially encoded 12S RNA comprises the sequence CAAGCACGCAGCAAUGCAGCUCAAAACGCUUAGCCUAGCCACACCCCCACGGG AAACAGCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAAGCUAUAC

UAACCCCAGGGUUGGUCAAUUUCGUGCCAGCCACACCG (SEQ ID NO:17). In some examples, the 3' UTR comprises the sequence CUCGAGCUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCUUUCCCGUCCUGGG UACCCCGAGUCUCCCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGC CCCACUCACCACCUCUGCUAGUUCCAGACACCUCCCAAGCACGCAGCAAUGCA GCUCAAAACGCUUAGCCUAGCCACACCCCCACGGGAAACAGCAGUGAUUAACC UUUAGCAAUAAACGAAAGUUUAACUAAGCUAUACUAACCCCAGGGUUGGUCA AUUUCGUGCCAGCCACACCGAGACCUGGUCCAGAGUCGCUAGCCGCGUCGCU (SEQ ID NO:13). In some examples, the poly(A) sequence comprises 120 adenine nucleotides. In some examples, the RNA comprises an RNA molecule comprising, in the 5'→3' direction: the polynucleotide sequence GGCGAACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACCAU GAGAGUGAUGGCCCCCAGAACCCUGAUCCUGCUGCUGUCUGGCGCCCUGGCCC UGACAGAGACAUGGGCCGGAAGC (SEQ ID NO:1); a polynucleotide sequence encoding the one or more neoepitopes resulting from cancer-specific somatic mutations present in the tumor specimen; and the polynucleotide sequence AUCGUGGGAAUUGUGGCAGGACUGGCAGUGCUGGCCGUGGUGGUGAUCGGAG CCGUGGUGGCUACCGUGAUGUGCAGACGGAAGUCCAGCGGAGGCAAGGGCGGC AGCUACAGCCAGGCCGCCAGCUCUGAUAGCGCCCAGGGCAGCGACGUGUCACU GACAGCCUAGUAACUCGAGCUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCU UUCCCGUCCUGGGUACCCCGAGUCUCCCCCGACCUCGGGUCCCAGGUAUGCUC CCACCUCCACCUGCCCCACUCACCACCUCUGCUAGUUCCAGACACCUCCCAAGC ACGCAGCAAUGCAGCUCAAAACGCUUAGCCUAGCCACACCCCCACGGGAAACA GCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAAGCUAUACUAACC CCAGGGUUGGUCAAUUUCGUGCCAGCCACACCGAGACCUGGUCCAGAGUCGCU AGCCGCGUCGCU (SEQ ID NO:2).

In one aspect, the present disclosure refers to a method for transferring pharmaceutical compositions using peristaltic pumps comprising: pumping a first composition from a first container through a first portion of tubing using at least one peristaltic pump; pumping a second composition from a second container through a second portion of tubing using at least one peristaltic pump; and dampening pulse in a fluid flow of the first composition in the first portion of tubing and dampening pulses in a fluid flow of the second composition in the second portion of tubing using a dampener fluid connected to the first portion of tubing and fluidly connected to the second portion of tubing. In some examples, a method for transferring pharmaceutical composition using peristaltic pumps includes: pumping a first composition from a first container through a first portion of tubing using at least one peristaltic pump; pumping a second composition from a second container through a second portion of tubing using at least one peristaltic pump; and dampening pulse in a fluid flow of the first composition in the first portion of tubing and dampening pulses in a fluid flow of the second composition in the second portion of tubing using a dampener fluid connected to the first portion of tubing and fluidly connected to the

second portion of tubing. In some examples, the method includes mixing the first composition from the first portion of tubing and the second composition from the second portion of tubing in a mixer fluidly connected to the first portion of tubing and the second portion of tubing. In some examples, the method includes depositing the mixture containing the first composition and the second composition into a mixture container that is fluidly connected to the mixture. In some examples, the first composition or second composition comprises a nucleic acid, one or more lipids, one or more proteins, or a buffer. In some examples, the first composition comprises a nucleic acid and the second composition comprises one or more lipids. In some examples, the first composition comprises RNA and the second composition comprises one or more lipids. In some examples, the RNA comprises one or more polynucleotides encoding 10-20 neoepitopes resulting from cancer-specific somatic mutations present in a tumor specimen. In some examples, the RNA is formulated in a lipoplex nanoparticle or liposome. In some examples, the lipoplex nanoparticle or liposome comprises one or more lipids that form a multilamellar structure that encapsulates the RNA. In some examples, the one or more lipids comprises at least one cationic lipid and at least one helper lipid. In some examples, the one or more lipids comprises (R)-N,N,N–trimethyl–2,3–dioleyloxy–1–propanaminium chloride (DOTMA) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). In some examples, at physiological pH the overall charge ratio of positive charges to negative charges of the liposome is 1.3:2 (0.65). In some examples, the RNA comprises an RNA molecule comprising, in the 5'→3' direction: (1) a 5' cap; (2) a 5' untranslated region (UTR); (3) a polynucleotide sequence encoding a secretory signal peptide; (4) a polynucleotide sequence encoding the one or more neoepitopes resulting from cancer-specific somatic mutations present in a tumor specimen; (5) a polynucleotide sequence encoding at least a portion of a transmembrane and cytoplasmic domain of a major histocompatibility complex (MHC) molecule; (6) a 3' UTR comprising: (a) a 3' untranslated region of an Amino-Terminal Enhancer of Split (AES) mRNA or a fragment thereof; and (b) non-coding RNA of a mitochondrially encoded 12S RNA or a fragment thereof; and (7) a poly(A) sequence. In some examples, the RNA molecule further comprises a polynucleotide sequence encoding an amino acid linker; wherein the polynucleotide sequences encoding the amino acid linker and a first of the one or more neoepitopes form a first linker-neoepitope module; and wherein the polynucleotide sequences forming the first linker-neoepitope module are between the polynucleotide sequence encoding the secretory signal peptide and the polynucleotide

sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule in the 5'→3' direction. In some examples, the amino acid linker comprises the sequence GGSGGGGSGG (SEQ ID NO:21). In some examples, the polynucleotide sequence encoding the amino acid linker comprises the sequence GGCGGCUCUGGAGGAGGCGGCUCCGGAGGC (SEQ ID NO:19). In some examples, the RNA molecule further comprises, in the 5'→3' direction: at least a second linker-epitope module, wherein the at least second linker-epitope module comprises a polynucleotide sequence encoding an amino acid linker and a polynucleotide sequence encoding a neoepitope; wherein the polynucleotide sequences forming the second linker-neoepitope module are between the polynucleotide sequence encoding the neoepitope of the first linker-neoepitope module and the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule in the 5'→3' direction; and wherein the neoepitope of the first linker-epitope module is different from the neoepitope of the second linker-epitope module. In some examples, the RNA molecule comprises 5 linker-epitope modules, and wherein the 5 linker-epitope modules each encode a different neoepitope. In some examples, the RNA molecule comprises 10 linker-epitope modules, and wherein the 10 linker-epitope modules each encode a different neoepitope. In some examples, the RNA molecule comprises 20 linker-epitope modules, and wherein the 20 linker-epitope modules each encode a different neoepitope. In some examples, the RNA molecule further comprises a second polynucleotide sequence encoding an amino acid linker, wherein the second polynucleotide sequence encoding the amino acid linker is between the polynucleotide sequence encoding the neoepitope that is most distal in the 3' direction and the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule. In some examples, the 5' cap comprises a D1 diastereoisomer of the structure:



. In some examples, the 5' UTR comprises the sequence UUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACC (SEQ ID NO:5). In some

examples, the 5’ UTR comprises the sequence GGCGAACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACC (SEQ ID NO:3). In some examples, the secretory signal peptide comprises the amino acid sequence MRVMAPRTLILLLSGALALTETWAGS (SEQ ID NO:9). In some examples, the polynucleotide sequence encoding the secretory signal peptide comprises the sequence AUGAGAGUGAUGGCCCCCAGAACCCUGAUCCUGCUGCUGUCUGGCGCCCUGGC CCUGACAGAGACAUGGGCCGGAAGC (SEQ ID NO:7). In some examples, the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule comprises the amino acid sequence IVGIVAGLAVLAVVVIGAVVATVMCRRKSSGGKGGSYSQAASSDSAQGSDVSLTA (SEQ ID NO:12). In some examples, the polynucleotide sequence encoding the at least portion of the transmembrane and cytoplasmic domain of the MHC molecule comprises the sequence AUCGUGGGAAUUGUGGCAGGACUGGCAGUGCUGGCCGUGGUGGUGAUCGGAG CCGUGGUGGCUACCGUGAUGUGCAGACGGAAGUCCAGCGGAGGCAAGGGCGGC AGCUACAGCCAGGCCGCCAGCUCUGAUAGCGCCCAGGGCAGCGACGUGUCACU GACAGCC (SEQ ID NO:10). In some examples, the 3’ untranslated region of the AES mRNA comprises the sequence CUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCUUUCCCGUCCUGGGUACCCC GAGUCUCCCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGCCCCACU CACCACCUCUGCUAGUUCCAGACACCUCC (SEQ ID NO:15). In some examples, the non-coding RNA of the mitochondrially encoded 12S RNA comprises the sequence CAAGCACGCAGCAAUGCAGCUCAAAACGCUUAGCCUAGCCACACCCCCACGGG AAACAGCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAAGCUAUAC UAACCCCAGGGUUGGUCAAUUUCGUGCCAGCCACACCG (SEQ ID NO:17). In some examples, the 3’ UTR comprises the sequence CUCGAGCUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCUUUCCCGUCCUGGG UACCCCGAGUCUCCCCCGACCUCGGGUCCCAGGUAUGCUCCCACCUCCACCUGC CCCACUCACCACCUCUGCUAGUUCCAGACACCUCCCAAGCACGCAGCAAUGCA GCUCAAAACGCUUAGCCUAGCCACACCCCCACGGGAAACAGCAGUGAUUAACC UUUAGCAAUAAACGAAAGUUUAACUAAGCUAUACUAACCCCAGGGUUGGUCA AUUUCGUGCCAGCCACACCGAGACCUGGUCCAGAGUCGCUAGCCGCGUCGCU (SEQ ID NO:13). In some examples, the poly(A) sequence comprises 120 adenine nucleotides. In some examples, the RNA comprises an RNA molecule comprising, in the

5’→3’ direction: the polynucleotide sequence GGCGAACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGAGAACCCGCCACCAU GAGAGUGAUGGCCCCCAGAACCCUGAUCCUGCUGCUGUCUGGCGCCCUGGCCC UGACAGAGACAUGGGCCGGAAGC (SEQ ID NO:1); a polynucleotide sequence encoding the one or more neoepitopes resulting from cancer-specific somatic mutations present in the tumor specimen; and the polynucleotide sequence AUCGUGGGAAUUGUGGCAGGACUGGCAGUGCUGGCCGUGGUGGUGAUCGGAG CCGUGGUGGCUACCGUGAUGUGCAGACGGAAGUCCAGCGGAGGCAAGGGCGGC AGCUACAGCCAGGCCGCCAGCUCUGAUAGCGCCCAGGGCAGCGACGUGUCACU GACAGCCUAGUAACUCGAGCUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCU UUCCCGUCCUGGGUACCCCGAGUCUCCCCCGACCUCGGGUCCCAGGUAUGCUC CCACCUCCACCUGCCCCACUCACCACCUCUGCUAGUUCCAGACACCUCCCAAGC ACGCAGCAAUGCAGCUCAAAACGCUUAGCCUAGCCACACCCCCACGGGAAACA GCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAAGCUAUACUAACC CCAGGGUUGGUCAAUUUCGUGCCAGCCACACCGAGACCUGGUCCAGAGUCGCU AGCCGCGUCGCU (SEQ ID NO:2).

[0010] In some examples, a method for transferring pharmaceutical compositions using peristaltic pumps includes: pumping a first composition from a first container through a first portion of tubing using at least one peristaltic pump head at a first flow rate; pumping a second composition from a second container through a second portion of tubing using at least one peristaltic pump at a second flow rate; and dampening pulses in a fluid flow of the first composition in the first portion of tubing and dampening pulses in a fluid flow of the second composition in the second portion of tubing using a dampener fluidly connected to the first portion of tubing and fluidly connected to the second portion of tubing, wherein the level of pulsation (LoP) of the flow rate of the first flow rate in the first portion of tubing after the dampener is less than 10 and the level of pulsation (LoP) of the flow rate of the second flow rate in the second portion of tubing after the dampener is less than 10.

[0011] In some examples, a method for manufacturing a pharmaceutical composition including a nucleic acid and one or more lipids includes: pumping a first composition comprising a nucleic acid from a first container through a first portion of tubing using at least one peristaltic pump head at a first flow rate; pumping a second composition comprising one or more lipids from a second container through a second portion of tubing

using at least one peristaltic pump head at a second flow rate; dampening pulses in a fluid flow of the first composition in the first portion of tubing and dampening pulses in a fluid flow of the second composition in the second portion of tubing using a dampener fluidly connected to the first portion of tubing and fluidly connected to the second portion of tubing; mixing the first composition comprising the nucleic acid from the first portion of tubing and the second composition comprising the one or more lipids from the second portion of tubing in a mixer fluidly connected to the first portion of tubing and the second portion of tubing; and depositing a composition comprising the nucleic acid and the one or more lipids into a container that is fluidly connected to the mixture.

[0012] In some examples, the first portion of tubing and the second portion of tubing are configured to be connected to pump heads of the same peristaltic pump. In some examples, the first portion of tubing, the second portion of tubing, the dampener, the mixer, and/or the mixture container are made up of single-use materials. the tubing kit or system is an aespetic, closed tubing kit or system; or the methods are performed in an aespetic, closed system.

[0013] In some examples, a tubing kit for forming a mixture includes: a first portion of tubing configured to be fluidly connected to a first container containing a first composition; a second portion of tubing configured to be fluidly connected to a second container containing a second composition; a tubing dampener comprising an enclosed volume of fluid fluidly connected to the first portion of tubing and the second portion of tubing; a mixer fluidly connected to the first portion of tubing and the second portion of tubing downstream from the fluid dampener and configured to mix the first composition from the first portion of tubing and the second composition from the second portion of tubing; a mixture container fluidly connected to the mixer and configured to collect the mixed first composition and second composition from the mixer, wherein the first portion of tubing is configured to be connected to a first peristaltic pump head upstream the tubing dampener for pumping the first composition from the first container to the mixture container, and the second portion of tubing is configured to be connected to a second peristaltic pump head upstream the tubing dampener for pumping the second composition from the second container to the mixture container.

[0014] In some examples, a system for forming a pharmaceutical composition or mixture of pharmaceutical compositions includes: a first container containing a first pharmaceutical composition; a second container containing a second pharmaceutical

composition; a first portion of tubing fluidly connected to the first container; a second portion of tubing fluidly connected to the second container; a peristaltic pump comprising a first peristaltic pump head connected to the first portion of tubing for pumping the first pharmaceutical composition from the first container and a second peristaltic pump head connected to the second portion of tubing for pumping the second pharmaceutical composition from the second container; a tubing dampener comprising an enclosed volume of fluid fluidly connected to the first portion of tubing and the second portion of tubing downstream from the peristaltic pump; a mixer fluidly connected to the first portion of tubing and the second portion of tubing downstream from the fluid dampener and configured to mix the first pharmaceutical composition from the first portion of tubing and the second pharmaceutical composition from the second portion of tubing; and a mixture container fluidly connected to the mixer and configured to collect the mixed first pharmaceutical composition and second pharmaceutical composition from the mixer.

[0015] In some examples, a method for transferring pharmaceutical compositions using peristaltic pumps includes: pumping a first composition from a first container through a first portion of tubing using a first peristaltic pump head; pumping a second composition from a second container through a second portion of tubing using a second peristaltic pump head; and dampening pulse in a fluid flow of the first composition in the first portion of tubing downstream the first peristaltic pump head and dampening pulses in a fluid flow of the second composition in the second portion of tubing downstream the second peristaltic pump head using a tubing dampener comprising an enclosed volume of fluid fluidly connected to the first portion of tubing and the second portion of tubing; mixing the first composition from the first portion of tubing and the second composition from the second portion of tubing in a mixer fluidly connected to the first portion of tubing and the second portion of tubing downstream from the fluid dampener; and depositing a composition comprising mixed first and second composition into a container that is fluidly connected to the mixer.

[0016] In some examples, a tubing kit for forming a mixture includes a first portion of tubing configured to be fluidly connected to a container containing a first composition; a second portion of tubing configured to be fluidly connected to a container containing a second composition; a first dampener fluidly connected to the first portion of tubing; a second dampener fluidly connected to the second portion of tubing; a mixer for mixing the first

composition from the first portion of tubing and the second composition from the second portion of tubing; a mixture container for collecting the mixed first composition and second composition from the mixer, wherein the first portion of tubing is configured to be connected to at least one peristaltic pump head for pumping the first composition from the container containing the first composition to the mixture container, and the second portion of tubing is configured to be connected to at least one peristaltic pump head for pumping the second composition from the container containing the second composition to the mixture container. In some examples, the first and/or second dampener comprises an enclosed volume of fluid. In some examples, the fluid is air. In some examples, the first and/or second dampener is a tubing dampener. In some examples, the dampener comprises a flexible membrane. In some examples, the tubing kit includes a first tee connector that fluidly connects the first dampener, the first portion of tubing, and a first mixer input portion of tubing, wherein the first mixer input portion of tubing fluidly connects to the mixer. In some examples, the tubing kit includes a second tee connector that fluidly connects the second dampener, the second portion of tubing, and a second mixer input portion of tubing, wherein the second mixer input portion of tubing fluidly connects to the mixer. In some examples, the first portion of tubing comprises a first segment of tubing and a second segment of tubing, wherein the first segment of tubing and the second segment of tubing are fluidly connected in parallel. In some examples, the first segment of tubing is configured to be connected to a first peristaltic pump head, and the second segment of tubing is configured to be connected to a second peristaltic pump head. In some examples, wherein the second portion of tubing comprises a third segment of tubing and a fourth segment of tubing, wherein the third portion of tubing and the fourth portion of tubing are fluidly connected in parallel. In some examples, wherein the third segment of tubing is configured to be connected to a third peristaltic pump head, and the fourth segment of tubing is configured to be connected to a fourth peristaltic pump head. In some examples, the mixer comprises an input fluidly connected to the first portion of tubing, an input fluidly connected to the second portion of tubing, and output fluidly connected to the mixture container. In some examples, the mixer comprises a Y-connector, a helical mixer, or a static mixer. In some examples, the tubing kit includes a first dampener connector that fluidly connects the first portion of tubing to the first dampener and to the mixer and a second dampener connector that fluidly connects the second portion of the tubing to the second dampener and to the mixer. In some examples, the mixture container is a bag, vessel, or bottle.

[0017] In some examples, a pulsation dampener for a fluid pump includes a bioprocessing bag comprising a fluid inlet and a fluid outlet, wherein the fluid inlet is configured to be fluidly connected downstream of a fluid pump; a housing configured to receive the bioprocessing bag, wherein the housing comprises a base and plurality of sidewalls forming a cavity for the bioprocessing bag and at least one sidewall comprises one or more notches configured to provide access to the fluid inlet and fluid outlet of the bioprocessing bag; and a housing lid configured to attach to the plurality of sidewalls and close the housing. In some examples, the bioprocessing bag comprises a gas inlet, wherein the gas inlet is configured to be fluidly connected to a gas source. In some examples, the one or more notches are configured to provide access to the gas inlet of the bio processing bag. In some examples, the base of housing comprises a window. In some examples, the window comprises an opening in the base of the housing or a transparent material in the base of the housing. In some examples, the housing lid comprises a window. In some examples, the window comprises an opening in the housing lid or a transparent material in the housing lid. In some examples, the dampener includes a front plate configured to be connected to at least one sidewall of housing and/or the housing lid, wherein the front plate comprises at least one aperture configured to receive the fluid inlet and fluid outlet. In some examples, the fluid pump is a cyclic pump. In some examples, the cyclic pump is a peristaltic pump. In some examples, the fluid outlet is configured to be fluidly connected to a fluid storage container. In some examples, the fluid outlet comprises a check valve.

[0018] Additional advantages will be readily apparent to those skilled in the art from the following detailed description. The examples and descriptions herein are to be regarded as illustrative in nature and not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Exemplary embodiments are described with reference to the accompanying figures, in which:

[0020] **FIG. 1** illustrates an example of an experimental setup to measure flowrates of a peristaltic pump in accordance with some embodiments disclosed herein.

[0021] **FIG. 2** illustrates the flowrate of water through the peristaltic pump achieved in Experiment 1.

[0022] **FIG. 3** illustrates an example of an experimental setup to measure flowrates of a peristaltic pump(s) with a dampener in accordance with some embodiments disclosed herein.