SUMMARY

[0012] Disclosed is a composition comprising (a) a plurality of intact minicells, each minicell of the plurality encompassing plasmid-free functional nucleic acid, and (b) a pharmaceutically acceptable carrier therefor. The category of "functional nucleic acid" is illustrated by single-, double-, or multi-stranded DNA or RNA. In one esample, minicells of the plurality contain plasmid-free functional nucleic acid that is regulatory RNA. Examples of such regulatory RNA include, but are not limited to,siRNA, miRNA, and shRNA.

[0012a] In one aspect, there is provided a composition comprising: (a) a plurality of intact, bacterial minicells, each minicell of the plurality comprising at least one exogenous~~plasmid-free~~ functional nucleic acid, wherein the exogenous functional nucleic acid is DNA, wherein the DNA is an antisense oligonucleotide; and (b) at least one pharmaceutically acceptable carrier, wherein (i) there is an absence from the minicells of a construct for *in situ* expression of the exogenous functional nucleic acid, and (ii) the plurality comprises a therapeutically effective amount of the exogenous functional nucleic acid.

[0013] The minicell-packaged functional nucleic acid may target the RNA transcripts encoding a protein that contributes to drug resistance, to apoptosis resistance, or to neoplasticity, *inter alia*. Also, a composition of the disclosure may further comprise a bispecific ligand comprised, for example, of a first arm specific for a minicell surface structure and a second arm specific for a non-phagocytic mammalian cell surface receptor.

[0014] Disclosed is a method for delivering a functional nucleic acid to a target mammalian cell. The inventive methodology comprises (a) providing a plurality of intact minicells in a pharmaceutically acceptable carrier, each minicell of the plurality encompassing plasmid-free functional nucleic acid, and (b) bringing minicells of the plurality into contact with mammalian cells such that the mammalian cells engulf minicells of the plurality, whereby the functional nucleic acid is released into the cytoplasm of the target cells. As mentioned, the functional nucleic acid, exemplified by regulatory RNA such as siRNA, miRNA and shRNA, can target RNA transcripts encoding a protein that contributes to drug resistance, apoptosis resistance or neoplasticity. In other examples, the methodology of the disclosure further comprises delivering a drug, distinct from the functional nucleic acid, to the target mammalian cell. The drug can be administered after or concurrently or even before the administration of the minicell composition.

[0014a] In another aspect, there is provided an *in vitro* method of delivering an exogenous ~~functional~~ nucleic acid to a target mammalian cell, comprising: (a) providing a plurality of intact, bacterial minicells in a pharmaceutically acceptable carrier, each minicell of the plurality comprising an exogenous ~~plasmid free~~ functional nucleic acid, wherein the exogenous functional nucleic acid is DNA, and wherein the DNA is an antisense oligonucleotide, and (b) contacting minicells of the plurality with the target mammalian cells such that the mammalian cells engulf minicells of the plurality, whereby the exogenous functional nucleic acid is released into the cytoplasm of the mammalian cells, wherein (i) there is an absence from the minicells of a construct for *in situ* expression of the exogenous functional nucleic acid, and (ii) the plurality comprises a therapeutically effective amount of the exogenous functional nucleic acid.

[0015] Disclosed is a method for formulating a minicell with a plasmid-free functional nucleic acid. The method comprises co-incubating a plurality of minicells with a functional nucleic acid, such as regulatory RNA like siRNA, miRNA or shRNA, in a buffer. In some examples, the co-incubation may involve gentle shaking, while in others the co-incubation is static. In some examples, the co-

incubation lasts about half an hour, while in others it lasts about an hour. In one example, the buffer comprises buffered saline, for example, a 1X phosphate buffer solution. In another example, the co-incubation is conducted at a temperature of about 4°C to about 37°C, about 20°C to about 30°C, about 25°C, or about 37°C. The co-incubation can comprise about 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12} or 10^{13} minicells.

[0015a] In another aspect, there is provided a method for formulating a minicell of the composition as described herein, comprising co-incubating a plurality of intact minicells with functional nucleic acid in a buffer.

~~**[0015b]** In another aspect, there is provided a composition comprising: (a) a plurality of intact minicells, each minicell of the plurality comprising functional nucleic acid, wherein there is an absence from the minicells of a construct for in situ expression of the functional nucleic acid, and (b) at least one pharmaceutically acceptable carrier.~~

[0016] Other objects, features and advantages will become apparent from the following detailed description. The detailed description and specific examples are given for illustration only since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. Further, the examples demonstrate the principle of the invention and cannot be expected to specifically illustrate the application of this invention to all the examples where it will be obviously useful to those skilled in the prior art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 depicts intact minicells packaged with Cy3 fluorophore-labeled siRNA. Figure 1A is from a light microscope, while Figure 1B shows the same slide but viewed under fluorescent light with a 515-560 excitation filter revealing strongly fluorescent siRNA molecules coincident with the minicells.

[0018] Figure 2 is an image captured by fluorescence confocal microscopy and shows the adhesion and internalization of EGFR-targeted, siRNA-Plk1-packaged minicells into human breast cancer cells *in-vitro*.

[0019] Figure 3 graphically shows a significant anti-tumor effect achieved by treating human breast cancer (MDA-MB-468) xenografts in nude mice with EGFR-targeted, KSP-siRNA-packaged minicells. Control group 1(–◆–) received sterile saline, while experimental group 2 (–⊖–) received $^{EGFR}\text{minicells}_{\text{siRNA-KSP}}.(10^9)$, four times per week.

[0020] Figure 4 graphically depicts a significant anti-tumor effect achieved by treating human colon cancer (HCT116) xenografts in nude mice with EGFR-targeted, KSP-siRNA-packaged minicells in conjunction with EGFR-targeted, carboplatin-packaged minicells. Group 1 (–◆–) mice received sterile saline, and mice of groups 2 (-△-), 3 (–■–) and 4

WE CLAIM:

1. A composition comprising:

(a) a plurality of intact, bacterial minicells, each minicell of the plurality comprising at least one exogenous ~~plasmid-free~~ functional nucleic acid, wherein the exogenous functional nucleic acid is DNA, wherein the DNA is an antisense oligonucleotide; and

(b) at least one pharmaceutically acceptable carrier,

wherein (i) there is an absence from the minicells of a construct for *in situ* expression of the exogenous functional nucleic acid, and (ii) the plurality comprises a therapeutically effective amount of the exogenous functional nucleic acid.

~~2. The composition of claim 1, wherein the functional nucleic acid is a regulatory RNA.~~

2. The composition of claim 1, wherein the exogenous functional nucleic acid is not chemically modified.

~~3. The composition of claim 2, wherein the regulatory RNA is selected from the group consisting of siRNA, miRNA, shRNA, antisense ssRNA, ribozyme, and decoy RNA.~~

~~4~~3. The composition of claim 1, wherein the ~~functional nucleic acid~~antisense oligonucleotide targets:

(a) an RNA transcript encoding a protein that contributes to drug resistance;

(b) an RNA transcript encoding a protein that contributes to apoptosis resistance; or

(~~b~~c) an RNA transcript encoding a protein that contributes to neoplasticity.

~~5~~4. The composition of claim ~~4~~ 3 wherein:

(a) the protein that contributes to drug resistance of subsection (a) is P-glycoprotein, MDR-2, or MDR-3;

(b) the antisense oligonucleotide targets the transcript of MRP2, BCR-ABL, STI-571 resistance-associated protein, lung resistance-related protein, cyclooxygenase-2, nuclear factor kappa, XRCCl, ERCCI, GSTPI, mutant f3-tubulin, or a growth factor;

(c) the ~~functional nucleic acid~~antisense oligonucleotide targets an RNA transcript encoding a protein that contributes to apoptosis resistance, wherein the transcript is of Bcl-2, Bcl-X_L, A1/Bfl 1, focal adhesion kinase or p53 protein; or

(~~b~~d) the ~~functional nucleic acid~~antisense oligonucleotide targets an RNA transcript encoding a protein that contributes to neoplasticity, wherein the transcript is of β-Catenin, PKC-α, C-RAF, K-Ras, DP97 Dead box RNA helicase, cdk1, DNMT1, FLIP, C-Sfc, 53BPI, Polycomb group protein EZH2, ErbB1, HPV-16 E5, HPV-16 E7, Fortilin & MCI1P, KSP, DIP13α, MBD2, p21, KLF4, tpt/TCTP, SPK1 & SPK2, P300, PLK1, Trp53, Ras, ErbB1, VEGF, or BAG-1.

~~6~~5. The composition of claim 1, further comprising a drug.

~~7~~6. The composition of claim ~~6~~5, wherein the exogenous functional nucleic acid targets a transcript encoding a protein that contributes to resistance to the drug.

~~8~~7. The composition of claim ~~6~~5, wherein the exogenous functional nucleic acid and the drug ~~is~~ are packaged ~~in an~~within different intact minicells.

~~9~~8. The composition of claim ~~8~~5, wherein the exogenous functional nucleic acid and the drug are packaged within the same minicell.

~~10~~9. The composition of claim 1, further comprising a bispecific ligand, wherein the bispecific ligand comprises:

(a) a first arm that carries specificity for a minicell surface structure, wherein the minicell surface structure is an O-polysaccharide component of a lipopolysaccharide on the minicell surface; and

(b) a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor.

~~11~~10. The composition of claim ~~10~~9, wherein the bispecific ligand comprises an antibody or an antibody fragment.

~~12~~11. The composition of claim 1, wherein the composition comprises:

(a) fewer than about 1 contaminating parent bacterial cell per 10^7 minicells;

(b) fewer than about 1 contaminating parent bacterial cell per 10^8 minicells; or

(c) fewer than about 1 contaminating parent bacterial cell per 10^9 minicells.

~~13~~12. An *in vitro* method of delivering an exogenous ~~functional~~ nucleic acid to a target mammalian cell, comprising:

(a) providing a plurality of intact, bacterial minicells in a pharmaceutically acceptable carrier, each minicell of the plurality comprising ~~plasmid-free~~an exogenous functional nucleic acid, wherein the exogenous functional nucleic acid is DNA, and wherein the DNA is an antisense oligonucleotide, and

(b) contacting minicells of the plurality with the target mammalian cells such that the mammalian cells engulf minicells of the plurality, whereby the exogenous functional nucleic acid is released into the cytoplasm of the mammalian cells,

wherein (i) there is an absence from the minicells of a construct for *in situ* expression of the exogenous functional nucleic acid, and (ii) the plurality comprises a therapeutically effective amount of the exogenous functional nucleic acid.

~~14. The method of claim 13, wherein the functional nucleic acid is a regulatory RNA.~~

~~15. The method of claim 14, wherein the regulatory RNA is selected from the group consisting of siRNA, miRNA, shRNA, antisense ssRNA, ribozyme, and decoy RNA.~~

~~16~~13. The method of claim ~~13~~12, wherein at least a portion of the plurality comprises a therapeutically effective amount of the functional nucleic acid.

~~17~~14. The method of claim ~~13~~12, wherein the ~~functional nucleic acid~~antisense oligonucleotide targets a transcript selected from the group consisting of:

(a) encoding a protein that contributes to drug-resistance;

(b) of P-glycoprotein, MDR-2 or MDR-3;

(c) of MRP2, BCR-ABL, STI-571 resistance-associated protein, lung resistance-related protein, cyclooxygenase-2, nuclear factor kappa, XRCC1, ERCC1, GSTP1, mutant β-tubulin, or a growth factor;

(d) of a protein that contributes to apoptosis resistance;

(e) of Bcl-2, Bcl-X_L, A1/Bfl 1, focal adhesion kinase or p53 protein;

(f) of a protein that contributes to neoplasticity; and

(g) of β-Catenin, PKC-α, C-RAF, K-Ras, DP97 Dead box RNA helicase, cdk1, DNMT1, FLIP, C-Sfc, 53BPI, Polycomb group protein EZH2, ErbB1, HPV-16 E5 and E7, Fortilin & MCI1P, DIP13α, KSP, MBD2, p21, KLF4, tpt/TCTP, SPK1 & SPK2, P300, PLK1, Trp53, Ras, ErbB1, VEGF, or BAG-1.

~~18~~15. The method of claim ~~13~~12, further comprising step (c) delivering a drug to the target mammalian cell.

~~19~~16. The method of claim ~~18~~15, wherein the exogenous functional nucleic acid targets the transcript of a protein that contributes to resistance to the drug.

~~20~~17. The method of claim ~~19~~15, wherein the exogenous functional nucleic acid and the drug ~~is~~ are packaged ~~in an~~within different intact minicells.

~~21~~18. The method of claim ~~20~~15, wherein the exogenous functional nucleic acid and the drug are packaged within the same intact minicell.

~~22~~19. The method of claim ~~18~~15, wherein step (c) occurs subsequent to steps (a) and (b).

~~23~~20. The method of claim ~~18~~15, wherein the drug is delivered concurrently with step (b).

~~24~~21. The method of claim ~~13~~12, wherein step (b) comprises contacting a bispecific ligand with at least some of the intact minicells and the target mammalian cell, such that the bispecific ligand causes minicells to bind to the mammalian cell.

~~25~~22. The method of claim ~~24~~21, wherein the target mammalian cell is a non-phagocytic cell.

~~26~~23. The method of claim ~~24~~21, wherein the bispecific ligand comprises:

(a) a first arm that carries specificity for a minicell surface structure, wherein the minicell surface structure is an O-polysaccharide component of a lipopolysaccharide on the minicell surface; and

(b) a second arm that carries specificity for a non-phagocytic mammalian cell surface receptor.

~~27~~24. The method of claim ~~26~~23, wherein the mammalian cell surface receptor is capable of activating receptor-mediated endocytosis of the minicell.

~~28~~25. The method of claim ~~24~~21, wherein the bispecific ligand comprises an antibody or antibody fragment.

~~29~~26. The method of claim ~~13~~12, wherein the mammalian cell is phagocytosis- or endocytosis-competent.

~~30~~27. A method for formulating a minicell of the composition of claim 1, comprising co-incubating a plurality of intact minicells with functional nucleic acid in a buffer.

~~31. The method of claim 30, wherein the functional nucleic acid is a regulatory RNA.~~

~~32. The method of claim 31, wherein the regulatory RNA is selected from the group consisting of siRNA, miRNA, shRNA, antisense ssRNA, ribozyme, and decoy RNA.~~

~~33~~28. The method of claim ~~30~~27, wherein the co-incubation comprises gentle shaking.

~~34~~29. The method of claim ~~30~~27, wherein the co-incubation lasts about 0.5 hour or about 1 hour.

~~35~~30. The method of claim ~~30~~27, wherein the buffer comprises:

(a) buffered saline;

(b) buffered saline in gelatin form; or

(c) a 1x phosphate buffer solution.

~~36~~31. The method of claim ~~30~~27, wherein the co-incubation is conducted at a temperature selected from the group consisting of:

(a) about 4° C to about 37° C.;

(b) about 20° C to about 30° C;

(c) about 25° C; or

(d) about 37° C.

~~37~~32. The method of claim ~~30~~27, wherein the co-incubation comprises about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12} or about 10^{13} minicells.

~~38. The composition of claim 1, wherein there is an absence from the minicells of a construct for *in situ* expression of the functional nucleic acid.~~

~~39. A composition comprising:~~

~~(a) a plurality of intact minicells, each minicell of the plurality comprising functional nucleic acid, wherein there is an absence from the minicells of a construct for in situ expression of the functional nucleic acid, and~~

~~(b) at least one pharmaceutically acceptable carrier.~~