TOWARDS PERSONALIZED DRUG DELIVERY
Preparation of an Encapsulated Multicompartment System

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Abstract: Single liposomes and vesicles are successfully utilized as delivery vehicles of pharmaceuticals. However limitations of these unilamellar, single compartments led to the development of encapsulated multicompartment systems that establishes the prospect of multicomponent or multifunctional drug delivery systems. So far compartmentalization is restricted to binary systems. To realize a personalized drug delivery, a programmable linkage of n-entities of different content will be needed. Here we present both a programmable DNA-mediated linkage of three distinct vesicle populations and a novel encapsulation protocol. We discuss how the techniques established in this study might be used in personalized healthcare based on custom-tailored encapsulated multicompartment vesicular drug delivery systems.

1 INTRODUCTION

Biological as well as artificial vesicles feature an aqueous compartment partitioned off an aqueous surrounding by a lipid membrane that is nearly impermeable for hydrophilic substances. The membrane organizes processes by compartmentalizing them. The compartmentalization enables segregation of specific chemical reactions for the purposes of increased controllability, observability, stability, and biochemical efficiency by restricted dissemination and efficient storage of reactants, and/or reaction products. Thus, wide usage of artificial vesicles is found in analytics (Hotani, Nomura and Suzuki, 1999; Jesorka and Orwar, 2008; Limozin, Roth and Sackmann, 2005; Luisi and Walde, 2000) and synthetics, where they are used as bioreactors (Bolinger, Stamou and Vogel, 2008; Michel et al., 2004; Noireaux and Libchaber, 2004), and drug delivery systems (Allen and Cullis, 2004; Bonacucina, Cespi, Misici-Falzi and Palmieri, 2009; Torchilin, 2005). Vesicles featuring biocompatibility, biodegradability, low toxicity, and structural variability are successfully utilized as therapeutic agents for the delivery of antibacterial, antiviral, and anticancer drugs, as well as of hormones, enzymes, and nucleotides (Eckstein, 2007; Lasic, Vallner and Working, 1999; Weissig, Boddapati, Cheng and D'souza, 2006).

Generally, single unilamellar vesicles apply in therapeutic systems. However premature content release in physiological environments limits their reliability (Bakker-Woudenberg, Schiffelers, Storm, Becker and Guo, 2005). Extending the circulation time of vesicles that results in accumulation at tumors or inflammation sites due to the enhanced permeability and retention (EPR) effect (Allen and Cullis, 2004) is realized at the molecular level via monomer design (Torchilin, 2009) or at the mesoscopic level via encapsulation. The bilayer-within-a-bilayer structure of encapsulated vesicles not only prevents a premature degradation and content release (Boyer and Zasadzinski, 2007) but offers a division of different membrane functions (biocompatibility, cargo release, targeting, and protection) among several membranes of different compositions and dimensions. Encapsulated vesicles are frequently used in pharmaceutical and cosmetic applications (Lasic, 1993). The applicability of single vesicles is limited further by the need for a simultaneous entrapment of a given set of (pharmaceutical) components in one single compartment, which is

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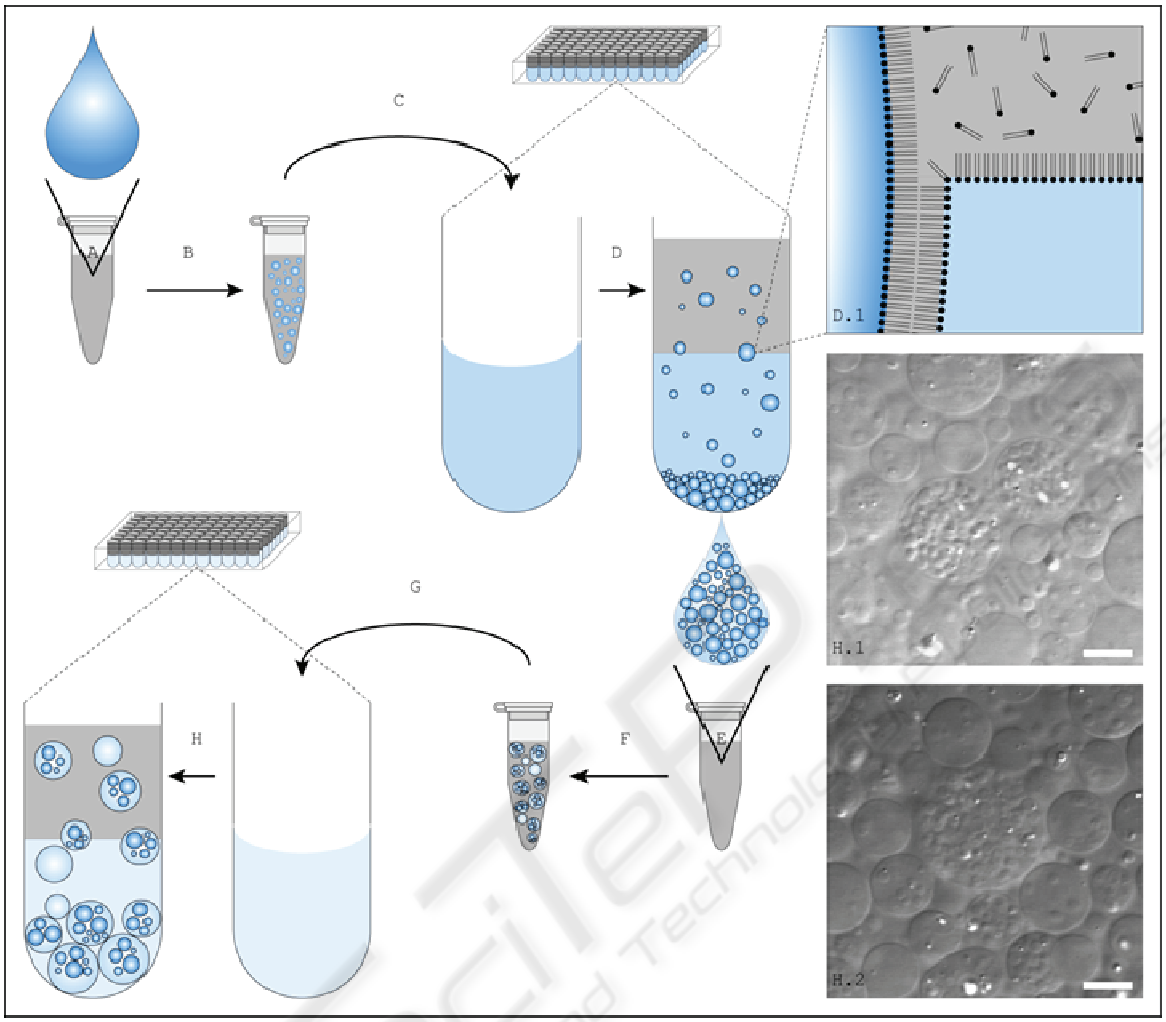

Figure 1: Schematic representation of the vesicle formation/encapsulation procedure and micrographs of internally compartmentalized vesicles. (A) A water droplet (blue) is added to a phospholipid suspension (light gray, cp. D.1). (B) A water-in-oil emulsion is produced by mechanical agitation and sonication. (C) The emulsion is placed over an aqueous solution. Vesicles are produced in 96-well microtiter plates, providing parallel formation of up to 96 distinct vesicle populations. (D) Induced by centrifugation, the droplets pass the oil/water interface. Due to the density difference of the *inter*- and *intra*vesicular fluid and the geometry of the microplate bottom, vesicles pelletize in the centre of the well and become easily accessible for pipetting. (D.1) Amphiphilic phospholipids, solved in mineral oil, stabilize water-oil interfaces by forming monolayers. Two monolayers form a bilayer when a water droplet passes the interface. (E) A droplet of the aqueous solution that hosts the vesicles is added to a phospholipid suspension. After mechanical agitation (F) and placing over an aqueous solution (G), internally compartmentalized vesicles are produced by centrifugation. Lower color saturation indicates lower density of the aqueous solution. (H.1, H.2) Differential interference contrast micrographs of internally compartmentalized vesicles. Scale bar represents 10µm.

"not an easy matter" (Luisi, de Souza and Stano, 2008, p. 14660). Multicompartment systems can overcome this limitation by conciliating smaller subsets of components entrapped in different compartments. Thus, encapsulated multicompartment systems could provide stable vehicles for a multicomponent or multifunctional drug delivery.

Zasadzinski et al. established a protocol to encapsulate a multicompartment system of tethered liposomes (Boyer and Zasadzinski, 2007; Kisak, Coldren, Evans, Boyer and Zasadzinski, 2004; Walker, Kennedy and Zasadzinski, 1997). Both tethering and encapsulation of these vesosomes are based on the molecular recognition process of the biotin-streptavidin complex. Like most of the current tethering strategies (Berti, Baglioni, Bonaccio, Barsacchi-Bo and Luisi, 1998; Chiruvolu et al., 1994; Constable, Meier, Nardin and Mundwiler, 1999;

Marchi-Artzner et al., 2001; Menger, Seredyuk and Yaroslavov, 2002; Paleos, Sideratou and Tsiourvas, 1996; Sideratou et al., 2002; Vermette, Taylor, Dunstan and Meagher, 2002; Weikl, Groves and Lipowsky, 2002), tethering is based on single ligand-receptor pairs and result in systems binary at most. DNA-mediated linkage (Beales and Vanderlick, 2007; Chan, van Lengerich and Boxer, 2009) offers multiple ligand-receptors, as needed in a multicomponent or multifunctional drug delivery system. However neither a coupling of more than two vesicle populations is realized, nor a procedure to encapsulate such a system is established.

In this study, we present both a programmable DNA-mediated linkage of three distinct vesicle populations and a novel encapsulation mechanism. Based on the results of this study, we formulate a scenario how encapsulated multicompartment systems might be used to realize custom-tailored vesicular drug delivery systems.

2 MATERIALS AND METHODS

Technical modifications of the vesicle formation protocol reported by Pautot et al. (Pautot, Frisken and Weitz, 2003) were: (i) the introduction of 96-well microtiter plates U96 to increase procedural manageability in laboratory experimentation and (ii) a density difference between *inter*- and *intra*vesicular solution induced by isomolar solutions of monosaccharids (glucose: *inter*) and disaccharids (sucrose: *intra*). For a description of the modified vesicle protocol see Figure 1A-D. For the membrane composition of the vesicles used in the encapsulation and the self-assembly experiments see Table 1. All phospholipids were solved in mineral oil.

Table 1: Membrane composition of vesicles used in experimentation.

Encapsulation	
100%	PC(16:0/18:1(Δ9-Cis)) = 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine
Self-Assembly	
99%	PC(16:0/18:1(Δ9-Cis)) = 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine
0.75%	methyl-PEG2000-PE(18:0/18:0) = 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy (Polyethylene glycol)-2000]
0.25%	biotin-PEG2000-PE(18:0/18:0) = 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Biotinyl (Polyethylene Glycol) 2000]

For details of the encapsulation procedure of untethered vesicles see Figure 1. Encapsulated vesicles exhibited quick random motion within the boundaries of the surrounding vesicle.

In the compartmentalization experiments, three distinct vesicle populations were prepared by doping their surface with binary combinations of six ssDNA populations (1st population: α, β; 2nd: α', γ; 3rd: β', γ'; for the sequence of biotinylated ssDNA strands see Table 2). The DNA strands were biotin-labeled and anchored to biotinylated vesicular membrane via streptavidin as a cross-linking agent. For a detailed protocol of the surface doping and the self-assembly procedure see Figure 2. The sequences of the ssDNA were produced by a genetic algorithm and optimized for minimal DNA-DNA-hybridization among the three pairs. The specificity was verified on a commercial program.

Light and confocal laser scanning microscopy was performed using an inverted Leica DMR IRE2 SP2 confocal laser scanning microscope.

Table 2: Sequence of complementary biotinylated DNA single strands used in the self-assembly experiments.

α	biotin-TGTACGTCACAACTA-3′
α'	3′-ACATGCAGTGTTGAT-biotin
β	biotin-AGAAAGAGCCCTCCA-3′
β'	3′-TCTTTCTCGGGAGGT-biotin
γ	biotin-AAAGATTACACACGA-3′
γ'	3′-TTTCTAATGTGTGCT-biotin

3 RESULTS

The density difference between the *inter*- and *intra*vesicular solution induced vesicle pelletization at the centre of the well. The size distribution of vesicles produced in the first round of vesicle formation (Figure 1 A-D) was shifted to the left when compared to vesicles produced in the second round (Figure 1 E-H). The two vesicle formation protocols differed only in the presence (Figure 1 B) or absence (Figure 1 F) of the sonication of the water-in-oil emulsion. To indicate independence of the tethering and encapsulation process, vesicles to be encapsulated were not tethered. Tethered assemblies were encapsulated without any modification of the encapsulation procedure (results not shown). As seen in Figure 1 H.1 and H.2 the ratio of vesicles internally compartmentalized to vesicles uncompartmentalized was high. Most of the vesicles produced in the first round were found to be enclosed – encapsulation efficiency was high.

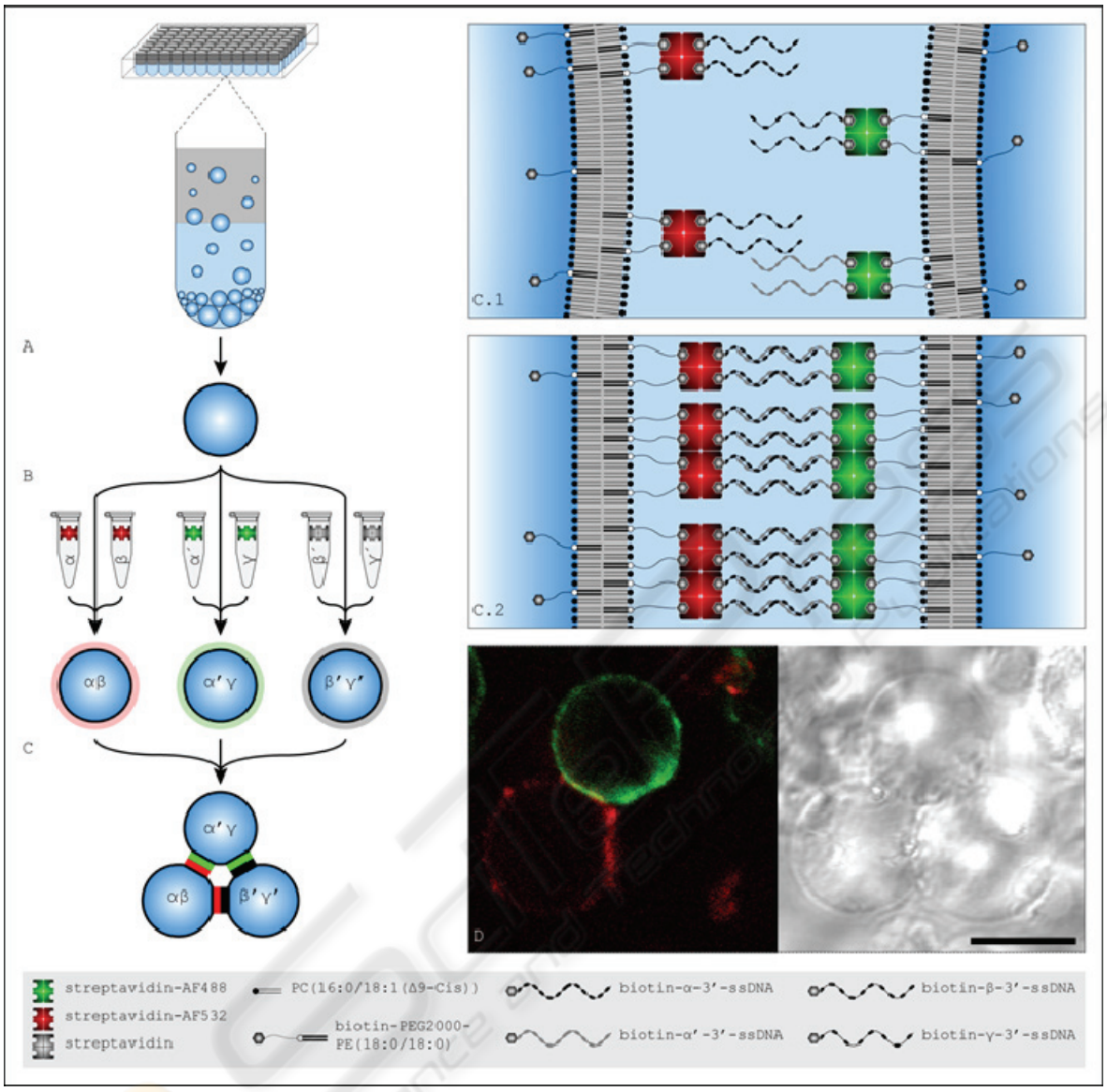

Figure 2: Schematic representation of the self-assembly process and micrographs of adhesion plaques. (A) For vesicle formation see Figure 1.(B) Vesicle populations become distinct by incubating them with single stranded DNA (ssDNA) of different sequence (α, α', β, β', γ, γ') and streptavidin differing in fluorescence labeling (Alexa Fluor 488 / 532 conjugate (AF488 / AF532) or unlabeled). Monohomophilic oligonucleotide-doping of streptavidin is provided by separated incubations. (C) The vesicle populations are merged. (C.1) The lateral distribution of linkers in the lipid membrane is homogeneous. Vesicles doped with complementary ssDNA come into contact. (C.2) Hybridization of DNA strands results in double stranded DNA and induces the assembly process. Due to their lateral mobility, linkers accumulate in the contact zone forming an adhesion plaque – the lateral distribution of linkers in the outer leaflet becomes inhomogeneous (situation shown for α-AF532, α'-AF488). (D) CLSM (confocal laser scanning microscope) and DIC (differential interference contrast) micrograph of a vesicular aggregate that emerged in real-world experimentation. Accumulation and depletion of linkers are clearly visible in the CLSM micrograph. Scale bar represents 10μm.

When vesicles doped with complementary ssDNA came into contact, hybridization of single DNA strands resulted in double stranded DNA. Linkers accumulated in the contact area of the two vesicles formed an adhesion plaque (Figure 2 D). Adhesion plaques were found exclusively, when DNA strands were complementary and inorganic ions were present (data of control experiments not shown). No transfer of linkers between the membranes of different vesicles was observed (data not shown).

4 DISCUSSION

Multicomponent or multifunctional custom-tailored vesicular drug delivery systems have to fulfil several requirements: (i) the actual drug containing system should be encapsulated to prevent premature degradation and content release, (ii) the drug containing system should consist of more than two distinct compartments, and (iii) the proper composition of the drug containing system should be controlled.

4.1 Encapsulation

The *in vitro* vesicle formation procedure (Noireaux and Libchaber, 2004; Pautot et al., 2003; Träuble and Grell, 1971) enables independent tailoring of chemical material properties of the *inter*- and *intra*vesicular fluid as well as of the inner and outer membrane leaflet composition. To our knowledge, the entrapment efficiency of this vesicle formation procedure is not analyzed so far. However one may speculate that its entrapment efficiency is better than for vesicle formation procedures currently used (for an overview of the current vesicle formation procedures see Jesorka and Orwar (2008)). The potential of an asymmetric leaflet composition was exemplified by the production of phospholipid and polymer hybrids combining biocompatibility and mechanical endurance in single vesicles (Pautot et al., 2003). We increased procedural manageability of the formation procedure by introducing microtiter plates and vesicle pelletization (due to density differences in the *inter*- and *intra*vesicular fluid). By introducing sonication of the water-in-oil emulsion, we could shift the size distribution of the vesicles formed. By refeeding the vesicle containing solution, we established a novel method to produce multivesicular assemblies. The protocol provides encapsulation of either tethered or untethered vesicular assemblies. The interdependence of tethering and encapsulation, faced in vesosome formation, is therefore resolved.

4.2 Compartmentalization

Single stranded DNA provides programmability, specificity, and high degrees of complexity (Licata and Tkachenko, 2006). Streptavidin offers the strongest noncovalent biological interaction known (Green, 1990), an extensive range of possible vesicle modifications, component modularity, and availability off the shelf. Phospholipid-grafted biotinylated PEG tethers feature lateral mobility (Singer and Nicolson, 1972), high detachment resistance (Burridge, Figa and Wong, 2004), and no intermembrane transfer of linkers. The combination of phospholipid-grafted biotinylated PEG tethers and streptavidin allows fast production of vesicles differently doped and avoids problems encountered in other approaches using cholesterol-tagged DNA to specifically link different vesicle populations by the hybridization of membrane-anchored DNA (Beales and Vanderlick, 2007; Benkoski and Hook, 2005; Chan et al., 2009): (i) Because the processes of vesicle formation and vesicle modification are not separated (the cholesterol-tagged ssDNA has to be present during vesicle formation), the formation procedure has to be adjusted anew for each change in the vesicle modification. The procedural manageability in laboratory experimentation is therefore reduced. (ii) As discussed by Beales and Vanderlick (2007) the cholesterol anchors of the cholesterol-tagged ssDNA spontaneously leave the lipid bilayer and incorporate randomly into (other) lipid bilayers. Thus, the specificity of the linking system is lost over time.

We presented a DNA-mediated tethering of three distinct vesicle populations. Linkage of more than two distinct vesicle populations is realized for the first time. Thus, restriction to binarism faced in current donor-acceptor mechanisms is resolved. The DNA-mediated linkage mechanism offers programmability of composition of multicompartment systems. Thus, custom-tailored vesicular drug delivery systems seem feasible.

4.3 Composition Control

By loading the vesicular membranes of tethered assemblies by ligand groups not used in the aggregation process, a column chromatographic purification procedure of aggregates may be realized. The ligand groups would be used to purify aggregates from single vesicles (for details see Figure 3). Figure 3 depicts the minimal situation of tethered assemblies of two vesicle populations and two columns in series. If the tethered assemblies consist of three different vesicle populations bearing three different ligand groups not used in the aggregation process, purification of aggregates of proper composition both from single vesicles and incomplete aggregates might become possible.

By a downstream fluorescence activated cell sorting (FACS; for a review of techniques used in cell separation see Pappas and Wang (2007)) inter nally compartmentalized vesicles might be purified from vesicles not equipped properly (Figure 3G). Based on the fluorescence signal, the chromatographic separation procedure (Figure 3 B-D) might

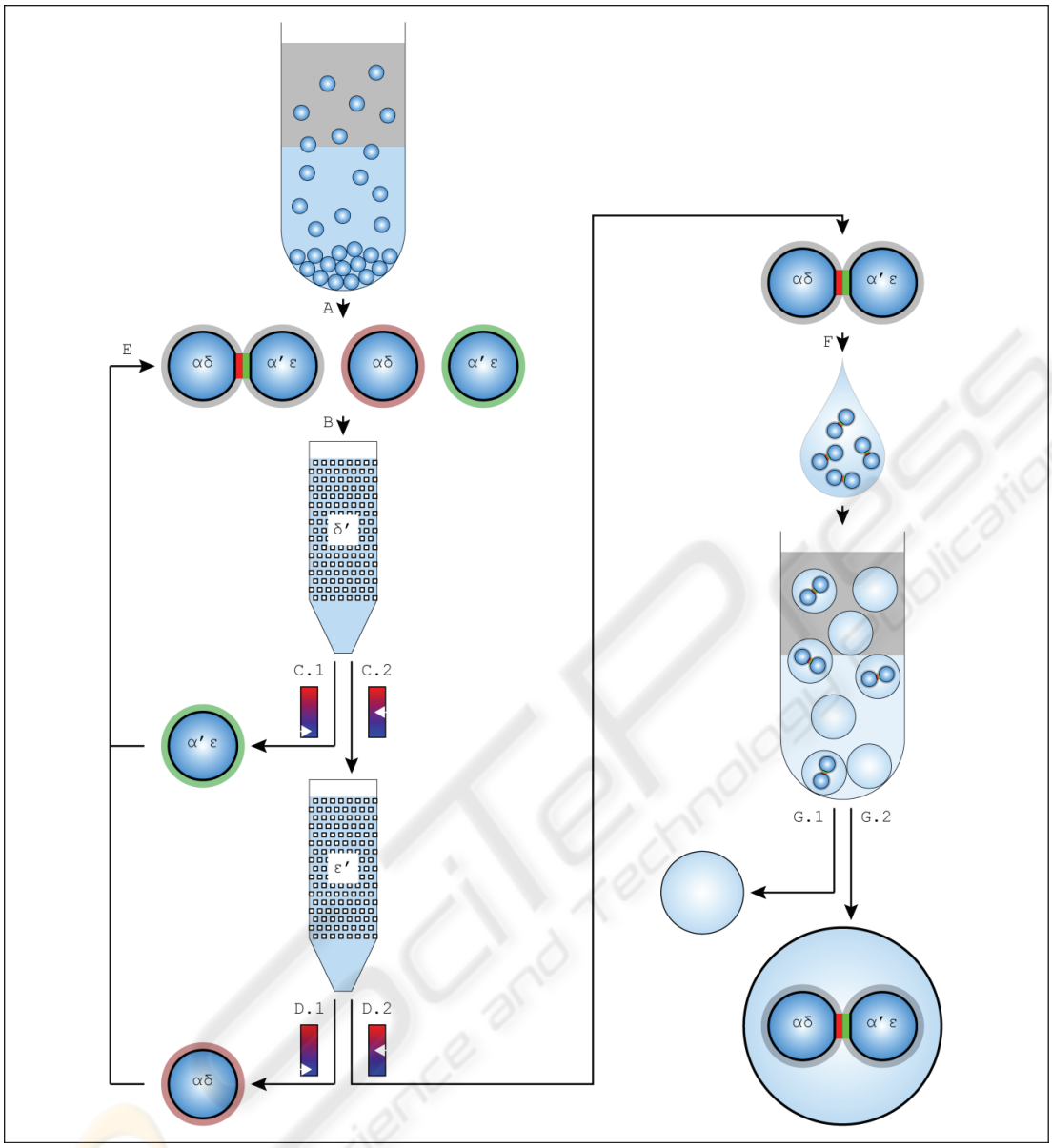

Figure 3: Schematic representation of the processes to form and purify internally compartmentalized vesicles. (A) For details concerning the self-assembly process resulting in vesicular aggregates see Figure 2. Vesicle populations become distinct by incubating them with single stranded DNA (ssDNA) of different sequence (α, α', δ, ε) and streptavidin differing in fluorescence labeling (Alexa Fluor 488 (green) / 532 (red) conjugate (AF488 / AF532) or unlabeled (black)) resulting in a surface doping of (α-AF532, δ-unlabeled; α'-AF488, ε-unlabeled). The DNA strands differ in their melting temperature ($T_m^{\alpha} > T_m^{\delta} = T_m^{\varepsilon}$). Due to the hybridization of DNA strands linkers α-AF532 and α'-AF488 accumulate in the contact zone forming an adhesion plaque. Lateral distribution of linkers δ-unlabeled and ε-unlabeled is not affected by the assembly process. (B) To dispose vesicles not assembled the mixture is fed to a column whose stationary phase is doped with single stranded DNA (δ', ε'). Hybridization of DNA strands results in a retention of aggregates and single vesicles doped with ssDNA (δ). (C.1) Single vesicles not doped with ssDNA (δ) are eluted at low temperature ($T < T_m^{\alpha}, T_m^{\delta}, T_m^{\varepsilon}$). (C.2) An increase in temperature ($T_m^{\delta}, T_m^{\varepsilon} < T < T_m^{\alpha}$) results in an elution of aggregates and single vesicles doped with ssDNA (δ). (D.1, D.2) The procedure of C.1 and C.2 is repeated for a column doped with ε'. (E) Single vesicles can be fed back in the self-assembly procedure (see Figure 2.C). (F) The purified aggregates are encapsulated in vesicles (for details see Figure 2 E-H). (G.1, G.2) The fluorescence signal of the internal compartments is exploited to purify internally compartmentalized vesicles from vesicles not equipped properly.

be replaced by a final FACS for proper composition of the tethered assemblies. By introducing an intermediate separation process, a feedback of single vesicles and incomplete assemblies into the self-assembly process may be realized before they become encapsulated (Figure 3 E). This may increase encapsulation efficiency and therefore may economize the production of custom-tailored vesicular drug delivery systems.

Encapsulation provides an extended circulation time resulting in accumulation at tumors or inflammation sites due the EPR effect, without the need of specific targeting. On the other hand, multiple compartments offer segregation of multicomponent pharmaceuticals that might be released only when and where they are needed. Permeability control might be realized either by exploitation of stimuli inherent to target site (pH, redox potential, temperature) or externally induced (temperature, magnetic field, ultrasound). For a recent review on stimuli-sensitive pharmaceutical nanocarriers see Torchilin (2009).

5 CONCLUSIONS

Encapsulated multicompartment systems may provide stable vehicles for a multicomponent or multifunctional personalized drug delivery. In this work, we established a novel encapsulation technique and provide evidence for the first stable DNA-mediated linkage of more than two vesicle populations. We discussed how these techniques may personalize the individual healthcare by providing custom-tailored vesicular drug delivery systems.

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REFERENCES

Allen, T. M. and Cullis, P. R. (2004). Drug delivery systems: Entering the mainstream. *Science*, 303(5665), 1818-1822.

Bakker-Woudenberg, I., Schiffelers, R. M., Storm, G., Becker, M. J. and Guo, L. (2005). Long-circulating sterically stabilized liposomes in the treatment of infections, *Liposomes, Pt E* (391: 228-260). San Diego: Elsevier Academic Press Inc.

Beales, P. A. and Vanderlick, T. K. (2007). Specific binding of different vesicle populations by the hybridization of membrane-anchored DNA. *Journal of Physical Chemistry A*, 111(49), 12372-12380.

Benkoski, J. J. and Hook, F. (2005). Lateral mobility of tethered vesicle - DNA assemblies. *Journal of Physical Chemistry B*, 109(19), 9773-9779.

Berti, D., Baglioni, P., Bonaccio, S., Barsacchi-Bo, G. and Luisi, P. L. (1998). Base complementarity and nucleoside recognition in phosphatidylnucleoside vesicles. *Journal of Physical Chemistry B*, 102(1), 303-308.

Bolinger, P. Y., Stamou, D. and Vogel, H. (2008). An integrated self-assembled nanofluidic system for controlled biological chemistries. *Angewandte Chemie-International Edition*, 47(30), 5544-5549.

Bonacucina, G., Cespi, M., Misici-Falzi, M. and Palmieri, G. F. (2009). Colloidal Soft Matter as Drug Delivery System. *Journal of Pharmaceutical Sciences*, 98(1),1-42.

Boyer, C. and Zasadzinski, J. A. (2007). Multiple lipid compartments slow vesicle contents release in lipases and serum. *Acs Nano*, 1(3), 176-182.

Burridge, K. A., Figa, M. A. and Wong, J. Y. (2004). Patterning adjacent supported lipid bilayers of desired composition to investigate receptor-ligand binding under shear flow. *Langmuir*, 20(23), 10252-10259.

Chan, Y. H. M., van Lengerich, B. and Boxer, S. G. (2009). Effects of linker sequences on vesicle fusion mediated by lipid-anchored DNA oligonucleotides. *Proceedings of the National Academy of Sciences of the United States of America*, 106(4), 979-984.

Chiruvolu, S., Walker, S., Israelachvili, J., Schmitt, F. J., Leckband, D. and Zasadzinski, J. A. (1994). Higher-order self-assembly of vesicles by site-specific binding. *Science*, 264(5166), 1753-1756.

Constable, E. C., Meier, W., Nardin, C. and Mundwiler, S. (1999). Reversible metal-directed assembly of clusters of vesicles. *Chemical Communications*,(16), 1483-1484.

Eckstein, F. (2007). The versatility of oligonucleotides as potential therapeutics. *Expert Opinion on Biological Therapy*, 7(7), 1021-1034.

Green, N. M. (1990). Avidin and streptavidin. *Methods in Enzymology*, 184, 51-67.

Hotani, H., Nomura, F. and Suzuki, Y. (1999). Giant liposomes: from membrane dynamics to cell morphogenesis. *Current Opinion in Colloid & Interface Science*, 4(5), 358-368.

Jesorka, A. and Orwar, O. (2008). Liposomes: Technologies and Analytical Applications. *Annual Review of Analytical Chemistry*, 1, 801-832.

Kisak, E., Coldren, B., Evans, C., Boyer, C. and Zasadzinski, J. (2004). The vesosome-A multicompartment drug delivery vehicle. *Current medicinal chemistry*, 11(2), 199-220.

Lasic, D., Vallner, J. and Working, P. (1999). Sterically stabilized liposomes in cancer therapy and gene delivery. *Current opinion in molecular therapeutics*, 1(2), 177.

Lasic, D. D. (1993). *Liposomes : from physics to applications*. Amsterdam; New York: Elsevier.

Licata, N. A. and Tkachenko, A. V. (2006). Errorproof programmable self-assembly of DNA-nanoparticle clusters. *Physical Review E (Statistical, Nonlinear, and Soft Matter Physics)*, 74(4), 041406.

Limozin, L., Roth, A. and Sackmann, E. (2005). Microviscoelastic moduli of biomimetic cell envelopes. *Physical Review Letters*, 95(17), 4.

Luisi, P. and Walde, P. (2000). *Giant vesicles*. Chichester: John Wiley & Sons, Ltd.

Luisi, P. L., de Souza, T. P. and Stano, P. (2008). Vesicle Behavior: In Search of Explanations. *Journal of Physical Chemistry B*, 112(46), 14655-14664.

Marchi-Artzner, V., Gulik-Krzywicki, T., Guedeau-Boudeville, M. A., Gosse, C., Sanderson, J. M., Dedieu, J. C. and Lehn, J. M. (2001). Selective adhesion, lipid exchange and membrane-fusion processes between vesicles of various sizes bearing complementary molecular recognition groups. *Chemphyschem*, 2(6), 367-376.

Menger, F. M., Seredyuk, V. A. and Yaroslavov, A. A. (2002). Adhesive and anti-adhesive agents in giant vesicles. *Angewandte Chemie-International Edition*, 41(8), 1350-1352.

Michel, M., Winterhalter, M., Darbois, L., Hemmerle, J., Voegel, J. C., Schaaf, P. and Ball, V. (2004). Giant liposome microreactors for controlled production of calcium phosphate crystals. *Langmuir*, 20(15), 6127-6133.

Noireaux, V. and Libchaber, A. (2004). A vesicle bioreactor as a step toward an artificial cell assembly. *Proceedings of the National Academy of Sciences of the United States of America*, 101(51), 17669-17674.

Paleos, C. M., Sideratou, Z. and Tsiourvas, D. (1996). Mixed vesicles of didodecyldimethylammonium bromide with recognizable moieties at the interface. *Journal of Physical Chemistry*, 100(33), 13898-13900.

Pappas, D. and Wang, K. (2007). Cellular separations: A review of new challenges in analytical chemistry. *Analytica Chimica Acta*, 601(1), 26-35.

Pautot, S., Frisken, B. J. and Weitz, D. A. (2003). Engineering asymmetric vesicles. *Proceedings of the National Academy of Sciences of the United States of America*, 100(19), 10718-10721.

Sideratou, Z., Foundis, J., Tsiourvas, D., Nezis, I. P., Papadimas, G. and Paleos, C. M. (2002). A novel dendrimeric "glue" for adhesion of phosphatidyl choline-based liposomes. *Langmuir*, 18(13), 5036-5039.

Singer, S. J. and Nicolson, G. L. (1972). Fluid mosaic model of structure of cell-membranes. *Science*, 175(4023), 720-&.

Torchilin, V. (2009). Multifunctional and stimuli-sensitive pharmaceutical nanocarriers. *European Journal of Pharmaceutics and Biopharmaceutics*, 71(3), 431-444.

Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*, 4(2), 145-160.

Träuble, H. and Grell, E. (1971). Carriers and specificity in membranes. IV. Model vesicles and membranes. The formation of asymmetrical spherical lecithin vesicles. *Neurosciences Research Program bulletin*, 9(3), 373.

Vermette, P., Taylor, S., Dunstan, D. and Meagher, L. (2002). Control over PEGylated-liposome aggregation by NeutrAvidin-biotin interactions investigated by photon correlation spectroscopy. *Langmuir*, 18(2), 505-511.

Walker, S. A., Kennedy, M. T. and Zasadzinski, J. A. (1997). Encapsulation of bilayer vesicles by self-assembly. *Nature*, 387(6628), 61-64.

Weikl, T. R., Groves, J. T. and Lipowsky, R. (2002). Pattern formation during adhesion of multicomponent membranes. *Europhysics Letters*, 59(6), 916-922.

Weissig, V., Boddapati, S., Cheng, S. and D'souza, G. (2006). Liposomes and liposome-like vesicles for drug and DNA delivery to mitochondria. *Journal of Liposome Research*, 16(3), 249-264.