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Preparation of size tunable giant vesicles from cross-linked dextran(ethylene glycol) hydrogels†

Néstor López Mora[a], **Jesper S. Hansen**[b], **Yue Gao**[a], **Andrew A. Ronald**[b], **Roxanne Kieltyka**[a], **Noah Malmstadt**[b], and **Alexander Kros**[a]

Noah Malmstadt: malmstad@usc.edu; Alexander Kros: a.kros@chem.leidenuniv.nl

[a]Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands. [b]Mork Family Department of Chemical Engineering and Materials Science, University of Southern California, Los Angeles, CA 90007, USA.

Abstract

We present a novel chemically cross-linked dextran–poly(ethylene glycol) hydrogel substrate for the preparation of dense vesicle suspensions under physiological ionic strength conditions. These vesicles can be easily diluted for individual study. Modulating the degree of cross-linking within the hydrogel network results in tuning of the vesicle size distribution.

The growth of high-quality giant (1–100 μm diameter) unilamellar lipid vesicles (GUVs) under physiologically relevant conditions (>300 mOsm kg^{-1}) is generally difficult using most common GUV fabrication methods.[1] To this end, the most widely used methods are gentle hydration and electroformation.[2] Gentle hydration involves the deposition of a lipid on a glass substrate and swelling of the lipid lamella into vesicles by rehydration in aqueous solutions. To adapt this method to grow vesicles at a moderate ionic strength (200 mOsm kg^{-1}), it is necessary to include negatively charged lipids and heat the lipids above their phase transition temperatures.[3] Most often, the vesicle yield of this method is variable and low. However, the addition of non-electrolytic monosaccharides in the dry lipid film promotes lamellar lipid repulsion to increase the vesicle yield.[4] Electroformation can provide higher yields and more homogeneous GUVs through the application of an electric field during GUV growth. However, to grow GUVs under high ionic strength conditions, high field frequencies and longer hydration times are required with the main drawback that lipid hydrolysis and peroxidation can occur.[5]

More recently, hydrogel forming polymer substrates have been employed for the preparation of GUVs in order to reach physiological ionic strength conditions. These substrates include agarose gels,[6] polyacrylamide[6] and thin films of poly(vinyl alcohol).[7] While these methods have allowed GUV formation at moderate ionic strengths (~200–280 mOsm kg^{-1}), they

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Correspondence to: Noah Malmstadt, malmstad@usc.edu; Alexander Kros, a.kros@chem.leidenuniv.nl.

afford minimal ability to control the characteristics of the GUV in terms of morphology and size distribution.

Here, GUVs were formed on a covalently cross-linked hydrogel substrate. We demonstrate that control over crosslink density can alter the size distribution of the GUVs formed. We used dextran polymers cross-linked by poly(ethylene glycol) (PEG) chains using Michael addition to simultaneously prepare the hydrogel (Dex–PEG) and anchor it to a glass surface. Our hypothesis is that an anchored covalent hydrogel cannot be dissolved during the GUV formation process to potentially contaminate the lipid bilayer, which may be a concern with non-covalently crosslinked hydrogels.[6] Moreover, covalent hydrogel matrices enable the possibility for control over GUV size distributions through modulation of cross-linker density and network topology. Dextran (MW = 70 kDa) was modified with *N*-maleoyl-β-alanine following a previously described protocol to provide polymer 1 to be used for Michael addition with reactive thiols on the PEG polymer and the glass surface.[8] The degree of substitution (DS) on the polymer was controlled by the molar ratio of *N*-maleoyl-β-alanine relative to dextran and subsequently validated by ^{1}H-NMR. Concurrently, microscope slides were prepared for anchoring of the chemically cross-linked hydrogel directly to the glass surface. A reactive thiol moiety was introduced on the glass slide surface with 3-mercaptopropyl trimethoxysilane. The thiol coated microscope slides were cross-linked to the hydrogel by drop-casting the dextran solution (2 wt%) and PEG dithiol 2 at various molar ratios at 40 °C (Fig. 1, step 2) until a homogeneous hydrogel film was formed. Following the formation of the hydrogel, the desired lipid mixture was deposited on the hydrogel surface and the solvents were evaporated in a vacuum oven for 30 minutes at 35 °C or at room temperature under a gentle stream of nitrogen gas to prevent lipid oxidation (Fig. 1, step 3). In a final step, the hydrogel and lipid film on the glass slide were rehydrated in aqueous buffer solutions for 1–2 hours to form free-floating vesicles (Fig. 1, step 5). In all cases, hydration of the lipid film was performed above the T_m of all the lipids used. To validate the widespread applicability of the Dex–PEG chemically cross-linked network for GUV growth, several buffers, lipid compositions, and cross-linking ratios were tested.

First, the effect of physiological ionic strength conditions on vesicle growth was examined. Hence, two buffers used ubiquitously in biological cell studies were examined for vesicle growth: phosphate buffered saline (PBS) and HEPES potassium chloride saline buffer (HBS). The osmolality of these buffers was experimentally determined by measuring the freezing point depression for both PBS (310 mOsm kg^{-1}) and HBS (320 mOsm kg^{-1}). In general, qualitative differences were noted between both buffers; HEPES buffer produced slightly greater yields and larger sized vesicles (see ESI[†]). However, in both of these buffers high yields of free floating vesicles were easily obtained.

The capacity of the Dex–PEG network to support vesicle growth using various lipid compositions was then explored. Binary mixtures of lipids containing both anionic and

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neutral lipids and ternary mixtures of cholesterol were examined. The lipid compositions A–H (see ESI,† Table S1) were designed to examine the effect of three different parameters on vesicle formation: cholesterol (CH) content, negatively charged lipid content (POPG and DOPS), and liquid ordered lipid phases (DPPC). Free floating GUVs using lipid mixtures (Fig. 1A, B and, F) with up to 25% CH provided a good yield of GUVs with a spherical morphology in PBS and HBS buffers. This result is in contrast to previous reports where gentle hydration methods showed exclusive formation of tubular morphologies and liposome networks connected by tubes using POPC/CH with 5–30% CH in HEPES.[9] Using the Dex–PEG system, we found numerous spherical GUVs alongside such morphologies.

Another parameter that was explored was the effect of increase in the anionic lipid content on GUV formation. Previously, it was found that lipid mixtures containing more than 10–20% anionic lipids were difficult to grow by gentle hydration methods.[10] Hence, we selected lipid mixtures ranging from 10–50% in POPG (Fig. 1C and D). Using our method, we were able to obtain high yields of spherical vesicles under high ionic strength conditions.

Moreover, we examined mixtures with DOPS (10%), a lipid that is known to decrease vesicle yields under high ionic strength conditions. Consistent with this notion we also observed high yields of spherical GUVs with 10% DOPS (Fig. 1E). Finally, lipid mixtures known to undergo phase separation through phase coexistence of liquid ordered (L_o) and liquid disordered (L_d) phases were tested.[11] We found, using the Dex–PEG system, that phase-separated GUVs consisting of 30–50% DPPC can be easily grown at 50 °C (Fig. 2). Moreover, we can prepare such kinetically trapped GUVs by the Dex–PEG method without the use of additional additives under high ionic strength conditions. Overall, all lipid compositions using the additive free Dex–PEG method to grow GUVs under high ionic strength conditions yielded high quantities of free floating vesicles. A Gaussian size distribution centered between 10 and 15 μm was found after 1 hour of GUV preparation for all lipid compositions upon using an equimolar ratio of the dextran polymer and the cross-linker (see ESI†). This result suggests a relationship between the method of vesicle growth and size.

Excitingly, a correlation between cross-link density and vesicle size distribution was observed (Fig. 3). On average, a population of a hundred GUVs were sized for each experiment. Decreasing the molar ratio of PEG to 75 and 50 mol% with respect to dextran polymer resulted in a concomitant increase in the vesicle size distribution in a single step. Current methods used to gain control over vesicle size require a minimum of two steps to achieve similar results whereas vesicle size can be tuned within the Dex–PEG system through modifying the composition of the chemical network in a single step.[12] Hence, the success of the Dex–PEG method in enabling growth of GUVs based on various lipid compositions under high ionic strength conditions arises from several pertinent chemical features and properties of materials.

Based on our data we hypothesize that the driving force for generating free floating GUVs is the high swelling behavior of the hydrogel upon hydration. Specifically the water content differs within the hydrogel from 2% in the dry state to 90% in the wet state. The ability of the Dex–PEG hydrogel to imbibe a high percentage of an aqueous solution on the order of

less 1 hour most likely contributes to interlamellar repulsion that generates the necessary forces to facilitate efficient growth of giant vesicles under physiological ionic strength conditions. In addition, the starting water content of the film plays an important role; vesicles are not formed without a pre-hydrated gel. Moreover, chemical ligation to the glass surface is essential due to the rapid growth of the hydrogel layer on the glass surface upon exposure to buffer. Earlier experiments showed that unligated hydrogels resulted in simultaneous detachment from the glass substrate during vesicle formation.[6] To examine whether hydrogel components were dissociated from the surface or incorporated in vesicles, we synthesized a fluorescently labeled dextran polymer with 1 and 2.5 mol% of methoxycoumarin-3-carboxylic acid (Dex–PEG–C) to be tracked by two-photon fluorescence microscopy. Analysis of free floating GUVs produced from Dex–PEG–C showed no fluorescence either in the membrane or inside the formed GUVs at room temperature (see ESI†).

In conclusion, we present a widely applicable method that facilitates the additive free growth of GUVs under physiological ionic strength conditions based on various lipid compositions. The high swelling capacity of the Dex–PEG promotes the formation of high yields of spherical, free-floating GUVs. Additionally, this method enables the growth of GUVs possessing phase separated domains under physiological conditions. Finally, modulating the cross-link density of the Dex–PEG network provides a handle to tune the vesicle size. This Dex–PEG hydrogel system is a powerful method that can be exploited to grow vesicles for applications such as membrane interactions, drug delivery, molecular recognition, lipid raft organization, and membrane fusion studies.[13]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Morales-Penningston NF, Wu J, Farkas ER, Goh SL, Konyakhina TM, Zheng JY, Webb WW, Feigenson GW. Biochim. Biophys. Acta, Biomembr. 2010; 1798:1324–1332.
2. (a) Reeves JP, Dowben RM. J. Cell. Physiol. 1969; 73:49–60. [PubMed: 5765779] (b) Angelova MI, Dimitrov DS. Faraday Discuss. 1986; 81:303–311.
3. Akashi K, Miyata H, Itoh H, Kinosita K Jr. Biophys. J. 1996; 71:3242–3250. [PubMed: 8968594]
4. Tsumoto K, Matsuo H, Tomita M, Yoshimura T. Colloids Surf., B. 2009; 68:98–105.
5. (a) Pott T, Bouvrais H, Meleard P. Chem. Phys. Lipids. 2008; 154:115–119. [PubMed: 18405664] (b) Montes LR, Alonso A, Goni FM, Bagatolli LA. Biophys. J. 2007; 93:3548–3554. [PubMed: 17704162]
6. Horger KS, Estes DJ, Capone R, Mayer M. J. Am. Chem. Soc. 2009; 131:1810–1819. [PubMed: 19154115]
7. Weinberger A, Tsai F-C, Koenderink GH, Schmidt TF, Itri R, Meier W, Schmatko T, Schröder A, Marques C. Biophys. J. 2013; 105:154–164. [PubMed: 23823234]

8. (a) Peng K, Tomatsu I, van den Broek B, Cui C, Korobko AV, van Noort J, Meijer AH, Spaink HP, Kros A. Soft Matter. 2011; 7:4881–4887.(b) Peng K, Tomatsu I, Korobko AV, Kros A. Soft Matter. 2010; 6:85–87.(c) Peng K, Tomatsu I, Kros A. Chem. Commun. 2010; 46:4094–4096.(d) Peng K, Cui C, Tomatsu I, Porta F, Meijer AH, Spaink HP, Kros A. Soft Matter. 2010; 6:3778–3783.
9. Nomura SIM, Mizutani Y, Kurita K, Watanabe A, Akiyoshi K. Biochim. Biophys. Acta, Biomembr. 2005; 1669:164–169.
10. Akashi K, Miyata H, Itoh H, Kinosita K. Biophys. J. 1998; 74:2973–2982. [PubMed: 9635751]
11. Veatch SL, Keller SL. Phys. Rev. Lett. 2002; 89:268101. [PubMed: 12484857]
12. Nishimura K, Suzuki H, Toyota T, Yomo T. J. Colloid Interface Sci. 2012; 376:119–125. [PubMed: 22444482]
13. (a) Zope HR, Versluis F, Ordas A, Voskuhl J, Spaink HP, Kros A. Angew. Chem., Int. Ed. 2013; 52:14247–14251.(b) Versluis F, Voskuhl J, van Kolck B, Zope H, Bremmer M, Albregtse T, Kros A. J. Am. Chem. Soc. 2013; 135:8057–8062. [PubMed: 23659206] (c) Zheng T, Voskuhl J, Versluis F, Zope HR, Tomatsu I, Marsden HR, Kros A. Chem. Commun. 2013; 49:3649–3651.(d) Robson Marsden H, Elbers NA, Bomans PHH, Sommerdijk NAJM, Kros A. Angew. Chem., Int. Ed. 2009; 48:2330–2333.

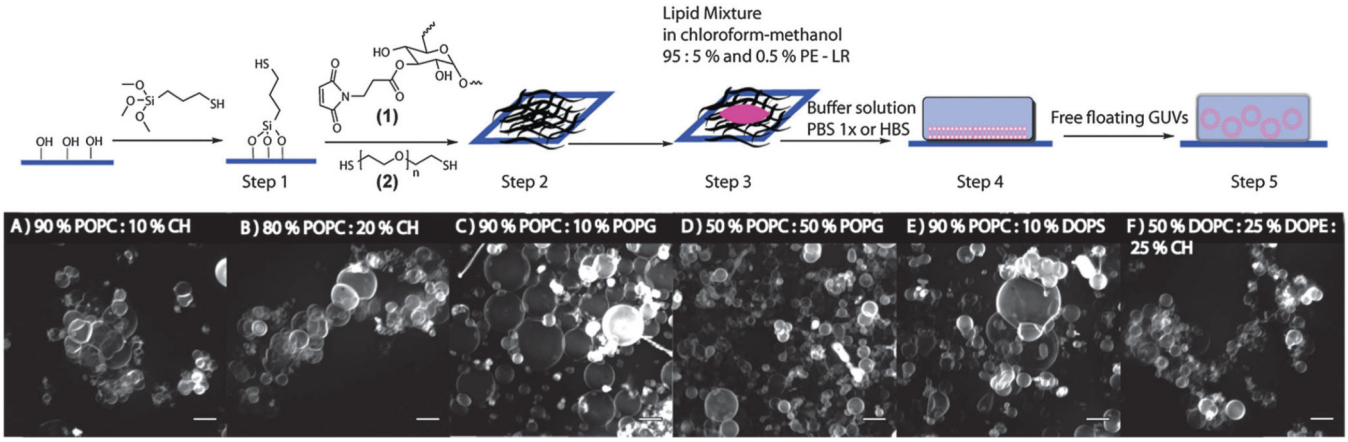

Fig. 1.

Formation of giant vesicles from dextran(ethylene glycol) hydrogels. The method used to produce GUVs from covalently cross-linked hydrogels is shown at the top. Confocal microscopy *Z*-projection images of GUVs formed with various lipid compositions are shown (see ESI† for further details. scale bar = 10 μm).

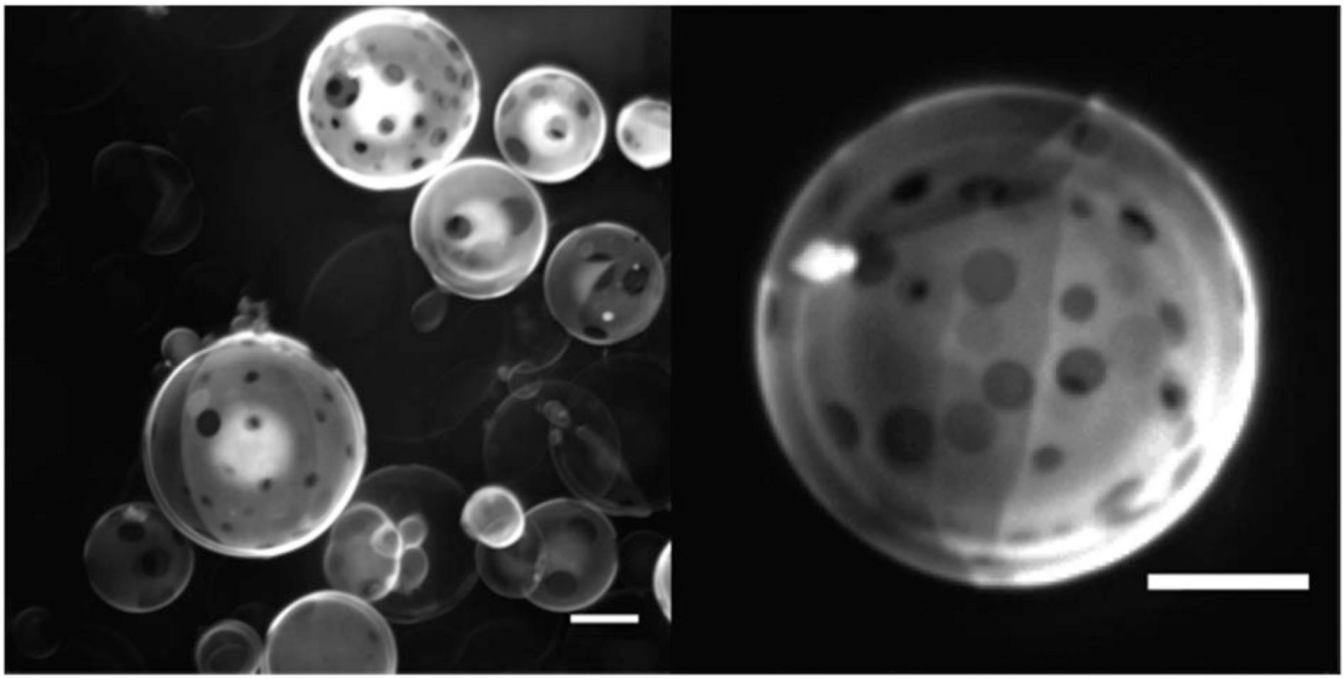

Fig. 2.

Confocal microscopy *Z*-projections showing phase separated GUVs with the lipid composition 40% DPhPC/40% DPPC/20% CH (mol%) in PBS at physiological ionic strength. The scale bars are 10 μm.

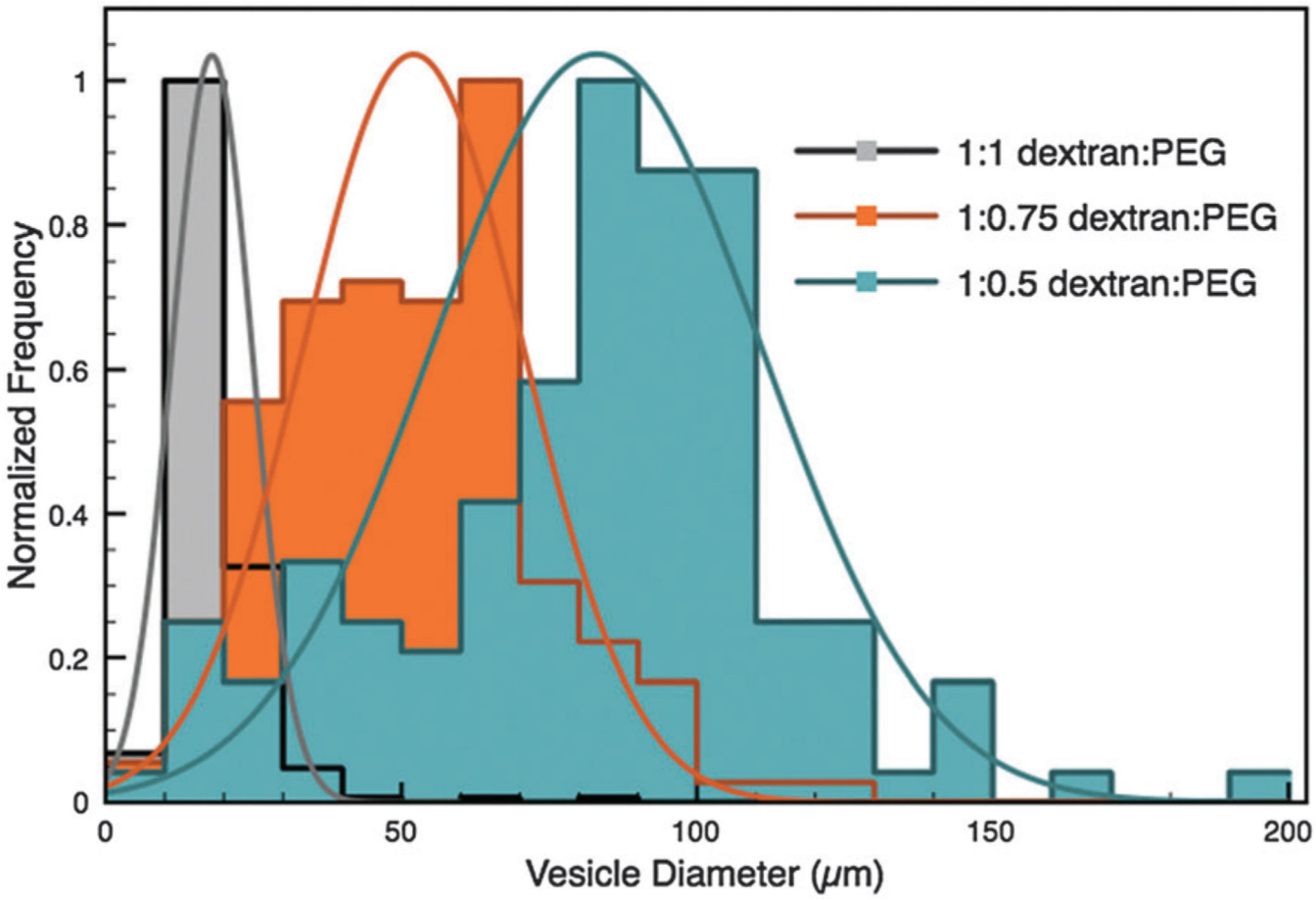

Fig. 3.

Tuneability of the size distribution of GUVs (50% DOPC/25% DOPE/25% CH) as a function of cross-link density of PEG relative to dextran in PBS. Average diameters were found to be 18 ± 8 μm (N = 215) for 1 : 1 ratio of dextran–PEG, 52 ± 22 μm (N = 171) for a 1 : 0.75 dextran–PEG and 83 ± 33 μm for a 1 : 0.5 dextran : PEG (N = 139).