Anisotropic Supported Lipid Bilayers for Spatially Dynamic Surface Protein Presentation

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Statement of Purpose: There has been interest in the development of lipid polymer hybrid particles (LPHPs) for various biomedical applications. Combining the stability of a polymeric core with the biomimetic nature of a fluidic supported lipid bilayer (SLB) this platform is a promising technology for therapeutics and diagnostics.[1] To our knowledge, to date all LPHPs have been synthesized utilizing a spherical core nanoparticle with a lipid shell. Recent evidence has suggested that ellipsoidal particles enable superior interaction with biomedical systems including reduced non-specific cellular uptake and increased targeted binding compared to equivalent spherical particles.[2] In addition, ellipsoidal microparticles and nanoparticles have shown superior cellular mimicry in the setting of immunoengineering applications.[3] To realize the potential of union of non-spherical particles and SLBs, we have developed an anisotropic, biodegradable LPHP for enhanced cellular biomimicry and therapeutic drug delivery.

Methods: Spherical poly(lactic-co-glycolic acid) (PLGA) microparticles were synthesized by a standard single emulsion technique. Spheres were then converted to ellipsoids utilizing the previously described thin film stretching method.[4] Preformed liposomes consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), maleimide functionalized DOPC, and cholesterol were then fused to spherical or non-spherical microparticles under agitation by sonication. Functionalization was achieved through the conjugation of a thiolated avidin to the maleimide lipids. Biotinylated proteins and fluorophores were assessed for specific and localized binding to the surface of the particles. SLB fluidity was evaluated through fluorescence recovery after photobleaching (FRAP) utilizing a confocal microscope. Cellular uptake rates of spherical and ellipsoidal SLBs were compared through encapsulation of a fluorophore and incubation with RAW 264.7 macrophages.

Results: The generated PLGA microparticles were determined to be 3.2 µm in size utilizing Image J analysis of SEM images of the particles. After stretching, the ellipsoidal particles had an aspect ratio of 3.3. Lipid/particle fusion was determined by confocal analysis of fluorophore labeled lipids combined with fluorophore labeled particles. Profile analysis of confocal images revealed an enrichment of the lipid signal at the borders of the particles confirming efficient coating of both spherical and ellipsoidal particles by lipid. Incubation of of maleimide SLBs with thiolated avidin pre-bound to fluorescent biotin resulted in efficient fluorescence capture on the surface of the SLBs, and this was verified through confocal analysis of avidin functionalized SLBs with a biotinylated fluorophore (Figure 1a and 1b). Biotinylated antibody conjugation was verified through incubation with avidin functionalized SLBs and subsequent evaluation of particle protein content. Analysis revealed 50%-70% protein conjugation efficiency across a variety of protein doses. Lipid bilayers were determined to have biomimetic fluidity by FRAP analysis (Figure 1c and 1d), as the lateral diffusion constants of both spherical and ellipsoidal SLBs were determined to be 10^{-10} cm^2/s. Confocal and flow cytometry analysis of macrophage particle uptake demonstrated a statistically significant reduction in internalization of ellipsoidal SLBs compared to spherical SLBs across a variety of particle/cell ratios.

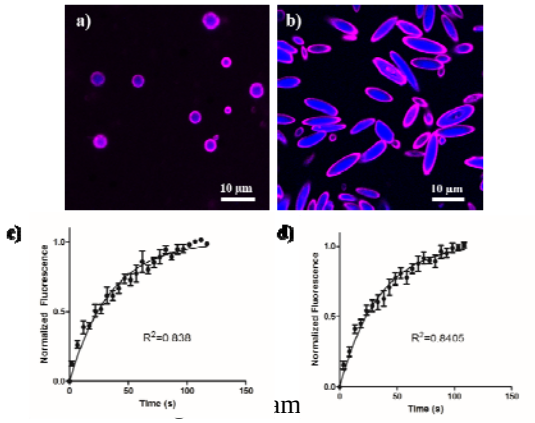

Figure 1: Functionalized (a) spherical and (b) ellipsoidal lipid bilayers (pink) can be synthesized using anisotropic polymeric supports (blue). (c) Spherical and (d) ellipsoidal SLBs demonstrate membrane-like fluidity.

Conclusions: Through the combination of the thin film stretching method for ellipsoidal microparticle generation and the liposome fusion method for supported lipid bilayer synthesis, a robust procedure for the development of ellipsoidal SLBs has been developed. Anisotropic SLBs were successfully synthesized and demonstrated to be capable of binding to multiple biotinylated entities via an avidin functional intermediate. Continued development of this technology will allow for more accurate biomaterials-mediated mimicry of natural cells.

References

1. Hadinoto K *et al*. Eur J Pham Biopharm. 2013:85:427-443.
2. Canelas DA *et al*. WIREs Nanomed Nanobiotechnol. 2009:1:391-404.
3. Sunshine JC *et al*. Biomaterials. 2014:35:269-277.
4. Ho C *et al*. Colloid Polym Sci. 1993:271:469-479.