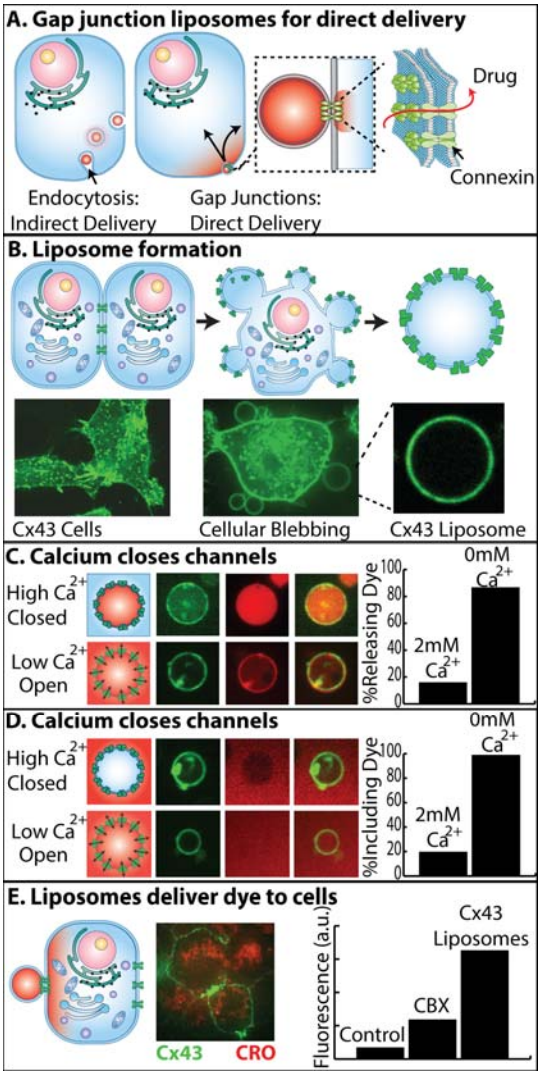
Introduction: Encapsulating chemotherapeutics within targeted particles such as liposomes achieves localization to tumors. However, to have their effect, encapsulated drugs must reach the cellular cytoplasm. Most particles are taken up by tumor cells through endocytosis, giving drugs a narrow window of time to escape from endosomes before they are expelled via exocytosis or degraded [1]. At present, poor control of endocytic pathways greatly limits the efficiency of particle-based drug delivery to the cytoplasm [2], reducing chemotherapeutic efficiency. Our work aims to address this problem with a *new strategy – a chemotherapeutic delivery approach that uses the cellular gap junction network to deliver drugs directly to the cytoplasm of tumor cells (**Fig A)***. Specifically, we have developed liposomes that contain functional gap junction pores (connexin 43), and are able to form gap junctions with target cells to deliver drugs and other small molecules directly to the cellular cytoplasm.

Materials and Methods: As transmembrane proteins, connexins are notoriously difficult to isolate and manipulate, making their functional incorporation in liposomes a challenge. To address this hurdle, our laboratory has developed a new method in which liposomes that contain a high concentration of gap junction proteins can be harvested directly from the plasma membrane of mammalian donor cells. In brief, by genetically manipulating cells to overexpress junction proteins and then inducing cells to expel large portions of their plasma membranes through cellular blebbing [3, 4], we produce membrane liposomes with functionally embedded gap junction proteins (***Fig B***).

Results and Discussion: Several key results have demonstrated that our approach yields a homogenous population of liposomes that contain functional gap junction pores (Cx43YFP) and are capable of delivering drugs directly to the cellular cytoplasm. (i) Over 90% of liposomes contain fluorescent connexin embedded in their membranes. (ii) Liposomes formed in the presence of fluorescent dye retain the dye in high calcium concentrations, which are known to close gap junctions. When calcium is removed from solution, the gap junctions open and the liposomes release their dye (***Fig C***). (iii) When dye is added to the outside of liposomes in high calcium concentrations, the liposomes exclude the dye. When calcium is removed from solution, the liposomes retain the dye. (***Fig D***). (iii) Liposomes exposed to cells expressing Cx43YFP associate preferentially with cellular interfaces enriched in Cx43YFP. This association is substantially reduced when cells are pre-incubated with carbenoxolone, a gap junction inhibitor. (iii) Dye-loaded liposomes exposed to cells transfer the dye to the cellular cytoplasm. Addition of carbenoxolone substantially reduces the dye transfer (***Fig E***). (iv) Finally, liposomes can be loaded with gemcitabine, and exposure of tumor cells to gemcitabine-loaded liposomes kills the cells. Current studies are quantifying the efficiency of drug transfer to the cellular cytoplasm using gap junctions in comparison to endocytic routes.

Conclusions: The cellular gap junction network provides a relatively unexplored and potentially highly efficient conduit for the delivery of drugs to the cytoplasm of tumor cells. We have created liposomes containing functional gap junction pores and demonstrated their ability to form junctions with cells and deliver dye to the cellular cytoplasm. Liposomes containing these gap junctions could in principle be used to deliver a wide range of water-soluble drugs or combination therapies including multiple classes of chemotherapeutics, siRNA, and peptides, each of which have been demonstrated previously to pass from cell to cell through gap junctions [5]. In each case, the gap junction network provides the key advantage of direct delivery to the cytoplasm, a promising alternative to the complex and poorly controlled process of delivery via endocytosis.



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