



CALIFORNIA SCIENCE & ENGINEERING FAIR 2019 PROJECT SUMMARY

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| Name(s) Seeta Patel; Hyunjin Rheem | Project Number S1218 |
| Project Title Surface-Modified Loaded Human Red Blood Cells for Targeting and Delivery of Drugs | |
| <p style="text-align: center;">Abstract</p> <p>Objectives The fabrication of nanoparticles derived from red blood cells is accompanied with the expression of an apoptotic marker which creates the problem of ineffective nanoparticles. Thus, the project serves to create the most efficient nanoparticles with the lowest outward expression of the apoptotic marker done by testing different fabrication processes and analyzing the results.</p> <p>Methods The methods of this experiment were separated into two distinct procedures: fabricating the four different nanoparticles and setting up the nanoparticles for flow cytometry in order to analyze the data. Human erythrocytes were utilized in order to fabricate erythrocyte ghosts. 1x PBS, 5 mM NaH₂PO₄/Na₂HPO₄ (3) solutions were utilized in order to wash erythrocytes, 0.5X PBS (contains 50 micromolar ICG) also used in order to resuspend particles after each centrifuging session. Hemoglobin depletion forms erythrocyte ghosts, and the addition of different solutions: PIGPA-C (pyruvate, inosine, glucose, phosphate), which serves as a rejuvenating solution, and PIGPA-NaCl solution, which has NaCl as a base. The fabrication process includes using 10 microliter cells (RBCs, EGs, NETs, or P-NaCl NETs) to 100 micro liter Annexin binding buffer (~ 1x10⁶ cells/mL). After 5 microliter Annexin V - AF488 is added, the cells are mixed and incubated, then analyzed using a flow cytometer.</p> <p>Results Through flow cytometry, the resulting experimental data indicated very low expression of PS for both RBCs and P-NaCl NETs. Meanwhile the number of PS+ cells were significantly higher for both EGs and NETs. Approximately, 60-65% of EGs and NETs expressed PS on the outer leaflet of the RBC membrane, whereas in RBCs and P-NaCl NETs only 1-2% of the cells were PS+.</p> <p>Conclusions Due to the fact that the translocation of PS is an apoptotic marker, exposed PS drastically lowers the circulation time of the RBC-derived drug delivery vehicles. P-NaCl NETs had significantly lower amounts of exposed PS than the EGs and NETs, quantified by the use of an Annexin V Alexa Fluor 488 conjugate that would bind to PS on the outer leaflet of the membrane. They were also the most similar to human RBCs in relation to the amount of translocated PS. These results, caused by PIGPA rejuvenation make the use of P-NaCl NETs most ideal for targeting and delivery of drugs in the human vasculature as these particles have the longest circulation time.</p> | |
| Summary Statement We have determined that the most efficient loading method for erythrocytes in order to serve as potential drug delivery vehicles is by the utilization of PIGPA-NaCl . | |
| Help Received We give our gratitude to Dr. Anvari and Mr. Tang for mentoring us with this project and allowing us to use UCR's materials and facilities. Mr. Tang assisted us in the data collection and the interpretation of the data that was collected. However, the analysis and conclusion was reached by ourselves. | |