Creation of an Artificial Golgi Organelle: Glycosaminoglycans in Microfluidics

by

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ABSTRACT

Studies were conducted to construct a prototype artificial Golgi organelle through the use of digital microfluidics, recombinant enzymes, computer automation, and magnetic nanoparticle technologies. Experiments were performed using the artificial Golgi to demonstrate the ability of the device to enzymatically redesign immobilized glycans. Fluorescence based protein binding studies were performed on these immobilized glycan products to determine their bioactivity.

Heparan sulfate, the glycosaminoglycan chain of the heparan sulfate proteoglycan was immobilized on magnetic nanoparticles using streptavidin-biotin and modified on this artificial Golgi using 3-*O*-sulfotransferase-1 in the presence of 3'-phosphoadenosine-5'-phosphosulfate. The resulting 3-*O*-sulfonated product gained the ability to bind antithrombin III demonstrating this conversion was successful. Next, *N*-sulfoheparosan was covalently coupled to amine magnetic nanoparticles through reductive amination and analyzed using heparin lyase treatment followed by liquid chromatography mass spectral analysis to determine disaccharide composition. This demonstrated that magnetic nanoparticles could be prepared with sufficient glycosaminoglycan capacity (greater than 100 ng/ μg of nanoparticles) for online spectroscopic detection and analysis. A high throughput system including a controller, droplet detector, software, and a robotic analytical interface was developed in collaboration with Justin Gullotta, an electrical engineer and William Fergus, a computer scientist at Rensselaer.