101d A Highly Integrated on-Chip Glycoprotein Processor for Rapid Sialic Acid Content Monitoring

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A highly integrated and efficient on-chip glycosylation analysis is for the first time demonstrated on a disposable plastic microfluidic chip. This process includes on-chip sample purification using packed affinity channel followed by on-chip liquid-phase reactions realized on a multiple reagent delivery and circulating structures. This approach will potentially enable real-time and multiple data on-line bioprocess monitoring including sample purification and spectroscopic or other means of analysis. To demonstrate this concept, a fluorimetric thiobarbituric acid (TBA) assay assessing the sialic acid content (moles sialic acid/mole protein) of therapeutic glycoproteins produced by Chinese Hamster Ovary was conducted using this integrated glycoprotein processor.

Glycosylation is a post-translational processing event has been reported to be important for protein folding, stability, solubility, biological activity, antigenicity, and immunogenicity. Specifically, terminal sialic acid improves therapeutic proteins' value by extending their half-life in circulation. Protein sialic acid content is an important parameter to assess product quality during the development and operation of bioprocesses. Conventional fluorescence - high performance liquid chromatography and fluorophore-assisted carbohydrate electrophoresis can quantify the sugar content and differentiate minute structural difference. The speed and throughput of these methods are however seriously limited by the lengthy sample preparations which take up to a number of days to be completed. Additionally, for the purpose of therapeutic protein production, the extent of sialylation, rather than the detailed glycosylation pattern, can be used as the criterion for product quality assessment. We are developing a highly integrated on-chip glycoprotein processor which can purify glycoproteins of interest out of cell fluid complex and quantify change of sialic acid content. A fluorimetric TBA assay was adapted to quantify the sialic acid content. This fluorimetric TBA assay took less than 2 hours to complete. As small as 20% change of sialic acid content was detected at a protein concentration of 20 μg/mL. An affinity purification column and a liquid phase reactor were fabricated on a polystyrene chip. On-chip programmable pumps were also integrated to deliver and mix fluid. We expect to connect this integrated on-chip glycoprotein processor to bioreactors for real time monitoring of the sialic acid content of recombinant glycoprotein. Furthermore, it is expected that the knowledge and solutions developed in this project will be extended to rapid detection of other sugars added during glycosylation.