

Enzyme Column with Trypsin Coated Polymer Nanofibers for Protein Digestion

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We constructed a trypsin column for rapid and efficient protein digestion in proteomic analysis. Electrospun and ethanol-dispersed polymer nanofibers were used for the fabrication of highly stable trypsin coatings, which was prepared by a two-step process of covalent attachment and enzyme crosslinking. It has been observed that a simple step of ethanol dispersion improved not only the enzyme loading but also the performance of protein digestion. In-column digestion of enolase was successfully performed in less than twenty minutes. By applying the ethanol dispersion of polymer nanofibers, the bypass of samples was reduced by filling up the column with well-dispersed nanofibers, yielding more rigorous and reproducible digestions. Regardless of ethanol-dispersion or not, trypsin coatings showed better digestion performance and improved performance stability under recycled uses than covalently-attached trypsin, in-solution digestion, and commercial trypsin beads. The combination of highly stable trypsin coatings and ethanol-dispersion of polymer nanofibers has opened up a new potential to develop a trypsin column for on-line and automated protein digestion.