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## Adhesion Control of Human Umbilical Vein Endothelial Cells Using Clickable Poly(2-oxazoline)-Grafted Biosynthesized Extracellular Matrix Protein

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**Key Words:** T7 expression system; *in vivo* incorporation; artificial extracellular matrix protein (aECM); *p*-azidophenylalanine; alkyne-containing poly(2-oxazoline), click reaction, ring-opening polymerization

**Abstract:** A bacterial expression host was used for *in vivo* incorporation of *p*-azidophenylalanine (*p*-N<sub>3</sub>Phe), a phenylalanine (Phe) analog, into an artificial extracellular matrix protein (aECM-CS5-ELF). The host harbors the mutant phenylalanyl-*t*RNA synthetase (PheRS) which has an enlarged binding pocket, where the Ala294Gly/Thr251Gly mutant PheRS (PheRS\*\*) was expressed under the control of T7 promoters. Biosynthesized aECM-CS5-ELF containing *p*-N<sub>3</sub>Phe (aECM-CS5-ELF-N<sub>3</sub>) was coupled with alkyne-containing poly(2-oxazoline) prepared via ring-opening polymerization of 2-oxazolines (2-methyl-2-oxazoline, 2-ethyl-2-oxazoline, and 2-*iso*-propyl-2-oxazoline) using methyl triflate (TfOMe) and propiolic acid as the initiator and terminator, respectively. A copper-catalyzed alkyne-azide click reaction was used to graft the poly(2-oxazoline)s onto the aECM. Examination of the solubility in organic and aqueous media, and the lower critical transition temperature (LCST), revealed a dependence on the incorporation ratio of *p*-N<sub>3</sub>Phe and graft chain species; the LCST behavior was markedly altered when poly(2-oxazoline) moieties were present as side chains. Circular dichroism measurements indicate that grafting was not responsible for conformational changes, because the conformation was retained both at below and above the LCST. Specific adhesion and subsequent temperature-sensitive detachment of human umbilical vein endothelial cells (HUVECs) onto the cross-linked aECM-CS5-ELF-N<sub>3</sub>-*graft*-poly(2-oxazoline) surfaces was also demonstrated.

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