# Click-Chemistry Customizable Polyhydroxyalkanoate Nanoparticles for Drug Delivery

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**Statement of Purpose:** Numerous materials have been proposed as drug delivery systems for improved treatment of cancer and inflammatory diseases. Successful clinical translation of materials designed for drug delivery is dependent upon biocompatibility and biodegradability, as well as ease of processing. Although a number of polyesters, particularly poly(lactic-co-glycolic acid) (PLGA), have already received FDA approval for use in drug delivery, polymer synthesis requires a large number of steps to introduce targeting moieties. Thus, a window of opportunity exists for the production of biocompatible and biodegradable materials that are amenable to rapid modification to further enhance delivery of therapeutics to diseased sites. Previously, our lab engineered *Escherichia coli* to produce polyhydroxyalkanoates (PHAs) with specifically defined repeating units.[1] In this study, we extend this research to include repeating units with functional moieties suitable for click chemistry reactions and describe the development of click chemistry-customizable, PHA-based nanoparticles for drug delivery.

**Methods:** Previous research using engineered *E. Coli* to produce PHA copolymers was extended beyond saturated fatty acids to include 10-undecenoic, 10-undecynoic acid, and 10-azidodeanoic acid monomers (Fig. 1).[1] These copolymers were used to prepare functionalized nanoparticles by miniemulsion. 4.0%wt solutions of copolymers in chloroform were added to a 0.42 %wt sodium dodecyl sulfate (SDS) solutions in water (2.4:1 water:chloroform), and the mixtures were stirred at room temperature for one hour. Ultrasonication at 50% amplitude, pulse cycle 0.6 with a Branson sonifier for 5 minutes yielded the desired emulsions. The chloroform was evaporated off at 65°C for 90 minutes, and nanoparticle characterization was conducted immediately.[2] The concentrated emulsions were diluted with aqueous SDS and size measurements were obtained via dynamic light scattering. For comparison, PLGA nanoparticles were prepared in an identical manner.

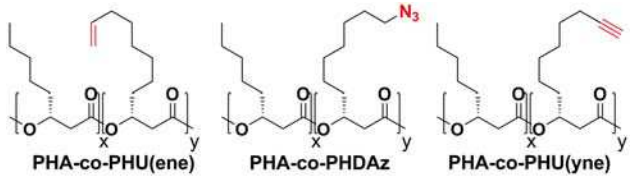


**Fig. 1.** PHA copolymers with functional side chains were synthesized by engineered E. coli.

To verify that functionalized PHA nanoparticles can be used as substrates for click chemistry, alkyne containing nanoparticles were reacted with Chromeo™ 642 Azide (Active Motif) in the presence of copper iodide catalyst and triethylamine following a literature protocol.[3] The fluorescently tagged nanoparticles were purified by dialysis against 1X PBS. This solution was used immediately for cellular uptake experiments. NIH3T3 cells on collagen-coated glass coverslips were exposed to the fluorescently labeled PHA nanoparticles for 45 min. After rinsing with PBS, the cells were imaged with a Nikon Eclipse Ti inverted microscope.

**Results:** PHA biopolymers with chemically modifiable side chains were successfully produced by engineered *E. coli* and subsequently used to generate nanoparticles. As shown in Table 1, the miniemulsion method consistently yielded nanoparticles with low polydispersity in the desired size range for drug delivery. Of note, PLGA yielded nanoparticles of the same approximate size.

**Table 1.** Nanoparticles were generated by miniemulsion of functionalized PHA copolymers or PLGA.

| Polymer | Functional Group | Size (nm) | PDI |
|---|---|---|---|
| **PHA** | Alkene | 88.4 | 0.09 |
| | Azide | 83.9 | 0.15 |
| | Alkyne | 136.8 | 0.09 |
| | Azide:Alkyne (1:1) | 133.5 | 0.16 |
| **PLGA** | None | 95.0 | 0.04 |

Nanoparticles prepared with PHA copolymers that included terminal alkynes were reacted with an azide-containing fluorescent tag via click chemistry. Size measurements verified that the PHA nanoparticles remained stable through the course of the reaction and dialysis. As shown in Fig 2, NIH3T3 cells were able to internalize the fluorescently tagged nanoparticles.

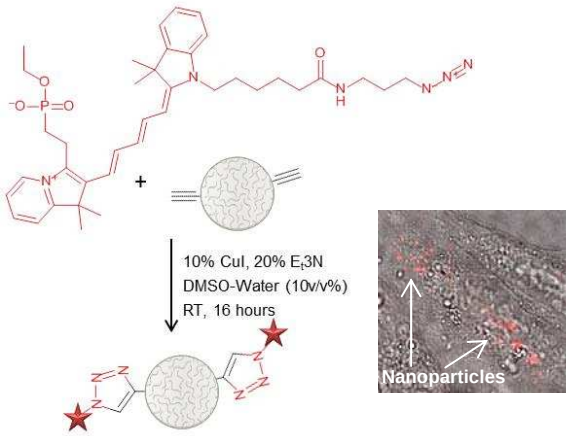


**Fig. 2.** NIH3T3 cells were able to uptake PHA-based nanoparticles fluorescently tagged via click chemistry.

**Conclusions:** Click chemistry-customizable, PHA-based drug delivery platforms were successfully developed. Future studies will aim towards modifying the functionalized nanoparticles with active targeting groups via click chemistry.

**References:**

1. Tappel RC. J. Biosci Bioeng. 2012;113:480-486.
2. Landfester K. Adv Mater. 2002;14:651-655.
3. Kele P. Org Biomol Chem 2009;7:3486-90