Grafted Block Copolymer Nano-Assembly (GNA) Drug Carriers

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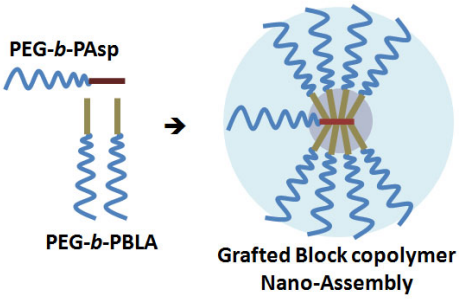
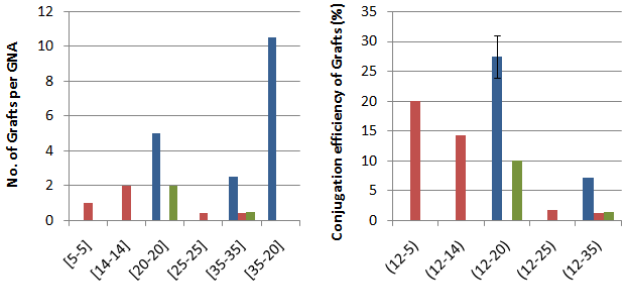
Statement of Purpose: In an effort to develop a platform that may integrate advantages of various drug carriers currently available, we develop grafted block copolymer nanoassembly (GNA) drug carriers in this study. GNAs consist of multiple poly(ethylene glycol)-*b*-poly(amino acid) (PEG-*b*-PAA) copolymers (grafts), which are tethered onto a flexible polymer backbone (scaffold) as represented in Scheme 1. In comparison to existing water-soluble, dendric or self-assembling polymer drug carriers, GNAs are expected to show improved in vivo stability without compromising integrity of themselves or their supramolecular assemblies upon chemical modifications for controlled entrapment and release of drug payloads.



Scheme 1. Grafted Block copolymer Nano-Assembly

Methods: PEG-*b*-PAA block copolymers were prepared by ring-opening polymerization of β-benzyl L-aspartate N-carboxy anhydride (BLA-NCA) with methoxy-PEG-amine (MW = 12,000) as a macroinitiator in anhydrous DMSO at 40°C for 2 days (PEG-*b*-PBLA).[1] Products were purified and collected by ether precipitation and freeze-drying from benzene, respectively. Compositions of PEG-*b*-PBLA were controlled by changing the amount of BLA-NCA. GPC and ^{1}H-NMR were used for analysis. PEG-*b*-PBLA was divided into two groups. One group was used as grafts by using an active amino group at the ω-end of PEG-*b*-PBLA block copolymer backbone. The other group was used to prepare scaffolds, removing benzyl ester groups on the side chain of PEG-*b*-PBLA, providing PEG-poly(aspartic acids) (PEG-*b*-PAsp) block copolymers. PEG-*b*-PAsp scaffolds and PEG-*b*-PBLA grafts were conjugated to prepare GNAs through various reaction conditions (e.g. mixing ratios, temperature, coupling agents and solvents). GNAs were purified by dialysis in DMF and DI water, followed by freeze-drying. Molecular weight (MW) of GNAs was determined by GPC measurements (10 mM PBS, pH 7.4).

Results and Discussion: A series of PEG-*b*-PBLA block copolymers were successfully synthesized in various chain lengths, denoted as X-Y where X is PEG MW×10^{-3} and Y is number of aspartic acids (5, 14, 20, 25, 35), respectively: PEG-*b*-PBLA 12-5, 12-14, 12-20, 12-25 and 12-35. Optimal grafting conditions were determined by calculating the number of grafts per either GNA or PAsp repeating unit. Results were summarized in Figure 1.



- **Red**: 40 °C, 48 h, Asp as -COONa, HBTU (1.5 fold of binding sites), DMF
- **Blue**: 40 °C, 48 h, Asp as -COOH, HBTU (3 fold), DIPEA (3 fold of HBTU), DCM
- **Green**: 40 °C, 48 h, Asp as -COOH, DIC (5 fold), DMAP (0.1 fold of HBTU), DCM
- **(12-X)**: PEG-*b*-PBLA composed of PEG (MW = 12,000) and PBLA with DP of X
- **[Y-Z]**: GNA prepared from 12-Y as a backbone and 12-Z as grafts

Figure 1. Molecular characterization of GNAs

GNAs from scaffolds and grafts of the same chain lengths were prepared using four copolymers; PEG-PBLA 12-5, 12-14, 12-25, and 12-35. The number of grafts per GNA was subsequently determined. Intriguingly, grafting yields (Figure 1, left) and efficiency (Figure 1, right) increased as chain length increased from 5 to 20, yet decreased as the chain length increased to 35. These results indicate that more grafts were conjugated to each scaffold with a longer chain length while higher grafting efficiency was obtained with a shorter graft. Our GNA library revealed that grafting efficiency increased up to 30 % by changing reaction conditions. Maximum 11 grafts were conjugated per GNA between 12-35 scaffolds and 12-20 grafts.

A great deal of attention has been paid to development of drug carriers based on diverse soft materials. These soft materials may include water-soluble polymers, liposomes, dendrimers, and self-assembling polymer nanoassemblies represented by polymeric micelles.[2,3] Despite potential, each carrier still requires extensive improvement of intrinsic drawbacks in regards to structural uniformity, controlled drug release, high drug-loading efficiency, and in vivo stability, respectively. Difficulties in designing 'ultimate' carrier that resolves these drawbacks simultaneously are attributed to the fact that there is no suitable drug carrier platform currently available. Therefore, it is exciting to compare physicochemical properties of GNAs with other existing drug carriers in drug-loading/release efficiency and stability in the future.

Conclusions: Grafted Block Copolymer Nano-Assembly (GNA) was proposed as a novel drug delivery carrier in this study. Methods to prepare the optimal GNA from PEG-*b*-PBLA were investigated by applying various reaction conditions and combinations of scaffolds and grafts. GNA with 12-35 PEG-*b*-PAsp scaffolds appeared to possess 10 or 11 chains of PEG-*b*-PBLA grafts at 30 % grafting efficiency. Therefore, GNAs would provide a unique drug carrier platform that may show properties of water-soluble polymers, dendrimers and polymer micelles.

References: [1] Bae Y. Angew. Chem. Int. Ed. 2003;42: 4640-4643. [2] Bae Y. Adv Drug Deliver Rev. 2009;61:768-784. [3] Duncan R Nat. Rev. Cancer 2006:6, 688-701.