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DNA-functionalised blend micelles: mix and fix polymeric hybrid nanostructures†

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We report the formation and characterisation of easily functionalisable mixed micelles with DNA/PEO corona and PPO core which can be loaded with hydrophobic molecules and stabilised by the formation of a cross-linked semi-interpenetrating network. Furthermore, the corona is functionalised by hybridisation either with dye-modified complementary DNA, with demonstrable distance control, or with DNA-labelled gold nanoparticles.

Amphiphilic block copolymers have long been the subject of intense study for their supramolecular aggregation properties, especially with regard to potential pharmaceutical applications.^{1,2} One family of these materials, the Pluronic™ block copolymers, composed of poly(ethyleneoxide) (PEO) and poly(propyleneoxide) (PPO) blocks with triblock structure PEO_n-PPO_m-PEO_n, have received particular attention for their tunable, thermally responsive aggregation behavior. The micellisation and gelation behaviour of Pluronic block copolymers has been described elsewhere;³ here it is sufficient to note that the process is governed by a critical micellisation concentration (CMC) and critical micellisation temperature (CMT) specific to each Pluronic species. In part due to the known biocompatibility of PEO chains, Pluronic block copolymers are strong candidates for biomedical applications, both for the bioactivity of the unimers and the possibility to load hydrophobic drugs into the micelle core.⁴ The micelles can be cross-linked either at the periphery of the corona⁵ or within the core⁶ to stabilise them against dilution and low temperature. The primary limitation to Pluronic-based drug delivery is the difficulty of targeting—Pluronic micelles can only be equipped with targeting moieties through chemical modification of the terminal hydroxy groups of the polymer.⁷

Another class of amphiphilic block copolymers with great biomedical potential is the DNA block copolymers (DBC), consisting of single- or double-stranded DNA covalently attached to polymer units.⁸ In aqueous media, amphiphilic DBCs also self-assemble into aggregates or micelles with a hydrophobic core and a hydrophilic DNA corona, which can be used for addressable functionalisation through simple hybridisation with complementary DNA (cDNA) covalently linked to the desired moiety.^{9,10} A powerful potential application for this technique within the pharmaceutical sphere is targeting, as has been demonstrated with tumor cells.¹¹ *In vivo*

applications are limited, though, by the instability of the micelles against dilution. Another potential drawback of pure DBC micelles for drug delivery might be the strong stimulation of immune responses, due to the high local DNA concentration, surrounding these aggregates with PEO chains would potentially implement a desirable kind of “stealth” function.

We report here on the combination of these two classes of materials (Fig. 1), represented by DNA-*b*-PPO (**22PPO**), a PPO block ($M_w = 6800 \text{ g mol}^{-1}$) covalently connected to the 5'-end of a 22-base single-stranded DNA (5'-CCT CGC TCT GCT AAT CCT GTT A-3'), and Pluronic F127. The latter was selected for the comparable sizes of the hydrophobic block and micellar structure to those of **22PPO**.^{12,13} These aggregates combine the potential for intramicellar cross-linking of Pluronic with the facile functionalisability of DBCs, allowing the formation of stable micelles with easy targeting capabilities and thereby addressing two of the current drawbacks of the individual components. It is also expected that the PEO corona should shield the DNA backbone and thus improve immunocompatibility. The resulting mixed micelles were characterised with UV/Vis and fluorescence spectroscopy, atomic force microscopy (AFM), Förster resonance energy transfer (FRET) and transmission electron microscopy (TEM).

Before forming blend micelles, we investigated the stabilisation of the individual components through UV-induced cross-linking of pentaerythritol tetraacrylate (PETA) using a pyrene solubilization method.¹⁴ F127 and **22PPO** were prepared and stabilised as per reported procedures,^{6,15} and stored overnight at 4 °C. Only the stabilised F127 samples retained well-resolved

Fig. 1 Schematic of the mixed micelle architecture and chemical structures of the polymeric components. (A) PEO block of Pluronic. (B) DNA block of DBC. (C) PPO blocks of Pluronic or DBC. (D) and (E) Probes at 5'- and 3'-ends of the complementary DNA, respectively. (F) Hydrophobic compound loaded into the hydrophobic core. (G) Cross-linked nanodomains of PETA in the core.

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pyrene fluorescence; though **22PPO** can be loaded with pyrene or other hydrophobic molecules, PETA uptake and/or polymerisation is ineffective in pure DBC micelles (see ESI 2† for sample preparation and absorption and UV/Vis spectra).

The same procedure was subsequently repeated with a mixture of F127 and **22PPO** solutions in a 5 : 1 molar ratio (final concentrations 800 μM F127 and 160 μM **22PPO**). The micelles were loaded with PETA and pyrene and photo-crosslinked. Whereas pure **22PPO** micelles could not retain pyrene after storage below the CMT, the blended solution showed characteristic pyrene fluorescence peaks, *i.e.* micelles were successfully stabilised (see Fig. S1B, ESI†). It should be noted that this cross-linking represents only a kinetic barrier to dissociation—after several weeks of storage, the stabilised blend micelle solutions began to form a white precipitate, and pyrene fluorescence could no longer be observed (data not shown).

All of the materials utilised in this study were analysed using AFM in Tapping Mode in air (see ESI† for sample preparation), to confirm the micellar structure and incorporation of DBC. Pure F127 micellar solution in the absence of PETA showed only shapeless smears (data not shown), in stark contrast to the well-resolved spherical aggregates found in internally cross-linked F127 micelles (Fig. 2A). As expected, immobilisation was much more effective for DNA-containing materials than for the neutral Pluronic micelles; in the images of **22PPO**, markedly more material is visible on the surface in spite of the 80% reduction in molar concentration (Fig. 2B). Relatively few well-formed micelles could be observed in these samples because the materials could not be stabilized with PETA. The combination of both materials results in a sharp increase in the population of well-defined, and thus stabilised, aggregates (Fig. 2C). This high coverage cannot be attributed to **22PPO** alone, and pure F127 micelles adhere poorly to the surface even when stabilised, thus these AFM results indicate the formation of stabilised micelles blending **22PPO** and F127.

The mixed nature of the micelles and the accessibility of the DNA in the corona were investigated with intermolecular FRET experiments between TAMRA and FAM (see ESI 5† for the full names and reference spectra of the fluorescent probes). To this end, F127 was labelled with TAMRA (ESI 4†) and mixed micelles were formed without PETA stabilisation

Fig. 2 Tapping Mode AFM images of micellar materials. (A) Stabilized F127, 160 μM , coverage: 39 aggregates per μm^2 . (B) Pure **22PPO**, 32 μM , coverage: 87 aggregates per μm^2 . (C) Stabilized blend, 160 μM F127 and 32 μM **22PPO**, coverage: 562 aggregates per μm^2 . Concentrations refer to solutions incubated on mica slide. Scale bars are 100 nm and vertical scale is 20 nm.

Fig. 3 FRET analysis of blend micelles. (A) FAM-3'-cDNA with TAMRA-F127 (dashed) and TAMRA-F127-**22PPO** blend (solid). (B) FAM-5'-cDNA with TAMRA-F127 (dashed) and TAMRA-F127-**22PPO** blend (solid). In all cases $\lambda_{\text{ex}} = 440 \text{ nm}$.

using an 8 : 1 : 2 ratio of F127 : F127-TAMRA : **22PPO**. Fluorescence was monitored under excitation at 440 nm after the addition of equimolar cDNA (relative to TAMRA) functionalised with FAM at the 3'- or 5'-end (Fig. 3A and B). In a control with no **22PPO** present and thus only diffusion controlled encounters between TAMRA and FAM, the intensity ratio of TAMRA to FAM fluorescence was $I_{583}/I_{523} = 0.81$. For mixed micelles and FAM-3'-cDNA, which affords the closest average approach of cDNA and F127 functionalities, this intensity ratio increased to 1.06, a significant enhancement of relative TAMRA fluorescence ($\sim 30\%$) for such a dynamic micellar system. In contrast, the same system with FAM-5'-cDNA, which results in a greater and more variable TAMRA-FAM separation on average, yielded no change in relative intensities: in the presence and absence of **22PPO**, we found $I_{583}/I_{523} = 0.60$.[‡] As such, these data are strongly indicative not only of successful mixing of F127 and **22PPO** in single micelles, but also of the distance control attainable through functionalisation *via* DNA hybridisation. This degree of control is due to the ability of **22PPO** to link between two key interactions for supra-molecular organization. The first is the hydrophobic interaction between the PPO blocks of F127 and **22PPO**, which allows **22PPO** to be incorporated into F127 micelles. The other is Watson-Crick base pairing between the DNA block of **22PPO** and its functionalised complement, enabling the positioning of FAM at the outer edge of the corona or at the hydrophobic-hydrophilic interface, as in the case of FAM-3'-cDNA.

As a final demonstration of the versatility of this system, we introduced markedly larger moieties—5 nm gold nanoparticles (Au-NPs)—to stabilised mixed micelles using the same simple hybridisation procedure. Au-NPs conjugated with single cDNA molecules were prepared according to a published protocol (ESI 6†).¹⁶ The resulting hybridised structures were investigated with AFM and TEM. As expected, the AFM results, and particularly the phase data (Fig. 4A), showed clear grouping of gold particles into numerous tight aggregates with sharp internal boundaries not detected in pristine micelles. This observation was supported with TEM results (Fig. 4B),

Fig. 4 Characterisation of mixed micelle-Au-NP aggregates. (A) Tapping Mode AFM phase image. Inset: $2\times$ zoom phase image showing distinct boundaries between aggregate members. (B) Representative TEM image showing high-contrast Au-NPs and weak contrast due to micelles. Inset: $2.5\times$ zoom image of the same region. (C) Nearest-neighbour separation histograms and percentage of total particle count extracted from TEM data (left bar, square) and randomly generated particle distribution (right bar, triangle). Scale bars are 200 nm in full images and 100 nm in insets.

where the high contrast of Au allowed unambiguous confirmation of inorganic nanoparticle clustering. Indeed, the distribution of nearest-neighbour separations of the particles visible in this scan sharply differs from the same distribution for simulated random particles, exhibiting a stronger tendency for small separations (Fig. 4C and ESI†). Furthermore, this aggregation is clearly centered on the micelles—fully 38% of the Au-NPs are located in the immediate vicinity of the 106 best-defined micelles (dark shadows visible under negative staining conditions), covering only 10% of the total scan area (Fig. S7B, ESI†). In short, DNA in the corona allows the combination of soft and hard materials, including high-contrast agents (e.g. gold) for TEM imaging, with potential implications for particle tracking.

In summary, we have generated micelles containing a mixture of both Pluronic F127 block copolymer and a DNA block copolymer. These micelles were stabilised by the UV-induced formation of a semi-interpenetrating network in the core, as confirmed by fluorescence studies with pyrene loading and AFM measurements of mixed micelles. AFM images also indicated the successful inclusion of DNA materials into the micelles. The mixed nature of these micelles was further demonstrated with FRET studies between FAM-cDNA conjugates and TAMRA-labelled F127. These results and the

controlled aggregation of Au-NPs through DNA hybridisation also showed the general availability of the DNA in the corona for further functionalisation. Indeed, such functionalisation even affords a degree of distance control, as demonstrated by the marked improvement in FRET efficiency when micelles are labelled with FAM-3'-cDNA versus FAM-5'-cDNA. The demonstrated incorporation of **22PPO** allows the combination of facile functionalisation and targeting, the hallmark of DBC micelles, with the biocompatibility and ease of stabilisation of F127. This novel pairing addresses fundamental obstacles to the application of both constituent materials and paves the way to further *in vivo* studies of immunogenicity, circulation lifetime, drug release and targeted delivery with this material.

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Notes and references

† The discrepancy between the fluorescence intensity ratios in the control experiments can be attributed to the different local electronic environment of the dye in 5'- versus 3'-functionalisation and the resulting changes in quantum efficiency and spectral characteristics.

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