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Functional hydrophobic and hetero-grafted block comb polymers *via* a combination of spontaneous zwitterionic copolymerisation and redox-initiated RAFT polymerisation†

Ayaat Mohamed Mahmoud,[a] Alexander Rajakanthan[a,b] and Kristian Kempe *[a,b]

The SZWIP of 2-oxazolines enables the one-pot synthesis of *N*-acylated poly(amino ester) based macromonomers with tuneable hydrophobicity. Their redox-initiated RAFT (co-)polymerisation yields hydrophobic homo- or amphiphilic block comb polymers with pendant carboxy groups, which can be self-assembled into nano-sized pH-responsive aggregates. The availability for further functionalisations in combination with their non-toxicity highlights their future potential for biomedical applications.

Poly(ester amide)s (PEAs) have attracted significant attention as advanced materials for numerous applications,[1,2] as they combine the properties of two classes of polymers, namely polyesters and polyamides. This fact makes them highly functional and also allows the materials to be tailored by combining building blocks with different properties, typically in a polycondensation or polyaddition process.[3] An intriguing polymerisation technique for the synthesis of tuneable PEAs simply by mixing an electrophilic (M_E) and a nucleophilic (M_N) monomer in the absence of any catalyst or initiator was described by Saegusa and coworkers in the 1970s.[4,5] This spontaneous polymerisation was termed spontaneous zwitterionic copolymerisation (SZWIP) as it is believed to involve zwitterionic species formed by the reaction of M_E and M_N. In this context, a range of monomer combinations have been tested with cyclic imino ethers (CIEs) being the most well studied M_N's. CIEs are well known monomers for the preparation of advanced polymeric materials.[6,7] These heterocyclic compounds exhibit an unique reactivity as they can be ring-opened by electrophiles as well as nucleophiles. Specifically, 2-substituted-2-oxazolines have been studied as monomers in the living cationic ring-opening polymerisation (CROP)[8,9] and particularly in the last two decades the resulting poly(2-oxazoline)s have emerged as modular and functional polymer class due to the ease of tuning their properties by changing the 2-substituent of the 2-oxazoline monomer and the biocompatibility of the water-soluble homologues.[10–13] However, 2-oxazolines can also react in a Michael-addition type reaction with electron-deficient compounds such as (meth)acrylates. Acrylic acid has emerged as particularly interesting M_E as after a proton transfer it forms a reactive zwitterion which can polymerise in a SZWIP manner (Scheme 1A).[14] Recently, we reported the synthesis of dual responsive comb polymers from telechelic *N*-acylated poly(amino ester)s (NPAE) based macromonomers obtained by SZWIP of acrylic acid (AA) and different short aliphatic 2-oxazolines, such as 2-methyl-2-oxazoline (MeOx) and 2-ethyl-2-oxazoline (EtOx).[15,16] Despite numerous early reports in the 1970s, to date, mainly 2-oxazolines which result in water-soluble systems, have been investigated. Here, we demonstrate the potential of the longer 2-oxazoline homologues, 2-propyl-2-oxazoline (PropOx) and 2-butyl-2-oxazoline (ButOx) for the preparation of hydrophobic NPAE macromonomers and subsequently homo and amphiphilic hetero-grafted block comb polymers. Moreover, the ability of the presented NPAE comb polymers to form stable and tuneable nano-sized aggregates with promising properties for future biomedical applications is reported. Strikingly, the approach presented enables the straightforward synthesis of macromonomers and comb polymers with tailored hydrophilic/hydrophobic balance from one polymer class. Typically, heterografted comb or brush polymers are obtained by combining macromonomers from different polymer classes.[17–19]

First, PropOx and ButOx were reacted with AA *via* SZWIP to yield the two macromonomers oligo(PropOx-*alt*-AA)$_n$A ($\mathbf{M}_{\mathrm{PropOx/AA}}$) and oligo(ButOx-*alt*-AA)$_n$A ($\mathbf{M}_{\mathrm{ButOx/AA}}$), respectively (Scheme 1B). ^{1}H NMR spectra showed the typical signals obtained for NPAE macromonomers (Fig. S1†). The degree of polymerisation was determined by comparison of the acrylate signals (5.7–6.5 ppm) with the methyl group of the 2-oxazoline (0.8–1.0 ppm) and the AA derived signals (2.5–2.75 ppm), respectively. In both cases a degree of polymerisation (DP) of

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[a] *ARC Centre of Excellence in Convergent Bio-Nano Science & Technology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia. E-mail: kristian.kempe@monash.edu*

[b] *Department of Chemistry, University of Warwick, CV4 7AL Coventry, UK*

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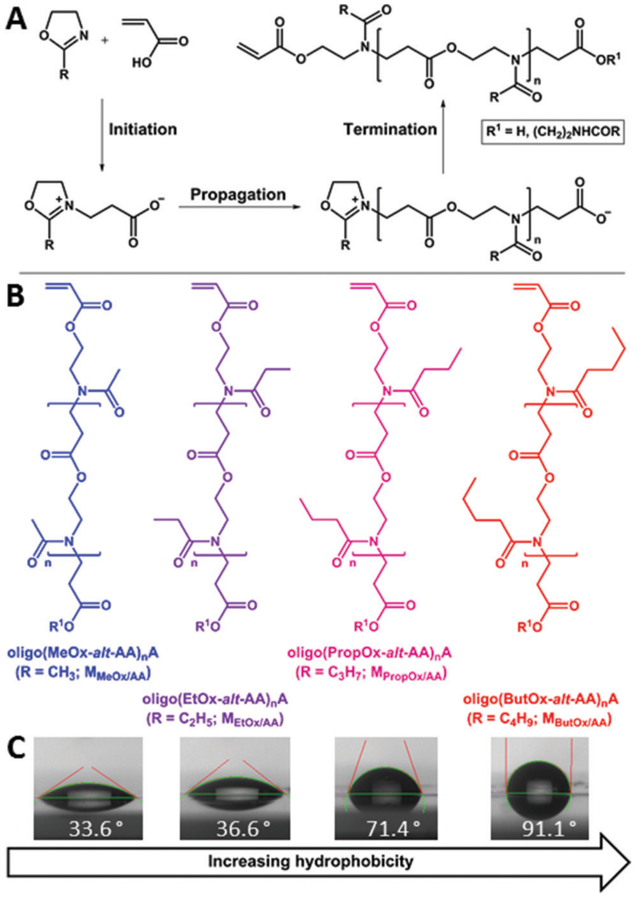

Scheme 1 Schematic representation of the spontaneous zwitterionic copolymerization (SZWIP) of 2-substituted-2-oxazolines and acrylic acid (A), macromonomers of different oxazoline units (B) and their corresponding water contact angle images (C).

2 was obtained. As for previously reported MeOx ($\mathbf{M}_{\text{MeOx/AA}}$) and EtOx ($\mathbf{M}_{\text{EtOx/AA}}$) based macromonomers, similar integral values for 2-oxazoline and AA indicate the equimolar incorporation of both monomers and thus an alternating composition.[16] Moreover, size exclusion chromatography (SEC) revealed the synthesis of rather defined macromonomers with low dispersities ($Đ$ < 1.25, Table S1†).

Previously, redox-initiated reversible addition–fragmentation chain-transfer (RRAFT) polymerisation[20] has been identified as suitable polymerisation technique to prepare comb-shaped polymers from NPAE macromonomers at ambient temperature in a mixture of water and dioxane.[16,21] In the case of $\mathbf{M}_{\text{PropOx/AA}}$ and $\mathbf{M}_{\text{ButOx/AA}}$ the solvent ratio had to be adjusted towards a higher percentage of dioxane due to the higher hydrophobicity of the two new macromonomers. Nonetheless, well-defined homo comb polymers were obtained in both cases. Similar to $\mathbf{M}_{\text{MeOx/AA}}$ and $\mathbf{M}_{\text{EtOx/AA}}$ macromonomers a complete disappearance of the acrylate signals in ^{1}H NMR and macromonomer traces in SEC demonstrated the quantitative introduction of the α-acrylate functionalities during the SZWIP. This enabled the synthesis of homo comb polymers with different DP simply by changing the monomer-to-chain transfer agent-ratio. SEC showed an increase of the molecular weight of the comb polymers by increasing the DP from 50 to 100 (Table 1), while maintaining narrow molecular weight distributions ($Đ$ < 1.31). In all cases, symmetrical monomodal SEC traces were observed (Fig. S2†). Further characterisation of the homo comb polymers with regard to their thermal and surface properties was performed by differential scanning calorimetry (DSC) and contact angle (CA) measurements, respectively. $\mathbf{P3a/b}_{\text{ButOx/AA}}$ showed lower glass transition temperatures (T_g's) than $\mathbf{P1a/b}_{\text{PropOx/AA}}$, which can be attributed to the length and thus flexibility of the side chain (Table 1).[22] In each group only minor differences in the T_g's were observed when doubling the DP. Compared to poly(oligo(MeOx-*alt*-AA)$_n$A)$_{100}$ ($\mathbf{P}_{\text{MeOx/AA}}$, T_g ~ 33.8 °C) and poly(oligo(EtOx-*alt*-AA)$_n$A)$_{100}$ ($\mathbf{P}_{\text{EtOx/AA}}$, T_g ~ 25.6 °C) a gradual decrease of the T_g values can be observed when increasing the side chain length. Moreover, extending the side chain length of the oxazoline units also had a significant effect on the wettability of the respective polymer films. Water contact angle measurements showed an increase from <40° for $\mathbf{P}_{\text{MeOx/AA}}$ and $\mathbf{P}_{\text{EtOx/AA}}$ to 71.4° and 91.1° for $\mathbf{P1b}_{\text{PropoOx/AA}}$ and $\mathbf{P3b}_{\text{ButOx/AA}}$, respectively, indicating a more hydrophobic character of the latter polymers (Scheme 1C). This observation stimulated the use of this novel more hydrophobic macromonomers for the preparation of heterografted block comb polymers $\mathbf{P2}_{\text{MeOx/PropOx/AA}}$ and $\mathbf{P4}_{\text{MeOx/ButOx/AA}}$ with presumably amphiphilic properties (Fig. 1A). To this end, a hydrophilic macro-chain transfer agent

Table 1 Characterization of the homo comb polymers and hetero-grafted block comb polymers based on the macromonomers (MM): oligo(MeOx-*alt*-AA)$_n$A ($\mathbf{M}_{\text{MeOx/AA}}$), oligo(PropOx-*alt*-AA)$_n$A ($\mathbf{M}_{\text{PropOx/AA}}$), and oligo(ButOx-*alt*-AA)$_n$A ($\mathbf{M}_{\text{ButOx/AA}}$)

Code	MM$_A$	MM$_B$	DP^a (MM$_A$)	DP^a (MM$_B$)	$M_{n,\text{ NMR}}$^{b} [g mol^{-1}]	$M_{n,\text{ SEC}}$^{c} [g mol^{-1}]	$Đ^c$	T_g^{d} [°C]
$\mathbf{P1a}_{\text{PropOx/AA}}$	$\mathbf{M}_{\text{PropOx/AA}}$	—	50	—	22 300	33 400	1.28	18.9
$\mathbf{P1b}_{\text{PropOx/AA}}$	$\mathbf{M}_{\text{PropOx/AA}}$	—	97	—	43 100	56 000	1.31	19.5
$\mathbf{P2}_{\text{MeOx/PropOx/AA}}$	$\mathbf{M}_{\text{MeOx/AA}}$	$\mathbf{M}_{\text{PropOx/AA}}$	100^e	48	60 050	61 100	1.23	—
$\mathbf{P3a}_{\text{ButOx/AA}}$	$\mathbf{M}_{\text{ButOx/AA}}$	—	50	—	23 740	26 100	1.22	9.9
$\mathbf{P3b}_{\text{ButOx/AA}}$	$\mathbf{M}_{\text{ButOx/AA}}$	—	95	—	44 890	35 600	1.22	10.9
$\mathbf{P4}_{\text{MeOx/ButOx/AA}}$	$\mathbf{M}_{\text{MeOx/AA}}$	$\mathbf{M}_{\text{ButOx/AA}}$	100^e	49	61 870	47 900	1.27	—

[a] Calculated by ^{1}H NMR (CDCl$_3$, 400 MHz) from theoretical DP and conversion. Theoretical DPs: a, DP 50; b, DP 100; blocks, MM$_A$ DP 100, MM$_B$ DP 50. [b] Calculated from DPs and molar masses of the macromonomers. [c] Determined by SEC (eluent: DMAc + LiBr, standard: PS). [d] Determined by DSC (second heating run). [e] MacroCTA SEC data: PMeOx$_{/AA}$: M_n = 38 900 g mol^{-1}, $Đ$ = 1.17. n.d. not determined.

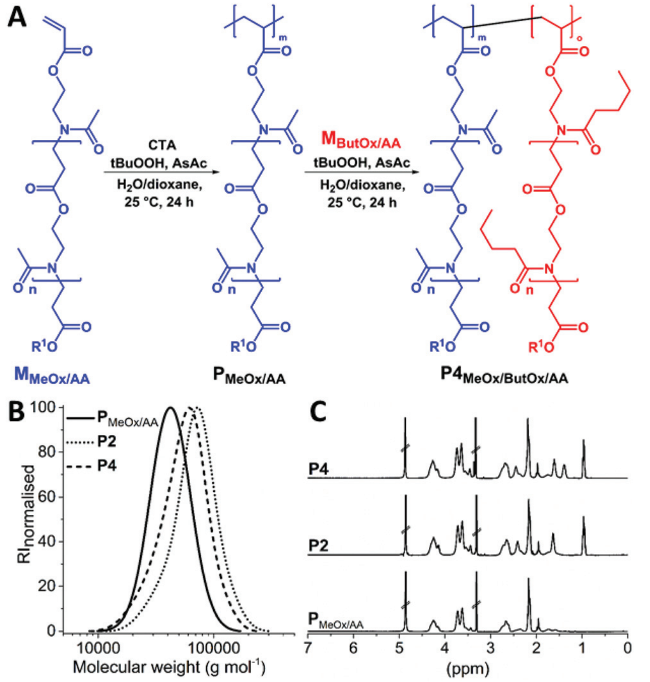

Fig. 1 Synthesis and characterisation of $\mathbf{P2}_{MeOx/PropOx/AA}$ and $\mathbf{P4}_{MeOx/ButOx/AA}$. Schematic representation of the redox-initiated RAFT (RRAFT) polymerisation of $\mathbf{M}_{MeOx/AA}$ and the chain extension with $\mathbf{M}_{ButOx/AA}$ (A), and SEC (B) and ^{1}H NMR (C) spectra of $\mathbf{P2}_{MeOx/PropOx/AA}$ and $\mathbf{P4}_{MeOx/ButOx/AA}$ in comparison to the macro-CTA $\mathbf{P}_{MeOx/AA}$.

(macro-CTA), namely $\mathbf{P}_{MeOx/AA}$ with a DP of 100 was synthesised and chain extended with the two hydrophobic macromonomers targeting DP 50. Noteworthy, it was found that the order of solvent addition was crucial to obtain well-defined block comb polymers based on $\mathbf{M}_{ButOx/AA}$. Due to the high water-solubility of the macro-CTA it was important to dissolve it first in water before the addition of the macromonomer in dioxane. Conversions of all macromonomers were near quantitative (Fig. S3 and S4†). The successful chain extension was observed by a shift of the SEC trace of the macro-CTA to higher molar masses (Fig. 1B, Table 1) and the appearance of signals corresponding to the two hydrophobic macromonomers in the respective ^{1}H NMR spectra (Fig. 1C). The comparison of the methyl group signals of the ring-opened MeOx (~2.1 ppm) and PropOx/ButOx (~0.9 ppm) units confirmed the targeted composition with a twice as long $\mathbf{P}_{MeOx/AA}$ block compared to the hydrophobic block.

Due to their hydrophobic and amphiphilic nature both homo and block comb polymers were expected to be good candidates for the formation of self-assembled structures in aqueous solution. Two different approaches were exploited to study the self-assembly of the polymers. While the amphiphilic block comb polymers were self-assembled by directly exposing them to aqueous media, aggregates of the hydrophobic homo comb polymers were prepared by the nanoprecipitation technique. To this end, **P1a**/**P1b** and **P3a**/**P3b** were dissolved in MeOH and mixed with water before removal of MeOH by evaporation. Addition of the polymer solution to water, slow evaporation of MeOH at room temperature and higher DP polymers led to smaller particles with lower PDI values. In all cases sub-100 nm aggregates were obtained as observed by dynamic light scattering (DLS) (Table 2). All particles were found to exhibit a negative zeta potential, which can be attributed to pendant carboxylic acid groups of the comb polymer side chains.[15] The latter is expected to not only stabilise the aggregates but also to endow them with a pH responsive behaviour. Adjustment of the aqueous solution pH value to 7.4 had a different effect on the two homo comb polymers. $\mathbf{P1b}_{PropOx/AA}$ particles disassembled (Fig. S5†), whereas $\mathbf{P3b}_{ButOx/AA}$ particles (Fig. 2) remained intact suggesting that deprotonation of the carboxyl

Table 2 Overview of hydrodynamic sizes of nanoparticles

Code	*z*-Average d^a, [nm]	PDI^a	Zeta potential^b [mV]
$\mathbf{P1a}_{PropOx/AA}{}^c$	68	0.16	n.d.
$\mathbf{P1b}_{PropOx/AA}{}^c$	59	0.19	−46 ± 13
$\mathbf{P2}_{MeOx/PropOx/AA}{}^d$	59	0.11	−27 ± 7
$\mathbf{P3a}_{ButOx/AA}{}^c$	62	0.18	n.d.
$\mathbf{P3b}_{ButOx/AA}{}^c$	56	0.19	−48 ± 14
P3b-PDA^c	101	0.16	−41 ± 8
P3b-PEtOx^d	14	0.13	−5.8 ± 1.3
$\mathbf{P4}_{MeOx/ButOx/AA}{}^d$	92	0.08	−25 ± 10

^{a} Determined by DLS (solvent: MilliQ water). ^{b} Determined by DLS (dispersant: 100 mM $NaCl_{aq}$). ^{c} Prepared by nanoprecipitation (MeOH into MilliQ water). ^{d} Prepared through direct dissolution in MilliQ water. n.d., not determined.

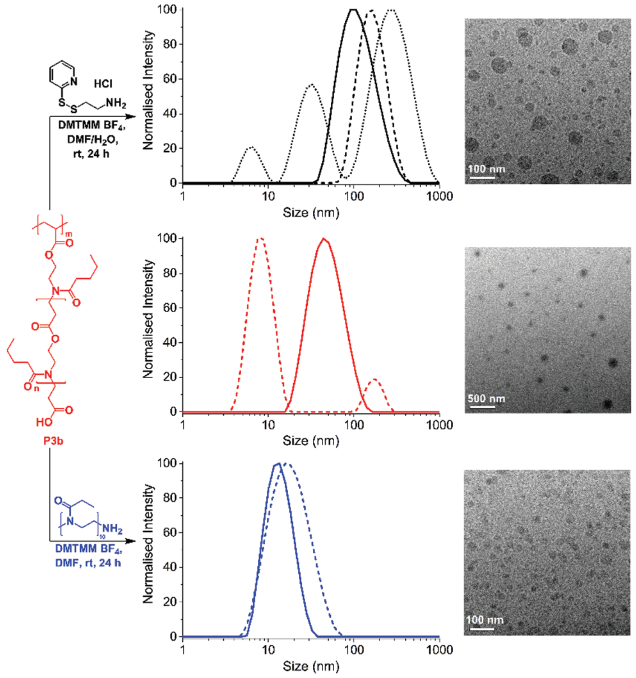

Fig. 2 Simplistic representation of the modification of $\mathbf{P3b}_{ButOx/AA}$ with PDA (**P3b-PDA**; top) and PEtOx-NH_2 (**P3b-PEtOx**; bottom) and their respective DLS spectra and TEM images. DLS: Full and dashed lines represent measurements in MilliQ water and PBS (pH 7.4), respectively. The dotted line in the **P3b-PDA** spectrum is obtained after the addition of DTT.

groups of **P1b**$_{\mathrm{PropOx/AA}}$ renders the polymer hydrophilic enough to be molecularly dissolved. In contrast, the higher hydrophobicity of comb polymers composed of ButOx units presumably prevents this hydrophobic/hydrophilic switch.

In order to gain more information about the behaviour of the **P3b**$_{\mathrm{ButOx/AA}}$ aggregates, they were exposed to different aqueous media (Fig. S6†). The aggregates were found to be stable in 100 mM NaCl and 100 mM urea solution, whereas a size decrease of the aggregates was observed in Hank's Balanced Salt Solution (HBSS) and PBS (Fig. 2, middle) suggesting a concerted pH and salt responsiveness. In order to stabilise the individual aggregates, the potential of the pendant carboxylic groups for amidation reaction was exploited as recently reported for fully water-soluble P$_{\mathrm{MeOx/AA}}$.[15] The introduction of disulphide moieties *via* the reaction with 2-(pyridyldithio)-ethylamine hydrochloride (PDA, Fig. S7†) enabled the cross-linking of the larger aggregates (Fig. 2, top). To achieve this, after nanoprecipitation of **P3b-PDA** free thiols were generated by treatment with dithiothreitol (DTT), which were left to oxidise after removal of excess DTT by dialysis. Washing the cross-linked particles into PBS resulted in marginally larger particles. Moreover, this modification endowed the nanoparticles with redox responsiveness, which allowed their disassembly in the presence of a reducing agent as exemplarily demonstrated with DTT (Fig. 2, top, dotted line) in PBS. The modification of the carboxylic groups with amine-terminated poly(2-ethyl-2-oxazoline)[23] (PEtOx$_{10}$NH$_2$, Fig. S7†) on the other hand resulted in combs with amphiphilic character and at the same time neutralised the negative charge of the acid groups. Nanoparticles formed of **P3b-PEtOx** possessed a significantly higher zeta potential (Table 2) and only showed a marginal size change when switching the aqueous media from water to PBS (Fig. 2, bottom).

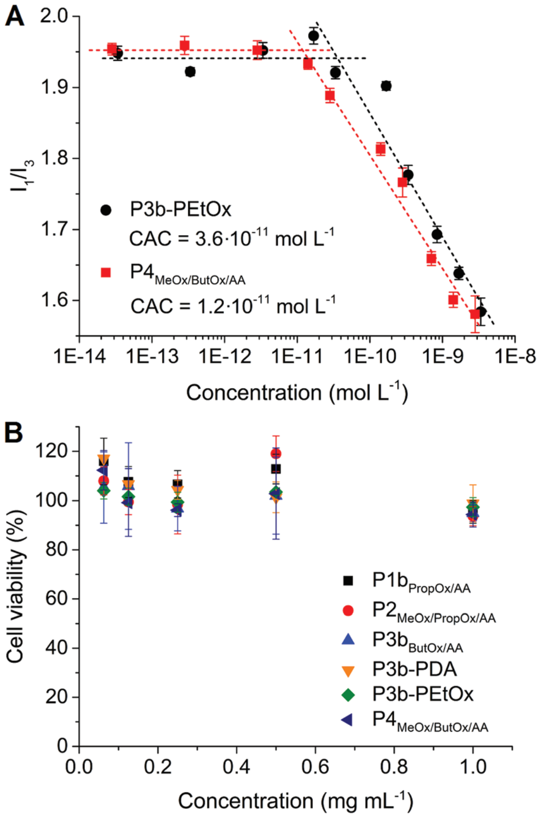

Fig. 3 Determination of CACs of amphiphilic systems **P3b-PEtOx** and **P4**$_{\mathrm{MeOx/ButOx/AA}}$ using purene as probe (5×10^{-7} M) at 25 °C in PBS (A); cytotoxicity of homo and hetero-grafted block comb polymers against NIH3T3 cells as determined by AlamarBlue® assay. Data presented as mean ± sd; *n* = 3 (B).

Further information about the assembly of the two amphiphilic systems, **P3b-PEtOx** (hydrophilic PEtOx block in the side chain) and **P4**$_{\mathrm{MeOx/ButOx/AA}}$ (hydrophilic **P**$_{\mathrm{MeOx/AA}}$ block in the main chain) in PBS was obtained from critical aggregation concentration (CAC) studies exploiting the solvatochromic properties of pyrene.[24,25] Low CACs were found for both systems with a three times higher value for **P3b-PEtOx** (3.6×10^{-11} M *vs.* 1.2×10^{-11} M), demonstrating the formation of aggregates and the capability of both systems to encapsulate hydrophobic cargo even at low concentrations (Fig. 3A).

In order to evaluate the future potential of the homo and hetero-grafted block comb polymer particles for biomedical applications, a first assessment of their interaction with cells was performed. To this end, the viability of NIH3T3 cells after the treatment with different polymer concentrations was investigated by an AlamarBlue® assay. No toxicities were observed with cell viabilities of about 100% for all polymers with concentrations up to 1 mg mL^{-1} (Fig. 3B).

Conclusions

In summary, the spontaneous zwitterionic copolymerisation (SZWIP) was identified as facile polymerisation method for the preparation of acrylate macromonomers with varying hydrophilic/hydrophobic balance. Novel hydrophobic NPAE macromonomers provided access to hydrophobic homo and amphiphilic block comb polymers, which are available for post-polymerisation modifications due to their pendant carboxylic acids inherently introduced during SZWIP. Self-assembly of the comb polymers in aqueous solution yielded responsive nano-sized aggregates of negligible toxicity. Detailed investigations of the self-assembly behaviour and the switchability of the particle size, as well as the use of the particles for targeted drug delivery applications with particular focus on their biocompatibility and ability to reversibly encapsulate cargo are currently performed in our laboratory.

Conflicts of interest

There are no conflicts to declare.

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