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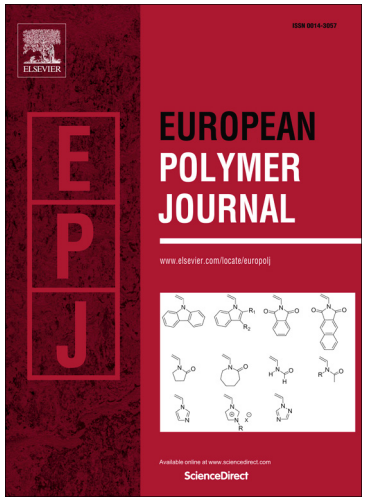

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Xin Liao, Grace Walden, Noelia D Falcon, Simon Donell, Mike Raxworthy, Michael Wormstone, Graham P Riley, Aram Saeed

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A direct comparison of linear and star-shaped poly(dimethylaminoethyl acrylate) polymers for polyplexation with DNA and cytotoxicity in cultured cell lines

Xin Liao[a], Grace Walden[a], Noelia D Falcon[a], Simon Donell[b], Mike Raxworthy[c], Michael Wormstone[d], Graham P Riley[d] and Aram Saeed[a]*

[a] School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom
[b] Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom
[c] School of Mechanical Engineering, University of Leeds, Leeds, LS2 9JT, United Kingdom
[d] School of Biological Science, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom
*Corresponding author at: School of Pharmacy, University of East Anglia. E-mail: Aram.Saeed@uea.ac.uk

Abstract

Poly[2-(Dimethylamino) ethyl acrylate] (PDMAEA) based polymers have been studied as potential gene delivery system. However, few reports emerging in literature suggesting that star-shaped PDMAEA based polymers are performing better in polyplexation with DNA, cytotoxicity and transfection, as compared to linear counterparts. Nonetheless, little evidences exist on direct comparison between the linear and star-shaped polymer structures. To address this, a series of new star-shaped PDMAEA polymers with linear counterparts were synthesised and directly compared their polyplexation with DNA and cytotoxicity in culture cell lines. The star-shaped PDMAEA polymers were synthesised using pentaerythritol tetrakis [2-(dodecylthiocarbonothioylthio)-2-methylpropionate] (4-arm DDMAT) RAFT agent in a "core-first" approach, whereas 2-(dodecylthiocarbonothioylthio)-2-methylpropionate was used to synthesise linear PDMAEA polymers. In order to investigate the effect of molar mass, both star-shaped and linear PDMAEA were synthesised in low (10kDa) and high (20kDa) molar mass. It must be noted here that the overall molar mass of the star-shaped polymer was equal to that of the linear counterparts. Interestingly, we found that the star-shaped polymer has slightly smaller hydrodynamic diameter (more compact) relative to linear counterparts, and importantly, star-shaped PDMAEA binds to DNA at much lower nitrogen to phosphate ratio (N/P ratio). However, the cytotoxicity studies in cultured 3T3 murine cell lines demonstrated that both star-shaped and linear counterparts have no toxicity at low 10kDa, but significantly toxic at higher 20kDa molar mass, this finding confirmed that the molar mass of PDMAEA play a key role in cytotoxicity effect, not variable polymer structures. Taken together, star-shaped PDMAEA binds more effectively to DNA than linear counterparts and showed no toxicity at 10kDa molar mass at variable polymer concentrations.

Keywords: cationic polymer, gene delivery, star-shaped polymer, cytotoxicity, polyplex

1. Introduction

There is a growing evidence that although viral gene delivery systems are the most efficient transfecting agent, their use often induce sever toxicity and immunogenicity [1, 2]. This has led to a surge in use and development of non-viral polycation-based synthetic gene delivery vectors as alternatives to achieve high transfection efficiency with less toxicity, both *in vitro* and *in vivo*[3, 4]. Among which, branched and linear poly (ethylene imine) (PEI) are the most extensively studied polycations used in transfection assay[5]. PEI with a wide range of primary, secondary and tertiary amines groups in its structure which can protonate to efficiently bind to DNA to form into a condense toroidal and globular nanostructures[6], known as polyplex. The PEI/DNA polyplex can then be internalised into cells by endocytosis mechanism[4]. And, it is generally postulated that following internalisation process, the PEI destabilise the endosomal membrane through its buffering capacity (pK_a) to result in membrane rupture which then leads to escaping of the polyplex into cytoplasm. However, PEI is also associated with severe toxicity and some studies suggested that the mechanism by which PEI induce toxicity is related to its ability to cause disruption of cell membrane and its integrity followed by activation of mitochondrial mediated apoptosis program[7-9]. These unwanted side effects have spurred the design and engineering of new polycation-based gene delivery systems having different structures and compositions.

Poly(dimethylaminoethyl acrylate) (PDMAEA) has emerged as potential substitute for PEI. This cationic polymer has a buffering capacity higher than that of PEI (pK_a = 7.8) and demonstrates a relatively lower cytotoxicity in cultured cells line[10]. Importantly, its synthesis from the *N, N*-dimethylaminoethyl acrylate (DMAEA), using advanced controlled/living polymerisation techniques, is relatively straightforward [11, 12]. And, numerous PDMAEA polymers with different structure have been reported in literature including linear, branched and dendritic, as transfecting agents. Recently, Georgiou et al