Environmentally Responsive Block Copolymers for Intracellular Delivery of Genes and Drugs

C. E. de Castro,[a] L. J. C. Albuquerque,[a] F. A. de Oliveira,[a] I. C. Belletinni,[b] C. Giacomelli[c] and F. C. Giacomelli[a*]
[a] *Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, Brazil*
[b] *Departamento de Ciências Exatas e Educação, Universidade Federal de Santa Catarina, Blumenau, Brazil*
[c] *Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, Brazil*
fernando.giacomelli@ufabc.edu.br

The extracellular and subcellular compartments are characterized by specific pH levels which can be substantially modified by pathophysiological states. Accordingly, whenever pH-stimuli nano-assemblies are exposed to such milieus, they may respond by physicochemical changes of their structure and/or surface charge. The structural changes of polymeric assemblies induced by environmental conditions can be used towards the delivery of therapeutic drugs and genes into specific sites of action.

In this framework, we highlight in this abstract the potential use of pH-responsive block copolymers for intracellular delivery of DNA fragments and hydrophobic therapeutics. We evaluated the block copolymers $POEGMA_{70}$-*b*-P($OEGMA_{10}$-*co*-DEA_{47}-*co*-DPA_{47}) and PEO_{113}-*b*-$PDEA_{50}$ as nonviral gene carriers.[1] The block copolymers are able to properly condense DNA into nano-sized particles however, biological assays evidenced that DNA delivery is negligible meaning that intracellular trafficking hampers efficient release. Subsequently, we demonstrated that cellular uptake and particularly the quantity of GFP-positive cells are substantially enhanced when the block copolymer polyplexes are produced and further supplemented by BPEI chains (branched polyethyleneimine)[2] as cartooned in Figure 1.

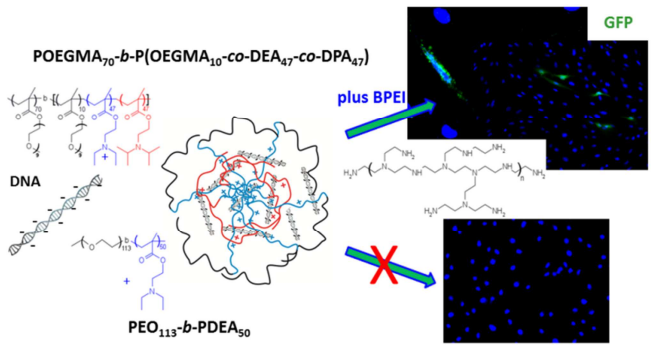

Figure 1. *This cartoon demonstrates that polyplexes produced using $POEGMA_{70}$-b-P($OEGMA_{10}$-co-DEA_{47}-co-DPA_{47}) and PEO_{113}-b-$PDEA_{50}$ efficiently delivery DNA chains at the intracellular environment, but only in the presence of free (uncomplexed) BPEI chains.*

These investigations pointed out that the transfection efficiency *vs*. cytotoxicity issue can be balanced by a mixture of BPEI chains and less cytotoxic cargo agents. We further demonstrated that the cytotoxicity of BPEI/DNA polyplexes can be reduced by the derivatization of BPEI with small molecules such as lactose. The sugar functionalization substantially reduced the cytotoxicity of the assemblies with a balanced effect in gene expression.[3]

Similarly, the enhanced cell internalization of drug-loaded nanoparticles is of due relevance since the cytosolic delivery usually potentialize the effect of active agents. Hence, the development of nanovehicles for pH-induced intracellular drug delivery is strongly bound to the understating and control over cellular uptake, which in turn is governed by the surface chemistry of the nanoparticles. Taking it into account, we also explored the cellular uptake of block copolymer assemblies consisting of a pH-responsive poly[2-(diisopropylamino)ethyl methacrylate] (PDPA) core stabilized by three different biocompatible hydrophilic shells: zwitterionic type poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), highly hydrated poly(ethylene oxide) (PEO) and an also proven non-toxic and non-immunogenic poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA). The produced nanoparticles had a spherical core-shell structure. The largest particles with the thickest hydrophilic stabilizing shell obtained from $PMPC_{40}$-*b*-$PDPA_{70}$ were internalized to higher level than those smaller in size and stabilized by PEO or PHPMA and produced from PEO_{122}-*b*-$PDPA_{43}$ or $PHPMA_{64}$-*b*-$PDPA_{72}$, respectively. The behavior was attributed to the preferred affinity of PMPC to cell membranes and particularly to scavenger receptors.[4]

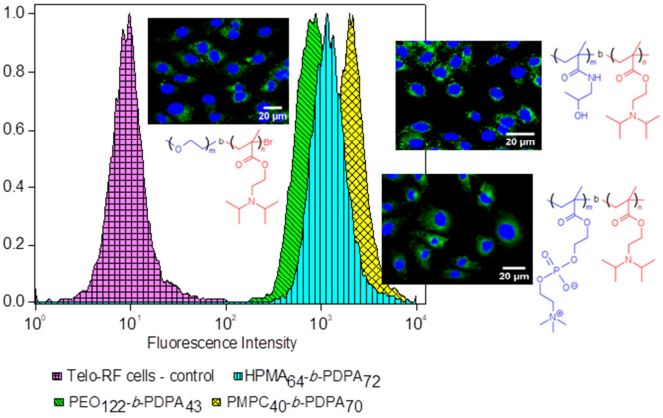

Figure 2. *This cartoon demonstrates that the $PMPC_{40}$-b-$PDPA_{70}$ nanoparticles are internalized to higher extent as compared to PEO_{122}-b-$PDPA_{43}$ or $PHPMA_{64}$-b-$PDPA_{72}$.*

In this context, we also recently demonstrated that not only the chemical nature but also the thickness of the stabilizing shell remarkably influences the cellular uptake of polymeric assemblies.[5]

Acknowledgements
These investigations were sponsored by FAPESP (Grants 2017/00459-4 and 2014/22983-9). L.J.C.A and C.E.C thank the fellowships granted by FAPESP (Grant No. 2016/23844-8 and 2015/24686-4, respectively).

References

1. Albuquerque, L. J. C. et al. *Langmuir*, 32, 577-586, **2016**.
2. Albuquerque, L. J. C. et al. *Biomacromolecules*, 18, 1918-1927, **2017**.
3. Albuquerque, L. J. C. *Macromolecular Bioscience*, 18, 1700299-1700299-9, **2018**.
4. de Castro, C. E. et al. *Langmuir*, 34, 2180-2188, **2018**.
5. de Castro, C. E. et al. *Macromolecular Bioscience*, 16, 1643-1652, **2016**.