Mesoporous silica nanoparticles as dual delivery platform for the efficient drug delivery and CRISPR-Cas9 editing machinery to one-shot treatments

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Abstract

The new CRISPR-Cas9 technology has been established quickly as an easy genome-editing method. This system presents two main components; the non-specific CRISPR associated endonuclease (Cas9) and the guide RNA (gRNA). The Cas9 targets the specific sequence in the genome to be edited guided by the gRNA. The most extended CRISPR application is for knocking out genes, being a potential technology to correct genetic defects in diseases [1]. To take advantage of this technology in therapeutic applications, the development of carriers to deliver the CRISPR-Cas9 system efficiently to human cells represents an important challenge.

Among the delivery protocols to introduce the CRISPR-Cas9 into the cells, the current non-viral used methods are lipofection, microinjection and electroporation.

However, these protocols are complex to apply in *in vivo* settings and the use of nanoparticles for CRISPR-Cas9 efficient delivery has become a research area of great interest. At this respect, new delivery methods has been described using nanomaterials based on polymers, lipid nanoparticles, cell-penetrating peptides and inorganic nanoparticles to deliver the CRISPR-Cas9 machinery [2-4].

From another point of view, several studies showed the use of surface-functionalized mesoporous silica nanoparticles (MSNs) as an efficient and safe carrier for bioactive molecules [5]. Indeed, previous published works described the use of MSNs to deliver nucleic acids into the cells [6]. In this scenario, MSNs could be a potential platform for the efficient co-delivering of the CRSIRP-Cas9 technology, as well as, to deliver an entrapped payload at the same time.

Based on these premises, we described here a novel vehicle that simultaneously delivers the CRISPR-Cas9 technology and an entrapped cargo. As a proof of concept, we used MSNs to edit GFP in human cells and, simultaneously, deliver an entrapped dye. The MSNs were loaded with a dye and then its external surface coated with a polyethylenimine (PEI) layer. Finally, CRISPR-Cas9 system is adsorbed, through electrostatic interactions, onto the PEI layer. This work demonstrated the possible use of MSNs as dual platforms to gene editing and drug delivery. MSNs represent a potential tool in the preparation of advanced nanodevices for one-shot treatments to simultaneously edit genes and release drugs by double-hit strategies, in particular to combine synergistic therapies or to overcome drug resistance in tumours.

References

[1] P.D. Hsu, E.S. Lander, F. Zhang, *Cell,* 157 (2014) 1262.
[2] C.E. Nelson and C.A. Gersbach, *Annu. Rev. Chem. Biomol.* Eng. 7 (2016) 637.
[3] E. Oude Blenke, MJ. Evers *et al.*, *J. Control Release,* 244 (2016) 139.
[4] M. Li, Y-N. Fan, Z-Y. Chen, Y-L. Luo, Y-C. Wang, Z-X. Lian, C-F. Xu, J. Wang, *Nano Res*. 11 (2018) 6270.
[5] E. Aznar, M. Oroval, L. Pascual et. al., *Chem. Rev*. 116 (2016) 561.
[6] Y. Zhang, Z. Hou, Y. Ge, K. Deng, B. Liu, X. Li, Q. Li, Z. Cheng, P. Ma, C. Li, J. Li, *ACS Appl Mater Interfaces. 7 (*2015) 37.

Figures

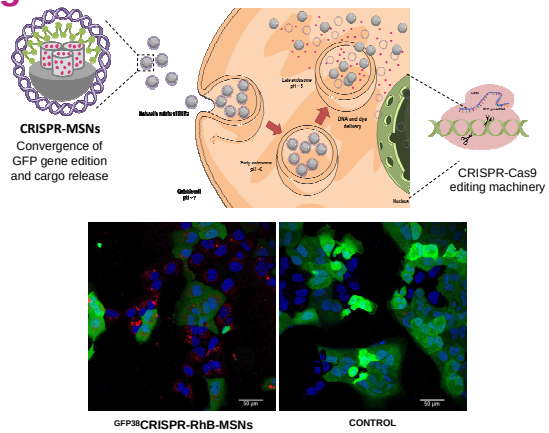

Figure 1. MSNs for the efficient co-delivery of CRISPR-Cas9 editing machinery and an entrapped cargo.