Microfluidic Manufacture of Cationic Lipid/DNA complexes

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Recent advances in biotechnology have been achieved through the employment of microfluidic devices, both for the development of diagnostic tools and the preparation of nanomedicines. In this regards, the microfluidic mixing of therapeutic agents with biomaterials yields remarkably small systems, which can be designed for drug and gene delivery. Here we compared the ability of lipid vectors made of the same lipid species but prepared by means of two different techniques, to transfect Chinese Hamster Ovarian (CHO) cells. The techniques employed are: microfluidic mixing of the components[1] and self-assembling process. In detail, we measured the transfection efficiency (TE) as the detected luciferase signal per mass unit of cell proteins, at different lipid-to-DNA mass ratios. Results are expressed in terms of luciferase signal l, amount of cellular proteins p and DNA concentration c, for each of the investigated samples. Fig. 1A shows the measured transfection efficiency, as l/p at different c-values. For complexes prepared both by self-assembling and microfluidic mixing, linear trends can be recognized and quantified by fitting the experimental data. Furthermore, similar patterns are exhibited by the absolute luminescence l (Fig 1B), which represents the total detected signal, without any information about the number of living cells in the sample. Hence, if l=p and l vary linearly with c, their trends can be described as follows:

$$\begin{aligned} l/p &= A_0 + A_1 c \\ l &= B_0 + B_1 c \end{aligned} \qquad (1)$$

where the coefficients A_0, A_1, B_0 and B_1 depend on the preparation technique. In other words, both the luminescence signal per cell and the total luminescence increase with the DNA amount administrated to cells. Despite this represents a reasonably predictable outcome, the relationships expressed in Eq. 1 describe coupled variables and thus can be easily managed to obtain the expected behavior of the cellular protein amount, as a function of c, which reads

$$p = (B_0 + B_1 c)/(A_0 + A_1 c) \qquad (2)$$

Therefore, p(c) describes a hyperbola, with intercept $p_0=B_0/A_0$ and horizontal asymptote $p_1=B_1/A_1$. In this regard, Fig. 1C shows experimental data and corresponding fitting curves.
Although for both self-assembling and microfluidic mixing the experimental data follow the aforementioned relationships, each procedure has its specific trends. As an instance, both l/p and l are higher for the former technique, thus suggesting that at any DNA concentration those systems transfect more than those prepared by microfluidic mixing (Fig. 1A, 1B). The measured control values are (1.26±0.6) 10^2 RLU/μg and (1.20±0.5) 10^3 RLU respectively, i.e. at least three orders of magnitude lower than the curves. From this perspective, we can infer that both the techniques provide effective systems for transfection experiments and the self-assembling procedure has slightly superior performances than the microfluidic mixing. However, this scheme is inverted for the curves describing the amount of cellular proteins (Fig. 1C). Indeed, the decreasing trend of p(c) is much more steeper for complexes prepared through self assembling, which induces a remarkable fall of the p-values at high DNA concentrations. This represents a noteworthy result, since the detected amount of cellular proteins is strictly related to the number of living cells in the sample and thus it gives information about the cytocompatibility of the complexes.[2] This relationship between "therapeutic" effect and side effect of the complexes can be viewed also in a representation of the involved variables, decoupled and plotted in a (l; p)-parameter space (Fig. 1D). Furthermore, we point out that the measured transfection efficiencies are comparable with those of other lipid formulations on the same cell line and slightly lower than that of Lipofectamine, a gold standard of the transfection reagents. Thus, the most notable difference between self-assembling and microfluidic mixing relies on the cytocompatibility of the resulting systems.

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[2] M. Rasoulianboroujeni et al., Materials Science and Engineering: C 13 (2016) 1377–1387.

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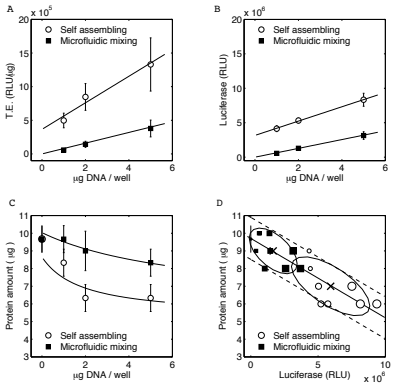

Figure 1. Transfection Efficiency (TE) of lipoplexes and DNA-lipid NPs, synthesized by self-assembling and microfluidic mixing, respectively. (A) Luciferase signal per protein amount as a function of the DNA concentration, (B) total detected luminescence signal and (C) measured amount of proteins. The lines show fitting curves of the results. (D) Resulting scatter plot of the aforementioned variables. The lines indicate a linear regression trend (within experimental errors), larger marker sizes correspond to higher DNA concentrations, ellipses are centered at the average values (”X”) of the distributions and are evaluated by diagonalizing the corresponding covariance matrices.

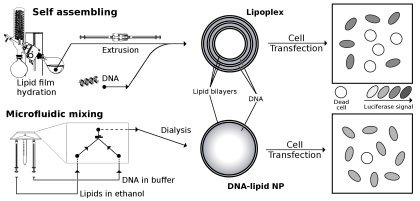

Figure 2. Representative scheme of the main outcomes. Liposomes are synthesized by lipid film hydration, then extruded and mixed with DNA to obtain lipoplexes. Conversely, DNA-lipid NPs are prepared by microfluidic mixing of the components, followed by an overnight dialysis. The transfection efficiency of lipoplexes is slight higher than DNA-lipid NPs, however the latter complexes are remarkably more cytocompatible.