NANOEMULSIONS AS NUTRACEUTICALS ABSORPTION ENHANCERS

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Introduction

Recently a lot of studies have been performed on nanocarriers able to enhance the absorption of nutraceuticals. Among them an ideal candidate for lipophilic nutraceuticals is represented by oil-in-water (O/W) nanoemulsions (NEs), which consist in small oil droplets dispersed in an aqueous phase.
Polymer coating is a known strategy helpful in preventing the thermodynamic driven phase separation. In our lab, by adding a shell of chitosan we obtained ultra-stable emulsions, which we call "Monolayer", able to keep their size distribution constant for over 1 year;[1] additionally, they are stable even in digestion fluids.[2]
A further polymer shell, made of hyaluronic acid (HA) can be added to the first one due to electrostatic interaction, which we call "Bilayer".
In order to mimic the intestinal epithelium a simil-3D contest is needed. Such a system is often reproduced by using transwells, systems in which intestinal cells can proliferate forming villi and that can be used to understand the passage of nutrients across the intestinal barrier.[3,4]

Experimental

A mixture of Caco2 and HT29 cells cultured in transwells are used in order to mimic the intestinal barrier. Different kinds of NEs, varying in the external shell polymer, are taken in contact for 1 hour with the cells layer and then washed, mimicking the intestinal washout.
Then, fluorescent dextran of two different sizes, namely 3 kDa and 40 kDa, was put in the donor phase and allowed to pass through the cell layer and the membrane, to understand passive and active transport, respectively. Furthermore, vitality tests are performed to verify the toxicity of such systems.

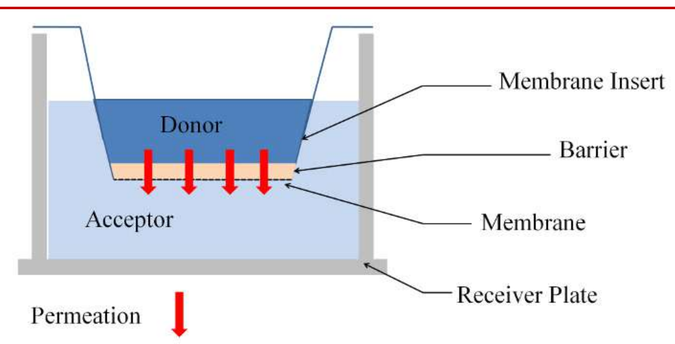


Fig. 1 Schematization of a transwell.
Cells are cultured on the upper part of the membrane. Dextran passes from donor to acceptor phase through the confluent cell layer. Images with the focus on the cell layer were taken at 0, 30, 60 and 120 minutes.

Results

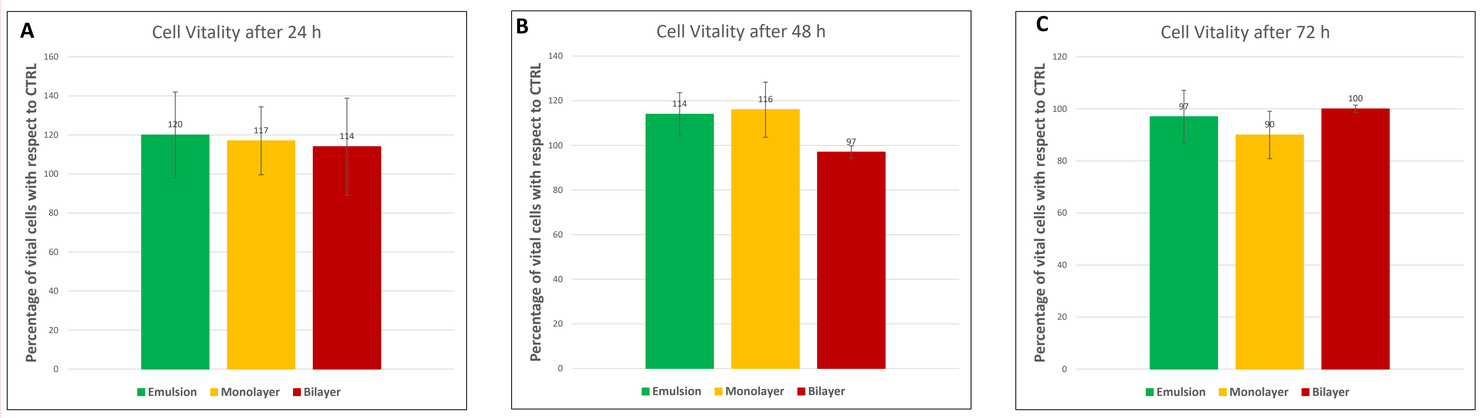
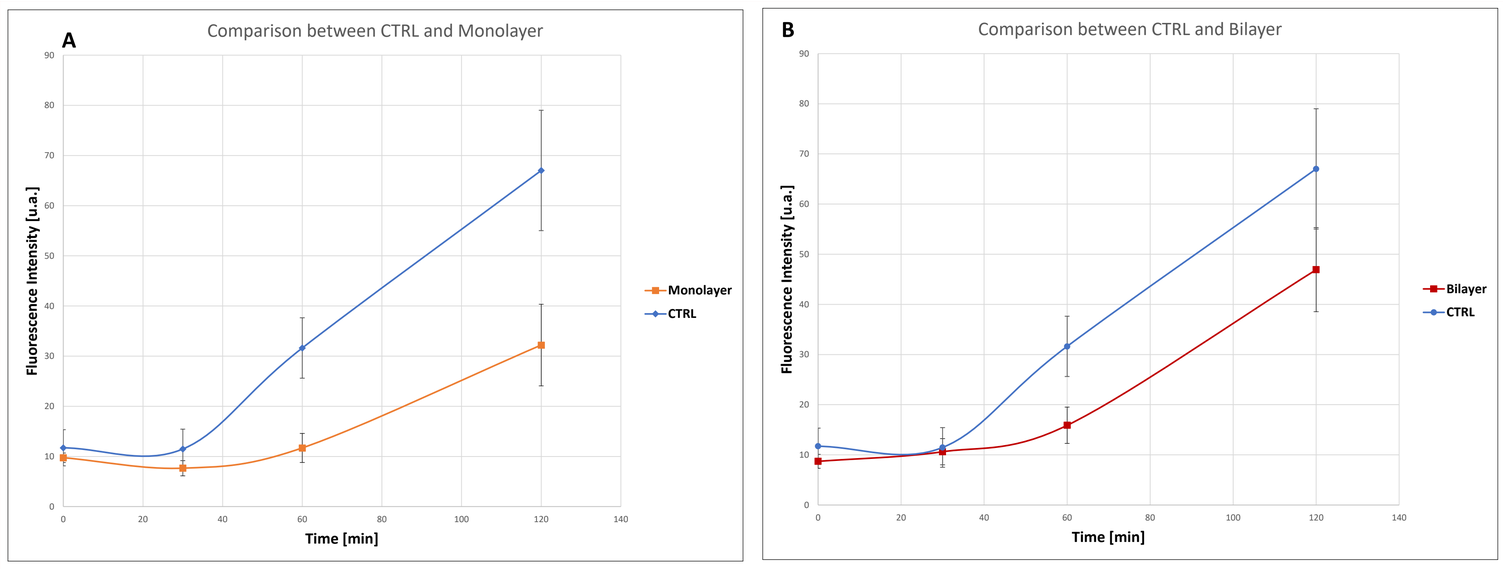


Fig. 2 Vitality tests (MTT) for cells in contact with different kinds of NEs.
A) Results after 24h. B) Results after 48h. C) Results after 72h.
The NEs concentrations were chosen as to obtain a final oil percentage of 0,25% v/v, the same used in the following experiments. Cell vitality is unaffected by the NEs, meaning that they show no toxic effect.



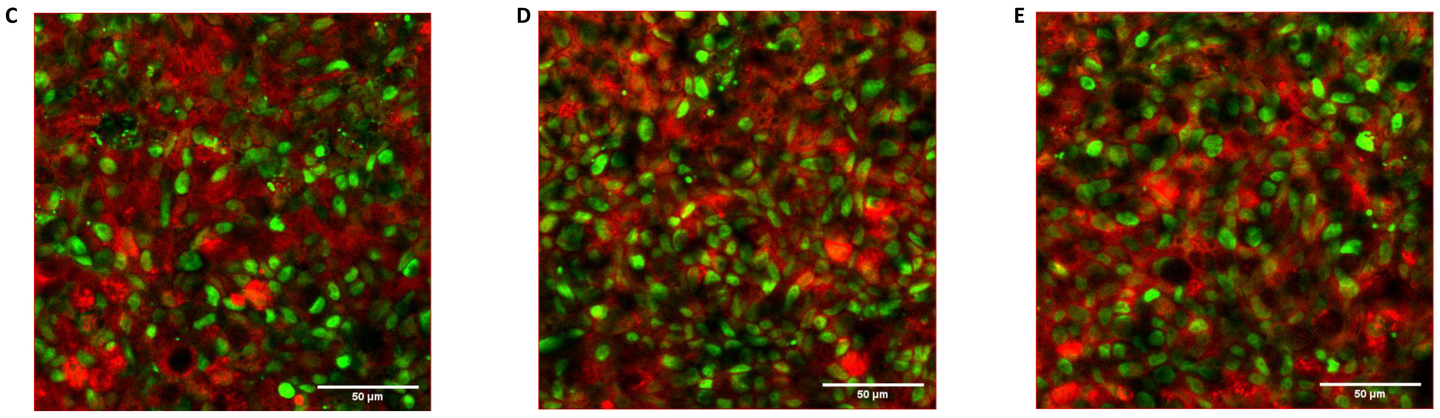
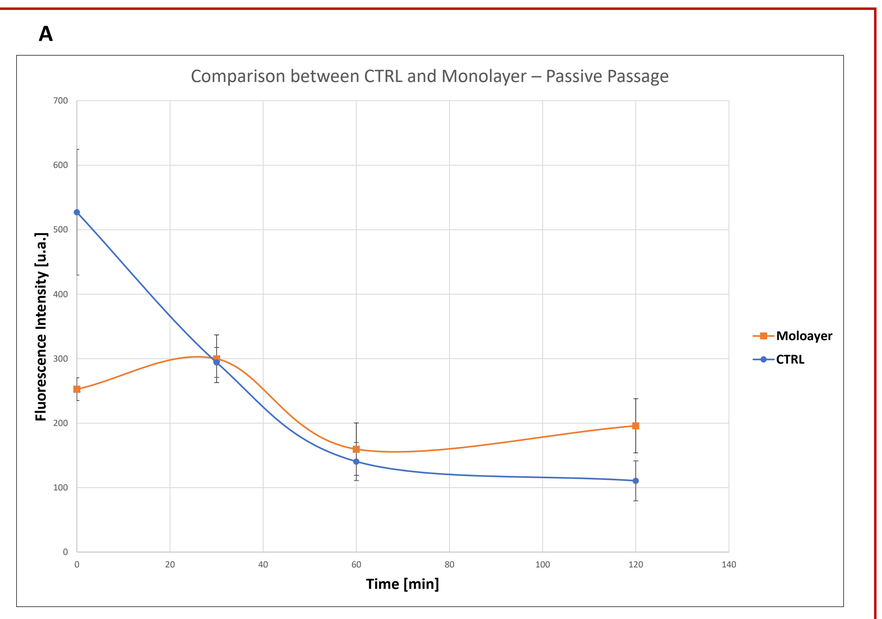


Fig. 4 Comparison between the concentration of **dextran 40 kDa**, seen as fluorescence intensity, in control and cells in contact with Monolayer (A) and Bilayer (B).
Confocal images of cells (green) and dextran (red) for the control (C), Monolayer (D) and Bilayer (E).
Overall, dextran concentration increases with time due to an accumulation on the cell layer.
For smaller periods of time the situation is similar for both formulations.
For larger periods of time the concentration of dextran is reduced, meaning that there is a higher dextran absorption through the intestinal layer, especially in the case of Monolayer.



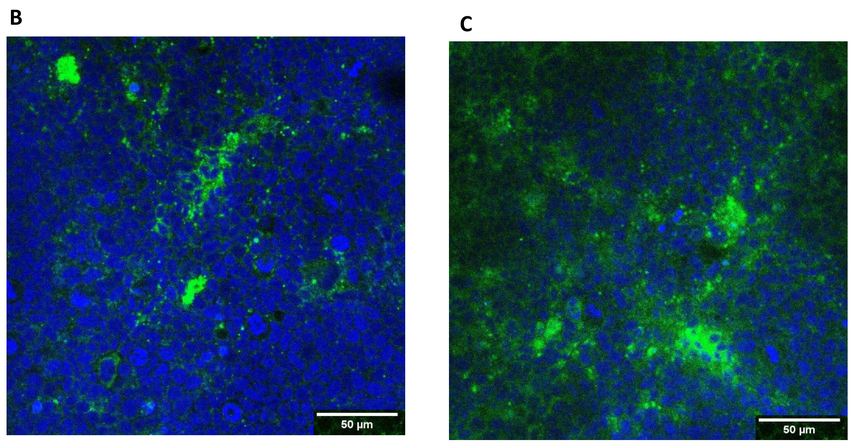


Fig. 3 Comparison between the concentration of **dextran 3 kDa**, seen as fluorescence intensity, in control and cells in contact with Monolayer (A).
Confocal images of cells (blue) and dextran (green) for the control (B) and Monolayer (C)
Overall, dextran concentration decreases with time due to the more facilitate passive passage through the membrane.
At time 0 the absorption of dextran is enhanced for Monolayer, which may be due to an interaction with the tight junctions that labilizes the membrane.
The situation is stabilized for intermediate periods of time.
At 120 min the absorption is slightly reduced for Monolayer, may be due to an accumulation of dextran on the cell layer.

Conclusion

The passage of nutraceuticals is enhanced, both for active transport especially for long times and passive transport for short times, by using NEs. Furthermore, the enhancement is not due to toxicity effects, indeed, cells are still vital after the contact with NEs.

- NEs can be loaded with specific molecules, like curcumin, which passage, as we demonstrated, is in this way favored.

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