PLGA Nanoparticles for Selective Neuroprotection of Neural Progenitor Cells During Chemotherapy

L. Cerutti[1,2], A. Borraccini[1], P. F. Ferrari[3,4], N. Fantini[1], P. Canepa[5], R. Marotta[6], M. Tedesco[1], P. Massobrio[1], M. Brofiga[1,2]

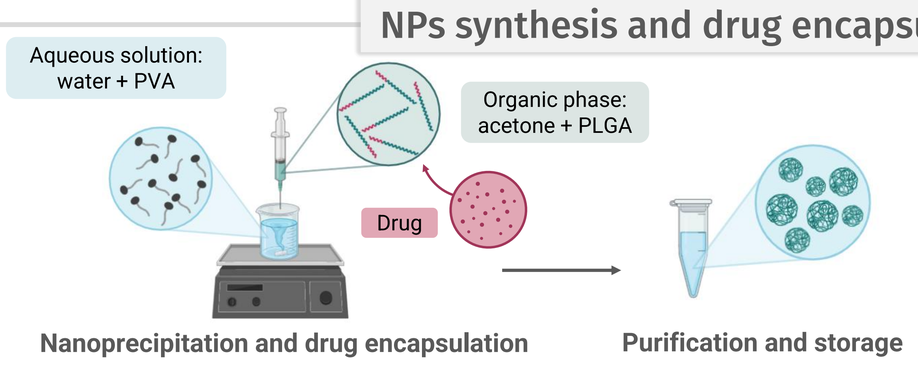
1 Department of Informatics, Bioengineering, Robotics and Systems Engineering (DIBRIS), University of Genova, Genova, Italy
2 Neurofacility, Italian Institute of Technology (IIT), Genova, Italy
3 IRCCS Ospedale Policlinico San Martino, Genova, Italy
4 Department of Civil, Chemical and Environmental Engineering (DICCA), University of Genova, Genova, Italy
5 OPTMATLAB, Department of Physics, University of Genova, Genova, Italy
6 Italian Institute of Technology (IIT), Genova, Italy

Abstract

One of the key challenges in oncology is developing therapeutic strategies that eliminate cancer cells while preserving healthy neural tissue. Conventional chemotherapies, although effective, lack sufficient selectivity and often damage neural progenitor cells (NPCs), compromising regenerative niches in the brain and impairing recovery processes. To overcome this limitation, we designed a nanoparticle-based system optimized for nose-to-brain delivery, specifically intended to protect hippocampal neural stem cells during chemotherapy, enabling targeted transport of therapeutic agents. Biodegradable PLGA nanoparticles were engineered, characterized, and evaluated for their compatibility with neural cells, with the aim of creating a platform that reduces neurotoxic effects and preserves NPC viability throughout aggressive anticancer treatments.

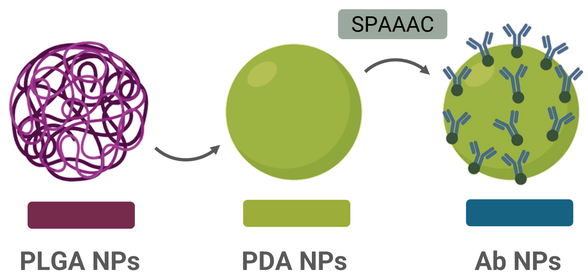
Materials and Methods

NPs synthesis and drug encapsulation



Synthesis of PLGA nanoparticles. 1. **Nanoprecipitation and encapsulation**: the drug is mixed in the organic phase, formed by PLGA dissolved in an organic solvent. This solution is then injected into an aqueous phase containing deionized water and PVA. 2. **Solvent evaporation**: the suspension is agitated in a fume hood to evaporate the organic solvent. 3. **Purification and storage**: the mixture is centrifuged to remove macroaggregates and purify the nanoparticles, which are then stored in deionized water or PBS at 4°C.

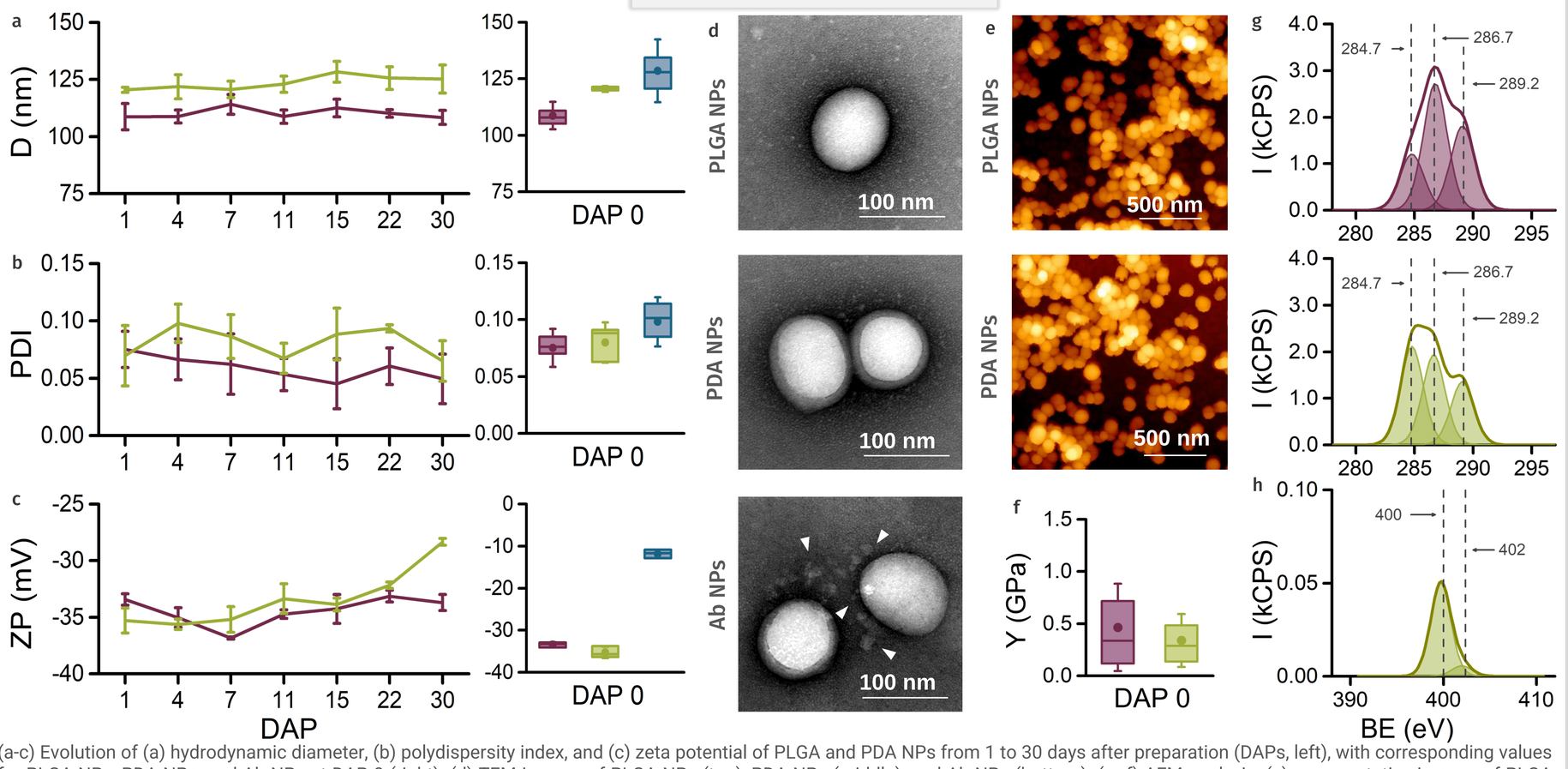
NPs functionalization



Nanoparticle Functionalization. Following synthesis, nanoparticles undergo surface modification and biofunctionalization. 1. **Polydopamine coating**: nanoparticles are coated with polydopamine by stirring in a dopamine solution for 2.5 hours at room temperature. 2. **Antibody functionalization** (nestin-targeting): nanoparticles are first modified with an NHS–PEG4–N3 linker to introduce azide groups; subsequently, DBCO-conjugated anti-nestin antibodies are attached via click chemistry (SPAAC), enabling rapid and specific conjugation.

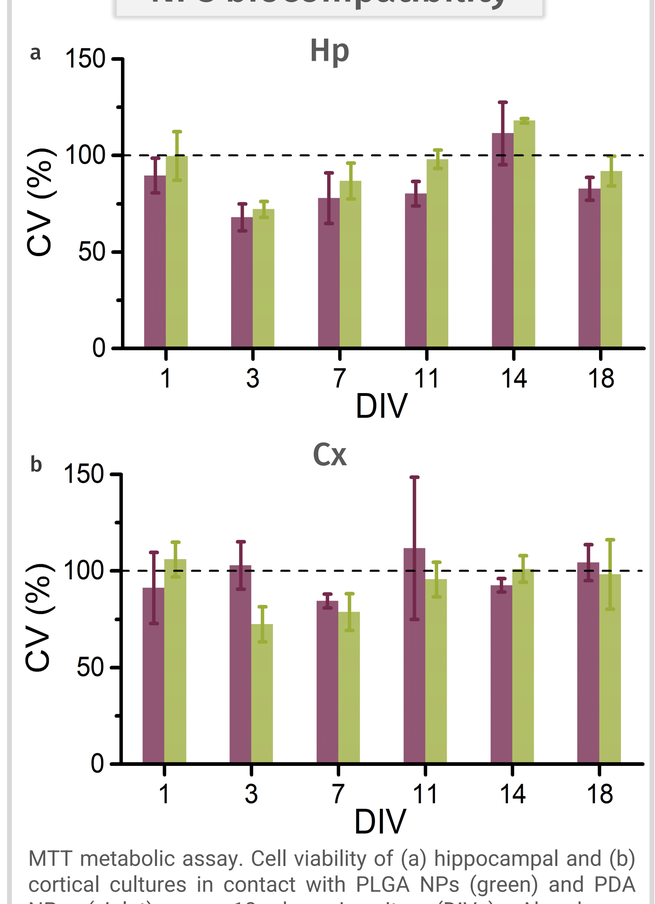
Results

NPs characterization



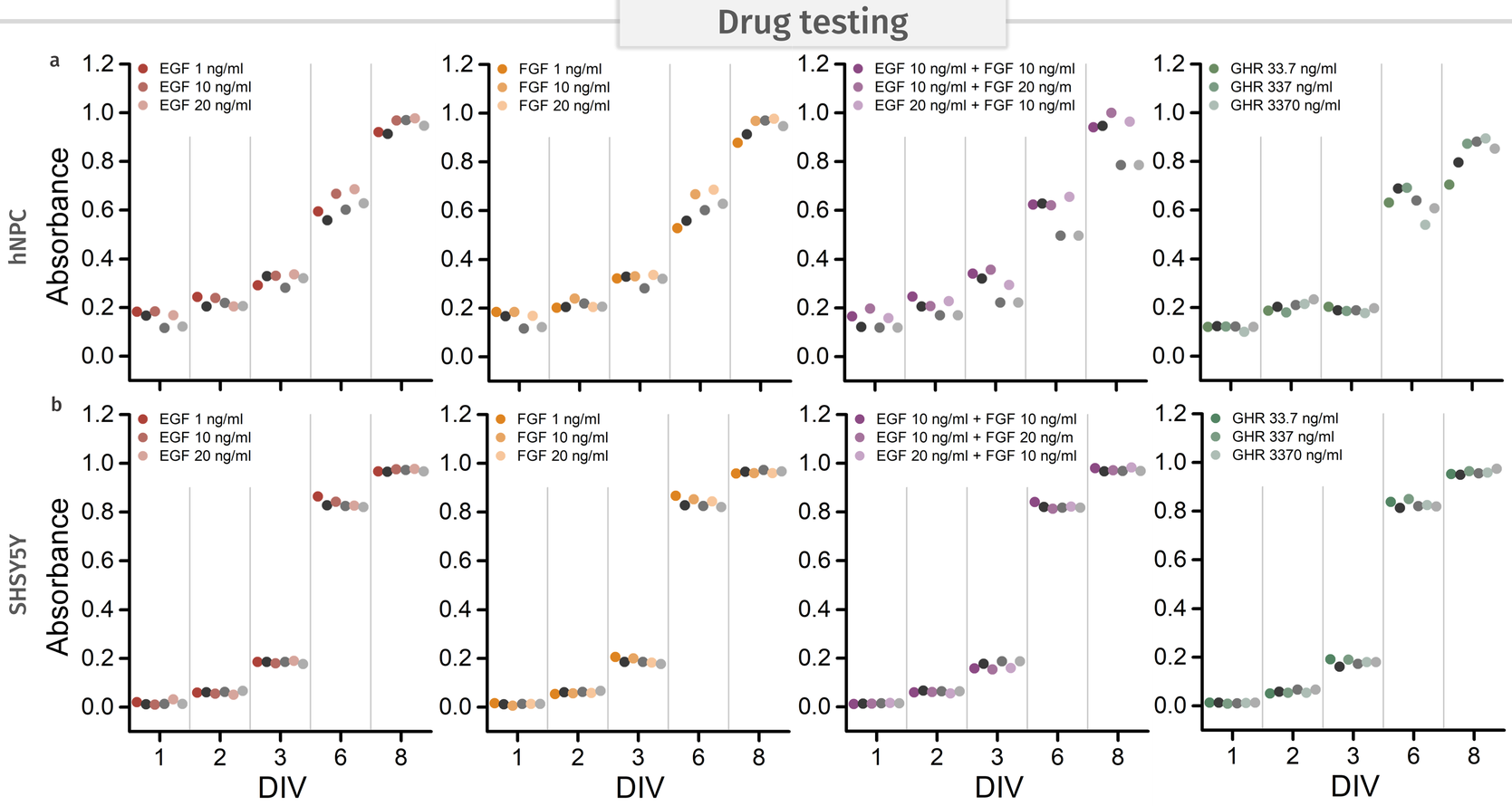
(a-c) Evolution of (a) hydrodynamic diameter, (b) polydispersity index, and (c) zeta potential of PLGA and PDA NPs from 1 to 30 days after preparation (DAPs, left), with corresponding values for PLGA NPs, PDA NPs, and Ab NPs at DAP 0 (right). (d) TEM images of PLGA NPs (top), PDA NPs (middle), and Ab NPs (bottom). (e–f) AFM analysis: (e) representative images of PLGA NPs (top) and PDA NPs (bottom), and (f) corresponding Young's modulus derived from force–distance maps. (g–h) XPS spectra: (g) C 1s spectra of PLGA NPs (top) and PDA NPs (bottom), and (h) N 1s spectrum of PDA NPs.

NPs biocompatibility



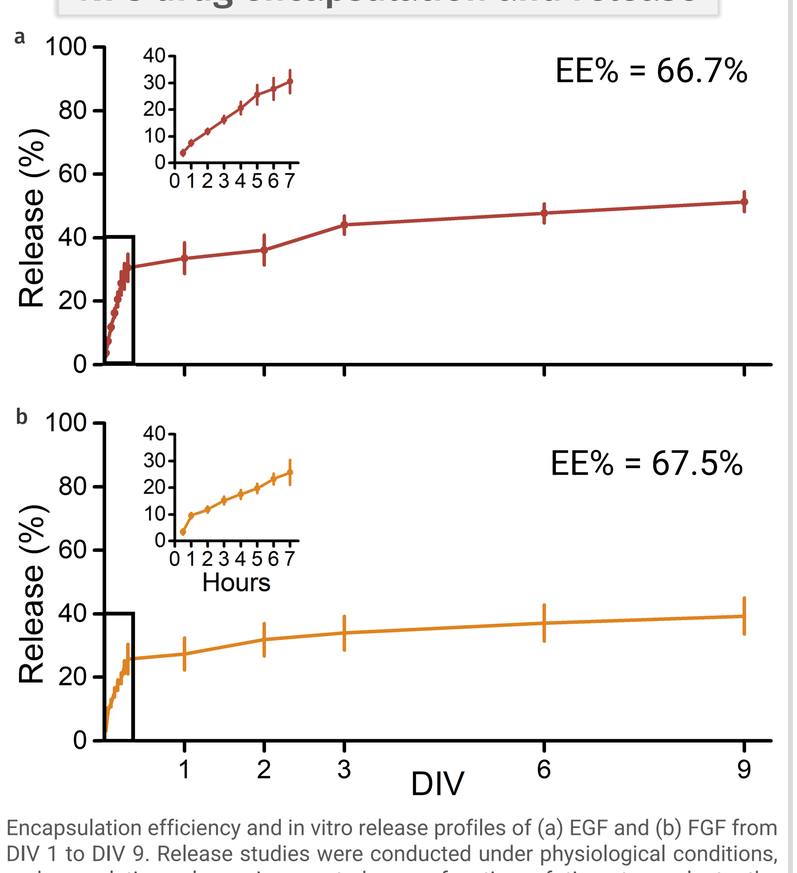
MTT metabolic assay. Cell viability of (a) hippocampal and (b) cortical cultures in contact with PLGA NPs (green) and PDA NPs (violet) over 18 days in vitro (DIVs). Absorbance measurements of cultures with NPs were normalized over the corresponding cultures without NPs.

Drug testing



Evaluation of drug candidates and concentrations for optimal nanoparticle loading in (a) hNPC and (b) SHSY5Y cell cultures: absorbance values obtained from the MTT assay from DIV 1 to 8. Colors correspond to different drug conditions, while gray denotes the PBS controls. Shades refer to different concentrations of the same drug/PBS.

NPs drug encapsulation and release



Encapsulation efficiency and in vitro release profiles of (a) EGF and (b) FGF from DIV 1 to DIV 9. Release studies were conducted under physiological conditions, and cumulative release is reported as a function of time to evaluate the sustained delivery behavior of both growth factors.

Conclusions

Results show that the designed NPs are non-cytotoxic for both cortical and hippocampal networks. With an average size of 110±5 nm, they are suitable for intranasal delivery, and when functionalized with nestin antibody, can recognize hNPCs. Moreover, the combination of EGF and FGF proved to selectively stimulate hNPC proliferation while minimally affecting cancer cells. Therefore, this approach offers a promising strategy for the delivery of therapeutic agents within the central nervous system, with the potential to protect and sustain NPC niches during oncological interventions.

letizia.cerutti@edu.unige.it

Via All'Opera Pia 13, 16145, Genova (GE), Italy