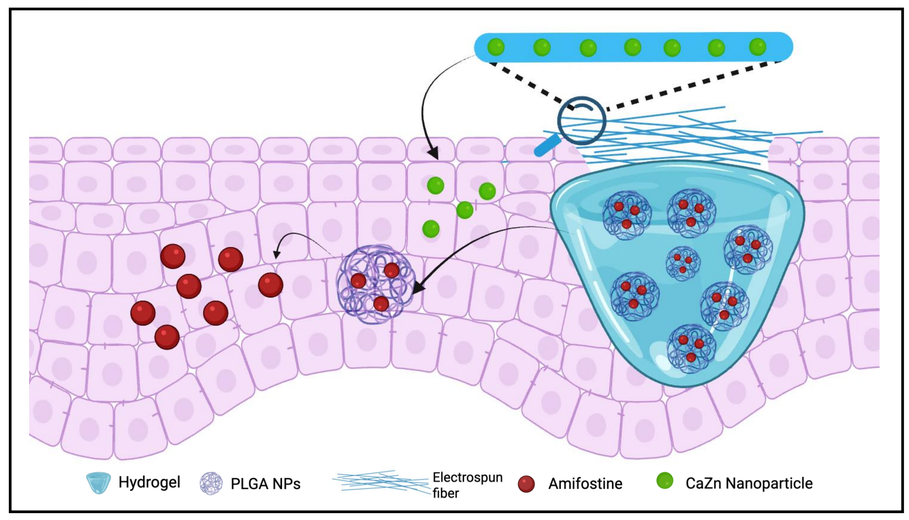
Injectable thermosensitive hydrogel for localized delivery of amifostine to prevent radiation-induced side effects in head and neck cancer

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Graphical Abstract

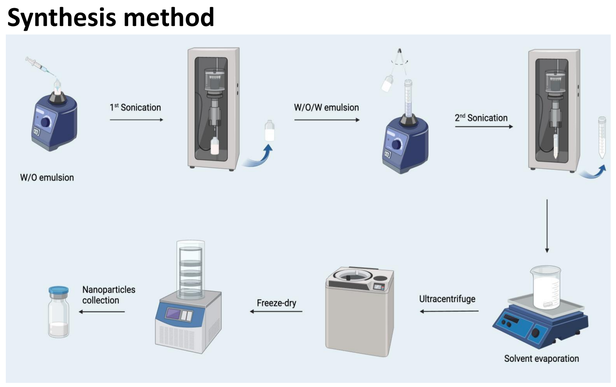


Introduction

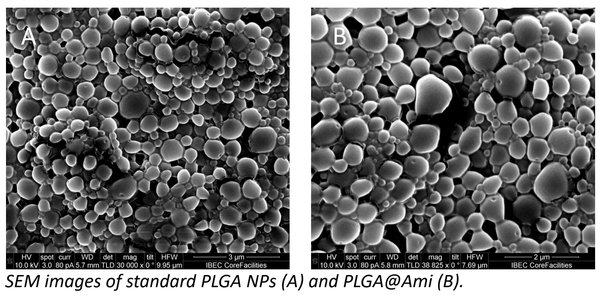
Head and Neck Squamous Cell Carcinoma (HNSCC) was the 7th most diagnosticated cancer in 2023 [1]. Standard treatment typically involves X-ray radiotherapy that effectively targets tumor cells but it also damages surrounding healthy tissues, leading to severe side effects. Currently, Amifostine is the only FDA-approved radioprotective drug [2]. We aim to develop a **thermosensitive drug delivery platform** designed for implantation in the surgical resected site. This system will enable the **localized release of Amifostine**, encapsulated in PLGA nanoparticles (NPs), to provide **in-situ radioprotection**. In parallel, the platform will be reinforced with PLA electrospun fibers loaded with CaZn NPs, supporting wound healing and tissue regeneration in the ablated area.

Amifostine nanocarriers – PLGA NPs

Synthesis method

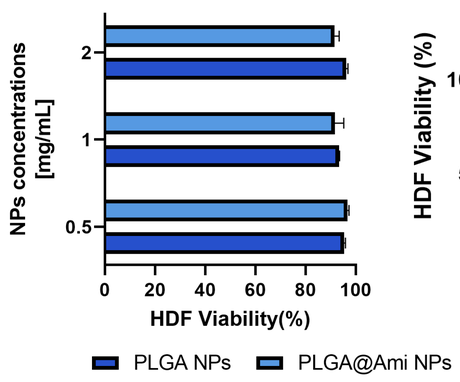


SEM

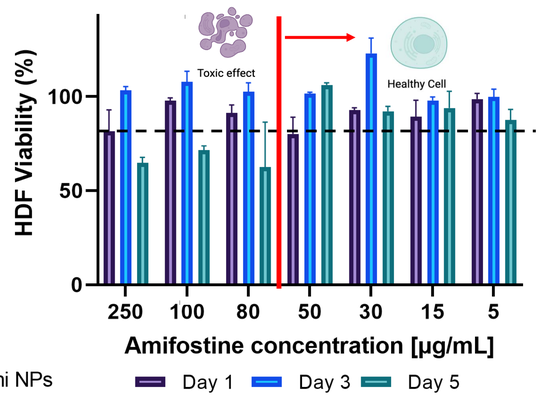


SEM images of standard PLGA NPs (A) and PLGA@Ami (B).

Nanocarriers Toxicity

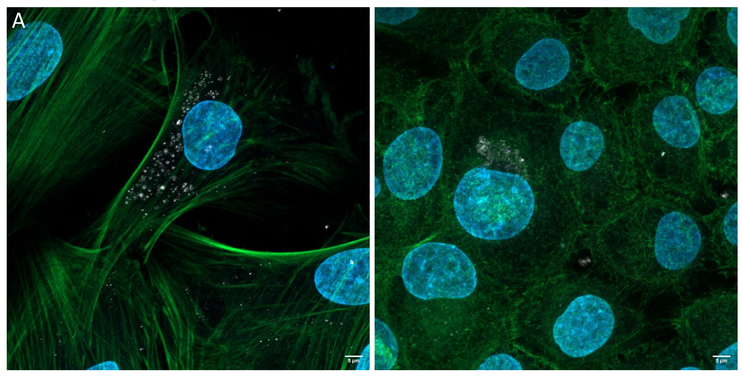


Amifostine Toxicity



DLS Data – PLGA NPs	Z-size (nm)	Z-Pot (mV)	PDI
PLGA NPs	213,45 ± 5,6	-21,2 ± 0,2	0,19
PLGA@Ami	229,1 ± 8,3	-9,1 ± 0,2	0,21

PLGA nanoparticles internalization



Microscope images of NPs internalization in HDF (A) and Cal27 (B) 24h after administration. Phalloidin in green, nuclei in blue, NP in white. Scale bar is 5µm.

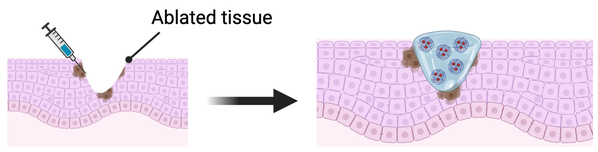
Key Points

- Synthesis of Amifostine carriers
- ~80% Encapsulation efficacy
- Amifostine administered at conc. ≤ 50µg/mL
- Proved NPs' internalization in HDF

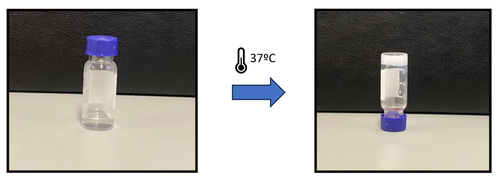
Thermosensitive Hydrogel as Drug Delivery Platform

Materials – Requirements

- Injectable polymeric solution
- Thermosensitive polymer
- Suitable as NPs' carrier

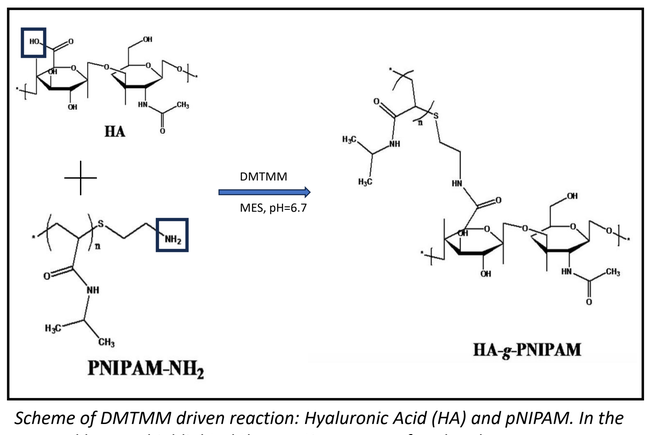


Schematic representation of hydrogel application at physiologic temperature



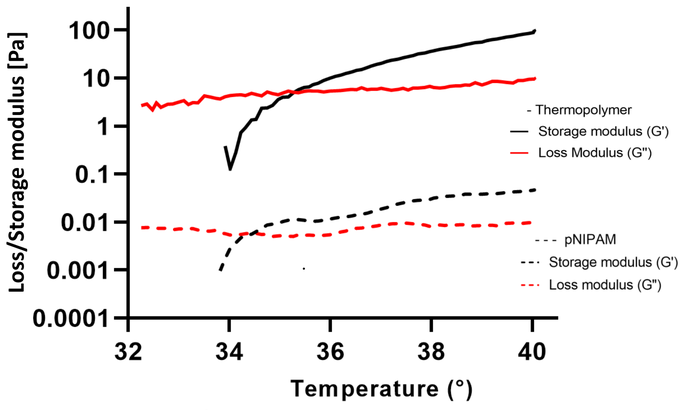
Picture of gelation of HA-pNIPAM solution at 37℃

Synthesis Reaction



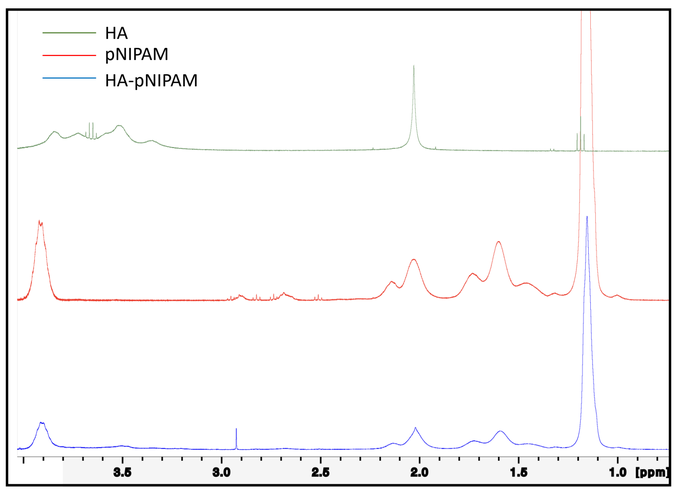
Scheme of DMTMM driven reaction: Hyaluronic Acid (HA) and pNIPAM. In the squared box are highlighted the reactive groups of each polymer.

Rheologic measure



Rheological measure of the thermopolymer showed gelation process at 35ºC, bearing a stiffness of about 10Pa.

H-NMR

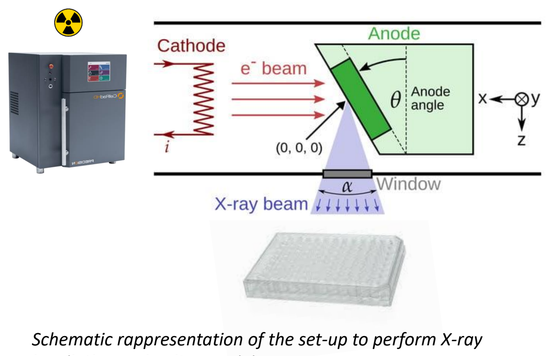


Key Points

- Grafted pNIPAM-NH_2 to Hyaluronic acid
- Thermopolymer - gelation at 35ºC
- Synthesis of drug delivery platform

X-Ray irradiation - In vitro model

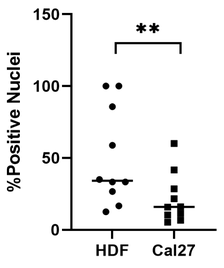
X-Ray treatment illustration



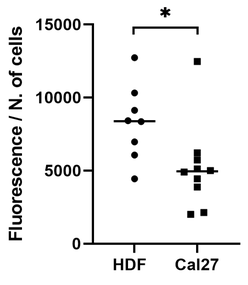
Schematic rappresentation of the set-up to perform X-ray irradiation on in-vitro models

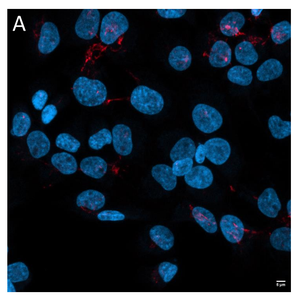
- Irradiation regime: single irradiation at 5Gy
- Amifostine is activated by alkaline phospatases (ALP)
- Cells are characterized to investigate ROS level and proliferation activity

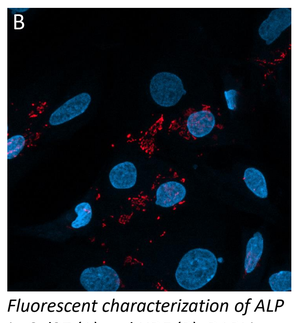
ALP Positive nuclei



Fluorescence - ALP

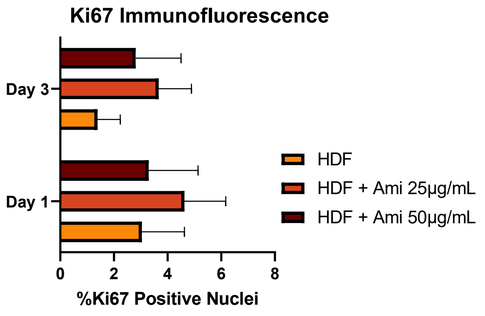




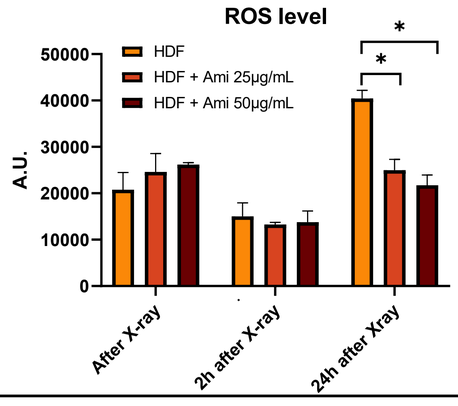


Fluorescent characterization of ALP in Cal27 (A) and HDF (B). DAPI in blue, ALP in red. Scale bar is 5µm.

Ki67 Immunofluorescence



ROS level



Conclusion

- ✓ We generated Amifostine's nanocarriers
- ✓ We synthetized injectable thermosensitive polymers
- ✓ We optimized Amifostine dosage for treatment

Future Step

- ⏳ Determine Amifostine kinetic release
- ⏳ Validation of radioprotection effect upon PLGA NPs subministration
- ⏳ Improve mechanical properties of the hydrogel

Acknowledgment

Project REGOMUC PID2021-128412OB-I00

Bibliography [1] Barsouk, A. et al. 2023 Med. Science. DOI: 10.3390/medsci11020042 [2] https://www.cancer.gov/about-cancer/treatment/drugs/amifostine