Preliminary *in vitro* evaluation of a e-beam cross-linked doxorubicin loaded hybrid hydrogel as a potential therapy strategy for melanoma

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Keywords: collagen-based hydrogels; melanoma cells; e-beam crosslinking; cancer therapy

Polymeric drug delivery systems have gained attention, with hydrogels emerging as promising candidates due to their tunable properties, controllable degradation, and ability to stabilize labile drugs [1]. Literature suggests that hybrid hydrogels, combining synthetic and natural polymers, offer optimal chemical and mechanical properties for medical use [2]. Among crosslinking strategies, electron beam (e-beam) radiation stands out [3], providing precise network control and inherent sterilization without toxic reagents [4]. In this context, hybrid hydrogels of bovine collagen and sodium carboxymethylcellulose (natural) with poly(vinylpyrrolidone) and poly(ethylene oxide) (synthetic) were obtained via e-beam (25 kGy), with doxorubicin loaded before or after irradiation.

The cytotoxic and anti-proliferative effects of these hydrogels, designed to release doxorubicin at its IC50 concentration (10 ng/mL), were evaluated on A375 melanoma cells. The anti-tumour efficiency was assessed via indirect contact studies, using the extraction method (ISO 10993-5 standards), in terms of cell viability (Live/Dead test), proliferation (CCK-8 assay) and morphology (actin staining with Alexa Flour 488 – phalloidin).

The results revealed significant differences in cell proliferation among the tested hydrogels, with the lowest OD values observed for the cells grown in the extraction media from the PD81'DOX*/PD81'DOX** samples, and closely followed by the cells maintained in the extraction media from the PD81' and PD81'DOX samples. This observation, combined with the absence of the red stained cells, indicates either a suppression of metabolic activity or a restriction in cell proliferation. Similarly, cytoskeleton examination showed a decrease in cell density and a more spread-out morphology in cells grown in the PD81'DOX* and PD81'DOX**extraction media, further highlighting the anti-proliferative effect of these hydrogel formulations.

In conclusion, the doxorubicin-loaded hybrid hydrogels exhibit promising anti-tumour potential and could serve as effective drug delivery platforms in melanoma therapy.

Acknowledgements: The authors gratefully acknowledge CNCS—UEFISCDI, project number PN-III-P1-1.1-PD2021-0552 for financial support.

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