Anticancer and immunological properties of selenium nanoparticles in 3D Breast Cancer Spheroids

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Introduction: Selenium Nanoparticles (SeNPs) have shown antitumor properties while having high biocompatibility, although a stabilizing agent is required to prevent their aggregation.[1,2]. BSA-SeNPs have also been demonstrated not only to induce apoptosis in cancer cells but also to function as an immunostimulant [3]. Since solid tumours develop in a 3-D shape, the use of 3-D cultures preserves better the biological characteristics of the original tumour than conventional 2-D monolayers, especially when including primary cancer cells removed from a mice tumour [4]. Therefore, the aim of this work was to develop 3D multicellular spheroids of breast cancer to characterize the anti-tumour immune-modulatory effect of SeNPs.

Methods: SeNPs were produced using sodium selenite, ascorbic acid as reducing agent and bovine serum albumin (BSA) as stabilizing agent, adjusting the pH reaction environment to 1. The stability of BSA-SeNPs was performed in human plasma, cell medium, pH 7.4 and 5.5.
Spleen was removed from C57BL/6J mice and the splenocytes were extracted and cultured with BSA-SeNPs for 48 h. The BSA-SeNPs effect on the viability of splenocytes was evaluated by flow cytometry.
A breast cancer orthotopic model was established by inoculating 1 × 10^6 E0771 cells in the fourth inguinal mammary fat pad of C57BL/6J mice, being divided in non-vaccinated and vaccinated with KRAS-loaded PLGA-Mannose NPs on days 7 and 14 after tumor inoculation. After 3 weeks, the mice were sacrificed and the tumour of the non-vaccinated mouse was extracted, while the splenocytes from the vaccinated mouse were isolated as well. 3D spheroids created using E0771 murine cells, were embedded in Matrigel. The splenocytes were divided into non-stimulated and stimulated with CD3 and KRAS peptide. Finally, the 3D-spheroids were treated with BSA-SeNPs and co-cultured with splenocytes, non-stimulated and stimulated according to the Scheme in **Figure 1**. The spheroids were observed under a microscope for 120 h.

Results: BSA-SeNPs demonstrated a size below 50 nm, low polydispersity, and a positive surface charge. The nanosystem produced was stable at cell medium, human plasma, and physiologic pH, although degraded at acidic pH, characteristic of the tumour microenvironment. The BSA-SeNPs at 20 ng/mL did not affect the viability of splenocytes after 48 h of incubation. Furthermore, BSA-SeNPs at 20 ng/mL presented synergic activity with both non-stimulated and stimulated T cells in inhibiting 3D-spheroids sprouting (**Figure 2**).

Conclusion: Stable SeNPs at physiologic pH and plasma were produced. These NPs aggregated at acidic pH. *Ex vivo* studies indicated that the BSA-SeNPs potentially present an immune-modulatory effect, as their anti-tumor effect was potentiated by activated T-cells , being thus an interesting approach for cancer therapy.

References

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Figures

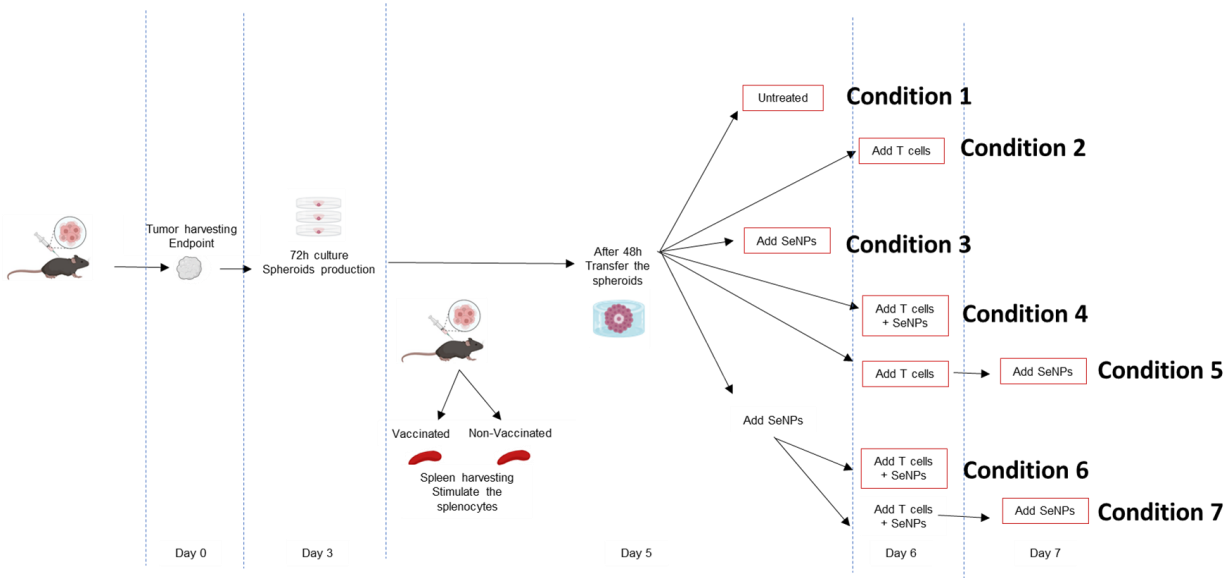

Figure 1. – Experimental design of the spheroids production, and the time schedule of the several treatment conditions (BSA1-SeNPs and splenocytes)

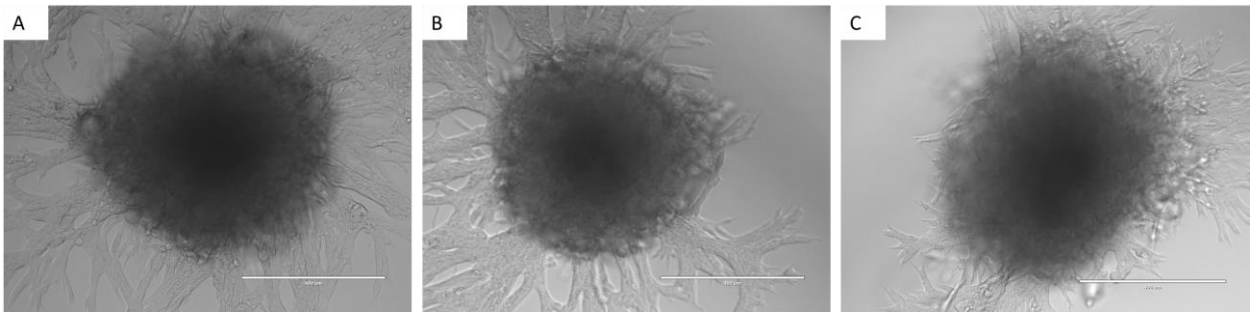

Figure 2. – Spheroids and sprouting formation after 120h in reduced growth factor Matrigel: (A) untreated, (B) incubated with BSA-SeNPs and (C) treated with BSA-SeNPs and co-cultured with splenocytes.