A HYDROGEL FOR LOCAL T LYMPHOCYTE DELIVERY TOWARDS ENHANCED CANCER IMMUNOTHERAPIES

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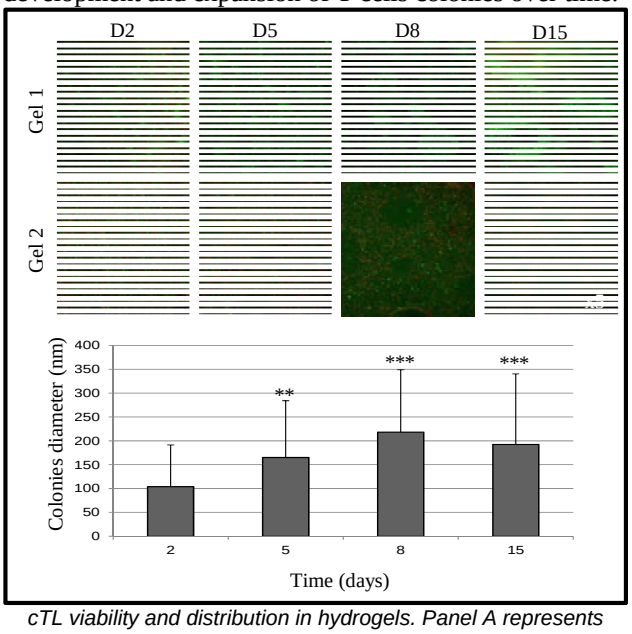
Introduction: Systemic adoptive T lymphocyte transfer is an emerging cancer immunotherapy showing tremendous promise in clinical studies, and its success lies in the ability of the antigen-experienced cytotoxic T lymphocytes (cTL) to access and to persist in the tumour microenvironment [1]. The mimicking of tertiary lymphoid structures (TLS) that persist and promote a protective immune response against cancer can be achieved using a locally implantable material releasing anti-tumour proliferating cTL. Chitosan is a natural polymer that can be combined with a weak base to form a thermosentive hydrogel which is liquid at room temperature and is able to gelify at 37°C [2]. We are working on the development of novel chitosan hydrogel formulations having high potential in terms of biocompatibility, mechanical properties and porosity for cell encapsulation and release. More particularly, new gelling agents composed by a combination of phosphate buffer (PB) and sodium hydrogen carbonate (SHC) permitted us to generate injectable thermogels that lead to iso-osmotic, strong and porous structures at physiological pH. Though proliferating T cells have never been cultured in biogels for this purpose, we hypothesize that the implantation TLS-like structures composed of these new hydrogels and cTL into the tumour microenvironment will provide a means for the delivery of a continuous feed of these cells against the tumour for the reprogramming of inflammation mechanisms and the reduction of tumour burden. Therefore, our aim was to fine-tune a chitosan-based hydrogel formulation that would provide an environment allowing the three-dimensional (3D) proliferation and release of cTL whose activation state can be influenced by the surrounding conditions. For such an application, the hydrogel should present good compatibility with cells, as well as adequate rheological properties allowing it to be injectable and to rapid solidify into a 3D scaffold of adequate porosity.

Method: Chitosan (Mw 250kDa, DDA 94%) thermogels were prepared with novel combinations of gelling agents (composed with PB and SHC) with the goal to reduce total salts concentration. Their rheological properties and mechanical strengths were evaluated by rheometry and unconfined compression tests (50% of stain) respectively. The pH and osmolilaty of the hydrogels were measured, and their morphology was observed by scanning electron microscopy. Two formulations (PB0.04M/SHC0.075M : Gel1 and PB0.04M/SHC0.05M : Gel2), presenting appropriated gelation kinetic, mechanical properties and low ionic strength were selected for further experiments. Their biocompatibility with T cells was determined using a 15 day time course of encapsulated primary T cells. Gel- and supernatant-derived cells were followed over time, where cell numbers, viability, phenotype and activation status were recorded utilizing microscopy and flow cytometry.

Results: For both formulations, rheological properties were compatible with injection purpose and provide good mechanical properties after gelation. Scanning electron microscopy revealed an interconnected macroporosity susceptible to provide a 3D support allowing cell proliferation and migration. However, minute differences in hydrogel formulation have dramatic effects on the porosity, survival and proliferation potential of encapsulated primary T cells *in vitro*. Flow cytometry and microscopy demonstrated that T cells encapsulated in Gel 2 had lower viability than those encapsulated in Gel 1. In addition, only Gel 1 permitted the growth of cells, where flow cytometry provided evidence of increased cellular proliferation and escape from the gel over time, along with the maintenance of cellular phenotype and activation status. Finally, microscopy proved the continuous development and expansion of T cells colonies over time.



cTL viability and distribution in hydrogels. Panel A represents fluorescent microscopy after Live/dead staining. Panel B represents the size of colonies formed in Gel 1 in function of time.

Discussion & Conclusion: We have developed a hydrogel formulation that acts as a scaffold for the growth of a 3D T cell culture that maintains its phenotype and is influenced by surrounding conditions, suggesting that encapsulated cells will retain their therapeutic functions. This locally injectable hydrogel can serve as a TLS-like T cell delivery vehicle to complement adoptive cell transfer therapies.

References: 1. Restifo *et al.*, Nat Rev Immunol, 2012. 12(4): p. 269-81. **2**. Chenite, *et al.*, Biomaterials, 2000. 21(21): p. 2155-61. **Funding:** CRC in biomaterials and endovascular implants to S.L. and the CCSRI to R.L. E.A also acknowledges FRQ-NT scholarship.