Standardization in tissue disaggregation minimizes differences in cells by fresh and frozen tissue and focuses relevant variations on both surface marker detection and functional parameters

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Fig. 1: Medimachine II

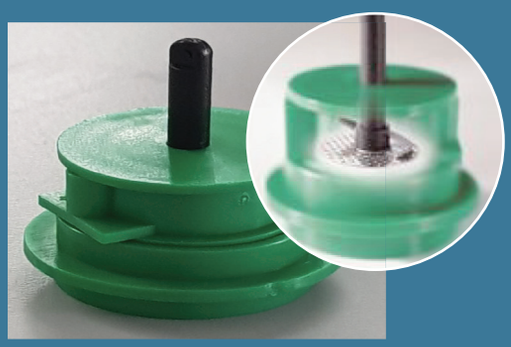

Fig. 2: Medicons chambers

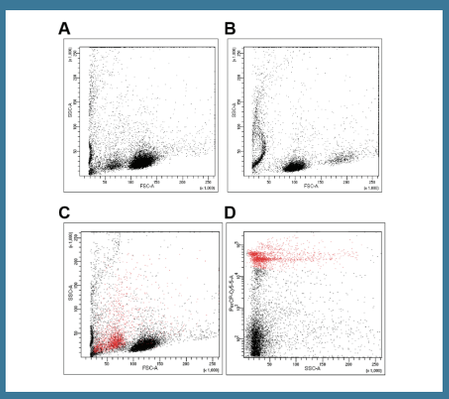

Fig. 3: Morphological features and cell death rate. FSC vs SSC in fresh (A) and frozen (B) lymph nodes after Medimachine II processing. PI positivity in fresh lymph nodes (C, D).

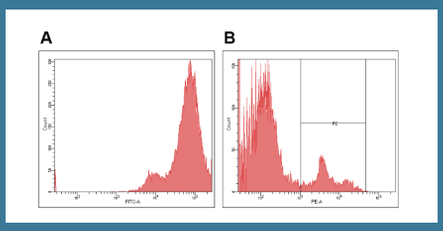

Fig. 4: Histograms for Lysotracker green (A) and Mitosox (B) in fresh lymph nodes.

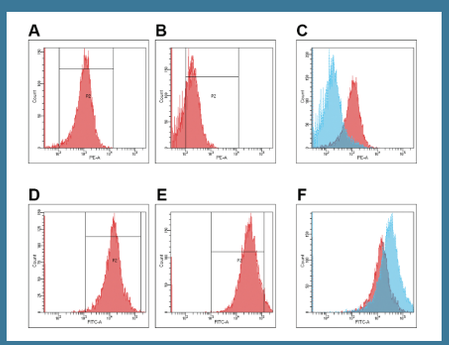

Fig. 5: (A, B, C) TMRE histograms for uninfected (A) and retrovirus- infected (B) lymph nodes. Overlay of (A) and (B) is shown in (C). (D, E, F) DCFDA histograms for uninfected (D) and retrovirus- infected (E) lymph nodes. Overlay of (D) and (E) is shown in (F).

Introduction

Tissue dissociation and its problems were described and defined over 80 year ago. More recent reviews have revealed newer methods for creating single-cell suspensions. Here we tested a mechanical tissue disaggregation method for the subsequent confocal and flow cytometric evaluation of cells from mouse tissues. In particular, the aim of the present study was to determine the feasibility to employ frozen lymph nodes, as well as the fresh ones: an issue of great practical importance for the huge number of samples to be tested that sometimes a postponed analysis at a later time is required.

Material and methods

Lymph nodes were collected from mice (uninfected and retrovirus-infected), then immediately processed (fresh uninfected) or frozen and maintained at 80°C. Furthermore, each lymph node was processed using Medimachine II (a mechanical disaggregation system working independently to operator's ability) (Fig. 1), adding 1ml PBS into the Medicons chamber's (Fig. 2). Protocol is 30s of working, whereas the cell suspension obtained was filtered using a 30µm
FILCONS filter, labelled with different cellular markers (TMRE, DCFDA, LYSOTRACKER) and different mAbs and analysed by flow cytometry.

Results

Both fresh and frozen samples showed similar physical characteristics (Fig. 3), although microscopy focuses a higher degree of aggregation in frozen tissues. Furthermore, all functional probes demonstrated to adequately work: in fact, not only fluorescence is well- detectable in both types of tissues (Fig. 4, 5), but also relevant variations are visible in the uninfected and retrovirus-infected samples. Finally, the good mAb staining obtained revealed that frozen tissue did not possess a higher background.

Conclusion

Standardization in tissue disaggregation (STD) is an important goal to be achieved for all researchers working with the different tissue typologies. Our preliminary results show that STD minimizes differences in cells by fresh and frozen tissue and focuses relevant variations on both surface marker detection and functional parameters.

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