# Dynamic 3D culture promotes lymphoid tissue maturation and allows the study of Chronic Lymphocyitc Leukemia cells dissemination *in vitro*

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Chronic Lymphocytic Leukemia (CLL) is a dynamic disease characterized by the accumulation of mature B cells in peripheral blood and lymphoid tissues. Circulating leukemic cells are resting and tend to home within lymphoid tissues where they acquire an activated phenotype and start to proliferate. Our aim is to establish an *in vitro* macroscale model of lymphoid tissues, in which recirculate CLL cells and study their behaviour in an *in vivo*-like environment.

We used and characterized a collagen-based scaffold on which we seeded human bone marrow stromal cells or lymph node fibroblasts with endothelial cells. The scaffolds were maintained in a millifluidic system (IVTech; Massarosa, Italy) and the dynamic settings were defined based on *in silico* computational studies. A leukemic cell line was used for circulation experiments. We analysed tissue viability and maturation by comparing static and dynamic cultures and evaluated leukemic cells immunophenotype at different timepoints.

Through the analysis of viability and specific functional markers (*e.g.* Collagen IV, CD31), we observed that the dynamic condition promotes a viable and compact tissue-like architecture, stimulating the organization of endothelial structures, thus reducing the risk of necrotic core. We then recirculate CLL cells in the matured lymph node and bone marrow tissues, comparing pre- and post-circulation conditions. Neoplastic cells efficiently home in both compartments, and preliminary data show regulation in the expression of functional markers (*e.g.* CXCR4, CD49d), resembling the *in vivo* situation.

We here demonstrated the feasibility and advantages of using a 3D dynamic culture to obtain viable, organized, and vascularized 3D lymphoid tissues to study leukemia cells dissemination *in vitro*. Moreover, this model opens the possibility to increase its complexity by adding other relevant cell types and to interconnect the different tissues to obtain a multiorgan system for CLL and other haematological malignancies.

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