THROMBOCYTOPENIAS

A SILK-BASED 3D BONE MARROW MODEL FOR CHEMOTHERAPY-INDUCED THROMBOCYTOPENIA

C. A. Di Buduo, M. Migliavacca, A. Malara, A. Balduini

Department of Molecular Medicine, University of Pavia, Italy

Introduction. Accurately modeling the human bone marrow (BM) microenvironment in vitro remains a major challenge, limiting investigation of chemotherapy-induced bone marrow suppression, the primary dose-limiting toxicity of many anticancer therapies. Conventional 2D cultures fail to sustain primary human hematopoietic stem and progenitor cell (HSPC) function, restricting the study of hemotoxicity and mechanisms of marrow recovery.

Aim. Here, we developed and validated a silk-based bioink to generate 3D bioprinted bone marrow constructs that recapitulate key cellular, biochemical, and mechanical features of the hematopoietic niche.

Materials and Methods. Primary human $CD34^+$ cells were encapsulated within mechanically stable, customizable 3D matrices that support long-term hematopoiesis, with a particular focus on megakaryocyte differentiation and platelet production. The chemotherapeutic agent 5-fluorouracil (5--FU) was administered transiently at defined stages of differentiation, either alone or in combination with the thrombopoietin receptor agonists eltrombopag or romiplostim.

Results. Stage-timed 5-FU exposure revealed pronounced differentiation-dependent chemosensitivity along the megakaryocytic lineage. Treatment at the early stem/progenitor stage largely preserved subsequent megakaryocyte viability, polyploidization, and proplatelet formation. In contrast, exposure during intermediate differentiation resulted in a dose-dependent depletion of high-ploidy megakaryocytes, reduced viability, and severe impairment of proplatelet formation, whereas late-stage exposure produced comparatively modest effects. These findings identify a window of heightened vulnerability following lineage commitment but before terminal maturation. Both eltrombopag and romiplostim partially rescued 5-FU–induced defects, restoring megakaryocyte maturation, proplatelet network complexity, and platelet output toward control levels. Integrated proteomic and single-cell transcriptomic analyses supported a role for the 3D microenvironment in preserving stemness while coordinating cell-cycle regulation, endomitosis, and DNA damage response pathways.

Conclusions. Overall, this silk-based 3D bone marrow model provides a reproducible and physiologically relevant platform for investigating chemotherapy-induced thrombocytopenia and therapeutic rescue strategies, with potential relevance for translational and patient-tailored applications.