Mimicking the Bone Marrow Niche using Tissue Engineered Extracellular Matrix Scaffolds supports Human Acute Myeloid Leukemia Cell Survival in Extended Ex Vivo Cultures

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Abstract: Stem cells reside in specialized and dynamic microenvironments called stem cell niches. An integral part of a stem cell niche is the extracellular matrix (ECM), which supports cells structurally and provides cells with chemical cues to regulate their behaviour. The composition and organization of ECM plays a crucial role in maintaining tissue homeostasis; thus deviations from its properties may lead to diseased conditions such as acute myeloid leukemia (AML). As recent studies suggest, myeloid malignancies modify the hematopoietic stem cell niche into a leukemic niche, whereby the altered niche can support leukemic proliferation. This evidence highlights the importance of understanding interactions between cells and their ECM niche. Without bone marrow niche support, primary AML cells differentiate and undergo apoptosis during the first 72 hours of *in vitro* cultures. To address this challenge, this research focused on screening different combinations of ECM to define an effective culture assay for primary AML samples to recreate the tumour niche. Patient derived mononuclear cells were seeded on matrix constructs incorporated with ECM components; collagen, elastin and hydroxyapatite in specific ratios and incubated for 72 hours. Colony-forming unit assay was used before and after incubation to determine whether biological functions can be maintained after recovering cells. We found some conditions that can increase the mean number of AML blast colonies. These findings may enable *ex vivo* studies on AML cells in the future and can provide insight into underlying niche mechanisms in human bone marrow in both healthy and diseased states

Background

Acute Myeloid Leukemia (AML) is a hematopoietic malignancy with a heterogeneous phenotype. It is characterized by an unrestrained expansion of undifferentiated myeloid blasts. Self-renewal and stalled differentiation lead to the expulsion of the normal hematopoietic system and the depletion of the healthy hematopoietic stem cell (HSC) pool. Patients usually present with symptoms of bone marrow failure, such as anemia, and initial therapy typically entails ablation of the native bone marrow with chemotherapy and healthy bone marrow transplantation.

Recent discoveries indicate that myeloid malignancies transform the HSC niche into a leukemic niche, where the alterations promote proliferation of the malignant cells while impairing the self-renewal and differentiation capacities of HSC's ability to function normally. Due to these complex interactions of the cancer cells with its microenvironment, research is now shifting towards understanding the underlying mechanisms of the niche, which will not only allow us to develop more effective culture systems in order to study these cells, but will also provide us with new potential therapeutic approaches. A model system employed by our laboratory involves seeding the cells on matrix constructs made with natural ECM components. The bone environment predominantly consists of collagen type I, as well as non-collagenous protein. A collagen scaffold with natural non-collagenous proteins incorporated inside would provide an ideal system to mimic the natural environment of the cells and support the cells. Using a functional scaffold system, we can analyze and identify the components that can contribute to cell niches which are important for cell survival, proliferation, and differentiation. This approach allows us to study the way the cells interact with their environment and gain a broader understanding of the system.

Methodology

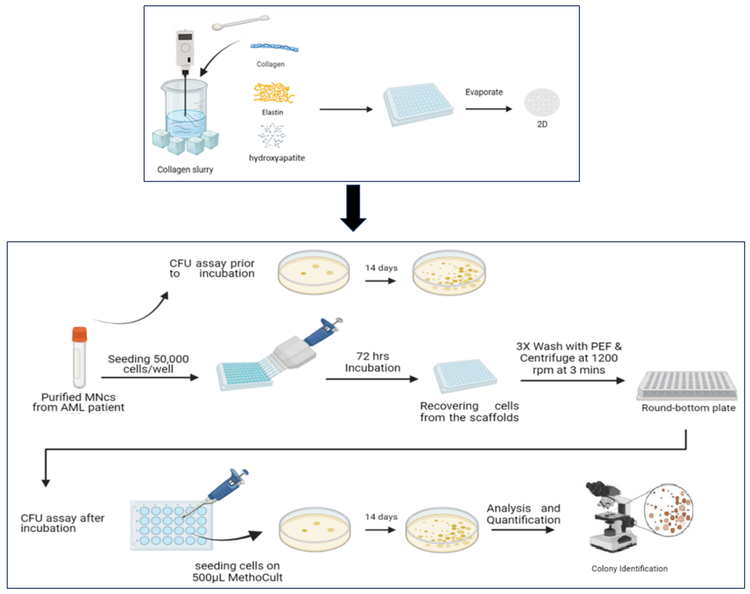

Figure 1: 2D Scaffold Fabrication Process and Workflow of scaffold Efficacy Assay. A homogenized slurry made from collagen type I and different ratios of ECM proteins were made and air-dried to make 2D combinatory scaffolds. PMNCs derived from AML patients were seeded on combinatory scaffolds and CFU Assay was used to observe colony growth to quantify stem cell and progenitor potential.

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Results

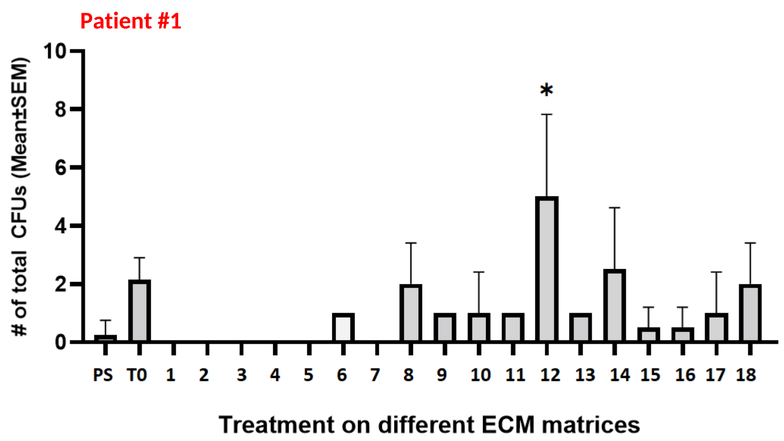

Figure 2: Number of Total CFUs Counted when Treating 3 Different AML patient PMNCs on different ECM matrices. Purified mononuclear cells (MNCs) derived from the peripheral blood collected from AML patients were seeded on combinatory scaffolds. After 72 hours of incubation, cells recovered from each scaffold were seeded on MethoCult™ H4434 for CFU assay. After 14 days of incubation, number of CFUs were counted under the microscope. Results indicate that some conditions can increase the mean number of AML blast colonies when compared to T0. (n=2.)

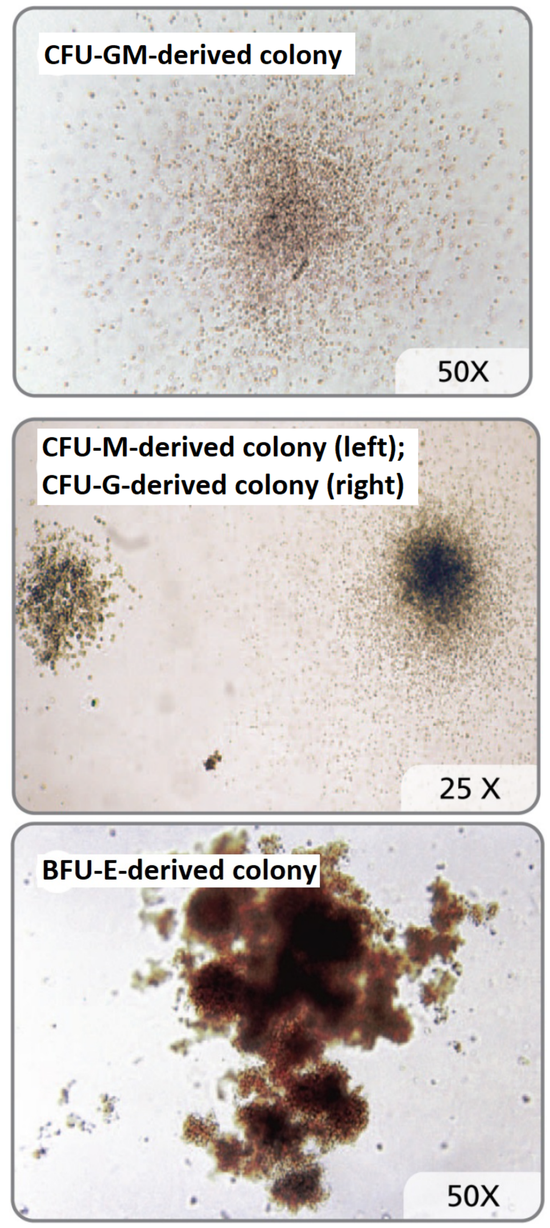

Figure 3: Photographs of Colonies in MethoCult™ Media. After culture in MethoCult™ for 14 days, colonies derived from BFU-E, CFU-GM, and CFU-G can be distinguished on the basis of their size and morphology.

Discussion

Scaffold efficacy is going to be determined using CFU assay as a functional test for HPSCs and leukemic stem and progenitor cells. In the next phase of the study, cells will be challenged again and volumetric counting will be done. This will allow us to analyze the phenotype of recovered leukemic cells to determine what ECM conditions are conducive to the proliferation of leukemic cells.

Ex vivo primary AML cultures provide useful data for studying AML cells *in vitro* and provide new directions for drug development, biomarker discovery, identification of drug response, and combination treatments.