

## **Tense your Megas! Structural rigidity is key**

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In this issue of *Blood*, Aguilar *et al* demonstrate that megakaryocytes adapt to the ‘stiffness’ of their microenvironment and that recreating the physical constraints encountered in native bone marrow dramatically improves *in vitro* megakaryocyte differentiation and proplatelet formation<sup>1</sup>.

Megakaryocytes are one of the rarest cells in bone marrow and yet collectively generate  $\sim 10^{11}$  platelets each day<sup>2</sup>. One platelet transfusion unit contains  $\sim 3 - 4 \times 10^{11}$  platelets, and significant advances have been made in the large-scale production of megakaryocytes and platelets from human pluripotent stem cells<sup>3</sup>. Despite the incredible physiological capacity for platelet production, *ex vivo* platelet biogenesis from megakaryocytes differentiated in standard liquid culture systems from hematopoietic progenitors remains inefficient. One explanation for this is that key environmental components and/or mechanical forces encountered in native bone marrow are lacking in standard “2D” liquid culture systems.

In addition to secreted factors, metabolic components and extracellular matrix factors, bone marrow provides a mechanical environment that is shaped by local hydrostatic pressure, shear stress and viscosity (Figure 1)<sup>4</sup>. The structural scaffolds provided by specific bone marrow ‘niches’<sup>5</sup> regulate the differentiation of all blood cell lineages and are especially important for megakaryopoiesis and thrombopoiesis. Developing megakaryocytes migrate from the bone lining to be close to specialized marrow sinusoids, where they reach “proplatelet” extensions through the vessel wall and release platelets into the circulation in response to hydrodynamic shear<sup>6,7</sup>. As megakaryocytes mature *in vivo*, they massively increase their cell size and nuclear content by endomitosis and polyploidization<sup>2</sup>. A distinct network of cytoplasmic intracellular membranes develops—the demarcation membrane system (DMS)—from which cytoplasmic territories and subsequently proplatelet extensions and platelet buds are eventually formed. Low ploidy megakaryocytes (2 – 4N) generate fewer platelets than do higher ploidy megakaryocytes (32 – 64N). Megakaryocytes cultured *in vitro* both fail to achieve

the high ploidy levels observed *in vivo* and also do not efficiently generate platelets, suggesting that the mechanical forces of the environment in which megakaryocytes differentiate are important for megakaryocyte maturation.

Previous attempts to design 3D *in vitro* systems mimicking the native environment in which megakaryocytes mature and produce platelets have incorporated artificial scaffolds and shear forces in addition to cytokines, matrix components and/or endothelial cells<sup>8,9</sup>. For example, Balduini's group developed a 3D bioreactor using silk-based vascular tubes<sup>9</sup>. Italiano *et al* designed a microfluidic bioreactor incorporating shear forces, matrix interactions and simulating bone marrow stiffness that also enabled high-resolution live-cell microscopy<sup>8</sup>. In the current study, Aguilar *et al* compared murine megakaryocytes differentiated *in vitro* in liquid medium and in MC hydrogels with megakaryocytes harvested directly from mouse bone marrow<sup>1</sup>. They tested hydrogels consisting of 2% and 2.5% MC, as these concentrations had a mechanical stiffness similar to that estimated for bone marrow (15 – 300 Pa), although the stiffness of 2.5% MC was 10-fold higher than 2% MC (300 – 600 Pa *vs.* 30 – 60 Pa respectively).

Electron microscopy showed that while liquid-cultured megakaryocytes had an abnormally developed DMS with poor demarcation of cytoplasmic territories, those grown in 2% MC developed a DMS that closely resembled that seen in megakaryocytes isolated from bone marrow. Four-fold fewer megakaryocytes grew in 2.5% than 2% MC gels, and 2.5% MC gel-derived megakaryocytes were smaller with a poorly developed DMS, indicating that megakaryocytes are highly sensitive to the specific viscoelastic properties of their microenvironment. Cells cultured in 2% MC also achieved higher ploidy, and importantly, the percentage of cells extending proplatelets was almost doubled in 2% MC as compared to liquid cultures.

Remarkably, the DMS rapidly disordered and cytoplasmic territories were lost within two hours when megakaryocytes were removed from MC hydrogels and resuspended in liquid culture. Two interesting hypotheses are raised by these observations. Firstly, differences in the mechanical stiffness between regions of bone marrow (e.g. perivascular *vs.* endosteal niches) may create areas that are more or less conducive to platelet production *in vivo*. Secondly, the rapid changes observed when megakaryocytes were switched from liquid culture to 2% MC hydrogels may occur physiologically, with dynamic remodelling

of the DMS occurring as megakaryocytes experience fluctuations in extracellular tension and mechanical forces.

What is the relevance of these findings for patients with platelet disorders? A group of inherited thrombocytopenias are caused by mutations in the gene encoding myosin heavy chain 9 (MYH9), a cytoskeletal contractile protein, leading to defects in platelet production as well as characteristic inclusion bodies in leukocytes, renal and hearing abnormalities. Paradoxically, while *Myh9*<sup>-/-</sup> megakaryocytes show impaired proplatelet formation within bone marrow, *in vitro* cultured MYH9-deficient megakaryocytes show an *increased* capacity for proplatelet formation<sup>10</sup>, suggesting that the defect caused by myosin deficiency is masked by *in vitro* liquid culture conditions. Aguilar *et al* found that myosin expression was absent from the cytoplasm in liquid-cultured megakaryocytes and *Myh9*<sup>-/-</sup> murine megakaryocytes did not increase proplatelet formation in response to 2% MC gels as did wild-type megakaryocytes<sup>1</sup>, indicating that myosin is required for megakaryocytes to adapt to the stiffness of their microenvironment. Further, nuclear accumulation of the transcription factor MKL1 occurred in response to culture in 2% MC, and treatment with a MKL1 inhibitor completely eliminated the increased proplatelet formation observed in the MC gels<sup>1</sup>, suggesting a role for the MKL1 pathway.

Bone marrow is a unique and dynamic mechanical environment – a viscous organ confined by solid bone. Mechanical forces exerted on differentiating hematopoietic cells are likely to be dynamic, changing in response to blood flow rates and volume and therefore factors such as physical activity and temperature (Fig. 1). This study emphasizes the importance of considering the viscoelastic properties of any *in vitro* culture system. This may be especially relevant for the study of megakaryocyte/platelet disorders due to abnormal cytoskeletal proteins. Further, it highlights interesting questions regarding how physiological fluctuations, as well as pathologies such as myelofibrosis, alter physical forces within bone marrow and thereby may influence hematopoiesis.

## **FIGURE LEGEND**

**Figure 1. Elements that influence the structural rigidity of the bone marrow microenvironment.** Model shows a developing megakaryocyte migrating from the endosteal bone lining to a bone marrow sinusoid. Cellular expansion, polyploidization and development of the intracellular demarcation membrane system (DMS) is shown, with shedding of platelets from proplatelet extensions within the vessel lumen. Factors that may influence the mechanical forces (block arrows) within bone marrow include marrow fat content, extracellular matrix components (e.g. collagen, fibronectin, vitronectin) and the hydrostatic pressure of sinusoids, which in turn is determined by blood pressure, flow rate, blood viscosity and temperature. Abbreviations: HSC/HPC – hematopoietic stem cells/progenitor cells; MSC – mesenchymal stem cell.

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