Development of a 3D culture system for an in-vitro hematopoietic stem cell niche

Christian Jose Garcia Abrego,1,2,† Samantha Saunz,2,† Burak Toprakhisar,1,2 Ramesh Subramani,1,† Olivier Deschaume,2 Stijn Jooken,2 Manmohan Bajaj,3 Herman Ramon,4 Catherine Verfaillie,4 Carmen Bartic5 and Jennifer Patterson6,7

1 Department of Materials Engineering, KU Leuven, 2 Department of Photonics and Astronomy, KU Leuven, 3 Others Cell Institute – KU Leuven, 4 Department of Biotechnologies – KU Leuven, 5 Department of Food and Processing Technology and Management, FSGII, Kosmательское College for Women, India, 6 IMDEA Materials Institute, Spain, 7 Contributed equally

Hematopoietic stem/progenitor cells (HSPCs) are responsible for blood cell production throughout life. Biophysical cues affect several stem cell behaviours. Various studies have investigated the effects of bone marrow (BM) niche elasticity on HSPCs behaviour, however, the effects of other niche sites like the fetal liver (FL) were not studied before. In this study, we investigated the mechanical properties (elastic modulus) of murine fetal liver niche via AFM at embryonic day 14.5. Fibrin hydrogels were then designed to mimic as close as possible the FL elasticity. Finally, BM-HSPCs were encapsulated in these fibrin gels to evaluate the effect of elasticity on HSPC viability and expansion.

AFM nanomechanics on fetal liver and hydrogels

We characterised the stiffness of FL tissue from murine embryos on embryonic day 14.5 via AFM. The FL modulus were very low (E = 0.2 kPa). Fibrin hydrogels were then designed in an attempt to mimic the elasticity of FL. The Soft hydrogel (0.6 mg/ml fibrin) was the closest to the FL stiffness (E = 0.8 kPa), followed by the Hard (1.8 mg/ml) with E = 2.72 kPa. Softer fibrin hydrogels were too fragile to be handled for cell culture conditions.

HSPC expansion in fibrin hydrogels

After 10 days of culture, TNCs and progenitors (LSK) cells expanded significantly in all culture conditions relative to day 0. Normoxia supported a greater expansion for TNC than hypoxia. LSKs expanded more in suspension cultures with no difference observed between soft and hard hydrogels for this population. Lastly, soft gels and suspension cultures provided better maintenance of the the hematopoietic stem cells (LSK-SLAM cells) than hard hydrogels.

3D culture of HSPCs in fibrin gels

Soft and Hard hydrogels were used to evaluate the influence of hydrogel elasticity on the expansion/maintenance of HSPC population and compared against suspension cultures (Susp). Lin/ckit+ cells isolated from murine bone marrow were in the 3 above-mentioned conditions under hypoxia (H) and normoxia (N). Cell expansion was monitored for 10 days at the level of total nucleated cells (TNC), Lin/ckit+ cells, multipotent progenitors (LSK cells) and hematopoietic stem cells (LSK-SLAM cells).

Acknowledgements

This work has been published and reproduced with permission from:


References

This research was funded by the KU Leuven Research Fund (grant no. IDO:13/016), the Consejo Nacional de Ciencia y Tecnología (CONACYT-Mexico) reference no. 326227/440074, The Hercules Foundation (grant no. HER/09/021, AKUL/1537_GOH1816N) and FWO (grants no. G.0947.17N, 11C3821N, G.0929.15).

Figure 1. AFM elasticity maps of FL (a), soft (b) and hard (c) hydrogels with their respective representative force-indentation curves (d). Box plots showing the Young’s modulus distribution with the mean presented with the plus sign (e).

Figure 3. Quantification of Total Nucleated Cells (TNCs), Lin/ckit+ cells, multipotent progenitors (LSK cells) and hematopoietic stem cells (LSK-SLAM cells) cultured in soft and hard hydrogels as well as suspension (Susp) culture under hypoxia (H) and normoxia (N). TNC were quantified via electronic cell counter while Lin/ckit+ cells, LSK and LSK-SLAM cells via flow cytometry.

Figure 2. 3D fibrin culture system containing encapsulated HSPC with cells observed via scanning electron microscopy (SEM). Scale bar 2 µm.