Modelling The Mesenchymal Stem Cell Niche In Bone Marrow

Jennifer Bara¹, Ursula Menzel¹, Mauro R. Alini, PhD², Martin Stoddart¹.
¹AO Research Institute Davos, Davos Platz, Switzerland, ²AO Research Insitute Davos, Davos Platz, Switzerland.

Disclosures:

Introduction: Mesenchymal stem cell (MSCs) are subject to significant phenotypic changes following in vitro expansion in monolayer. Current MSC-based therapies for orthopaedics rely on this in vitro expansion phase, which may not only compromise the therapeutic potential of these cells but is a lengthy, costly process. Manipulation of MSCs in the mononuclear cell (MNC) fraction of bone marrow, using growth factors or gene transfer, could negate these cellular and clinical challenges. In order to develop such therapies, we must establish suitable culture systems to support MNCs which would then allow us to investigate how aspects of naive MSC behaviour are regulated and how these cells differ from traditionally monolayer expanded MSCs. Here, we describe the use of an in vitro culture system to model the MSC niche in bone marrow. Additionally, we investigated the susceptibility of MNC subpopulations from freshly isolated cells to viral transduction by two commonly used viral gene vectors, adenovirus and adeno-associated virus. This information is required in order to make informed choices as to which vector and which transgene for later use with freshly isolated MNCs.

Methods: Bone marrow aspirates from the iliac crest or vertebral body were obtained from patients undergoing reconstructive joint surgery or spinal fusion respectively, with full ethical approval, MNCs were isolated from bone marrow using Ficol™ density centrifugation and the relative haematopoietic and non-haematopoietic cell fractions characterised by cell surface marker profiling. Flow cytometry was performed using a BD Aria III and data analysed using BD FACS Diva 6.1.3. MNCs were seeded into fibrin gels and cultured statically for 7 vs. 14 days. Cells were recovered from enzymatic treatment and the relative proportions of haematopoietic vs. MSCs assessed by cell surface marker profile for CD45 and CD73. Cell cycle analysis was assessed by EdU incorporation and DAPI staining. The susceptibility of freshly isolated MNCs to adenoviral (Ad) and adeno-associated viral (AAV) transduction was investigated using Ad-GFP and AAV-RFP.

Results: Freshly isolated MNCs were 58.3+/−3.5% CD45+, 15.5+/−9.4% CD73+, 41.5+/−4% CD45-CD73- with the naive MSC population (CD45-CD73+) representing 0.58+/−0.37%. Similar proportions of haematopoietic cells and MSCs were recovered following 1 week culture in fibrin (60.3+/−8.2% CD45+, 5.8+/−2.3% CD73+, 34.8+/−9.8% CD45-CD73-, 0.45+/−0.13% CD45-CD73+) (Figure 1A). After 2 weeks, cell proportions had altered yielding 39.3+/−11.58% CD45+, 14.38+/−2% CD73+, 49.5+/−7.7% CD45-CD73-, and 7.36+/−4% CD45-CD73+, indicating loss of short-lived haematopoietic cells as part of their natural life cycle and expansion of the MSC population (Figure 1B). Proliferation rate of MNCs in fibrin remained low with 4.1% and 3.8% of the total MNC cell population EdU+ following 1 and 2 weeks of culture respectively. After 2 weeks, 74.6+/−8.3% of the actively proliferating cell population were CD45- and 89.0+/−18.9% of CD45-CD73+ cells were EdU+ indicating proliferation of the MSC fraction. Freshly isolated MNCs demonstrated susceptibility to adenoviral and adeno-associated viral transduction with 2.9% Ad-GFP+ and 4.5% AAV-RFP+ after 12 days static suspension culture. AAV appeared to preferentially transduce CD56+ cells with 82.6% and 92.2% of RFP+ cells also CD56+ from donors where the CD56+ fraction represented 19.6 and 4.3% of the total respective cell populations (Figure 1C). In these donors, 36.2+/−5.3% of CD56+ cells were transduced with AAV. In the same donors, adenoviral transduction appeared more promiscuous with 20.8% and 81.0% of GFP+ cells also CD56+, and overall 16.9+/−4.5% of CD56+ cells were transduced (Figure 1D).
Discussion: Here, we describe the use of a 3D culture system which maintains relative proportions of heterogeneous bone marrow derived MNCs, including the discrete naive MSC population. In the presence of FGF2, the MSC fraction proliferated during extended in vitro culture whilst the surviving haematopoietic fraction remained relatively quiescent. The preferential transduction of CD56+ MNCs by AAV suggests the potential for targeted gene transfer therapies whereby a haematopoietic cell subset could be utilised to modulate MSC behaviour. Future work using this system to apply other candidate stem cell regulatory growth factors will allow us to investigate how MSCs can be activated in their niche environment. This knowledge may support both the development of clinically and economically viable MNC-based therapies for orthopaedic medicine and pharmacological...
based approaches that take advantage of the innate homing ability of MSCs, to direct intrinsic tissue repair.

Significance: In light of the growing interest in using freshly isolated cells for cell-based repair therapies, a greater understanding of their biology is required. Furthermore, protocols originally developed for monolayer expanded MSCs will likely require modification when applied to the entire MNC fraction.

Acknowledgments: This work was funded by the AO Foundation. The fibrin used in these experiments was generously provided by Baxter Biosurgery (Vienna, Austria).

References: None

ORS 2014 Annual Meeting
Poster No: 1474