

Mesenchymal stem cells find their niches in skeletal regenerative medicine

Cosimo De Bari, MD, PhD, MRC Fellow

Division of Applied Medicine, University of Aberdeen, Scotland, UK

Mesenchymal stem cells (MSC) are very attractive for skeletal regeneration because they are easily accessible and expandable, and have ability to form skeletal tissues such as cartilage and bone. We previously reported the isolation and characterization of MSCs from adult human synovium (1-3) and periosteum (4-6).

The use of culture-expanded MSC populations requires assessment of potency for quality control and to ensure efficacy. Indeed, donor-associated factors and MSC preparation protocols influence the potency of MSCs and MSCs isolated from different tissues have distinct differentiation properties. The resulting variability limits standardization of MSC-based tissue repair approaches. There is, therefore, an unmet clinical need for assays that allow quantitative estimation of the potency of MSC preparations. Clinically relevant potency assays and related quality control measures will provide a solid basis for establishing batches of certified stem cell products with specific clinical indications. This will increase consistency and decrease costs of therapies employing MSCs.

In a proof-of-concept study, we developed a mathematical model that predicts osteogenic potency of adult human clonal MSC preparations from synovium and periosteum, independent of donor and tissue source (6). We quantified the bone-forming potency of matched human MSCs from synovium and periosteum and analyzed the sources of variability in osteogenic outcome. We identified the tissue of origin of MSCs as the main source of variability, since MSCs from periosteum had significantly greater osteogenic potency than MSCs from synovium. A second source of variability was related to the individual donor, within each tissue. We measured the basal expression levels of osteoblast-lineage genes in clonal MSCs prior to osteogenic treatment, identified biomarkers that correlated with osteogenic outcome and developed a mathematical model that predicts bone-forming potency of clonal MSC

preparations, independent of donor and tissue source (6).

An approach to joint surface regeneration could rely on the activation of intrinsic repair mechanisms via pharmacological targeting of joint stem cell niches. This would also be ideal in the prevention of secondary osteoarthritis. However, the lack of specific markers has so far impeded the prospective identification of MSCs within their native joint tissues *in vivo*.

We set out a double-nucleoside labelling strategy to identify stem cells within the synovium of knee joints *in vivo* using a novel validated mouse model of joint surface injury (7). We detected slow-cycling long-term retaining IdU+ cells in the synovium of uninjured animals. After joint surface injury, there was marked proliferation of IdU+ cells, which co-stained positive for CldU. IdU+ cells were negative for the pan-haematopoietic marker CD45. In uninjured animals a proportion of IdU+ cells stained positive for MSC markers, increasing after injury. At 12 days after injury, a subset of IdU+ and CldU+ cells stained positive for chondrocyte-lineage markers and were located within newly formed cartilage. Our findings clearly demonstrate for the first time the existence *in vivo*, within the postnatal knee joint synovium, of slow-cycling cells with a phenotype compatible with MSCs, which in response to injury proliferated and differentiated into cartilage. Our work opens new exciting opportunities for pharmacological manipulation of joint stem cell niches for treatment of joint surface defects and prevention of post-traumatic osteoarthritis.

REFERENCES: 1. De Bari et al, *Arthritis Rheum* 2001; 44:1928-42. 2. De Bari et al, *J Cell Biol* 2003; 160:909-18. 3. De Bari et al, *Arthritis Rheum* 2004; 50:142-50. 4. De Bari et al, *Arthritis Rheum* 2001; 44:85-95. 5. De Bari et al, *Arthritis Rheum* 2006; 54:1209-21. 6. De Bari et al, *Arthritis Rheum* 2008;58:240-50. 7. Eltawil et al, *Osteoarthritis Cartilage in press*.

ACKNOWLEDGEMENTS: Cosimo De Bari is supported by the Medical Research Council, UK.