Device Based Enrichment of Knee Joint Synovial Cells To Drive MSC Chondrogenesis without Prior Culture Expansion in vitro - A Step Closer to One Stage Orthopaedic Procedures

Appendix

**Figure A1:** Experimental design and sample distribution for before after use of STEM device; 2ml from the 10 ml concentrated synovial cells were subjected to CFU-F for each petri dish and 2 ml for each chondrogenic pellet. And Experimental design for synovium suspended culture. This figure created with BioRender.com
Figure A2:
Correlation of CFU-F numbers and sGAG production for SF-MSCs and Sm-MSCs

Figure A3: Gene expression of minimally manipulated SF-MSCs and Sm-MSCs chondrogenic pellets. A-B synovial origin MSCs marker. C: Adipogenic marker. D: Osteogenic marker.
Figure A4: Gating strategies of CD90$^{\text{high}}$CD45$^{\text{low}}$ and macrophages subpopulations in non-expanded initial aspiration and mobilized joint aspirates (n=6).
Supplementary Methods

Retrieval of MSCs from irrigation fluid and synovial MSCs mobilization aspirating:

The first sample contained resident SF-MSCs and was collected by the initial saline used to irrigate the joint cavity. The second sample of irrigation fluid collected after agitation of the synovium to mobilize MSCs (Sm-MSCs) as previously described. Briefly, the synovial membrane was brushed using a device to that biophysically augments release of stem cells into the synovial fluid.

The first sample was containing SF-MSCs was procured following injection of normal saline irrigation fluid as previously described whereby approximately 45mLs of fluid on average could be retrieved. The second sample was collected after agitation of the bristled surface of the synovial surface for 1 minute. synovium using the stem cell mobilizing device (STEM device) releasing material containing synovial membrane MSCs (Sm-MSCs) about 43ml on average was retrieved, as previously described.

Colony-forming unit–fibroblast (CFU-F) assay

For the CFU-F assay 2mL of the freshly obtained SF cells (from the concentrate 10 mL total volume) were plated in duplicate 10cm² plastic Petri dishes (1mL each) containing 15mL of StemMACS MSCs expansion media supplemented 100units/mL penicillin and 100 mg/mL streptomycin (all from Gibco). Cells were incubated in a humidified atmosphere at 37°C and 5% CO₂, with a full media change after 48 hours, followed by half media changes three times a week. Colonies were stained with 1 % methylene blue after two weeks of culture for counting using ImageJ version 2.0.

1 Baboolal TG, Khalil-Khan A, Theodorides AA, et al. A novel arthroscopic technique for intraoperative mobilization of synovial mesenchymal stem cells. Am J Sports Med. 2018;46(14):3532-3540.