A xeno-free, serum-free defined medium to rapidly differentiate human mesenchymal stem cells to osteoblasts

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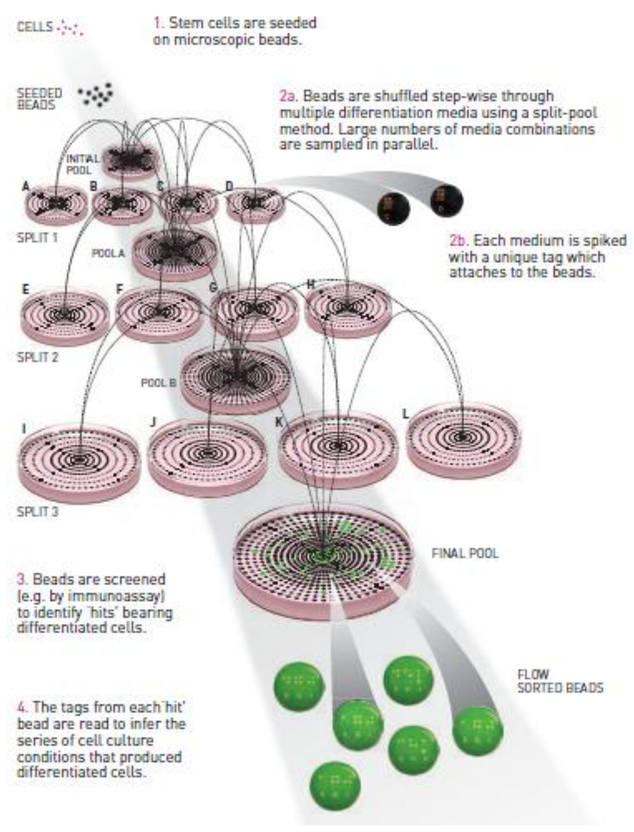
Abstract

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that have been traditionally isolated from bone marrow but have now been derived from adipose and placental tissue, and umbilical cord blood and can be generated in vitro from embryonic and induced pluripotent stem (iPS) cells. They harbor the capacity to differentiate into bone, cartilage and fat cells and are the subject of more than 120 clinical trials for a range of regenerative and inflammatory diseases. The considerable therapeutic potential of MSCs is hampered by current difficulties in directing the differentiation to a single cell-type specific lineage at sufficient high purity and functionality. Current protocols for osteogenic differentiation employ animal-derived serum, a source of lot-to-lot variations and a cause of concern for researchers contemplating a transition to clinical applications. These differentiation protocols require a long time frame, typically 2-3 weeks before the appearance of mineralization and the degree of differentiation is generally dependent upon the tissue source and intra-clonal variations of the stem cell lines derived.

Here we describe development of a defined xeno-free, serum-free osteogenic differentiation medium that can efficiently generate mineralized cultures in under 7 days and works consistently across all sources of MSCs tested, including bone marrow and adipose tissues and those derived from human embryonic stem cells (hESCs). The xeno-free formulation was discovered using a high-throughput combinatorial platform, termed the Combicult® system, which combines miniaturization of cell culture on micro-carriers, a pooling/splitting protocol, and a unique tagging system to allow multiplexing of thousands of experiments in one screen. This approach allowed testing of 3,375 unique differentiation protocols to identify a xeno-free, serum-free medium that promotes the selective differentiation of human MSCs to osteoblasts. A large percentage of mature osteocytes can be generated from all sources of MSC tested in 7-10 days as opposed to 21 days using standard culture methods. To assess stability, the medium was incubated at 37ºC for seven days and underwent two freeze-thaws. Results from the accelerated stability study indicated no loss of activity as compared to the unstressed control and was confirmed with data obtained from a one year real-time stability study. The stability of the raw material components at 37ºC, a temperature frequently used to culture MSCs and a long expiration dating of greater than two years at -20ºC is expected to help reduce the costs of scale-up manufacture. A reliable, cost effective, and rapid method to produce large amounts of human osteoblasts is an important advance towards wide-spread utilization of stem cells for cell therapy and drug discovery applications.

Materials and Methods



Results

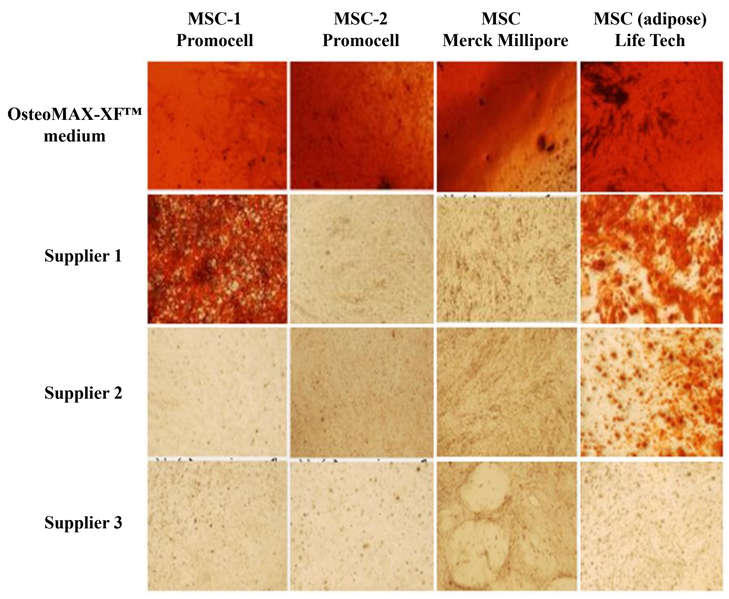


Figure 1. Validation and refinement of serum-free differentiation protocols determined using the CombiCult® system resulted in identification of protocols that drive MSC differentiation to mineralizing osteocytes. The novel protocols are more effective than commercially available kits and give consistent results across multiple cell lines. Alizarin red staining of MSC cultures differentiated for 28 days; cell lines 1-3; bone marrow-derived MSC cell line 4: adipose-derived MSC.

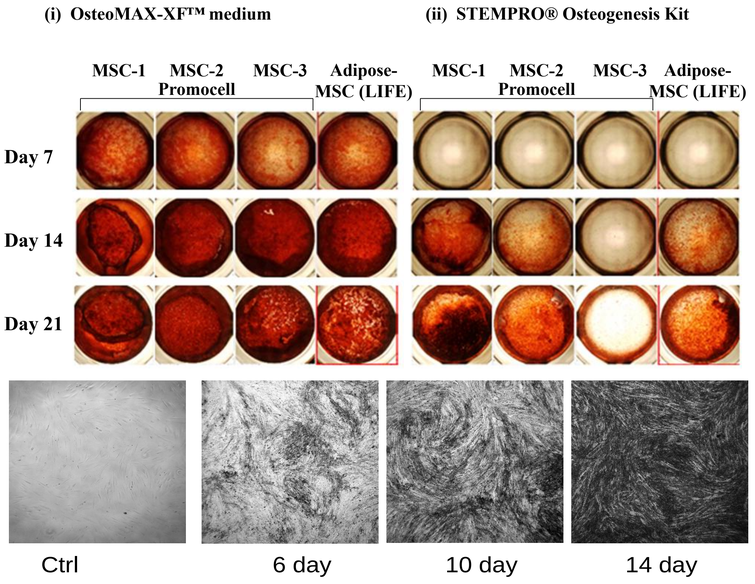


Figure 2. Differentiation of multiple MSC cell lines using (i) OsteoMAX-XF™ Differentiation Medium (Cat. No. SCM121); (ii) STEMPRO® Osteogenesis Differentiation Kit (Cat. No. A10072-01). Differentiation was induced over 21 days in 48-well plate cultures of 4 different human MSC cell lines (Promocell and Life Technologies). Alizarin red staining of representative wells at day 7, 14, and 21 are shown. (b) Mineralization kinetics of human bone marrow-derived MSC (Merck Millipore Cat. No., SCC034) differentiated in OsteoMAX-XF™ medium.

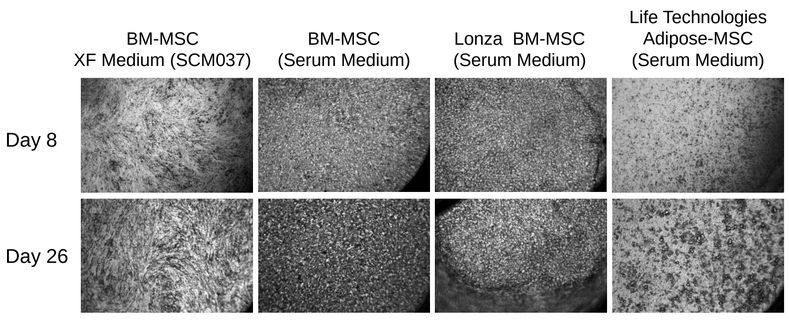


Figure 3. Rapid mineralization of multiple MSC lines in OsteoMAX-XF™ Differentiation Medium. Cell lines were expanded in serum-based medium or XF culture medium (Merck Millipore Cat. No. SCM037) before being exposed to OsteoMAX-XF™ medium.
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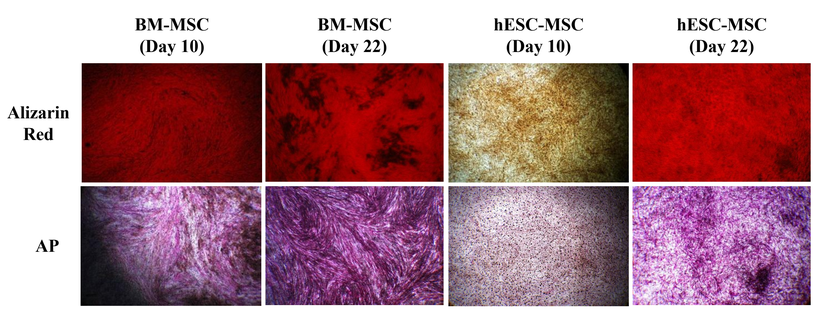

Figure 4. Differentiation kinetics of human BM-derived MSC (Cat. No. SCC034) and human ESC-derived MSC (Cat. No. SCC036) in OsteoMAX-XF™medium. Human ESC-derived MSC exhibited slower differentiation kinetics as compared to BM-derived MSC. However by day 22-24, maximal differentiation is observed in both cell types.

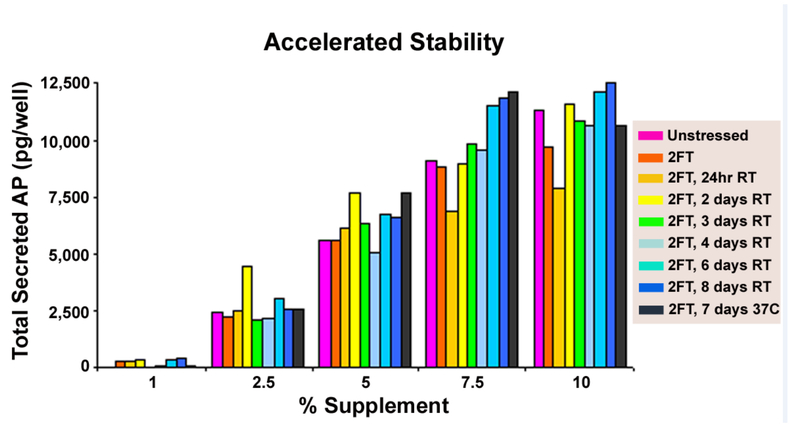

Figure 5. Quantitative alkaline phosphatase (AP) determination of OsteoMAX-XF™ medium stability subjected to stressed conditions ranging from 24 hours at room temperature to 7 days at 37ºC. Cells were exposed to varying concentrations of the stressed supplements for 7 days before the conditioned medium was collected for quantitative determination of AP activity. Merck Millipore's Quantitative Alkaline Phosphatase ES Characterization Kit (Cat. No. SCR066) was used. Duplicate reactions were performed for each condition. Stressed conditions were:
#1: One freeze thaw (control)
#2: Two freeze thaws (FT)
#3: Two freeze thaws, 24 hours at RT
#4: Two freeze thaws, 2 days at RT
#5: Two freeze thaws, 3 days at RT
#6: Two freeze thaws; 4 days at RT
#7: Two freeze thaws, 6 days at RT
#8: Two freeze thaws, 8 days at RT
#9: Two freeze thaws, 7 days at 37ºC

Figure 6. Scaled up differentiation: Human MSCs were differentiated using OsteoMAX-XF™medium in cell factories and stained with Alizarin Red at day 14. Differentiation in a standard T25 flask is shown for comparison.

Conclusions

- Xeno-free, serum-free defined medium: Suitable for drug discovery and translational research.
- Rapid, efficient differentiation: Rapidly generates mineralizing osteoblasts, as early as day 7.
- Robustly works across multiple MSC lines: Able to rapidly differentiate different types of MSCs (bone marrow, adipose, and hESC-derived) from multiple vendors (Merck Millipore, Lonza, Promocell, LIFE).
- Highly stable raw material components: Suitable for scale-up applications as components have long expiration dates to help reduce manufacturing costs.

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