APPLICATION NOTE

Dynamic Bladeless Bioreactor Culture Enables Scalable Production Of Osteogenic MSC Spheroids With Preserved Biological Performance

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1. INTRODUCTION

Currently, most laboratories culture MSCs on microcarriers because they are naturally adherent cells. However, with the development of new systems and protocols, some advanced laboratories have found ways to grow MSCs as aggregates.

Three-dimensional (3D) spheroid culture of mesenchymal stromal cells (MSCs) enhances cell–cell interactions, extracellular matrix (ECM) deposition, and differentiation potential compared to conventional 2D culture. For bone tissue engineering and organoid development, reproducible generation of compact, viable, and osteogenically competent spheroids is essential.

Dynamic culture systems improve nutrient exchange and scalability, but mechanical constraints must not compromise morphology, viability, or differentiation potential.
This study compares static Ultra-Low Attachment (ULA) culture with the SoftXS low shear dynamic system for the generation of osteogenic MSC spheroids.

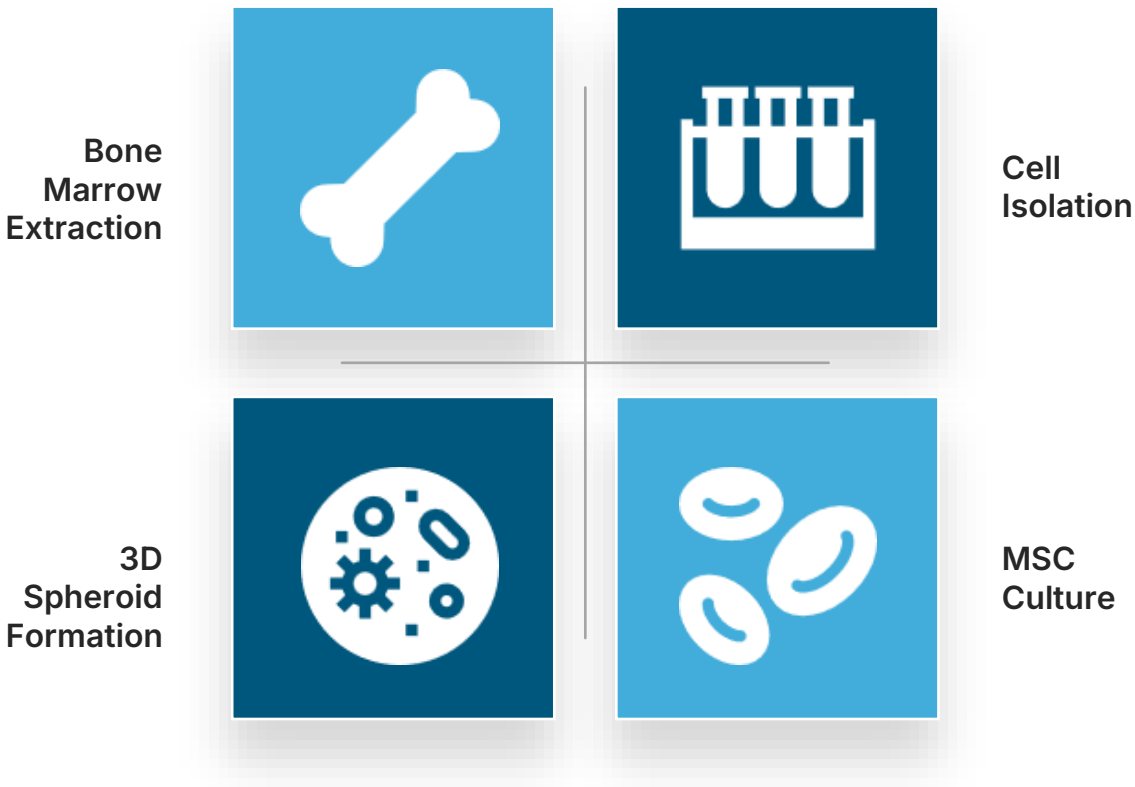
The SoftXS platform equipped with 50 mL, 125 mL, and 500 mL vessels, illustrating its modular design for scalable and low-shear cell culture applications.

2. MATERIAL & METHOD

Bone marrow was obtained from donations of human femoral heads (patients aged 61–84 years) after orthopedic surgery according to the guidelines of the ethical committee of Toulouse university under the N° AC-2014-2384. Human mesenchymal stromal cells (MSCs) were obtained after culture in expansion medium of mononuclear cells, separated by gradient centrifugation as described in our previous work (Bouacida et al, PlosOne. 2012). Then, 50,000 cells of MSCs per well were used to generate 3D spheroids under non-adherent conditions in Ultra Low Attachments (ULA) 96-wells plates with expansion medium (reaching 200µl of media at the end of the culture). During the culture (7 days (D0–D7) under standard culture conditions (37°C, 5% CO_2), the ULA plate was under gentle orbital agitation (75rpm). Spheroids were allowed to self-assemble and contract until reaching a size around 300µm.

To compare SoftXS automated technology with the 3D culture protocol with manual ULA plates, individual spheroids from a same donor were transferred at D1 by reversing the ULA plates in a Petri dish. Then, spheroids were gently transferred in the SoftXS dynamic culture system with a pipetboy.

In both systems (ULA or SoftXS), spheroids were maintained for 6 days (D1–D7) under standard culture conditions (37°C, 5% CO_2).



3.1. MORPHOLOGY AND VIABILITY

MSC spheroids cultured under 3D ULA plates and dynamic 3D SoftXS conditions displayed comparable morphology, structural integrity, and viability over 7 days. Brightfield imaging at D7 revealed compact, and well-defined spheroids in both systems, with no differences in overall size or architecture. Quantitative analysis confirmed similar circularity between conditions. Contractility measurements further demonstrated overlapping contraction kinetics and comparable percentage of area reduction for both fresh and thawed MSC-derived spheroids. Finally, viability staining showed similar cell distribution and no increase in necrotic core formation in SoftXS.

Overall, dynamic culture in SoftXS preserves spheroid morphology, compaction behavior, and cell survival at levels equivalent to conventional static ULA culture.

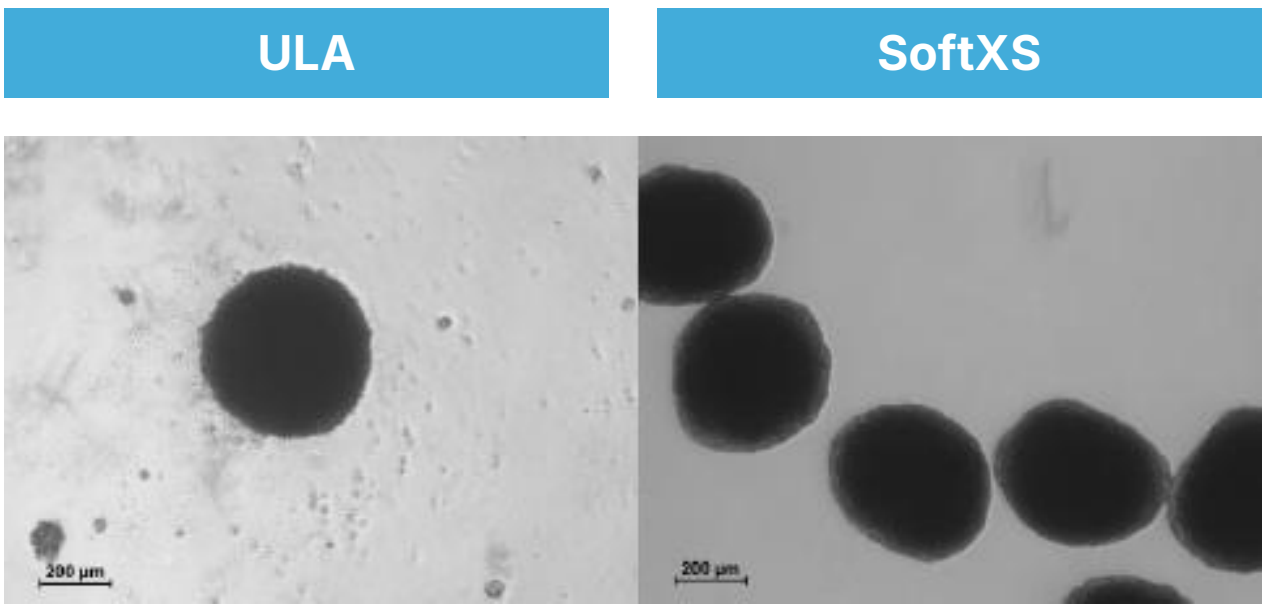


Figure 1 – Growth and morphology of MSC spheroids up to Day 6 (D6) in ULA versus SoftXS culture.

MSC-derived spheroids were cultured for 6 days either in Ultra-Low Attachment (ULA) plates or in the SoftXS dynamic system. Brightfield images were acquired after gentle tilting and aspiration using a pipet controller (pipetboy) to collect spheroids for observation. Scale bar: 200 µm. (N = 4 runs; n = 20 analyzed samples)

3.2. OSTEOGENIC COMMITMENT AND MATRIX MATURATION

The combined analyses of extracellular matrix organization, protein expression, and gene expression demonstrate a clear commitment of MSC spheroids toward osteogenic maturation in both ULA and SoftXS culture systems.

Second Harmonic Generation (SHG) imaging revealed the presence of organized fibrillar collagen within the spheroids, indicating active extracellular matrix deposition and structural tissue organization.

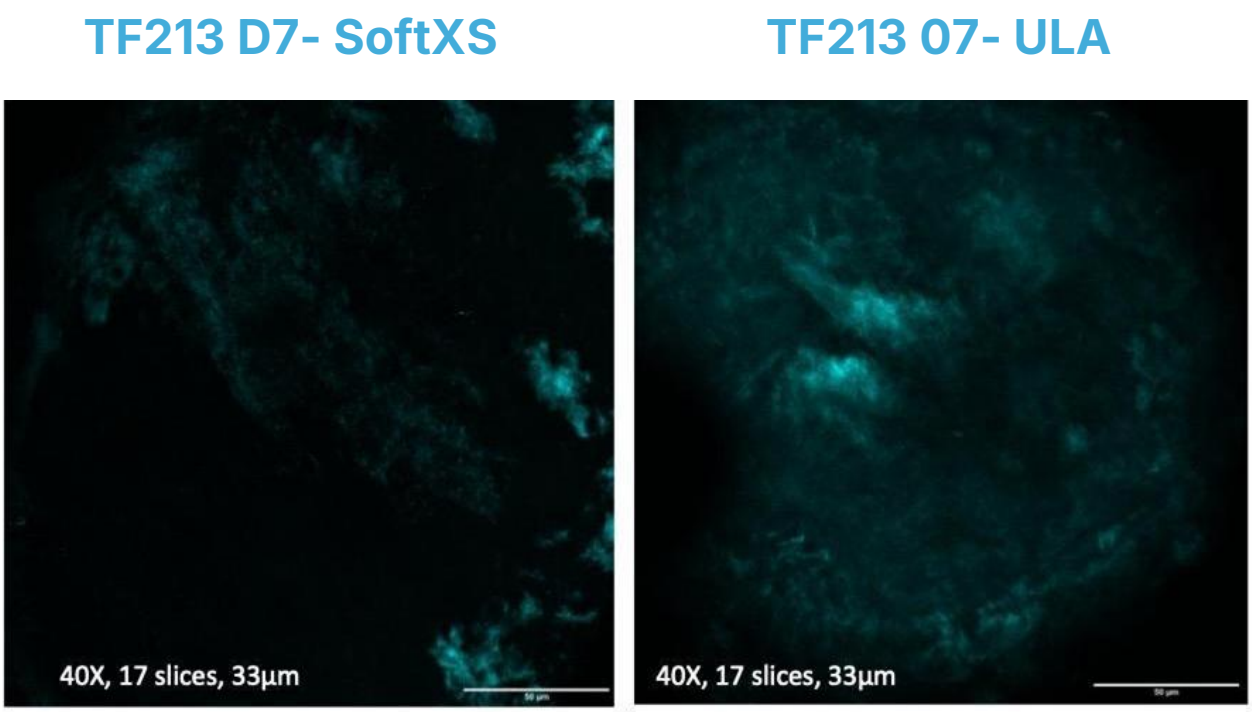


Figure 2 – Second Harmonic Generation (SHG) imaging of MSC spheroids at Day 7 (D7) cultured in SoftXS or ULA.

MSC-derived spheroids were cultured until Day 7 either in the SoftXS dynamic system or in Ultra-Low Attachment (ULA) plates. Extracellular matrix organization was visualized using multiphoton microscopy via Second Harmonic Generation (SHG), enabling label-free detection of fibrillar collagen fibers. Images were acquired at 40× magnification (17 optical sections, total depth: 33 µm). Scale bar: 50 µm.

Immunofluorescence confirmed expression of osteogenic markers (RUNX2, Osteocalcin), supporting progression along the osteoblastic pathway. In addition, endothelial-associated markers (CD31) showed comparable spatial distribution in both systems, indicating preserved cellular self-organization.

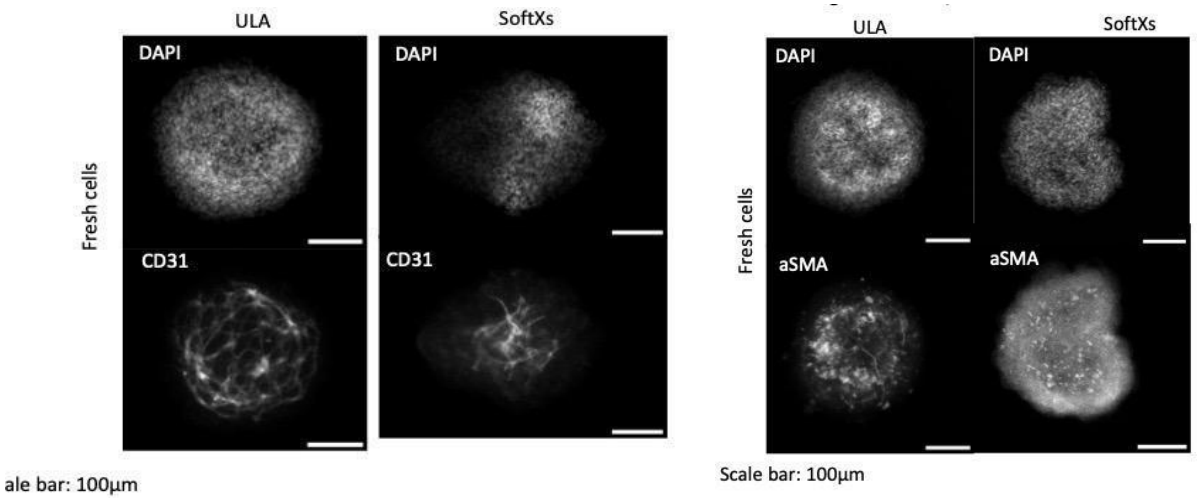


Figure 3 – Self-organization and expression of endothelial markers CD31 at D6 in MSC spheroids cultured in ULA plates or the SoftXS bioreactor.

MSC-derived spheroids generated from fresh cells were cultured either in ULA plates or in the SoftXS dynamic system. Spheroids were fixed and analyzed by immunofluorescence staining with DAPI (nuclear staining, upper panels) and antibodies against CD31 at D6 (endothelial-associated markers, lower panels).

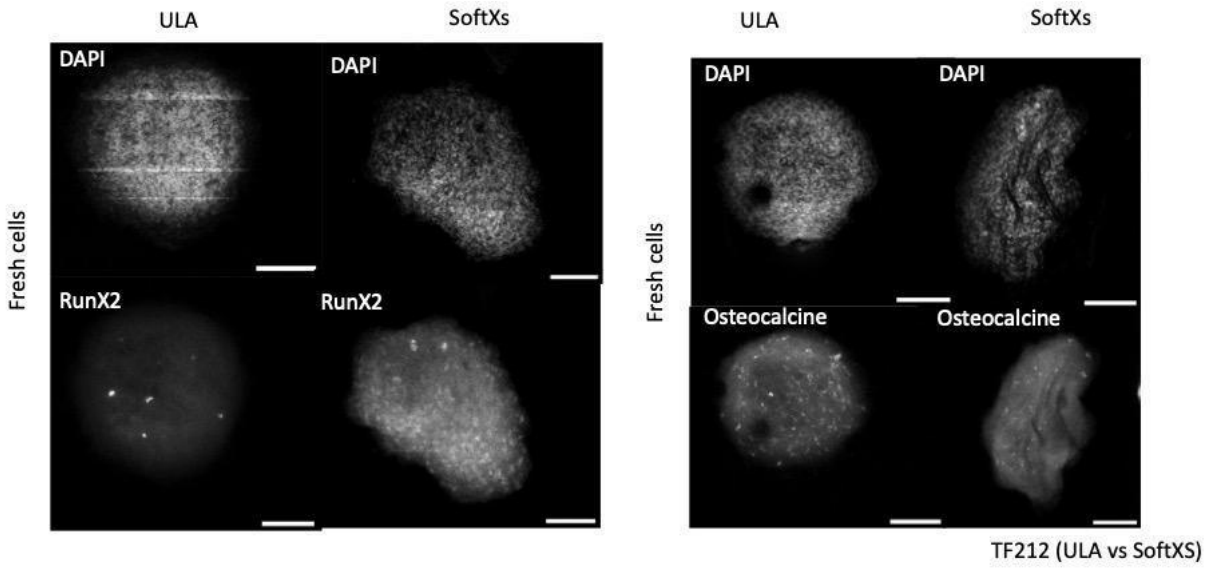


Figure 4 – Expression of osteogenic markers RUNX2 and Osteocalcin in MSC spheroids cultured in ULA plates or the SoftXS bioreactor.

MSC-derived spheroids generated from fresh cells were cultured either in Ultra-Low Attachment (ULA) plates or in the SoftXS dynamic system. Following fixation, spheroids were analyzed by immunofluorescence staining with DAPI (nuclear staining, upper panels) and antibodies against RUNX2 (early osteogenic differentiation marker, lower left panels) or Osteocalcin (late osteoblastic maturation marker, lower right panels).

qRT-PCR data further demonstrated the upregulation of key osteogenic transcription factors (RUNX2, DLX5) and the late matrix-associated marker IBSP over time. Expression levels were comparable between static and dynamic culture conditions.

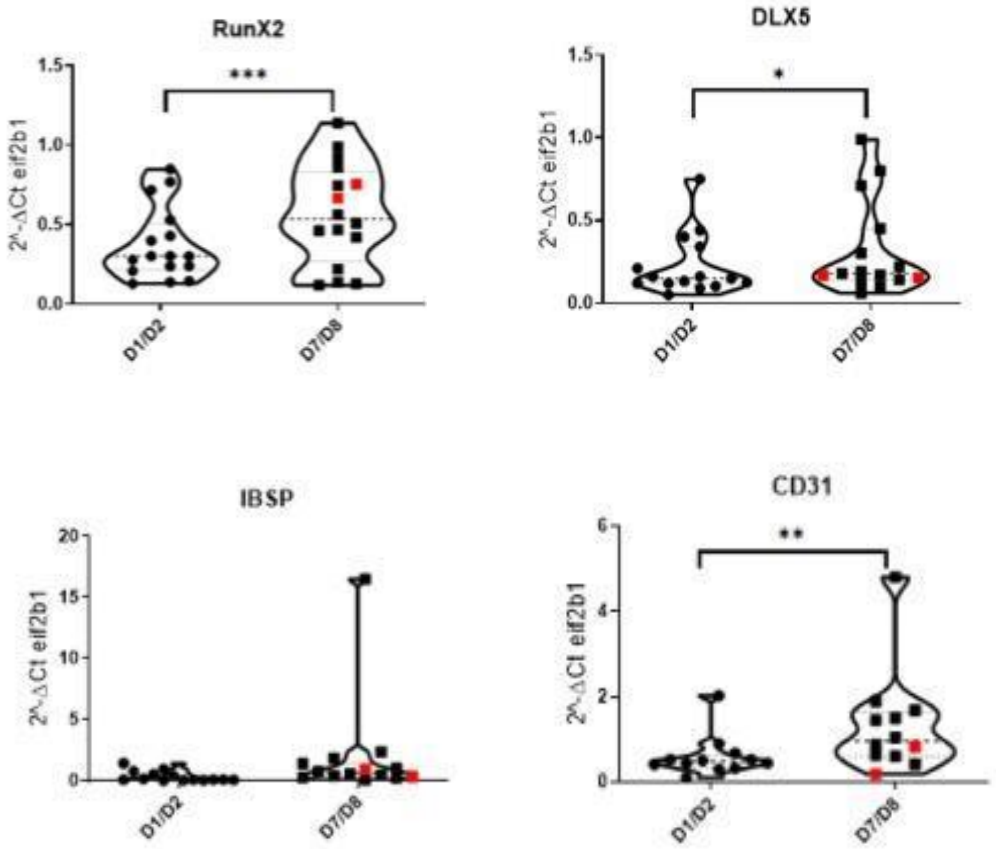


Figure 5 – qRT-PCR analysis of early osteogenic markers (RUNX2, DLX5), the late osteogenic marker (IBSP), and the endothelial marker CD31 in MSC spheroids cultured in ULA or SOFTXS.

*Gene expression levels are presented here and normalized to the reference gen. Violin plots represent data distribution. Red data points correspond to measurements obtained from spheroids cultured in the SoftXS system, whereas black data points represent spheroids cultured in ULA plates. Statistical comparisons are indicated (*p < 0.05; ***p < 0.001).*

Overall, these findings indicate that MSC spheroids undergo osteogenic differentiation and matrix maturation to a similar extent in both ULA and SoftXS systems.

4. CONCLUSIONS

The present study demonstrates that MSC spheroids cultured in the SoftXS dynamic system exhibit morphology, viability, self-organization, extracellular matrix deposition, and osteogenic gene expression levels that are highly comparable to those obtained under conventional static ULA conditions.

Quantitative analyses of circularity, roundness, viability, early and late osteogenic markers (RUNX2, DLX5, IBSP), as well as endothelial marker expression (CD31), revealed no significant differences between the two systems. Collagen fibrillar organization assessed by SHG imaging further confirmed preserved extracellular matrix assembly in SoftXS-cultured spheroids.

Importantly, while biological outcomes appear equivalent between ULA and SoftXS at this scale, the SoftXS platform provides the inherent advantages of bioreactor-based culture, including:

KEY ADVANTAGES OF SOFTXS

Scalability
Transition from research-scale to larger working volumes without changing culture principles

Process Standardization
Controlled and reproducible dynamic environment

Improved Mass Transfer
Enhanced nutrient and oxygen distribution in 3D culture vs ULA plates culture

Reduced Operator Variability
Increased robustness for translational and industrial applications

GMP-oriented Potential
Alignment with future clinical manufacturing requirements

In summary, SoftXS maintains biological performance equivalent to static ULA culture while offering the operational and translational benefits of a scalable bioreactor system. This positions SoftXS as a strong platform for advanced 3D MSC spheroid production, bone organoid development, and future clinical-scale applications.

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