Functional & Economic Comparability of Xeno-Free Stem Cell Bioprocess System

Lye Theng Lock, PhD., Iain Farrance, PhD., Jon Rowley, PhD. RoosterBio, Inc. 4539 Metropolitan Court, Frederick, MD 21704

ABSTRACT

Human Mesenchymal Stem Cells (hMSCs) are key raw material in Regenerative Medicine and are widely used for therapeutics, engineered tissues, and medical devices. Yet, achieving an economical bioprocess for hMSC production remains a significant challenge for industry. Bioprocess economic modeling highlights media as a major cost driver in cell manufacturing. Hence, the availability of efficient and robust xeno-free bioprocessing media will not only reduce manufacturing cost, but also decrease regulatory burden associated with bovine serum components found in traditional culture media. Here, we evaluated and compared hMSCs quality parameters in bovine serum-containing and 'xeno-free' bioprocess media formulations and assessed quality parameters such as cell identity, potency and functionality. Cells in xeno-free media maintained critical hMSC functional properties including angiogenic cytokine (FGF, HGF, IL-8, TIMP-1, TIMP-2, and VEGF) secretion, tri-lineage differentiation, and immunomodulatory potential. In addition, hMSCs cultured in xeno-free media expanded rapidly and achieved confluency within 4-5 days of culture without media exchange. The economics of hMSC expansion in this xeno-free media were modeled and compared to other competitive hMSC cell/media systems where it consistently outperformed traditional hMSC systems by >8 fold on the critical productivity metric of Million cells/ L, making it ideal for industrial-scale manufacturing of hMSCs.

MATERIALS & METHODS

High Performance hMSC Media

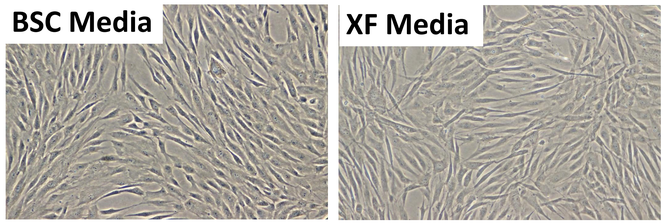
RoosterBio Media Name	General Term	Acronym	RoosterBio Part #
RoosterBio High Performance Media	Bovine Serum Containing Media	BSC Media	KT-001
RoosterBio High Performance Media XF	Xeno-Free Media	XF Media	KT-016

STUDY I: hMSCs were thawed and expanded in BSC or XF media, and further characterized for their expansion, and quality parameters

STUDY II: BM aspirate was processed and MSC isolated in XF or BSC media, and characterized for their quality parameters

STUDY 1: hMSC MAINTAINED MORPHOLOGY & QUALITY ATTRIBUTES IN XF MEDIA

(A) Cell Morphology



(B) Enhanced Cell Expansion in BSC and XF Media Compared to Media from Different Suppliers

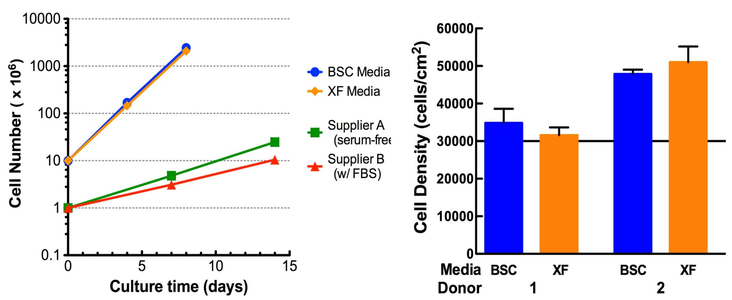
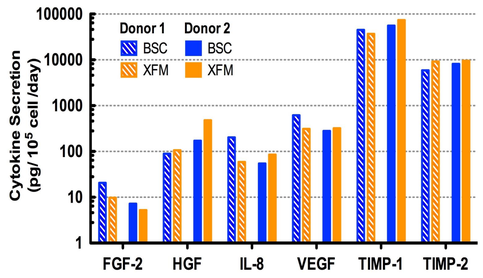


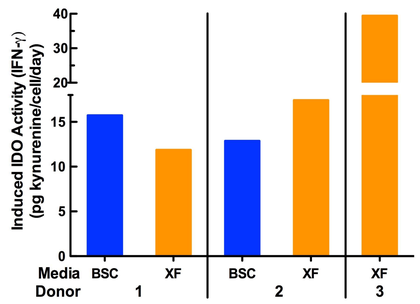
Fig 1: *RoosterBio BSC Media and XF Media (A) maintained their morphology and (B) quickly expanded to >100B hMSC.* RoosterBio cells in both BSC and XF Media showed a 200-fold expansion in 8 days vs. 10- to 20-fold expansion rate in 14 days (over 2 passages) for the other Suppliers. Donors 1 and 2 in XF Media expanded to a similar level as cells in BSC Media. All cell lots expanded at least 10-fold (>30,000 cells/cm^2) in XF Media. Other cell lots (donors) that were grown in XF Media, without a parallel expansion in BSC Media, also expanded greater than 10-fold (not shown). Data are mean of 3 replicates +/- SD.

hMSC Cultured in XF and BSC Media Maintained their Potency

I. Cytokine Secretion



II. IDO Activity



III. Surface Marker Expression

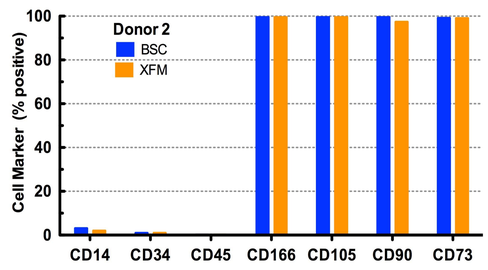


Fig 2. *(I) Comparable levels of (I) Angiogenic Cytokine Secretion, (II) Induced IDO Activity (Immunomodulatory) and (III) Surface Marker Expression of 2 hMSC donors in BSC and XFM media.* The IDO activity for untreated cells was from 0 to 0.12 pg kynurenine/cell/day (not shown). Both cells in BSC and XF media maintained >90% positive for CD166, CD105, CD90, and CD73 and <5% positive for CD14, CD34 and CD45 markers

IV. Tri-lineage Differentiation

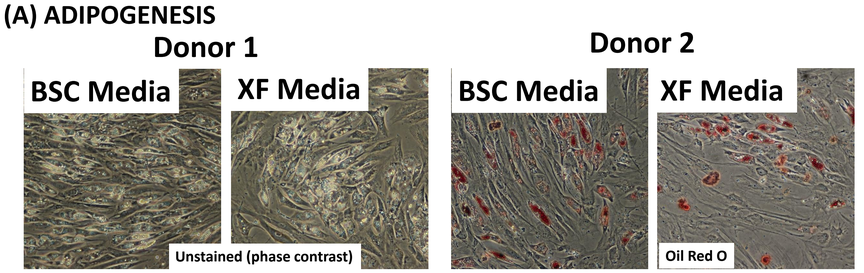
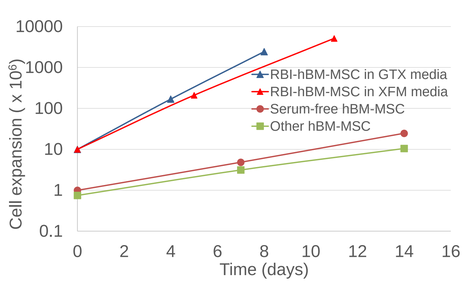


Fig 3. *hMSC grown in BSC and XF Media were capable of trilineage differentiation (adipo-, osteo- and chondrogenesis).*

STUDY 2: SUCCESSFUL ISOLATION & EXPANSION OF hBM-MSC IN XF MEDIA



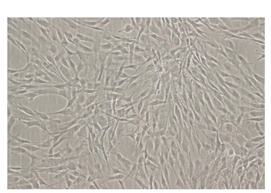
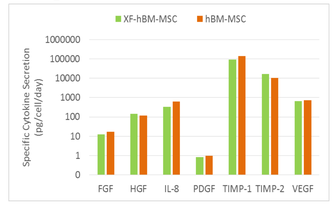


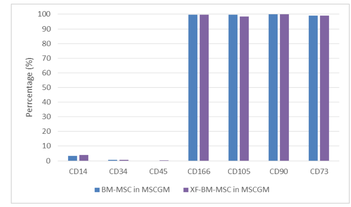
Fig. 4: *hMSCs isolated in XF media (A) maintained their fibroblastic cell morphology and (B) Inducible IDO Activity when treated with interferon-gamma (IFNg).* Cells reached conluency of >85% within 4-5 days of culture

hMSC Derived in XF Media Maintained their Potency

I. Cytokine Secretion



II. Surface Marker Expression



III. Differentiation & IDO Activity

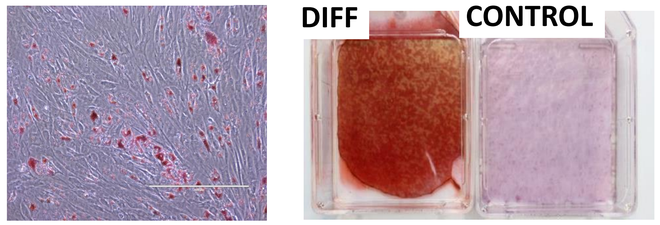


Fig 5: hBM-MSC derived in XF media maintained similar level of (I) Angiogenic cytokine secretion, (II) Surface marker expression as well as (III) Tri-lineage differentiation and IDO activity

ECONOMIC ADVANTAGE OF ROOSTERBIO XF BIOPROCESSING SYSTEMS

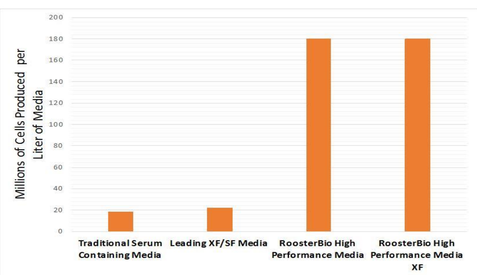


Fig 6: The standard metric of measurement for media efficiency demonstrate that both the RoosterBio BSC and XF media outperforms traditional serum and XF media by >8-fold for hMSC expansion.

CONCLUSIONS

- XF Media used for the manufacturing and derivation of MSCs resulted in cells with comparable expansion performance, potency and functionality.
- XF Media designed for bioprocessing of hMSCs outperformed the traditional media to efficiently produce 8-9-fold higher cells per Liter of media consumed.
- XF media will ease the translation of MSC therapeutics by eliminating the safety risk associated with the use of BSC media

RoosterBio
Radically Changing the Use of Stem Cells

www.RoosterBio.com www.Facebook.com/RoosterBio @RoosterBio