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Review Article

Serum-Free Expansion Media and Genomic Stability of Human Mesenchymal Stromal Cells: A Review

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Abstract

Background: Clinical doses of mesenchymal stromal cells require weeks of expansion, historically in fetal bovine serum. Replacing it raised a second question: whether culture destabilises the genome.

Objective: To review human platelet lysate and defined serum-free media as replacements for fetal bovine serum, and the evidence on genomic stability.

Methods: Narrative review of comparative culture studies and of cytogenetic, array and sequencing analyses of expanded MSCs.

Findings: Platelet lysate matches or exceeds fetal bovine serum for proliferation and supports clinical-scale production, with heparin and residual fibrinogen the technical constraints. The early reports of spontaneous malignant transformation reflected cross-contamination with tumour lines, and one was retracted; aneuploidy occurs without transformation.

Conclusions: The ceiling on expansion is replicative senescence, not malignant transformation.

Keywords: mesenchymal stromal cells; serum-free medium; platelet lysate; genomic stability; senescence

Introduction

A clinical dose of mesenchymal stromal cells is of the order of a hundred million cells, which cannot be obtained from a marrow aspirate without weeks of culture. That culture has traditionally used fetal bovine serum at 10%, and two objections have driven the search for alternatives.

The first is safety and supply. Bovine serum carries a risk of adventitious agents, varies between lots, and raises ethical and availability concerns. It is also immunologically consequential: internalised bovine antigens must be removed to prepare hypoimmunogenic cells [1], and patients given cells expanded in it develop anti-fetal calf serum antibodies [2].

The second is scientific. Serum is an undefined mixture, so a product expanded in it is not fully specified, and lot-to-lot variability propagates into it. The choice of serum determines proliferation, differentiation, gene expression and transcriptome stability [3] — a variable, not a background condition.

Both point toward defined media, and both raise the second question here: whether expansion under any medium leaves the genome intact. Cells throughout are defined by the standard identity criteria [4].

Materials and Methods

Studies were retrieved from PubMed, Europe PMC and Crossref for the terms *mesenchymal stromal cell*, *human platelet lysate*, *serum-free medium*, *genomic stability*, *aneuploidy* and *senescence*, without date restriction. Retraction and correction status was checked against Crossref update metadata for every paper reporting transformation. Bibliographic records were confirmed against Crossref or the publisher.

Human platelet lysate

Platelet lysate is the alternative that has actually displaced serum in practice. Lysates promote expansion and were proposed early as a safety substitute for animal serum [5], and they support clinical-scale expansion of functional cells [6]. Comparative work against fetal bovine serum and other human alternatives has broadly favoured them for proliferation while preserving phenotype and differentiation capacity [7,8].

Two technical constraints define its use. Platelet lysate contains fibrinogen and will clot, so heparin is added — and heparin concentration is critical, since excess inhibits cell growth [9]. Removing the fibrinogen rather than inhibiting clotting supports heparin-free propagation [10]. Both change the effective composition of the medium and therefore the comparability of results between laboratories.

Standardisation is accordingly the focus. Lysate is a pooled product whose composition depends on donor number, platelet concentration and preparation, and position statements from the International Society of Blood Transfusion [11] and jointly from the AABB and ISCT [12] set out quality requirements and the gaps that remain. Whether it is a gold standard has been examined directly [13].

Defined and xeno-free media

Fully defined media remove the remaining undefined component. A serum-free formulation was described and commercialised [14], and defined media supporting rapid isolation and expansion developed independently [15]. Both require a surface coating, since serum proteins otherwise mediate attachment.

GMP-compliant isolation and large-scale expansion has been demonstrated across several platelet lysate-based systems, all of which yielded at least one clinical dose of a hundred million cells; population doubling at passage 0 was 12.9 ± 1.4 in 12.0 ± 1.5 days, a doubling time of 22.4 ±

2.9 hours, from donors aged 18 to 37 [16]. That study also reported that osteogenic differentiation was significantly higher in serum than in platelet lysate cultures, while trilineage capacity was retained in all conditions.

Genomic stability: a corrected record

This part of the literature requires care: its most-cited early findings were wrong and the correction is not universally reflected in secondary sources.

Spontaneous transformation of human adult stem cells in long-term culture was reported in 2005 [17], and bone marrow-derived MSCs were subsequently reported to transform in 45.8% — 11 of 24 — of long-term cultures [18]. Both were taken seriously.

Both were then shown to be artefacts of cross-contamination with tumour cell lines. The authors of the 2009 report published a letter establishing that the apparent transformation reflected contamination [19]. The 2005 paper was retracted by *Cancer Research* in 2010. It therefore carries a formal retraction, whereas the 2009 paper was corrected by its own authors' letter rather than by a registered retraction — a distinction that is frequently reported inaccurately.

The corrected position is supported by work that predates the retractions. Human bone marrow-derived MSCs did not undergo transformation after long-term culture and did not exhibit telomere maintenance mechanisms [20], the absence of the latter offering a mechanistic reason for the former. Clinical-grade production shows aneuploidy occurring without transformation [21], which is the key distinction: a chromosomal change is not by itself evidence of malignancy, and such changes are often transient.

What does accumulate

Something does change with passage, and sequencing has characterised it. Whole-genome sequencing of MSC lines at successive passages found that 90% of all single-nucleotide variants in one line appeared at passage 9, and 70% in the second line at passages 7 to 9, with 33 coding variants and 283 indels across both lines, late-passage mutations enriched for C-to-A transversions, and — importantly — no mutations in Cancer Gene Census genes [22]. Copy-number alterations were mostly zero to two per passage. In the same lines, population doubling time was under 40 hours initially and increased markedly by passages 8 to 10, with telomere shortening after passage 7.

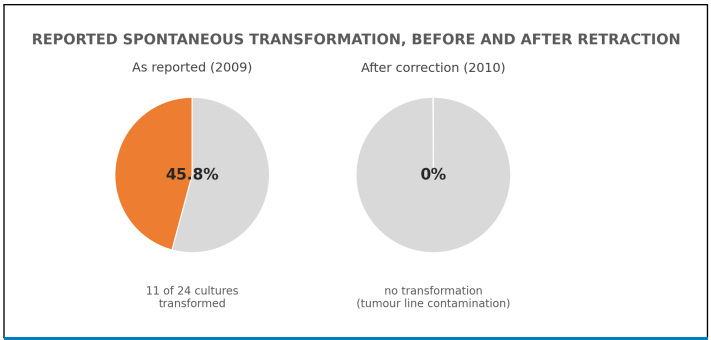

Figure 1: Frequency of spontaneous malignant transformation in long-term bone marrow mesenchymal stromal cell culture as originally reported, and in the same cultures once cross-contamination with a tumour cell line had been identified. Data from Røsland et al. [18] and Torsvik et al. [19].

That last observation identifies the real limit. Cells do not become malignant with extended passage; they become senescent, and do so before mutational burden reaches anything of concern. Senescence itself is continuous rather than abrupt: transcriptome profiling across 7 to 12 passages found over a thousand transcripts changed more than twofold, with adipogenic potential falling as osteogenic potential rose [23]. Culture at low oxygen tension improves growth and genetic stability by activating glycolysis and limiting oxidative stress, DNA damage and telomere shortening [24] — aimed at senescence rather than transformation.

The regulatory position has been articulated in these terms, bridging the scientific observations with what supervisory bodies require of tumorigenicity assessment

[25], and the stability evidence has been reviewed as a biosafety question in its own right [26].

Table 1: Reports of genomic instability in cultured human mesenchymal stromal cells, and their current status.

Report	Claim	Status	Ref.
Rubio 2005	Spontaneous transformation of adult stem cells	Retracted by the journal in 2010	[17]
Rosland 2009	Transformation in 45.8% of long-term cultures	Corrected by the authors' own 2010 letter	[19,18]
Torsvik 2010	Apparent transformation is cross-contamination	Stands; the corrective finding	[18]
Bernardo 2007	No transformation; no telomere maintenance	Stands	[20]
Tarte 2010	Aneuploidy occurs without transformation	Stands	[21]
Kim 2017	Passage-dependent somatic mutation accumulation	Stands; none in cancer genes	[22]

Discussion

Three conclusions follow. First, platelet lysate is a credible replacement for fetal bovine serum on proliferation and phenotype, and its remaining problems — heparin dependence, residual fibrinogen, pooled-donor variability — are being addressed through standardisation rather than being fundamental.

Second, the transformation scare was a contamination artefact. Reports of high-frequency spontaneous transformation of human MSCs do not survive; the corrected consensus is that human cells, unlike murine ones, rarely transform in culture, and that observed aneuploidy occurs without malignancy.

Third, the operative constraint on expansion is replicative senescence. Doubling time rises, telomeres shorten and mutations accumulate in a passage-dependent manner without touching cancer genes, so the practical rule is to limit passage number for reasons of potency and yield rather than of oncogenic risk.

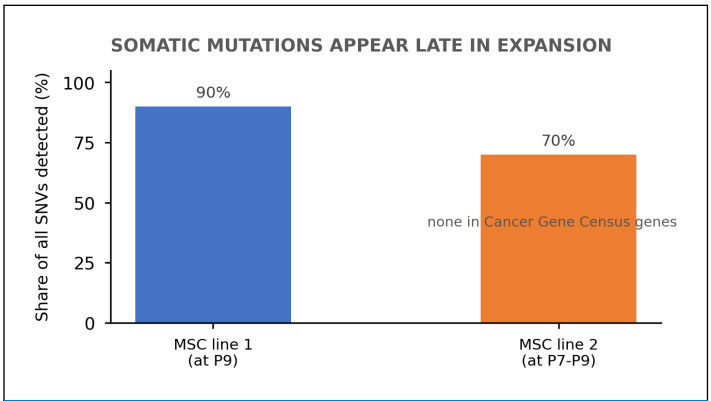

Figure 2: Proportion of all single-nucleotide variants detected across serial passages that first appeared at late passage, in two mesenchymal stromal cell lines analysed by whole-genome sequencing. Data from Kim et al. [22].

This is a narrative review without systematic search or risk-of-bias assessment. The sequencing evidence rests on a small number of donors, karyotype and array methods differ in resolution between studies, and quantitative claims are limited to values confirmed at source.

Conclusion

Serum-free and xeno-free expansion of human mesenchymal stromal cells is achievable at clinical scale, with human platelet lysate the most widely adopted route and defined media a fully specified alternative that requires surface coating. Medium choice is not neutral: it alters proliferation rate and differentiation propensity even where identity criteria are met. On genomic stability, the alarming early literature was corrected — one report retracted, another amended by its own authors after cross-contamination was identified — and the current evidence is that human MSCs accumulate passage-dependent somatic mutations outside cancer genes while becoming senescent rather than transformed. Manufacturing protocols should specify medium composition including heparin and fibrinogen handling, report passage number and cumulative population

doublings, and treat senescence rather than transformation as the reason to cap expansion.

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Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this review.

Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

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