Human platelet lysate as an alternative to FBS: production, characterization and regulation

Platelet Lysate Production and Characterization Under GMP Conditions: the Experience of an Academic Facility

Prof. (apl.) Dr. Karen Bieback
Institute of Transfusion Medicine and Immunology
Medical Faculty Mannheim, Heidelberg University
German Red Cross Blood Service Baden-Württemberg – Hessen
Platelets and Regeneration

1. Activated Platelet
2. Coagulation Cascade
3. Inflammation:
   - Macrophage
   - Neutrophil
4. Fibroblast Proliferation
5. Tissue Granulation Collagen Synthesis
6. Wound Contraction
7. Epithelization
8. Remodeling
9. Healed Tissue

www.prosysglobal.com
# Platelet Granule Content

<table>
<thead>
<tr>
<th></th>
<th>α-Granules</th>
<th>Dense granules</th>
<th>LDCVs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diameter</strong></td>
<td>200–500 nm</td>
<td>150 nm</td>
<td>150–300 nm</td>
</tr>
<tr>
<td><strong>Number</strong></td>
<td>50–80 per platelet</td>
<td>3–8 per platelet</td>
<td>~10,000 per cell</td>
</tr>
<tr>
<td><strong>Percentage of cell volume</strong></td>
<td>10</td>
<td>~1</td>
<td>13.5</td>
</tr>
<tr>
<td><strong>Contents</strong></td>
<td>Integral membrane proteins (e.g., P-selectin, αIbβ3, GPIbα)</td>
<td>Cations (e.g., Ca²⁺, Mg²⁺)</td>
<td>Structural proteins (e.g., granins, glycoproteins)</td>
</tr>
<tr>
<td></td>
<td>Coagulants/anticoagulants and fibrinolytic proteins (e.g., factor V, factor IX, plasminogen)</td>
<td>Polyphosphates</td>
<td>Vasoregulators (e.g., catecholamines, vasostatins, renin-angiotensin)</td>
</tr>
<tr>
<td></td>
<td>Adhesion proteins (e.g., fibrinogen, vWF)</td>
<td>Bioactive amines (e.g., serotonin, histamine)</td>
<td>Paracrine signaling factors (e.g., guanylin, neurotensin, chromogranin B)</td>
</tr>
<tr>
<td></td>
<td>Chemokines [e.g., CXCL4 (PF4), CXCL12 (SDF-1α)]</td>
<td>Nucleotides (e.g., ADP, ATP)</td>
<td>Immune mediators (e.g., enkelytin, ubiquitin)</td>
</tr>
<tr>
<td></td>
<td>Growth factors (e.g., EGF, IGF)</td>
<td></td>
<td>Opioids (e.g., enkephalins, endorphins)</td>
</tr>
<tr>
<td></td>
<td>Angiogenic factors/inhibitors (e.g., VEGF, PDGF, angiostatins)</td>
<td></td>
<td>Ions (e.g., Ca²⁺, Na⁺, Cl⁻)</td>
</tr>
<tr>
<td></td>
<td>Immune mediators (e.g., IgG, complement precursors)</td>
<td></td>
<td>Nucleotides (e.g., AMP, GDP, UTP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleotides</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyphosphates</td>
</tr>
</tbody>
</table>

[Image of platelet granules and cytoplasmic organelles]

Fitch-Tewkik, Frontiers Endocrinol. 2013
Effect of platelet lysate on growth and sulfated glycosaminoglycan synthesis in articular chondrocyte cultures.

“Human platelet lysate (PL) has a strong growth promoting action on rabbit articular chondrocytes in monolayer culture. The responsible factor is heat stable (56 degrees C, 30 minutes) and above 10,000 MW.”

Human platelet lysate contains growth factor activities for established cell lines derived from various tissues of several species.
Eastment CT, Sirbasku DA. In Vitro. 1980.

Platelets as a source of growth-promoting factor(s) for tumor cells.
Platelet Lysate for Cell Culture

Platelet-derived growth factors enhance proliferation of human stromal stem cells.

“The effect of platelet-rich plasma (PRP) released by platelet gel on SSC proliferation and differentiation was investigated”

Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications.

“In vitro expansion of MSCs is conventionally achieved in medium containing fetal calf serum (FCS) and is increased by addition of growth factors. However, for widespread clinical applications, contact of MSCs with FCS must be minimized since it is a putative source of prion or virus transmission. Therefore, because platelets are a natural source of growth factors, we sought to investigate in vitro MSC expansion in response to platelet lysates (PL) obtained from platelet-rich plasma.”
Mesenchymal Stromal Cells

- Differentiation
- Immune modulation
- Support of Hematopoiesis
- Trophic support

Angiogenic
Anti-apoptotic
Anti-scarring
Mitotic
Repair and Regeneration
Mobilisation?

local Injection
systemic Injection

Djouad et al
Nature Reviews Rheumatology 2009

Modified from Caplan & Dennis
JCB 2006
Towards Clinical-Scale Manufacturing

MSC are classically expanded in culture medium containing fetal bovine serum

• protein-uptake derived from FBS by MSC
• risk of transmission of prions and viruses
• possible immunological reactions by repeated exposition

→ Alternative supplements needed !!!
Human Blood Derived Components

- Human Plasma
- Human Serum
- Human Cord Blood Serum
- Human Platelet Derivates:
  - Platelet Releasate
  - Platelet Lysate
    - Pool Buffy Coat
  - Apheresis
    - fresh
    - outdated

→Autologous vs allogeneic pools

- Serum proteins
- Protease inhibitors
- Transport proteins
- Attachment and spreading factors
- Enzymes
- Hormones
- Growth factors, cyto- and chemokines
- soluble cell adhesion molecules
- Fatty acids and lipids
- Vitamins
- Carbohydrates
- Nonprotein nitrogens
- Essential amino acids
- Trace elements
- Exosomes, microparticles
- Micro RNAs
## Humanized Culture Conditions

- Precursor frequency
- Expansion
- Immune phenotype
- Differentiation potential
- Immuno suppression
- Secretome (174 cytokines)

<table>
<thead>
<tr>
<th></th>
<th>FBS Fetal Bovine Serum</th>
<th>HS Pooled Human Serum</th>
<th>tPRP Pooled Thrombin activ. Platelet Releasate Plasma</th>
<th>pHPL Pooled Platelet lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSC</strong> Bone Marrow</td>
<td>„gold standard“</td>
<td>n.s</td>
<td>n.s</td>
<td><strong>Highest expansion</strong></td>
</tr>
<tr>
<td><strong>ASC</strong> Adipose Tissue</td>
<td>„gold standard“</td>
<td><strong>Enhanced expansion</strong></td>
<td>Enhanced expansion</td>
<td>High expansion in early passage</td>
</tr>
</tbody>
</table>
Differences Bone Marrow to Lipoaspirate MSC

→ PL for BM, but tPRP for LA

Kinzebach et al, BMC Cell Biology 2013
Differences in Gene Expression

102 from 34,039 genes >2fold change
90 in FBS
12 in HS and tPRP

Bieback et al. Tissue Eng 2010

→ Fetal bovine serum induces small but possibly important changes in MSC gene expression
Platelet Lysate - MSC

- Promotes cell expansion, increases CFU size and frequency, retains differentiation potential and immunomodulative activity Doucet 2005, Lucarelli 2003

- Allows rapid clinical-scale expansion of 1.5 – 5ml bone marrow to yield 7.8 +/- 1.5 x 10^8 MSCs within a single 11- to 16-day primary culture Schallmoser 2008, Fekete 2012


- Enhances osteoblastic differentiation Chevallier 2010, Xia 2011. Lucarelli 2011

- Reduces adverse adipogenic differentiation Lange 2012


Differences in Function?

Bone Marrow Transplant. 2009

In our patient cohort, response to MSC transfusion was lower than in the series reported earlier. However, our experience supports the potential efficacy of MSC in the treatment of steroid-refractory aGVHD.

(Introna et al. 2014)

Safety?

Centeno et al: Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique.
Curr Stem Cell Res Ther 2011

Using both intensive high field MRI and complications surveillance in 339 patients, no neoplastic complications were detected at any stem cell re-implantation site. These findings are consistent with our prior publication and other published reports also show no evidence of malignant transformation in vivo following implantation of MSC for orthopedic use.
Platelet Lysate – Production and Characterisation

Human Platelet Derivates:
- Platelet Releasate
- Platelet Lysate
  - Pool Buffy Coat
  - Apheresis
    - fresh
    - outdated

→Autologous vs allogeneic pools: pools of 8 donor
Initially platelets blood group 0 in AB plasma, now = platelets in T-sol

Characterisation
Platelet count: 2-3x10⁶ platelets/µl
Average protein content 26 ± 5 g/L, with 62 ± 1% albumin

Kocaömer et al. Stem Cells 2007; Fekete et al. Cytotherapy 2012
Platelet Lysate – Characterisation

Fekete et al. Cytotherapy 2012
Platelet Lysate - Manufacturing

Buffy-Coat Pools in T-Sol or MacoFlex;
30 Gy irradiation;
Quarantine storage
Schrezenmeier et al

Plasma-free Strunk et al
(1) otherwise discarded buffy coats by (2) pooling multiple units of human buffy coat-derived PRP to reduce variations, (3) removing the plasma to exclude the risk of transmitting isoagglutinins, plasma factors and in particular plasma related diseases and allergic reactions and (4) substituting the removed plasma by virtually antigen- and antibody-free human albumin solution.

Maco Pharma, Bourin et al

→Viral Inactivation: Burnouf et al
→Pathogen Inactivation: Abedi et al
Conclusion

**FBS:** high lot-to-lot variability, risk of contamination and immunization

**PRESENT:** *xenogenic-free culture conditions* Human supplements
→ human plasma, serum, umbilical cord blood serum, platelet derivates are in direct clinical use since years; standardizable pools, quality control systems well established; effects on MSC are currently investigated; proteomic characterisation of human supplements

**FUTURE:** *Chemically defined media:* best standardized, but not yet approved for GMP and clinical use, effects on MSC not yet known, anticipated to have even higher impact than human supplements due to significant changes in the medium composition.

Any significant change of culture conditions can have an impact on cellular qualities and needs to be investigated!

Standardized manufacturing protocols, quality control parameters and potency assays are of utmost importance.
Acknowledgements

→ Asli Kocaömer, Susanne Kern, Andrea Hecker, Harald Klüter, Anh-Thu Ha, Melanie Grassl,
→ Katharina Schallmoser, Dirk Strunk
→ Hermann-Josef Thierse
→ Arnaud Scherberich, Laurent Tchang

Thank you for your attention!