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Evaluation of human platelet lysate versus fetal bovine serum for culture of mesenchymal stromal cells

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Abstract

Culture media for therapeutic cell preparations—such as mesenchymal stromal cells (MSCs)—usually comprise serum additives. Traditionally, fetal bovine serum is supplemented in basic research and in most clinical trials. Within the past years, many laboratories adapted their culture conditions to human platelet lysate (hPL), which further stimulates proliferation and expansion of MSCs. Particularly with regard to clinical application, human alternatives for fetal bovine serum are clearly to be preferred. hPL is generated from human platelet units by disruption of the platelet membrane, which is commonly performed by repeated freeze and thaw cycles. Such culture supplements are notoriously ill-defined, and many parameters contribute to batch-to-batch variation in hPL such as different amounts of plasma, a broad range of growth factors and donor-specific effects. The plasma components of hPL necessitate addition of anticoagulants such as heparins to prevent gelatinization of hPL medium, and their concentration must be standardized. Labels for description of hPL—such as "xenogen-free," "animal-free" and "serum free"—are not used consistently in the literature and may be misleading if not critically assessed. Further analysis of the precise composition of relevant growth factors, attachment factors, microRNAs and exosomes will pave the way for optimized and defined culture conditions. The use of hPL has several advantages and disadvantages: they must be taken into account because the choice of cell culture additive has major impact on cell preparations.

Key Words: *fetal bovine serum, fetal calf serum, serum, mesenchymal stromal cells, platelet lysate, platelet lysate gel*

Introduction

Overall, the composition of cell culture media still closely resembles formulas developed in the pioneering work of the 1950s: Harry Eagle described a basal medium (Eagle's minimal essential medium), which comprised of a mixture of 29 essential components including 13 amino acids, nine vitamins, D-glucose and six inorganic salts (1). In the early days of cell culture, this basal medium was supplemented with human or horse serum to support the *in vitro* growth of human carcinoma cells or murine fibroblasts. To date, fetal bovine serum (FBS; alternatively termed fetal calf serum [FCS]) is the most commonly used serum additive that is capable of supporting growth of a variety of cell types.

Over the past decades, mesenchymal stromal cells (MSCs) have received much attention for their potential role in regenerative medicine and cellular therapies (2,3). They can be easily culture-expanded, they harbor differentiation capacity toward mesodermal lineages and they reveal a variety of immunomodulatory features. The first clinical trial with the use of culture-expanded MSCs was performed in 1995 (4). In this study, MSCs were obtained from 23 patients who were then reinfused intravenously to demonstrate that these cells can be expanded *in vitro* and were then transplanted without toxicity (4). By 2013, the public clinical trials database (http://clinicaltrials.gov) registered 338 clinical trials that used MSCs for a wide range of therapeutic applications. MSCs can hardly be purified directly from tissue and therefore they are usually culture-expanded *in vitro* to attain sufficient cell numbers for clinical applications. Most of the protocols that have been reported in the literature use FBS-supplemented media to raise and expand human MSCs. However, since concerns have been raised regarding the safety of FBS-based culture media, protocols intended to raise cells for the clinical application should—according to Good Manufacturing Practice—avoid usage of animal sera.

Culture conditions exert major impact on cells cultured *in vitro*. Because of the wide range of

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therapeutic applications of MSCs and to their worldwide use, we aimed to discuss the relevance of serum substitutes for MSC cultures. Accordingly, this article summarises limitations and improvements of culture media with particular emphasis on human platelet lysate (hPL) (Table I).

Fully defined synthetic culture media: an unmet goal

Efforts have been made to replace serum supplements and to design more standardized and better-defined serum-free formulations. They must comprise all nutrients, amino acids, lipids hormones, vitamins, buffer substances and growth factors that are essential to maintain all physiological functions and to facilitate cellular proliferation. Over the past decade, various serum-free and animal-free media have been described (5–12). In fact, it has been suggested that specific serum-free culture media can be used for expansion of human MSCs (13). However, these media often failed to support the initial isolation and expansion steps: particularly on untreated culture flasks, the cell adhesion and initial outgrowth of fibroblastoid colony-forming units were largely impaired. Therefore, peptides and serum proteins have been used to coat the culture dishes upfront—at the expense of fully standardized and serum-free culture conditions. In general, precisely defined synthetic media provide a better controlled cell culture environment, but optimization of the concentration of individual compounds is a tedious and expensive task that is ongoing (14).

FBS: the gold standard for cell culture

To date, FBS is the most widely used serum supplement for *in vitro* culture of eukaryotic cells. It has been estimated that the annual worldwide production is approximately 500,000 L of raw FBS, which equates harvesting of more than one million bovine fetuses per year (15,16). Generally, FBS is produced from the blood drawn from a bovine fetus that is obtained from pregnant cows sent to slaughter. The fetus—usually at approximately 6 months of fetal development (17)—is separated at the abattoir, and the fetal blood is collected under aseptic conditions. This is usually performed by puncturing of the heart. The blood is chilled, allowed to clot and serum is then separated from the fibrin-clotted mass and red blood cells by centrifugation (Figure 1A). Thereby, FBS can be produced in relatively large quantities, and large batches of pretested serum can be generated and distributed on a commercial basis.

Because of its relatively easy production and rich content of growth factors, FBS became the "most universally applicable cell culture additive for the stimulation of cell proliferation and biological production" (9). Other advantages in the use of FBS in cell culture include the following (Table II): i) it is effective on most types of human and animal cells; ii)

Table I. Different types of culture media.

Type of culture media	Definition
Xeno-free media	All components are either synthetic or derived from the same species corresponding to the species of cellular origin and/or recipient of the transplant. For application with human cells, particularly in clinical therapy, "xeno-free" means that it comprises exclusively human components and chemically defined substances. Addition of recombinant proteins and/or recombinant growth factors is often considered as acceptable for this definition. Human serum or hPL are examples for xenogen-free supplements.
Serum-free media	Does not comprise any serum, either from animals or humans. Per definition, it may include recombinant growth factors and even animal components that are not serum-derived. In this context, hPL comprises plasma but not serum. However, definition of hPL to be "serum-free" might be misleading because plasma has a composition very similar to serum.
Animal-free media	Media are completely devoid of any animal substances. Animal-free media may contain recombinant proteins. Please note that coating of culture dishes is not necessarily animal-free. Often, coating is performed with serum or serum proteins; therefore culture conditions are not "animal-free." For hPL, the label "animal-free" may be misunderstood under the perception that humans are also mammals. However, many groups conventionally use this definition to discern culture condition with supplements derived from animals as opposed to humans.
Animal-free culture conditions	Culture conditions are completely devoid of animal substances, including potential surface coating. They may comprise recombinant proteins, such as growth factors, which are sometimes difficult to standardize and to define.
Fully defined synthetic culture media without growth factors	These media comprise only pure synthetic substances with known activities. They do not rely on recombinant growth factors, which may comprise traces of other proteins or vary in activity. Only such media can be completely standardized.