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REVIEW ARTICLE

A consensus introduction to serum replacements and serum-free media for cellular therapies

OHAD KARNIELI[1], ORYAN MAKLER FRIEDNER[2], JULIE G. ALLICKSON[3], NAN ZHANG[4], SUNGHOON JUNG[5], DAVID FIORENTINI[6], EYTAN ABRAHAM[5], SHANNON S. EAKER[7], TAN KAH YONG[8], ALLAN CHAN[8], SARAH GRIFFITHS[9], AMY K. WHEN[10], STEVE OH[8] & OHAD KARNIELI[1]

[1]Karnieli Ltd and ATVIO Biotech, Ticon, Israel, [2]Pluristem Therapeutics, Matam Park, Haifa, Israel, [3]Regenerative Medicine Clinical Center, Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA, [4]Hematology Branch, National Heart, Lung, and Blood Institute National Institute of Health, Bethesda, Maryland, USA, [5]Cell Therapy Research & Technology Lonza Walkersville, Walkersville, Maryland, USA, [6]Biological Industries Ltd., Beit Haemek, Israel, [7]GE Healthcare Cell Therapy Division, Marlborough, Massachusetts, USA, [8]Bioprocessing Technology Institute, Singapore, [9]Stimlabs, Roswell, Georgia, USA, and [10]Irvine Scientific, Santa Ana, California, USA

Abstract

The cell therapy industry is a fast-growing industry targeted toward a myriad of clinical indications. As the cell therapy industry matures and clinical trials hit their pivotal Phase 3 studies, there will be a significant need for scale-up, process validation, and critical raw material quality assurance. Part of the well discussed challenges of upscaling manufacturing processes there is a less discussed issue relating to the availability of raw materials in the needed quality and quantities. The FDA recently noted that over 80% of the 66 investigational new drug (IND) applications for mesenchymal stem cell (MSC) products analyzed described the use of FBS during manufacturing. Accumulated data from the past years show an acceleration in serum consumption by at least 10%-15% annually, which suggests that the global demand for serum may soon exceed the supply. Ongoing concerns of safety issues due to risks of various pathogen contaminations, as well as issues related to the aforementioned serum variability that can affect final product reproducibility, are strong motivators to search for serum substitutes or serum-free media. it is important to note that there are no accepted definitions for most of these terms which leads to misleading's and misunderstandings, where the same term might be defined differently by different vendors, manufacturer, and users. It is the drug developer's responsibility to clarify what the supplied labels mean and to identify the correct questions and audits to ensure quality. The paper reviews the available serum replacements, main components, basic strategies for replacement of serum and suggests definitions.

Key Words: *serum replacement, serum free, defined media, cell therapy, animal component free, platelet lysate, cGMP media, fetal bovine serum, human serum, xeno free*

Introduction

The cell therapy industry is a fast-growing industry targeted toward myriad clinical indications. Most cell therapy companies are currently conducting clinical trials in phase 1 or 2 with several that are more mature in phase 3 or even early market approvals [1]. As the cell therapy industry matures and clinical trials hit their pivotal phase 3 studies, there will be a significant need for scale-up, process validation and critical raw material quality assurance [1]. Addressing this need might result in changes to culturing technologies, methods, raw material sourcing and testing. Because of the sensitivity of cells to their microenvironment, any change in culture conditions can affect cellular physiology, thereby altering physical and performance characteristics and critical quality attributes. Thus, uncontrolled scale-up may affect the final product's activity or attributes and prevent it from being comparable to the original smaller-scale product [2,3]. Other challenges of

Correspondence: **Ohad Karnieli**, PhD, MBA, Karnieli Ltd and ATVIO Biotech, 57 Tamar Street, Ticon 36015, Israel. E-mail: Ohad@karnieli.com

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upscaling manufacturing processes relate to the availability of raw materials in the needed quality and quantities. Difficulties in keeping up with the supply of raw materials while maintaining their quality and minimizing variability may prevent progression of large-scale production.

One of the most critical raw materials in the cell therapy industry is serum. Serum serves as a media supplement used for culture and enables cell growth stimulation. The most common type of serum used in the cell therapy industry is fetal bovine serum (FBS). The U.S. Food and Drug Administration (FDA) recently noted that more than 80% of the 66 investigational new drug applications for mesenchymal stromal cell (MSC) products analyzed described the use of FBS during manufacturing [4]. The concentration of FBS in media ranged from approximately 2 to 20%, with 10% FBS the most common concentration. The most common alternative noted for these MSC investigational new drugs was human platelet lysate. However, there are also other sources for bovine sera, such as newborn calf serum or donor bovine serum, which are obtained from animals by repeated bleeding. Serum from other animals (e.g., horse, sheep) are also in use [5].

Serum manufacturing

Currently, 90% of all serum used in the cell therapy industry is supplied from three countries: the United States, Australia and New Zealand [2]. A large quantity of serum may be derived from any number of animals and can range from a few hundred liters to several thousand liters [2]. Therefore, serum will often be derived from a large number of animals. The production of FBS begins with removal of the calf fetus from the slaughtered pregnant cow in the abattoir [6]. Next, blood is removed from the heart to prevent microbial contamination and then centrifuged to remove the clot and blood cells from the serum. The serum is then passed through a 0.1-μ filter to remove even the smallest bacterial or fungal contaminants and mycoplasmas, effectively "sterilizing" the product. Additionally, the serum may undergo gamma irradiation sterilization to eradicate viruses and other pathogens small enough to pass through the filter. Between all manufacturing steps, the serum is stored frozen to maintain potency by minimizing degradation of biological moieties. Representative samples from pooled batches of serum are taken for various testing, including sterility to ensure safety of the product. The final FBS product is also frozen until sampling for quality control release to buyers [7]. For current Good Manufacturing Practices (cGMP) applications, regulators including the World Health Organization require that serum be traceable throughout the supply chain, and it must originate from cattle that are free of bovine spongiform encephalopathy and have not have ingested prohibited animal feeds [8]. Several ethical issues centered on potential suffering of the fetus have also been raised regarding the production of FBS [6,9]. Additionally, because the serum industry is a byproduct of the meat industry, there is no real apparent economic incentive for cattle ranchers to increase herds to source more FBS, limiting again the quantities.

Future availability of serum

In 2002, an estimated 600 000 L of FBS were produced, of which only one-third met the regulatory requirements in cell therapy [6,9]. Accumulated data from the past years show an acceleration in serum consumption by at least 10–15% annually, which suggests that the global demand for serum may soon exceed the supply [2,10,11]. Evidence of this growing demand is a threefold price increase in clinically used serum over the past few years [2]. As already noted, FBS is currently a byproduct of the cattle industry, and it would not be economical for cattle to be bred solely for the production of serum. Currently, approximately 15 companies are audited and certified by the International Serum Industry Association for the Traceability Audit Checklist that supports use of serum for the cell therapy industry [2]. Therefore, it is unlikely that serum production in its current form will be able to keep up with future demand, which may be a key limitation in the commercialization of cell-based medicine.

Inherited serum variation and quality assurance

Although FBS has been used for more than 50 years, the knowledge is incomplete regarding the connections between serum components and cell product attributes influenced by culturing in the presence of serum [11]. FBS is a complex medium supplement; subtle variation in its composition can influence key properties of cells due to their sensitivity to culture conditions and might even cause alterations in the final product [12]. This variation in serum composition therefore becomes a challenge for generating consistent and quality-assured cells in clinical-scale production [2]. Potential causes of serum variation include genetic diversity of source herds and animal diet as well as the serum manufacturing process itself and the fact that there are about some 1800 proteins and 4000 metabolites present in serum [11]. Furthermore, the current specifications for serum manufacturing and release are under a broad acceptable range, leading to inherent variation. Additional modifications and optimizations are needed to narrow the ranges of these respective specifications and standardize manufacturing methodologies. A procedure of serum screening from various lots before purchas-