Efficacy of Fetal Bovine Serum Alternatives

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Abstract

Fetal bovine serum (FBS) is one of the leading cell culture supplements in laboratory studies across the world. Although its constituents are not well-defined, it is estimated to house more than one thousand components, including but not limited to growth factors, carbohydrates, and other proteins. Its use provides an optimal environment for cultured cells to grow. However, FBS is a byproduct of the meat industry, making its availability unpredictable and costly. To combat this issue, new sera have been developed from alternative sources in hopes of saving costs. Studies are ongoing to investigate the effectiveness of these alternatives, namely human platelet lysate (HPL), chemically defined serum replacements (CDSRs), and other bovine products such as newborn calf serum (NBCS), bovine calf serum (BCS), and Fetalgro. Cell viability and proliferation rate are key variables used to compare the functionality between sera and will be the primary focus of this review.

1 Introduction

Fetal bovine serum (FBS) has long reigned as the golden standard of supplemental media and is used extensively in wet laboratories. Serums such as FBS have never been fully characterized, bringing into question its validity for experimental procedures [1]. FBS is an animal-derived serum that has proven to be less effective for clinical testing [2, 3]. However, its plentiful reservoir of constituents, including various carbohydrates, proteins, lipids, hormones, and electrolytes among others, contribute to an optimal cell growth environment. They function to protect cells from damaging chemicals and agents as well as pH shifts within the media [4]. In addition, its relatively low levels of immunoglobulins and clotting factors make it an even more useful component to promoting cell proliferation [5].

There have been some ethical considerations concerning the origin of FBS. Many have advocated for the use of FBS alternatives to avoid the unnecessary suffering of animals. However, if cows are found to be pregnant with a fetus at the time of slaughter, serum is harvested via cardiac puncture. Thus, the case could be made that the product would go to waste, thereby increasing utility of the animal products.

Despite the benefits, FBS comes with a major drawback—it is a very expensive serum due to factors relating to its source. During meat production, if female cows are found to be pregnant at the time of slaughter, then blood is extracted from the bovine fetus via a cardiac puncture [4]. The blood is then allowed to clot, thereby eliminating fibrin and other clotting factors [5]. The remaining liquid is frozen and sold as a consumer product, usually for experimental purposes [4]. Thus, serum availability is solely dependent on the meat industry and results in fluctuating costs [4]. For example, at the time of this writing, a 500 mL FBS product from Rocky Mountain Biologicals ranged from $399 to 860$. Limited funding given to researchers restricts their ability to purchase this serum at elevated prices.

To combat this issue, researchers have experimented with alternative sera in developing cell lines. Among these include various forms of chemically defined serum replacements (CDSRs), human platelet lysate (HPL), Fetalgro and other bovine products. In contrast to the exorbitant price of FBS, 500 mL Fetalgro serum from Rocky Mountain Biologicals ranged from $125 to 177.30$, roughly less than half the amount at which FBS is priced at the same company. Similarly to FBS, all these sera are allowed to clot to remove coagulation factors; the remaining plasma becomes the product [4, 5]. It is a relatively new area of study with other alternatives being tested, but the reagents listed previously will be the focus of this review. We will evaluate how alternative sera—including bovine varieties, HPL, and CDSRs—perform in comparison to FBS across multiple studies in terms of cell viability and proliferation rate.

2 Review

Newborn calf serum (NBCS) and bovine calf serum (BCS) have been frequently studied to observe each performance relative to FBS in promoting cell growth.

NBCS is generally taken from young calves under 14 days of age while BCS comes from calves under 6 months of age [4]. In a study observing the cell proliferation and morphology of head and neck cancer lines, NBCS did not support adequate proliferation times in carcinoma cell lines [6]. In contrast, BCS provided an environment for suboptimal growth, but still not as favorable as FBS [6].

In the same study, several additional carcinoma cell lines were introduced in a different series of passages along with a noncarcinogenic line. Again, proliferation time was measured as a ratio in comparison to FBS. Several additional calf sera were included as well, including iron-supplemented calf serum (ICS), cosmic calf serum (CCS), FetalClone III, and Fetalgro. ICS and BCS did not perform as well as FBS in doubling time [6]. CCS, FetalClone III and Fetalgro performed similarly to FBS in some cell lines and outperformed FBS in others [6].

Fetalgro has been a product of interest as of late. As a new serum to enter the market, it is advertised by its manufacturer as an optimal alternative to FBS in terms of proliferation rate and cell viability. An in-house study found that Fetalgro shortened doubling-time and exhibited comparable growth characteristics in a handful of cell lines [7]. However, another study has demonstrated that cell viability was less in cell lines cultured with Fetalgro compared to those cultured with FBS [1].

Human-derived sources of sera have been popular substitutes for FBS in cell growth and differentiation. For example, HPL has become popular in clinically related studies as opposed to utilizing animal-derived sera [2, 8–10]. Some researchers claim that using reagents of animal-derived serums are inadequate for clinically related research [3]. Consequently, sera such as HPL should provide an advantage under certain parameters. However, results from several studies are conflicting. Regarding doubling time, some studies show no improvement when comparing HPL to FBS [5, 9, 10] while others observe superior performance with HPL [2, 3, 8, 11]. In terms of cell viability, HPL performs similarly to FBS [12]–however, other important aspects of cell culture growth may be impaired resulting in abnormal cell morphology and enzyme production [3, 8–10].

Other studies have aimed to experiment with serum-free alternatives, or chemically defined serum replacements (CDSR). These are commercially manufactured to contain similar constituents that are also present in FBS [13]. A huge advantage to using this type of media is that it improves experimental reproducibility, in essence eliminating all variations that typically occur in animal-derived batches of sera [14]. Several studies found adequate cell differentiation and growth [13, 14] while another showed enhanced doubling time [13]. In any case, cell viability does not seem to be altered when utilizing CDSR [13, 14], but individual CDSR sera can be manufactured to have a formula specific to a certain cell line which may provide opportunities for enhanced performance [14]. However, the use of serum-free media is limited in laboratory settings due to its high-cost of production [13].

3 Discussion and Conclusions

Since FBS and other similar alternatives are not clearly defined, studies directed towards analyzing FBS in its entirety would greatly benefit the advancement of serum research. However, wide variability between batches presents an obstacle that would need to be considered when approaching this type of study [4, 15–17]. Discovering a consistently reliable serum might help explain differences in cell differentiation, viability, and protein expression in many cell lines. However, developing sera with different chemical formulas—such as CDSRs—is costly. could provide optimal cell conditions for a cell line.

A new serum to enter the market that has not been extensively proven is Fetalgro, a form of bovine growth serum. Very few studies have investigated this serum as an alternative to FBS, and even then, the few studies performed have conflicting outcomes and competing interests. However, Fetalgro does show potential as a cost-effective alternative serum. Thus, further studies incorporating Fetalgro into FBS alternative sera studies in a variety of cell lines has yet to be performed.

A reservoir of potential serum extract that is studied very little is donor bovine serum (DBS). In this case, DBS is extracted from a donor animal, thus eliminating its association as a byproduct of the meat industry. Donor equine serum (DES) is currently available for purchase at a cost-effective price, suggesting that other animals could act as serum reservoirs. One study investigating the effects of this serum found that DBS performed relatively similar to FBS in terms of cell proliferation rate [18], however, scientific literature surrounding this serum alternative is scarce.

Human-derived sources of sera, such as human platelet lysate (HPL), have been frequently tested as alternatives to FBS. They are relatively inexpensive and widely applicable in clinical practice. For example, many dental offices use patients' own plasma and red blood cells to help facilitate regrowth and healing after bone grafts or extractions. Because FBS is deemed by some as less clinically friendly [2, 8], utilizing HPL can provide a financial as well as a

functional advantage [5, 9, 10].

Additional research has been conducted to find the optimal concentration of FBS and other alternative sera in cell cultures. Most studies tend to use a 10% concentration of FBS (or some other alternative serum) when developing cell lines, however, one study focusing solely on optimal serum concentrations found 5% to be more effective than 10% at promoting cell growth [11]. Therefore, finding the optimal concentration of supplemental media can further enable researchers to save money and utilize funds elsewhere.

Benefits to using FBS alternatives in cell culture work is evident in cell proliferation rate and viability. For many of these alternatives, it also presents the opportunity to save institutions valuable resources. By utilizing a cost-effective serum that sufficiently provides an optimal environment for cell lines, researchers and organizations can utilize funds more efficiently to further their research in other capacities.

Abbreviations

FBS: fetal bovine serum
HPL: human platelet lysate
CDSR: chemically defined serum replacement
NBCS: newborn calf serum
BCS: bovine calf serum
ICS: iron-supplemented calf serum
CCS: cosmic calf serum
DBS: donor bovine serum
DES: donor equine serum.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability

Not applicable.

Conflicts of Interest

The authors declare that they have no competing interests.

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Author Contributions

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