

INTERVIEW

Advances in Media Formulations for Clinical-Scale Expansion of Cells



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Q Fetal bovine serum has long been the gold standard medium supplement for cell expansion **in vitro**. What factors make it undesirable for human applications?

TB: There are several reasons as to why fetal bovine serum (FBS) is undesirable for any protocol that is intended to be used in human therapy.

The primary concern is patient safety, for two different reasons. First is a general safety concern regarding the use of animal-derived components for any product or procedure that is going to be applied to treat human patients.

The safety concern originates from the fact that FBS can be contaminated by viruses, known or unknown, as well as prions. Although some viruses can be tested for, the detection threshold of these tests may not be sensitive enough to remove the infectious risk. In addition, there may be contamination with new viruses, which cannot be tested for. Additionally, prions are very difficult to detect and/or inactivate in biological materials. Second, is the risk of immunological impact on the patient, who would receive stem cells grown in a medium containing FBS. Some studies have shown that between 7 and 30 milligrams of bovine protein may be integrated and internalized by the human cells in culture. When the cells are transplanted to the patient, this can induce immunological reactions in the patient, which may lead to failure of the cell transplant procedure.

There are additional concerns that factor into the industry's drive to move away from using FBS. One is ethical, as there may be some pain induced to the fetus at the time the blood is collected in order to prepare the serum. Another is technical, as there are variations in the quality of the FBS from batch to batch. This can substantially impact the expansion capacity of the cells from one batch to another causing variability in the cell-based product, which can lead to inconsistent clinical results and regulatory challenges. The last concern is a supply issue. As the cell therapy industry continues to mature, and demand for FBS increases, there is an inherent risk that there will be shortages of good quality, well-controlled and trusted sources of this material.

Q What's the rationale for using humanized culture formulations, in particular human platelet derivatives, in regenerative medicine?

TB: The cell therapy industry is able to gain from all the expertise and knowledge that has developed in the field of blood transfusion. In the past, blood transfusions have been associated with transmission of several viruses. Therefore, this field has been under the scrutiny of regulatory agencies for a long time. To address the viral transmission issue, donors are required to undergo rigorous screening and testing, and pathogen inactivation techniques have been developed.

Platelets can be separated from the blood prior to transfusion and the platelet concentrate can be used for therapeutic applications. The advantage of using platelets in regenerative medicine is that the starting material has already been subjected to a lot of scrutiny and testing by the blood establishment following regulations imposed by regulatory authorities. Consequently, the starting material is compliant with clinical use.

Q How is human platelet lysate (HPL) produced? Are there different starting materials used for HPL production compared to quality of lysates?

TB: The first type of production method used to make HPL for stem cell expansion as substitute for FBS originated a little over 10 years ago. At this time, the source material to prepare the platelet ly-

“Animal-derived components such as FBS pose significant safety concerns, thus making them undesirable for human applications”

sate was fresh platelet concentrates kept for 5 days at 20-24°C after the collection. Now there's a good number of publications showing that the platelet concentrate does not have to be fresh. Instead, frozen platelet concentrate, which is

not used for the transfusion, can be frozen and subsequently used to make HPL.

Platelets contain a lot of growth factors that need to be released in order to produce the HPL. There are two main procedures used to release the growth factors. One approach is to perform several (3) cycles of freeze and thaw, at -80 to +37 degrees, to break the platelet membrane. The result is a platelet lysate containing platelet growth factors and other platelet and plasma components, including fibrinogen. Another way to release the content of the platelets is to perform an activation with calcium chloride. Calcium chloride is used to counterbalance the anti-coagulant that is used at the time of blood or platelet collection to avoid clotting by calcium complexation. Providing and increasing the content of calcium leads to coagulation and degranulation of the platelets to ultimately release their content. In this case, the platelet lysate does not contain fibrinogen because calcium chloride also leads to conversion of fibrinogen into a fibrin clot (fibrinof ormation). In either case, cellular debris must be removed from the liquid through filtration, and then the product is filled into the final container and can be frozen.

Ensuring standardization of HPL is quite important, because the platelet lysate from donor to donor may have differences in composition such as content of growth factors. Some blood centers in Europe are already producing pooled HPL from pools of platelet concentrates coming from 40–50 donors, but in other countries like Germany the regulations do not allow pooling over 16 donations when there is no dedicated virus inactivation treatment. And some commercial companies are producing batches with larger pool size, around 250 donors, under certain conditions to enhance virus safety.

According to recent data, regardless of the way HPLs are produced, by freeze/thaw, calcium chloride activation, or even other techniques such as sonication or solvent/detergent treatment, cells are typically found to expand better in the presence of HPL compared to FBS. Studies show that supplementing the growth medium with 5–10% HPL generally induces a similar cell expansion of mesenchymal stromal cells (MSC) as 10–15% FBS. Under HPL conditions, cells maintain their stem cell immunophenotype, remain immunosuppressive and can be differentiated into the three main standard cell lineages (osteocytes, chondrocytes and adipocytes). However, whether specific types of HPL may be preferable to expand particular types of stem cells or differentiated cells is still under investigation.

Q What do you see as the key variables in HPL production that could influence cell expansion, and what efforts have been put to develop a standardized method to produce and characterize HPL?

TB: There are several key variables in HPL production that can lead to variability in HPL composition and ultimately influence cell expansion. With respect to equipment, the methodology used to prepare the platelet concentrate can lead to different levels of leukocytes (white blood cells) in the platelet concentrate. In some collection procedures, the platelet concentrates can be subjected to leukoreduction through filtration, which can also affect the HPL composition.

Similarly, new developments in platelet concentrate preparation leads to partial removal of the plasma and its replacement by a platelet additive solution (PAS). When plasma is removed, and replaced by this PAS, the total protein content of the HPL is decreased substantially. This can ultimately have an impact on the percentage of human platelet lysate needed in the growth medium to supply sufficient nutrients for expanding the cells. In addition, removal of part of the plasma will lower the content of insulin-like growth factor (IGF), which may be important for cell expansion. But otherwise the concentration of the other growth factor will not be changed, because they come from the platelets themselves.

Another variable that is still under study is the impact of the pathogen inactivation treatment, which may be performed on the platelet concentrate for transfusion. The first data appearing in the literature seems to indicate that pathogen inactivation treatment does not have an observable or quantifiable impact on the capacity to expand MSCs.

Q What strategies are used to remove pathogens in HPL?

TB: In my opinion, the regulators who will be making decisions to ensure the safety of HPL, are going to follow the rules existing for pooled therapeutic plasma products; one can expect that implementing dedicated inactivation treatments during the manufacturing process of HPL will become mandatory.

Pathogen inactivation can be done either on the starting platelet concentrate or on the pooled platelet lysate during the process of production. There are technologies for pathogen inactivation of platelet concentrates for transfusion that are already used by some blood establishments: typically these are based on a photo-inactivation process using either psoralen or vitamin B2, combined with UV irradiation. These techniques may satisfy the regulators in particular if the pool size is not too large. However, if the economic situation pushes towards a larger pool size of HPL, then regulators will likely be asking for two dedicated and complementary ('orthogonal') steps of viral activation or removal. One that is performed on the starting platelet concentrate for transfusion, and another implemented on the industrial pool. Alternatively, if the starting platelet concentrates are not subjected to a pathogen inactivation step, the platelet lysate pool could be subjected to two distinct virus reduction steps, if technically achievable.

Q HPL-expanded mesenchymal stromal cells are being tested in clinical trials now. Is HPL being studied for expansion of other cell types as well?

TB: There is less work at this time done on differentiated cells; however, a number of recent publications indicate that the HPL works very well for other cell types. In general, cells that can be grown in medium containing FBS can be grown in a medium where FBS is replaced by HPL. So, I think HPL should be beneficial for isolation and expansion of primary cells for transplant into patient under completely xeno-free conditions.

Q What are the challenges ahead for use of HPL in clinical-scale expansion of cells?

TB: If cell therapy continues to develop in a way some people expect or believe, one challenge will be HPL supply and cost. To meet the demands of the industry, it will be important to have continuous supply of HPL for expanding the cells, at an affordable cost. Right now, in blood establishments, about 10–15% of the platelet concentrates initially collected for transfusion end-up being discarded. If they are not transfused they are discarded because the platelet concentrates for transfusion are stored at 20–24°C with a shelf-life that is limited to 5 to 7 days due to concerns of risks of bacterial contamination. These ‘expired’ platelet concentrates are suitable to make HPL for cell expansion. In addition, not all collected blood is used to make platelet concentrate. So there is a potential to produce more platelet concentrate dedicated to make HPL, but this will have an impact on the cost.

Another challenge is the fact that yet only a limited number of blood establishments in industrialized countries have interests in producing HPL for cell therapy. One can expect that big blood establishments or commercial companies producing HPL will contribute to avoiding a wastage of expired platelet concentrates.

Finally, regulatory hurdles may present yet another challenge. As previously discussed, viral inactivation treatment will be important and not all producers may be currently capable to perform viral inactivation treatments, especially on pooled platelet lysates.

Q What other human blood products are being studied for cell expansion protocols?

TB: There has been research carried out with serum. Serum is blood collected without anticoagulant, and left to clot for a few hours to yield a supernatant containing plasma, no fibrinogen, and growth factors released by the platelets that have been activated. However, when blood is collected to make serum, neither red blood cells or plasma can be produced for transfusion. Therefore, I do not see the blood establishment collecting blood to make serum at a large scale. They will prefer to use the licensed blood processing system already in place to prepare platelet concentrate and suitable for cell expansion.

Plasma has been tested as well. Some groups have found it suitable for growing cells *in vitro*, but it is essentially deprived of many platelet growth factors and cannot be used to expand the cells. Plasma is also used for

transfusion or can be used to make a lot of other therapeutic products by fractionation. Therefore it is not an ideal material for cell expansion.

Q Where do you see the next opportunities for advancing the large-scale expansion of cells?

TB: One opportunity that requires further investigation is to fractionate the HPL into different types of growth factor fractions, to make 2–3 products instead of 1, from the same HPL batch. Perhaps specific or particular fractions of platelet lysate will open the potential

“Implementing dedicated virus inactivation treatments during HPL manufacturing will become mandatory to ensure safety of HPL.”

for direct clinical applications of HPL in regenerative medicine. For example, HPL-derived therapies for bone regeneration, wound healing or treatment of neurodegenerative disorders may benefit from dedicated,

specialized platelet fractions with a very well controlled composition in specific growth factors.

Another area where HPLs may provide benefit at the research and clinical level is in tissue engineering. Currently, FBS is still typically used at research scale to design and develop novel constructs or scaffolds. Using HPL instead of FBS from the start, at the research scale, may be important to better understand how cells react and grow in contact with a particular type of tissue engineering construct.

Finally, the last opportunity, which is probably more provocative, would be to use HPLs as a growth medium supplement for expressing recombinant proteins or vaccines. Right now, recombinant proteins are most often produced by cell lines in a chemically defined medium that typically does not contain proteins from animal or human origin. It is conceivable that genetically modified human or mammalian cells would have higher productivity if grown in a medium supplemented with human HPL. One could also speculate that recombinant proteins for substitutive therapy would exhibit a glycosylation pattern mimicking better that of the natural human proteins, thereby potentially decreasing the risks of development of inhibitors in treated patients.

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