Recombinant Human Albumin

With industry advancements in cryopreservation and stem cell therapies, the manufacturing, formulation, and handling challenges of these therapies need new considerations

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Stem cell and gene therapies are some of the most cutting-edge and sophisticated therapeutic developments. They offer attractive alternative approaches to widely used treatments in areas such as multiple sclerosis, metabolic diseases, cardiovascular disease, and liver disease. The field is burgeoning, but developers still face challenges, some of which can be addressed by using recombinant human albumin (rHA).

As a long-established ingredient of cell culture media, albumin is well recognised for its ability to facilitate growth of many cell types. With the industry expanding the use of high-quality, fully recombinant, cGMP excipients in cryopreservation and formulation of stem cell therapies, the manufacturing, formulation, and handling challenges associated with the development of cell therapies must be explored. The authors will also assess how the use of rHA in the culture, expansion/differentiation, cryopreservation, and formulation of stem cells compares with alternative approaches such as albumin(s) derived from human (human serum albumin [HSA]) or bovine (FBS) serums.

Overcoming Cell Therapy Challenges

Stem cell therapy, a type of regenerative medicine, promotes the repair response of diseased, dysfunctional, or injured tissue using stem cells or their derivatives. Cell therapy can face many challenges in regard to reproducibly optimising, controlling and scaling cell isolation, expansion, transformation, cryopreservation, and final formulation. From a processing angle, cell viability, growth rate, and reproducibility can all present serious issues for manufacturers. These can be affected by aggregation of the cells, shear stresses, and contact of the cells with surfaces. Additionally, the maintenance of cell identity and multipotency and the assurance of cell survival during transformation can also lead to setbacks during development. Regarding formulation, quality control of final products, storage and transportation, apoptosis, loss of identity, multipotency, and safety all present considerable challenges. The cost of goods to manufacture a cell therapy can also have an enormous impact on the long-term viability and sustainability of the therapy as a commercialised product.

Serum and HSA have been found to be useful stabilisers in cell culture and preservation media. Albumin is one of the earliest and most widely used proteins in the pharmaceutical field. Historically, serum has been an important component of cell culture methodology as a provider of complex biological molecules such as hormones, growth factors, attachment factors as well as numerous low molecular weight nutrients. In the development of stem cell culture, albumin has been shown to contribute a variety of functions (1). As the most ubiquitous protein in blood, human albumin is present at amounts of approximately 40g/L. It acts as a buffer or reservoir for smaller entities such as metals, hormones, fatty acids, and toxins. Combined with its ubiquitous presence in many tissues and body compartments, and its free flow between these compartments, albumin shuttles these smaller entities from areas of high concentration to low concentration. Additionally, albumin constitutes about 75% of the oncotic pressure of blood, and the single free cysteine of albumin makes up most of the reducing equivalents in blood.

Several of albumin's biological properties have been proven valuable across the cell therapy value chain, making it a useful component in the development of stem cell therapies, including:

- Transportation and complexation with metals or other beneficial molecular entities, creating an optimal microenvironment for sustained cell viability
- Acting as a rich nutrient source ensuring optimal conditions, particularly during cell proliferation
- Functioning as a pH buffer to prevent unwanted effects during differentiation

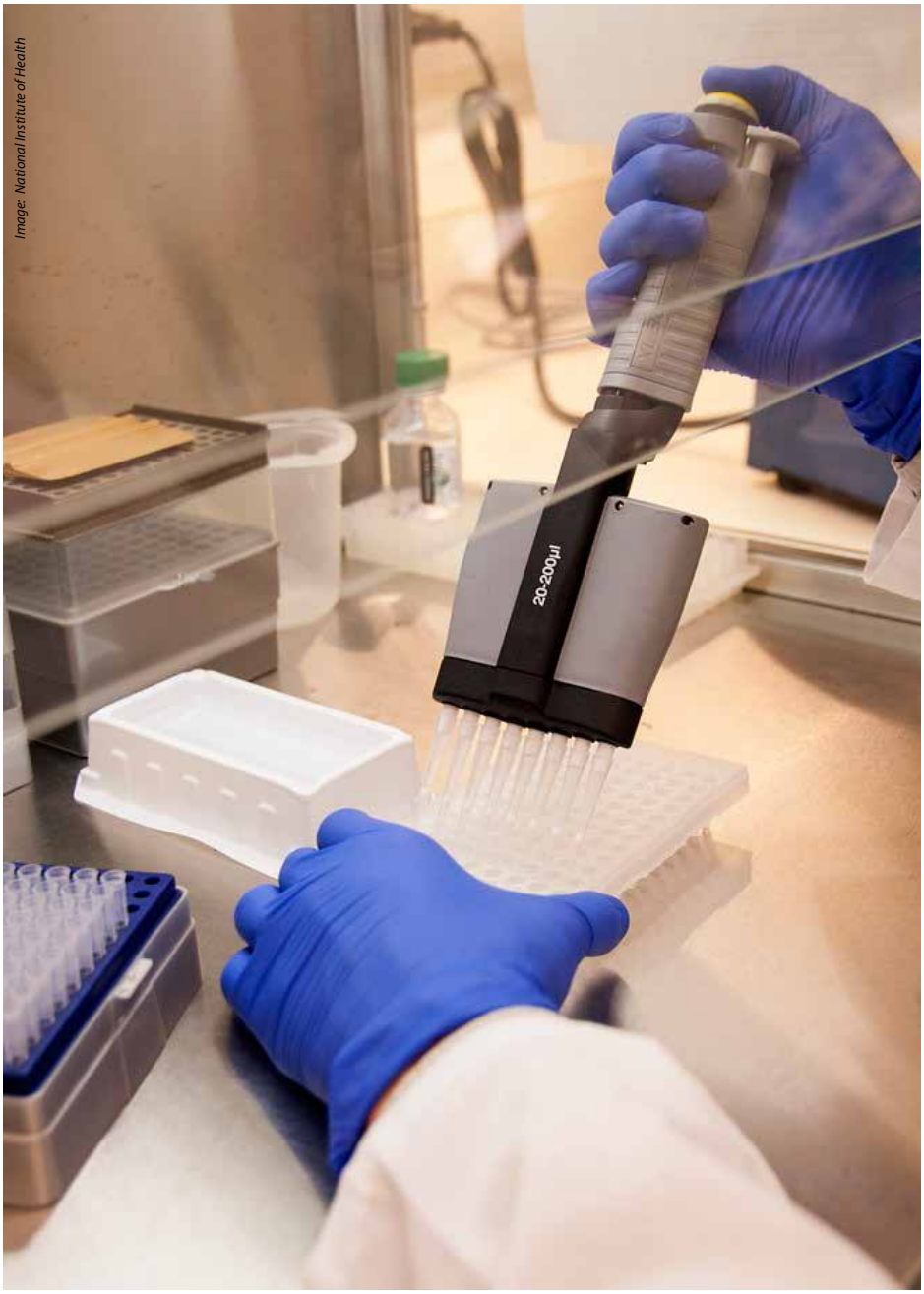

Image: National Institute of Health

- Maintaining cell viability during cryopreservation – driven by the high purity of recombinant albumin
- Acting as a scavenger of toxins and other reactive oxygen species, albumin protects cells against chemical stress all the way from culture to patient
- Providing an insulation effect in media due to its propensity to distribute evenly throughout solutions

Why Recombinant Human Albumin?

Albumin has a long history of medical use dating from the 1940s. Originally prepared from pooled plasma, its unique properties have been exploited in a range of medical applications, including plasma volume expansion, the treatment of sepsis, detoxification, imaging and diagnostics, medical device coating, and therapeutic peptide and protein stabilisation. Regardless of the numerous advantages provided by albumin, regulatory concerns over blood-borne contaminants (mycoplasma, viruses, and prions), potential problems with reliability of supply, and the performance variability between batches of HSA and undefined serum has seen an intensification in industry demand for more well-defined, well characterised, and easily controlled media. The use of rHA enables complex culture media to be chemically defined, as it provides a serum-free, cGMP raw material of high consistency and purity. Moving towards chemically defined media is favoured by the regulatory authorities as comprehensive quality information must be available for all their constituents.

Performance, cost, and regulatory compliance are the three most referenced factors that are considered when choosing an albumin source to use in stem cell culture with a therapeutic endpoint. In the context of technical and performance necessities, the use of rHA should support the beneficial growth of undifferentiated stem cells in a vigorous and reproducible fashion. rHA contributes many useful traits in the development of stem cell therapies in both upstream and downstream processing. In addition to improving the regulatory pathway of these types of therapies, the use of rHA can bring considerable improvements in the viability of cells while controlling batch-to-batch variability through higher tolerance to stress and contributing significant improvements to cell viability. These attributes call for cell therapy and stem cell media developers to now look at recombinant albumin in a new way and consider its potential for the clinical development of cell-based therapies. Due to cryopreservation and expansion improvements, recombinant albumin has been shown as a very important tool to improve the functional performance of these cutting-edge stem cell therapies, in addition to the intrinsic safety and regulatory advantages. Let's take a closer look.

Addressing Formulation Challenges

Formulation typically occurs directly after stem cell generation or thawing. As with any pharmaceutical, the right formulation is important to reproducibly deliver a functional cell therapy. Prior to a product being administered to patients, several time-consuming release assays must be successfully performed following formulation. As a result, the longer that cells can be maintained in a stable state, the greater the applicability and flexibility of the therapy.

A stem cell formulation prepared in a controlled medium will generally make for a smoother release of the therapy as analysis can focus on the therapy itself rather than the potential effects and impurities from the medium. The controlled nature of the formulation should reduce variation in the background of biological assays. However, due to carry over between process steps, it is not enough to employ controlled media in only the generation of the final formulation. If a controlled final formulation is desired, this must be designed into the process sufficiently upstream to ensure enough dilutions and exchanges have taken place to mitigate any risks from uncontrolled substances. Therefore, components such as rHA need to be considered and introduced upstream of the final formulation to minimise additional scrutiny of the media by the regulatory bodies.

Addressing Cryopreservation Challenges

Cryopreservation is an important and often necessary step in the development of commercial stem cell therapies. It removes the necessity for a continuous process, generating improved flexibility and logistical benefits. Cryopreservation generally takes place at two stages in the manufacturing process, leading to the differentiated stem cell therapy dose. The first stage is typically at an upstream point, usually as close to the harvest or generation of the stem cells as possible. The second stage takes place as far downstream in the process as possible, preferably after the production of the differentiated stem cell therapy dose.

The initial stage in the cryopreservation process can provide economies of scale, as multiple batches can be produced either in parallel or in combination depending on the nature of the therapy. The second phase permits flexibility in distribution and administration of the therapy. These advantages are what make the process highly beneficial in the development of commercially viable products.

During cryopreservation, stem cells are normally removed from their growth medium, which supports their biological needs for growth, and placed in a cryopreservation medium. This environment generally contains no small molecular nutrients,

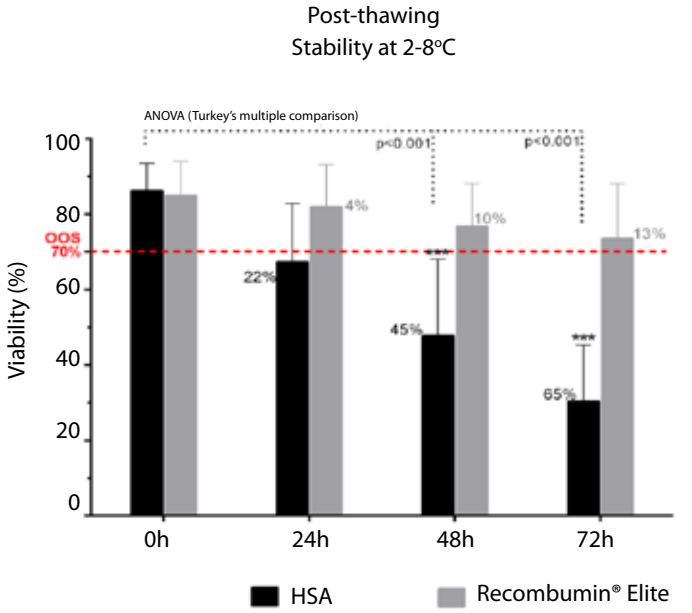

Figure 1: Increasing product shelf life

but some salts and buffers to maintain pH and isotonic conditions, and various cryopreservation agents such as DMSO and albumin. After formulation, stem cells are quickly frozen. Once thawed, they are either returned to a growth medium supporting further processing or to a final formulation solution depending on whether they are to be further processed or administered.

Albumin gives numerous distinctive improvements to the cryopreservation process that help stem cells to tolerate media change and transition, including its buffering capability and its capacity to stabilise entities in solution. Figure 1 shows the viability of hMSC post-thaw from cryopreserved stocks, comparing Albumedix recombinant albumin (Recombumin® Elite) to HSA. When relying on HSA, the viability drops significantly below the release criteria of 70% viability, whereas Recombumin® Elite maintained viability of the cells after thawing considerably better compared to HSA. The Recombumin® Elite product still met the acceptance criteria at 72 hours while products conditioned with HSA were out of specification at 24 hours. This study was carried out in collaboration with University of Barcelona, Xcelia and Banc de Sang I Teixits, Barcelona.

The purity and source of albumin also plays a significant role as it can prevent mesenchymal stem cells from progressing to late-state apoptosis compared to plasma-derived albumin (2). Cells do not only have higher viability post thaw, but even after injection, the general state of the cells is better as they are not already on the track of cell death, which, it could be argued,

results in a more effective final therapy. This higher-viability post thaw of stem cells cryopreserved with rHA ensures a longer shelf life than was formerly attainable using other cryopreservation media.

Enhancing Efficiencies

In countless instances, cell therapies are modified to alter their function, to activate in the case of immune cells, or to express certain proteins or antigens on their surface. When modifications are performed by means of viral vector gene therapies to deliver recombinant DNA, chimeric antigen receptors, or CRISPR-Cas9 modifications, recombinant albumin has the potential to improve gene therapy. A recent paper by the University of North Carolina, US, showed that adding HSA prior to cryopreservation or during formulation improved adeno-associated virus (AAV) vector transduction 5-7 fold resulting in a concomitant increase in expressed and active protein (3). Additionally, mechanism studies suggest that "human albumin increased AAV vector binding to the target cell surface and resulted in faster blood clearance after systemic administration, but did not impact AAV infection pathway". Therefore, whether such vectors are used to modify cell therapies, or to serve as standalone therapeutics, the addition of albumin can significantly improve the performance of the modified cells. It is not clear if the use of albumin would improve other viral vectors at this time, thus, further exploration would be warranted. In this endeavour, rHA has the potential to be superior to HSA for such cutting-edge technologies.

The Future of Stem Cell Therapies

The progress of stem cell therapies poses numerous development and formulation challenges. Therapy heterogeneity is one of the leading concerns and is a function of the complete value chain design, from harvesting, upstream culture, and cell preservation to cell therapy administration. Accordingly, pharma companies are determined to understand and control all steps in order to optimise cell viability and variability. rHA enhances cell therapy applications making it a proficient way for cell therapy developers to get superior results from their product.

From operating as a nutrient carrier, to safeguarding optimal growth conditions, commanding stabilisation of cell membranes and co-formulated proteins, working as a scavenger of free radicals, and acting as a viscosity moderator, it proffers a safe solution for optimised cell performance. Albumin readily coats surfaces so any unwanted interaction between cell and surface is eliminated. It can also protect cells from shear stress during processing and stabilise cells during freeze and thaw. Importantly, it does not contain any blood-derived impurities that otherwise could activate unwanted cell pathways. Additionally, recombinant albumin can help maintain the safety and efficacy of products during storage. Overall, rHA products are specifically developed to enhance the functional properties of albumin in cell culture, cryopreservation, and stem cell formulation, bestowing developers more confidence in their cell therapy.

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About the authors

Dr Phil Morton is the Chief Technology Officer heading up Albumedix's Technology Group. He has over 20 years' experience in the biopharmaceutical industry within process and product development both in R&D and manufacturing environments. His experience ranges from developing and transferring purification processes to formulation development and characterisation of these processes and products. Phil holds a PhD in Biochemical Engineering from Birmingham University, UK, and followed this with post-doctoral studies at Cambridge University, UK.

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