The use of albumin in stem cell therapy

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Introduction

The regenerative medicines field encompasses areas of cell- and tissue-based therapies, as well as gene therapy. While human albumin can be used in other areas of regenerative medicine, its major application to date has been in the field of stem cell therapies. The therapies of regenerative medicine hold much promise in treatment of a wide range of diseases including multiple sclerosis, cardiovascular disease, liver disease and cancer.

Albumin is a long-established ingredient of cell culture media and is well known to facilitate growth of many cell types. Historically it has been used in the context of serum. Later studies have shown that serum can successfully be removed from stem cell cultures, but that the presence of albumin then is favorable. In recent years, the positive properties of albumin in cell culturing have in some instances been expanded to its use in cryopreservation of, or formulation buffers for, stem cell therapies.

This document aims to give the reader an overview of albumin's use in the culturing, cryopreservation and formulation of stem cells and will discuss both practical and regulatory considerations.

Albumin at a glance

Human albumin is the most ubiquitous protein in blood and is present at amounts around 40 g/L. It is a single chain protein of 585 amino acids of about 66.4 kDa in mass [1]. Albumin has a compact heart-shaped structure with 17 internal di-sulfide bridges. Its role in blood is to act as a buffer or reservoir for numerous smaller entities such as metals, hormones, fatty acids and toxins. Combined with its ubiquitous presence in many tissues and body compartments, and the free flow of albumin between these compartments, albumin shuttles these smaller entities from areas of high concentration to low concentration and *vice versa*. Additionally, albumin also constitutes about 75% of the colloidal oncotic (or colloidal osmotic) pressure of blood and the single free cysteine of albumin (at position 34) makes up most the reducing equivalents present in blood. All these properties are traits which are functional in employing albumin in stem cell therapies.

Albumin in stem cell culture

Originally, crude human serum or fetal bovine serum was employed as a source of, amongst other substances, albumin in stem cell culture. As time has progressed the desire to have more well-defined, well characterized and easily controlled media has shifted the field towards using media comprised of individually added proteins. This shift has not always been favorable for the growth of the stem cell given that serum contains many constituents, some of which were beneficial for the stem cells in question. Therefore, when replacing serum, it was found that albumin in combination with several growth enhancers had to be added to the media for satisfactory performance. In some cases, people have struggled to achieve as good a growth as in the absence of serum, despite adding albumin and testing a wide range of growth factors. The unidentified factor(s) causing this lack of effect have often humorously been referred to as "pixie dust".

Many academic laboratories still use bovine serum albumin for stem cell culture, but the clear majority of industry prefers to avoid cross-species media and thus employ human albumin when in need of an albumin product to cultivate human stem cells. However, the human serum albumin product derived from human plasma is not a pure protein. Human serum albumin specifications often allow for up to 5% of non-albumin proteins to be present and accordingly, serum-derived products cannot be used as components in media claiming to be chemically defined. Therefore, recombinant human albumin must be employed to achieve this additional level of control and safety reassurance. Like the case of serum vs serum-derived albumin, it has in some cases been found that exchanging recombinant human albumin for human serum albumin results in slower growth, indicating that the serum-derived albumin can at times impart some "pixie dust" on the media [2,3]. But the opposite has also been found where the increased recombinant albumin purity improves the cell culture performance [4].

The exact role of albumin in stem cell culture is not known [5]. But over the years albumin has been ascribed several biological properties, such as; being a transporter and reservoir/sink for metals or other beneficial molecular entities; being a nutrient source; functioning as a pH buffer; acting as a scavenger of toxins and reactive oxygen radicals; covering surfaces to minimize direct interaction and acting as an "insulator" in media due to its propensity to distribute evenly throughout solutions. The actual function of albumin in stem cell cultures is probably a combination of all these properties. It is likely that it is the specific stem cell and exact culture conditions, which determines what properties of albumin are used and to what extent they are needed. This multifaceted functionality of albumin could explain why it is so often a good base protein to add to a medium, and why its use has caught on so widely, not just in stem cell culture, but in biology in general.

When considering what albumin source to use in stem cell culture with a commercial endpoint, three parameters usually come into play: technical requirements, cost and regulatory compliance. The technical requirements were touched upon above; the albumin product used should support good growth of undifferentiated stem cells in a robust and reproducible manner. For the technical requirements, there is strictly no bias for any of the albumin sources. But sources which have a high batch to batch variability will be difficult to handle in a GMP environment and if a choice must be made between slightly lower but reproducible growth compared to a higher yield, but with high variation, the first option will typically be favored. For the two other parameters, there is roughly a contrasting relationship, see **Figure 1**. In terms of the cost of goods for albumin, the price increases from human serum through serum-derived animal albumin and serum-derived human albumin to recombinant human albumin. However, the acceptability from a regulatory perspective also increases in parallel with serum-derived animal albumin being unacceptable in a commercial setting and human sources being acceptable to the regulators. Over the past few years, recombinant human albumin has entered the field of stem cell cultivation. As an animal-free, GMP raw material of high consistency and purity, the use of recombinant human albumin enables the culture media to be better defined and controlled. These media not only afford higher cell growth reproducibility, but are also favored by the regulatory authorities as comprehensive quality information is available for all their constituents.

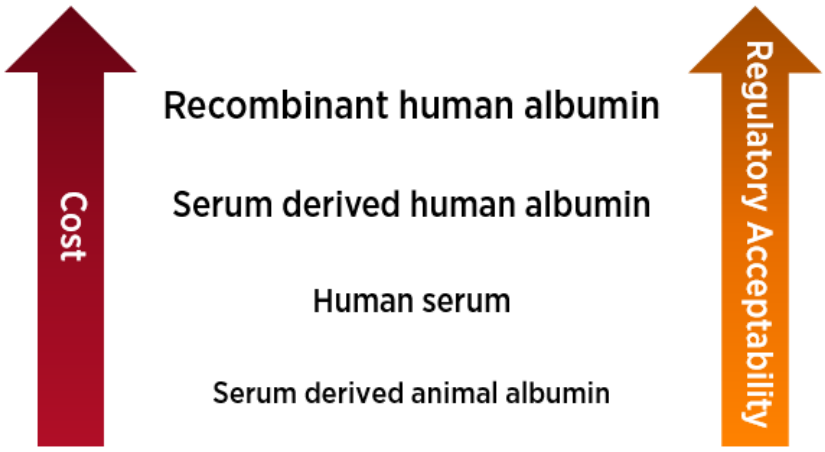

Figure 1 Considerations for choice of albumin

Albumin in stem cell cryopreservation

Cryopreservation is a crucial step for commercial stem cell therapies as it alleviates the need for a continued process thus allowing for flexibility [6].

Typically, it is advantageous to have cryopreservation steps at a minimum of one of two positions in the process leading to the differentiated stem cell therapy dose. The first position is somewhere upstream, often as close to the harvest or generation of the stem cells as possible. The second position is as far downstream in the process as possible, ideally after the production of the differentiated stem cell therapy dose. The first cryopreservation step potentially enables economy of scale as multiple batches can be produced either in parallel or in combination depending on the nature of the therapy. The second cryopreservation step allows for flexibility in distribution and administration of the therapy. Both these possibilities are highly desirable to have if a commercially viable product is to be developed.

We have demonstrated recombinant albumin to be beneficial for the survival of stem cells during cryopreservation, more so than plasma derived albumin. For cryopreservation, stem cells are usually removed from their growth medium, which supports their biological needs for growth or otherwise, and placed in a cryopreserving medium. Normally, this medium contains no small molecular nutrients, but some salts and buffers, to maintain pH and isotonic conditions, and various cryopreservation agents such as DMSO etc. and albumin. Once the stem cells are formulated they are quickly frozen. Upon thawing, stem cells are either quickly 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.

The advantage of albumin in cryopreservation is probably due to a combination of its properties, including the ability to cover surfaces, its buffering capacity and its ability to stabilize entities in solution, i.e. all functions that help the stem cells to endure the media change and phase transition during the cryopreservation. But the purity and source of albumin also plays a role as we have found that, yeast-derived recombinant albumin in cryopreservation can prevent mesenchymal stem cells from progressing in to late state

apoptosis compared to plasma derived albumin. This effectively makes for a higher viability post thaw of stem cells cryopreserved with recombinant human albumin compared to human serum albumin.

Considerations akin to those above in **Figure 1** are also relevant for the albumin source for cryopreservation use. Again, the paramount decider will be the technical requirements. But beyond this, now the cost of goods will be of less importance here as the volumes needed in cryopreservation are much lower than those needed for cell culture.

Albumin in stem cell formulation

The formulation used for stem cell products is crucial for the ability to reproducibly deliver a functional therapy. Typically, stem cell therapies will be formulated immediately after either their generation or thawing. After formulation, several time-consuming release assays must be successfully performed prior to administration to the patient. Therefore, the longer the stem cells can be maintained in a stable state at this point, the greater the applicability and flexibility of the therapy. A crude rule of thumb is that one-day stability will allow for onsite administration, two-day stability will allow for nationwide distribution and three-day stability in theory allows for worldwide use.

A stem cell formulation prepared in a controlled medium will generally make for a smoother release of the therapy, as the analysis can focus on the stem cell therapy itself rather than potential effects and impurities from the medium. Furthermore, 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 only to employ controlled media in the generation of the final formulation. If a controlled final formulation is desired, this must be designed into the process sufficiently upstream to ensure that enough dilutions and exchanges have taken place to mitigate any risks from uncontrolled substances. Thus, controlled substances, such as recombinant human albumin, should be considered introduced well in advance of the final formulation.

Practical considerations for using albumin in stem cell therapies

The many abilities of albumin mentioned above are likely to need different amounts of albumin to be effective.

In general, it is advantageous to try two different concentrations of albumin, a high and a low concentration, to cover its different biological properties experimentally. What exactly high and low entail will depend on factors relating to the cell (e.g. type, concentration, phase etc.) and production process step. Typically, it is advisable to commence with the high concentration being in the order of 2-5% of albumin and the lower concentration being about ten times lower. Then, if some beneficial effects are seen, the albumin content can be further optimized by further experimentation.

Recombinant human albumin supplied by Albumedix is in a ready-to-use liquid form with the albumin at 10% concentration or higher depending on product. Due to the high concentration of the albumin material, a simple addition/dilution of the liquid albumin is usually workable and easily implemented in both formulation studies and at later large scale production.

If you have any further questions regarding the use of recombinant human albumin products from Albumedix, please do not hesitate to contact us. We have dedicated albumin scientists who can guide and support you in the use of albumin in stem cell therapies.

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