Stem Cell Research & Therapeutics
SCRT, 1(1): 30-40
www.scitcentral.com

Original Research: Open Access

Quality Control Considerations for Clinical Use of Stromal Vascular Fraction (SVF) Based Therapies in the United States

Ryan A. Lockhart[2], Cloe S. Hakakian[2], Zoe Birnbaum[2] and Joel A. Aronowitz[1,2,3]

[1]*Cedars-Sinai Medical Center, Los Angeles, CA, USA*

[2]*University Stem Cell Center, Los Angeles, CA, USA*

[3]*USC Keck School of Medicine, Los Angeles, CA, USA*

Received April 29, 2016; Accepted June 15, 2016; Published June 29, 2016

INTRODUCTION

With a variety of SVF based therapies beginning to translate into the clinical setting, it is important for clinicians to be aware of the regulatory considerations and quality control measures which need to be in place in order to build reliable data and ensure patient safety. Important factors to consider are the composition of the SVF output from the isolation method being employed, the dosing scheme, route of administration appropriate for each specific treatment, as well as adequate infection control protocols. The methodology described in this review is only applicable to the autologous use of freshly isolated SVF cells. Additional quality control and regulation measures are necessary for other, non point of care uses, such as in a wound care matrix or expansion of cells in culture.

Brief Regulatory Overview

Current regulation of the clinical use of autologous stromal vascular fraction cells in the United States remains somewhat convoluted and uncertain. Use of autogenous cells at the point of care was long considered to fall within the scope of the practice of medicine and therefore regulated at the state level. However it is now clear that the field is heading towards an era of significantly higher regulation at a federal level. The FDA in the United States issued a series of draft guidance announcements beginning in October 2014 concerning the use of adipose-derived human cellular, tissue, and cellular and tissue-based products (HCT/Ps) [1-4]. These draft guidance documents are not binding but are used to express current FDA thinking. A reading of these draft guidance publications readily reveals the FDA's position that the clinical use of autologous SVF cells does not fall under the practice of medicine or the same surgical procedure exemption. FDA has concluded that the SVF isolation process, whether enzymatic or purely mechanical, represents more than minimal manipulation. In this view, the isolation of autologous SVF at the point of care by whatever means creates a tissue product subject to FDA oversight. This emerging FDA view means the clinical use of SVF will be subjected to a rigorous regulatory pathway and enforcement methods unfamiliar to most clinicians.

A three-tiered system based on level of risk was established by the FDA to classify the use of HCT/Ps in clinical practice. Table 1 summarizes the FDA recognized regulatory categories. Previously, it was assumed that the use of SVF was regulated only under 21 CFR part 1271 and Section 361 of the Public Health Service Act (PHS), and as such deemed "361 HCT/Ps" [5,6]. When SVF is considered a 361 HCT/P, it's use is subject to very little to no FDA regulation under the assumption that SVF prepared under these criteria qualified for almost every exemption when used autologously in the same surgical procedure using surgical practices meeting the local standard of care. The "more than minimally manipulated" classification of SVF cells subjects them to additional regulation under Section 351 of the PHS act and/or the Food, Drug and Cosmetics Act (FD&C), making them "351 HCT/Ps" [6,7]. As a 351 HCT/P, clinical use of SVF cell-based therapies will be required to be conducted in formal FDA-approved clinical trials under the regulatory approval of an Investigational New Drug (IND) application or an Investigational Device Exemption (IDE) in addition to institutional approval from an Institutional Review Board (IRB).

Corresponding author: Joel A. Aronowitz, Cedars-Sinai Medical Center, Los Angeles, CA, USA, E-mail: dra@aronowitzmd.com

Citation: Lockhart A R, Hakakian S C, Birnbaum Z & Aronowitz A J. (2016) Quality Control Considerations for Clinical Use of Stromal Vascular Fraction (SVF) Based Therapies in the United States. Stem Cell Res Th, 1(1): 30-40.

This is because ultimately these therapies will have to acquire a Biologics License Application (BLA) before they can reach the market. FDA-approved clinical trials require extensive amounts of preclinical data and product characterization as well as a set of quality control measures in place to ensure patient safety. The cost of the additional analysis as well as the costs associated with designing, initiating and conducting a clinical trial can be quite substantial and put an undue financial burden on smaller practitioners and essentially prevent them from being able to participate in the clinical research process without government or industry support. The 351 HCT/P classification of SVF cell based therapies excludes a significant portion of clinicians from offering these therapies to patients because they lack the time, money and regulatory knowledge to successfully navigate the complex regulatory framework involved with establishing a formal clinical trial and dealing with the FDA.

Infection Control

When it comes to a clinical treatment using SVF cells, infection control is one of the primary concerns in regards to ensuring patient safety. There are 2 main tests which need to be run in order to adequately assess the sterility of the product for human administration. These tests are a STAT gram stain and aerobic/anaerobic cultures, usually run for 3-5 days [8]. **Figure 1** and **Table 2** summarizes the suggested quality control assays as well as the clinical workflow.

Table 1. Summary of Regulatory Categories [5,44]

Category	Risk Level	Definition	Characteristics	Examples
Practice of Medicine	Lowest	Involves diagnosis, treatment, or correction of human conditions, ailments, diseases, injuries, or infirmities whether physical or mental, by any means, methods, devices, or instruments.	Not subject to FDA pre-approval Regulated at the state level	Organ transplant, Bone marrow transplant, Blood derived products
361 HCT/Ps	Mid-level	Any human tissue derived from a human body and intended for transplantation into a human that is minimally manipulated, intended for homologous use, not combined with another agent (with exceptions), and does not have a systemic effect and is not dependent up on the metabolic activity of living cells for its primary function, or if it has such effect, is intended for autologous use or allogeneic use in a close relative.	Regulated solely under 21 CFR part 1271 Exempt from FDA pre-market approval and clearance processes Must be minimally manipulated Intended for homologous use only	Acellular dermal matrices for wound care Fat grafting to the hands/face Cryopreserved vein/artery grafts for hemodialysis
351 HCT/Ps	High	Any human tissue derived from a human body and intended for transplantation into a human that does not meet the criteria for a 361 HCT/P and does not meet any of the exemptions laid out in section 361 of the PHS Act	Regulated under 21 CFR part 1271 and the FDCA and/or Section 351 of PHS Require full pre-approval biologics license applications (BLA) if not exempt under Section 361 of PHS Act	SVF cell therapies Fat grafting to the breast Lyophilized amniotic membrane (powder)

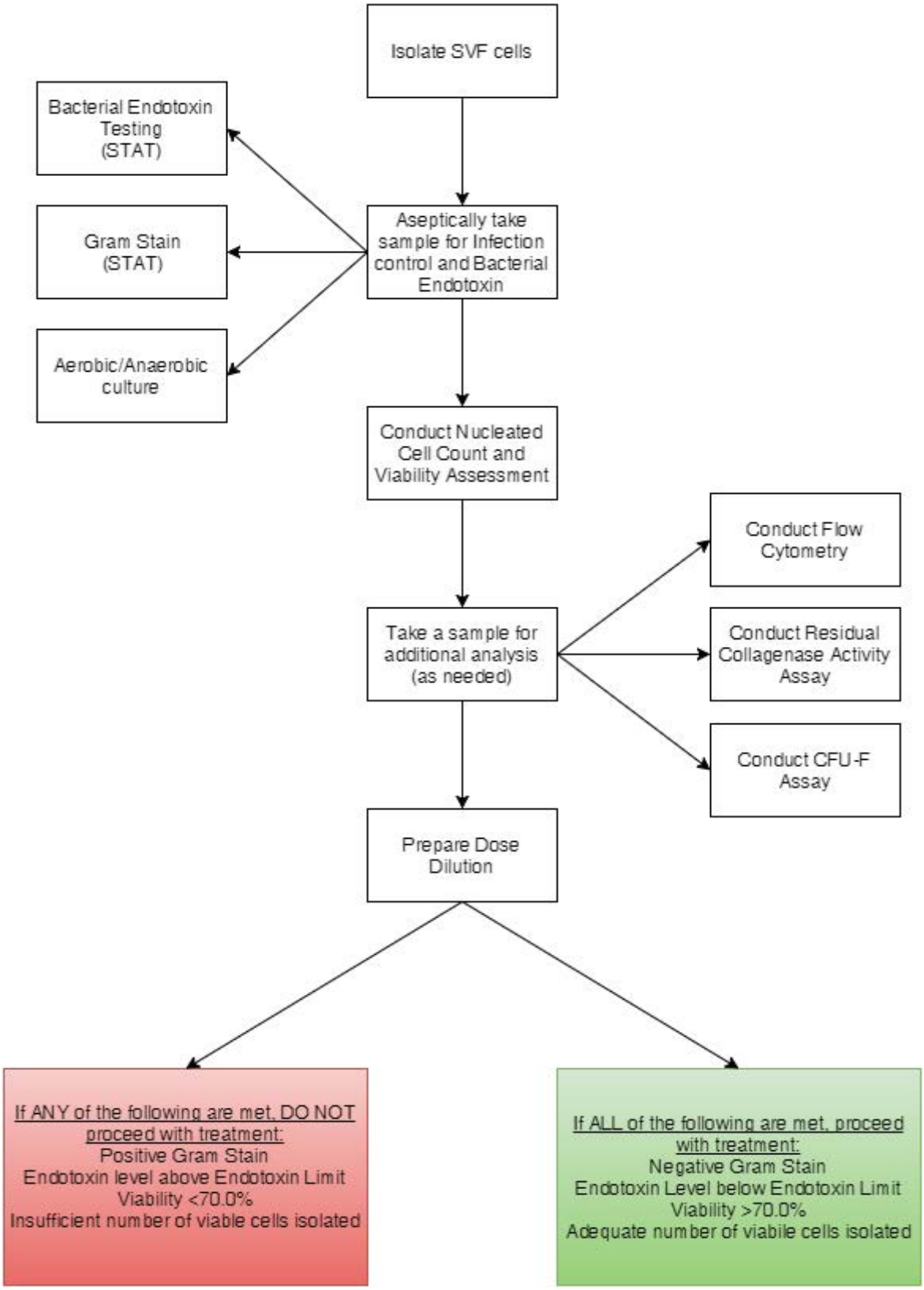

Figure 1. Summary of Clinical workflow

Table 2. Summary of Quality Control Assays

QC Aspect	Leading Assays	Rationale	Acceptance Criteria
Infection Control	Gram Stain AND Long Term aerobic/ anaerobic cultures	Gram stain detects for immediate signs of contamination. Culture detects presence of any slow growing bacteria that may not have been seen in gram stain	No bacteria seen in gram stain
Bacterial Endotoxin Testing	Limulus Amoebocyte Lysate (LAL) assay OR Rabbit Pyrogenicity Test	Ensures that sample is free of significant amounts of endotoxins and pyrogens.	Endotoxin level below acceptable endotoxin limit
Nucleated Cell Count and Viability	Various methods	To assess the efficacy of the isolation process and determine that an acceptable number of viable cells have been isolated in order to adequately prepare the required therapeutic dose	Cell count and viability above the acceptable threshold
Residual Collagenase Levels	Wunsch Assay OR FALGPA Assay	To ensure that there is not an excess of proteolytic enzymes remaining in the sample	Product is previously qualified as having low levels or levels are below acceptable Threshold
Product Characterization	Flow Cytometry AND CFU-F Assay	Flow cytometry determines the relative cellular composition of the SVF cell population CFU-F assay provides information on the quantity of stem cells present in a sample, as well as the growth characteristics of those cells.	Not included in lot release criteria. An adequate profile for one or both of these assays should be established prior to clinical initiation for a given isolation method.

The STAT Gram stain and anaerobic cultures will test for the presence of microbial contaminants. From a practical standpoint, once the final SVF is isolated, a sample will immediately be sent over to a local pathology lab (or done in house if proper staffing, facility certifications and equipment is in order) for gram staining and cultures. The turnaround time for a STAT gram stain is usually about an hour, but this can differ based on various factors such as the proximity, workload and staffing available at the pathology lab. Administration of the therapeutic product cannot proceed until the results of the gram stain are received and are negative for the presence of bacteria. If a positive gram stain result is returned from the pathology lab, then the procedure should not proceed (**Figure 2**).

The anaerobic and aerobic cultures can be most easily carried out using a standard blood culture set such as a BD BACTEC aerobic and anaerobic culture bottle set (Becton, Dickinson and Company, Franklin Lakes, NJ). These can easily be aseptically inoculated and then sent off to a local pathology lab to maintain and analyze. As the results from these cultures will not be received before the treatment occurs, these are not intended to be a clinical deterrent. Even in the event of a positive culture result, the subject does not necessarily need to be withdrawn from participation in a clinical trial, but rather should be monitored closely for potential infection. A positive aerobic or anaerobic culture does not necessarily mean that the patient is going to develop an infection. Additionally, pathology or microbiology labs typically offer microbial identification as well as specificity testing which can help guide clinicians to the proper measures such as which antibiotics to use in the event of an infection.

Bacterial Endotoxin Testing

Bacterial endotoxin testing is another important quality control measure for SVF as well. The US FDA will require this for all SVF and ASC based therapies [9,10]. Bacterial endotoxins are lipopolysaccharides present in the cell membrane of gram negative bacteria. Endotoxins are pyrogenic, meaning that they can potentially cause fever or disease if present at high enough levels *in vivo*. While bacterial endotoxins are associated with gram negative bacteria, the bacteria does not have to be viable in order to exert pyrogenicity, meaning that even membrane fragments of gram negative bacteria can cause fever or disease at high enough levels. This is an important parameter to track in terms of patient safety, especially if the therapeutic product is to be injected or administered intravenously. SVF can potentially have higher levels of endotoxin as a result of the tissue dissociation enzyme mixture used to dissociate the lipoaspirate. The GMP grade tissue dissociation enzyme (TDE) mixtures tend to contain proteolytic enzymes from bacterial origins. As a result, there are residual levels of endotoxin present in the final lyophilized product as a result of the manufacturing process [11]. While there are acceptable standards for endotoxin levels in the TDE products in order to be GMP grade, clinicians should be aware of these endotoxin levels which in combination with other potential endotoxin sources can potentially raise sample levels above that acceptable threshold for treatment. The certificate of analysis should provide the endotoxin level present in the TDE product.

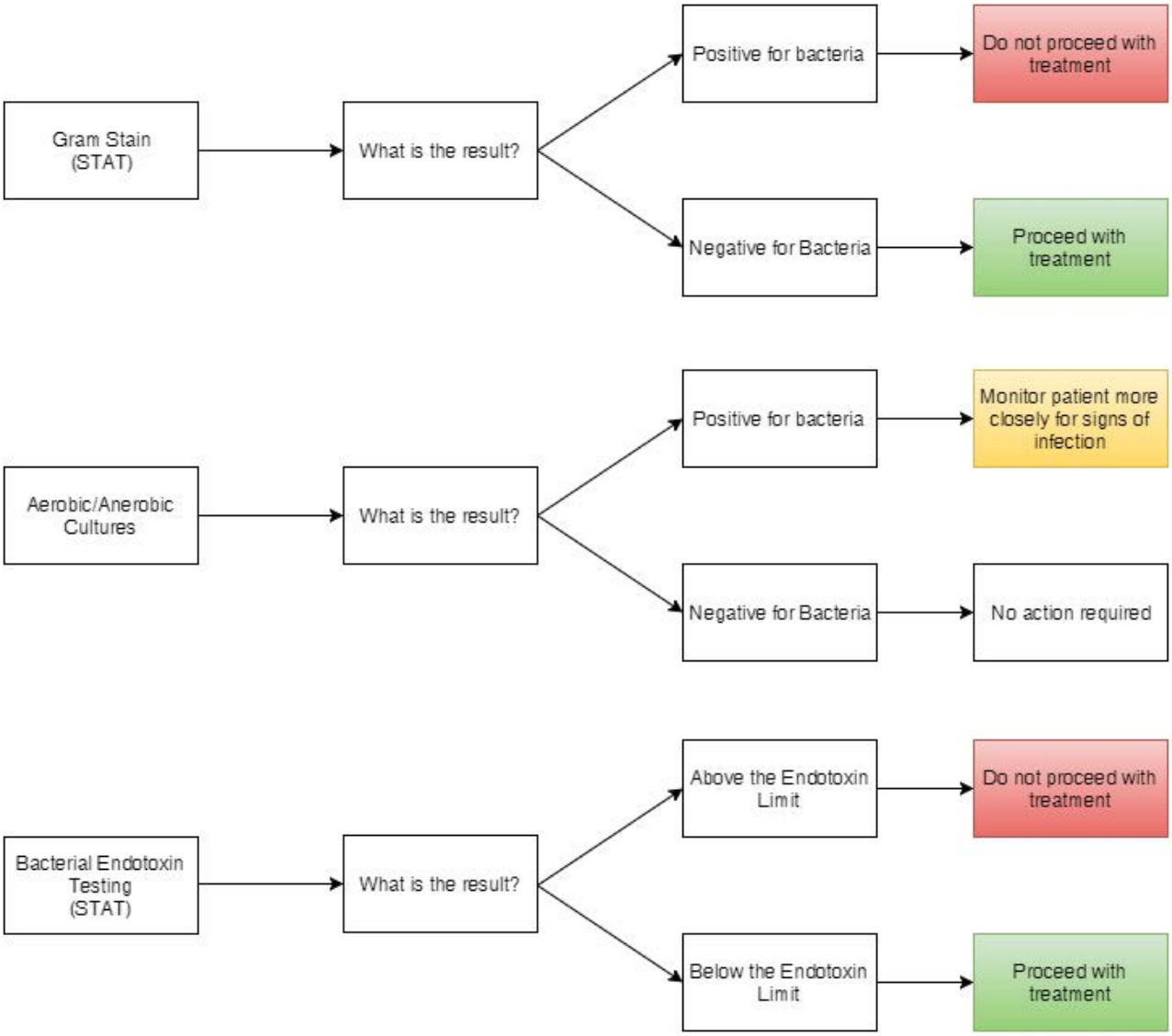

Figure 2. Summary of infection control and Bacterial endotoxin testing outcomes.

Ideally, a STAT endotoxin test sent out to a local pathology laboratory would be the easiest way to have this conducted because no specialized equipment would need to be acquired. Unfortunately many microbiology/pathology labs do not offer a STAT format for this test, but rather the test would have a turnaround time ranging from a few days to a week. This leaves the alternative of running this test at the point of care in house. The leading assay used to assess endotoxin levels is the Limulus Amoebocyte Lysate (LAL) assay. There are systems available for the point of care endotoxin testing, such as the EndoSafe-PTS100 or EndoSafe NexGenPTS, (Charles River Laboratories International, Inc., San Diego, CA). These two systems use FDA approved single-use test cartridges to run point of care endotoxin testing with a 15 minute turnaround time. The alternative to purchasing the system or sending out the sample to a pathology lab is to run the LAL assay in house. The necessary reagents may be purchased and the assay can be run using a microplate reader and minimal other equipment. There are 3 versions of the LAL assay which can be run: the gel-clot method, the chromogenic method and the turbidimetric method. All three methods are approved by the United States Pharmacopeia for use with injectable drugs and other products [12].

The lot release specifications pertaining to endotoxin are based on the endotoxin limit. The endotoxin limit is the threshold established for a product to be safe enough for human use. If the product measures an endotoxin level above the threshold, clinical treatment should not proceed (**Figure 2).** The endotoxin limit varies from patient to patient and is a measurement calculated based on the weight of the patient, route of administration and the maximum amount of the therapeutic agent which will be injected. For a detailed explanation of how to calculate the endotoxin limit, see Chapter 85 of the United States Pharmacopeia [13].

Nucleated Cell Counting

Nucleated cell counting is arguably one of the single most important aspects of quality control. It will typically be the first checkpoint reached in terms of lot release specifications for clinical use. From a clinical workflow perspective, after completing isolation, a sample should be taken and sent out or set aside for infection control testing and endotoxin testing followed immediately by cell counting. There will be some downtime before the results of these tests are received, leaving time to complete cell counting and other required analysis.

The isolation of SVF cells can be somewhat variable from isolation to isolation and depends on many factors, some of which cannot be control, mainly patient to patient variation. Setting lot release specifications is an essential part of a proper manufacturing process and should not be ignored as they will be required by the FDA [8] and ensure a minimum level of quality for cell-based therapies. The SVF cell isolation will need to hit a certain threshold of viable nucleated cells while also being above the threshold of acceptable cellular viability (usually ≥70%). If an isolation does not meet the predetermined lot release criteria, then the clinical treatment should not proceed.

Nucleated cell counting typically does not require a large sample volume, typically only a few hundred microliters. Nucleated cell counting can be conducted in a variety of ways, but it helps to uses those which the FDA has deemed valid, such as the Chemometec NC-100 or NC-200, which effortlessly can provide nucleated cell counts and cellular viability in a matter of minutes. A sample cell count is shown in figure 3. These systems are expensive, so if funding is not available a cheaper alternative such trypan blue staining and manual counting using a hemocytometer can be employed. There are a variety of options available for determining nucleated cell counting and viability, but typically the ease of use is inversely proportional to the cost to operate.

Dosing Schemes

The FDA will require all clinical trials to contain calculated dosing schemes [8]. Depending on the indication of use, the dosing pattern will vary. For example, if using SVF cells for the treatment of a chronic wound or hair growth, the dose will be calculated as cells per area (ie 500,000 viable nucleated cells/cm^2). Other indications, such as intra-articular injections for the treatment of osteoarthritis might be established as a set number of nucleated cells (ie 10 million viable nucleated cells). The difference between the two being that for the former, the minimum number of cells required to be obtained from the isolation process can vary based on the size of the treatment area (a 10cm^2 wound will require more cells than a 5cm^2 wound), whereas the latter will remain constant. Another aspect which should be considered is the combination of SVF cells with lipoaspirate tissue for cell-assisted lipotransfer (CAL) and cell-enhanced fat grafting procedures. For this, the dose would ideally be calculated as a density of cells per volume of graft material (ie 10,000 viable nucleated cells per mL of graft material). It is also important to note that there is very little information currently available in relation to dose-versus-effect in terms of SVF cell based therapies. That being so, the FDA will almost certainly require some amount of dose exploration for all trial, for example a high dose (40 million viable nucleated cells) and a low dose (20 million viable cells). Controlling the dose and instituting an aspect of dose exploration is the only way that a valid dose-vs-effect relationship can be developed. **Table 3** summarizes appropriate dosing schemes.

Another aspect to consider when preparing the dose is that you must take into account the volume of sample being taken for analysis and not used for treatment. Typically 1-2 ml of the final output will be used for analysis, including cell counting, gram staining, 5 day aerobic/anaerobic culture and

potentially flow cytometry and CFU-F assays if being conducted. This will reduce the number of available viable nucleated cells that clinicians have access to for use in treatment. There are ways to avoid this which can be built into the isolation process, such as generating a larger volume of SVF output. It is important to use a little sample as possible while still meeting the minimum requirements for each specific test, so as to avoid not being able to meet the dosing requirement.

Table 3. Summary of Dosing Schemes

Dosing Method	Dose Calculation
Intradermal/Intramuscular injection	Viable nucleated cells/area Example: 1,000,000 cells/cm^2
Intravenous injection/infusion	Viable nucleated cells/ unit of body mass; Example:1,000,000 cells/kg
Cell-enhanced fat grafting	Viable nucleated cells/ vol of fat graft; Example:100,000 cells/ mL of fat
Intra articular injections	Total viable nucleated cells; Example:10,000,000 cells

Residual Proteolytic Enzymes

If the SVF cell isolation method employs the use of proteolytic enzymes, there will be a risk of excess levels of residual proteolytic enzymes in the final isolate, ie collagenase. The use of proteolytic enzymes is an easy way to drastically increase the yield of nucleated cells recovered during an isolation process, an aspect which is extremely beneficial if using the isolate immediately at the point of care [14]. Residual enzymes are a risk because in theory they can result in allergic reaction or unwanted tissue degradation *in vivo* if not adequately removed. The primary enzymes used for isolation of SVF cells are collagenase and neutral protease, both of bacterial origin.

Collagenase has been fairly well studied in terms of safety for human use in the two FDA approved iterations of the enzyme, Xiaflex and Collagenase Santyl. Santyl (250U/g) is a topical wound ointment for use in the nonsurgical debridement of wounds [15,16]. Xiaflex (3600 U per dose) is a highly concentrated mixture of type I and type II clostridial collagenases for the treatment of Dupuytren's contracture and Peyronie's disease [17-22].

The clinical and non-clinical evidence overwhelmingly supports the safety of clostridial collagenases. Collagenase was shown to have no systemic toxicity after local injection and systemic exposure was only observed if injected into highly vascularized areas [23-26].

Collagenase was also shown to be removed from the system rapidly, with neither isoform I nor II being detectable 2 hours after injection. Overall, collagenase has a very low level of toxicity [27-30]. In a previous study from our group in which we conducted 174 clinical isolations using collagenase, we noted no adverse events which could be attributed to excess residual activity of collagenase or neutral protease. As a note, we conduct 3 washing steps on our SVF isolate [31].

The easiest way to control for excess residual enzymes is to neutralize all activity of these enzymes. This can be done using autologous serum which can be isolated from the patient concurrently to the SVF isolation protocol. Serum has been shown to neutralize the activity of collagenase. This is because serum contains alpha-2-macroglobulin, which acts as an antiproteinase and inactivates a variety of proteolytic enzymes, including collagenase [32,33]. Alternatively, a 2013 study by Chang et al. 27 showed that so long as 3 washing steps are conducted of the resulting SVF isolate, that there is negligible risk associated with proteolytic enzymes because they are found in such low concentrations.

This is important because it will be required by the FDA for the isolation process being used to be evaluated in some way for safety of residual proteolytic enzymes before an IND/IDE will be granted [8]. This may entail animal studies or a qualification study measuring the residual enzyme levels in the final output. Obviously a small qualification study is more favorable than animal studies for a number of reasons, mainly cost and time. The most common assays used to assess collagenase activity are the FALGPA assay and the Wunsch Assay [34,35]. There are a variety of assays which can be used to assess neutral protease activity [36,37]. The residual enzyme levels in samples can be easily

measured using commercially available kits or manually by purchasing appropriate reagents. The goal of a qualification study would be to prove that the levels present in the final output are so low that they are not clinically significant and do not pose any significant risk to patients.

Flow Cytometry

Product characterization is very important aspect of quality control as well. Flow cytometry is important for clinical applications because it allows for identification of the abundance of the various cell types present in the therapeutic product. For SVF, being that it is a heterogeneous population of cells, this is important since every SVF isolation is different due to a variety of factors including differences in isolation techniques, differences in amount of tissue processed and patient to patient variation. While flow cytometry is not included in lot release criteria, the cellular composition will need to be established prior to clinical initiation of a later stage clinical trial (ie Phase 3) or established as a result of an early stage clinical trial (ie Phase 1/2) as part of the identity test for the therapeutic product [38].

There are a number of ways that flow cytometry can be conducted, but generally the more specific the flow cytometry analysis is (ie more surface markers), the more information that is ultimately learned. Ideally, through flow cytometry, the goal would be to identify the portion of the SVF which is actually composed of adipose-derived stem cells, which generally is the target therapeutic agent for clinical use. This is a small fraction of the SVF cell isolate, usually <2%. To do this, a more targeted flow cytometry protocol is required. Typically additional markers screened for are CD73, CD90 and CD105 [39-41]. It is crucial to note that the surface

markers for cultured ASCs and uncultured SVF cells differ. Typically, as SVF cells are cultured, they stop expressing CD34, and therefore express a different phenotype. Potential clinicians should be cautious not to make this mistake when reviewing pertinent literature on expected outcomes of cellular populations and surface markers used to identify them.

Figure 3. A representative readout from the Chemometec Nucleocounter NC-200. The sample tested was a 10% dilution of the final SVF output. The final volume of SVF was 12 mL total. 400 mL of lipoaspirate was processed. The total nucleated cell count for the whole SVF sample was 128.4 million nucleated cells. 321,000 nucleated cells were isolated per mL of washed lipoaspirate processed with 90.6% viability.

Colony Forming Unit-Fibroblast (CFU-F) Assay

The CFU-F assay is a valuable tool and a definitive method for quantifying the number of adipose-derived stem cells in a sample of SVF cells. It offers a more specific method for quantification than using a standard cell counting device, which non-specifically quantifies nucleated cells. This helps to further characterize the final product. The CFU-F assay

involves culturing a set number of cells (ie 1,000 and/or 2,500 viable nucleated cells) for usually 10-14 days and observing the growth characteristics. This assay will assess the number of colonies formed, which is an overall indicator of the frequency of adipose-derived stem cells. Additionally, this assay determines the population doubling time which assesses the overall growth characteristics of the adipose stem cells present within the stromal vascular fraction. Generally, a faster doubling time is preferred because it means that fewer days can be spent in culture in order to reach confluence. This saves money, as culture supplies can be expensive, and suggests higher metabolic activity of the cells. One of the most important considerations when conducting CFU-F assays is that the culture conditions must be identical from assay to assay. Results from assays conducted under different culture conditions are not comparable from an analytical standpoint because cells grow differently under different growth conditions. Hicok and Hendrick published a method for conducting a CFU-F assay on SVF cells [42].

This assay is not meant to be a clinical deterrent, but rather to further develop the identity of the therapeutic product. As mentioned earlier, the FDA will require the presence of an identity test in order to proceed with any clinical trial which is phase 3 or later. Doing a CFU-F in tandem with a 6 marker flow cytometry panel will give an accurate assessment of the composition of the SVF isolate once the data set is large enough.

With a large enough data set pertaining to stem cell content of the SVF, a profile can be generated when comparing a treatment subject's CFU-F frequency with the overall success rate of the procedure. This has been demonstrated with bone marrow aspirates used for treatment of tibial nonunions. In 2005, Herniguo et al. [43] reported a relationship between the numbers of CFU-F present in grafted bone marrow aspirates with the success of the grafting procedure to obtain bone-healing of tibial nonunions. They observed that more CFU-Fs correlated with increased volume of mineralized callus formation at 4 months, and low CFU-F counts correlated with longer healing times. With increasing amounts of data, Herniguo et al. were able to roughly estimate a relative threshold for success based on the number of CFU-F present in an aspirate, where a CFU-F level below a certain number was a strong indicator of slower healing time. The CFU-F assay should be included in all clinical analysis of SVF in order to build the data in this manner, not just for the FDA, but for the benefit of the clinician as well.

CONCLUSION

In summary, the regulatory status of SVF based therapies in the United States suggests that an IND or IDE is required in order to proceed clinically. As such, a variety of quality control and analytical measures are required. The goal of these measures is to ensure that clinicians definitively know what they are treating patients with and know that they are not introducing any added risk to the patient in doing so.

REFERENCES

1. FDA (2014) Minimal Manipulation of Human Cells, Tissues, and Cellular and Tissue-Based Products: Draft Guidance for industry and food and drug administration staff.
2. FDA (2014) Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) from Adipose Tissue: Regulatory Considerations; Draft Guidance for Industry.
3. FDA (2014) Same surgical procedure exception under 21 CFR 1271.15(b): questions and answers regarding the scope of the exception. Draft Guidance for industry.
4. USFDA (2015) Homologous use of human cells, tissues, and cellular and tissue-based products. Draft Guidance for Industry and FDA staff..
5. USFDA (2015) 21 CFR part 1271.
6. USFDA (2013) Public Health and Service Act.
7. USFDA(2015) Food, Drug, and Cosmetic Act. U.S. Food and Drug Administration.
8. USFDA (2008) Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug applications (INDs).
9. USFDA (2015) Bacterial Endotoxins/Pyrogens. Inspection Technical Guides. U.S. Food and Drug Administration.
10. USFDA (2012) Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers. U.S. Food and Drug Administration.
11. Williams KL (2007) Pyrogens, LAL Testing and Depyrogenation. (3rd edn), Informa Healthcare.
12. Joiner TJ, Kraus PF, Kupiec TC (2002) Comparison of endotoxin testing methods for pharmaceutical products. Int J Pharm Comp 6: 408-409.
13. United States Pharmacopeia (2012) Bacterial Endotoxins Test. Chapter 85, United States Pharmacopeia.
14. Aronowitz JA, Lockhart RA, Hakakian CS (2015) Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. Springer Plus 4: 713.

15. Shi L, Carson D (2009) Collagenase Santyl ointment: A selective agent for wound debridement. J Wound Ostomy Continence Nurs 36: 512-516.

16. Smith, Nephew (2014) Exploring Clinical Data for Collagenase Santyl Ointment.

17. Nunn AC, Schreuder FB (2014) Dupuytren's contracture: emerging insight into a viking's disease. Hand Surg 19: 481-490.

18. Gilpin D, Coleman S, Hall S, Houston A, Karrasch J, Jones N (2010) Injectable collagenase clostridium histolyticum: a new nonsurgical treatment for Dupuytren's disease. J Hand Surg Am 35: 2027-2038.

19. Sood A, Therattil PJ, Paik AM, Simpson MF, Lee ES (2014) Treatment of Dupuytren's contracture with injectable collagenase in a veteran population: A case series at the department of veterans affairs new jersey health care system. Eplasy 14: e13.

20. Egui Rojo MA, Moncada Iribarren I, Carballido Rodriguez J, Martinez-Salamanca JI (2014) Experience in the use of collagenase histolyticum in the management of peyronie's disease: Current data and future prospects. Ther Adv Urol 6: 192-197.

21. Gelbard M, Goldstein I, Hellstrom WJ, et al. (2013) Clinical efficacy, safety and tolerability of collagenase clostridium histolyticum for the treatment of peyronie disease in 2 large double-blind, randomized, placebo controlled phase 3 studies. J Urol 190: 199-207.

22. AAC (2009) Briefing Document for Collagenase Clostridium Histolyticum (AA4500).

23. Miyabayashi T, Lord PF, Dubielzig RR, Biller DS, Manley PA (1992) Chemonucleolysis with collagenase. A radiographic and pathologic study in dogs. Vet Surg 21: 189-194.

24. Friedman K, Pollack SV, Manning T, Pinnell SR (1986) Degradation of porcine dermal connective tissue by collagenase and by hyaluronidase. Br J Dermatol 115: 403-408

25. Bromley JW, Hirst JW, Osman M, Steinlauf P, Gennace RE, et al. (1980) Collagenase: an experimental study of intervertebral disc dissolution. Spine 5: 126-132.

26. Bromley JW (1982) Intervertebral discolysis with collagenase. Arzneimittelforschung 32: 1405-1408.

27. Chang H, Do BR, Che JH, et al. (2013) Safety of adipose-derived stem cells and collagenase in fat tissue. Aesthetic Plast Surg 37: 802-808.

28. Zook BC, Kobrine AI (1986) Effects of collagenase and chymopapain on spinal nerves and intervertebral discs of Cynomolgus monkeys. J Neurosurg 64: 474-483.

29. Sussman BJ, Bromley JW, Gomez JC (1981) Injection of collagenase in the treatment of herniated lumbar disk: Initial clinical report. JAMA 254: 730-732.

30. Garvin PJ (1974) Toxicity of collagenase: the relation to enzyme therapy of disk herniation. Clin. Orthop Relat Res 101: 286-291.

31. Aronowitz JA, Lockhart RA, Hakakian CS, et al. (2015) Clinical Safety of Stromal Vascular Fraction Separation at the Point of Care. Ann Plast Surg 75: 666-671.

32. Curry TE Jr, Mann JS, Estes RS, et al. (1990) Alpha 2-macroglobulin and tissue inhibitor of metalloproteinases: collagenase inhibitors in human preovulatory ovaries. Endocrinol 127: 63-68.

33. Pedersen JZ, Franck C (1986) Increased serum levels of alpha-2-macroglobulin in severe chronic airflow obstruction. Eur J Respir Dis 68: 195-199.

34. Wünsch E, Heidrich HG (1963) On the Qualitative Determination of Collagenase, Z. Physiol. Chem 333: 149.

35. Van Wart HE, Steinbrink DR (1981) A continuous spectrophotometric assay for Clostridium histolyticum collagenase. Analytical Biochem 133: 356-365.

36. Breite AG, Dwulet FE, McCarthy RC (2010) Tissue dissociation enzyme neutral protease assessment. Transplantation Proceedings 42: 2052-2054.

37. Lockhart RA, Hakakian CS, Aronowitz JA (2015) Tissue dissociation enzymes for adipose stromal vascular fraction cell isolation: A review. J Stem Cell Res Ther 5: 12

38. USFDA (2013) Guidance for industry: Preclinical assessment of investigational cellular and gene therapy products.

39. Mitchell JB, McIntosh K, Sanjin Z, et al. (2006) Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 24: 376-385.

40. Bourin P, Bunnell BA, Casteilla L, et al. (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the

International Society for Cellular Therapy (ISCT). Cytotherapy 15: 641-648.

41. Lin G, Garcia M, Ning H, et al. (2008) Defining stem and progenitor cells within adipose tissue. Stem Cell Dev 17: 1053-1064.
42. Hicok KC, Hendrick MH (2001) Automated isolation and processing of adipose-derived stem and regenerative cells. Methods Mol Biol 702: 87-105.
43. Herniguo P, Poignard A, Beaujean F, et al. (2005) Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 87: 1430-1437.
44. Bipartisan Policy Center (2015) Advancing regenerative cellular therapy: Medical innovation for healthier americans.