cell therapy sciences
Developing Regenerative Medicine for Vets

Stem Cell yields are less than 10 % from Canine Stromal Vascular Fraction and further reduced after Cryopreservation

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ABSTRACT:

Objectives:
Mesenchymal stem cells (MSCs) can be isolated from adipose tissue by enzyme extraction using collagenase. The resultant cell mixture is called the stromal vascular fraction (SVF) and is a heterogeneous mixture of cells and cellular debris. Various systems are available to produce SVF as a same-day "stem cell therapy" from adipose tissue. This study estimated the MSC yields from fresh SVF and investigated the effects of cryopreservation of the SVF on MSC numbers.

Methods:
Adipose samples were processed to form SVF using a standard protocol using collagenase. The resultant SVF was split into two equal portions. One was placed directly into culture and the second was cryo-frozen and then thawed and cultured. Cultures were photographed on day 2 and adherent cell numbers estimates. Statistics were estimated by comparison of means using a two tailed T-test.

Results:
Fresh SVF comprised > 50% dead cells/cells of miscellaneous mixed cell type, collagen and capillary fragments. Of the living cells, even after 2 days in culture < 10% were MSCs.
The mean number of MSCs per gram of adipose tissue was 51,872 (+/- 23,648) cells per gram. Cryo-freezing of SVF further reduced the number of viable stem cells down to < 2% of the total cells.

Statement:
The low % of MSC yield, low viability and wide variation in MSC yield from SVF makes it impossible for veterinary practitioners to treat osteoarthritis in dogs with a standardised number of MSCs when using a same day SVF therapy. Culture expanded MSCs allow for a standardized, quality controlled, viability tested cell product.

OBJECTIVES:

To calculate mesenchymal stem cell (MSC) yields from fresh Stromal Vascular Fraction (SVF) as compared with stem cells grown using validated cell culture methods and to investigate whether SVF could be cryopreserved without impacting MSC viability.

INTRODUCTION:

Stem cell and regenerative therapies have a wide variety of clinical uses, including for bone marrow transplantation, irritable bowel syndrome and degenerative joint diseases such as osteoarthritis[1]. In veterinary medicine, as regenerative medicine grows in popularity, SVF is sometimes sought as a quicker alternative to cultured-expanded stem cells. Various devices and methods are available to provide SVF as a same-day, patient-side form of "stem cell therapy" from adipose tissue, as opposed to using validated stem cell culture techniques[2]. SVF is known to be fundamentally inferior to cultured stem cell therapies in terms of the numbers of available MSCs and whilst it has not yet been fully characterised, SVF is known to be a heterogenous mix of stromal and haematopoietic cells; endothelial cells, white blood cells, including lymphocytes, monocytes and macrophages, adipose mesenchymal stem cells, haematopoietic stem cells, other progenitor stem cells, fibroblasts, erythrocytes, collagen and other extracellular matrix proteins[3].

This study has investigated the proportion of MSCs available in canine adipose tissue SVF therapy using enzymatic processing, compared with culture-expanded stem cell therapy and has compared the efficiency of culture expansion from freshly prepared or cryopreserved SVF.

MATERIALS AND METHODS:

12 canine adipose samples were processed using a standard protocol with 300CDU/ml collagenase produced enzymatic SVF.[4]
The total mass of the starting tissue was recorded and the resultant SVF was examined using the Trypan Blue exclusion method to estimate viable and non-viable cells.

The total SVF product was subsequently split into two equal portions with one half placed directly into culture and the second cryogenically-frozen before being thawed and cultured. Cultures were photographed every day using the same photofield. The cells were removed from the culture flasks when 90% confluent by incubation for 5-10 minutes in trypsin (TripLE from Life Technologies) and resuspended in phosphate buffered saline supplemented with 10% fetal calf serum to stop the trypsin action. Estimates of the total number of cells and the percentage of viable and non-viable cells were repeated using the Trypan Blue exclusion method. The number of cells present at each day of culture was estimated using the number of cells visible in the photo field as a ratio of the total yield of cells from the culture flask on the harvest day. Population doubling times were calculated from the log phase growth of each cell culture.

RESULTS:

Figure 1: Composition of Stromal Vascular Fraction (SVF) Compared with Mesenchymal Stem Cells (MSCs) Grown in Culture

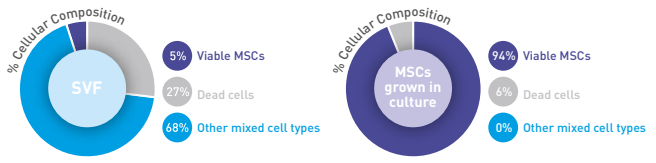
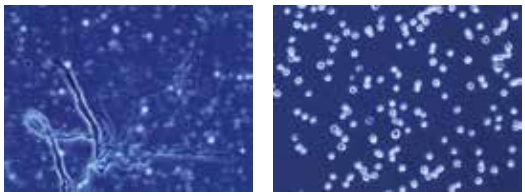


Figure 1 shows the proportion of viable MSCs, other mixed cell types and dead cells present in SVF when compared with culture-expanded MSCs. The data clearly shows the very low percentage of viable stem cells available from SVF (percentage of viable MSCs from SVF = 5% compared with 94% from MSCs grown in culture; N=12, p-value p < 0.001). SVF comprises 95% other cell types and dead cells.

Figure 2: Photomicrographs showing the Composition of Fresh Stromal Vascular Fraction (SVF) compared with Mesenchymal Stem Cells (MSCs) grown in Cell Culture for 5 days

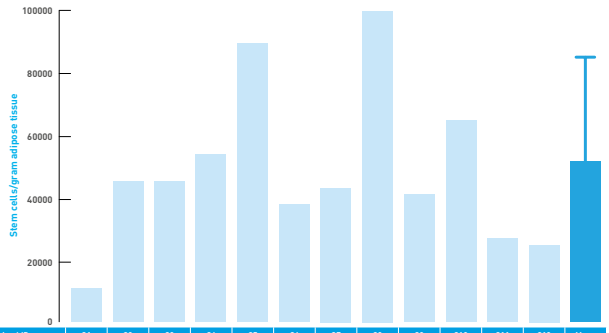


SVF | MSC grown in culture

Dark blue cells = Dead Cells | **Bright cells = Living Cells**

Figure 2 Photomicrographs taken under phase contrast after Trypan Blue staining show fresh SVF compared with culture-expanded MSCs. The SVF can be seen to contain a lot of connective tissue debris, plus many dead cells (dark blue) whereas the cultured stem cells are uniformly bright, living and active cells with very few dark blue dead cells and no debris present.

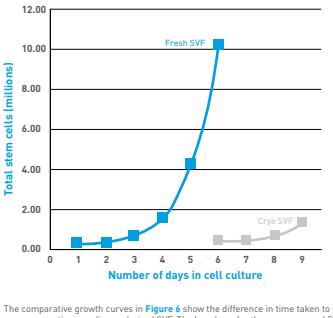
Figure 3: Variation in numbers of stem cells present in SVF from 12 different dogs

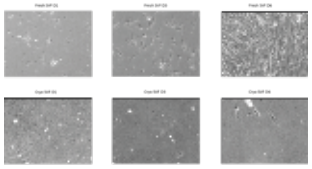


Animal ID	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	Mean
Same-day SVF therapy: no. of grams of adipose tissue required to produce 5 million stem cells	553.85	111.53	110.72	90.04	55.86	159.02	126.54	49.86	142.73	83.52	176.81	204.17	106.58 (+/- 57.29 SD)
Animal's age when adipose tissue harvested	7 months	2y 11m	10y 5m	6 years	11y 4m	7 years	5 years	2 years	5 years	1y 4m	3y 2m	4y	4 yr 1.7 m
Breed	Border collie	Dogue de Bordeaux	English Springer Spaniel	Labrador	English Springer Spaniel	Cross-breed	Labrador	English Springer Spaniel	Labrador	Labrador	Rottweiler	Labrador	

Figure 3 shows the number of stem cells in same-day SVF from 12 different dogs and calculates the number of grams of tissue that would be required to deliver a standard dose of 5 million stem cells. Adipose samples from individual dogs demonstrate a very large variation in the number of stem cells available in the resultant SVF. To deliver the same number of stem cells per treatment via SVF as delivered via culture-expanded MSC therapy, the number of grams of adipose tissue required from each dog ranges from 49.86 grams up to > 500 grams, mean 107 grams. This is in sharp contrast with the number of grams of adipose tissue required for culture-expanded stem cell therapy, which requires 5 to 10 grams to generate a standard dose of 5 million cells.

Figure 6: Change in Stem Cell Number over Time in Culture: Comparison of Fresh and Cryopreserved SVF





These photos show the progression of cell growth from day 1-6. Cells are visible on day 1 from fresh SVF but none are visible from the frozen SVF until day 6. Fresh SVF is confluent and ready to harvest by day 6.

The comparative growth curves in **Figure 6** show the difference in time taken to grow cells in culture and further demonstrate the impact of cryopreservation on adipose-derived SVF. The lag phase for the cryopreserved SVF is six days compared with only one day for the fresh SVF preparation. Five out of twelve of the cryopreserved SVF samples had no cells visible after four days in culture and one out of twelve had no cells visible after 9 days in culture and was therefore presumed to contain no viable cells at all in the cryopreserved SVF.

Effects of Freezing (Cryopreservation) on SVF:

Figure 4: Effect of Cryopreservation on Viability of MSCs from SVF compared with MSCs grown in culture

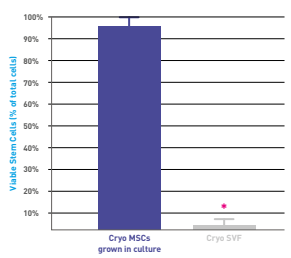


Figure 4 shows the percentage of viable MSCs in SVF after cryopreservation and thawing compared with culture-expanded MSCs after the same cryopreservation and thawing protocol [SVF = 1.09% (+/- 2.15%) viable stem cells vs culture-expanded MSCs = 94.75 % (+/- 5.05%) viable stem cells]. This difference was highly significant (N=12, p< 0.001).

Figure 5: Effect of Cryopreservation on Number of MSCs in SVF (per gram of Adipose Tissue)

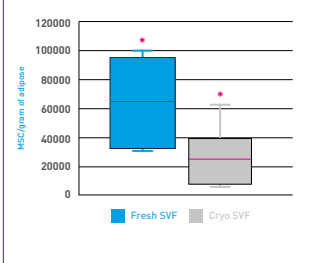


Figure 5 Fresh SVF was shown to have a significantly higher number of stem cells per gram of adipose tissue compared with cryopreserved SVF [67,261 cells (SD 28,535) vs 21,579 cells (SD 20, 148)]. This difference was highly significant [n = 5, p < 0.05].

DISCUSSION:

- The data presented in this poster has been accumulated as part of regular QA and QC processes undertaken by CTSL during the preparation of culture-expanded stem cell therapies for the treatment of dogs with degenerative joint disease.
- The data demonstrates that SVF contains a large amount of cellular and connective tissue debris, whereas MSC culture expansion can select for viable, adhesive MSCs and allows debris to be washed off after two days in culture.
- Furthermore, while culture-expanded MSC therapies provide a homogenous population of 94% viable stem cells and no tissue debris, SVF injections will contain not only tissue debris but also comprise ~ 68% mixed living cells that are not MSCs, 27% dead cells and only 5% viable MSCs.
- SVF cannot be frozen, even using validated cryopreservation techniques, without further reducing the numbers of viable MSCs down to ~ 1% of the injected cells and reducing the ability of the SVF cells to grow in culture.
- Therefore, SVF therapy cannot be cryostored for subsequent treatments or pending microbiological testing and would require an additional adipose harvest for each treatment. In contrast, the viability of culture-expanded MSCs is not adversely affected by cryopreservation, with typical viability levels of 95%.
- Finally, the number of MSCs in adipose tissue is highly variable in different dogs and it is impossible for veterinary practitioners to provide a standard dose of active cells for all cases when using SVF.
- Typically, SVF protocols ask for 30 to 50 grams of adipose, although based on our data this will not provide adequate numbers of MSCs, which would need on average > 100 grams; clearly removing such a large mass of tissue is not practical and would be associated with significant morbidity and recovery time as compared with the small sample (5-10 grams) required for culture-expanded stem cells.

CONCLUSION:

Mesenchymal stem cell yields from SVF therapy are very low at 5%, with 95% of SVF comprising dead cells or other cell types. The wide variation in yield from different dogs makes it impossible for veterinary practitioners to treat osteoarthritis in their patients with a standardised number of MSCs when using a same day SVF therapy. Culture expanded MSCs allow for a standardized, quality controlled stem cell product containing 95% viable cells, that can undergo micropathology testing and be safely cryopreserved for future use.

REFERENCES:
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ACKNOWLEDGEMENTS: Alex Georgiou (Coventry University) ; Dr Christine Standen (CTSL)

AFFILIATIONS AND CONFLICT OF INTEREST: Both authors work in Research and Development at CTSL