Viable Stem Cells Are in the Injury Effusion Fluid and Arthroscopic Byproducts From Knee Cruciate Ligament Surgery: An In Vivo Analysis

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Purpose: To examine the number of viable stem cells contained in the postinjury effusion fluid and the waste byproducts of arthroscopic cruciate ligament surgery. **Methods:** This study included patients older than 18 years of age with acute (<5 weeks old) cruciate ligament injuries requiring arthroscopic surgery. The postinjury effusion fluid (effusion fluid), fat pad and cruciate ligament stump debridement tissue (byproduct tissue), and arthroscopic fluid collected during fat pad and/or stump debridement (byproduct fluid) were collected at the time of surgery from 30 individuals. Specimens were analyzed, investigating cell viability, nucleated cell counts, cell concentrations, colony-forming unit assays, and flow cytometry. Samples from the first 20 individuals were collected in small specimen containers, and samples from the last 10 individuals were collected in larger specimen containers. **Results:** Cells of the injury effusion exhibited the greatest viability (86.4 ± 1.31%) when compared with the small volume harvest byproduct tissue (50.2 ± 2.5%, $P = .0001$), small volume harvest byproduct fluid (48.8 ± 1.88%, $P = .0001$), large volume harvest byproduct tissue (70.1 ± 5.6%, $P = .0001$), and large volume harvest byproduct fluid (60.3 ± 3.41%, $P = .0001$). The culture analysis of fibroblast colony-forming units found on average 1916 ± 281 progenitor cells in the effusion fluid, 2488 ± 778 progenitor cells in the byproduct tissue, and 2357 ± 339 progenitor cells in the byproduct fluid. Flow cytometry confirmed the presence of immature cells and the presence of cells with markers typically expressed by known stem cell populations. **Conclusions:** Viable stem cells are mobilized to the postinjury effusion at the time of cruciate ligament injury and can be found in the byproduct waste of cruciate ligament surgery. **Clinical Relevance:** The methodology around effusion fluid and byproduct tissue capture during cruciate ligament surgery should be investigated further. Cell amounts available from these tissues with current technologies are not sufficient for immediate evidence-based clinical application.

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The 4 fundamental stem cell capabilities are the ability to reproduce (proliferative potential), the ability to differentiate into a different number of end-stage cell lines (multipotentiality), the ability to mobilize in situations of injury, and the ability to monitor and/or affect an environment with the release of growth factors, cytokines, chemokines, and messenger RNA (paracrine functions).[1-8] Although initial focus was on multipotentiality, recent interest has centered on paracrine function, mobilization, and potential application on orthopaedics.[9-16]

Recent studies have investigated the application of stem cells to facilitate graft incorporation in anterior cruciate ligament (ACL) reconstruction.[17-19] The authors have advocated the harvest of stem cells from the fat pad inside the knee joint to improve arthroscopic procedures.[20] Cells with stem capabilities reside in the synovium of the joint, the fat pad, injured ACL stump tissue, and in synovial fluid in the setting of arthritis.[1-4,17,21-27] Synovial-derived stem cells, which have been harvested, expanded in culture, and injected into the joint

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after tissue injury, have shown encouraging results in animal models involving the potential for cartilage and meniscal regeneration.[24,25,28]

The most practical and cost-effective way to harvest and clinically apply stem cells has not been established, because these cells reside in many tissues and multiple donor sites are available. Although the mobilization of stem cells in instances of cardiac injury, skin injury, and hypoxia has been established, the mobilization of stem cells in the setting of an acute knee injury has not been clarified.[1-4] After knee injury, an effusion is common and can produce pain. Some clinicians routinely aspirate and discard the effusion in the clinic setting before surgery. Similarly, cruciate ligament surgery creates tissue and fluid byproducts consisting of synovial tissue, fat pad tissue, ACL stump tissue, and arthroscopic fluid, which are discarded. Orthopaedic clinicians have begun to use bone marrow aspirate to augment the biology of healing, with studies investigating the optimal methods of harvest.[29-32] Additional interest has been focused on pharmaceutical and environmental mobilization of stem cells from the bone marrow to the blood stream,[14,33,34] and new processing disposables are under development that immediately cause cells to preferentially release anti-inflammatory proteins such as Interleukin 1 receptor antagonist and soluble receptor for tumor necrosis factor-α.[35]

The purpose of this study was to examine the number of viable stem cells contained in the effusion fluid and the waste byproducts of arthroscopic cruciate ligament surgery. If found in large quantities, stem cells harvested at the time of cruciate ligament surgery have the potential to be applied clinically; alternatively, if found in small quantities, stem cells combined with developing technologies around the preferential release of anti-inflammatory proteins may also be of benefit. Our hypothesis was 2-fold: (1) the cruciate injury knee response involves mobilization of stem cells from the synovium and fat pad to the synovial fluid as components of an effusion and (2) the cruciate injury knee effusion along with the byproducts of cruciate reconstruction contains useful quantities of viable stem cells.

Methods

Participants

Institutional review board approval was obtained. Patients eligible for inclusion were male and female patients older than 18 years with acute (<5 weeks old) cruciate ligament injuries. Enrollment was discussed with patients presenting to the primary institution over a period of 24 months requiring isolated ACL reconstruction, isolated posterior cruciate ligament (PCL) reconstruction, and combined ACL/PCL reconstruction. The exclusion criteria were as follows: time between injury and surgery greater than 5 weeks, preoperative knee aspiration of the injury effusion, any signs concerning for local or systemic infection, a history of immunosuppression, or a history of chronic steroid use. A total of 30 patients were chosen because previous studies regarding stem cell quantification have found significance with cohorts ranging in size from 5 to 15.[31,33,34] A power analysis was impossible to perform before the study because the number of cells mobilized with knee injury has not been previously evaluated, and the therapeutic dosage of stem cell technologies has not been established. After data collection in 20 patients, the study was amended to add a large volume harvest method in 10 patients to see if the collection yield would improve. We hypothesized that a method involving one large volume container would capture stem cells more efficiently than a method involving multiple small volume containers. The study in bone marrow aspirate has found an effect dependent on the volume of fluid involved in harvest.[31]

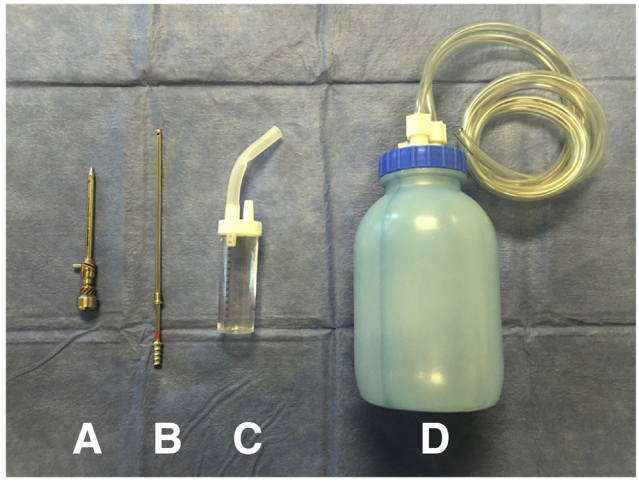

Fig 1. Tissue collection involved (A) the arthroscopic trochar, (B) a metal suction tube, and either (C) an 80-mL fluid collection container or (D) a 1.9-L specimen container.

Tissue Collection

Tissue was collected intraoperatively at the time of cruciate ligament surgery. On the first initiation of intra-articular access, the knee effusion fluid was collected. This involved an arthroscopic trocar, a metal suction tube, an 80-mL fluid specimen container (Medline, Mundelein, IL), and suction (Fig 1). The byproduct tissue, including infrapatellar fat pad tissue and cruciate stump tissue, was collected concomitantly with byproduct fluid during the initial steps of cruciate reconstruction. This involved the arthroscopic shaver and either small volume harvest with multiple 80-mL fluid specimen containers or large volume harvest involving a single large 1.9-L specimen container (VWR, Radnor, PA) (Fig 1). Tissue collection for the first 20 patients involved small volume harvest, and tissue collection for the last 10 patients involved large volume harvest. The specimen containers were interposed between the suction tubing and the suction

generator (Neptune 2, Stryker, Kalamazoo, MI) at the suction generator. The extent of tissue debridement was limited to the typical amount for the arthroscopic approach and visualization at the discretion of the operating surgeon.

Tissue specimens were prepared for shipment to a third-party laboratory facility. This involved following a standard operating procedure provided by the laboratory facility. The sample volume and color were collected at the time of packing of each sample. The specimen containers were sealed with waterproof tape, labeled, placed in a TheraPak biohazard bag (Therapak, Buford, GA) with absorbent material, and sealed. The specimen containers were placed in a foam-lined shipping container with Phase 5 change panels (Cryopak, Edison, NJ), small blocks that contain a special phase change polymer that freezes at 5°C instead of 0°C.

Tissue Characterization

Volume, Viability, and Total Nucleated Cell Counts. Tissue samples were analyzed after processing in a tissue culture lab under a sterile biosafety cabinet. For the small volume samples and for the knee effusion fluid samples, the samples were filtered with a 70-μm cell strainer and collected into labeled 50-mL sterile, conical tubes. For the large volume samples, the most fluid from the large volume container was first extracted into 250-mL sterile containers, and then the remainder of the material was filtered through 70-μm cell strainers. Fat pad tissue collected in the strainers was placed in a separate sterile tube. The volumes of all samples were recorded during the process.

A collagenase enzyme (Vitacyte; Indianapolis, IN) was heated to 37°C and added to the fat pad samples. The fat pad samples were then placed in a 37°C rocker for 30 minutes. After digestion, the fluid was then passed through a 40-μm cell strainer into a fresh 50-mL tube. The samples were washed and centrifuged at 900 *g* for 5 minutes and resuspended in phosphate-buffered saline. The samples were analyzed with an automated cell counter (Nucleocounter Eppendorf/ChemoMetec, Allerod, Denmark) to determine the number of total nucleated cells and viability. The total cell concentration was calculated by dividing the total nucleated cell count by the collected sample volume. Cell viability was reported as a percentage of total nucleated cells (%) by the automated cell counter.

Culturing Methods

Colony-forming unit assays were performed to quantify progenitor cell populations and document multipotentiality of the progenitor cell population, that is, the ability of cells to colonize as fibroblasts and chondrocytes. Colony-forming unit of fibroblast (CFU-F) assays were performed by the following steps: dilution series of each cell preparation in culture medium (ScienCell, Carlsbad, CA), plating onto appropriately labeled 12-well plates, and incubation at 37°C, 5% CO_2, and 100% humidity. The medium was replaced every 3 days, and the plates were examined after 10 days. Colony quantification was performed at the end of the culture period by fixing the colonies and/or cells with formaldehyde, staining the attached cells with Crystal Violet, washing, and air-drying the plates. Visualization and counting of the colonies was performed using a "20-cell or above" cutoff for being counted as a colony.

Colony-forming unit of chondrocyte (CFU-C) assays were performed identically to the CFU-F assay for the first 10 days. After the 10th day, the cells were changed to a chondrogenic medium (ScienCell). The medium was replaced every 3 days, and the plates were examined after 20 days total. Colony quantification was performed at the end of the culture period with the following steps: aspirating the medium, washing the colonies with phosphate-buffered saline, fixing the colonies and/or cells with formaldehyde, staining the attached cells with Alcian Blue, washing and/or staining with nuclear fast red stain, repeat washing, and air-drying the plates. Visualization and counting of the colonies was performed using a "20-cell or above" cutoff for being counted as a colony. The concentration of connective tissue progenitor cells was reported as the number per 1.0 mL of aspirate.

Flow Cytometry

Flow cytometry was performed with a BD Accuri C6 flow cytometer (BD Biosciences, MD). The samples for flow cytometry analysis were washed and then diluted based on the sample concentration. The samples were incubated for 30 minutes with a binding inhibitor (eBiosciences, San Diego, CA) to block nonspecific binding sites. The samples and compensation beads (eBiosciences) were labeled and incubated for 30 minutes with antibodies for a Lineage panel, CD34, CD146, and CD31 (Invitrogen, Carlsbad, CA). Isotype control samples were simultaneously prepared with the following isotypes: FITC Iso, PE Iso, PE-Cy7 Iso, and APC Iso (Invitrogen). The samples were washed, centrifuged, and then fixed with 10% formaldehyde. The samples were then run and data collected.

Lineage-positive cells are a mixture of all cells expressing mature cell lineage markers. Lineage-negative cells are not stained by the lineage antibodies, and immature cell populations with stem potential are considered to be within the lineage-negative population. CD34 is a hematopoetic stem cell marker.[36] CD146 is a marker found on perivascular cells exhibiting stem cell function, that is, pericytes.[37] CD31 is a marker for endothelial cells and immune

Table 1. Volume, Viability, and Total Nucleated Cell Counts

Sample	Average Viability, %	Average Total Nucleated Cells	Average Nucleated Cell Concentration, cells/mL
Knee effusion	86.4 ± 1.31	$10.1 \pm 1.80 \times 10^6$	$4.86 \pm 0.65 \times 10^5$
Byproduct tissue			
Small	50.2 ± 2.5	$6.89 \pm 0.79 \times 10^5$	$1.63 \pm 0.18 \times 10^5$
Large	70.1 ± 5.6	$5.93 \pm 1.86 \times 10^5$	$2.49 \pm 0.77 \times 10^4$
Byproduct fluid			
Small	48.8 ± 1.88	$2.68 \pm 0.26 \times 10^6$	$1.81 \pm 0.18 \times 10^4$
Large	60.3 ± 3.41	$3.68 \pm 0.64 \times 10^6$	$2.71 \pm 0.44 \times 10^3$

NOTE. This table captures viability and results of the automated cell counter (± standard deviation).

cells.[38] We also ran a panel selected for a scenario where all of these markers were present in a manner favorable to represent a stem cell population, that is, a CD marker panel including lineage negative (Lin−), CD146+, CD31−, and CD34+.

Data sets are reported as mean ± 95% confidence interval and the significance level was set at a probability value of less than .05. All data sets were checked for normality of distribution using the Lilliefors test, and an unpaired *t*-test was chosen to evaluate for statistical significance between groups. All statistical calculations were preformed using the Matlab computational software.

Results

Patient Sample Characteristics

The study population included 22 male participants and 8 female participants with an average age of 27 ± 11 years, with a range of 18-57 years. The level of participation included 8 recreational athletes, 8 collegiate athletes, 7 professional athletes, and 7 high school athletes. Injuries included 23 isolated ACL injuries, 4 combined ACL and meniscus injuries, 2 combined ACL and medial collateral ligament injuries, and 1 combined ACL, medial collateral ligament, and PCL injury. Fluid color at the time of collection was amber in 12 cases, red in 12 cases, yellow in 4 cases, and white in 1 case. Fluid color was not recorded for one sample. The average volume of effusion fluid collected was 21 ± 13 mL, with a range of 1 to 45 mL. The small volume harvest method averaged 174 ± 51.8 mL, with a range of 54 to 267 mL. The large volume harvest method averaged 939 ± 392 mL, with a range of 500 to 1893 mL.

Volume, Viability, and Total Nucleated Cell Counts

Table 1 presents the viability, average total nucleated cells, and average nucleated cell concentration of the effusion fluid, byproduct tissue, and byproduct fluid. Comparing the viability of the byproduct tissue with the byproduct fluid, we found that there was a statistically significant difference for the large volume harvest ($P = .0079$), but no statistically significant difference for the small volume harvest ($P = .3802$). A comparison of the small volume harvest with the large volume harvest showed better viability for the large volume harvest ($P = .0001$, $P = .001$). The effusion fluid illustrated the best viability when compared with the small volume harvest for the byproduct tissue and byproduct fluid ($P = .0001$, $P = .0001$), and when compared with the large volume harvest for the byproduct tissue and byproduct fluid ($P = .0001$, $P = .0001$). Comparing the average total nucleated cells of the byproduct tissue and byproduct fluid, we found that there were more cells in the byproduct fluid than the byproduct tissue for the small volume and large volume ($P = .0001$, $P = .0001$). For byproduct fluid collection, a comparison of the small volume harvest with the large volume harvest showed more cells for the large volume harvest ($P = .0021$). For the byproduct tissue, a comparison of the small volume with large volume harvest showed no statistical difference ($P = .28$). The effusion fluid illustrated the highest average total nucleated cells when compared with the small volume harvest for the byproduct tissue and byproduct fluid ($P = .0001$, $P = .0001$), and when compared with the large volume harvest for the byproduct tissue and byproduct fluid ($P = .0001$, $P = .0003$). Comparing the average nucleated cell concentration of the byproduct tissue and byproduct fluid, we found statistically significantly higher concentrations in the byproduct tissue for the small volume and large volume harvests ($P = .0001$, $P = .0001$). For the byproduct tissue, a comparison of the small volume harvest with large volume harvest showed more cells for the small volume harvest ($P = .0001$). For the byproduct fluid, a comparison of the small volume with large volume harvest showed more cells for the small volume harvest ($P = .0001$). The effusion fluid illustrated the highest average nucleated cell concentration when compared with the small volume harvest for the byproduct tissue and byproduct fluid ($P = .0001$, $P = .0001$), and when compared with the large volume harvest for the byproduct tissue and byproduct fluid ($P = .0001$, $P = .0001$). One of the knee effusion fluid samples did not have sufficient cell numbers to run nucleated cell analysis. One of the byproduct tissue samples did not have sufficient cell numbers to run nucleated cell

Table 2. CFU-Fibroblast Assay

Sample	CFU-F Total Count	CFU-F Counts/mL
Knee effusion	1,916 ± 281	135 ± 22.6
Byproduct tissue		
Small	2,488 ± 778	429 ± 99.8
Large	273 ± 111	18 ± 9.01
Byproduct fluid		
Small	2,357 ± 339	14 ± 6.14
Large	515 ± 157.4	0.39 ± 0.34

NOTE. This table captures the results of the colony-forming unit of fibroblast (CFU-F counts) assays (± standard deviation).

analysis or CFU-F assays. On the analysis of the large volume harvest, 2 of the byproduct tissue samples did not have enough cells to perform the analysis on them.

CFU-F Results

Table 2 shows the average CFU-F assay counts found in the samples. Comparing the CFU-F total counts of the effusion fluid with the byproduct tissue and byproduct fluid methods with the small volume harvest, we found no statistically significant difference ($P = .1244$, $P = .0555$). A comparison of the small volume harvest with the large volume harvest showed more progenitor cells on the small volume harvests ($P = .0015$, $P = .001$). Comparing the CFU-F counts/mL of the effusion fluid with the byproduct tissue and byproduct fluid methods with the small volume harvest, we found higher counts in the byproduct tissue and lower counts in the byproduct fluid ($P = .0001$, $P = .0001$). A comparison of the small volume harvest with the large volume harvest showed more progenitor cells on the small volume harvest ($P = .0001$, $P = .0002$). One of the knee effusion fluid samples and one of the byproduct fluid samples did not have sufficient numbers to run an analysis. Two of the byproduct tissue samples in the large volume harvest did not have enough cells to perform the analysis on them.

CFU-C Results

Table 3 shows the average CFU-C assay counts in the samples. Comparing the CFU-C total counts of the effusion fluid with the byproduct tissue and byproduct fluid methods with the small volume harvest, we found higher counts in the effusion fluid ($P = .0012$, $P = .067$), although not statistically significant in the case of the small volume harvest digest. A comparison of the small volume harvest with the large volume harvest showed no difference in the byproduct tissue and more progenitor cells on the small volume harvest in the byproduct fluid group ($P = .1443$, $P = .0001$). Comparing the CFU-C counts/mL of the effusion fluid with the byproduct tissue and byproduct fluid methods with the small volume harvest, we found higher counts in the byproduct tissue and lower counts in the byproduct fluid ($P = .0001$, $P = .0001$). A comparison of the small volume harvest with the large volume harvest showed more progenitor cells on the small volume harvest ($P = .0007$, $P = .0001$). The CFU-F assays were given priority when the number of cells in a sample were limited, and if there were enough cells remaining, then the CFU-C assay was performed. One of the knee effusion fluid samples had high CFU-C counts, which could not be calculated as the colonies were too dense. Statistical comparison of the small volume harvest method with the large volume harvest method showed no significance.

Flow Cytometry Results

Table 4 reports the flow cytometry results. Priority was given to providing enough cells from the samples to perform the colony-forming unit assays, so a limited number of samples were analyzed by flow cytometry. None of the byproduct digest samples had sufficient cells for flow cytometry.

Discussion

This study confirmed the presence of viable stem cells in a postinjury knee effusion and in the waste byproducts of cruciate ligament surgery, suggesting that stem cells are mobilized to the synovial fluid with a cruciate injury event and can be harvested at the time of arthroscopic surgery. Cells of the injury effusion exhibited the greatest viability, and we hypothesize that these cells are mobilized from the synovium and fat pad. The culture analysis of CFU-F found on average 1,916 ± 281 progenitor cells in the effusion fluid, 2,488 ± 778 progenitor cells in the byproduct tissue, and 2,357 ± 339 progenitor cells in the byproduct fluid. The harvest with a small volume container consistently produced more colony-forming units than the harvest with a large volume container. Flow cytometry confirmed the presence of immature cells and the presence of cells with markers typically expressed by known stem cell populations.

When considering whether knee injury effusion fluid and surgery byproduct tissue represents a useful source for stem cells, it is important to compare other tissue sources. Quantification studies evaluating bone marrow

Table 3. CFU-Chondrocyte Assay

Sample	CFU-C Total Count	CFU-C Counts/mL
Knee effusion	2,436 ± 629	146 ± 40.5
Byproduct tissue		
Small	499 ± 118	215 ± 85.2
Large	347 ± 152	25 ± 13.2
Byproduct fluid		
Small	1,704 ± 285	10 ± 1.91
Large	501 ± 115	0.37 ± 0.08

NOTE. This table captures the results of the colony-forming unit of chondrocyte (CFU-C counts) assays (± standard deviation).

Table 4. Flow Cytometry Results

	Lineage Negative, %	CD 146+, %	CD 31+, %	CD 34+, %	Stem Cell Panel, %
Knee effusion fluid (n = 9)	83.0 ± 9.28	6.6 ± 5.22	86.1 ± 16.2	2.3 ± 1.11	0.14 ± 0.03
Small volume byproduct fluid (n = 5)	88.9 ± 8.77	2.8 ± 1.31	96.8 ± 1.58	2.9 ± 1.05	0.10 ± 0.06
Large volume byproduct fluid (n = 4)	96.0 ± 2.74	1.7 ± 1.67	98.1 ± 1.76	2.1 ± 2.16	0.16 ± 0.28

NOTE. The first column shows the frequency of lineage-negative cells in the samples (± standard deviation). The second to fourth columns show the frequency of other specific markers within the lineage-negative cell population (± standard deviation). CD34 is a hematopoietic stem cell marker. CD146 is a marker found on perivascular cells exhibiting stem cell function, that is, pericytes. CD31 is a marker for endothelial cells and immune cells. The Stem Cell Panel column represents a panel test investigating for a combination of markers most favorable to represent a stem cell population, that is, a CD marker panel including lineage negative, CD146+, CD31−, and CD34+.

aspirate are variable with estimates ranging from 1 stem cell per mL of tissue collected to 300,000 stem cells per mL of tissue.[39] Lipoaspirate has also established variability with studies estimating 5,000 stem cells per mL of tissue collected to 1,500,000 stem cells per mL of tissue collected.[39] This study found significant variation in progenitor cell availability based on the individual and the collection technique, suggesting that with the current techniques and understanding it is not a useful source. However, as technologies around cell manipulation progress, this may become a useful source in the future.

Although we theorized a greater harvest with a large volume container, the harvest with a small volume container consistently produced more colony-forming units than harvest with a large volume container. This may be attributed to the dilution effect of using a larger harvest container. This phenomenon has been illustrated in the bone marrow aspiration technique, with Hernigou et al.[31] documenting the superior harvest of progenitor cells with a small volume syringe technique compared with a large volume syringe technique.

Two studies allow for direct comparison: Hernigou et al.[32] analyzed cell counts when treating a series of patients with bone marrow aspirate from the iliac crest for tibial nonunions, and Beitzel et al.[30] analyzed cell counts of bone marrow aspirate obtained from the proximal humerus and distal tibia during arthroscopic procedures. In the Hernigou et al.[32] study, nucleated cell counts varied from 1 to 24 million cells/mL, with a mean of 18 ± 7 million cells/mL. Similarly, Beitzel et al.[30] found an average of 30 ± 16.7 million nucleated cells/mL when aspirating bone marrow from the proximal humerus and distal tibia. In our study, the knee joint effusion fluid exhibited a nucleated cell count of 486 ± 179 thousand cells/mL. This reflects the lower cell density of synovial fluid compared with bone marrow. On CFU-F analysis, the Hernigou et al.[32] study found bone marrow aspirations averaged 612 ± 134 progenitor cells/mL (range, 12-1,224 progenitor cells/mL). Evaluating colony-forming units after culture in an osteogenic medium, Beitzel et al.[30] found on average 766.3 ± 545.3 progenitor cells/mL from bone marrow aspirates. Our results, 135 ± 22.6 progenitor cells/mL of effusion fluid and 429 ± 99.8 progenitor cells/mL of byproduct tissue, suggest that the effusion fluid and byproduct tissue do not contain as dense a population of cells with stem potential as bone marrow.

The number of cells that are necessary to produce a therapeutic result for orthopaedic indications has not been established. In the Hernigou et al.[32] study regarding the treatment of tibial nonunion with bone marrow aspirate, the average number of stem cells injected at the nonunion site in patients who healed was approximately 55,000 ± 17,000, and the average number of stem cells injected in patients who did not heal was 19,000 ± 7,000. Other than this study, the therapeutic dosage of stem cell technologies has little evidence for clinical guidance. It is likely that, as studies clarify effective stem cell counts, dosage will vary with indication, and the methods and site of harvest will be chosen based on the clinical need. Our results suggest that approximately 1,900 progenitor cells are within the effusion fluid after a cruciate ligament injury and approximately 2,400 progenitor cells are available with harvest and processing of the fat pad and stump debridement tissue during cruciate ligament reconstruction. At this time, we cannot advocate any immediate clinical application of the cells from these sources. However, the cells of the effusion fluid can be concentrated at the time of surgery with simple density gradient methods, and new processing disposables are under development that immediately cause cells to preferentially release anti-inflammatory proteins such as interleukin 1 receptor antagonist and soluble receptor for tumor necrosis factor-α.[35] We theorize that an autologous injection of these anti-inflammatory proteins in the perioperative period would be beneficial.

Previous investigations have evaluated ACL stump tissue including quantifications of stem cells and their therapeutic potential.[17-19] In an animal study, cells cultured from ACL injury stump tissue have proven effective in augmenting graft incorporation.[18,19] Using flow cytometry analysis to evaluate ACL injury tissue from humans, Matsumoto et al.[17] confirmed the presence of cells with the cell surface marker CD34, a marker expressed by hematopoietic stem cells, and cells with the cell surface marker CD146, a marker

expressed by perivascular stem cells. These cells showed multipotentiality in vitro.[17] The CD34+ cells proliferated more rapidly than the CD 146+ cells, and significantly more stem cells were documented within injured ACL tissue than noninjured ACL tissue.[17] We identified a significant population of immature cells, 83.0% to 96.9%, within the samples collected, represented by the lineage negative populations. Of these immature cells, 2.1% to 2.9% were CD34+ and 1.7% to 6.6% were CD 146+, whereas a majority 86.1% to 96.8% expressed CD 31, a marker for endothelial cells and immune cells. The high percentage of the lineage negative marker and immune marker lead us to believe that the most of these cells are immature immune cells. Although cells with CD34 and/or CD 146 are consistent with cells expressing stem potential, it is hard to know if cells within the lineage negative that are CD 31 positive also have stem potential. When interpreting flow cytometry results, it is important to consider that cells change expression of cell surface markers dependent on the environmental niche that they occupy as well as after culture.[17,40]

Limitations

This study is limited by a small sample size. It sought to provide pilot data for a further analytical study of harvest methods while confirming the presence and viability of cells with stem potential within the effusion fluid and waste fluid of cruciate surgery. A power analysis was impossible to perform before the study because the number of cells mobilized with knee injury had not been previously evaluated, and the therapeutic dosage of stem cell technologies had not been established. When looking critically at the sample size, the study was likely underpowered with regard to the small volume harvest method compared with the large volume harvest method; therefore, conclusions between the harvest methods may be susceptible to type II error. In addition, some of the samples were not sufficient to run all the tests due to the variability of the sample harvest. A limitation of quantification studies involving assays is that the number of CFU-F and CFU-C progenitor cells present in a particular culture or the average in an individual study is highly dependent on the culturing techniques, conditions, and investigators' definition of a colony. Further testing comparing the effusion aspirate with bone marrow aspirate in the same individuals would provide better comparison data. This study sought to develop a methodology around byproduct tissue capture. Lessons learned will help us optimize and validate byproduct tissue capture as a source for viable stem cells further.

Conclusions

Viable stem cells are mobilized to the postinjury effusion at the time of cruciate ligament injury and can be found in the byproduct waste tissue of cruciate ligament surgery.

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