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Research

Evolution of the Push-2-Spin Fat Graft Processing Device: Enhancing Efficiency and Reducing Risk of Contamination

Shawn J. Loder, MD; Fuat Baris Bengur, MD; Phoebe L. Lee, MD; and Lauren Kokai, PhD

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Abstract

Background: Small-volume fat graft efficiency is a critical determinant of the cost and material effectiveness of aesthetic fat grafting in the clinical space. Recent development of devices, such as the Push-2-Spin (P2S) system (Pittsburgh, PA), has improved upon the process by yielding a rapid, handheld, multi-use system to minimize operative time and mess.

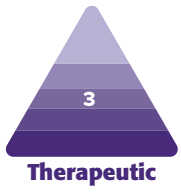
Objectives: In this study, the authors describe further technical innovations on the P2S prototype that improve operative ease of use, time, and safety.

Methods: Abdominoplasty samples were obtained as discarded tissue. Lipoaspirate was collected utilizing a 3.0 mm liposuction cannula and processed through centrifugation (Coleman technique), gauze (telfa) rolling, mesh straining, the tabletop P2S device (prototype), or the P2S handheld (P2S-H) device. Operative processing time, spin time, oil fraction, stromal vascular fraction (SVF) yield and viability, and adipocyte viability were assessed to compare the efficacy and viability of each device/technique. Blood agar smears of lipoaspirate were performed to assess for risk of contamination.

Results: The P2S-H device outperformed its prior iteration in rotary and processing speed and was significantly faster than each other technique assessed. Furthermore, the use of an inline system offered significant advantages over open-air techniques in terms of resistance to contamination. Serial use characteristics were assessed; under these conditions, oil yield as well as adipocyte and SVF number and viability was similar between all techniques.

Conclusions: The technical advancements to the P2S system which enable single-unit, handheld operation significantly improve operative time and minimize space requirements. This operative quality of life improvement comes at no cost to the efficacy of oil extraction, cellular yield, or cell viability.

Level of Evidence: 3



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The efficacy and availability of autologous fat grafting have pushed the technique well beyond its initial indications and limitations in the operative space. Fat grafting is now essentially ubiquitous in plastic surgery and across the spectrum of aesthetic and reconstructive care. Consequently, as the technique has evolved away from a purely operative procedure, there is a constant need to evaluate and improve upon the safety and efficiency of the tools used. As

From the Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, USA.

Corresponding Author:
Dr Lauren Kokai, Scaife Hall, Suite 6B 3550 Terrace Street, Pittsburgh, PA 15261, USA.
E-mail: kokail@upmc.edu

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opposed to larger and more complex cases, small-volume fat grafting is exceedingly well suited to the tertiary surgical, clinic, and office environment.[1,2] A majority of these procedures can be performed with minimal anesthetic requirements and high patient convenience, satisfaction, and consequently demand.[3-5] As indications expand, however, the needs of the practitioner shift in tandem and logistical and technical limitations, which had been negligible in the operating room, take on new importance in the clinic and office space.

Currently, small-volume lipoaspirate processing is highly variable with a range of techniques vying for the "gold standard." Traditional Coleman centrifugation, nonadherent gauze rolling, and mesh filtration are common and well-described methods to remove undesirable organic/oil and aqueous fractions from lipoaspirate.[1,2,4] While each technique is effective in achieving its goal, it carries several logistical and technical disadvantages when used outside of the operating room. In the clinic, time and space are limited resources and long processing, extensive use of counter space, peripherals, equipment, and assistants can increase time and overhead reducing the value of the technique. Similarly, use of open-air processing (gauze rolling, mesh filtration) requires processing space, multiple biohazardous disposables, and, when outside of the regulated clean-air environment of the operating room carries further risk of contamination.[6-8] Closed systems or use of a closed/sealed Coleman-style centrifugation process negate these risks; however, they often require costly equipment and a complex setup which mitigates their perceived benefits.

To address these issues in turn, Yang et al proposed a table-mounted, closed system that doubled as both a negative-pressure syringe and hand- or spring-powered rotary centrifuge.[9] This device, the Push-2-Spin (P2S; Pittsburgh, PA) system developed by the senior authors on that manuscript, Drs Jeffrey and Beth Gusenoff, was intended as an all-in-one device to minimize the time, mess, and need for costly peripherals described above. Their data suggest that the device was effective as described with improvements in time saved, minimal mess, and decreased need for peripherals to provide a cytologically viable product. However, successive evaluation of the device led to identification of several key areas of improvement: (1) the need to transition from tabletop to handheld device to better suit the limited sterile space available in the clinic; (2) improvement and standardization of rotary speed to more quickly and reliably enhance oil clearance; (3) improvement in internal mesh design to improved efficacy of a single device for multiple sequential uses in a single operative encounter; (4) enhanced scaling between 10 and 20 cc sizes to enhance utility and better improve fit for hand size/intrauser variability; and (5) a critical analysis on the safety and sterility of the device in direct comparison with other processing techniques under clinic-appropriate conditions. The P2S handheld (P2S-H) is the result of successive iterations to address these areas for improvement, and the results of our evaluation of this device are presented below (Figure 1A).

METHODS

Human Samples and Sample Processing

Human tissue samples ($n = 11$) were obtained as discarded tissue from elective body contouring procedures under the IRB exemption (PRO13090506) between June 2020 and June 2021 as full-thickness panniculectomy and/or abdominoplasty samples. All samples were collected under operative sterile conditions, transferred in a sterile insulated container, and processed within 6 h of resection. All samples are maintained under sterile conditions in a biological safety hood at ambient room temperature for all procedures. Exclusion criteria included evidence of active infection and/or panniculitis. No tumescent was infiltrated prior to lipoaspirate harvest. Under sterile conditions in a dedicated cell and tissue culture hood, lipoaspirate was obtained with 3 mm cannulas through syringe liposuction, utilizing either a 10 or 20 cc syringe for non-P2S groups or a 10 or 20 cc P2S device.

Device Details

Technical descriptions of the P2S technology as described in Yang et al. The transition from benchtop to handheld model is described in Figure 1A. Briefly, the P2S-H contains an indwelling suction device allowing for function as a syringe during liposuction with a male Luer-lock head to integrate with standard Luer-lock cannulas. Both 10 and 20 cc versions use similar suction mechanics. Lipoaspirate is drawn into a central filter-lined chamber that is then secondarily spun to allow for filtration of oil and aqueous components by filtration. Aqueous and organic effluent is then drained through a side port. Now processed lipoaspirate is immediately available for grafting and may be re-used after grafting for additional liposuction. The usage of the P2S-H device is showcased in Video.

Sample Processing

Samples in the Coleman group were centrifuged at 300*g* for 5 min, with the oil and aqueous fractions evacuated as described by Coleman. Samples in the nonadherent gauze (Telfa; Covidien, Dublin, Ireland) group were manually rolled over 2 pads per described techniques. Samples in the Mesh group were strained through a sterile mesh tea

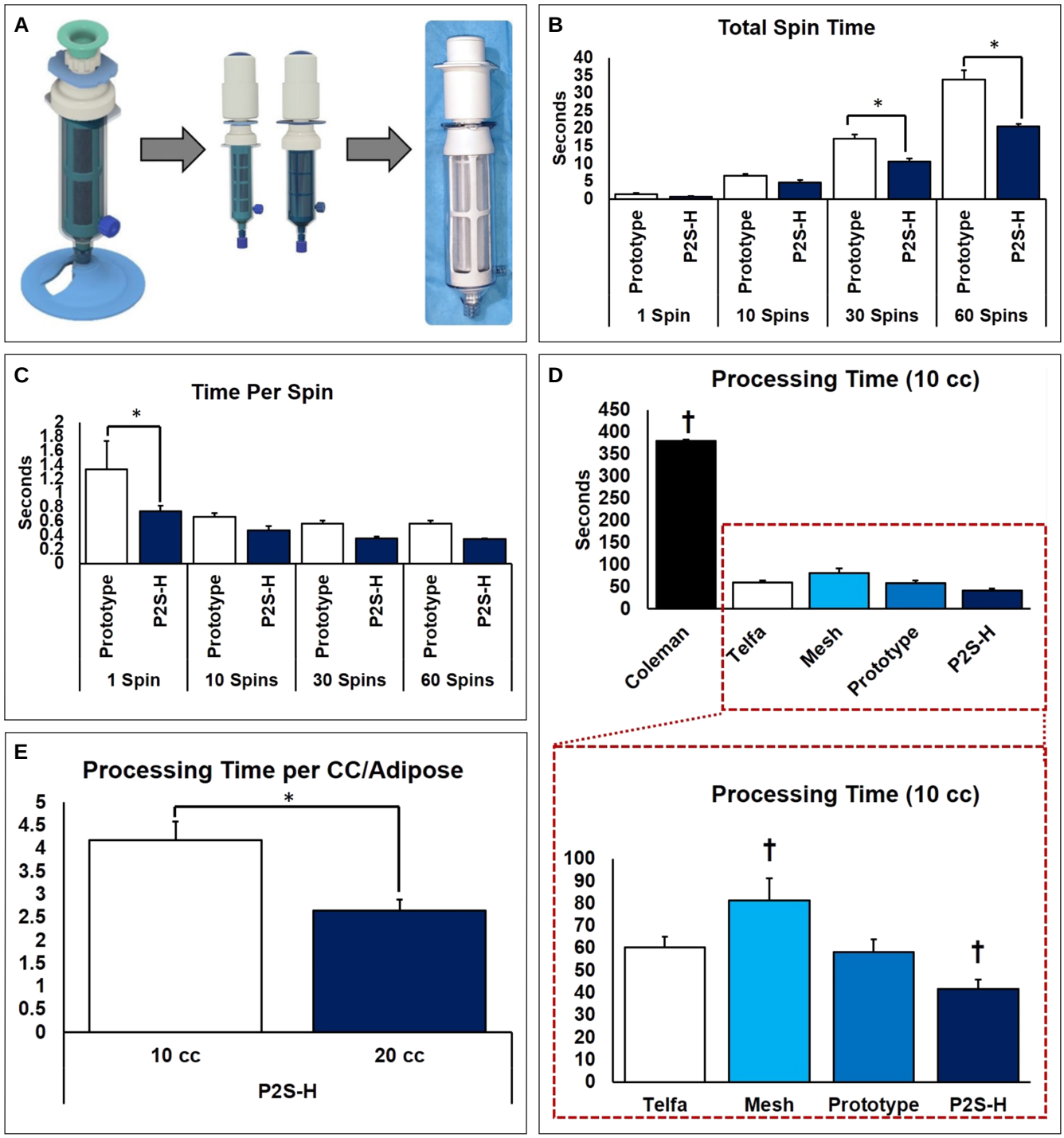
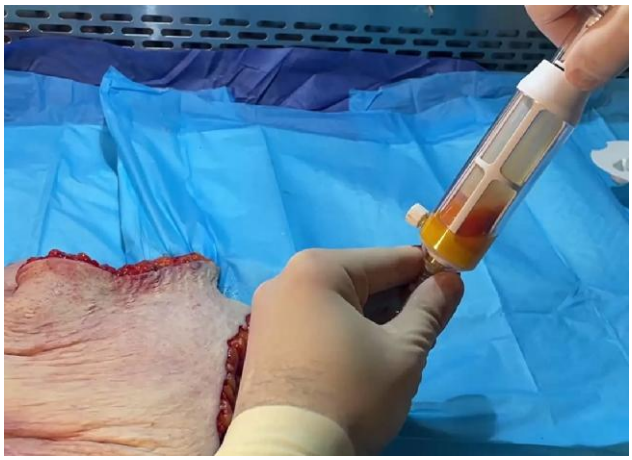


Figure 1. (A) Schematic representation of the Push-2-Spin handheld (P2S-H) device with comparison to the original benchtop system. (B) Total spin time is significantly decreased with the P2S-H vs the prototype. (C) Time to spin is decreased in the P2S-H vs prototype. (D) The P2S-H significantly improves spin time vs both the P2S prototype and all other techniques tested. (E) There is a significant improvement in processing time per cc of lipoaspirate in the 20 vs 10 cc version of the P2S-H system. All error bars represent SD. †Significant difference among all the other represented groups. *$P < .05$. For all timed trials, $n = 10$.

strainer with constant agitation using a sterile glass rod to minimize cellular injury or clumping. Samples in the P2S-H and P2S prototype groups were processed by pushing for 30 pushes unless otherwise specified. The remaining lipoaspirate was not processed further and served as the control.



Video. Watch now at http://academic.oup.com/asjopenforum/article-lookup/doi/10.1093/asjof/ojad093

Processing Time

Processing time was determined serially by measurement of discrete motions/tasks (ie, unscrewing nozzle, decanting oil, etc) inherent to each processing step. For centrifugation, a block of 5 min was added to all measurements per our standard lipoaspirate processing protocol. During processing from start to finish, the stopwatch was not stopped until completion of the discrete task. The average of 20 attempts at each discrete processing step was collected, and average times were summed to determine the overall time to complete each processing technique from start to finish.

Centrifugation and Oil Column Assessment

Oil columns were generated by collecting control or processed lipoaspirate into 5 mL polystyrene round bottom tubes. These were then initially spun at 300*g* for 5 min to separate the organic and aqueous fraction. Each layer was measured with a ruler with 1.0 mm minimum increments at the midpoint between the highest peak and trough of each given layer. The height of the aqueous fraction was excluded from further measurements.

Colony-Forming Assay

Each of the processing techniques described above was performed either in a laminar-flow hood to stimulate the regulated-air environment of a modern sterile operating room or in open air. For open air, a sterile field was created to simulate the clinic or office space with sterilization of all surfaces and placement of a sterile drape prior to the opening of any sterile packs and/or autoclaved materials. After lipoaspirate was processed, samples were returned to a sterile biosafety cabinet and mixed with sterile saline. An aliquot of sterile saline from each sample was smeared under standard technique across blood agar, and the blood agar plates were transferred to a 37° incubator for 48 h to assess for the formation of colonies. After 48 h, the plates were removed from the hood for photography. Representative photographs were presented as is for qualitative evaluation, and semi-quantitative evaluation was performed by division of each plate into 72 distinct high-powered fields (HPFs) for random quantification of 20 HPFs per sample.

Cell Counts and Viability

Processed lipoaspirate was transferred to 50 cc Falcon Conicals (Thermo Fisher Scientific, Waltham, MA) and digested in a 1:4 ratio of 0.1% collagenase Type I (Worthington Biochemical, Lakewood, NJ) in 1% bovine serum albumin (Sigma-Aldrich, St Louis, MO) and Hank's buffered saline solution (Gibco; Thermo Fisher Scientific) at 37°C for 60 min. Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific) was added for neutralization, and the mixture was centrifuged at 300*g* for 5 min to isolate a distinct stromal vascular fraction (SVF) pellet and a distinct adipocyte layer. The adipocyte layer was decanted away from the column for separate assessment as described below. The aqueous fraction below the adipocyte layer was then removed, and the SVF pellet was resuspended in red blood cell lysis buffer for 5 min before DMEM with 10% FBS was utilized for neutralization. This was centrifuged at 300*g* for 5 min to generate a purified cellular pellet which was resuspended for cell counting. Cell counting of SVF was performed using a hemocytometer. Twenty microliters of the cell suspension was collected and mixed with a 1:1 ratio of 0.4% trypan-blue solution (Gibco; Life Technologies). Cells were visualized utilizing an Axiovert 25 CFL Inverted Fluorescence Phase Contrast Microscope equipped with a 50 W mercury HBO fluorescence lamp with mbq52ac power supply (Zeiss).

Live, dead, and total nucleated cells were calculated to generate the viability:

$$\frac{\text{Live nucleated cells}}{\text{Total nucleated cells}}$$

Total cell counts were then determined by the following equation:

$$(\text{Total nucleated cells}) \times (\text{Dilution factor}) \times 10^4$$

where dilution factor equals:

$$(\text{Ratio of trypan dilution}) \times (\#\text{cc of 10\% DMEM suspension})$$

Adipocyte number and viability were quantified using Calcein-AM and propidium iodide visualization and a Cellometer automated cell counter (Thermo Fisher Scientific). Counts and images were collected through the use of onboard Nexelon software and presented as is.

Statistical Analysis

All appropriate data are presented as mean and standard deviation (SD) for all groups. The analysis of variance was performed to determine the statistical difference between groups, and Tukey's HSD was utilized to determine the relative *P*-value between any 2 groups. Statistically significant differences were determined using *P*-value <.05.

RESULTS

The P2S-H Device is Significantly Faster vs Other Lipoaspirate Processing Techniques Utilized in Clinical Practice

The P2S system utilizes centrifugal force to filter lipoaspirate against an internal mesh membrane for the purpose of removing aqueous and oil contaminants prior to engraftment. A key area of variability in clinical evaluation of the prototype tabletop system was user-dependent differences in rotary velocity. This affected both the speed and degree of purification with slower use translating to less efficacious clearance. To correct for this, the rotary system for the handheld device incorporated into it a free spinning mechanism such that the complete compression of the rotor in the handle would translate to continuous rotation upon release of the mechanism consequently compensating for variability in operator speed, resulting in a faster spin and ultimately a decrease in the overall time for a given number of rotations (Figure 1A-C). The decrease in overall spin time between the prototype device and the P2S-H resulted in an overall decrease in processing time per-unit adipose (10 cc; prototype: 58.25 ± 5.68 s vs P2S-H: 41.77 ± 4.07 s; $P < .05$) and translated to a statistically significant decrease in processing time vs both mesh (81.33 ± 9.85 s) and gauze/telfa rolling (60.25 ± 4.91 s; Figure 1D). Notably, traditional centrifugation techniques were notably slower vs both P2S iterations given the static centrifugation time of 5 min under standard conditions (380.32 ± 2.33 s; $P < .05$; Figure 1D). The P2S-H device was generated in 2 variants based on the size of the reservoir. The 20 cc version required slightly greater time than the 10 cc version for filtration of a full reservoir (52.98 ± 4.90 s); however, when correcting for volume, this actually resulted in a statistically significant decrease in time/cc adipose (10 cc: 4.18 ± 0.41 s vs 20 cc: 2.65 ± 0.25 s; $P < .05$; Figure 1E).

Adipose Purification is Rapid and Remains Reliable Across Multiple Runs of the P2S-H Device

The efficacy of oil and aqueous purification was assessed as the primary functional metric of the P2S system (Figure 2A). Total oil release preprocessing and postprocessing as determined by centrifugation was similar between all techniques tested (Figure 2B) with all techniques demonstrating significant improvement vs untreated controls. We noted that in the P2S devices, oil purification increased directly with the number of spins performed with the P2S system approaching total free-oil clearance between 30 and 60 spins regardless of iteration (Figure 2C, D). There was no significant difference between the 10 and 20 cc versions of the P2S in regards to purification efficiency (Figure 2E). When multiple processing runs were assessed serially, we noted consistent oil clearance for up to 2 successive uses of the mesh with a slight albeit statistically significant loss of efficacy starting with the third consecutive use of the internal mesh chamber (Figure 2F, G). Notably, even at fourth consecutive run, oil purification of the P2S-H was no less effective than standard centrifugation (Figure 2B, F, and G).

Use of an Inline Device Such as the P2S Enhances Operative Sterility and Minimized Contamination Risk to Lipoaspirate

In addition to the time saved and efficacy of purification, a key benefit of an inline system is the reduction of exposure to environmental contamination. While initially sterile, exposure to open air during processing during technical steps such as mesh processing or telfa rolling provides the opportunity for microbial contamination. While this can be mitigated to some extent in the operating room, open air is not reliably sterile in the outpatient clinic or office setting.[7,8] To evaluate the efficacy of an inline system in minimizing contamination, we evaluated open (mesh, telfa) and closed-processing (centrifugation) techniques performed within sterile fields under open-air and laminar-flow conditions utilizing a standard colony-forming unit (CFU) on blood agar assay (Figure 3A, C). These were compared with the use of the P2S device under open-air conditions (Figure 3B, C). We additionally tested whether repeat usage of the P2S device increased the risk of contamination (Figure 3D). We noted that when performed under laminar-flow conditions, contamination was absent and no significant differences were identifiable between techniques (Figure 3A, B). When exposed to open air, contamination was present in each of the traditional techniques with the use of a steel-mesh filter, demonstrating the

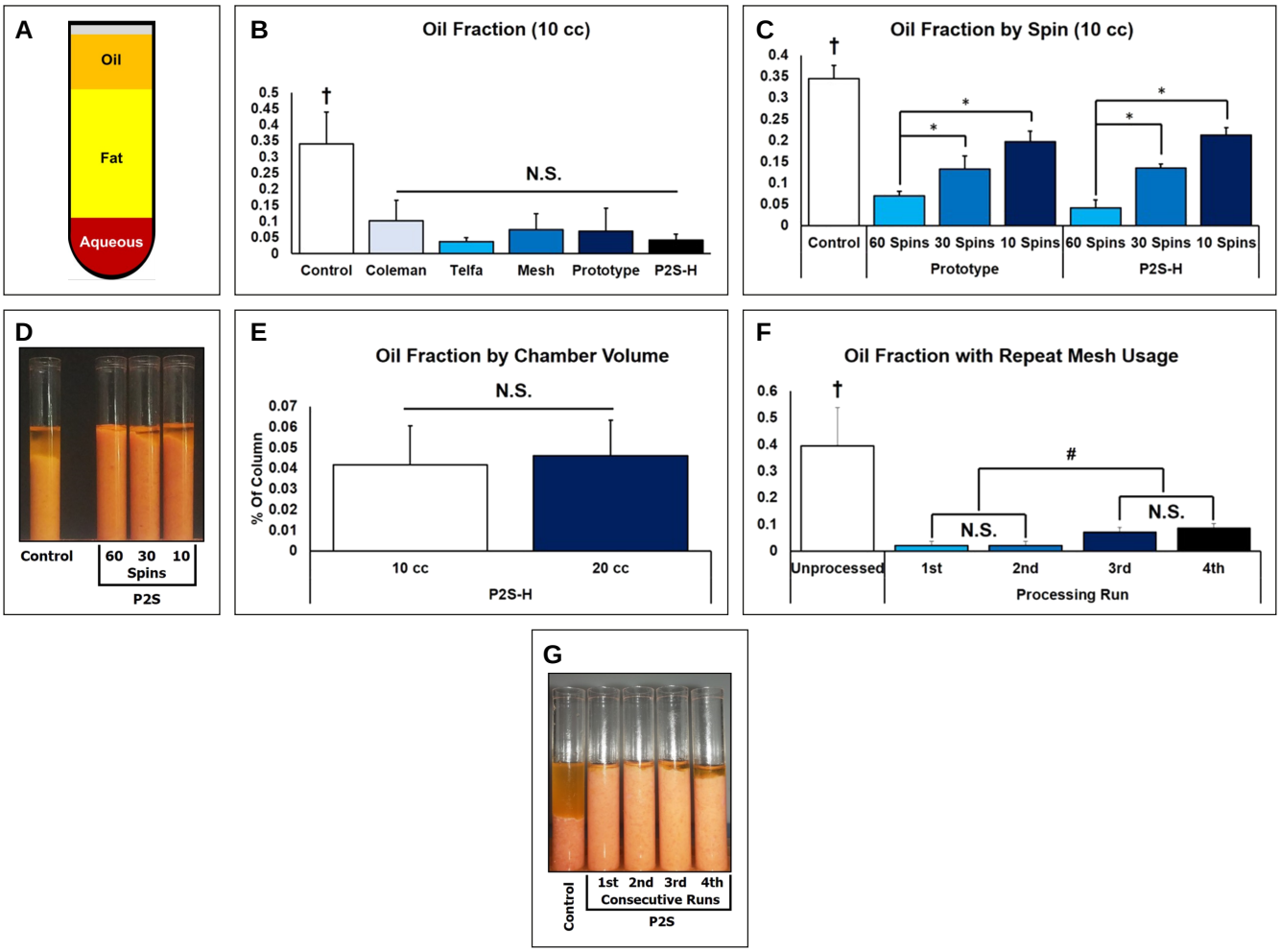


Figure 2. (A) Oil column schematic. (B) Oil is significantly purified with all techniques/devices tested vs unspun control lipoaspirate. (C) Purification increases with an increasing number of consecutive spins. (D) Representative oil columns of the effect of consecutive spins. (E) No significant difference was noted in the oil fraction between samples collected from 10 and 20 cc devices. (F) Purification decreases with an increasing number of sequential purification. (G) Representative oil columns of the effect of sequential purification of the Push-2-Spin handheld (P2S-H) system. All error bars represent SD. *$P < .05$. †Significant difference among all other represented groups. #Both first and second processing runs are significantly different when compared with both the third and fourth processing runs. For all adipose/oil columns assessed, $n = 5$.

greatest magnitude of CFUs formed (Figure 3A, C). On the initial run, no contamination was noted with the P2S-H system (Figure 3B, D); however, on successive runs, we noted the formation of 1 CFU on one of the plates tested (Figure 3B, D).

Cytologic Viability and Cell Density is Preserved Utilizing the P2S System

Cytologic density and viability provide a useful proxy for the overall health or quality of lipoaspirate. Mature adipocytes and heterogenous SVF are the 2 key cytologic populations regularly evaluated in human lipoaspirate and vary significantly in both number and viability between different harvest and processing techniques. To assess the postprocessing quality of lipoaspirate after usage of the P2S system, samples of lipoaspirate from each device iteration were enzymatically digested and compared with patient-matched unprocessed and centrifuged (Coleman-processed) controls. No loss of viability or reduction in SVF count by trypan-blue exclusion was noted with any variation of the P2S system vs controls (Figure 4A, C). No significant difference was noted between 10 and 20 cc variants of the device (Figure 4B, D). Mature adipocytes were simultaneously collected and evaluated using calcein/propridium iodide co-staining which demonstrated no loss in viability or qualitative change in morphology between the P2S system and controls (Figure 4).

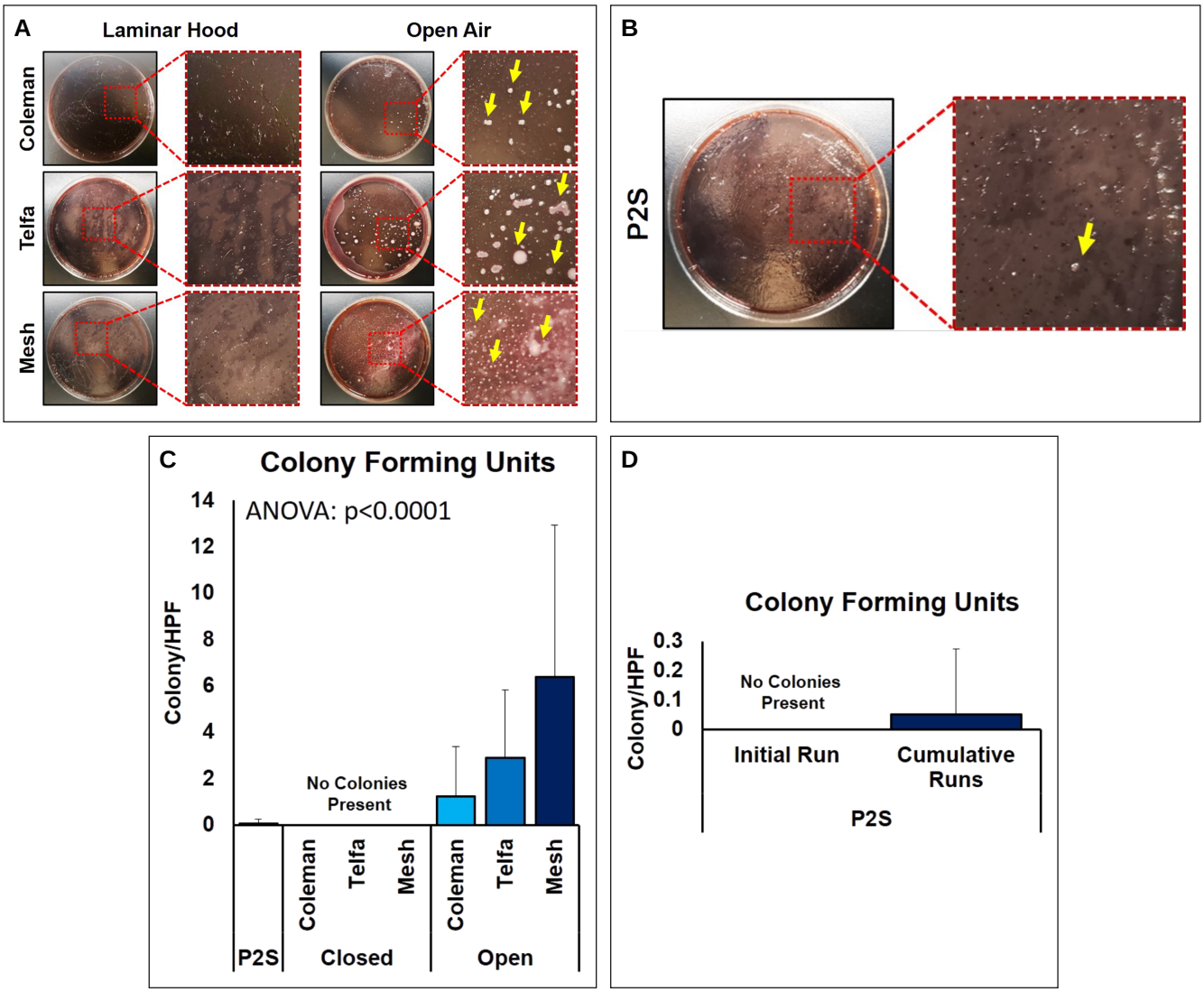


Figure 3. Representative image of blood agar plates inoculated after (A) traditional or (B) Push-2-Spin (P2S) techniques for adipose processing under laminar air-flow conditions (left) or under open-air sterile field (right). (C) Quantification of colony-forming units (CFUs) (arrows) from high-powered fields (HPFs) of blood agar plates inoculated after the conditions described for A or B. (D) Quantification of CFUs from HPFs of blood agar plates inoculated after either first pass or subsequent fat processing using the P2S device. All error bars represent SD. *$P < .05$. For all conditions assessed, $n = 20$ HPFs.

DISCUSSION

High patient demand for small-volume fat grafting will continue to drive its growth and spread for the foreseeable future, and as these techniques migrate from the operative setting and into the community clinic and office space, there is an abundant need to address the limitations of those settings. There are a myriad of tools and technologies available to filter and purify lipoaspirate each with their own advantages and disadvantages. The key to small-volume fat grafting is the need to balance price, speed, efficacy, and safety. Currently, open-air techniques, such as mesh filtration, gauze/telfa rolling, and some forms of open Coleman centrifugation, are highly penetrant in operative fat grafting given the efficiency and minimal risk of centrifugation-induced injury. These techniques, however, expose previously sterile lipoaspirate to the open air. While in the operative field, air circulation and negative-pressure technologies are in place to prevent contamination; however, the same cannot be said for every clinic or office where small-volume fat grafting is performed. Our data suggest even brief exposure to lipoaspirate outside of the sterile field risks contamination. A limitation of this approach is the question of the relevance of laboratory conditions to the clinical setting in terms of infection risk. Although a common metric in preclinical product evaluation, it is not

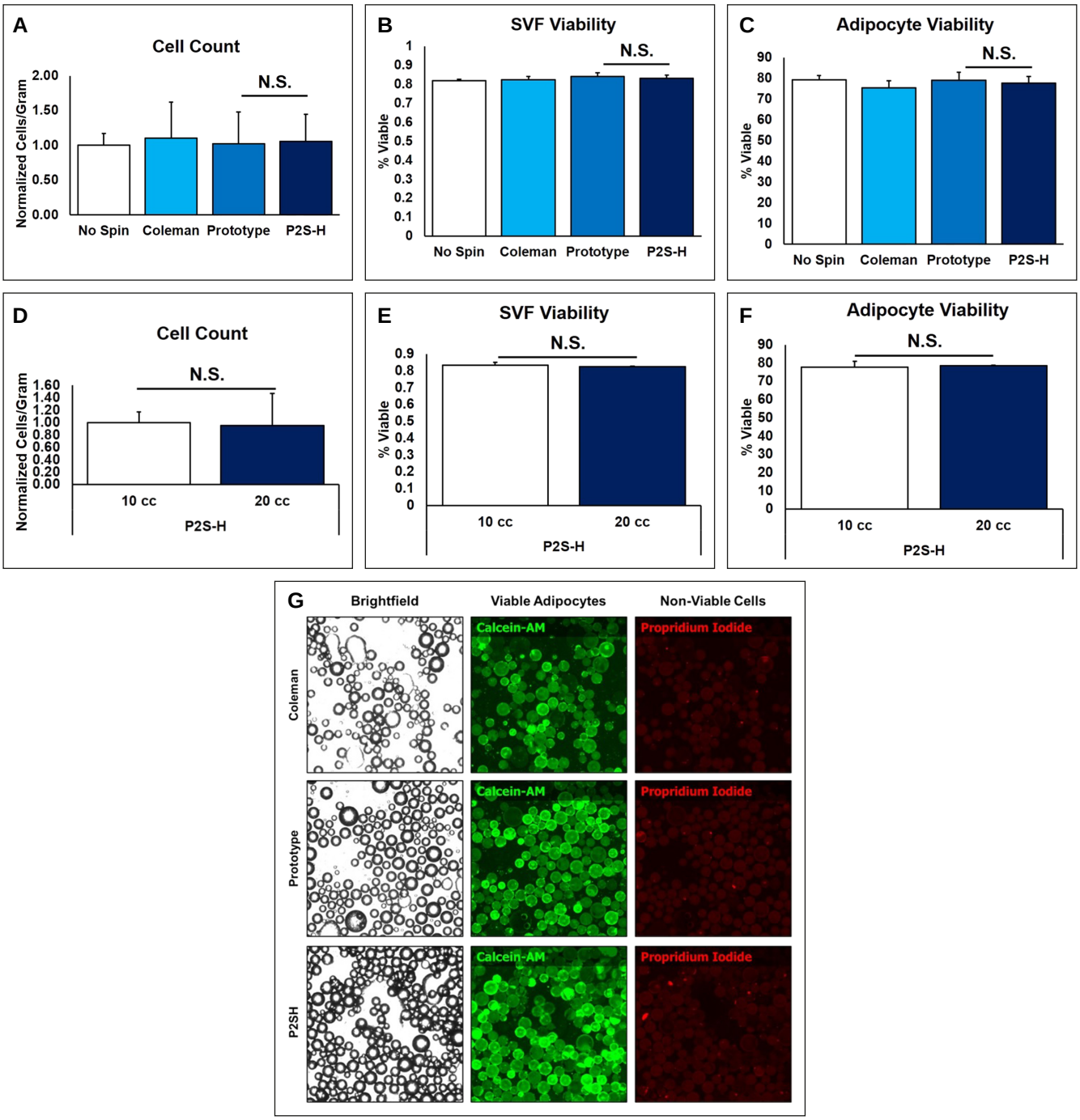


Figure 4. There is no significant difference in stromal vascular fraction (SVF) density (A) between any of the techniques tested ($n = 5$) or (B) between the 10 and 20 cc devices tested. There is no significant difference in SVF viability (C) between any of the techniques tested or (D) between the 10 and 20 cc devices tested. There is no significant difference in mature adipocyte viability (E) between any of the techniques tested or (F) between the 10 and 20 cc devices tested. (G) Representative images of mature adipocytes collected after either Coleman centrifugation or spinning using the 2 Push-2-Spin devices tested (Protoype and P2S-H). Viability was assessed by Calcein-AM (middle column); cell death was assessed by propridium iodide exclusion (right column). Images were obtained from an automated Cellometer readout utilizing onboard software. All images within a row represent the same high-powered field and scales are equal for all rows. All error bars represent SD. *$P < .05$. For comparisons between groups, $n = 5$.

clear that CFUs in vitro or ex vivo directly translate to gross infection, given the presence of an intact immune system and/or antibiotic prophylaxis in patients undergoing fat grafting, it is uncertain how subclinical colonization may contribute to graft-site inflammation, fibrosis, or graft resorption. We found that the inline P2S-H system minimizes these risks even in the setting of serial use with the same patient. Notably, there are other inline handheld systems, such as the LipiVage or Lipografter, which could presumably provide protection but were not evaluated in this study.[9,10] Similarly, the Puregraft and Dermapose devices are designed for closed-system adipose processing; however, we did not compare them with repeated washing/decantation processes because techniques such as centrifugation, mesh filtration, and gauze/telfa rolling are ubiquitous in the clinical space.[11] In our evaluation, we accepted this limitation to the work particularly given the current preclinical nature of the device and sought to compare its efficacy across multiple metrics vs traditional techniques.

After the risk of contamination, there are the logistical and technical concerns associated with small-volume fat grafting. Traditional Coleman-style centrifugation may be considered the most analogous technique to the P2S; however, there is nearly a log-fold difference in centrifugation time between the techniques. When compared with prior versions, we had previously noted variability in rotary efficacy and speed in the P2S prototype. Modifications during the transition from tabletop to handheld device resulted in an improvement in processing time of approximately 28% with the P2S-H system. Scalability to 20 cc further allowed for an additional approximately 36% improvement in time/cc adipose processed. This benefit caps with the reservoir size for the current P2S-H system which is not removable. For larger volumes, alternate techniques or technologies including centrifugation or other handheld devices such as the LipoGrafter or a larger scale P2S may be suitable. Rather than scaling down to a 10 mL device, larger 30 or 50 mL devices may be designed to further optimize larger grafting needs. A key limitation of these analyses is time for training and device familiarity. Investigators who assessed each of the devices were familiar with all the techniques tested including both P2S devices. A user whose familiarity with a given technique or device differs may find that their own results can vary from these posted norms. Finally, from a functional standpoint, we noted statistically similar purification efficiency and consequently potential yield of processed fat per cc of harvested tissue between all techniques tested supporting clinician selection of the tool or technique that supports their specific practice pattern. Fat was not noted to be grossly lost to the chamber and when unprocessed lipoaspirate was compared with the Coleman centrifugation standard or with either iteration of the P2S system we noted no loss in viability or count with processing for either SVF or mature adipocytes.

There are some additional limitations to the work as described. Primarily, lipoaspirate was collected from abdominoplasty samples collected as discarded tissue. Differences in patient body habitus, time from resection, tissue temperature, and storage/transfer techniques present both real and theoretical differences in the mechanical, cytologic, and biochemical characteristics of fat, which could influence results. Additionally, all research staff who performed analyses were trained on the device prior to use, and no blinding was performed during data collection. Consequently, these data are preclinical in nature, and there remains a need for direct clinical study to ensure the safety and efficacy of the P2S system prior to FDA approval and market.

CONCLUSIONS

Taken together, these data support the value of the P2S-H system in the setting of the small-volume fat graft. The transition to a handheld system represents an ease-of-use advancement particularly advantageous for the outpatient setting and consequently reduces the barrier to access for small-volume fat grafting. The P2S-H device offers a safe, rapid, easy to use, inline system for small-volume adipose purification. Contamination risk and space used/mess made are reduced compared with traditional techniques by the use of an all-in-one inline device. Furthermore, operative time is significantly improved upon the prototype device with no loss in purification efficiency and with equivalent viability and cell density to prior versions/controls; consistent across SVF and adipocytes. These findings are in favor of further translation for the use of this technology for testing in a clinical setting.

Supplemental Material

This article contains supplemental material located online at www.asjopenforum.com.

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Disclosures

None of the authors in this manuscript have financial stake or holding in Push2Spin Technologies, Inc. (Pittsburgh, PA). The original Push-2-Spin System (P2S) and successive iterations evaluated in this manuscript were donated by Drs Jeffrey and Beth Gusenoff, co-inventors of the P2S device

and CEO and CCO, respectively, of the Push2Spin Technologies, Inc.

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