

Composition assessment of drained fluids in the Puregraft® Drain Bag

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BACKGROUND

The success or failure of long-term fat graft retention is known to be affected by many different variables, including how well different graft preparation techniques are able to remove undesirable contaminants such as free lipid, residual tumescent solution, red blood cells, and leukocytes. In an earlier study, we demonstrated that graft tissue prepared using a washing and filtration method in a commercially available closed, sterile system contains less oil and blood cells, and more viable adipose tissue than grafts prepared by other graft preparation methods. This product, called the Puregraft® System (Puregraft LLC, San Diego, CA), can be used to successfully standardize and increase the reproducibility of graft quality, which in turn can be expected to improve long-term autologous fat graft retention. A common question raised is whether the graft tissue and its desirable components will pass through the Puregraft system and be lost in the Drain Bag. Here we study this possibility by investigating the composition of the “wash-out” fraction in the Drain Bag of the Puregraft System

METHODS

The use of laser in combination with suction harvest is known to generate very fine pieces of fat tissue, creating a worst case scenario for this investigation. Subcutaneous adipose tissue was acquired by the laser + suction method from four donors (n=4). Each donor tissue was divided into two aliquots for different processing methods: control

(no manipulation prior to analysis) and Puregraft (tissue was simultaneously washed and filtered in the Puregraft System). The “wash-out” fraction that had collected into the Drain bags was collected and centrifuged to identify the different components for further visual and microscopic evaluation.

RESULTS COMPOSITION OF THE “WASH-OUT” IN THE DRAIN BAG

In order to simulate a worst case scenario for losing tissue into the Drain Bag, we used fine particle graft tissue in this study. The “wash-out” from the Drain Bag (Figure 1) was concentrated by a low speed centrifugation at 400xg for 5 minutes.

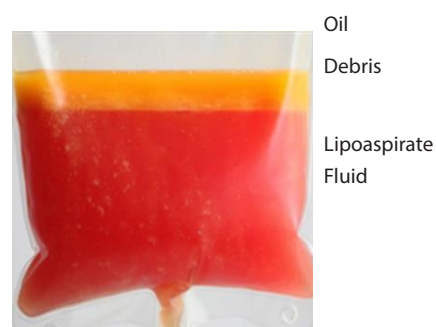


Figure 1. Representative example of the appearance of the Drain Bag after Puregraft processing. The drained fraction mainly contains washing solution (Lactated Ringer’s solution), blood, wetting solution, lipid, and tissue debris.

The “wash-out” was then separated by centrifugation into four component parts (oil, a white dense layer, aqueous fluid, and a bloody cell matrix pellet, from top to bottom) (Figures 2A and 2B).

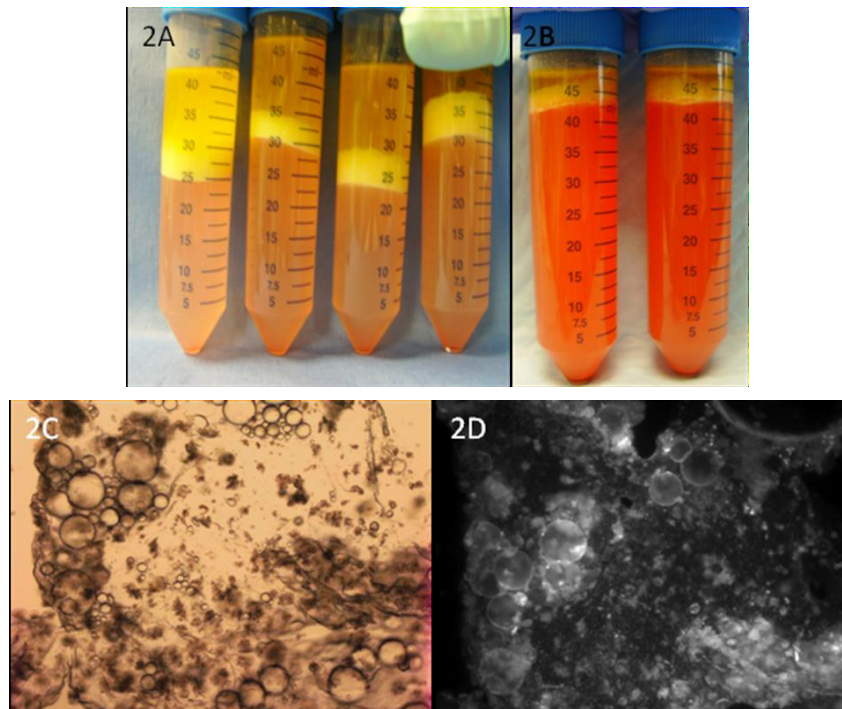


Figure 2. Composition of “wash-out”. (2A, 2B) Representative image of “wash-out” after centrifugation: bloody cell pellet (bottom), aqueous infranatant, dense adipocytes mixed with lipid layer (upper), and free lipid layer (top). (2C, 2D) Microscopic evaluation of “wash-out”. (2C) bright field image of white dense adipocyte layer; (2D), fluorescent image of “wash-out” labeled with “DAPI”. Only a few mature nucleated adipocytes were observed within the field; most of the samples were overwhelmed with smaller lipid droplets.

We collected the white dense layer and made a smeared slide to analyze its composition under a microscope. The slides primarily contained overcrowded lipid droplets (Figures 2C and 2D). Five randomly made smear slides from each donor sample was analyzed. After a thorough screening, only one single small clump of around 20 adipocytes was observed across all 20 slides. Thus, the probability of graft tissue being lost in the Drain Bag is extremely low. In a similar study, around 95% of RBCs and WBCs were removed from the graft by Puregraft. This study further demonstrated this reduction of RBCs and WBCs, as the analysis showed they were the main cell components in the “wash-out”.

CONCLUSION

Fat grafts prepared by Puregraft contain less aqueous liquid, oil, and blood cells, and more viable adipose tissue than grafts prepared by other methods¹. The amount of viable adipose tissue lost in the “wash-out” is insignificant in the four samples tested; only a few loosely attached adipocytes may be washed out. Rather, we have demonstrated the drained fluid consists almost exclusively of contaminants: lipid, tumescent fluid, red blood cells, and leukocytes. Thus, preparation of grafts by Puregraft can be used to successfully standardize and increase the reproducibility of graft quality without loss of desirable components during processing.

REFERENCES

- (1) Zhu M, Cohen R, Hicok KC et al. Comparison of three different fat graft preparation methods: gravity separation, centrifugation, and simultaneous washing with filtration in a closed system. *Plast Reconstr Surg.* 2013 Apr; 131(4):873-80.

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