

are well preserved when centrifuged at below  $3000\times g$ .<sup>13</sup> Thus, the adipocyte/ASC ratio can be increased after strong centrifugation (by 20% when centrifuged at  $1200\times g$ ; see Fig. 1B). Adequate centrifugation condenses tissue and ASCs, and also improves fat graft survival, although too strong centrifugation may worsen graft survival.<sup>13,14</sup>

There are some other attempts to further condense adipose graft tissue. Mechanical chopping, shredding, pureeing, or mincing, manually or with specific devices (like homogenizers or food processors), can further fragment aspirated fat tissue and rupture adipocytes. Appropriately, such mechanical processing can reduce substantially adipocytes, which become oil and can be removed by subsequent centrifugation. As a result, condensed fat tissue with a reduced volume can be obtained, although excessive processing can kill ASCs as well as endothelial cells, and has to be avoided. Ultrasonic cavitation may be also useful to damage selectively adipocytes in the future.

#### Adipose-Derived Stem/Stromal Cells Condensation by Supplementing Stromal Vascular Fraction or Adipose-Derived Stem/Stromal Cells

Another strategy is supplementing freshly isolated SVF or culture-expanded ASCs to aspirated fat tissue and called cell-assisted (enhanced) lipotransfer (Fig. 3). We can achieve SVF cells from lipoaspirates through collagenase digestion (processed lipoaspirate cells), although a much smaller number of SVF is also obtained from the fluid infranatant portion of liposuction aspirates (liposuction aspirate fluid cells).<sup>1</sup> Other nonenzymatic methods, such as mechanical processing and ultrasonic cavitation, have been attempted, but there are no established, efficient methods so far.

#### Stromal Vascular Fraction Isolation Procedures

For processed lipoaspirate cells, suctioned fat tissue is digested with 0.075% collagenase in phosphate-buffered saline for 30 minutes on a

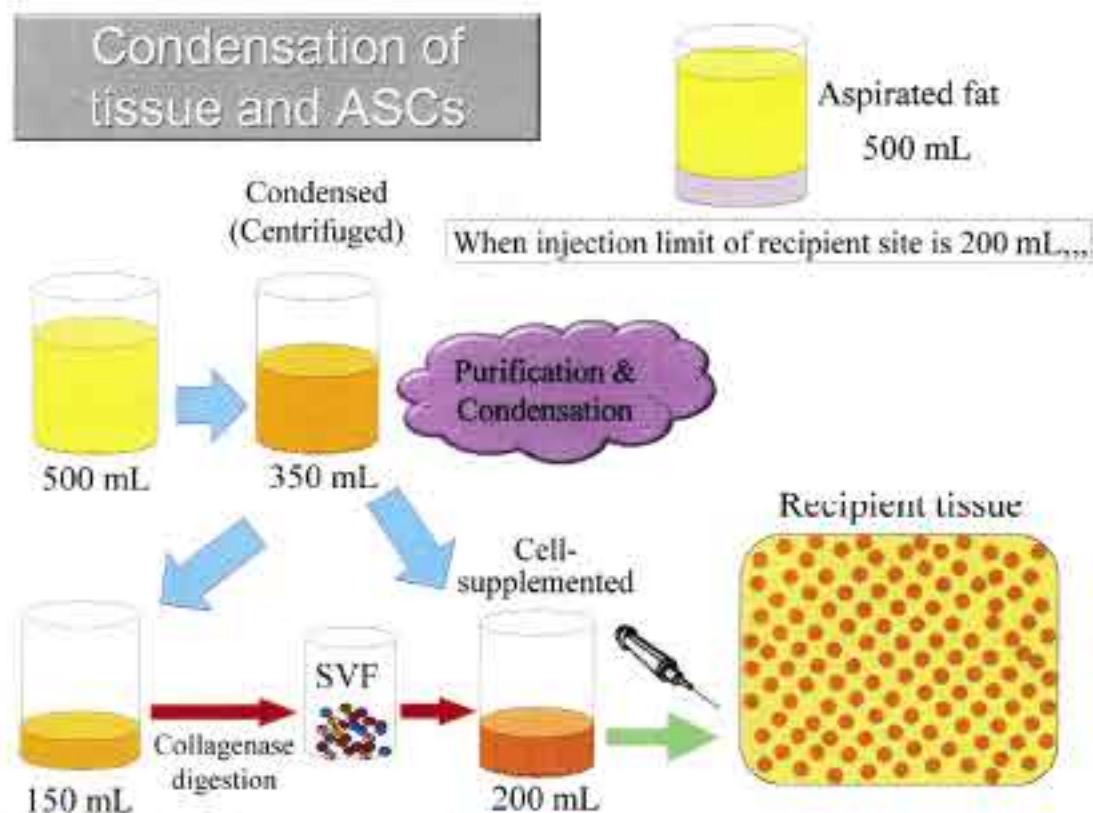


Fig. 3. An example of how to use aspirated fat. In this case, when we have 500 mL aspirated fat but the recipient tissue accepts a 200-mL injection at maximum (eg. owing to limited skin envelop), we can process and condense the graft tissue and adipose-derived stem/stromal cells (ASCs) before transplantation. Even after strong centrifugation, 350 mL of condensed fat tissue remains. Then the excessive 150 mL of centrifuged fat can be used for stromal vascular fraction (SVF) isolation for further condensation of ASCs in the graft material.