Editorial Commentary: Mesenchymal Stem Cell Preparation Methods Affect the Properties of Shoulder Subacromial Bursa-Derived Cells

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Abstract: Augmentation of mesenchymal stem cells (MSCs) from the subacromial bursa in the setting of irreparable rotator cuff tears is a powerful candidate for future regenerative medicine. However, little is known about whether the preparation technique affects the individual differences and potentials of subacromial bursa-derived MSCs. The yields obtained by chopping or cell-expansion techniques with a high-density of initial nucleated cells seem to be lower than those obtained with low-density cell-expansion techniques. Differences in cell-preparation methods may affect whether individual differences in their properties exist. Further studies are required using a low-density cell-expansion technique to establish a new regenerative treatment using subacromial bursa-derived MSCs for irreparable rotator cuff tears.

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Augmentation of mesenchymal stem cells (MSCs) from the subacromial bursa for rotator cuff tear surgery is a powerful candidate for future regenerative medicine. However, very little is known about the individual differences in the potential of subacromial bursa-derived MSCs. The article, "Subacromial Bursa-Derived Cells Demonstrate High Proliferation Potential Regardless of Patient Demographics and Rotator Cuff Tear Characteristics," reported by Muench, Baldino, Berthold, Kia, Lebaschi, Cote, McCarthy, and Mazzocca, investigated the individual differences and concluded that the proliferation potential of subacromial bursa-derived cells (SBDCs) was comparable regardless of the patient's characteristics, including the severity of the rotator cuff tear or osteoarthritis of the glenohumeral joint.[1]

Several studies reported that the properties of the synovium in terms of the yields of MSCs are associated with age and the severity of osteoarthritis. Mochizuki et al.[2] demonstrated that the nucleated cell number per synovial weight was greater in elderly donors than in young donors. Murata et al.[3] reported that the number of MSCs from the cotyloid fossa synovium of the hip joints was negatively correlated with age. They also revealed that the number of MSC colonies per nucleated cell was significantly greater from donors with severe hip osteoarthritis than from donors with femoroacetabular impingement without osteoarthritis.[4] Muench et al.[1] showed in their current study that the number of SBDCs per weight was not correlated with the severity of osteoarthritis or age.

It is worth discussing why the results of the knee/hip studies and the current shoulder study by Muench et al. are different and how these differences will affect future perspectives on regenerative medicine for rotator cuff tears. We discuss 4 points: (1) the rate of osteoarthritis in the target population, (2) the differences in the synovium-processing methods, (3) the confluence and ceiling effects, and (4) the cell number required for augmentation in human rotator cuff repair surgery.

First, the knee and hip joints are weight-bearing joints that differ from the shoulder. The previous studies from Mochizuki et al.[2] and Murata et al.[4] included patients with severe osteoarthritis, and the synovium was harvested during arthroplasty. In contrast, Muench et al.[1] included only 4.2% of cuff tear arthropathy patients with a Hamada radiographic classification grade of 4 (glenohumeral arthritis positive), which was likely a result of great efforts

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made by the authors to exclude patients who were not good candidates for arthroscopic shoulder rotator cuff repair surgery.[1] This finding indicates no significant difference in the number of SBDCs among patients who are good candidates for arthroscopic rotator cuff surgery.

Second, we found differences in the methods of processing the synovium. Muench et al.[1] expanded the SBDCs after chopping of the synovium. Here, Muench et al.[1] demonstrated that the average cell number of SBDCs obtained by 3-week expansion followed by the chopping technique was 1.3 million per gram. In contrast, in the study of MSCs from the shoulder by Utsunomiya et al.,[5] the average number of MSCs from subacromial bursa using the collagen digestion technique was 900 million per gram. This difference in yield varied by approximately 700 times. In previous studies by Mochizuki, Murata, and Utsunomiya et al., nucleated cells digested by collagenase were initially placed at a low density.[2-5] In the shoulder MSC study by Utsunomiya et al.,[5] 1.54% of the nucleated cells obtained by collagenase digestion formed MSC colonies. The reason why we used this low-density plating technique was to allow cells to form the largest colonies without colony-to-colony contact inhibition. It is also known that colony contact can lead to changes in other MSC characteristics.[6]

Third, in the current study by Muench et al.,[1] SBDCs were obtained after achieving full confluence. It is not fully understood how such a technical difference would affect the yield of MSCs from subacromial bursa, although Morikawa et al.[7] reported that the chopping technique showed a similar yield of SBDCs compared with the collagenase digestion technique. In the study by Morikawa et al.,[7] 50 mg of synovial tissue was digested using collagenase, and the digested cells were simply placed onto a dish. The number of nucleated cells obtained by collagenase digestion was not mentioned in the study. In the shoulder MSC study by Utsunomiya et al.,[5] 11,200 nucleated cells were obtained from 1 mg of subacromial bursa using collagenase digestion. This means that 50 mg of subacromial bursa consists of approximately 500,000 nucleated cells. With a colony formation rate of 1.54%, more than 7000 cells can form colonies. If the seeding density is high, the dishes will reach confluence, and cells cannot proliferate to the maximum number of cells predicted from the number of cells before culture. The method of Muench et al. may have caused a ceiling effect. After the chopping technique, if the plating cell density is high, the cells will reach confluence in the dishes. The ceiling effect during cell culture might affect the yield, and this was the main result of the current research.[1] If the authors placed the cells at a low density, the results might vary but may demonstrate a more accurate cell autonomous effect.

Fourth, one of the most important issues for the clinical application of MSCs for rotator cuff repair remains elusive and that is how many cells must be transplanted to be effective. Rothrauff et al.[8] applied 1 million adipose-derived stem cells to rotator cuff tears in a rat model. Dyrna et al.[9] used 150,000 MSCs from subacromial bursa synovial tissues for patellar tendon defects in mice. The number of cells required has been studied in animal models but has not been prospectively examined in clinical cases. If more than 1 million MSCs are required, the processing method using the chopping technique may result in a smaller than 1 million yield of MSCs, which is not optimal for regenerative medicine.

We suggest reconfirming whether the same findings regarding the yield of MSCs is found when the low-density cell-expansion method is applied. Further, it is still unknown whether the MSC-isolation technique affects the characteristics of SBDCs. After this reconfirmation, preparation techniques for subacromial bursa-derived MSC applications can be established as a powerful tool in regenerative medicine in the setting of irreparable rotator cuff tears, and human clinical trials can begin.

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