Regeneration of Foot Fat Pad with Autologous Adipose Tissue Derived Mesenchymal Stem Cells

Zijun Zhang, Reed Mitchell, Jeremy Molligan, Lew Schon.
MedStar Union Memorial Hospital, Baltimore, MD, USA.

Disclosures:  Z. Zhang: 5; Bioventus. R. Mitchell: None. J. Molligan: None. L. Schon: 1; Bioactive Surgical. 2; Zimmer, Biomed. 3B; Zimmer, Biomed. 4; Bioactive Surgical.

Introduction: The foot fat pad (FFP) is a unique fat tissue structure, consisting of clusters of adipocytes enclosed by elastin-containing septa. At a standing position, FFP ultimately bears body weight and absorbs shock. During aging, adipocytes in FFP gain a pro-inflammatory, tissue-remodeling, senescent-like state. Such an adipocyte phenotype may cause the FFP thinning or atrophy, which links with foot pain, affects the function of other joints in lower extremity and makes the foot more susceptible to pressure ulcer [1]. Recent advances in mesenchymal stem cell (MSC) biology offer great potential for the prevention of age-related FFP atrophy and the stimulation of FFP regeneration. Adipose tissue derived MSC (AT-MSC) transplantation has been used clinically for tissue reconstruction, such as breast augmentation [2]. Unlike autologous fat transplants that carry with the property of original tissue, the transplanted AT-MSCs may regenerate fat tissue that adapts a tissue phenotype of the recipient tissue [3]. It is promising for using AT-MSCs isolated from storage fat tissue, which is abundant, to regenerate weight-bearing FFP. In this study, we investigated the efficacy of autologous AT-MSC implantation for FFP regeneration in rats.

Methods: A total of 30 male Sprague-Dowley rats, at age of 14 weeks, were used for this study (approved by institutional IACUC). Under anesthesia, rats were operated for harvesting fat tissue from both sides of inguinal areas. Fat tissue from individual rats was carefully labeled and processed separately. The collected fat tissue was minced and digested in collagenase for one hour. After centrifugation, the vascular fraction of the fat tissue was collected and counted for cell number. The cells were seeded at in cultureware at a density of 3,000 cells/cm². Samples of the isolated cells were labeled with antibodies of CD73, CD95 and CD105 for flow cytometry. Cells were passaged at 75% confluency. At passage 3, cells were cultured in adipogenic differentiation medium, which contained insulin and 3-isobutyl-1-methylxanthine, for one week. Adipogenic differentiation was confirmed by the appearance and positive Oil Red O staining of lipid droplets in the cells. On the day of injection, the cells were trypsinized and labeled with Vybrant®Dil, a lipophilic membrane stain (Life Technologies). The efficiency of Dil labeling was subsequently confirmed by flow cytometry. The Dil-labeled adipogenically differentiated AT-MSCs (5x10⁴ suspended in 50ul saline) were injected into the second infradigital pad in right hind foot of the rat of origin. Saline only (50ul) was injected into the corresponding fat pad in the left hind paw of each rat.

Rats were not restricted from normal activity during the experimental period. A group of rats (n = 10) was euthanized at 1, 2, and 3 weeks and the second infradigital fat pads were dissected from both sides. The fat pads were fixed in 4% paraformaldehyde and sectioned with a cryostat, along the middle line. Slides were viewed under a fluorescent microscope for tracking the Dil-labeled AT-MSCs in the injected fat pads. The sections were also stained with hematoxylin and eosin (H&E) and imaged with compound...
microscopy. Fat pad units (FPUs) are the histologic hallmark of FFP and include a cluster of adipocytes and the surrounding septa. The total number of FPUs and FPU area in each fat pad was calculated with ImageJ (NIH).

**Results:** Flow cytometry confirmed that the isolated cells expressed a typical MSC profile of CD markers (data not shown). The injected fat pads showed no signs of swolenessness and infection. Rats appeared no difficulty to walk after the injections.

On cell tracking, the injected AT-MSCs were largely alive through the experimental period. By week 3, there were signs that the injected AT-MSCs migrated along the septa into a larger area of the fat pad. On histology, the FPU structure appeared intact on both AT-MSC injected FFPs and the control ones (Fig 1). The numbers of FPUs in the fat pads that received AT-MSC injections were greater than that in the control fat pads. The difference, however, was only significant at week 2. The areas of FPUs in fat pads that injected with AT-MSCs, increased as compared with the controls. The trend was persisted at 2 and 3 weeks and became statistically significant (Fig 2).

**Discussion:** FFP bears weight. This structural fat tissue is functionally different from other (energy) storage fat tissues and has unique biological and physical properties. It is unknown whether the fat tissue and MSC transplantations that succeed in other parts of the body could reconstruct or regenerate FFP. In this study, autologous AT-MSCs were induced for adipogenic differentiation in vitro preceded FFP implantation. Probably due to their adipose origin, AT-MSCs showed uniform and predominant adipogenic differentiation in one week of culture, as judged by the formation of intracellular lipid droplets. When the AT-MSCs were injected into FFP, as Dil cell tracking indicated, they spread into other areas of the fat pad along connective tissue septa of the FPU. Cell tracking in this study, however, could not confirm whether the injected AT-MSCs directly turned into adipocytes or formed new FPUs. Nevertheless, the numbers of FPUs in the fat pads that implanted with AT-MSCs were increased, comparing with the control fat pads. It is to be further validated, however, why the increased FPU number in the AT-MSC injected fat pads was significant only at week 2.

Another interesting finding was the increased FPU area in the AT-MSC injected fat pads. Given that aging-related FFP degeneration is primarily a result of decreased adipocytes per FPU [4], AT-MSC stimulation on individual FPUs may be more significant than regeneration of new FPUs for potentially to stop or reverse the aging process in FFP. The mechanism of the implanted autologous AT-MSCs in FFP regeneration is to be further investigated, but it is likely through the MSC trophic effect.

**Significance:** This is the first study to examine the utility of AT-MSCs for treating age-related FFP degeneration.
ORS 2015 Annual Meeting
Poster No: 1036