

A bioabsorbable implant seeded with adipose-derived stem cells for adipose regeneration

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Introduction

The increasing incidence of breast cancer among young women has intensified the demand for effective post-mastectomy reconstruction methods to improve patients' psychological health and quality of life^{1,2}. However, current treatments, including silicone implants, autologous flaps, and fat injections face significant limitations such as surgical complications, low fat retention, implant rupture, infection, and long-term risks like breast implant-associated anaplastic large cell lymphoma^{3,4}. To address these issues, we developed a bioabsorbable implant composed of poly-L-lactic acid (PLLA) mesh and collagen sponge (CS), designed to maintain internal space in vivo and promote autologous adipose tissue regeneration without additional adipose-derived stem cells (ASCs) or growth factors⁵.

While previous studies confirmed the implant's adipogenesis capabilities in animal models, adipose formation was predominantly limited to areas adjacent to native adipose tissue, with minimal regeneration within the implant's internal spaces⁵⁻⁷. ASCs possess extensive regenerative potential due to their multipotent differentiation abilities and secretion of various growth factors and cytokines⁸. Despite their promise, factors like mechanical stress from surrounding tissues and limited vascularization can hinder the survival and integration of transplanted ASCs, reducing their effectiveness in promoting fat formation^{9,10}. To overcome these challenges, we hypothesized that incorporating ASCs into our PLLA-CS implants would enhance adipogenesis within the implant itself. This study investigates the combined use of ASCs and PLLA-CS implants to promote adipose tissue formation and elucidates the underlying mechanisms involved.

Methods

Green fluorescent protein (GFP) - positive ASCs were isolated from transgenic rats' inguinal fat pads. Flow cytometry was used to verify the mesenchymal stem cell (MSC) phenotype, with CD90 and CD29 as positive markers and CD45 as a negative marker. The differentiation of ASCs into chondrocytes, osteoblasts, and adipocytes was respectively confirmed by Alcian blue, alkaline phosphatase (ALP), and Oil Red O staining. Perilipin staining was further utilized to verify adipogenesis.

The bioabsorbable implants were fabricated with a PLLA mesh as the external frame and CS as the internal filler. PLLA-CS implants seeded with GFP- positive ASCs were implanted into the rat inguinal area at a high concentration (1×10^6 cells/implant, H-ASC) and a low concentration (1×10^5 cells/implant, L-ASC). Unseeded implants were also inserted as controls.

Evaluations were conducted at 3, 6, and 12 months, including:

- (1) Evaluation of the weight and volume of all the newly formed tissues.
- (2) Histological assessment of the area of formed tissue and adipose tissue inside implants.
- (3) Assessment of GFP-positive cells inside implants.
- (4) Analysis of the transplanted cell morphology and differentiation within the

implants.

Results

1. Characterization of ASCs

The ASCs exhibited high expression of MSC markers CD90 and CD29 (99% of cells) and low expression of the hematopoietic marker CD45 (0.8% of cells), confirming their MSC phenotype. They successfully underwent chondrogenic differentiation, forming chondrogenic pellets with strong GFP expression; Alcian blue staining confirmed extracellular matrix deposition characteristic of chondrogenesis, indicating differentiation into chondrocytes. Osteogenic differentiation was evidenced by ALP staining on day 14, indicating early-stage osteogenic activity. Adipogenic differentiation was validated by Oil Red O staining, which revealed lipid accumulation within differentiated ASCs, and perilipin staining confirmed the presence of perilipin-positive lipid droplets, confirming successful adipogenesis.

2. Attachment of ASCs to PLLA-CS Implants

Fluorescence microscopy confirmed uniform distribution and attachment of ASCs on the PLLA-CS implants in both low (L-ASC) and high (H-ASC) ASC groups.

3. Evaluation of Weight and Volume of Newly Formed Tissues

No infections or tumors were observed postoperatively. All newly formed tissues increased in size over time. At 3 months, the H-ASC group showed greater weight and volume than the control group. At 6 and 12 months, weight and volume differences among groups were not significant. Across all groups, weight and volume at 6 and 12 months were greater than at 3 months, with values at 12 months exceeding those at 6 months.

4. Histological Assessment of Tissue Formation Inside Implants

H&E-stained sections showed gradual adipose regeneration in areas contacting native adipose tissue. At all time points, the H-ASC group had a significantly larger area of formed tissue inside implants compared to control and L-ASC groups. At 6 and 12 months, the adipose tissue area inside implants was significantly larger in the H-ASC group. The adipose tissue area at 6 and 12 months in both L-ASC and H-ASC groups was significantly larger than at 3 months. At 12 months, the adipose percentage in the H-ASC group was significantly higher than in the control group, and all groups showed increased adipose percentages over time.

5. Assessment of GFP-Positive Cells Inside Implants

Anti-GFP staining revealed spindle-shaped GFP-positive cells inside PLLA-CS implants in both L-ASC and H-ASC groups at all time points. In the H-ASC group, circular GFP-positive adipocytes were observed at 6 and 12 months. GFP-positive cells and adipocytes were predominantly located within collagen fiber regions. The H-ASC

group had significantly more GFP-positive cells than the L-ASC group at all time points.

6. Transplanted Cell Morphology and Differentiation

Immunofluorescence staining revealed that in the H-ASC group, GFP-positive adipocytes co-expressing Perilipin appeared yellow due to the overlap of green and red fluorescence. Additionally, GFP-positive spindle-shaped cells were observed in both the L-ASC and H-ASC groups, indicating the presence of undifferentiated ASCs or other cell types.

Discussion

This study demonstrated that combining PLLA and CS implants with ASCs significantly enhances adipose tissue formation. Transplanted ASCs persisted within the implants for up to one year and spontaneously differentiated into adipocytes *in vivo* without prior induction.

The isolated ASCs exhibited typical MSC markers, consistent with previous reports, and were capable of differentiating into adipocytes, chondrocytes, and osteoblasts¹¹. Although only a portion of ASCs differentiated into lipid droplets *in vitro*—likely due to inherent limitations where only a fraction possess multipotent potential—a higher concentration of ASCs notably promoted adipose tissue formation *in vivo*^{12–14}. This suggests that ASCs enhance adipogenesis not only through differentiation but also via paracrine effects¹⁵.

The PLLA-CS implants effectively maintained internal space, crucial for adipogenesis, and provided a supportive environment for ASCs. The PLLA scaffold ensured structural stability over one year⁵, while the collagen sponge facilitated cell attachment, proliferation, and extracellular matrix synthesis^{16,17}. The implants degraded gradually *in vivo* without causing infections or requiring secondary surgical removal, highlighting their safety and biocompatibility.

Incorporating ASCs into PLLA-CS implants proved to be a safe and effective strategy for enhancing adipogenesis without the need for pre-differentiation or exogenous growth factors. The efficacy of adipose tissue formation increased with higher doses of ASCs, indicating a dose-dependent relationship.

The mechanism of enhanced adipogenesis likely involves both the differentiation of transplanted ASCs into adipocytes and the secretion of growth factors that promote angiogenesis and the proliferation of surrounding tissues. The absence of tissue pressure within the PLLA scaffold may also contribute to the survival and function of ASCs^{18,19}.

Limitations of the study include the relatively low number of ASCs undergoing spontaneous differentiation, the diminishing advantage of increased weight and volume over time in the high-dose ASC group, and the inability to fully elucidate the specific molecular mechanisms involved. Additionally, a high concentration of ASCs was required for substantial adipose tissue formation. Future research will focus on investigating the characteristics of ASCs during long-term implantation and determining

the optimal ASC concentration to enhance adipose tissue formation more efficiently.

Conclusion

The study highlights the innovative application of ASCs seeded onto PLLA-CS implants to enhance adipose tissue proliferation and facilitate spontaneous differentiation into adipocytes in vivo, without the requirement for pre-differentiation or exogenous growth factors. Particularly, the group with implants seeded with a high dose of ASCs (1×10^6 cells per implant) demonstrated a superior outcome. These findings are expected to advance adipose tissue engineering, offering more effective regenerative medical options for clinical applications.

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