INTRODUCTION

Soft tissue loss is commonly caused by a loss of adipose tissue due to trauma or injury. Soft tissue loss is seen in soldiers suffering facial deformities due to gunshot wounds and breast cancer patients requiring mastectomies. Soft tissue reconstruction is a major challenge in plastic surgery. A standard therapy involves a fat graft from the patient taken from a fat-rich area and injected into the site of injury or defect. However, the outcome of this autologous fat grafting is widely unpredictable as the fat graft does not fully vascularize and therefore loses volume, leading to resorption. Resorbed fat grafts offer no longer offer any benefit to the patient. Healthy adipose tissue includes widespread vessel networks. Autologous fat grafts need to achieve a similar level of vascularization to retain volume and act as healthy adipose tissue. Therefore, alternate regenerative therapies are being considered for soft tissue reconstruction.

Adipose tissue engineering aims to repair damaged adipose tissue, as well as promoting natural regeneration of the tissue. Adipose tissue consists of adipocytes and a supporting extracellular matrix. Engineered adipose tissue must mimic the properties of these components. Materials commonly used for adipose tissue engineering include synthetic injectable materials, injectable biopolymers, and injectable extracellular matrix-based materials. Injectable materials can adapt to size and shape constraints, making them easily customizable to individual patient needs. Adipose tissue engineering aims to create an injectable material that does not provoke an immune response and encourages volume retention through formation of new fat. Volume retention of an injectable material is necessary to encourage the formation of new adipose tissue, known as adipogenesis. This would allow new, healthy, functional tissue to replace injured tissue or compensate for a lack of tissue. Of all the materials currently available, materials derived from adipose extracellular matrix hold the most potential for encouraging new fat formation and have been proven successful in vitro.

The method by which extracellular matrix (ECM) materials are prepared have significant effects on their structural properties and their ability to encourage adipose tissue growth. The materials must not elicit an immune response when implanted in humans; therefore, the cells of the adipose tissue are stripped such that only the ECM remains, in a process known as decellularization. Decellularized adipose matrix materials have not been investigated in vivo for their adipogenic properties.

OBJECTIVE

The overall project objective is to test a novel adipose matrix material in vivo for the material’s ability to induce new fat formation through adipogenesis and ability to retain volume. The adipose matrix will be injected in the form of implants in a mouse model. The short-term objective is to quantify the volume retention of the implants in two experimental groups through gas pycnometry and view the infiltration of adipocytes using Masson’s Trichrome staining.

HYPOTHESIS/SUCCESS CRITERIA

The adipose extracellular matrix (adECM) implants are expected to perform better than the negative control, synthetic ECM implants, in terms of increased volume retention and increased adipocyte infiltration into the site of implantation.

In terms of volume retention, 100% of the volume of the implant should be retained over the total implantation time of 12 weeks.

In terms of adipocyte infiltration, new adipocytes should be mixed throughout the implant at all study time points throughout 12 weeks.

METHODS

First, adECM implants were prepared at two different concentrations, 15% adECM and 33% adECM. The implants were dissolved in PBS prior to implantation. The volume of the implant was measured using a gas pycnometer before implantation.

5 groups each of 5 mice at each concentration were implanted. The implants were injected subcutaneously into the flanks of the mice. The implants were excised after 3, 6, and 12 weeks. The implants were compared to a negative control of synthetic ECM (HyStem™, Sigma-Aldrich, St. Louis, MO). The negative control was implanted in 5 groups of 3 mice each.

After excision, the volume of the implant was again measured using a gas pycnometer. After volume measurement, the implant was stained using a standard Masson’s Trichrome stain. The implant was subsequently paraffinized and sectioned into slides 5-7 µm thick for imaging.

RESULTS

Over 12 weeks, the 33% adECM retained more volume than the 15% adECM group as seen in Figure 1. In the 15% adECM group, 47% volume was retained after 3 weeks and 51% volume was retained after 12 weeks. In the 33% adECM group, 110% volume was retained after 3 weeks and 65% volume was retained after 12 weeks.
Figure 1. Percent volume retention in 15% adECM and 33% adECM groups at 3, 6, and 12 weeks. Overall, the 33% adECM group retained more volume than the 15% adECM group.

As seen in Figure 2, through Masson’s Trichrome staining, adipocyte infiltration was evident in both experimental groups at all timepoints. After 12 weeks, evidence of vascularization was seen in the 33% adECM group.

Figure 2. Results of Masson’s Trichrome staining and imagine in 15% adECM and 33% adECM over 3, 6, and 12 weeks. Adipocyte infiltration is evident at all time points in both groups.

DISCUSSION

100% volume retention of the implants was not seen in either experimental group. However, the 33% adECM retained more volume than the 15% adECM group. The increased concentration of extracellular matrix likely led to increased volume retention because of its increased ability to promote adipogenesis. Adipocyte infiltration was seen in both groups at all time points.

This study is limited by several factors. Firstly, an in vivo rat model is not comparable to humans. Regardless of how well these materials work in rats, they are not directly translatable to immediate use in humans. Also, the extracellular matrix implants injected into the mice were on the order of several millimeter and were of a mostly spherical shape. Soft tissue defects in humans are generally several centimeters big. The implants are not of adequate size and shape to properly mimic a human soft tissue defect. The scale of the implants must be considered.

In the future, bigger implants of appropriate three-dimensional structure should be tested. Soft tissue defects in humans are abnormally shaped. Various shapes and other three-dimensional structures must be considered.

Also, varying concentrations of adECM should be tested. Currently, 25% adECM implants are being tested. Eventually, the entire range of 15% - 33% adECM should be tested to specifically pinpoint the optimal concentration for adipose tissue repair.

With all of this further testing and studies, engineered adipose tissue can become the preferred treatment option for soft tissue replacement.

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REFERENCES