TISSUE ENGINEERING: Part A
Volume 18, Numbers 7 and 8, 2012

DOI: 10.1089/ten.tea.2011.0250

Adipose Tissue Engineering Using Injectable, Oxidized Alginate Hydrogels

Woo Seob Kim, M.D., Ph.D.,[1,2,3] David J. Mooney, Ph.D.,[2,3] Praveen R. Arany, B.D.S., M.D.S., M.M.Sc., Ph.D.,[2,3,4] Kangwon Lee, Ph.D.,[2,3] Nathaniel Huebsch, Ph.D.,[2,3,5] and Jaeyun Kim, Ph.D.[2,3]

Current treatment modalities for soft tissue augmentation which use autologous grafting and commercially available fillers present a number of challenges and limitations, such as donor site morbidity and volume loss over time. Adipose tissue engineering technology may provide an attractive alternative. This study investigated the feasibility of a degradable alginate hydrogel system with commercially available cryopreserved human adipose stem cells (hADSCs) to engineer adipose tissue. hADSCs were differentiated into adipogenic cells, and encapsulated in alginate hydrogels made susceptible to hydrolysis by partial periodate oxidation of the polymer chains. Cell laden gels were subcutaneously injected into the chest wall of male nude mice, and a cell suspension without alginate served as control. After 10 weeks, specimens were harvested and analyzed morphologically, histologically, and with immunoblotting of tissue extractions. Newly generated tissues were semitransparent and soft in all experimental mice, grossly resembling adipose tissue. Analysis using confocal live imaging, immunohistochemisty and western blot analysis revealed that the newly generated tissue was adipose tissue. This study demonstrates that degradable, injectable alginate hydrogels provide a suitable delivery vehicle for preconditioned cryopreserved hADSCs to engineer adipose tissue.

Introduction

Contour defects due to loss of soft tissue, mostly subcutaneous adipose tissue, are associated with trauma, tumor resection, and congenital abnormalities. These not only affect patients cosmetically, but also affect the emotional well being of patients and may impair functions such as range of motion. Currently, several surgical approaches, including the use of autologous tissue flaps, free fat grafting, and the implantation of commercially available prosthetic materials are used to restore or replace a volume of adipose tissue.[1]

Injectable soft tissue fillers are in high demand because there is a shorter recovery time, results are immediate, and injection is both safer and more cost-effective than surgical implantation. Autologous fat is the ideal soft-tissue filler; it is readily available, easy to harvest, and safe. However, consistent results are lacking because a majority of the injected fat tissue is resorbed.[2] Fat graft survival is dependent on the number of viable adipocytes at the time of transplantation, host physiology, and the recipient site environment.[3] The goal of fat grafts is to maximize assimilation at the recipient site. Increasing the surface area to volume ratio and maximizing the nutrient supply is thought to promote long-term viability.

Hyaluronic acid, collagen, polymethyl methacrylate spheres, and calcium hydroxyl apatite, and poly-L-lactic acid have all been used as tissue surrogates, due to the limitations of autologous solutions.[4,5] But these commercially available artificial fillers also present potential limitations, including foreign body reaction, fibrous capsule contraction, distortion, suboptimal mechanical properties, migration, and long–term resorption.[1] Engineered tissue fillers can potentially improve on these results by incorporating autologous cells in a delivery vehicle that provides structure, shape, and the proper 3-dimensional matrix for tissue and vascular ingrowth while the biomaterial degrades to leave a natural tissue.[6]

Adipose-derived stem cells (ADSCs) are in many ways an ideal transplant cell because they are highly expandable in monolayer culture, and can be readily induced to differentiate into adipocytes *in vitro* in response to well-established inductive conditions. They are easy to obtain from the

[1]Department of Plastic Surgery, College of Medicine, Chung-Ang University, Heuk Seok-Dong, Dong Jak-Gu, Seoul, Korea.
[2]School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts.
[3]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, Massachusetts.
[4]Program in Oral and Maxillofacial Pathology, Leder Human Biology and Translational Medicine and Biological Sciences in Dental Medicine, Harvard School of Dental Medicine, Boston, Massachusetts.
[5]Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge, Massachusetts.

subcutaneous fat of the patient, and are available in relatively large quantities at harvest using a minimally invasive procedure.[7] In addition to providing a ready and abundant source of seed cells for tissue engineering strategies, these cells can be readily cryopreserved for later use. Cryopreservation confers many advantages for practitioners engaged in cell-based therapies, including allowing transport of cells, pooling of cells to reach a therapeutic dose, and allowing time for the completion of safety and quality control testing.[8,9] A potential limitation of these cells in engineering tissues, though, is that they can readily spread from the recipient site if introduced without a carrier.

Alginates, a family of naturally occurring polysaccharides comprised of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues organized into copolymers, form hydrogels due to rapid cross-linking in the presence of divalent cations. Alginates are one class of hydrogel-forming material and have been widely utilized in tissue engineering and drug delivery application[10,11] because they are biocompatible, injectable, and easily processed into a desired shape during gelation. These polymers have been used safely as a nonprescription medication for the treatment of heartburn and acid reflux (Gaviscon, Bisodol, Asilone), as wound dressing materials (Algicell™, AlgiSite M™, Comfeel-Plus™, Kaltostat™, Sorbsan™, and Tegagen™), and as an appetite suppressant for long-term weight loss. Degradability is a critical material property for many applications in tissue engineering,[12] but typical alginate hydrogels degrade very slowly and in a poorly controlled manner as no hydrolytic or enzymatic chain breakages occur within alginate chains under physiological conditions in mammals. However, previous studies have shown that alginate hydrogels can be made susceptible to hydrolysis, and thus readily degraded, by using a combination of partial oxidation of the polymer chains and a biomodal molecular weight distribution of the polymer forming the gels.[13–15] These gels demonstrate ~80% mass loss in 40 days *in vitro* with only small gel fragments remaining *in vivo* at 4 weeks.[16,17] The purpose of this study was to investigate the feasibility of these degradable alginate hydrogels as a vehicle for preconditioned cryopreserved human adipose stem cells (hADSCs) to engineer adipose tissue *in vivo*.

Materials and Methods

hADSCs: preparation and adipogenic differentiation in vitro

Passage 1 hADSCs were purchased from Lonza. hADSCs were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL) supplemented with 10%(v/v) fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Invitrogen), and used between passages 5–6. Cells were maintained at lower than 80% confluency in culture, and trypsinized and replated on T225 flasks (Falcon) at a density of 4×10^6 cells per flask for predifferentiation. Cells were incubated in standard medium overnight to allow adherence to the bottom of flasks, then the medium was replaced with adipogenic differentiation medium containing DMEM with 10% FBS, 1 μM dexamethasone (Sigma), 10 μM human recombinant insulin (Sigma), 200 μM indomethacin (Sigma), and 0.5 μM 3-isobutyl-1-methylxanthine (IBMX; Sigma) for 2 weeks. The adipogenic medium was exchanged every 3 days. The adipogenic differentiation of hADSCs was assessed by the presence of intracellular lipid accumulation without staining, Nile Red staining, and indirect Immunostaining.

Nile Red staining

Nile Red staining was used to detect the presence of intracellular lipid-filled droplets. Briefly, cells were fixed in 4% paraformaldehyde (PFA) for 30 min, washed with phosphate buffered saline (PBS), and stained with 1 μg/mL Nile Red (Sigma) in PBS for 20 min at room temperature. The nuclei of cells were counter-stained with 4′, 6-diamidino-2-phenylindole (DAPI; Invitrogen), and stained cells were treated with Prolong Gold antifade reagent (Invitrogen). After two intensive rinses with deionized water, photomicrographs were taken using a fluorescence microscope (Nikon Eclipse E800).

Immunostaining for peroxisome proliferating antigen receptor gamma (PPAR-γ)

After 10 days of adipogenic culture in Lab-Tek chamber slides (Thermo scientific), cells were rinsed in PBS and fixed in 4% PFA. The samples were blocked in a solution containg 10% normal goat serum, 0.5% BSA (bovine serum albumin), and 0.1% Triton in PBS for 30 min, incubated overnight in primary PPAR-γ rabbit antibody (Cell Signaling) diluted at 1:1000 in 1% BSA-PBS solution, and then incubated for 2 h in Daylight 549-conjugated anti-rabbit antibody (Jackson Immunolabs) as secondary antibody diluted 1:500 in 1% BSA-PBS solution. After samples were counterstained with DAPI, and treated with Prolong Gold antifade reagent, photomicrographs were taken using the camera system described above.

Alginate modification

Ultrapure alginates were purchased from ProNova Biomedical, and chemically modified as previously described.[13,14] In brief, MVG alginate (M/G:40/60) was used as the high molecular weight (HMW) component to prepare gels. Low molecular weight (LMW) alginate was obtained by γ-irradiating HMW alginate with a cobalt-60 source for 4 h at a γ-dose of 5.0 Mrad (Phoenix Lab, University of Michigan, Ann Arbor, MI), as specified by Kong *et al.*[14] Both alginate polymers were diluted to 1% w/v in double–distilled H_2O, and 2% of the sugar residues in the polymer chains were oxidized by treatment with sodium periodate (Sigma). Oxidation was performed in the dark for 17 h at room temperature, as previously described.[15] An equimolar amount of ethylene glycol (Fisher) was added to stop the reaction and the solution was subsequently dialyzed (MWCO 1000, Spectra/Por®) over 3 days. Following oxidation, the adhesion peptide sequence G4RGDASSKY-OH (Peptides International) was coupled to both HMW and LMW alginate using published carbodiimide chemistry.[18] The concentration of peptides and polymer was adjusted to yield two peptides per polymer chain. The efficiency of this reaction was previously characterized.[18] Following peptide modification, alginate was dialyzed, treated with activated charcoal, filter sterilized (0.22 μm), free-dried, and stored at −20°C. To prepare gels, each molecular weight alginate was reconstituted at 4% w/v in media without serum and phenol red. Gels were formed using a combination mixture of the two different molecular weight polymers at a ratio of 3:1 (low:high).

Injection of differentiated hADSCs-alginate hydrogel mixture In vivo *and harvest*

To form injectable hydrogels containing cells, 0.2 mL of a suspension containing predifferentiated cells (2×10^6/mL) was mixed with 0.2 mL of the 4% (w/v) alginate solution using two syringes coupled by a connector. 0.4 mL of the resulting 2% (w/v) alginate solution was gelled by combining with an aqueous slurry of calcium sulfate (0.21g $CaSO_4$/mL distilled H_2O) at a ratio of 25:1 (40 μL of $CaSO_4$/1 mL of 2% w/v alginate solution) in the syringe subsequently used to inject the mixture. This cell concentration was chosen based on a previous reports,[19] although a lower total cell number was transplanted in the current study.

Under inhalation general anesthesia using methoxyfluorane, hydrogels were subcutaneously injected into two locations on either the chest wall or abdomen of each male nude mice (Jackson Laboratory) using a 23 gauge needle. The cell-encapsulating hydrogels (Experimental Group) were on one side, a cell suspension without alginate hydrogel (Control Group) was on the other side. Mice ($n=5$) were euthanized 10 week after injection in a CO_2 gas chamber. Newly formed tissue specimens were harvested for gross and histological evaluation.

Histology, immunostaining, western blot analysis, scanning electron microscopic examination

Specimens were sequentially sectioned at 7–10 μm thickness and stained with hematoxylin and eosin, oil red O (ORO), or subject immunostaining. For immunostaining, PPAR-γ (Cell Signaling Technology) was used as the primary antibody for tissue analysis, as described above. For western blot analysis, 100 mg samples of the newly generated tissue were homogenized and lysed in Radio Immunoprecipitation Assay buffer (Sigma) with Minitab Protease Inhibitors (Roche) on ice for 20 min. The lysate was collected and centrifuged at 12,000 rpm for 10 min at 4°C. Aliquots of the supernatants containing 20 μg of protein (determined using the Pierce BCA protein assay kit) were subjected to protein gel electrophoresis using 4%–20% Tris-Glycine gel (Invitrogen), transferred onto a nitrocellulose membrane (Amersham), and treated with 20% methanol in Tris-glycine buffer. After being blocked with PBS containing 3% BSA for 1 h at room temperature, the membranes were incubated with 1:1000 diluted primary monoclonal PPAR-γ (Cell Signaling Technology), Adiponectin (Cell Signaling Technology), C/EBP alpha/beta (Cell Signaling Technology), and β-actin (Sigma) antibodies at 4°C overnight and then with 1:10,000 diluted HRP-conjugated monoclonal secondary antibody (Jackson Immunolabs) for 1 h at room temperature. Signals were detected by bioluminescence using X-ray films (Kodak MR, Sigma-Aldrich) and the Enhanced Chemiluminescence substrate system (Thermo Scientific). For scanning electron miscroscopic (SEM) examination, newly generated tissues were fixed in 2.5% glutaraldehyde (Sigma) at 4°C for 1 h, postfixed for 2 h in 1% osmium tetraoxide (Polyscience), and dehydrated in a series of ethanol solutions (30%, 50%, 70%, 90%, 100%: Sigma). After completion of critical point drying, samples were coated with platinum by sputtering at an accelerating voltage of 15 kV, and then examined by SEM (FESEM Supra55VP; Zeiss) to evaluate the morphology of the newly generated tissue.

Whole-mount staining of living tissue

Visualization of newly generated living tissue was performed as described.[20] Briefly, freshly harvested tissue was cut into 2–3 mm pieces within 2 h after sacrifice, and incubated for 1 h with three different colored immunofluorescent reagents: 5 μM boron-dipyrromethene (BODIPY) 493/503 (Invitrogen) to stain adipocytes, 2 mM Alexorfluor 568-congugated isolectin GS-IB_4 (Invitrogen) to stain endothelial cells, and 40 μM hoechst 33342 (Molecular Probes) to stain all nuclei. Samples were then washed and directly observed with a confocal microscope system (Olympus IX81) equipped with a Coolsnap HQ2 camera (Prior Scientific) and a Carv II Niplow-type Spinning Disc Confocal Attachment (BD bioscences).

Results

In vitro *culture of preadipocytes*

The original number of cryopreserved hADSCs was 1.2×10^6, and after primary culture in the control medium, hADSCs demonstrated the typical elongated fibroblast-like morphology. hADSCs incubated in the adipogenic medium for 10 days exhibited accumulation of small lipid vacuoles in the cytoplasm (Fig. 1A), and the intercellular lipid vacuoles positively stained with Nile Red (Fig. 1B). Immunostaining for PPAR-γ protein revealed a nuclear localization of PPAR-γ protein in differentiated cells (Fig. 1C,1D).

Analysis of newly generated tissue

After injection of cell-encapsulating hydrogels, the volume of the grafts gradually decreased through the subsequent 3–4 weeks to a volume ~½ the initial. The volume then remained stable until sacrifice. The gel constructs did not migrate away from the injection location, or invade the surrounding tissue. There was no gross inflammation, swelling, or redness through the experimental period. The injection sites of the experimental group could be distinctively identified at tissue harvest, and the newly formed tissues obtained from cell-alginate constructs were harvested at 10 weeks after injection. Gel volumes ranging from 0.1–0.42 mL were injected into mice, and the average volume of engineered fat tissue was 50% ± 12% of the original injection volume at the 10 week time of harvest. The explants were pale yellowish, semi-transparent, soft on palpation, and exhibited evidence of neovascularization (Fig. 2). In constrast, injection of cells without gel carrier (control) resulted in no tissue formation at sacrifice; no new tissue could be identified.

Sections of tissues from the experimental group and native inguinal fat tissue were stained with hematoxylin and eosin (H&E) (Fig. 3A) and ORO (Fig. 3B), and demonstrated well-organized adipose tissue with small fragments of alginate hydrogel interspersed in the new tissue, but without any signs of inflammatory cell infiltration or evidence of tissue necrosis, cystic spaces, or fibrosis. Lipid staining revealed that the cells comprising the new tissue were largely adipocytes. Immunostaining for PPAR-γ revealed a concentration at the periphery of the cytoplasm of adipocytes in the newly formed tissue (Fig. 3C), confirming that adipose tissue was successfully regenerated from the adipogenically differentiated hADSC-alginate construct. Western blot analysis of lysates from newly formed adopose tissue and native inguinal

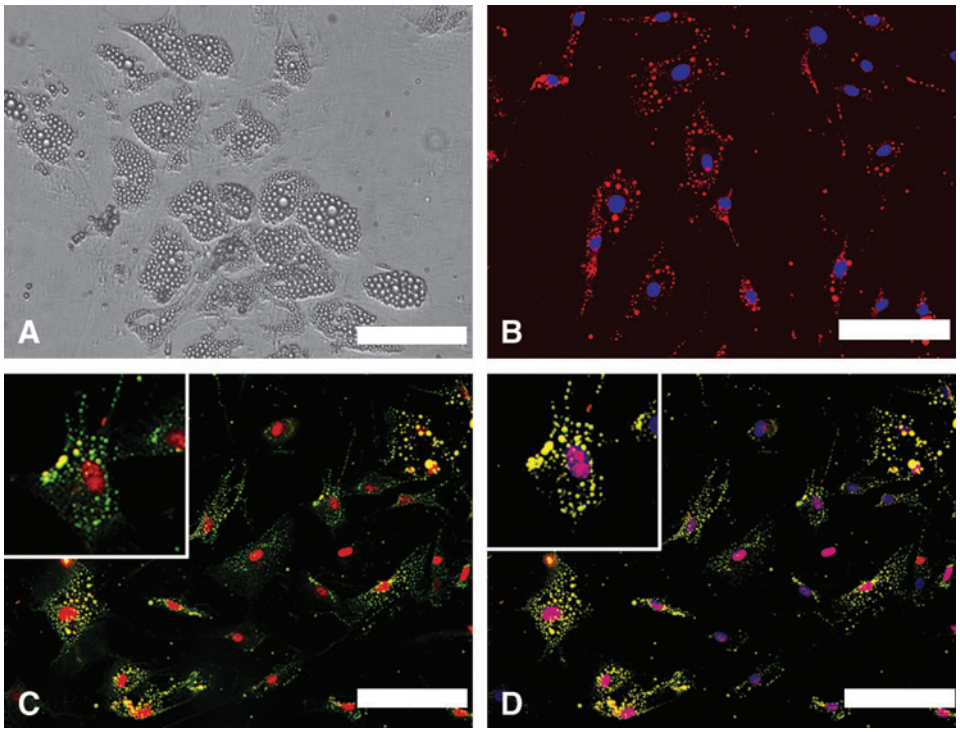

FIG. 1. Morphology of adipogenic differentiating hADSCs after treatment with induction medium for 10 days. **(A)** Intracellular accumulation of small lipid droplets in differentiated hADSCs (Phase contrast) **(B)** Small lipid droplets(red) and nucleus(blue) with Nile Red and DAPI staining **(C)** Immunostaining for detection of PPAR-γ protein(red) in the nuclei and BODIPY lipid staining (green) **(D)** Previous image merged with DAPI staining. Insets show higher magnification view of cells. All scale bars = 100 μm. hADSCs, human adipose stem cells; DAPI, 4′,6-diamidino-2-phenylindole; BODIPY borondipyrromethene. Color images available online at www.liebertonline.com/tea

fat tissue demonstrated expression of PPAR-γ, adiponectin, and C/EBP alpha/beta in the engineered tissues (Fig. 3D). SEM analysis of the tissue formed from cell-encapsulating hydrogels demonstrated intact adipocytes surrounded by extracellular matrix fibers (Fig. 4).

Whole-mount staining of living tissue

The overall structure of the engineered fat tissue was next analyzed with whole-mount staining. The overall morphology was very similar to native adipose tissue. Spherical adipocytes dominated the volume of the new tissue, supporting the histology analysis, and the majority of the cells in the tissue were adipocytes. An extensive series of capillaries was evident, running alongside adipocytes to form a well-organized network. Small capillaries could be appreciated growing out from larger vessels, suggesting an active remodeling of the vessel network (Fig. 5).

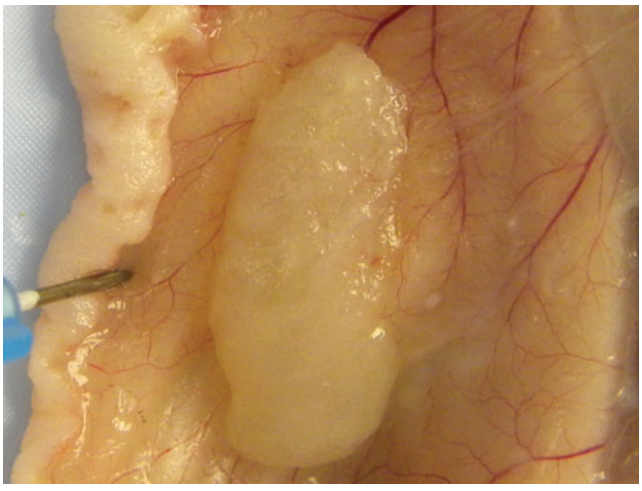

FIG. 2. Gross image of newly developed tissue at 10 weeks after implantation demonstrating pale yellowish, semi-transparent, soft tissue structure. Color images available online at www.liebertonline.com/tea

Discussion

This study demonstrated the ability of a degradable alginate hydrogel system with preconditioned cryopreserved hADSCs to generate living adipose tissue via minimally invasive injections, and these results highlight the potential clinical application of this approach for contour improvement. Adipose tissue engineering has been an area of relatively intense research because soft tissue replacement using autologous tissue flaps, autologous fat injections, and artificial fillers have significant pitfalls for both surgeons and patients. The limitations of current therapies include volume loss and donor site morbidity over time, leading to a broad clinical impetus for new approaches that can provide treatments for conditions ranging from trauma, congenital abnormality, and cosmetic improvement.[1]

Potential cell sources for adipose tissue engineering include terminally differentiated adipocytes, preadipocytes, adult mesenchymal stem cells, embryonic stem cell (ESCs), and induced pluripotent stem cells (iPS cells). Lineage-committed precursor cell populations, ESCs and iPS cells have been limited in use because of issues with availability, danger of tumor formation, or ethical issues. Adult mesenchymal stem cells, having a multipotent differentiation potential, are currently the best candidate for use as donor cell. Adult mesenchymal stem cell populations can be harvested from mature adipose tissue (hADSCs), bone marrow (hBMSCs), trabecular bone, periosteum, articular cartilage, synovium, synovial fluid, muscles, tendons, blood, blood vessels, skin, spleen, and thymus.[21,22] A comparison of hBMSCs to hADSCs has shown that both types of cells can

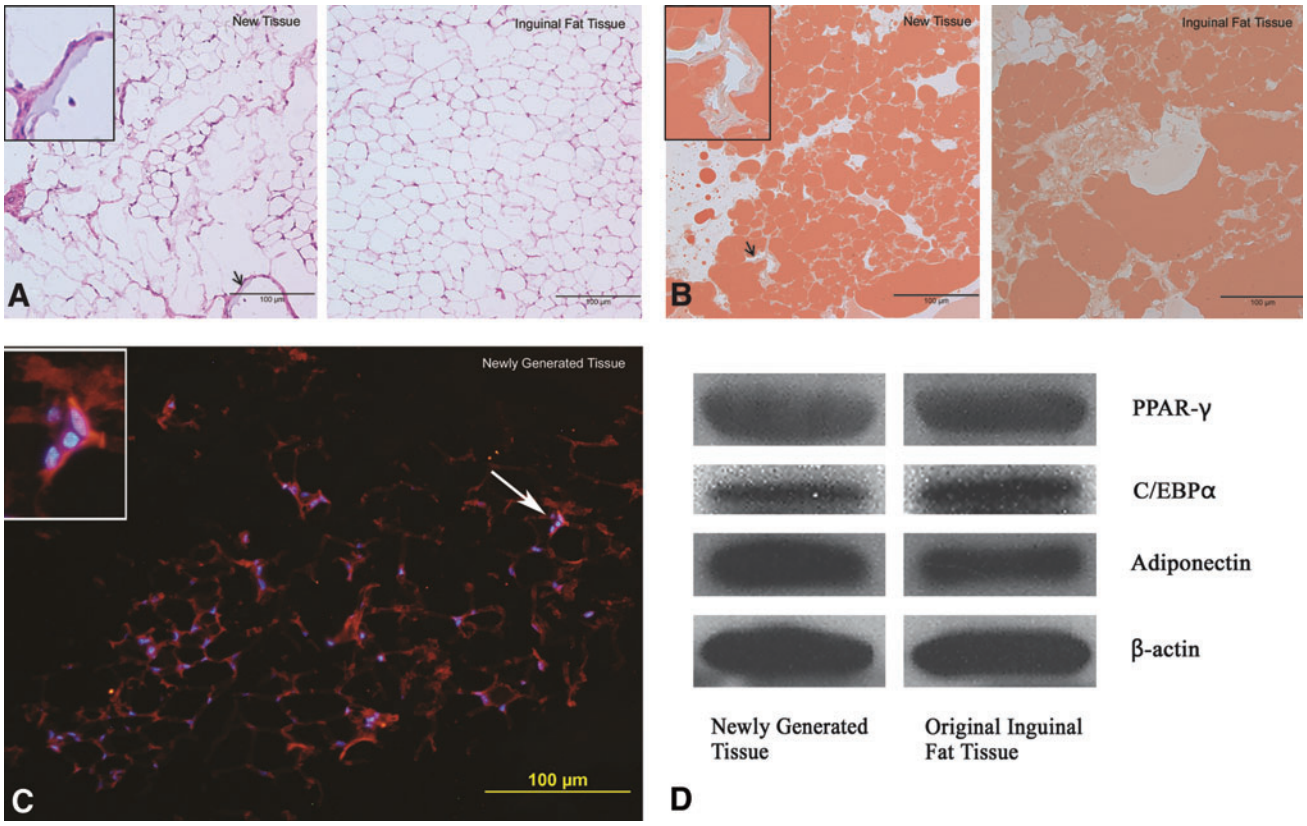

FIG. 3. Histological and immunohistochemical analyses of the newly formed tissue 10 weeks after subcutaneous injection and native adipose tissue. **(A)** H&E staining (left-new tissue/right-inguinal fat tissue), **(B)** ORO staining (left-new tissue/right-inguinal fat tissue), and **(C)** immunofluorescent staining for PPAR-γ (red) and DAPI (blue) of newly formed tissue at 10 weeks showed typical characteristic of adipose tissue. Arrows in **(A)** and **(B)** highlight residual gel in the engineered tissue, and insets in each panel highlight regions with residual gel (gel does not pick up stains used for analysis, and so appears clear). Arrow and inset in **(C)** highlights positive PPAR-γ staining. **(D)** Western blot results demonstrating expression of PPAR-γ (53 kDa), C/EBPα (42 kDa), and adiponectin (27 kDa) in the newly generated tissue (left), compared with native inguinal fat tissue (right). β-actin was used as loading control. ORO, oil red O; H&E, hematoxylin and eosin. Color images available online at www.liebertonline.com/tea

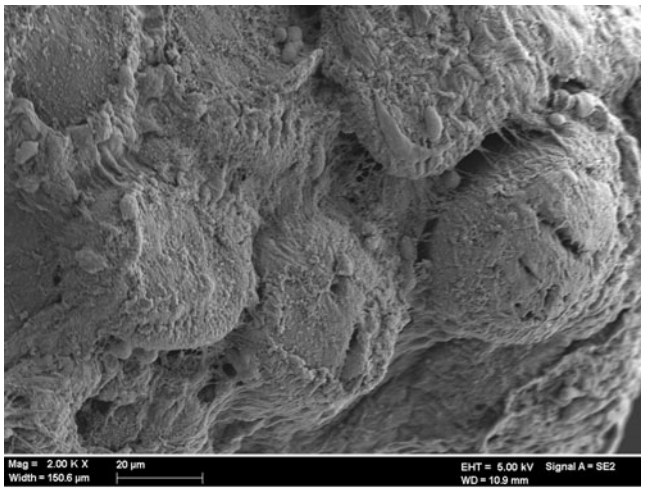

FIG. 4. Scanning electron micrograph of newly developed tissue at 10 weeks after implantation showing the adipocytes surrounded by extracellular matrix fibers.

be differentiated toward multiple lineages, and both exhibit similar cytokine secretory profiles.[23] Since adipose tissue is available in large quantities, is easy to obtain, and causes minimal patient discomfort and donor site morbidity when harvested, it provides an abundant reservoir of stem cells for clinical use. hADSCs were reported to maintain their ability to undergo adipogenesis over 160 population doublings,[24] providing a significant advantage to their use in cell therapies. Preadipocytes, found in the stromal vascular fraction of adipose tissue, are able to differentiate to mature adipocytes; these cells, however, lose their capacity to differentiate following elevated numbers of passages.[7] Nevertheless, preadipocytes and adipocytes have advantages for clinical application, as they can allow one to avoid the culture and predifferentiation processes. The source and purity of the stem cells are important variables, and those used in the current experiments were specified by the vendor (29 year old, female, manufacture date September 14, 2007, current experiments spanned 2010–2011, virus testing-negative, CD13, CD29, CD44, CD73, CD90 >90% positive, CD14, CD31, <5% positive).

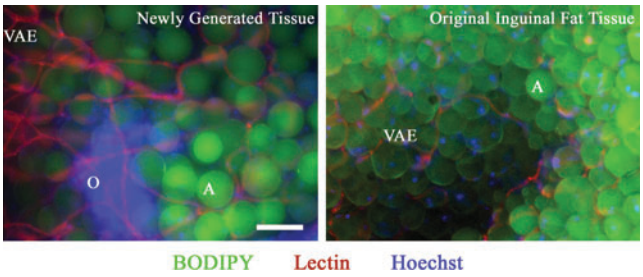

FIG. 5. Photomicrographs of living tissue revealed the presence of capillaries running alongside adipocytes to form a well organized network in newly generated tissue (left), compared with native inguinal fat tissue (right). A cell was regarded as an adipocyte (A) when the nucleus was localized within the BODIPY-positive lipid area (green). When the nucleus was within or attached to capillaries, they were regarded as a vascular associated cells, for example, adipose-derived stromal cells, endothelial cells, pericytes (red), and when localization of the nucleus (blue) did not meet either of these two condition, we regarded as other cell types(O), such as blood cells or fibroblasts. All scale bars = 100 μm. Color images available online at www.liebertonline.com/tea

These experiments demonstrated that cryopreserved hADSCs (>3 year) could be used to successfully engineer tissue. Freshly prepared cell populations are preferred, but from a clinical application point of view, the ready availability of cryopreserved hADSCs or preadipocytes has a significant advantage for the surgeon and patients because liposuction procedures would not have to be performed each time cells are needed. Further, for optimal contour improvement, serial touch up processes over a scheduled time period is likely be necessary.

Various synthetic materials such as poly(lactide), poly (glycolide), poly(lactide-co-glycolide), polyethylene glycol, and natural biomaterials such as adipose-derived extracellular matrix, collagen, gelatin, and hyaluronic acid have been explored for adipose tissue engineering. These materials demonstrate varying levels of biocompatibility, and different mechanical and chemical properties, and degradability. The ability to consistently control material properties of synthetics typically provides a considerable advantage over natural materials, but natural materials have been shown to provide advantages with regard to compatibility, extent of adipose tissue formation, and degradation properties.[25] Past experimental approaches for adipose tissue engineering showed considerable evidence of adipose tissue formation, but many of the tested materials may not be suitable for clinical application since many are not injectable. Alginate is commonly used in many tissue engineering applications because of its biocompatibility, safety, relatively low cost, and mild gelation behavior with divalent cations.[26] A previous study performed with collagen sponges and gelatin microspheres containing basic fibroblast growth factors also demonstrated a robust induction of adipose tissue.[27] As that study did not use any transplanted cells, the induction of new tissue generation was solely dependent on the viability and potency of cells in the surrounding host tissue and cells that home to the implant site.[28] Transplantation of exogenous cells, preferably autogenous, can overcome this host dependency and may significantly add to procedure consistency and provide an efficacious and predictable surgical outcome. Another study using cultured ADSCs encapculated in nonmodified alginate gels demonstrated the potential of alginate gel a as filler material.[29] Alginate gels with widely varying chemical, physical, and degradative properties have been developed over the past decade,[30] and these materials can be designed to tightly regulate the gene expression of encapsulated cells via manipulation of their cell binding capability and mechanical properties.[31] Since cell adhesion is a strict requirement for survival, we utilized alginate gels that contained covalently coupled RGD peptides to provide an adhesive cue from the gel. Previous studies, using a variety of cell types including MSCs, have demonstrated this modification enhances cell adhesion, survival, proliferation, and allows differentiation of MSCs to adipocytes.[18,32,33] The current experiment demonstrates that degradable alginate hydrogels can be useful injectable scaffolds for adipose engineering, following the ideal dictum of the artificial scaffold material being replaced over time by the newly regenerating tissue that it induces. Although the degradability of materials can be exactly measured *in vitro*, the *in vivo* degradation is less precisely predicted due to multiple tissue and subject variations such as the local tissue enzymes and proteases, physical forces, degree of immune response, among others. This requires that future studies more precisely predict the degradation rate of the gels used in the current study.

A key issue in adipose tissue engineering is the vascularization of the new tissue, as this is crucial to maintain the viability of the transplanted cells and prevent large-scale shrinkage of the tissue over time. The use of confocal microscopy allows one to determine that degradable alginate gels allowed significant ingrowth of capillaries that grew alongside the adipocytes in the newly generated adipose tissue by 10 weeks (Fig 5). Additional studies will be required to examine the kinetics of vascularization, and to determine if enhancing this process with delivery of angiogenic factors from the gels[16,34] in concert with cell transplantation would increase the volume of newly engineered tissue formed from transplanted cells.

In summary, the results of this study demonstrate that degrade alginate gels can serve as injectable cell delivery vehicles for adipose tissue engineering. The engineered adipose tissue demonstrated typical characteristics of adipose tissue, grossly and microscopically and at the molecular level.

Acknowledgment

The authors wish to thank Katie Parodi for administrative assistance.

Disclosure Statement

No competing financial interests exist.

References

1. Patrick, C.W., Jr. Tissue engineering strategies for adipose tissue repair. Anat Rec **263,** 361, 2001.
2. Kaufman, M.R., Bradley, J.P., Dickinson, B., Heller, J.B., Wasson, K., O'Hara, C., *et al.* Autologous fat transfer national consensus survey: trends in techniques for harvest, preparation, and application, and perception of short- and long-term results. Plast Reconstr Surg **119,** 323, 2007.

3. Har-Shai, Y., Lindenbaum, E.S., Gamliel-Lazarovich, A., Beach, D., and Hirshowitz, B. An integrated approach for increasing the survival of autologous fat grafts in the treatment of contour defects. Plast Reconstr Surg **104,** 945, 1999.
4. Dayan, S.H., and Bassichis, B.A. Facial dermal fillers: selection of appropriate products and techniques. Aesthet Surg J **28,** 335, 2008.
5. Hilinski, J.M., and Cohen, S.R. Soft tissue augmentation with ArteFill. Facial Plast Surg **25,** 114, 2009.
6. Coleman, S.R. Structural fat grafting: more than a permanent filler. Plast Reconstr Surg **118,** 108S, 2006.
7. Gomillion, C.T., and Burg, K.J. Stem cells and adipose tissue engineering. Biomaterials **27,** 6052, 2006.
8. Hubel, A. Parameters of cell freezing: implications for the cryopreservation of stem cells. Transfus Med Rev **11,** 224, 1997.
9. Gonda, K., Shigeura, T., Sato, T., Matsumoto, D., Suga, H., Inoue, K., *et al.* Preserved proliferative capacity and multipotency of human adipose-derived stem cells after long-term cryopreservation. Plast Reconstr Surg **121,** 401, 2008.
10. Tonnesen, H.H., and Karlsen, J. Alginate in drug delivery systems. Drug Dev Ind Pharm **28,** 621, 2002.
11. Marler, J.J., Guha, A., Rowley, J., Koka, R., Mooney, D., Upton, J., *et al.* Soft-tissue augmentation with injectable alginate and syngeneic fibroblasts. Plast Reconstr Surg **105,** 2049, 2000.
12. Lu, L., Zhu, X., Valenzuela, R.G., Currier, B.L., and Yaszemski, M.J. Biodegradable polymer scaffolds for cartilage tissue engineering. Clin Orthop Relat Res S251, 2001.
13. Boontheekul, T., Kong, H.J., and Mooney, D.J. Controlling alginate gel degradation utilizing partial oxidation and bimodal molecular weight distribution. Biomaterials **26,** 2455, 2005.
14. Kong, H.J., Kaigler, D., Kim, K., and Mooney, D.J. Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. Biomacromolecules **5,** 1720, 2004.
15. Bouhadir, K.H., Lee, K.Y., Alsberg, E., Damm, K.L., Anderson, K.W., and Mooney, D.J. Degradation of partially oxidized alginate and its potential application for tissue engineering. Biotechnol Prog **17,** 945, 2001.
16. Silva, E.A., and Mooney, D.J. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. J Thromb Haemost **5,** 590, 2007.
17. Hao, X., Silva, E.A., Mansson-Broberg, A., Grinnemo, K.H., Siddiqui, A.J., Dellgren, G., *et al.* Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. Cardiovasc Res **75,** 178, 2007.
18. Rowley, J.A., Madlambayan, G., and Mooney, D.J. Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials **20,** 45, 1999.
19. Mizuno, H., Itoi, Y., Kawahara, S., Ogawa, R., Akaishi, S., and Hyakusoku, H. *In vivo* adipose tissue regeneration by adipose-derived stromal cells isolated from GFP transgenic mice. Cells Tissues Organs **187,** 177, 2008.
20. Nishimura, S., Manabe, I., Nagasaki, M., Hosoya, Y., Yamashita, H., Fujita, H., *et al.* Adipogenesis in obesity requires close interplay between differentiating adipocytes, stromal cells, and blood vessels. Diabetes **56,** 1517, 2007.
21. Pountos, I., and Giannoudis, P.V. Biology of mesenchymal stem cells. Injury **36 Suppl 3,** S8, 2005.
22. Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J., *et al.* Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng **7,** 211, 2001.
23. Kilroy, G.E., Foster, S.J., Wu, X., Ruiz, J., Sherwood, S., Heifetz, A., *et al.* Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. J Cell Physiol **212,** 702, 2007.
24. Rodriguez, A.M., Elabd, C., Delteil, F., Astier, J., Vernochet, C., Saint-Marc, P., *et al.* Adipocyte differentiation of multipotent cells established from human adipose tissue. Biochem Biophys Res Commun **315,** 255, 2004.
25. Choi, J.H., Gimble, J.M., Lee, K., Marra, K.G., Rubin, J.P., Yoo, J.J., *et al.* Adipose tissue engineering for soft tissue regeneration. Tissue Eng Part B Rev **16,** 413, 2010.
26. Thornton, A.J., Alsberg, E., Albertelli, M., and Mooney, D.J. Shape-defining scaffolds for minimally invasive tissue engineering. Transplantation **77,** 1798, 2004.
27. Kimura, Y., Tsuji, W., Yamashiro, H., Toi, M., Inamoto, T., and Tabata, Y. *In situ* adipogenesis in fat tissue augmented by collagen scaffold with gelatin microspheres containing basic fibroblast growth factor. J Tissue Eng Regen Med **4,** 55, 2010.
28. Lee, C.H., Cook, J.L., Mendelson, A., Moioli, E.K., Yao, H., and Mao, J.J. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. Lancet **376,** 440, 2010.
29. Moyer, H.R., Kinney, R.C., Singh, K.A., Williams, J.K., Schwartz, Z., and Boyan, B.D. Alginate microencapsulation technology for the percutaneous delivery of adipose-derived stem cells. Ann Plast Surg **65,** 497, 2010.
30. Augst, A.D., Kong, H.J., and Mooney, D.J. Alginate hydrogels as biomaterials. Macromol Biosci **6,** 623, 2006.
31. Huebsch, N., and Mooney, D.J. Inspiration and application in the evolution of biomaterials. Nature **462,** 426, 2009.
32. Huebsch, N., Arany, P.R., Mao, A.S., Shvartsman, D., Ali, O.A., Bencherif, S.A., *et al.* Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat Mater **9,** 518, 2010.
33. Alsberg, E., Anderson, K.W., Albeiruti, A., Rowley, J.A., and Mooney, D.J. Engineering growing tissues. Proc Natl Acad Sci U S A **99,** 12025, 2002.
34. Silva, E.A., and Mooney, D.J. Effects of VEGF temporal and spatial presentation on angiogenesis. Biomaterials **31,** 1235, 2010.

Address correspondence to:
Woo Seob Kim, M.D., Ph.D.
Department of Plastic Surgery
College of Medicine
Chung-Ang University
224-1 Heuk Seok-Dong
Dong Jak-Gu
Seoul 156755
Republic of Korea

E-mail: kimws@cau.ac.kr

Received: May 3, 2011
Accepted: October 17, 2011
Online Publication Date: January 13, 2012