INTRODUCTION:
Arthritis is the leading cause of chronic disability, and affects approximately 46 million adults in the U.S. alone. Currently, the predominant approach for cartilage tissue engineering invariably involves cell transplantation. However, cell transplantation for cartilage repair is confronted with critical drawbacks of cell availability, donor site morbidity, and excessive cost associated with cell manipulation. Stem cell homing is an emerging concept, and may circumvent the need for cell transplantation. The objective of the current study was to devise a cartilage regeneration approach without cell transplantation by 1) homing stem/progenitor cells into an acellular scaffold, and 2) inducing concurrent chondrogenesis of the homed cells. A cytokine delivery system was devised to recruit surrounding stem/progenitor cells and differentiate them into chondrocytes. Prevalent cell types adjacent to an articular cartilage defect include: bone marrow derived mesenchymal stem cells (MSCs), adipose derived stem cells (ASCs) from nearby fat pads, synovial cells, and native chondrocytes. Of these cell types, ASCs and MSCs are being studied for their recruitment by controlled release of cytokines. Our data demonstrate that MSCs and ASCs were homed into acellular scaffolds and concurrently differentiated into chondrogenic cells. This novel approach using concurrent cell homing and chondrogenesis strategies has potential applications for cartilage regeneration.

METHODS:
Stromal cell-derived factor-1 (SDF1) and transformation growth factor β3 (TGFβ3) were microencapsulated in gelatin microspheres that were fabricated using a water-in-oil emulsion technique and chemically cross-linked with glutaraldehyde (Fig. 1A). Following lyophilization, the microspheres were rehydrated in a solution containing 300 ng/ml TGFβ3, 100ng/ml SDF1 or PBS. Acellular scaffolds were fabricated with two separate but integrated layers consisting of a layer of cross-linked 4% (w/v) calcium alginate containing 30 mg (dry wt) of embedded microspheres and an underlying collagen sponge (Fig. 1B). Human bone marrow MSCs and human ASCs were isolated from adult donors and seeded in 6-well plates (100,000 cells per well). Four conditions were tested: TGFβ3 alone, SDF1 alone, SDF1+TGFβ3 and cytokine free. Each scaffold was placed in the center of the well. Scaffolds were harvested after 3 hours, 1 week and 3 weeks, fixed in 10% formalin, embedded in paraffin and sectioned. DAPI staining was used to assess the number of cells per scaffold section and toluidine blue to assess chondrogenesis. H&E staining was done as a confirmation of DAPI and to evaluate the morphology of homed cells. Multivariate ANOVA and Bonferroni tests were used for statistical analysis (p<.05).

RESULTS:
A layer of glistening white tissue was visible in the collagen portion of the scaffolds following 3 wks of cell homing (Fig. 1C). DAPI staining confirmed that both MSCs and ASCs were homed into the collagen scaffold (Fig. 2). Delivery of combinatorial SDF1 and TGFβ3 was most effective in recruiting both ASCs and MSCs into scaffolds upon 3 wk cell homing, generating significantly greater cell numbers than the other three conditions (Fig. 3). SDF1+TGFβ3 homed twice as many MSCs as ASCs in 3 wks (Fig. 2B vs. 3A). SDF1 recruited significantly more ASCs by 1 and 3 wks than TGFβ3 alone or cytokine free (Fig. 3). For the MSCs, only the SDF1/TGFβ3 condition resulted in a significantly greater cell number than the other three conditions (Fig. 3B) at 3 wks. These results were confirmed with H&E staining (data not shown). Toluidine blue revealed darker staining for TGFβ3 alone and SDF1+TGFβ3 conditions after 3 wk cell homing (Fig. 4B,D & 4F,H). In contrast, there was minimal blue staining for cytokine-free or SDF1 alone conditions (Fig. 4A,C & 4E,G).

DISCUSSION:
We discovered that the SDF1/TGFβ3 delivery system not only homed stem/progenitor cells, but also induced chondrogenesis of the homed cells in vitro. While SDF1 was able to home ASCs and MSCs, its action alone did not seem to be sufficient for inducing chondrogenesis. In contrast, TGFβ3 showed moderate cell homing effects, but was capable of inducing MSC differentiation into chondrocytes. Combinatory delivery of SDF1 and TGFβ3 was most effective in homing both ASCs and MSCs, in addition to generating cartilage matrix, as shown by toluidine blue staining. MSCs and ASCs may act synergistically in vivo. Ongoing experiments are addressing in vivo effects of cell homing and chondrogenesis, as well as other target cell lineages present in a cartilage defect, which include synovial cells, and hematopoietic cells. These experiments are motivated by our recent findings (in a separate study) that an entire synovial joint condyle can be regenerated by cell homing in vivo to function and bear weight. We continue to investigate the sources and interactions of multiple cell lineages present near a cartilage defect and continue to devise cell homing and chondrogenesis approaches to regenerate cartilage without the need for cell transplantation.

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