Mesenchymal Stem Cell Therapy Targeted To Damaged OA Cartilage Using Type I Collagen Microspheres Linked To Monoclonal Antibody For Type II Collagen

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Introduction Post traumatic and chronic osteoarthritis (OA) share the underlying feature of damaged hyaline cartilage and exposure of type-II collagen (CII) in the damaged tissue due to erosive proteolytic degradation triggered by low grade ongoing inflammation. Adipose tissue derived mesenchymal stem cells (ADSC) have shown efficacy in the treatment of osteoarthritis through their ability to mitigate the host inflammatory response through paracrine signaling and possess capability for chondrogenic differentiation. However in vivo, very few ADSC bind to the damaged cartilage or differentiate in the OA lesion. Our study offers a solution to these obstacles through the creation of injectable type I collagen (Col I) microspheres loaded with ADSC and linked to an E4D4 IgG monoclonal antibody that specifically targets the type II collagen (MabCII) unmasked in osteoarthritic hyaline cartilage. The ADSC microspheres promote chondrogenic differentiation during culture in vitro showing an increase in Alcian blue staining. Upon binding to damaged cartilage, the ADSC carried by the microsphere rapidly migrate onto the damaged cartilage surface. We propose that MabCII linked to Col I microspheres can serve as an intraarticular carrier for transport and targeting large numbers of ADSC to osteoarthritic tissue while promoting chondrogenic differentiation.

Methods:

Isolation and culture of ADSC: The ADSC were isolated from subcutaneous fat tissue of 3-4 months old pigs as described previously (1). All animal protocols and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center. The adipose tissue was digested with 200 µg/mL collagenase II solution (Sigma) to yield ADSC that were cultured in Dulbecco's Modified Eagle Medium-High Glucose (DMEM-HG) (Gibco) supplemented with antibiotics at 37°C and 5% CO_2. The ADSC were characterized by flow cytometry and for multigenic differentiation before experimental use.

Preparation of MabCII targeted Col I scaffold: Lyophilized purified native Col I from fetal calf skin was the gift of Dr. David Brand, Memphis VA Medical Center. The E4D4 IgG monoclonal antibody was isolated and characterized as described previously (2). The experimental group of MabCII targeted scaffolds were constructed using a two-step EDC/NHS crosslinking reaction (ThermoFischer) that bound hydroxyl groups on Col I to primary amine groups on MabCII in a 2:3 molar ratio. The efficiency of conjugation of MabCII conjugation on the Col I scaffold was measured by protein assay of the supernatant after pelleting the collagen by centrifugation at 1000g's for 10 minutes. Cellular scaffolds were assembled in a solution of DMEM-HG using 4mg/ml homogenized MabCII-Col I and 16 million cells/ml ADSC.

Experimental group and analysis: 5ul droplets of the cells (80,000 cells) in a 4 mg/ml collagen suspension were plated on a non-tissue culture petri dish to form microsphere pellets and then cultured in DMEM-HG supplemented with antibiotics at 37°C and 5% CO_2 for three days. At this time point, microsphere pellets were either collected for experimentation or left on tissue culture plate and imaged under light microscopy after 4, 6, and 12 days (Figure 1A, B, C). Control groups were established using pelleted ADSC (80,000 cells/ 5 microliters) without any Col I and ADSC integrated in a Col I scaffold without linked MabCII similarly cultured. Chondrogenic differentiation was analyzed by Alcian Blue staining of all pellets and scaffold composites at 1 and 2 weeks to visualize proteoglycan accumulation. To test the migration of ADSC from the scaffold onto damaged cartilage tissue, ADSC were stained with a fluorescent dye (Vivotrack680, PerkinElmer) before pellet formation. A 5ul MabCII linked ADSC microsphere was subsequently placed on to a 5 mm trypsinized cartilage explant and imaged after 6 days using a Keyence BZ-X800 microscope with a Cy5.5 filter (NIKON). To demonstrate targeting efficiency of our MabCII linked scaffolds, microspheres of fluorescent ADSC, pelleted alone or with Col I or MabCII/Col I were suspended in a 2 ml test tube on a rocker table with 5 mm damaged (trypsinized) cartilage explants. IVIS images were obtained at 4 and 24 hours and total fluorescence of ROI was calculated by Living Image 4.2 software.

Results: ADSC integrated in Col I microspheres and cultured demonstrated release from microsphere and growth on tissue culture plate (Figure 1). Furthermore, the ADSC integrated in Col I microspheres stained positive for proteoglycan synthesis with Alcian Blue after 7 days indicating chondrogenesis (Figure 2). Col I microspheres with no cells did not stain, evidence that the ADSC themselves produced the blue-staining proteoglycans. ADSC alone also demonstrated positive staining of Alcian Blue as well. Additionally, over 6 days in culture, ADSC migrated from their microsphere attached to the cartilage explant and spread on damaged cartilage tissue as seen by the spread of fluorescence outside of the stationary ADSC/MabCII microsphere (Figure 1D). Finally, IVIS imaging of quantified fluorescence showed targeting of only ADSC/Col I/ MabCII microspheres to damaged cartilage at 4 hours, while the explants incubated alone or with ADSC or ADSC/Col I exhibited no florescence at this time point (Figure 3). ADSC/Col I linked MabCII pellets remained fluorescent on damaged cartilage at thru the 6 days of culture.

Discussion: Adipose tissue derived mesenchymal stem cell regenerative therapy for regrowth of damaged cartilage for osteoarthritis relies on ADSC's ability to differentiate into chondrocytes on damaged hyaline cartilage tissue. We demonstrate in vitro that the use of a CII targeting MabCII linked to a Col I scaffold induced chondrogenesis of ADSC and increased the specificity and rapidity whereby ADSC can be delivered to osteoarthritic tissue. Our targeted pellets not only bound more rapidly to damaged cartilage, but they also remained attached after 24hrs-6 days and migrated onto the damaged cartilage. These results show promise for an in vivo study using our MabCII targeted Col I microspheres as an intra-articular injectable therapeutic treatment for OA.

Significance: The creation of an injectable cellular scaffold with ability to target ADSC to type-II collagen on damaged hyaline cartilage increases the localization of mesenchymal stem cells to an osteoarthritic lesion even at early stages.

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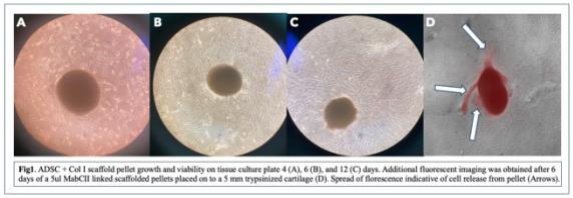

Fig1. ADSC + Col I scaffold pellet growth and viability on tissue culture plate 4 (A), 6 (B), and 12 (C) days. Additional fluorescent imaging was obtained after 6 days of a 5ul MabCII linked scaffolded pellets placed on to a 5 mm trypsinized cartilage (D). Spread of florescence indicative of cell release from pellet (Arrows).

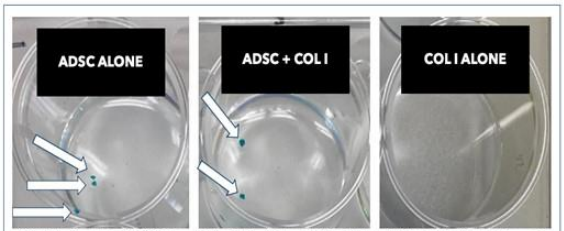

Fig2. Alcian Blue staining of ADSCs alone, ADSCs + Col I scaffolds, and Col I pellet alone. ADSCs + Col I scaffolded pellets are of larger size than ADSC pellet alone.

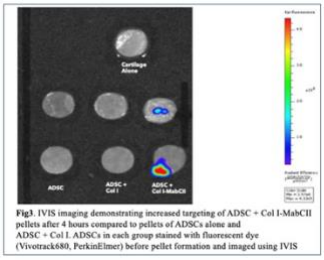

Fig3. IVIS imaging demonstrating increased targeting of ADSC + Col I-MabCII pellets after 4 hours compared to pellets of ADSCs alone and ADSC + Col I. ADSCs in each group stained with fluorescent dye (Vivotrack680, PerkinElmer) before pellet formation and imaged using IVIS