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

Nathan Beda, Hongsk Song Cho, Evan Porter, David Brand, Karen A. Hasty
Department of Orthopaedic Surgery and Biomedical Engineering, University of Tennessee Health Science Center- Campbell Clinic, Lt. Col. Luke Weathers, Jr. VA Medical Center, Memphis, TN, USA
nbeda@uthsc.edu

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Introduction: Post traumatic and chronic ostearthritis (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% CO2. 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 proteoglycan synthesis with Alcian Blue after pelleting the collagen by centrifugation at 1000g’s for 7 days indicating chondrogenesis

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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