Matrix-Specific Anchors: A Novel Concept for Targeted Delivery and Retention of Therapeutic Cells

Mark L. Wang, MD, PhD\textsuperscript{1,2}, Pedro K. Beredjiklian, MD\textsuperscript{1,2}, Maulik D. Shah\textsuperscript{3}, Ryan Hoffman\textsuperscript{4}, Andrzej Fertala, PhD\textsuperscript{2}.

\textsuperscript{1}Rothman Institute at Thomas Jefferson University, Philadelphia, PA, USA, \textsuperscript{2}Thomas Jefferson University, Philadelphia, PA, USA, \textsuperscript{3}Rowan University School of Osteopathic Medicine, Stratford, NJ, USA, \textsuperscript{4}Drexel University College of Medicine, Philadelphia, PA, USA.


Introduction: Excessive fibrosis after tendon injury and repair remains a challenging clinical problem impacting patient functional outcome. Current molecular strategies to improve tendon healing have focused on modulating cellular responses at the injury site. Limitations of previously explored methods include inefficient therapeutic cell delivery, failure to retain cells within the injury site, and potential safety concerns of cellular-based treatment. The purpose of this study is to address this problem of site-specific cell delivery by exploring a new approach to target, bind, and retain cells within collagen I-rich tissues, including tendon, bone, ligament, and skin. We propose that targeted delivery and retention of cells can be achieved by employing a novel Artificial Collagen-Specific Anchor (ACSA) capable of specific targeting and binding to collagen I-rich injury sites. We hypothesize that, following delivery to the injury site, when the ACSA is expressed on the surface of therapeutic cells, rather than dispersing into surrounding areas, these cells will remain within the targeted site, thereby improving the efficiency of restoration to the damaged tissue. Potential benefits of the ACSA include an inducible collagen-targeting anchor, controlled delivery and retention of cells within an injury site, decreased requirement of therapeutic cells and duration of treatment, and enhanced tissue healing with improved safety and efficacy.

Methods: This engineered construct includes the following features: Murine fibroblasts (NIH 3T3), specific anchoring to human type I collagen (C-terminus, α2 chain), tetracycline (Tet)-responsive promoter, and GFP tag for localization (Fig. 1A). The stable expression of the ACSA-GFP construct was confirmed by culturing the selected transfected cells in the presence or the absence of doxycycline (Dox). The presence of the ACSA-GFP was confirmed by Western blot and microscopy. Cell attachment assays were performed on cells expressing the ACSA construct (Tet-On) and non-induced cells (Tet-Off). Cells were seeded on human collagen I-coated plates. Proliferation assays were performed colorimetrically. Western blot assays were performed to evaluate the effect of the ACSA-GFP construct on collagen-dependent signaling mediated via native collagen-specific receptors, specifically integrins and discoidin domain receptors (DDRs). Migration assays were executed on transduced cells cultured in the absence or the presence of Dox. At designated time points, cell migration was analyzed by measuring the distance of the outermost cells from the edge of an agarose drop.

Results: GFP-tagged ACSA is expressed on the surface of engineered cells in a Tet promoter-dependent fashion. Specific antibodies identified the following: the extracellular fragment of the construct (RED), the GFP portion (GREEN), and DAPI-stained nuclei (BLUE; Fig. 1B). ACSA enhances the attachment of the
cell construct to collagen, and this expression does not interfere with cell proliferation (Fig. 1C and D). Additionally, ACSA expression did not disrupt collagen-dependent intracellular signaling, as the phosphorylation patterns in transduced cells expressing the ACSA-GFP construct, in the presence of Dox, were similar to that seen in transduced NIH/3T3 cells, cultured in the absence of Dox, in which the expression of the ACSA-GFP was blocked (Fig. 2). At Day 2, the average migration distance of cells, without expression of the ACSA-GFP, was 0.28 mm (±0.02), while the average migration distance for cells expressing this construct was 0.12 mm (±0.03) (Fig. 3). The corresponding values after three days of incubation were 0.5 mm (±0.01), and 0.3 mm (±0.01), respectively. The difference in migration distances was statistically significant for both days (p<0.0001). No difference in migration was observed between non-transduced NIH/3T3 control cells cultured in the presence or the absence of Dox (not shown), and the migration patterns of these groups were similar to those patterns seen in transduced cells cultured in the absence of Dox.

**Discussion:** Expression of the ACSA-GFP construct enhances the ability of the cell construct to bind collagen without adversely impacting cellular proliferation. Additionally, the ACSA did not disrupt collagen-dependent intracellular signaling, or the ability of engineered cells to contract collagen gels. These findings suggest that this artificial anchor does not interfere with cellular binding via native collagen-specific receptors. The dynamics of contraction did not differ between the group of cells expressing the ACSA-GFP, and when expression was inhibited, it appears that the ACSA-GFP does not actively participate in gel contraction. Considering that collagen-specific Integrins and DDR1 recognize well defined specific sites within the collagen I triple helical domain and that the ACSA targets the distant α2Ct region, the collagen gel contraction assays indicates the high binding specificity of the novel collagen I-binding construct that does not interfere with the binding of native receptors.

The novel collagen specific cell-anchoring system proposed here may have a wide-ranging impact on developing improved repair processes of collagen I-rich tissues, including tendon, ligament, bone, intervertebral disc, and skin. Moreover, a successful ACSA approach to deliver and retain cells can potentially provide a blueprint for the development of additional cell-delivery methods that can target other elements within the extracellular matrix, including laminin and fibronectin. Another potential advantage of this concept is that the engineered cells can be designed to carry a therapeutically relevant molecule to the site of injury, with controlled delivery. Thus, ACSA-expressing cells can be engineered to produce a growth factor that enhances the repair process, allowing the precise binding and retention of therapeutic cells at the injury site, with controlled release of therapeutic growth factors. Future experiments in preclinical models may determine the clinical utility of the proposed method to improve the site-specific delivery and retention of therapeutic cells.

**Significance:** Fibrosis after tendon injury remains a challenging clinical problem, and current molecular strategies for improving tendon healing are limited by inefficient and non-specific therapeutic delivery. This novel cellular construct enables the controlled expression of collagen-targeting anchors at the surface of therapeutic cells, enhancing cellular attachment and retention to collagen-rich sites, without disrupting cell proliferation or collagen-dependent intracellular signaling. Potential benefits may include the specific targeting and controlled binding of therapeutic cells to the injury site, the reduced dispersion of cells into surrounding tissue, and enhanced tendon healing with improved safety and efficacy.
Figure 1:
A: Schematic of the proposed arrangement of the ACSA-GFP in employed NIH3T3 cells. Symbols: TM: transmembrane domain, VH and VL: variable domains of the heavy and the light chain of the archetypical anti-G2C antibody, GFP: green fluorescent protein. B: Immunofluorescence of cells expressing the ACSA-GFP construct in cells cultured in the presence of Dox. Specific antibodies identified the extracellular fragment of the construct (RED), the GFP portion (GREEN), and DAPI-stained nuclei (BLUE). Western blot confirming sub-cellular localization of the ACSA-GFP construct in transduced cells cultured in the presence (+) or absence (-) of Dox. C: Cellular proliferation assay of cells expressing the ACSA-GFP and non-transduced NIH/3T3 cultured in the presence (+) or absence (-) of Dox. D: Cell attachment of ACSA-GFP construct in transduced cells cultured in the presence (+) or absence (-) of Dox and seeded on collagen I or BSA-coated surfaces.

Figure 2:
Western blot assays of selected proteins participating in integrin and DDR-mediated collagen-cell interactions. The non-phosphorylated and phosphorylated (P) forms of these proteins were detected in cell lysates with the use of specific antibodies. Cells harboring the ACSA-GFP construct and not transformed NIH/3T3 cells cultured in the presence (+) or absence (-) of Dox were analyzed. GAPDH was employed as an internal control.
Figure 3:
Migration assay of transduced cells cultured in the absence or the presence of Dox. Dotted lines delineate the edges of agarose drops with encapsulated cells and denote the position of the migrating cell front present on the days indicated. Arrows show the overall migration distance in the depicted experimental groups.