SCIENTIFIC ARTICLE

Tendon Tissue Engineering: Mechanism and Effects of Human Tenocyte Coculture With Adipose-Derived Stem Cells

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Purpose Adipose-derived stem cells (ASCs) are a potential candidate for cell-based therapy targeting tendon injury; however, their therapeutic benefit relies on their ability to interact with native tenocytes. This study examines the mechanism and effects of coculturing human tenocytes and ASCs.

Methods Tenocytes (T) were directly cocultured with either ASCs (A) or fibroblasts (F) (negative control) in the following ratios: 50% T/50% A or F; 25% T/75% A or F; and 75% T/25% A or F. Cells were indirectly cocultured using a transwell insert that allowed for exchange of soluble factors only. Proliferation and collagen I production were measured and compared with monoculture controls. Synergy was quantified using the interaction index (II), which normalizes measured values by the expected values assuming no interaction (no synergy when II = 1). The ability of ASCs to elicit tenocyte migration was examined *in vitro* using a transwell migration assay and *ex vivo* using decellularized human flexor tendon explants.

Results Compared with monoculture controls, II of proliferation was greater than 1 for all tenocyte and ASC direct coculture ratios, but not for tenocyte and fibroblast direct coculture ratios or for tenocyte and ASC indirect coculture. The ASCs elicited greater tenocyte migration *in vitro* and *ex vivo*. The II of collagen I production was greater than 1 for direct coculture groups with 25% T/75% A and 75% T/25% A.

Conclusions Direct coculture of ASCs and tenocytes demonstrated synergistic proliferation and collagen I production, and ASCs elicited tenocyte migration *in vitro* and *ex vivo*. These interactions play a key role in tendon healing and were absent when ASCs were replaced with fibroblasts, supporting the use of ASCs for cell-based therapy targeting tendon injuries.

Clinical relevance When ASCs are delivered for cell-based therapy, they directly interact with native tenocytes to increase cell proliferation, collagen I production, and tenocyte migration, which may enhance tendon healing. *(J Hand Surg Am. 2017;■(■):1.e1-e9. Copyright © 2017 by the American Society for Surgery of the Hand. All rights reserved.)*

Key words Coculture, flexor tendons, tendon repair, tendon tissue engineering, tissue engineering.

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Tendon injuries, which afflict approximately 17 million people in the United States, present a significant source of morbidity.[1] Currently, the surgeon's options for acute injuries include primary surgical repair or secondary tendon reconstruction with tendon autograft. Despite significant advances in the past few decades, the intrinsic healing of tendons remains poor; thus, leaving patients with biomechanically suboptimal repairs that are inevitably weaker than the native, uninjured tendon.[2,3] This, coupled with the scarcity of donor tendons and concerns regarding donor site morbidity, presents a need for alternative options in tendon repair.

Cell-based therapy, a tissue engineering strategy, is a promising solution[4] that can potentially address both the scarcity and the suboptimal functional outcomes of using autologous donor tendons.[2] Delivering tenocytes could facilitate therapeutic action because tenocytes, through depositing collagen and remodeling the extracellular matrix (ECM),[5] are responsible for intrinsic tendon healing.[1,6] However, tenocyte use has been limited by the scarcity of donor tendons from which tenocytes can be cultured, the difficulty of isolating tenocytes, and the loss of phenotype of isolated tenocytes during *in vitro* expansion.[7,8]

In contrast, adipose-derived stem cells (ASCs) can be harvested with low donor site morbidity, are easily isolated, are found in abundance, and have the ability to differentiate along several lineage pathways.[9,10] This makes them an attractive option for regenerative cell-delivery therapies, with various potential applications in the field of reconstructive surgery.[11] The ASCs are particularly promising for tendon repair because they have been shown to enhance primary tendon healing,[12] exhibit tenocyte-like phenotype *in vitro* and *in vivo*,[13] modulate inflammatory responses during tendon healing,[14] and upregulate tenocytic markers when cocultured with tenocytes.[15]

Because ASCs remain viable for a relatively short period of time when delivered *in vivo*,[16,17] even in an immunocompromised model,[18] the therapeutic benefit of delivering ASCs to sites of tendon injury depends on ASCs' interactions with native tenocytes.[19] One method to examine these interactions is through coculture systems that allow for different forms of cell-to-cell interaction. In these coculture systems, there is building evidence that ASCs can not only differentiate along desirable lineages but also secrete paracrine signals that target surrounding cells, promoting proliferation, synthetic activities, and migration.[20–22] These interactions facilitated by stem cell coculture have already been explored in various tissues including cartilage,[23–25] bone,[26–29] vasculature,[10,30] lung,[22,31] kidney,[32] liver,[33–35] and nerve.[36–38]

Because these interactions have been well documented in other tissues, we hypothesize that ASCs will similarly promote proliferation, synthetic activities, and migration when cocultured with tenocytes. Although ASC-tenocyte coculture has been shown to promote ASCs' differentiation toward a tenogenic lineage,[15] there is limited understanding of the nature, mechanism, and potential therapeutic benefit of the interaction between these 2 cell types. Thus, in this study, we aimed to explore the nature of this interaction by examining proliferation, collagen production, and cell migration in an *in vitro* and *ex vivo* environment and the mechanism of this interaction by comparing 2 different coculture systems. In addition, human cells were used to expedite eventual translation. The goal is to optimize cell-based therapy as a therapeutic option for augmenting intrinsic tendon healing.

MATERIALS AND METHODS

Cell culture

Human ASCs (Lonza, Walkersville, MD) were cultured in adipose-derived stem cell growth medium (ASC-GM) (Lonza). Human tenocytes were primarily harvested from human flexor tendon specimens obtained through a surgical biopsy (institutional review board–approved protocol) of an unscarred area to avoid cell harvest from pathological specimens. Briefly, specimens were minced into 1-mm segments, trypsin-bathed (Sigma-Aldrich, St. Louis, MO) samples were incubated at 37°C on a rotator, the suspension was centrifuged, and the supernatant was removed. Tendon samples were then incubated in collagenase type I (Sigma-Aldrich), the suspension was passed through a cell strainer, the filtrate was centrifuged, and the remaining cell pellet was resuspended and cultured in F-12 media (Invitrogen, Waltham, MA). Adult skin derived Normal Human Dermal Fibroblasts (NHDF-Ad) (Lonza) were grown in fibroblast basal media (Lonza). All media were supplemented with 10% fetal bovine serum, and all cells were grown to 90% confluence at 37°C in a humidified tissue culture chamber with 5% carbon dioxide content.

Direct coculture

Tenocytes (T) and ASCs (A) were directly cocultured in triplicate with 10,000 cells per well in a 24-well plate at various ratios (50% T/50% A; 25% T/75% A; 75% T/25% A) and compared to monoculture controls (100% T, 100% A). The F-12 media and