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Research Paper

A preliminary technical study on sodium dodecyl sulfate-induced changes of the nano-structural and macro-mechanical properties in human iliotibial tract specimens

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ABSTRACT

Introduction: Acellular scaffolds are frequently used for the surgical repair of ligaments and tendons. Even though data on the macro-mechanical properties related to the acellularization process exist, corresponding data on the nano-structural properties are still lacking. Such data would help identify target proteins of the formed extracellular matrix that are chemically altered by the acellularization. In this study we examined the altered structure by comparing molecular properties of collagens from native and acellular iliotibial tract samples to the macroscopic stress–strain behavior of tract samples.

Material and methods: Matched pairs of five human iliotibial tract samples were obtained from five donors (mean age 28.2±4.7 years). One of each pair was acellularized using 1 vol% sodium dodecyl sulfate (SDS) for 7 days. ^{13}C magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP MAS NMR) was utilized to compare the collagen overall secondary structure and internal dynamics of collagen-typical amino acid proteins. The resulting data was compared to age-matched stress–strain data of tract samples obtained in an uniaxial tensile setup and histologically.

Results: Typical and nearly identical collagen ^{13}C CP MAS NMR spectra were found in the tract samples before and after acellularization with SDS. The characteristic collagen backbone remained intact in the native and acellular samples. Collagen molecular composition was largely unaltered in both conditions. Furthermore, a similar dynamic behavior was found for the amino acids Hyp γ, Pro α/Hyp α, Ala α, Gly α and Ala β. These

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minute alterations in the collagens' molecular properties related to acellularization with SDS were in line with the similarly minute changes in the macro-mechanical tensile behavior, such as the elastic modulus and ultimate stress. Histology showed intact type I collagens, minute amounts of elastins before and after acellularization and evidence for acellularization-induced reductions of proteoglycans.

Discussion: Nano-structural properties of collagens are minutely affected by SDS treatment for acellularization, indicated by the molecular composition and dynamics. The lacking acellularization-related changes in the molecular structure properties of collagens in iliotibial tract samples are in line with the small alterations in their macro-mechanical tensile behavior. Though the given setup approaches soft tissue mechanics from both scaling extremes of mechanical testing, further structural analyzes are needed in a larger sample size to substantiate these findings.

1. Introduction

Acellular scaffolds are frequently used for the surgical repair of ligament tears or ruptured tendons (Barber and Aziz-Jacobo, 2009; Branch, 2011; Chen et al., 2009; Rao et al., 2012; Rubin and Schweitzer, 2005) serving the principal purpose of reinforcing injury sites (Barber and Aziz-Jacobo, 2009; Rubin and Schweitzer, 2005). These scaffolds are allogeneic or xenogeneic tissues with reduced immune response due to the lack of cells and cellular proteins (Chen et al., 2009; Tischer et al., 2010), allowing for ample biocompatibility by means of host cell ingrowth and physiological remodeling (Byrom et al., 2013; Dohmen et al., 2014; Dong et al., 2015). A number of tissues (Adams et al., 2006; Chen et al., 2009; Ellis and Kulber, 2012; Gilbert et al., 2006; Hulsmann et al., 2012) have been utilized for the purpose of ligament (Mascarenhas et al., 2015; Robayo et al., 2011; Tischer et al., 2010), cartilage (Hwang et al., 2007), muscle and fascia (Chung et al., 2003; Ma et al., 2011; Rao et al., 2012) as well as nerve reconstruction (Ma et al., 2011). Given its wide field of application in reconstructive surgery, it is necessary to describe acellular scaffolds on a corresponding micro- and macro-structural level, especially since some mismatch exists between the *in-vivo* experiments and the clinical results regarding resilience and biocompatibility (Chen et al., 2009). Though mechanical testing of human acellular scaffolds has been performed extensively *in vivo* (Adams et al., 2006) and *ex vivo* (Barber and Aziz-Jacobo, 2009; Ehsan et al., 2012; Hammer et al., 2014; Hulsmann et al., 2012; Song et al., 2010), corresponding micro-structural data are lacking in the current literature (Li and Cao, 2010).

Such data would help clarify how acellularization chemicals alter tissue properties found on a macroscopic level (Adams et al., 2006; Barber and Aziz-Jacobo, 2009; Ehsan et al., 2012; Hammer et al., 2014; Hulsmann et al., 2012; Rubin and Schweitzer, 2005; Tischer et al., 2010) and identify their target proteins on the extracellular matrix (ECM)(Li and Cao, 2010). Furthermore, this information would facilitate in optimizing acellular scaffolds as "perfect" conduits concerning their biomechanics and biocompatibility (Byrom et al., 2013; Chen et al., 2009; Gilbert et al., 2006). Combining ^{13}C solid-state magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy to determine molecular properties with macro-mechanical tensile data of human tissues may help address this issue. It has been shown that natural abundance ^{13}C MAS NMR spectroscopy is a very useful tool for the characterization of molecular collagen and elastin composition in cartilage, bone, skin and other tissues (Hagenau et al., 2009; Huster, 2008; Penk et al., 2013; Reichert et al., 2004; Saito and Yokoi, 1992; Schulz et al., 2007; Zernia and Huster, 2006). In addition to the verification of collagen fibrils in the sample due to their characteristic ^{13}C cross-polarized (CP) MAS NMR spectrum, it is also possible to determine the dynamic properties of different molecular groups inside the collagen fibrils. In our preliminary technical study, we aimed at establishing a protocol capable of comparing the molecular properties of collagen in human iliotibial tract specimens to their macro-mechanical behavior, combining uniaxial tensile testing with ^{13}C MAS NMR to address the following hypotheses:

Hypothesis 1. Molecular collagen composition and dynamics are altered in iliotibial tract specimens acellularized with sodium dodecyl sulfate, as compared to their native counterparts.

Hypothesis 2. Changes in the molecular composition and dynamics of collagen in tract specimens resemble their macro-mechanical tensile behavior.

2. Materials and methods

2.1. Sample acquisition and processing

Five iliotibial tract specimens were obtained from five body donors (3 males, 2 females, mean age 28.2±4.7 years, range 22–33 years; Table 1) during autopsy at the Institute of Forensic Medicine, University of Leipzig, Germany. None of donors had a history of connective tissue disease and the tissues were removed in a fresh and chemically unfixed condition with a post-mortem delay of two days or less. The university's ethics committee approved the given study (protocol number 156-10-1207-2010). To remove the tract specimens, an incision was made between the greater trochanter and the lateral femoral epicondyle, measuring