Longitudinal, 3D Imaging of Collagen Remodeling in Murine Hypertrophic Scars In Vivo Using Polarization-Sensitive Optical Frequency Domain Imaging

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Hypertrophic scars (HTS), frequently seen after traumatic injuries and surgery, remain a major clinical challenge because of the limited success of existing therapies. A significant obstacle to understanding HTS etiology is the lack of tools to monitor scar remodeling longitudinally and noninvasively. We present an in vivo, label-free technique using polarization-sensitive optical frequency domain imaging for the 3D, longitudinal assessment of collagen remodeling in murine HTS. In this study, HTS was induced with a mechanical tension device for 4–10 days on incisional wounds and imaged up to 1 month after device removal; an excisional HTS model was also imaged at 6 months after injury to investigate deeper and more mature scars. We showed that local retardation and degree of polarization provide a robust signature for HTS. Compared with normal skin with heterogeneous local retardation and low degree of polarization, HTS was characterized by an initially low local retardation, which increased as collagen fibers remodeled, and a persistently high degree of polarization. This study demonstrates that polarization-sensitive optical frequency domain imaging offers a powerful tool to gain significant biological insights into HTS remodeling by enabling longitudinal assessment of collagen in vivo, which is critical to elucidating HTS etiology and developing more effective HTS therapies.

Journal of Investigative Dermatology (2016) **136,** 84-92; doi:10.1038/JID.2015.399

INTRODUCTION

Hypertrophic scars (HTS) are conspicuous, potentially disfiguring lesions that remain a major therapeutic challenge. In fact, its incidence rate after surgery in burn injury is particularly high (>90%) (Gauglitz et al., 2011). Apart from the cosmetic disfigurement, HTS frequently recur and can cause pruritus, pain, functional impairment, and profound psychological effects.

The pathogenesis of HTS is not fully understood. Its formation typically involves an abnormal wound healing response to trauma, such as burns, inflammation, or surgery, especially when the wound crosses joints or skin creases at right angles (Wolfram et al., 2009). Among other factors, tension is known to be critical to the formation of HTS and, indeed, the well-established surgical treatment—Z-plasty or W-plasty (English and Shenefelt, 1999)—works by relieving tension along the scar. Decreased cellular apoptosis was observed in murine models in which mechanical stress was applied to healing wounds (Aarabi et al., 2007). In addition, overproduction of transforming growth factor-β and platelet-derived growth factor suggests the pathologic persistence of wound healing signals (transforming growth factor-β1 and -β2 stimulate collagen synthesis) in HTS (Gauglitz et al., 2011). Histologically, HTS are characterized by the presence of nodular structures with fine, randomly organized collagen bundles (Ehrlich et al., 1994).

To understand scar etiology and assess treatment outcome, imaging is needed. Several imaging techniques have been investigated to assess collagen ex vivo and in vivo. Historically, picrosirius red staining with polarization microscopy, which enhances tissue birefringence (Junqueira et al., 1979), was used to visualize collagen ex vivo. More recently, second-harmonic generation and two-photon excited fluorescence techniques have been used to analyze collagen and

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Abbreviations: DOP, degree of polarization; HTS, hypertrophic scars; LR, local retardation; OCT, optical coherence tomography; PS, polarization-sensitive; PS-OFDI, polarization-sensitive optical frequency domain imaging

Received 24 January 2015; revised 28 September 2015; accepted 29 September 2015; accepted manuscript published online 8 October 2015

elastin, respectively, in ex vivo HTS and keloid specimens (Chen et al., 2009; Vogler et al., 2011). Unfortunately, these techniques only offer a limited field of view and are challenging to use in vivo. Optical coherence tomography (OCT) offers fast imaging speed, increased field of view, and imaging depth, at a spatial resolution suitable for biological applications, which makes it particularly amenable to in vivo imaging. OCT has been investigated for the imaging of skin (Alex et al., 2010; Gambichler et al., 2011), and in particular for the imaging of burn scars (Gong et al., 2013) and skin cancer (Mogensen et al., 2009).

Polarization-sensitive OCT is an extension of OCT that also measures the polarization state of the light backscattered by the sample. It enables the measurement of tissue birefringence, primarily caused by fibrillar collagen, in the skin. Polarization-sensitive OCT has been shown to provide intrinsic contrast in thermally damaged tissue, thereby providing a tool for burn depth assessment (De Boer et al., 1998; Park et al., 2001; Pierce et al., 2004a, 2004b) and mapping of dermal birefringence in photoaged skin (Sakai et al., 2008) in vivo.

In this study, we present the use of polarization-sensitive optical frequency domain imaging (PS-OFDI), a variant of polarization-sensitive OCT that offers improved imaging speed and sensitivity (Villiger et al., 2013), for the 3D imaging of a rat model of surgical HTS in vivo. This animal model employs a mechanical tension device placed on healing incisional wounds, which enables the systematic study of HTS formation (Aarabi et al., 2007). To validate PS-OFDI in imaging deeper scars, we also investigated an excisional wound scar placed under autologous tension, imaged at 6 months after injury. We evaluate both local retardation (LR) and degree of polarization (DOP), computed using our spectral binning algorithm (Villiger et al., 2013), for enhancing the contrast of HTS, which, to the best of our knowledge, has not been reported previously. Compared with cumulative retardation, LR expresses the rate of change of the measured polarization states with depth, and is a direct, more intuitive measure of tissue birefringence. DOP is a quantity related to the randomness of the detected polarization states of light, which scales from zero for completely random to unity for perfectly uniform polarization states. Unlike LR, DOP captures the cumulative effect of the tissue from the surface to a given depth. We observed that the combination of LR and DOP provides a robust optical signature to differentiate HTS given the presence of fine, densely packed collagen bundles in HTS compared with thicker collagen bundles in normal skin.

In addition, we demonstrate the use of PS-OFDI for studying HTS remodeling by imaging the incisional wound model longitudinally for 1 month and the excisional model at 6 months. Interestingly, we observed a progressive increase in LR and a persistently high DOP within the scar region over time, which corresponded well with the remodeling and thickening of collagen fibers histologically. Normalization of the scar was associated with an increased LR and decreased DOP back to baseline levels in normal skin. Our findings suggest that PS-OFDI can serve as a valuable 3D imaging tool for the noninvasive, longitudinal assessment of HTS in vivo, by providing significant biological insights into collagen remodeling central to understanding HTS etiology and monitoring therapeutic response to improve current therapies and investigate novel approaches.

RESULTS

Imaging aberrant collagen organization in HTS using PS-OFDI

Figure 1 shows the cross-sectional PS-OFDI images of a mechanical tension–induced HTS after 10 days of loading (imaged 1 month later) compared with normal rat skin. For intuitive visualization, the LR and DOP signals were merged with the intensity image (as a brightness channel) and displayed using an isoluminant colormap (Geissbuehler and Lasser, 2013). Whereas conventional intensity images show minimal contrast (only slightly increased backscattering), the local retardation (PS-LR) and degree of polarization (PS-DOP) images demonstrate significant differences in HTS compared with normal skin. HTS shows significantly reduced LR and increased DOP deep inside the dermis, compared with heterogeneous LR and low DOP beyond the epidermis in normal skin. This optical signature (reduced LR and high DOP) corresponds histologically to thin, less organized collagen bundles in HTS (Figure 1d), compared with thicker collagen bundles in normal skin (Figure 1h). These abnormal collagen bundles are cigar-shaped and orientated parallel to the surface of the skin along the tension lines of the scar tissue (Figure 1d). In addition, there is an increased cellularity of fibroblasts in HTS compared with normal skin (Figure 1d and h).

Histological correlation

We further analyzed the histological correlation of the PS-OFDI images in each animal group with varying duration of tension (Figure 2). In all cases, the scar region shows reduced LR and increased DOP. Overall, the PS-DOP images correlate well with the extent and shape of the scar as confirmed by hematoxylin and eosin histology (Figure 2c, f, i, and l), whereas the PS-LR images show more variability. The size of HTS also increased with the duration of tension, as expected, from a barely noticeable scar with minimal deposition of collagen in the 4-day group (Figure 2c) to a significantly larger scar extending all the way through the dermis that is characterized by aberrant collagen bundles and increased cellularity of dermal fibroblasts in the 10-day group (Figure 2l).

Longitudinal, 3D imaging of HTS in vivo

A major advantage of using PS-OFDI, compared with conventional histology processing, is the ability to assess HTS longitudinally and comprehensively in vivo (Figure 3), which is particularly important for studying HTS etiology and assessing response. By imaging the incisional HTS model (6-day group) at 1-week intervals after device removal, we observed rapid contraction of the scar in the first week, as indicated by the normalization of DOP and LR around the boundary of the scar to baseline levels in normal skin (increased LR and decreased DOP). From weeks 1 to 4, the scar continued to remodel progressively, leading to a further reduction in scar size and an interesting increase in LR, particularly in deeper regions. The DOP remained persistently high within the scar region. To investigate the evolution of the LR and DOP signals further, we analyzed the PS-LR and