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A three-dimensional analysis of the collagen network in rats treated with hyaluronic acid-based soft tissue filler

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Tissue Vision

Introduction

Hyaluronic acid (HA)–based soft tissue fillers are widely used to restore lost facial volume due to ageing and lipoatrophy. In addition to the space occupying effect of these devices, studies have shown additional benefits related to stimulation of collagen and other extracellular matrix proteins.

This study evaluates the tissue integration and collagen network effects of the HA-based soft tissue filler VYC-20L in a rat model using a novel three-dimensional (3-D) imaging platform and image analysis algorithms.

Methods

Tissue Preparation

Sprague Dawley rats were treated with a subcutaneous injection of 125 µL of the HA-based soft tissue filler VYC-20L (Juvéderm Voluma XC; Allergan Aesthetics, an AbbVie Company, Irvine, CA).

After either 4 weeks (n= 7) or 12 weeks (n= 6), rats were euthanized and skin samples were excised. Samples were immersion fixed in formalin for 48 hours at 4 °C and subsequently placed in 1X Phosphate Buffered Saline (PBS) and 0.1% sodium azide.

The fixed explants were then gently shaved with a razor to remove external hair to assist with sample processing.

Following, samples were embedded in an agarose block and polymerized in an embedding matrix to provide stability for sectioning.

STP² Imaging

Explants were sectioned serially with 100 um thickness and imaged with a TissueCyte Serial Two-Photon (STP²) system **(Figure 1)** to visualize tissue autofluorescence and collagen fibril signatures using second harmonic generation (SHG) imaging.

A 16X Nikon water immersion objective was utilized to produce 1.3 micron per pixel resolution data. An excitation wavelength of 920 nm provided an emission spectrum of > 560 nm (channel 1), 500-550 nm (channel 2), and 442-478 nm (SHG, channel 3).

Gross tissue anatomical features could be readily visualized from the autofluorescence (channel 1, channel 2). The collagen within the skin, both around and inside the injection, could be readily distinguished with SHG signatures (channel 3).

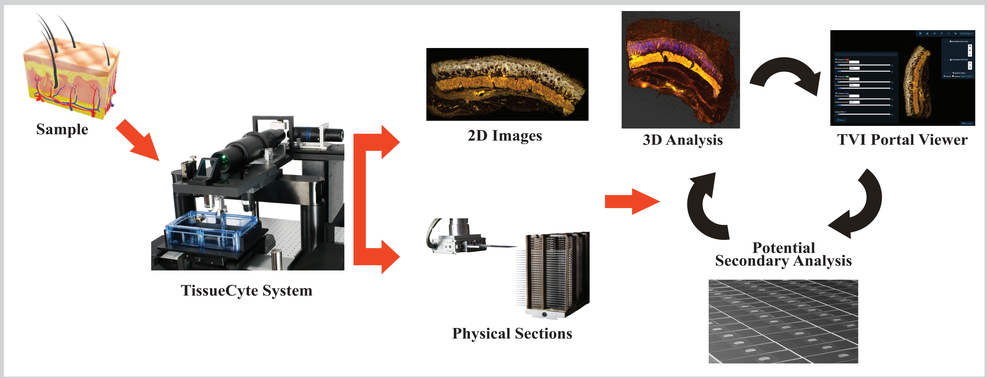

Figure 1. TissueVision's Serial Two-Photon Plus STP² processing pipeline.

Image Analysis

The extent of tissue integration and collagen quality metrics were evaluated through image analysis of 3D collagen integration into the filler bolus, collagen fiber remodeling (anisotropy), and collagen fiber bundle frequency. Manual annotation to mask the filler bolus region of interest (ROI) was performed on every 5th section. The interleaving section bolus masks were interpolated from the manual annotations to capture the full bolus ROI.

3D Integration Metric

The SHG signal within the bolus was masked, and the density (fractional area) of SHG collagen was determined within the bolus region. The quantification of the 3D distribution of the collagen density and integration was determined across the entirety of the bolus for each sample.

Collagen Remodeling

Normal collagen fibers in the dermis tend to arrange in a basket-weave pattern with random fiber orientations, whereas aged or scar damaged dermis results in an increased alignment of fibers. The alignment of collagen fibers may change over the tissue remodeling process.

To assess the relative degree of alignment of collagen fibers throughout the bolus, the local fiber orientations were computed across bolus regions, parsed into 250 x 250 µm windows. The degree of alignment can be represented by the fiber anisotropy, wherein tissues with low anisotropy have more random arrangements of orientation, and tissues with high anisotropy have more alignment of fiber orientations.

Collagen Bundle Frequency

Within the bolus, the relative distribution and density of collagen fibers can vary as fibers aggregate into fiber bundles. The collagen bundle frequency metric provides an estimate of the distance (in microns) between bundles of collagen within the regions of the injection, where higher values translate to more closely-packed collagen bundles.

Bundle frequency is computed from the segmented mask of the collagen fibers. The Euclidean distance transform of the background measures the distance from all background pixels to the nearest collagen fiber pixel. The median value of the distance transform image estimates the bundle spacing, and its reciprocal estimates the bundle frequency **(Figure 2)**.

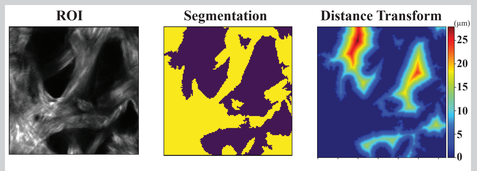

Figure 2. Euclidean distance transform computed for sample region.

Results

TissueCyte STP² imaging demonstrated a HA gel bolus within the samples, which is discernable from the surrounding tissue **(Figures 3, 4)**. Autoflourescence and SHG imaging show a robust fibrillar collagen network within the filler bolus at 4- and 12- weeks after injection **(Figures 5, 6)**. An image analysis algorithm that measured the density and uniformity of tissue throughout the bolus (3D integration metric) indicated a 2.1-fold increase ($p = 0.005$) in collagenous tissue integration, a 1.3-fold increase ($p = 0.035$) in collagen fiber bundle remodeling (anisotropy) and a 1.4-fold increase ($p = 0.035$) in bundle frequency throughout the bolus from 4 to 12 weeks, when assessed using the Mann-Whitney Two Sided test **(Figure 7)**.

Figure 3. HA gel bolus outlined (in red) for 4-week (A) and 12-week (B) samples.

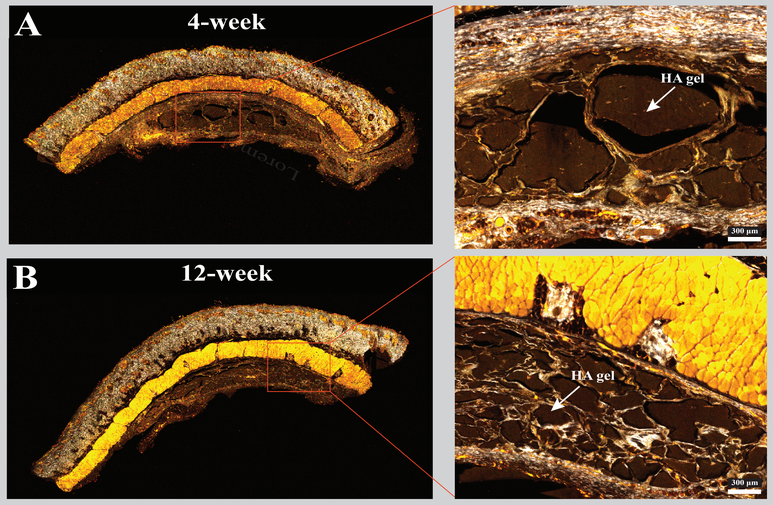

Figure 5. 2D planes produced by the TissueCyte system demonstrating tissue autofluorescence (channel 1; red, channel 2; green), and SHG collagen fibril signatures (channel 3; white) in 4-week (A) and 12-week (B) HA gel injections.

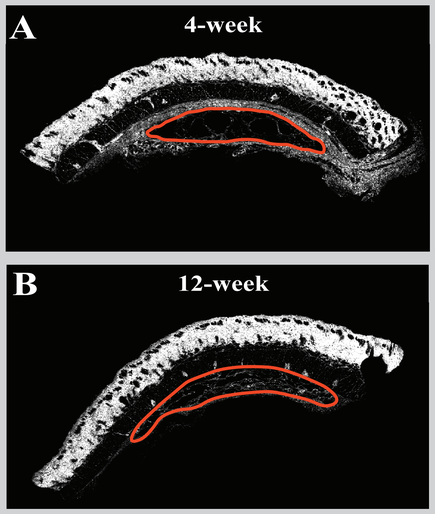

Figure 4. SHG collagen within the HA gel bolus outlined (in red) for 4-week (A) and 12-week (B) samples.

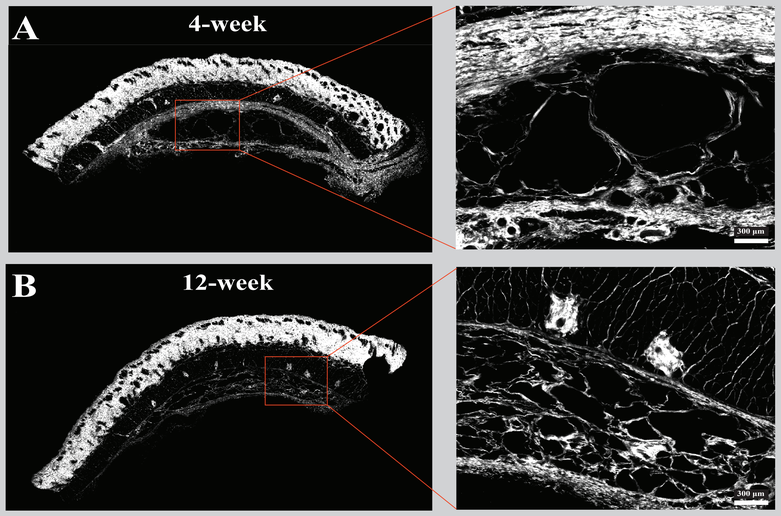

Figure 6. 2D planes produced by the TissueCyte system demonstrating SHG collagen fibril signatures (channel 3; white) in 4-week (A) and 12-week (B) HA gel injections.

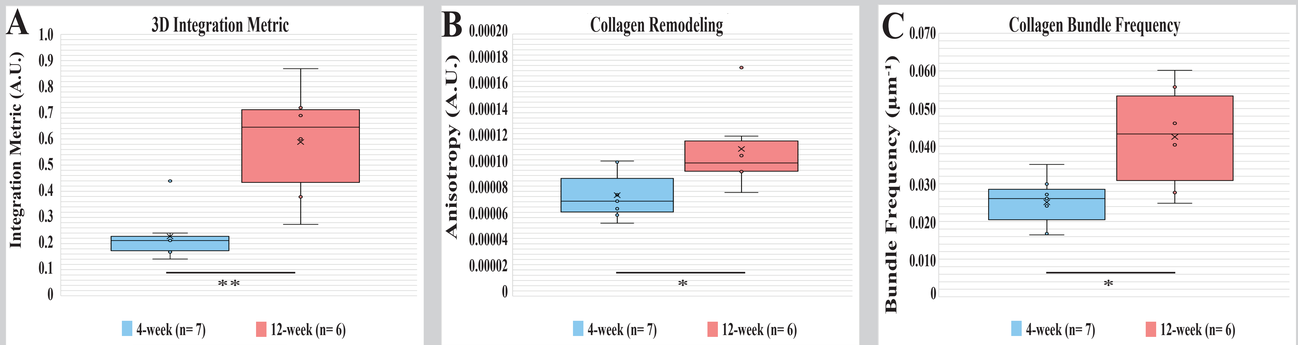

Figure 7. Results of 3D Integration Metric (A), Collagen Remodeling (Anisotropy) (B) and Collagen Bundle Frequency (C). Horizontal lines represent the lower bound, first quartile, median, third quartile, and upper bound of the data. The mean is indicated by an 'x'. Additional values inside the interquartile range (IQR) are indicated with a data point. Outlying values more than one and a half times outside the IQR are indicated with a data point beyond the lower and upper bound. Significance is indicated by *= $p < 0.05$ and **= $p < 0.01$.

Conclusion

This study employed a new method, the STP² imaging platform, to characterize the tissue integration and collagen structure throughout the entirety of a HA filler bolus. Using this method, it was demonstrated that the HA-based soft tissue filler VYC-20L supported the development of a 3D fibrillar collagen network that becomes more robust over the course of 12 weeks. 3D imaging and analysis methodologies enable a deeper understanding of the tissue response to HA-based fillers and hold promise for future evaluation of soft tissue filler formulations.

Disclosure

Financial arrangements of the authors with companies whose products may be related to the present report are listed as declared by the authors: A. Kutikov, A. Pierce, and L. Nakab are full time employees of Allergan Aesthetics, an AbbVie Company. G. Ferron, A. Knesis, S. Linehan, and T. Ragan are full time employees of TissueVision Inc. Allergan Aesthetics, an AbbVie Company was the study sponsor.