# Supporting Material

## Structural characterization of the fibrin network

### Fiber Extraction Algorithm

Image stacks of the fibrin network were loaded in MATLAB (The MathWorks, Natick, MA) and processed using custom-made image analysis algorithms. Pre-processing of the image stacks was performed in accordance with the approach described by D’Amore et al. [63]. Image contrast was enhanced via a histogram equalization algorithm (histeq function in MATLAB) and noise on the images was reduced by a 3 by 3 median filtering operation (medfilt2 function in MATLAB). Images were subsequently smoothed with a Gaussian filter (with a standard deviation of 1 pixel) and binarized by keeping a percentage of the maximal pixel intensities. These percentages were carefully chosen to obtain a good qualitative match between the original and binarized fiber network for the various hydrogel formulations, which was always the lowest possible value for the best representation without having a disrupted extracted fiber network. Fiber areas in the binarized images were then thinned and smoothed, and isolated pixels were removed using the bwmorph MATLAB function (with ‘thin’, ‘majority’, and ‘clean’ option, respectively). Fibers were refined via an image erosion operation on the binarized images (imerode function in MATLAB, using a disk element with size 1/12 of a representative fiber diameter, RFD). Estimations for RFD were obtained from Wufsus et al. [26], describing an average fiber radius of ~60 nm, which equals a size of 0.6 pixel. Pixel areas smaller than 100xRFD were removed via the bwareaopen function in MATLAB. Binarized fiber areas were dilated (via the imdilate MATLAB function with disk element size of RFD/6) and eroded again (imerode function with a disk size element of RFD/12).

The distance from a pixel contained in the fiber area to the nearest background pixel was calculated via a Euclidian distance transform on the binarized image stack. The 2D images contained in the image stack have an isotropic pixel aspect ratio and could be transformed via the bwdist MATLAB function. Along the z axis, an anisotropic voxel aspect ratio was present and therefore required the use of a line-scan algorithm to calculate the Euclidian distance transform [68]. The distance transform was smoothed with a Gaussian filter with standard deviation of 0.2 pixels.

Network topology was extracted with the fiber extraction (FIRE) algorithm described by Stein et al [49]. In brief, nucleation points were identified at local maxima of the distance transform (points were traced in a surrounding box with size of 11 by 11 pixels) that also exceeded a threshold value of 1.5 pixels. Next, local maximum points (LMPs) were searched on the surface of the surrounding box and were identified as local maxima when larger than a threshold value of 0.2. Extensions of nucleation points to these LMPs were defined as new fiber branches and were extended until they reached an end position. End positions of a fiber were reached at other nucleation points or if no new LMPs could be identified. New LMPs were obtained if a minimal distance of 2 pixels lay between 2 LMPs and if changes in fiber direction resulting from fiber extension were not larger than 70°. Identified fibers were removed when they were shorter than 15 pixels and had a minimal difference in angle of 10° with another fiber going through the same nucleation point. Fibers that had a similar orientation at the end were linked together to form a single fiber. Fiber ends that were separated by 25 pixels and had a minimum angle of 130° between them were connected to make a single fiber. The total fiber length in each composition was quantified from the extracted network by summation of all individual fiber segment lengths.

### Turbidimetry

C:\Users\u0084133\Desktop\GRAPHS POOL\FINAL IMAGES\raw_turbidity_PP.tif

*Fig. S1: (a) Data obtained from turbidity measurements for 20LL and 20HH compositions. Note the different spectral range used as a consequence of the high optical density of the 20LL hydrogel. (b)Turbidimetry analysis as explained earlier for the deduction of fiber structural parameters. Both samples show a linear response of τλ5 versus λ2.*

## Diffusivity of dextran molecules

*C:\Users\u0084133\Desktop\GRAPHS Revision\DvsPhi_pp.tif*

*Fig. S2: Diffusivity of 10 (a) and 40 kDa dextran (b) with respect to the fiber volume fraction for all hydrogel compositions.*

*C:\Users\u0084133\Desktop\GRAPHS Revision\DvsR_pp.tif*

*Fig. S3: Diffusivity of 10 (a and b) and 40 kDa dextran (c and d) with respect to the fiber radius for all hydrogel compositions (a and c) and all except the outliers 20LH and 20HH (b and d). Data in (d) are divided in a high-thrombin (empty circles) and a low thrombin concentration (solid circles).*

C:\Users\u0084133\Desktop\GRAPHS Revision\RawFRAP_pp.tif

*Fig. S4: Hankel transform and single exponential fit to the experimental data for the diffusion of 10 kDa (a and c) and 40 kDa dextran (b and d) in 5LL (a and b) and 20HH (c and d) hydrogels as obtained from the FRAP analysis program* [51]*.*

### Estimation of the predicted relative diffusivities for impermeable fibers

Fibrin fibers exhibit intrafibrous porosity which is dependent on the polymerization conditions. For the purpose of assessing whether the Ogston model would be applicable for fibrin fibers which are hypothetically entirely impermeable, the following approximations were made. First, the internal fiber density was hypothesized to be equal to fibrinogen density (ρfib = 1395 mg/mL) for all compositions, and new values for the fiber volume fraction were calculated again using equation (5). Second, the network conformations were assumed to remain unchanged and new fiber radii were then approximated by the following relationship:

With ρimp, Vimp and rimp the hypothetical fiber internal density, fiber volume and fiber radius of the impermeable fiber, respectively.

C:\Users\u0084133\Desktop\GRAPHS Revision\impermeable_pp.tif

*Fig. S5: Comparison of experimental relative diffusivity of dextran solutes in fibrin hydrogels with the predicted values from the Ogston model adapted for impermeable fibers. The dashed line has a slope equal to 1 (equal experimental and predicted values).*

## Shear rheology

The following calculations were performed using Matlab. Ġ was computed using a Savitzky–Golay filter (27-point window, cubic-spline approximation) on the raw G'-t curve and the maximum Ġ value was determined. The part of the Ġ curve lying to the right of the maximum was approximated by a two-term exponential decay function in order to determine ts (Fig. S6). Due to the very small changes in obtained values over time this approximation was needed to find the actual time point with high precision.

C:\Users\u0084133\Desktop\GRAPHS POOL\FINAL IMAGES\SG_PP.tif

*Fig. S6: Black lines indicate Ġ obtained by applying a Savitzky-Golay filter on averaged experimental G' results (data in Fig. 8a); red lines are a two-term exponential fit to the Ġ data.*

*C:\Users\u0084133\Desktop\GRAPHS Revision\DvsG_pp.tif*

*Fig. S7: (a) Storage shear modulus versus relative diffusivity and (b) loss modulus at high frequency (100 rad/s) versus diffusivity, for 10 kDa (solid circles) and 40 kDa (empty circles) dextran solutes.*