Geometry influences inflammatory host cell response and remodeling in tissue-engineered heart valves in vivo

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Material and Methods

Scanning electron microscopy (SEM)

SEM was used to assess and confirm the degree of endothelialization for the two TEHVs designs (JACC, Emmert). Briefly, samples were fixed in 2% glutaraldehyde (Sigma-Aldrich; Switzerland), dehydrated with a sequence of different EtOH concentrations, and embedded in a solution of EtOH with increasing concentration of plastic components. Sections were then cut in an ultramicrotome and finally platinum-sputtered for imaging.

Supplementary Table 1: Summary of the antibodies used for immunofluorescence.

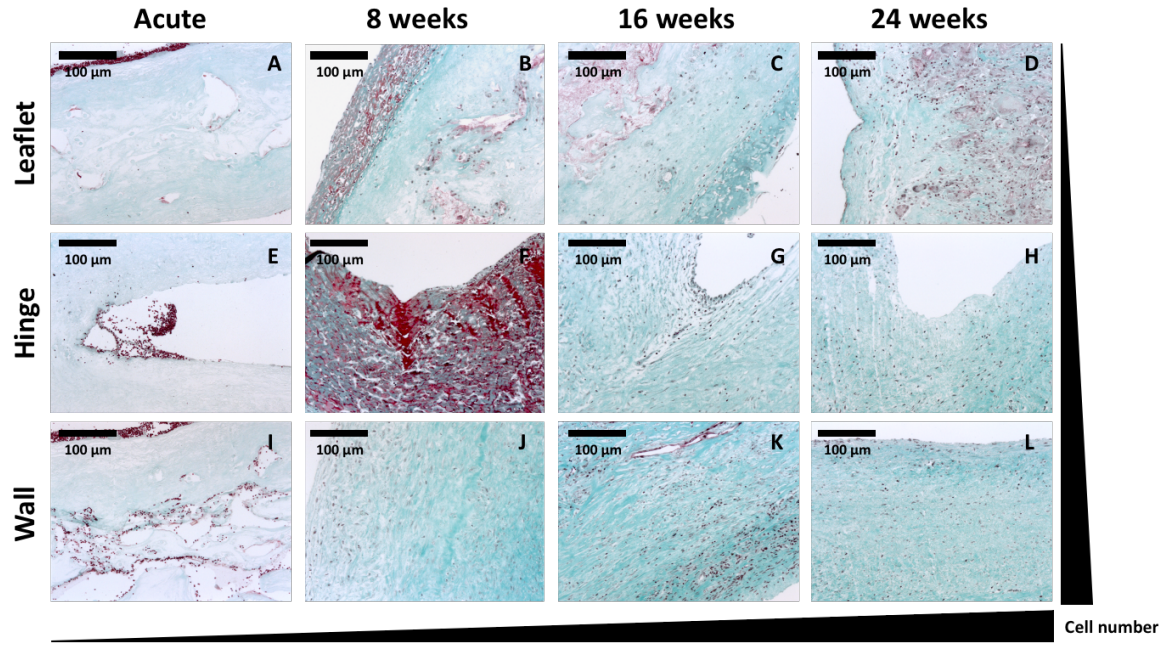
First-generation **TEHV**	**Primary AB**	**Secondary AB**
M1	Rabbit anti-CCR7 (Abcam, ab32527)	Goat anti-rabbit Alexa 568
M2	Mouse anti-rat CD163 (Abd serotec, MCA342GA)	Goat anti-mouse Alexa 488
Second-generation **TEHV**	**Primary AB**	**Secondary AB**
M1	Rabbit anti-CCR7 (Abcam, ab32527)	Goat anti-rabbit Alexa 568
M2	Mouse anti-rat CD163 (Abd serotec, MCA342GA)	Goat anti-mouse Alexa 488

Supplementary Table 2: *First-* and *second-generation* TEHVs leaflet lengths and hinge thickness measurements. Measurements are represented as mean value ± standard deviation

Follow-up [weeks]	***First-generation*** **TEHV**	
	Leaflet length [μm]	**Hinge thickness [μm]**
Control	11533.33 ± 1042.43	754.17 ± 12.81
8	9365.83 ± 273.14	1686.67 ± 784.39
16	6597.16 ± 1703.23	2715 ± 700.72
24	3612.916 ± 1848.99	2255.83 ± 355.41
	Second-generation **TEHV**	
	Leaflet length [μm]	**Hinge thickness [μm]**
Control	14451.43 ± 1081.86	870.57 ± 107.34
8	11415.55 ± 2017.84	1996.67 ± 1001.77
52	13538.33 ± 2014.32	651.33 ± 445.42

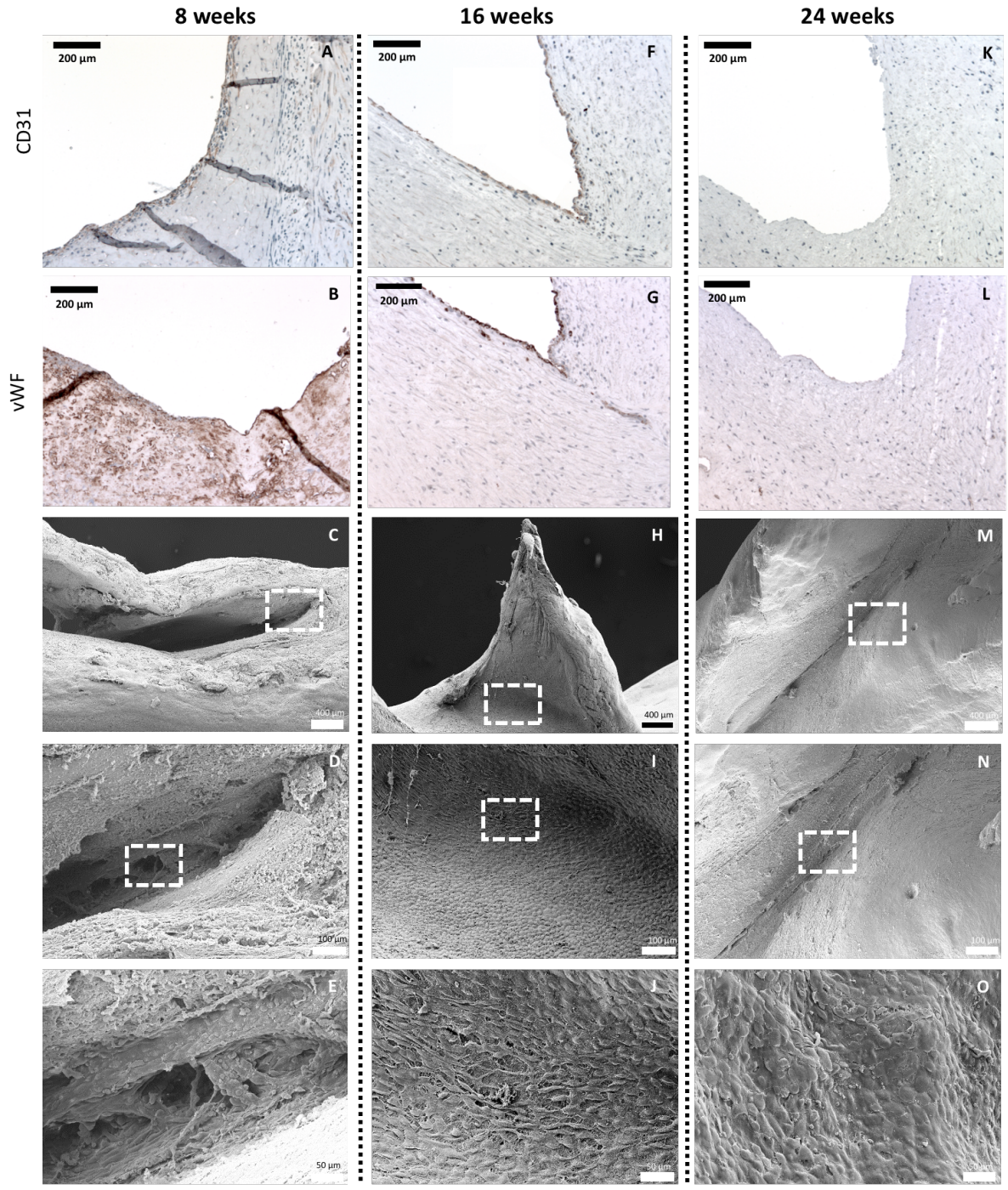
Results

Host cellular infiltration in *first-generation* TEHVs



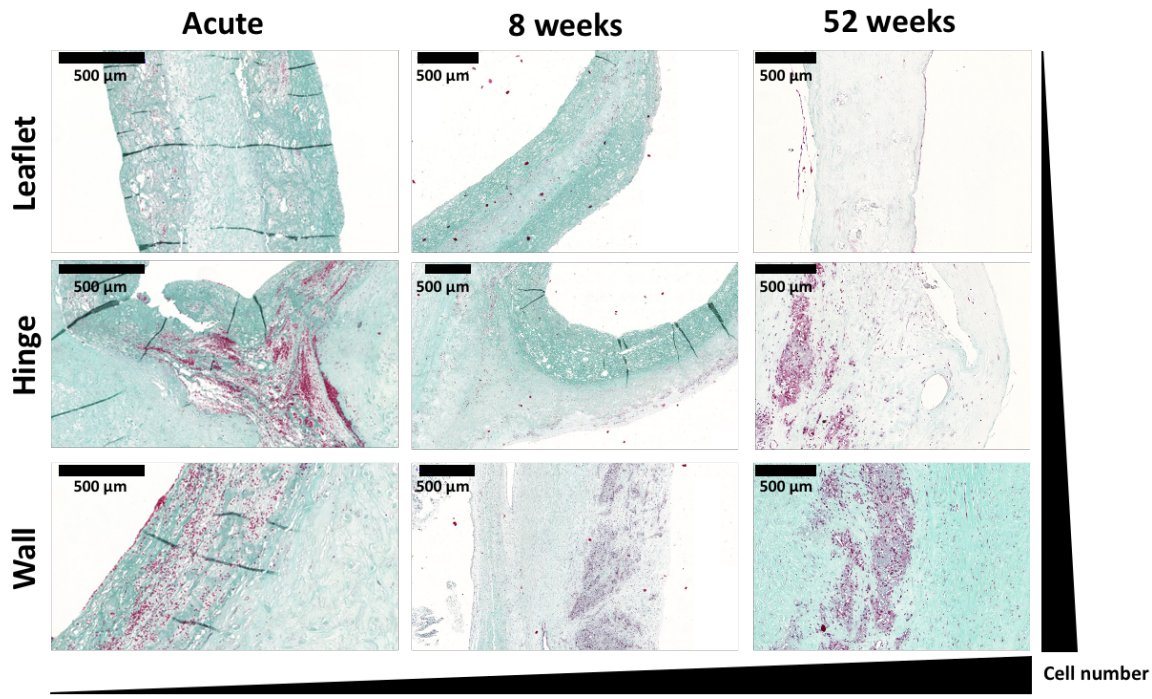
Supplementary Figure 1: Representation of endogenous cellular infiltration in *first-generation* TEHVs. Host cells gradually repopulate TEHVs over time starting from the wall and progressively migrating towards the hinge and the leaflet area (scale bars 100 µm).

Endothelialization of *first-generation* TEHVs



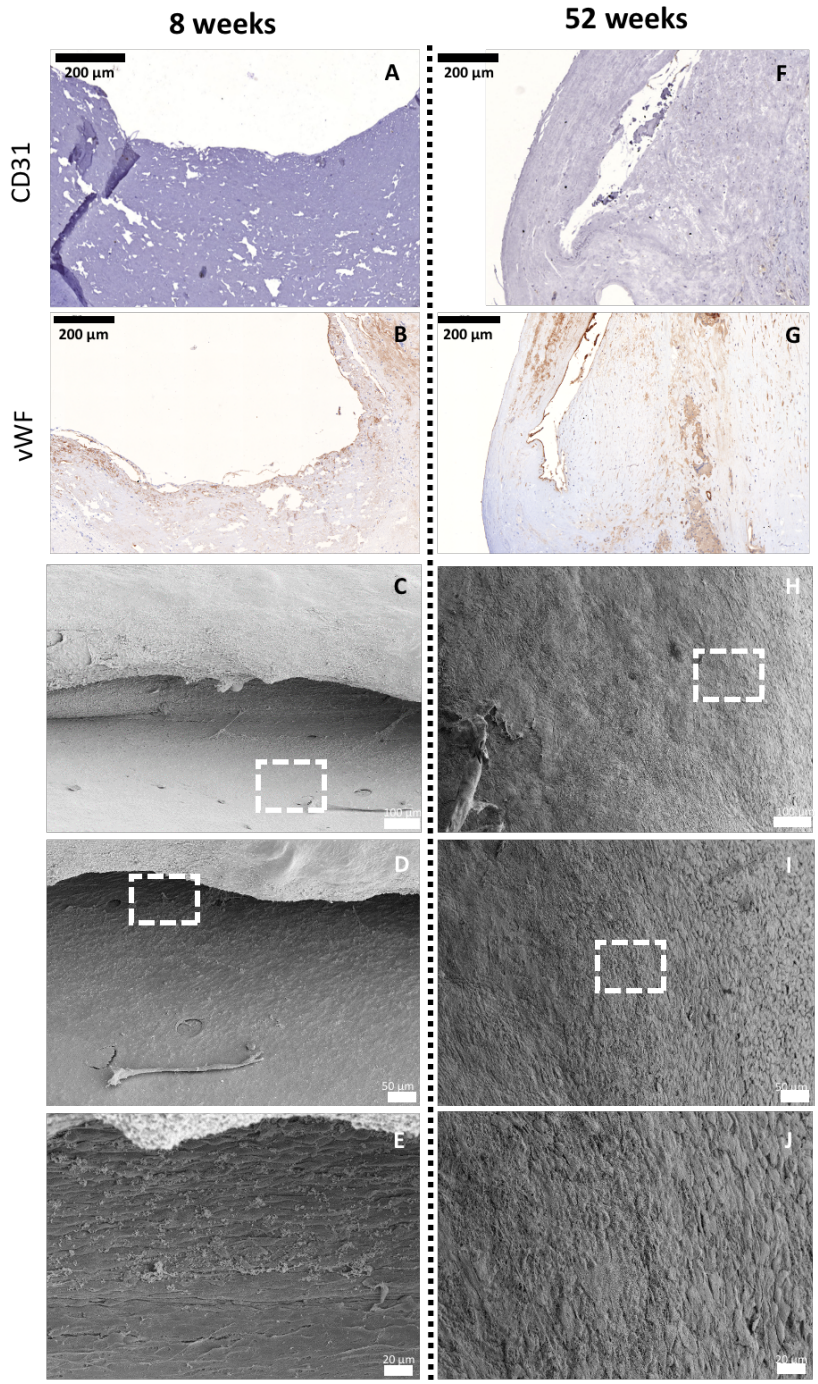
Supplementary Figure 2: Assessment of endothelialization of *first-generation* TEHVs via immunohistochemistry and SEM imaging. Images of the TEHVs hinge area stained for CD31 and vWF at different time-points (8, 16, and 24 weeks) (scale bars 100 µm). SEM images of the hinge area of at different time-points (8, 16, and 24 weeks) and at three different magnifications. Endothelial cells are visible already at 8 weeks, but they form a confluent layer only at later time-points.

Host cellular infiltration in *second-generation* TEHVs



Supplementary Figure 3: Representation of endogenous cellular infiltration in *second-generation* TEHVs. Host cells gradually repopulate TEHVs over time starting from the wall and progressively migrating towards the hinge and the leaflet area (scale bars 500 µm).

Endothelialization of *second-generation* TEHVs



Supplementary Figure 4: Characterization of endothelialization of *second-generation* TEHVs via immunohistochemistry and SEM imaging. Images of the TEHVs hinge area stained for CD31 and vWF at different time-points (8 and 52 weeks). Endothelial cells are visible and lining the surface of TEHVs. Cutting artefacts might prevent the correct representation of endothelial cells presence at 8 and 52 weeks for the CD31 staining (scale bars 200 µm). SEM images of the hinge area of *second-generation* TEHVs at different time-points (8 and 52 weeks) and at different magnifications. A confluent and oriented endothelium is already visible at 8 weeks and retained also after 52 weeks in-vivo.