Supplementary information:

Fluorescent nano-diamonds for detecting free radical generation in real time during shear stress in human umbilical vein endothelial cells

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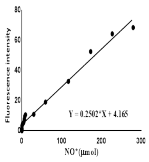
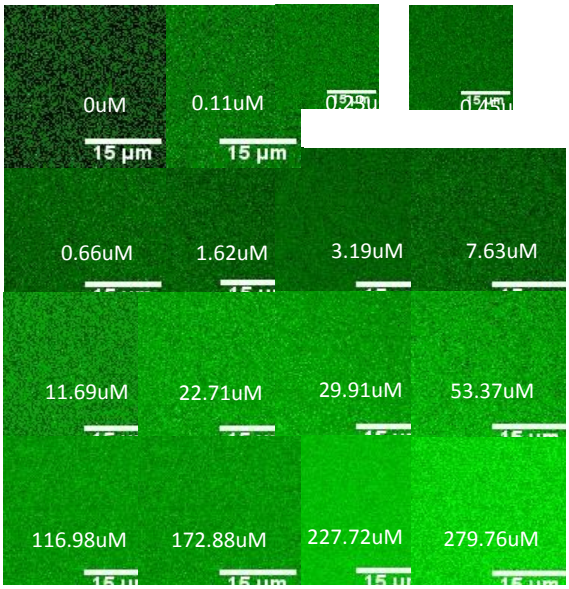
A



B

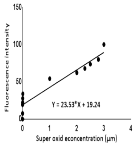
NO*(µmol)	Fluorescence intensity	NO*(µmol)	Fluorescence intensity
0.00	0.0000	7.63	9.6850
0.12	1.1250	9.02	10.6300
0.23	1.4380	29.91	10.9260
0.35	3.2933	59.37	19.0320
1.62	4.4033	116.98	32.7570
3.19	4.8790	172.88	52.4700
4.71	5.5500	227.12	64.2990
6.19	7.6580	279.76	68.4246

C



Supplementary Fig 1: Standard curve for nitric oxide radical (NO^*) quantification. Fluorescence intensity gradually increased with increasing NO^* concentration from 0.0µM to 279.76µM (A). Fluorescence intensity corresponding to NO* concentration has been shown in (B). Confocal images show fluorescence intensity of differently concentrated NO^* (produced by the decomposition of Spermine NONOate) after reacting with Non-acetylated DAF-FM (4-Amino-5-Methylamino-2',7'-Difluorofluorescein) (B). (C) shows representative images which were used to create the data in (A). The scale bar is 15 µm.

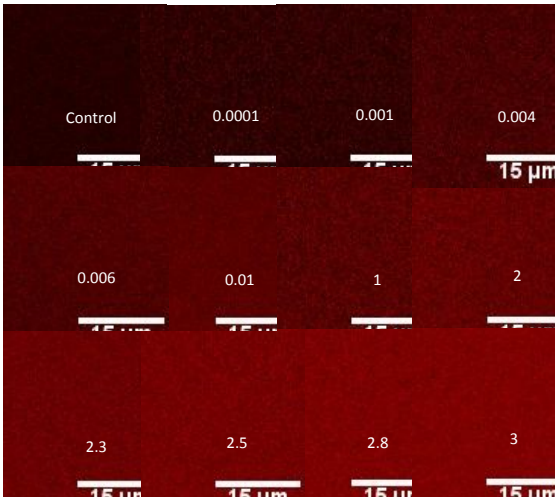
A



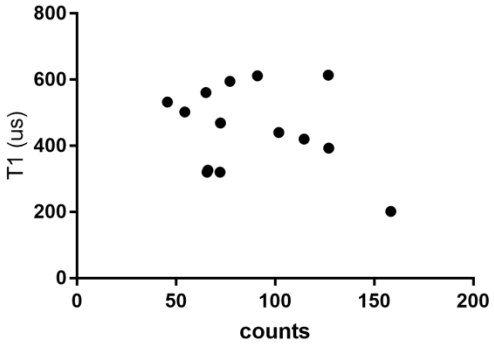
B

$O_2^{\cdot-}$ (µm)	Fluorescence intensity	$O_2^{\cdot-}$ (µm)	Fluorescence intensity
0.0000	0.00	1.0000	54.56
0.0001	7.68	2.0000	62.41
0.0010	19.54	2.3000	68.15
0.0040	22.45	2.5000	73.95
0.0060	27.99	2.8000	80.30
0.0100	34.01	3.0000	100.38

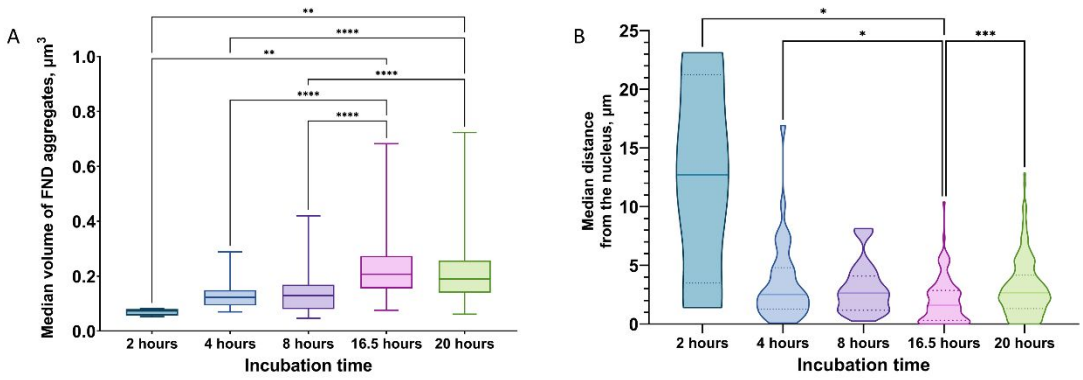
C



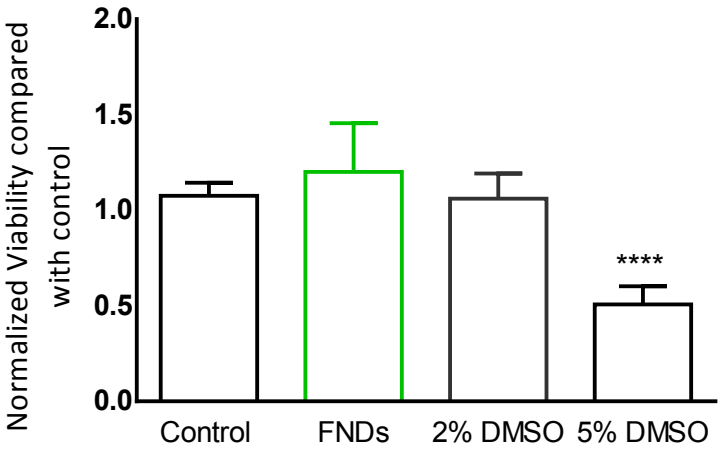
Supplementary Fig. 2: Standard curve for superoxide radicals ($\mathbf{O_2^{-\bullet}}$) quantification. Fluorescence intensity gradually increased with increasing superoxide radical concentration from 0.001µM to 3µM fig (S:2A). Fluorescence intensity corresponding to $\mathbf{O_2^{-\bullet}}$ concentration has been shown in the fig (S:1B). Confocal images show fluorescence intensity of superoxide produced by Xanthin and Xanthine oxidase's chemical reaction and the superoxide selective probe Dihydroethidium (DHE) fig: (S: 2C). The scale bar is 15 µm.



Supplementary Fig. 3. Testing correlations between size and T_1. To find any potential correlations between the size of the particles and the resulting T_1, we plotted the brightness (an approximate measure for the size) against T_1 of bare particles of different sizes. Using a pearson correlation test, we found that there is a bit of a negative coefficient. The P-value was 0.31, so the correlation is not significant.

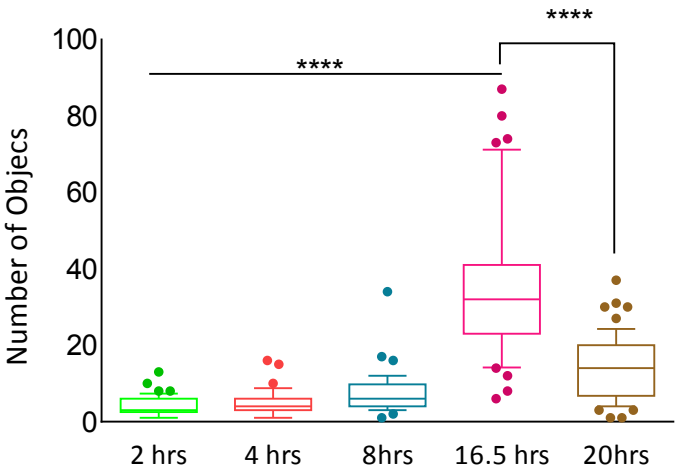


Supplementary Fig. 4 : At different time points, volume of FNDs aggregation and distance of FNDs from nucleus have been shown in fig A and B. The data are shown by box and whisker plot, where upper and lower lines represent the upper quartile and the lower quartile. The middle line represents the median with 95% confidence interval. Data were analyzed by using Kruskall-Wallis test followed by Dunn's multiple comparisons test (* P < 0. 05, ** P < 0. 01, *** P < 0.001, **** P < 0.0001). Each group represented 50 individual cells data.



Supplementary Fig.5: Viability of HUVECs cells after 12 hours of FNDs incubation. The control group was incubated only in EGM2 medium where FNDs suspension 2µg/ml with EGM2 medium was subjected to the FNDs group. In case of positive control groups, 2 and 5 % DMSO solutions were mixed with EGM2 medium. Error bars show the standard error of the mean. Significance was tested against the control group. Statistical analysis was done by a one-way ANOVA test followed by Tukey post hoc test (****p < 0.0001). This experiment was repeated 3 independent times.

A



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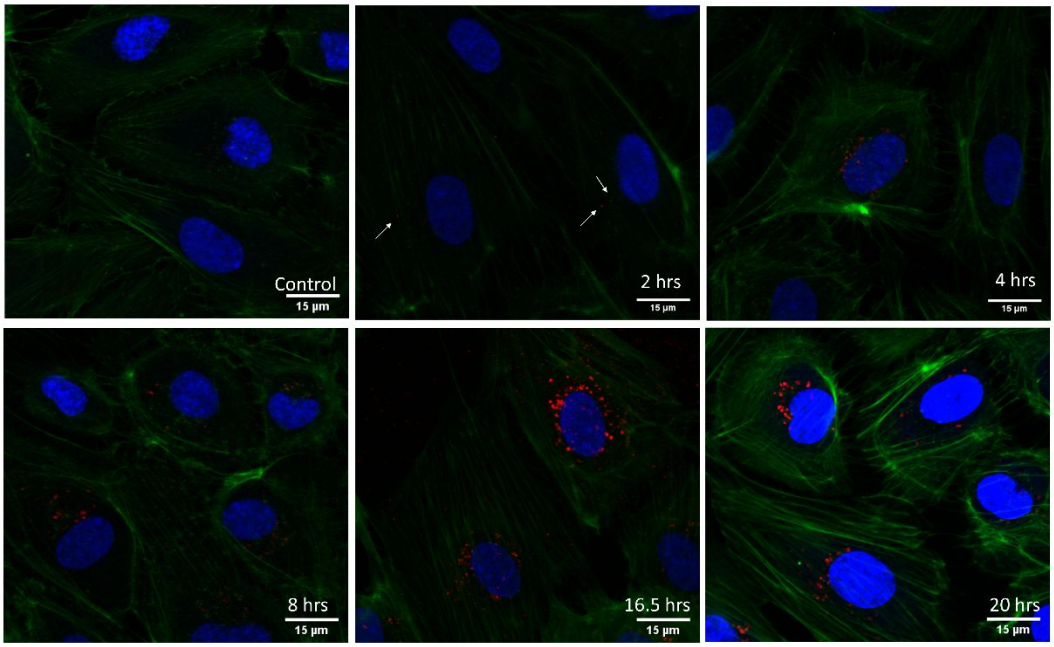
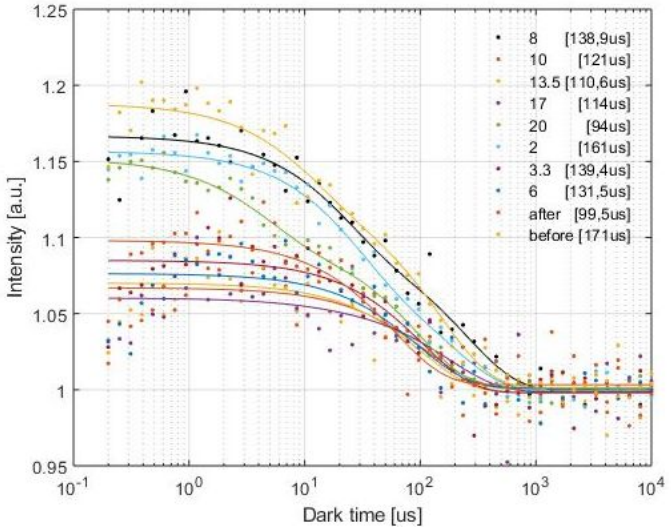


Supplementary Fig.6 Fluorescence nanodiamond particles (FNDs) internalization in HUVECs at different time points. 2µg/ml FNDs suspension was prepared with EGM2 (Endothelial cell growth) medium. Then cells were incubated for 2, 4, 8, 16.5 and 20 hours. (A) shows the number of objects we counted at each timepoint. We observed the maximum number of FNDs were internalized at 16.5 hours where, 20 hours incubation group showed significantly lower FNDs than 16.5 hours. From the data, it is clearly shown that HUVECs retained maximum number of FNDs inside the cell until 16.5 hours before excretion. (B) Confocal images show the FNDs uptake at different time points. Actin fibers stained by phalloidin are shown in green and nuclei stained by DAPI in blue. Bright red spots are Fluorescence nanodiamond particles inside the cells. White arrow in the 2 hours experiment indicate the FNDs. The data is shown as a box and whisker plot, where upper and lower dots represent upper values from upper quartile and lower values from lower quartile. The middle line represents the median of the data. Whiskers show 10–90 percentile. Data were analyzed by using one-way ANOVA and followed by Tukey post hoc test. The significance level was **** $P < 0.0001$ and each group represents 50 individual cells data.



Supplementary Fig. S7 Representative raw data for T1 curves. The decay curves are from independent measurements of particles within HUVECs before flow. The points show the measured points while the solid lines represent the fit. From these curves, T1s are calculated. The values in the legend represent the respective shear stress values and the T1 in μs.