# Superoxide Dismutase Detection on Silver Nanostructured Substrates through Surface-Enhanced Spectroscopic Techniques

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Figure S1. Image of the 24-well plate with the substrates on which the SOD assay was performed.
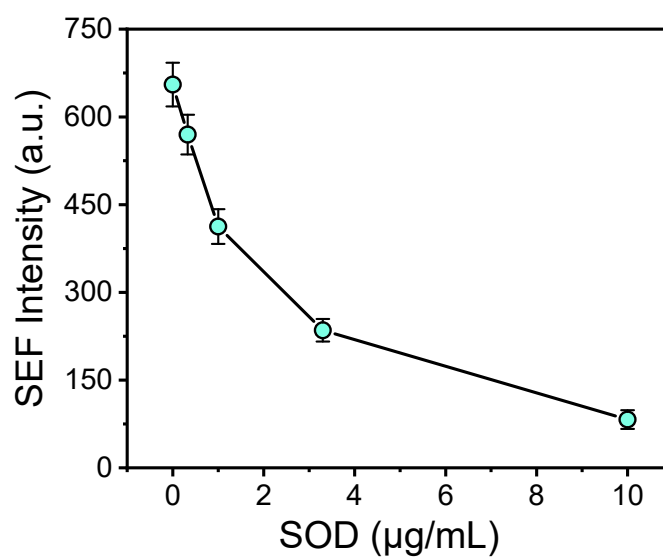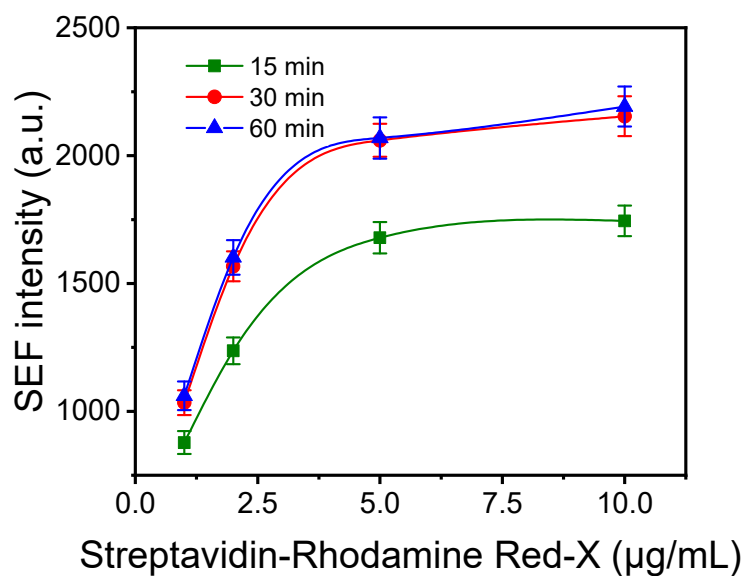
Figure S2. SOD calibration curve obtained from SEF substrates employing the 2-step configuration. Each point corresponds to the mean value of 5 measurements from 3 different samples ± SD
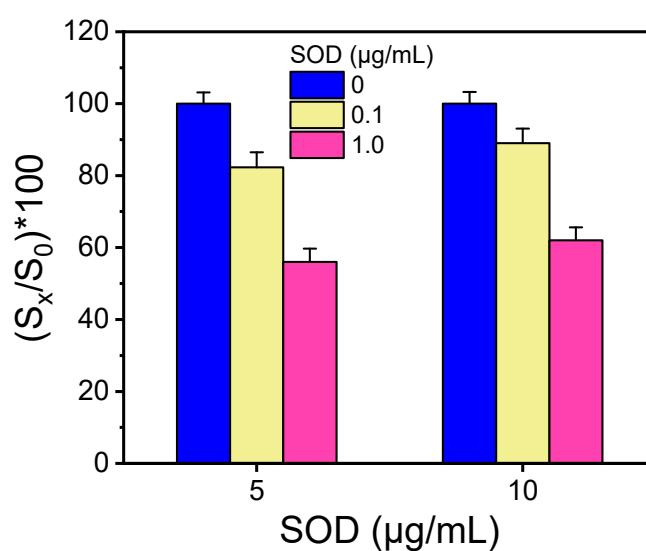

Figure S3. Percent signal values obtained for SOD calibrators with concentration 0.1 (yellow columns) and 1.0 µg/mL (pink columns) with respect to the zero calibrator value (blue columns). Each column represents the mean value of 5 measurements from 3 different samples ± SD.
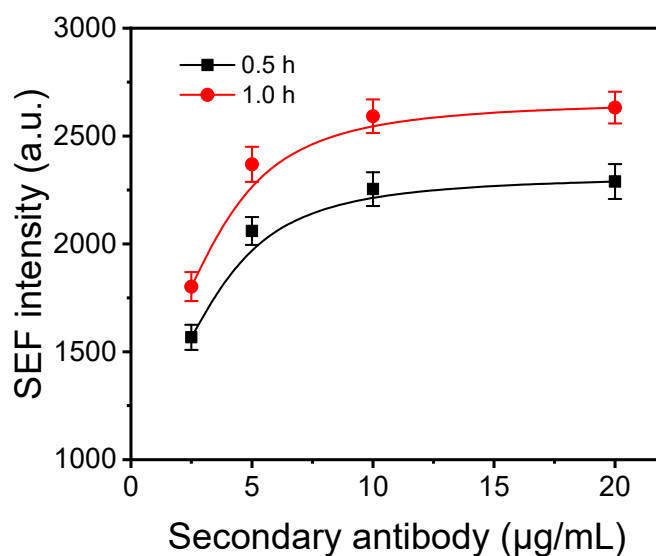
Figure S4. Zero calibrator SEF intensity values obtained from substrates coated with 5.0 µg/mL of SOD and assayed using a 0.5 µg/mL anti-SOD antibody solution with respect to the concentration of biotinylated secondary antibody for reaction duration of 0.5 (red circles) or 1.0 h (black squares). Each column represents the mean value of 5 measurements from 3 different samples ± SD.


Figure S5. Zero calibrator SEF intensity values obtained from substrates coated with 5.0 µg/mL of SOD and assayed using a 0.5 µg/mL anti-SOD antibody solution and a 5.0 µg/mL secondary antibody solution with respect to the concentration of streptavidin Rhodamine Red-X for reaction duration of 15 (green squares), 30 (red circles), and 60 min (blue triangles). Each column represents the mean value of 5 measurements from 3 different samples ± SD.
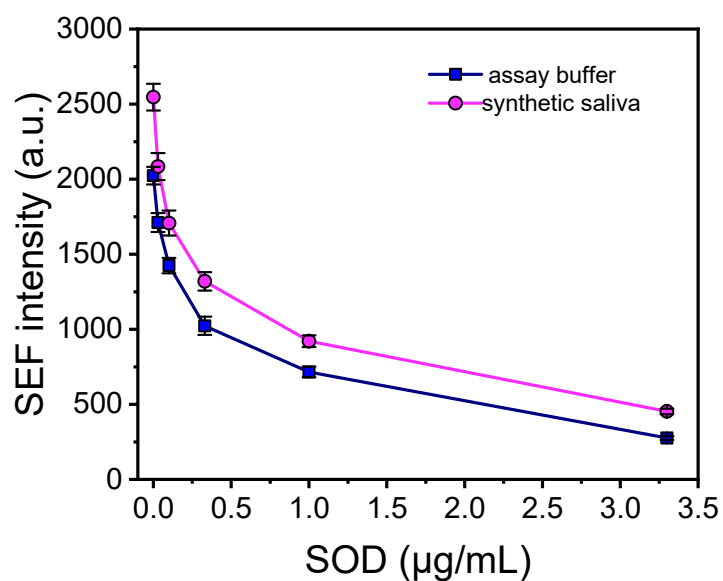
Figure S6. SOD calibration curves obtained from SEF substrates with calibrators prepared either in assay buffer (blue squares) or synthetic saliva (pink circles). Each point corresponds to the mean value of 5 measurements from 3 different samples ± SD.
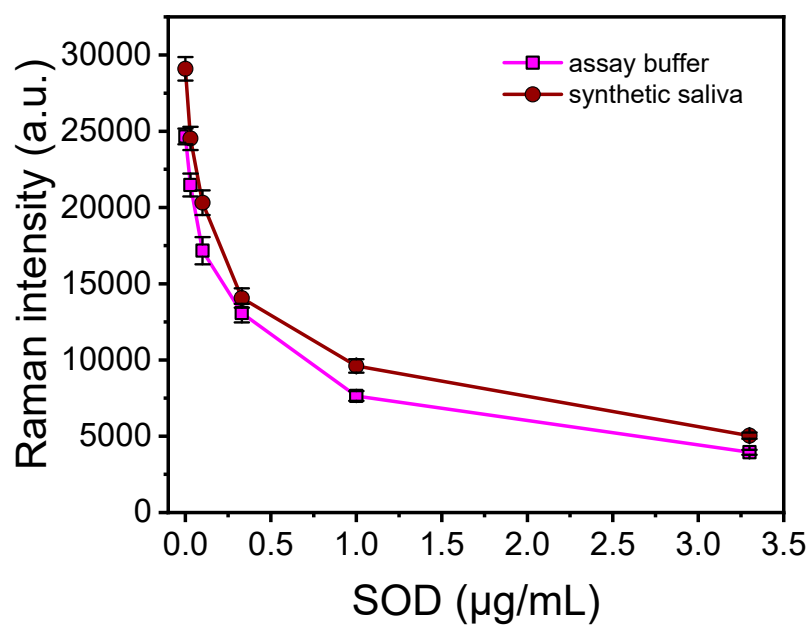
Figure S7. SOD calibration curves obtained from SERS substrates with calibrators prepared either in assay buffer (pink squares) or synthetic saliva (wine circles). Each point corresponds to the mean value of 5 measurements from 3 different samples ± SD.
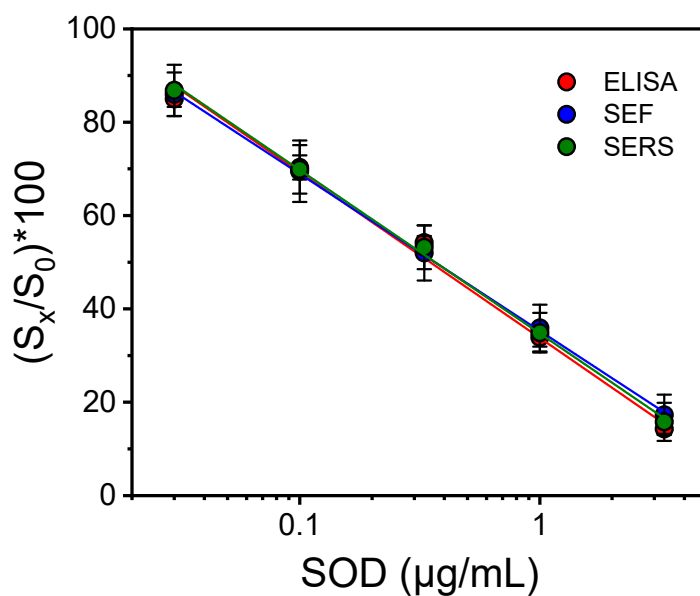
Figure S8. SOD calibration curves obtained with ELISA (red circles), SEF (blue circles) or SERS measurements (green circles). Each point corresponds to the mean value of 5 measurements from 3 different samples ± SD. The linear regression equations of the calibration curves and the respective coefficients of variation are as follows: i) ELISA curve: $\log Y = 34.2(\pm1.4) - 34.9(\pm1.6)\log X$; $R^2 = 0.994$, ii) SEF: $\log Y = 35.4(\pm0.4) - 33.6(\pm0.4)\log X$; $R^2 = 0.9997$, and iii) SERS: $\log Y = 34.8(\pm0.7) - 34.8(\pm0.8)\log X$; $R^2 = 0.998$.

Table 1. Percent recovery of SOD amounts added to saliva samples as determined by the SEF and SERS immunoassay methods developed.

Initial concentration (µg/mL)	Amount added (µg/mL)	Amount determined (µg/mL)		% Recovery	
		SEF	SERS	SEF	SERS
	0.07	0.21	0.22	86	100
0.15	0.1	0.26	0.24	110	90
	0.15	0.29	0.30	93	100
	0.20	0.36	0.34	105	95