Electronic supplementary information

Targeted removal of blood cancer cells from mixed cell populations by cell recognition with matching particle imprints

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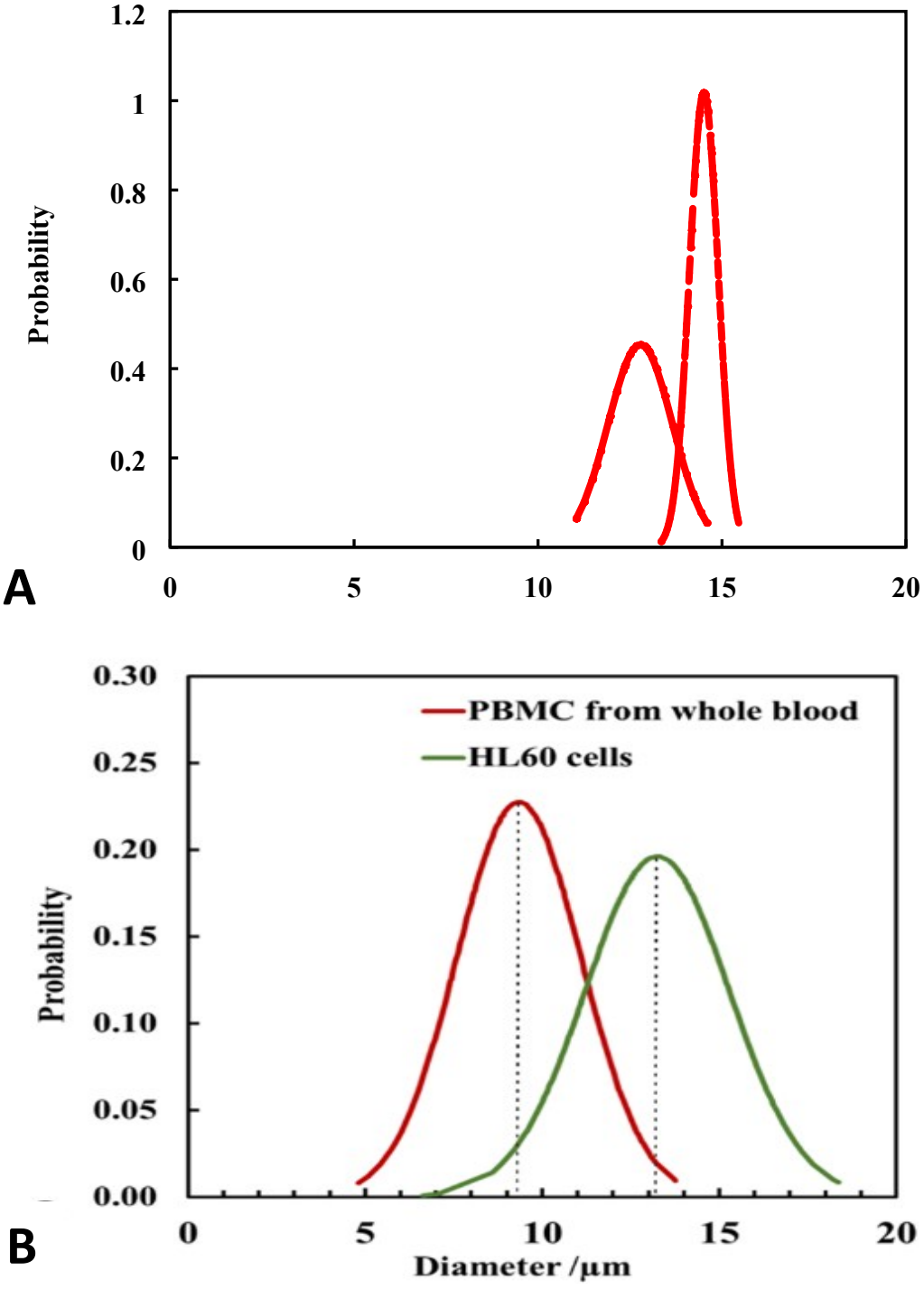

Figure S1. (A) Size distribution of CA15 Spheromer® (PMMA 15 µm particles) (dashed line) and the imprint cavities (solid line). (B) Size distribution of human PBMC and HL60 leukemic cells.

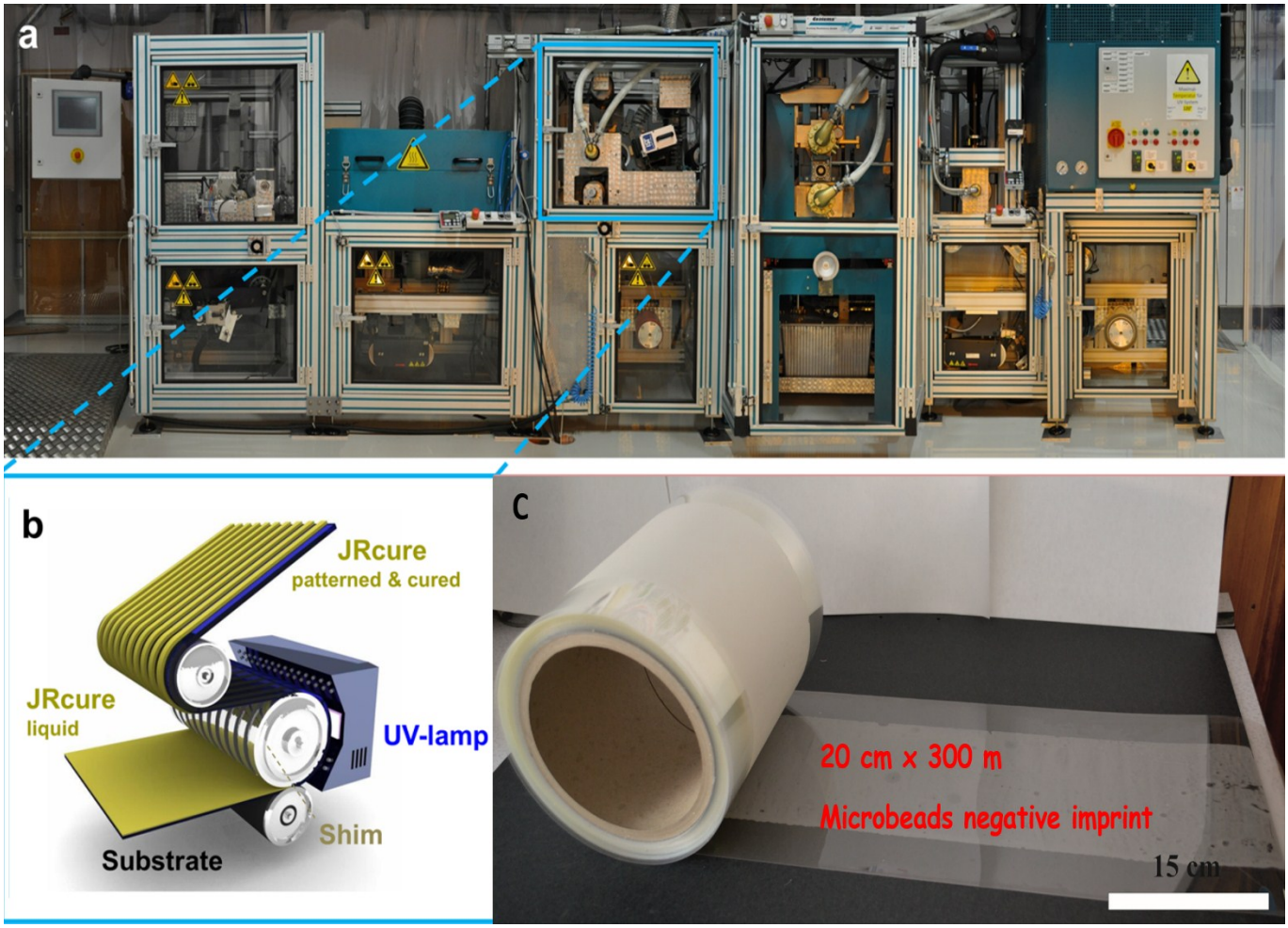

Figure S2. (a) The Roll-to-Roll-UV Nano-Imprinting Lithography (R2R NIL) machine at our collaborator Joanneum Research FmbH (Weiz, Austria) printing facility with a speed up to 30 m min^{-1}. For our application, a speed of 1 m min^{-1} was used. (b) Scheme of the R2R-UV-NIL unit. (c) Photographs showing the production of a roll of negative acrylate-based bioimprint on PET foil fabricated from positive PU imprint shims on PET foil. Figures S2a and S2b reprinted with permission from ref. 1. Copyright 2016 American Chemical Society.

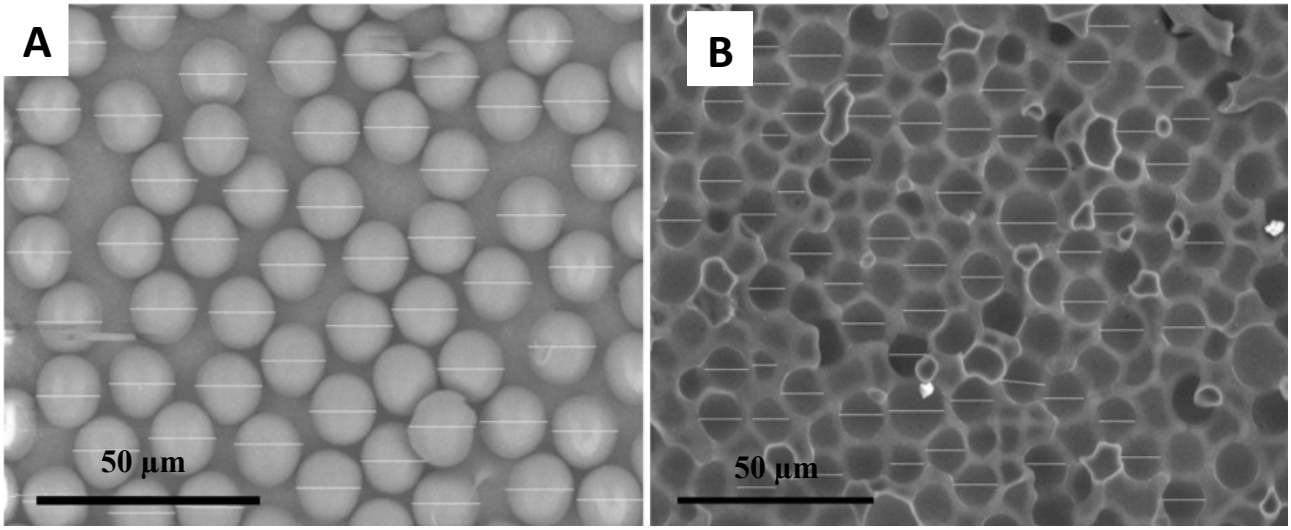

Figure S3 SEM images showing the length mean diameter measurements of (A) Spheromers® CA15 PMMA microbeads and (B) PDMS imprints of a layer of microbeads.

Table S1. Measurements of length mean diameter of Spheromer® CA15 PMMA microbeads and the imprint cavities produced by imprinting a layer of CA15 microbead suspension spread on a glass slide.

	Diameter (µm)			
	Minimum	Maximum	Length mean	Standard Deviation
CA15 microbeads	13.8	15.3	14.5	0.4
CA15 imprints	11.0	14.6	12.8	0.9

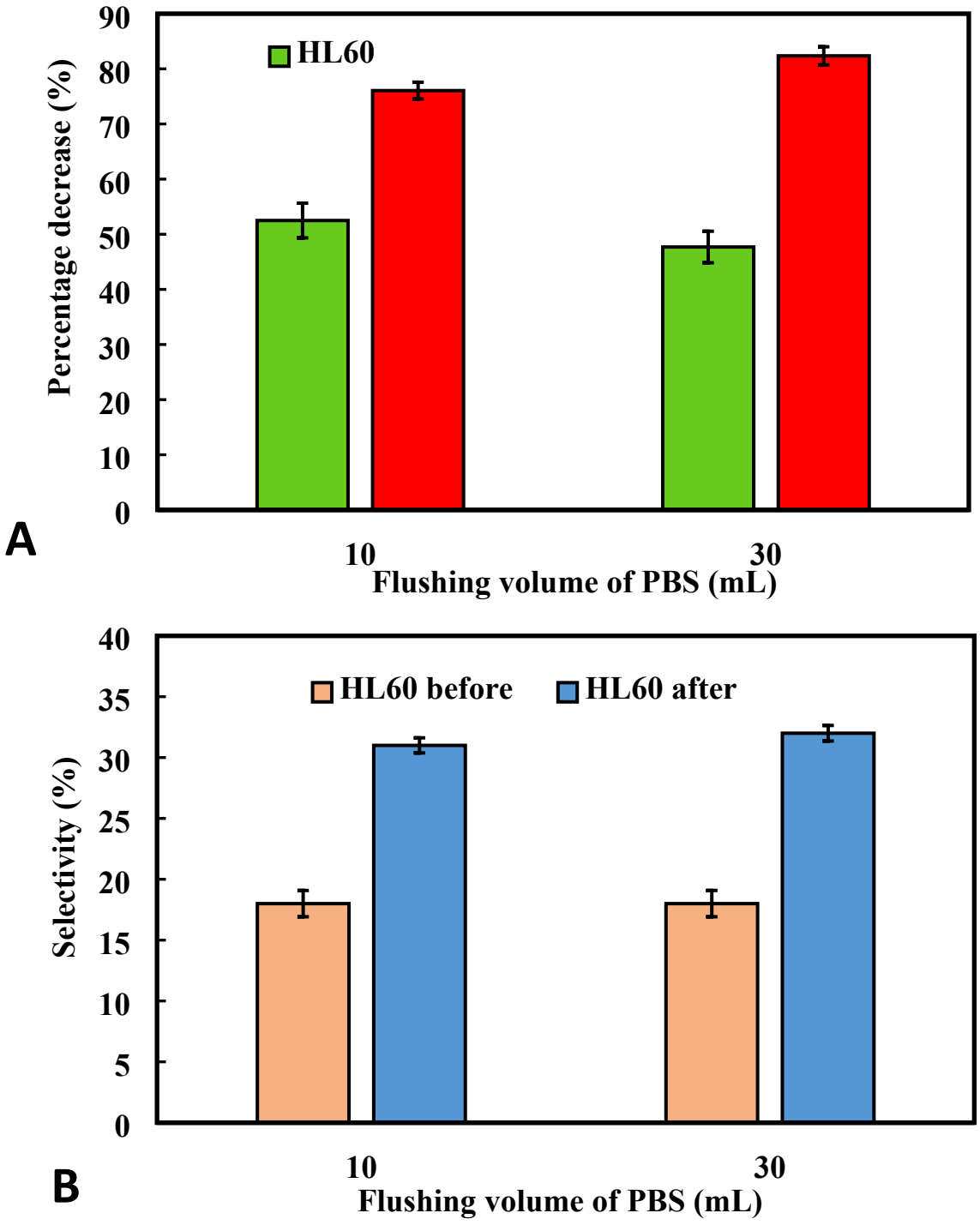

Figure S4 (A) The percentage decrease of cells and the HL60 selectivity before and after flushing the chip containing imprint of 12 cm coated with 3 wt% Poloxamer 407 functionalised, oxygen plasma treated and 0.015 wt% bPEI at a flowrate of 220 ml/hr, washed with 10 mL or 30 mL of PBS.

One sees that there is no effect of the flushing volume of PBS on the percentage decrease of both HL60 and PBMCs and the selectivity with respect to HL60.

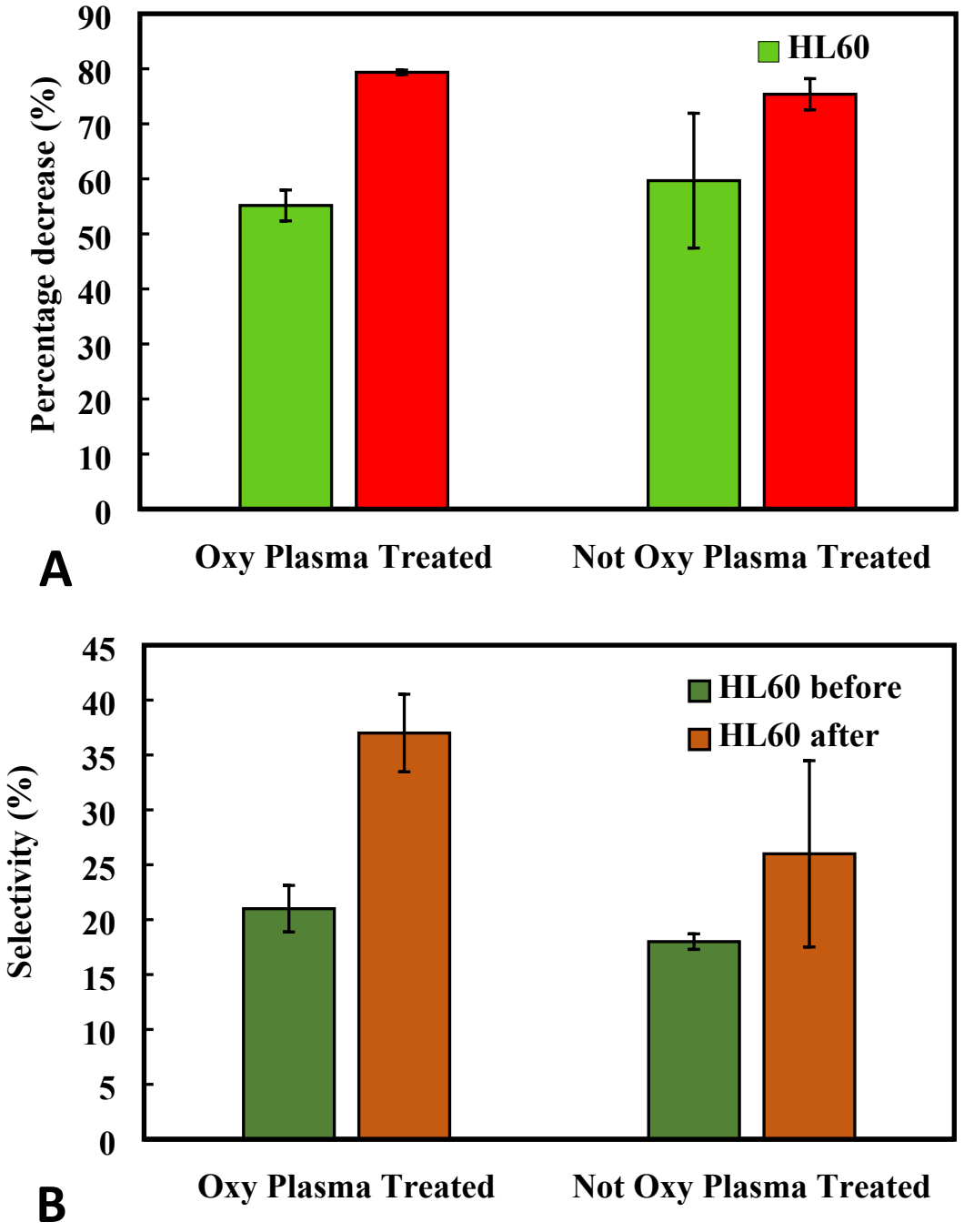

Figure S5: (A) The percentage decrease of cells and (B) the HL60 selectivity after flushing the chip containing 4 cm imprint coated with 3 wt% Poloxamer 407 functionalised, with and without oxygen plasma treated and 0.015 wt% bPEI at a flowrate of 220 mL/hr.

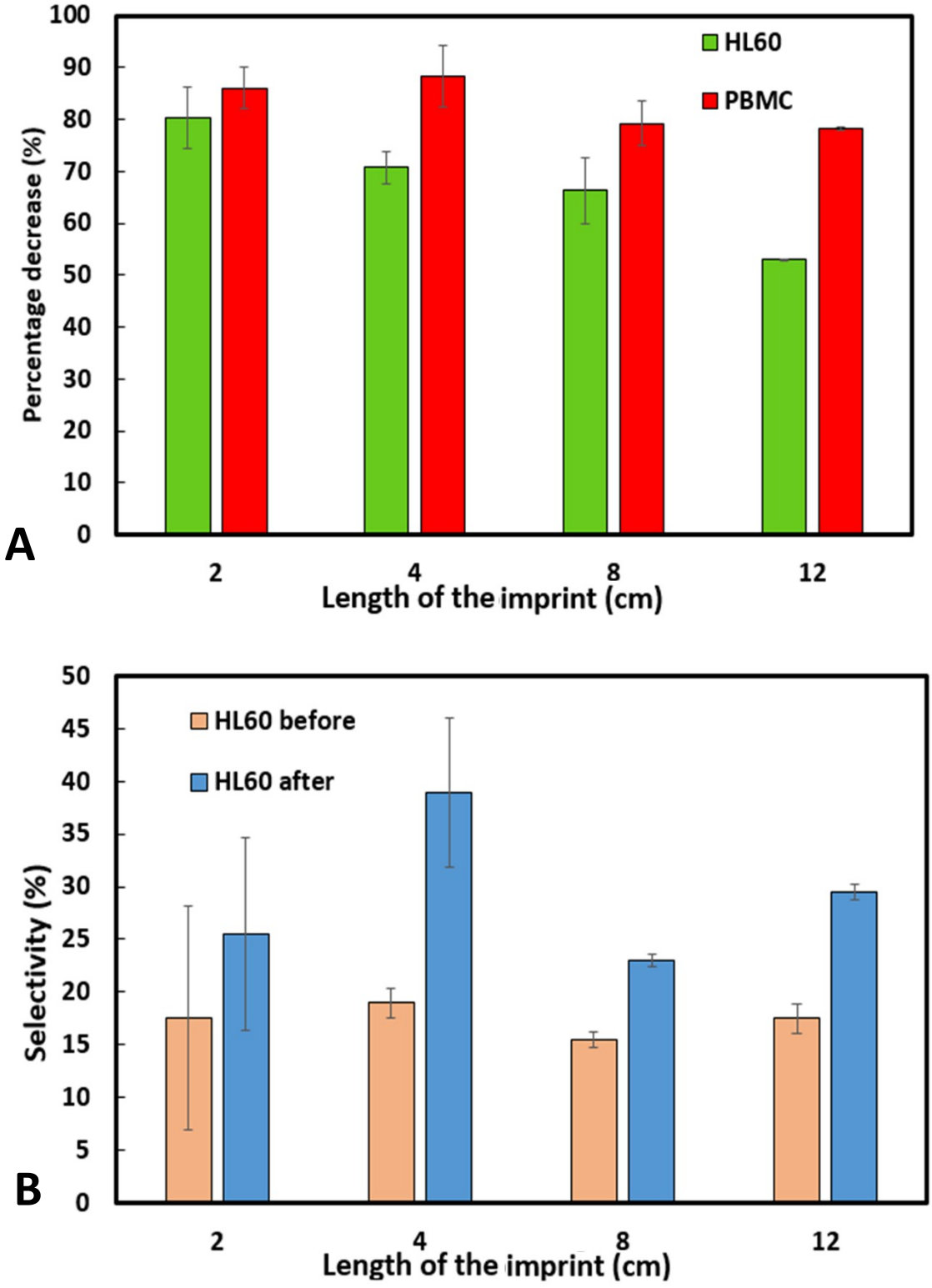

Figure S6: The percentage decrease of cells after washing (A) and the selectivity before and after flushing the chip (B) containing imprint of 2, 4, 8 and 12 cm length coated with 3 wt% POL 407, HL60/PBMC: 25%/75%, oxygen plasma treated and coated with 0.015 wt% bPEI at a flowrate of 218.9 mL/hr. Flushing volume was 10 mL PBS.

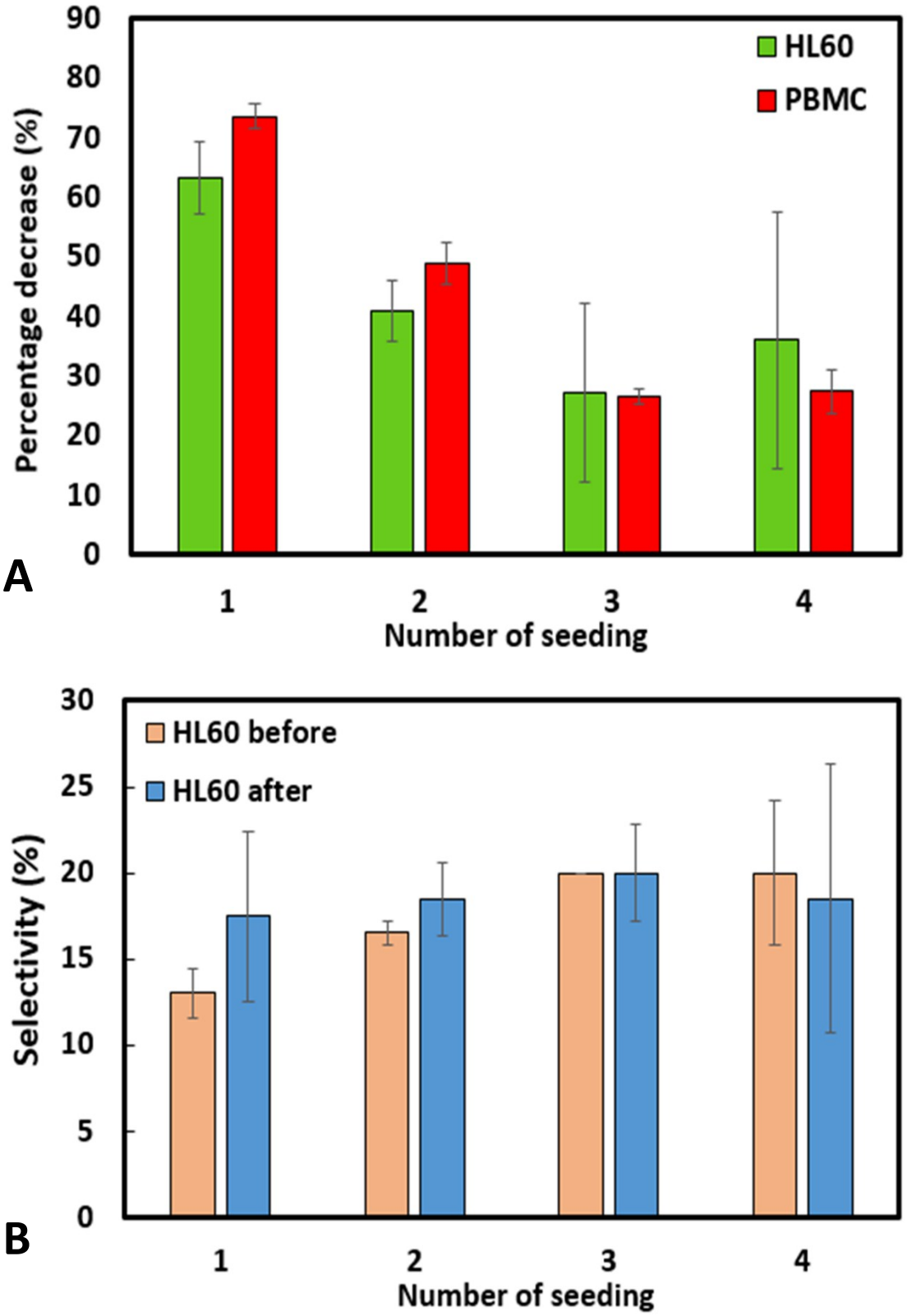

Figure S7. The percentage decrease of cells after washing (A) and the selectivity before and after flushing the chip (B) for multiple seedings on imprint of 4 cm coated with 3 wt% POL 407 functionalised, HL60/PBMC: 10/90, oxygen plasma treated and coated with 0.015 wt% bPEI at a flowrate of 218.9 mL/hr, number of cells per seeding: 6.54×10^5 cells. Flushing volume was 10 mL PBS after each seeding.

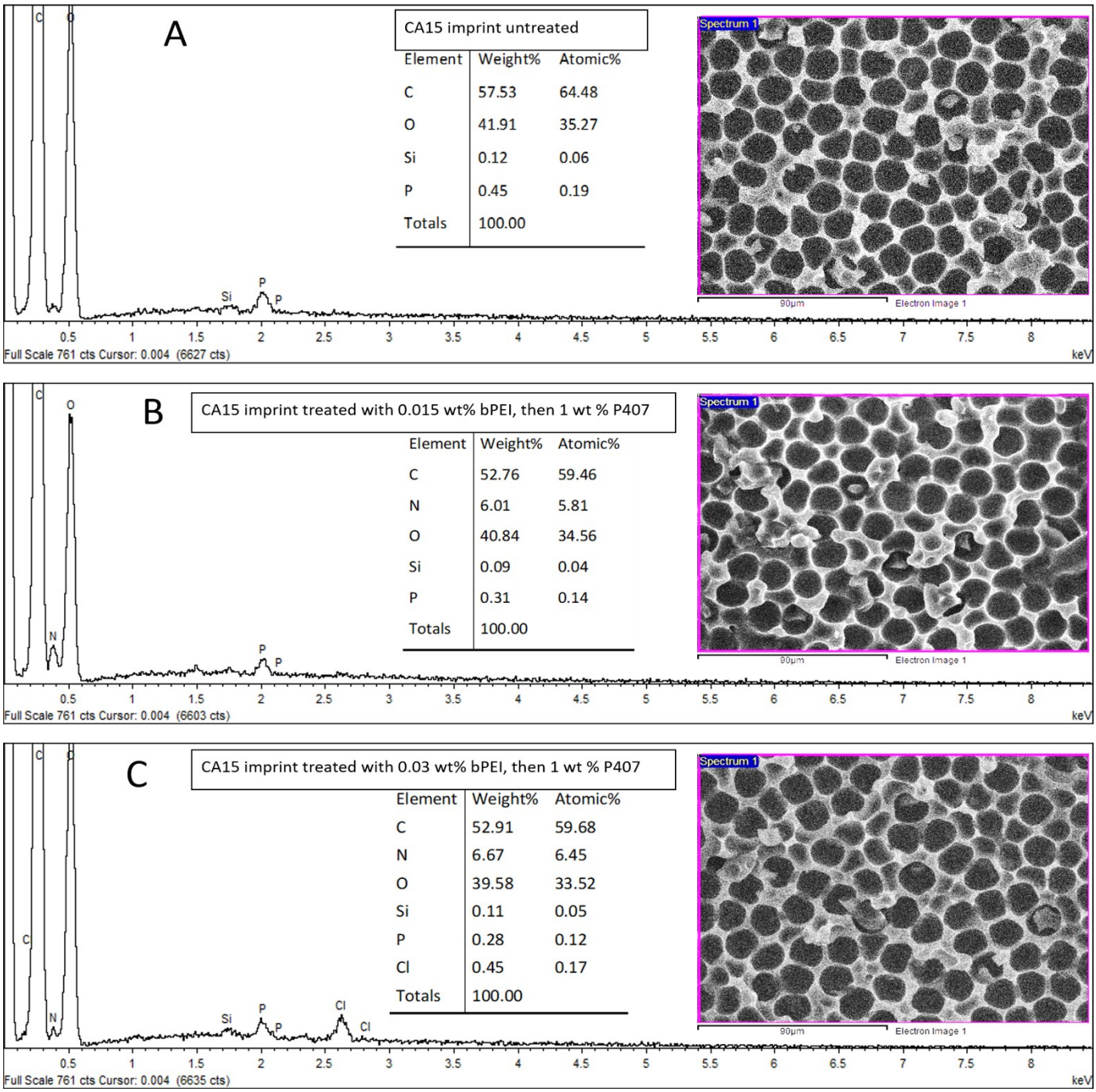

Figure S8. SEM images and EDX spectra of (A) non-treated and (B-C) treated CA15 microbeads imprint: (A) shows EDX spectra of the non-treated imprint; (B) shows the EDX spectra of CA15 imprint treated with 0.015 wt% bPEI followed by 1 wt% Poloxamer 407; (C) shows the EDX spectra of CA15 imprint treated with 0.030 wt% bPEI followed by 1 wt% Poloxamer 407. The measurement was taken at the bottom of the imprint cavities. Oxford Laboratories micF+ X-stream-2 EHX was used to take measurements, the results were analysed in Aztec One v.3.3. Images were acquired using a TM3030 Plus SEM. Scale bars represent 90 µm.

Untreated control sample contained no nitrogen, 0.015 wt% bPEI sample contained 6.67% nitrogen, and 0.03 wt% bPEI treated sample contained 6.01% nitrogen, which indicates deposition and stable attachment of bPEI. Each sample' surface layer, including the uncoated control, was found to contain traces of phosphorus and silica.

Statistical analysis for the effect of flowrate, bPEI and Poloxamer 407 treatment (Figure 5).

Table S2. Percentage decrease at different flowrates statistical analysis of PBMC cell-line. Data were expressed as average values ± standard deviations of the mean. *P*-values of less than 0.05 were considered significant. All Unpaired two-tailed T-tests were performed in GraphPad v7.0.4.

Figure 5A	Cell line	Multiple comparison	P-value	Significance
	PBMC	Flowrate – 50 ml/hr vs 125.7 ml/hr	0.4434	NS
	PBMC	Flowrate – 125.7 ml/hr vs 218.9 ml/hr	0.0100	*

< 0.05 is considered significant. *P <0.05, **P <0.01, ***P <0.001

Table S3. Selectivity change at different flowrates statistical analysis of HL60 cell-line. Data were expressed as average values ± standard deviations of the mean. *P*-values of less than 0.05 were considered significant. All Unpaired two-tailed T-tests were performed in GraphPad v7.0.4.

Figure 5B	Cell line	Multiple comparison	P-value	Significance
	HL60 after	Flowrate – 50 ml/hr vs 125.7 ml/hr	0.3427	NS
	HL60 after	Flowrate – 125.7 ml/hr vs 218.9 ml/hr	0.1603	NS

< 0.05 is considered significant. *P <0.05, **P <0.01, ***P <0.001

Table S4. Percentage decrease at different bPEI concentrations statistical analysis of HL60 and PBMC cell-lines. Data were expressed as average values ± standard deviations of the mean. *P*-values of less than 0.05 were considered significant. All Unpaired two-tailed T-tests were performed in GraphPad v7.0.4.

Figure 5C	Cell line	Multiple comparison	P-value	Significance
	HL60	0.01 w/w% vs 0.015 w/w%	0.6788	NS
	HL60	0.015 w/w% vs 0.02 w/w%	0.1588	NS
	HL60	0.02 w/w% vs 0.025 w/w%	0.0018	**
	HL60	0.025 w/w% vs 0.03 w/w%	0.0005	***
	PBMC	0.01 w/w% vs 0.015 w/w%	0.0023	**
	PBMC	0.015 w/w% vs 0.02 w/w%	0.0003	***
	PBMC	0.02 w/w% vs 0.025 w/w%	0.0259	*

	PBMC	0.025 w/w% vs 0.03 w/w%	0.1086	NS

< 0.05 is considered significant. *P <0.05, **P <0.01, ***P <0.001

Table S5. Selectivity change at different PEI concentrations statistical analysis of HL60 cell-line. Data were expressed as average values ± standard deviations of the mean. *P*-values of less than 0.05 were considered significant. All Unpaired two-tailed T-tests were performed in GraphPad v7.0.4.

Figure 5D	**Cell line**	**Multiple comparison**	**P-value**	**Significance**
	HL60 after	0.01 w/w% vs 0.015 w/w%	0.0012	**
	HL60 after	0.015 w/w% vs 0.02 w/w%	0.0004	***
	HL60 after	0.02 w/w% vs 0.025 w/w%	0.0356	*
	HL60 after	0.025 w/w% vs 0.03 w/w%	0.0023	**

< 0.05 is considered significant. *P <0.05, **P <0.01, ***P <0.001

Table S6. Percentage decrease at different Poloxamer 407 concentrations statistical analysis of HL60 and PBMC cell-lines. Data were expressed as average values ± standard deviations of the mean. *P*-values of less than 0.05 were considered significant. All Unpaired two-tailed T-tests were performed in GraphPad v7.0.4.

Figure 5E	**Cell line**	**Multiple comparison**	**P-value**	**Significance**
	HL60	0.0 w/w% vs 0.25 w/w%	0.0159	*
	HL60	0.25 w/w% vs 0.5 w/w%	0.0163	*
	HL60	0.5 w/w% vs 1 w/w%	0.0417	*
	HL60	1 w/w% vs 2 w/w%	0.2269	NS
	HL60	2 w/w% vs 3 w/w%	0.9099	NS
	PBMC	0 w/w% vs 0.25 w/w%	0.0001	***
	PBMC	0.25 w/w% vs 0.5 w/w%	0.0048	**
	PBMC	0.5 w/w% vs 1 w/w%	0.0172	*
	PBMC	1 w/w% vs 2 w/w%	0.0249	*
	PBMC	2 w/w% vs 3 w/w%	0.7828	NS

< 0.05 is considered significant. *P <0.05, **P <0.01, ***P <0.001

Table S7 Selectivity change at different Poloxamer 407 concentrations statistical analysis of HL60 cell-line. Data were expressed as average values ± standard deviations of the mean. *P*-values of less than 0.05 were considered significant. All Unpaired two-tailed T-tests were performed in GraphPad v7.0.4.

Figure 5F	**Cell line**	**Multiple comparison**	**P-value**	**Significance**
	HL60 after	0.0 w/w% vs 0.25 w/w%	0.6158	NS
	HL60 after	0.25 w/w% vs 0.5 w/w%	0.0488	*
	HL60 after	0.5 w/w% vs 1 w/w%	0.58719	NS
	HL60 after	1 w/w% vs 2 w/w%	0.7855	NS
	HL60 after	2 w/w% vs 3 w/w%	0.6912	NS

< 0.05 is considered significant. *P <0.05, **P <0.01, ***P <0.001

Image J macro used to automatically count the stained cells from the fluorescence microscope images of the imprint.

```
//run("Threshold...");
run("8-bit");
setOption("BlackBackground", false);
run("Make Binary");
run("Watershed");
run("Analyze Particles...");
run("Set Scale...", "distance=0 known=0 pixel=1 unit=pixel global");
run("Analyze Particles...", "size=180-Infinity show=Masks display clear summarize");
run("Close");
run("Close");
```

The equation for calculation of percentage decrease is shown below. The Image J macro was used to calculate the area of fluorescence for each stained cell type.

Plate reader $(\text{Fluorescence Intensity}_{after}/\text{Fluorescence Intensity}_{before}) \times 100$

or

$\text{Microscopy} = 100\times[(\text{Number of cells/m}^2)_{before} - \text{Number of cells/m}^2)_{after}] / (\text{number of cells/m}^2)_{before}$

References

1. M. Leitgeb, D. Nees, S. Ruttloff, U. Palfinger, J. Götz, R. Liska, M.R. Belegratis and B. Stadlober, *ACS Nano*, 2016, **10**, 4926-4941.