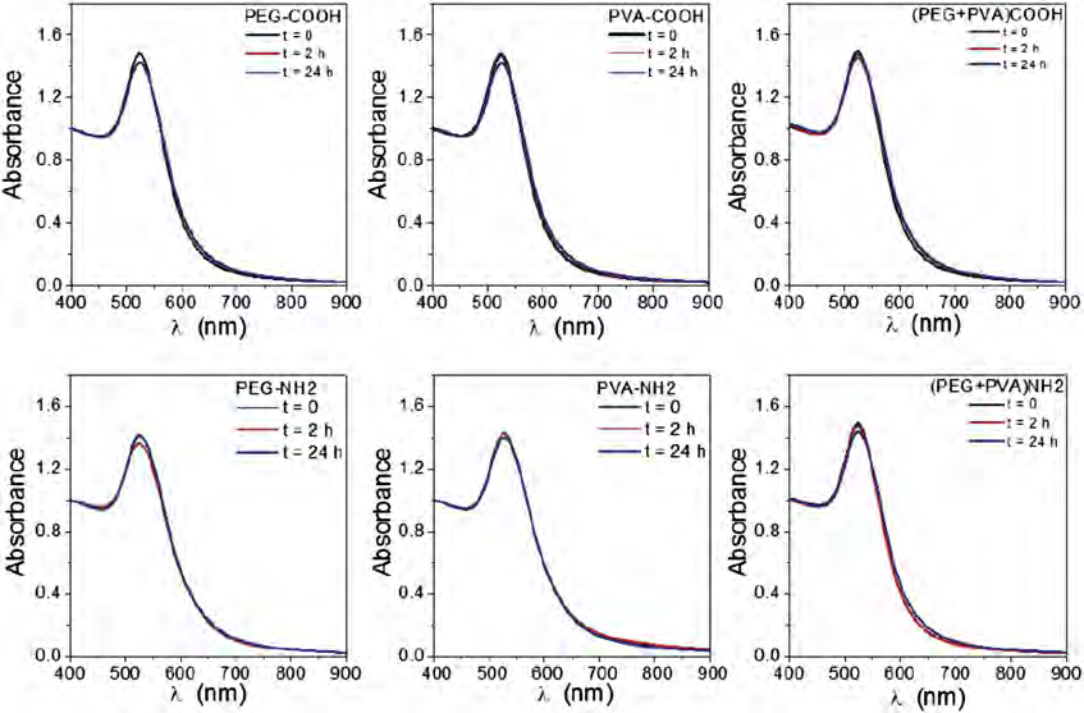
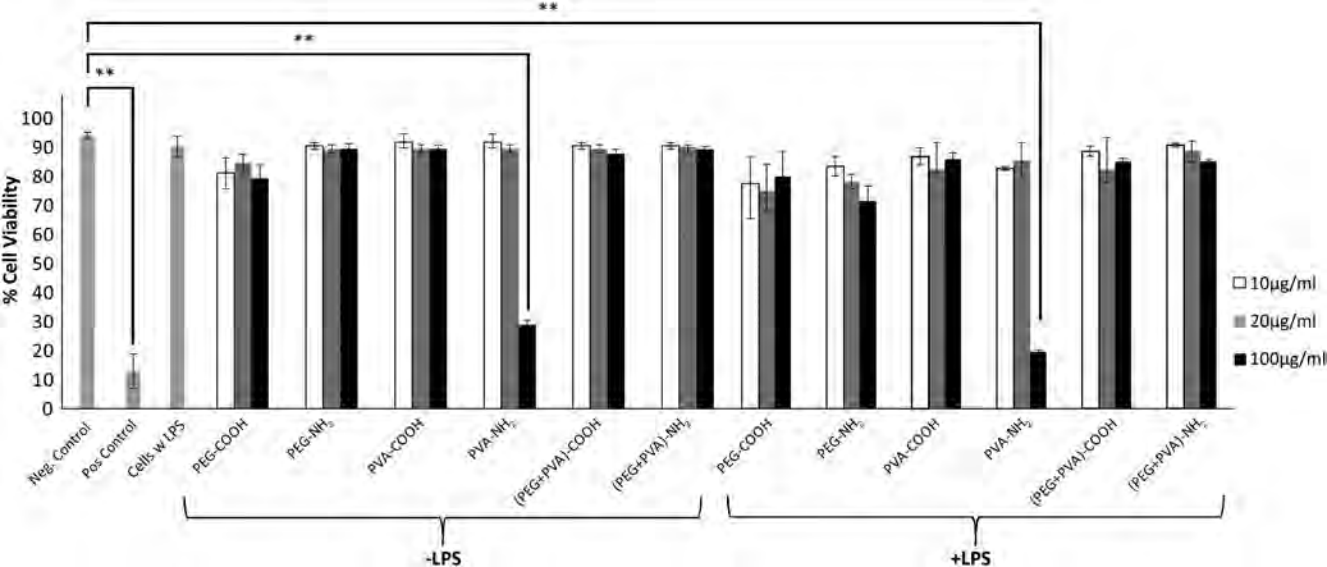
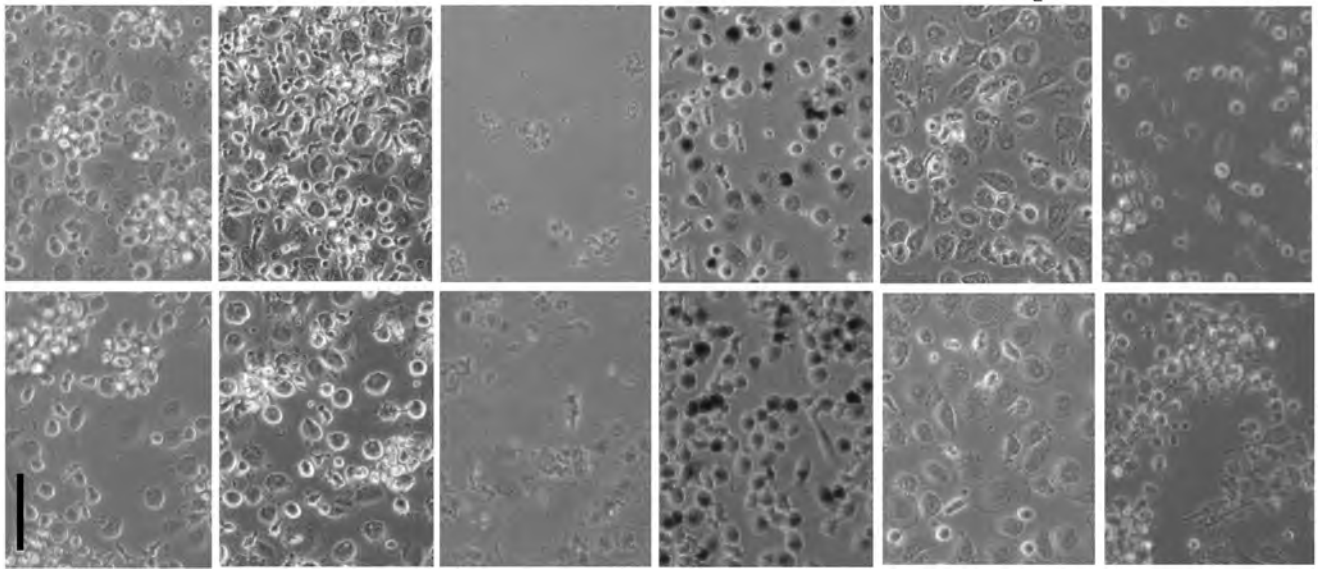
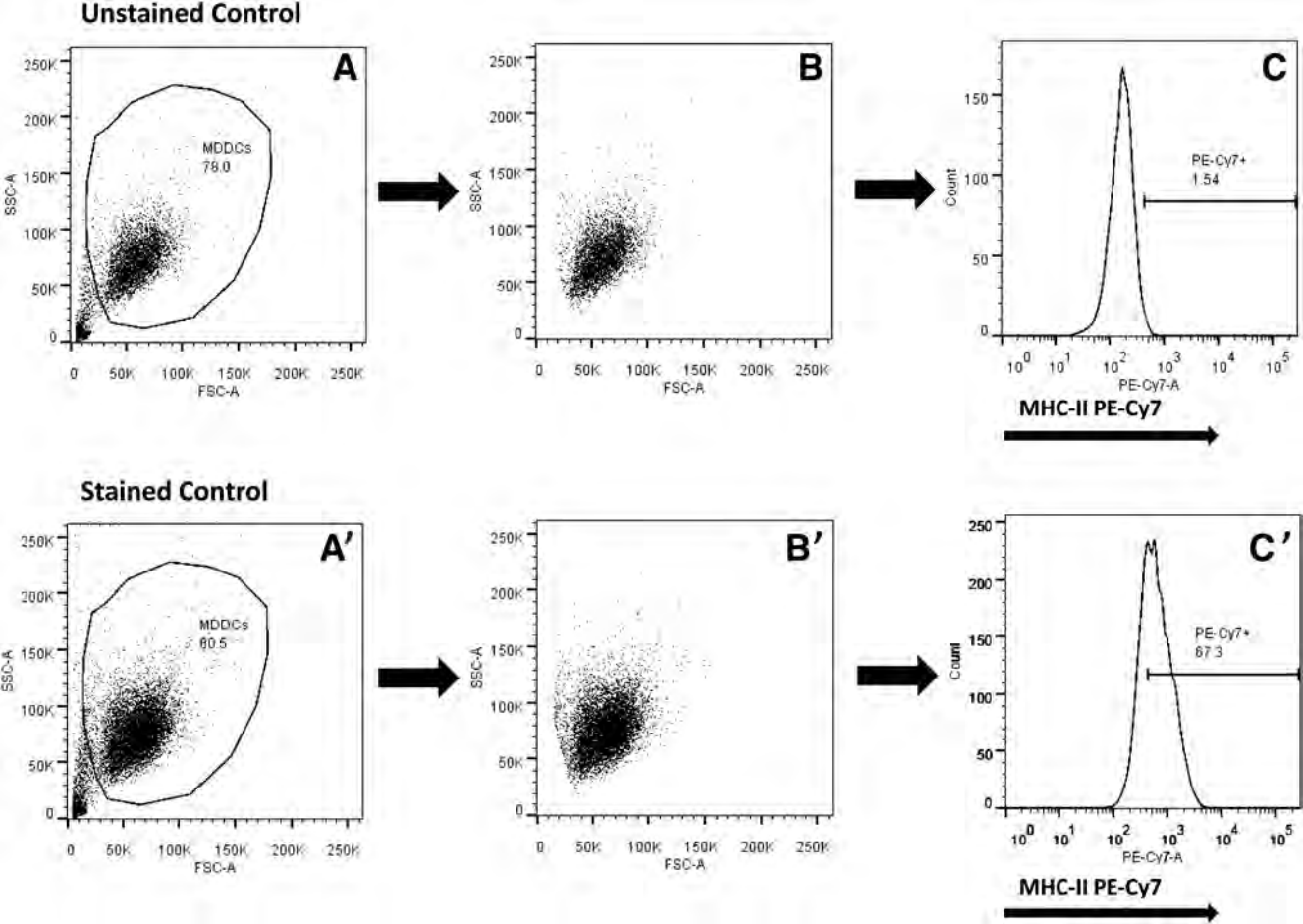
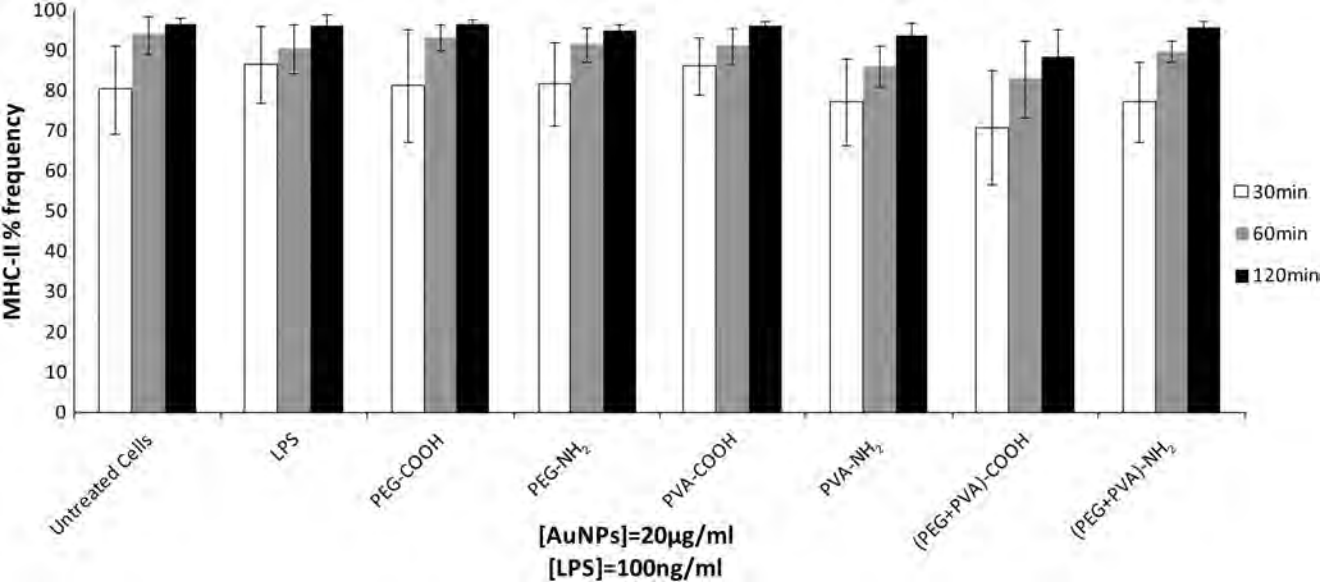
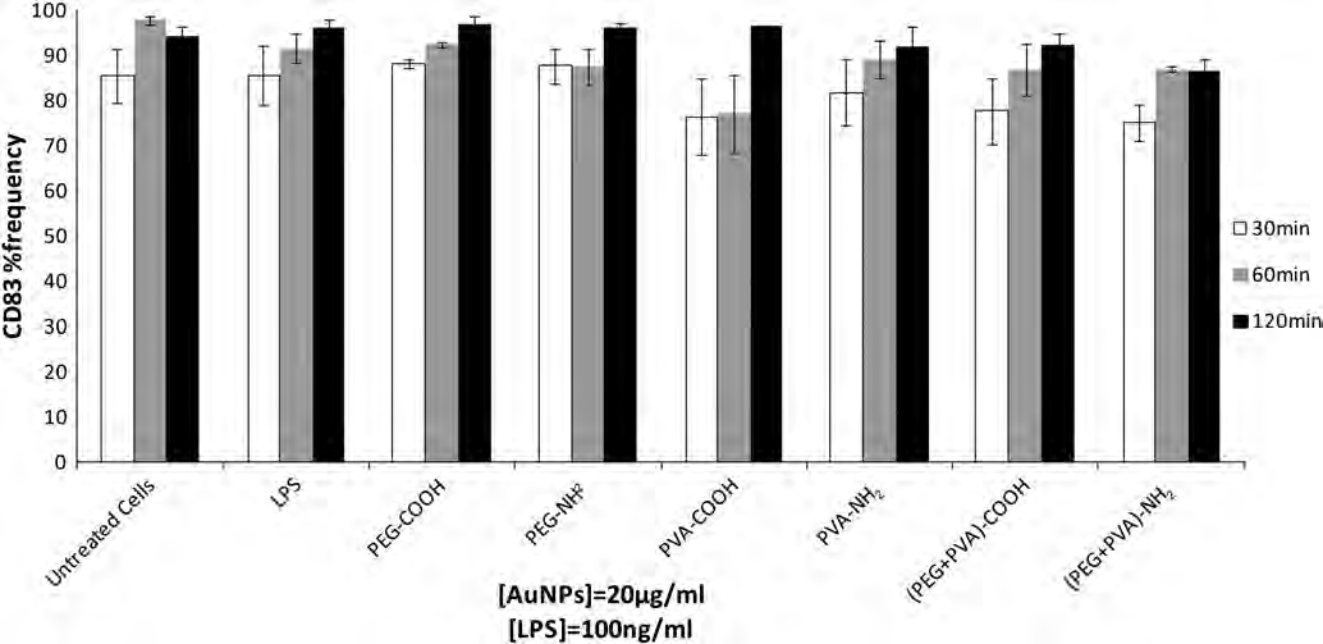

PEG-COOH
t = 0
t = 2 h
t = 24 h
PVA-COOH
t = 0
t = 2 h
t = 24 h
(PEG+PVA)COOH
t = 0
t = 2 h
t = 24 h
PEG-NH2
t = 0
t = 2 h
t = 24 h
PVA-NH2
t = 0
t = 2 h
t = 24 h
(PEG+PVA)NH2
t = 0
t = 2 h
t = 24 h
Absorbance
λ (nm)
0.0
0.4
0.8
1.2
1.6
400
500
600
700
800
900

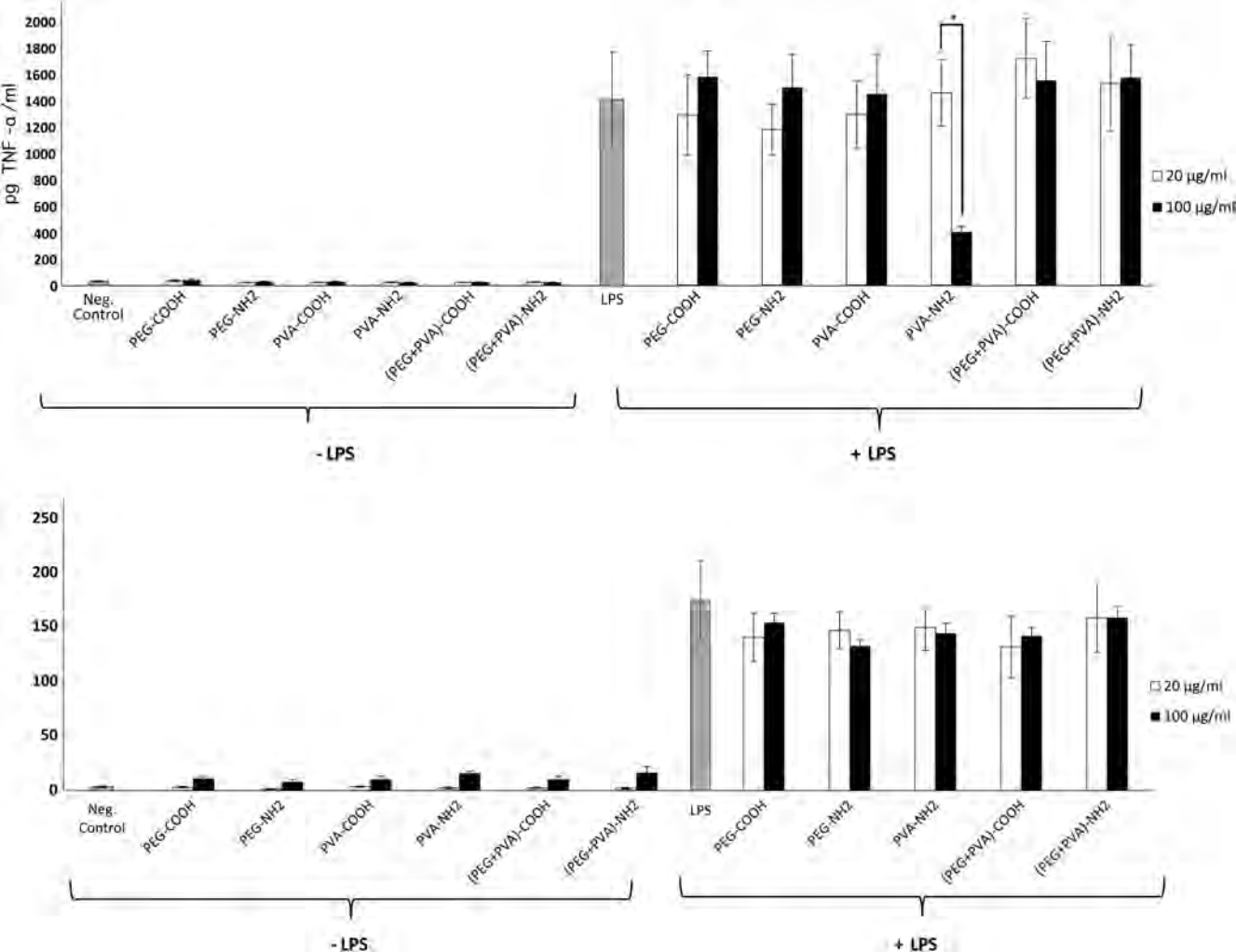

**
**
**
% Cell Viability
100
90
80
70
60
50
40
30
20
10
0
10µg/ml
20µg/ml
100µg/ml
Neg. Control
Pos Control
Cells w LPS
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
-LPS
+LPS

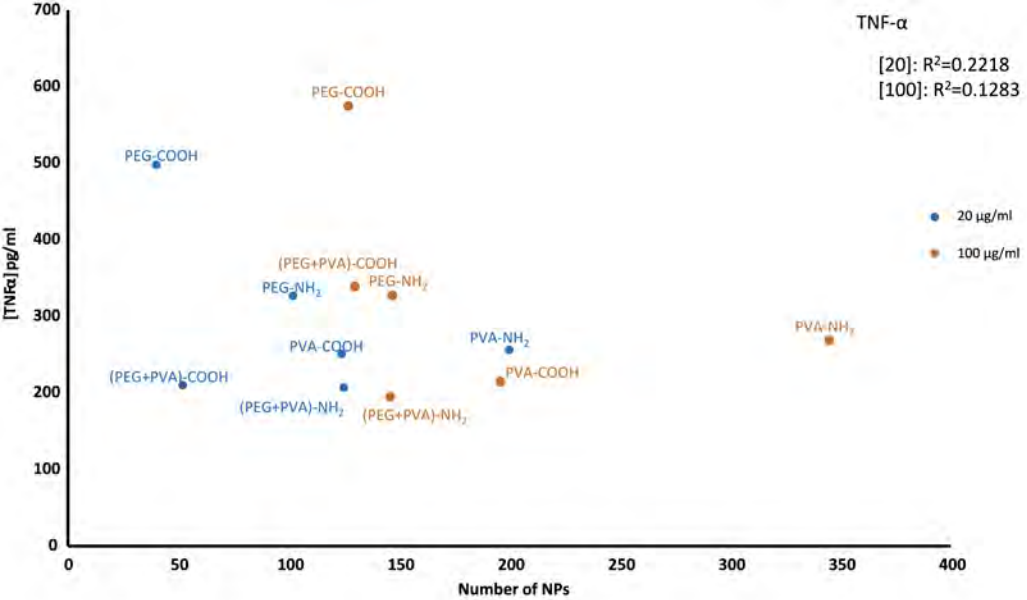

(PEG+PVA)-NH2
(PEG+PVA)-COOH
PVA-NH2
PVA-COOH
PEG-NH2
PEG-COOH
-LPS
+LPS


Unstained Control
A
MDDCs
78.0
SSC-A
FSC-A
B
C
PE-Cy7+
1.54
Count
PE-Cy7-A
MHC-II PE-Cy7
Stained Control
A'
MDDCs
60.5
B'
C'
PE-Cy7+
67.3


MHC-II % frequency
100
90
80
70
60
50
40
30
20
10
0
30min
60min
120min
Untreated Cells
LPS
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
[AuNPs]=20µg/ml
[LPS]=100ng/ml


CD83 %frequency
100
90
80
70
60
50
40
30
20
10
0
30min
60min
120min
Untreated Cells
LPS
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
[AuNPs]=20µg/ml
[LPS]=100ng/ml


pg TNF -α /ml
2000
1800
1600
1400
1200
1000
800
600
400
200
0
*
20 µg/ml
100 µg/ml
Neg. Control
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
LPS
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
- LPS
+ LPS
250
200
150
100
50
0
20 µg/ml
100 µg/ml
Neg. Control
PEG-COOH
PEG-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
LPS
PEG-COOH
PEG-NH2
PVA-NH2
(PEG+PVA)-COOH
(PEG+PVA)-NH2
- LPS
+ LPS


TNF-α
[20]: R²=0.2218
[100]: R²=0.1283
20 µg/ml
100 µg/ml
[TNFα] pg/ml
Number of NPs
PEG-COOH
PEG-COOH
(PEG+PVA)-COOH
PEG-NH2
PEG-NH2
PVA-NH2
PVA-COOH
PVA-NH2
(PEG+PVA)-COOH
PVA-COOH
(PEG+PVA)-NH2
(PEG+PVA)-NH2

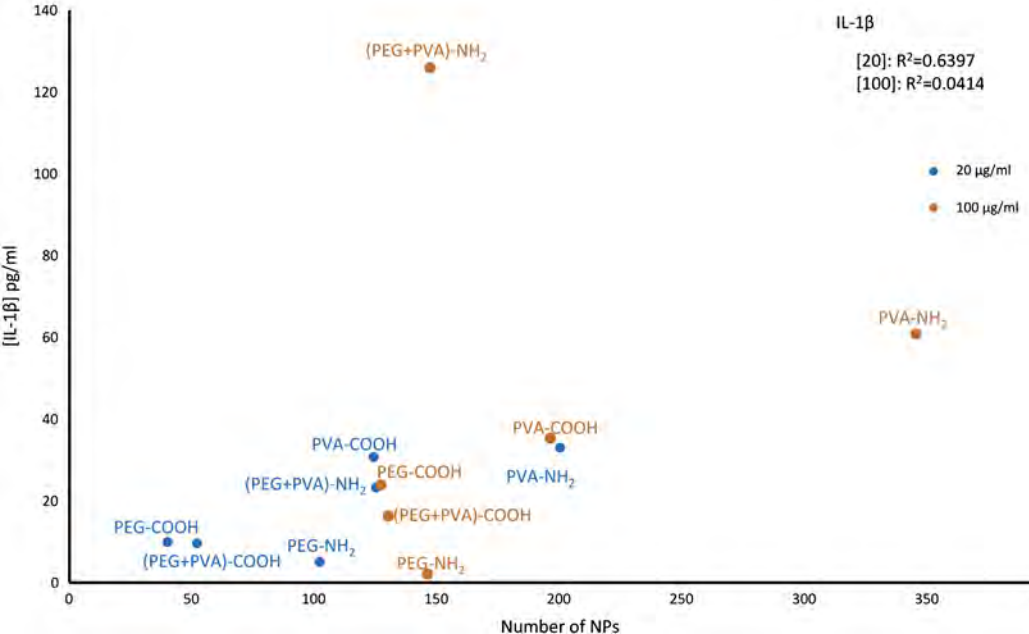

IL-1β
[20]: R²=0.6397
[100]: R²=0.0414
20 µg/ml
100 µg/ml
[IL-1β] pg/ml
Number of NPs
(PEG+PVA)-NH2
PVA-NH2
PVA-COOH
PVA-COOH
PEG-COOH
(PEG+PVA)-NH2
PVA-NH2
(PEG+PVA)-COOH
PEG-COOH
PEG-NH2
(PEG+PVA)-COOH
PEG-NH2

Table S1. Endotoxin quantification in the AuNPs suspensions. Results are expressed as Mean±SD (n=3).

AuNPs	Concentration [EU/mL]
LPS control	0.863 ± 0.025
PEG-COOH	0.045 ± 0.036
PEG-NH_2	0.020 ± 0.011
PVA-COOH	0.053 ± 0.018
PVA-NH_2	0.075 ± 0.0544
(PEG+PVA)-COOH	0.028 ± 0.003
(PEG+PVA)-NH_2	0.063 ± 0.025

Table S2. Size of polymer coated AuNPs

Au-NPs	– LPS		+ LPS	
	D_h (nm)[a]	Polydispersity (%)	D_h (nm)[a]	Polydispersity (%)
PEG-COOH	40.18	18.54	37.82	17.41
PEG-NH_2	26.13	8.82	27.07	9.15
(PEG+PVA)-COOH	21.85	8.93	21.86	8.92
(PEG+PVA)-NH_2	36.35	15.74	37.70	16.98
PVA-COOH	23.71	11.37	23.69	11.40
PVA-NH_2	62.02	26.13	62.69	26.64

Supporting Information

Cell viability

Trypan Blue exclusion assay and phase contrast pictures

The experiment was performed according to the manufacturer's protocol (Sigma Aldrich, T8154-100ML). Briefly, 100μL of cell suspension was mixed with 100μL of Trypan Blue dye and incubated at RT for 3min. After the incubation period, 10μL of the mixture was applied to a haemocytometer (Blau Brand, Ref. 717805, Germany) and a cell count was performed (a total of 100 cells were counted for each sample). The percentage viability in relation to the negative control (i.e. cells not treated with AuNPs) was subsequently calculated. Cells that were incubated at -80^{o}C for 30min were used as a positive control. Complementary to that, phase contrast pictures at a x40 magnification were captured (Motic, AE2000 Inverted Microscope Motic Deutschland GmbH, Wetzlar, Germany) in order to characterize the morphology of the cells following AuNP exposure. Results (Fig. S3) indicate the PVA-NH_2 AuNPs at 100μg/ml induce significant decrease in cell viability ($p<0.01$) compared to the negative control (untreated cells).

Polymer mediated Cytokine secretion

In order to examine if the polymers that were used as coating for the AuNPs are associated with cytokine secretion, MDDCs were exposed to 20 and 100μg/ml of the polymers, without the presence of AuNPs. TNF-α and IL-1β ELISA measurements were also performed as mentioned on the Methods section (Fig. S7)

Polymer synthesis

PEG-COOH

The PEG-COOH solutions are prepared by dissolving 0.6mg of COOH-PEG (Creative PEGWorks) in 1mL of PBS pH 7.4, followed by sonication of the solution to RT for 20min.

PEG-NH_2

The PEG-NH_2 solutions are prepared by dissolving 0.6mg of NH2-PEG (Creative PEGWorks) in 1mL of PBS pH 7.4, followed by sonication the solution to RT for 20min.

PVA-COOH

The polymer solutions are prepared by dissolving 0.7mg of COOH-PVA (Kuraray Poval KL-506), and 1.2mg of PVA (INV-000019, Mowiol 3-85) in 1mL of PBS pH 7.4, followed by rapidly heating the solution to 90°C for 15min and cooling to RT.

PVA-NH_2

The polymer solutions are prepared by dissolving 0.2mg of NH_2-PVA (Vinyl amine/vinyl alcohol co-polymer M12), and 1.1mg of PVA (INV-000019, Mowiol 3-85) in 1mL of PBS pH 7.4, followed by rapidly heating the solution to 90°C for 30 min and cooling to RT.

(PEG+PVA)-COOH

The polymer solutions are prepared by dissolving, 0.4mg of COOH-PEG (Creative PEGWorks) and 0.4mg of COOH-PVA (Kuraray Poval KL-506) in 1mL of PBS pH 7.4, followed by sonication the solution to 40°C for 30min and cooling to RT.

(PEG+PVA)-NH_2

The polymer solutions are prepared by dissolving 0.4mg of NH_2-PEG (Creative PEGWorks) and 0.4mg of NH_2-PVA (Vinyl amine/vinyl alcohol co-polymer M12) in 1 mL of PBS pH 7.4, followed by sonication the solution to 40°C for 30min and cooling to RT.

ELISA

MDDCs were exposed to 20 and 100μg/ml of polymer with/without the addition of 100ng/mL LPS. After 16h of incubation, supernatants were collected and the TNF-α and IL-1β ELISA experiments were performed, as described in the Methods section.

Endotoxin Testing

Initially, the quantification of endotoxin content of all AuNPs solutions was performed by using the PYROGENTTM - 5000 Limulus Amebocyte Lysate assay (Lonza, USA). The results were observed to be below the limit of detection, <0.003EU/mL, (Courtesy of Dr. Huber at the Institute of Pharmacy, Inselspital, Bern, Switzerland). Since there is evidence that AuNPs interfere with endotoxin assays, we have used the Pierce LAL Chromogenic Endotoxin Quantification kit (Cat. No.:88282, Thermo Scientific, Waltham, MA, USA) was utilized according to the kit's instruction. According to the literature **[1-3]** this kit is suitable for endotoxin testing of nanoparticles, since the interference effect is brought to a minimum. Results indicate that all the used AuNPs suspensions have an endotoxin content of less than 0.1EU/mL. The EU limits for medical devices are defined by the US FDA with the limit of 0.5 EU/mL for products that directly or indirectly contact the cardiovascular system and lymphatic system **[4]**.The exact values measured in our samples are shown in Table S1.

LPS-AuNPs interference testing

In order to examine potential interference between LPS and AuNPs, 20μg/ml AuNPs and 100ng/ml LPS were incubated for 24h at RT. DLS (scattering angle of 90^o for 5min, at RT. Each treatment was run 3 times) measurements were performed in order to observe potential differences in the size of the LPS-treated AuNPs. Non-LPS treated AuNPs suspensions were used as a control. Table S2 demonstrates that no alterations in size take place. These results can be explained by the lack of electrostatic and hydrophobic interactions between LPS and the polymer on the Au surface, which does not favor the LPS adsorption on AuNPs. Gao et al. **[5]** have previously demonstrated that only when electrostatic attraction and hydrophobic stacking are both present, the binding of LPS on AuNPs can be not only highly efficient, but also positively cooperative. In a complementary study, Lan et al **[6]** reported a new colorimetric sensor, which is capable of detecting picomolar concentrations of LPS. The sensor performance was demonstrated to originate from multiple electrostatic and hydrophobic cooperative interactions.

Correlation calculations

In order to calculate correlation between the cell-associated Au and the secreted cytokines, the Pearson's correlation method was used. According to the experimental design that was followed, each experiment was repeated three times (n=3) and all treatments were measured in triplicates. This gives a 3X3=9 (N=9). For the Pearson's correlation the degrees of freedom (df) is given from the formula df=N-2. According to our experimental design df=9-2=7. According to the two-tailed Probabilities Table at the statistical significance level of 0.05, the R value is equal to 0.666. None of the obtained values is higher than 0.666 ($p>0.05$), so there is no statistical relevance.

Figure Legends

Figure S1. Colloidal stability in serum supplemented cell culture medium. Extinction spectra of polymer-coated AuNPs kept at 37°C and 5% CO_2 in RPMI 1640 medium with 10% FCS, 1% L-Glu, 1%, Pen-Strep, 10ng/mL GM-CSF and 10ng/mL IL-4. The spectra were normalized based on their absorbance at 400nm.

Figure S2. Phase Contrast Images of MDDCs exposed to 100μg/ml of AuNPs, with/without LPS. MDDCs were exposed to 100μg/ml of AuNPS with different surface functionalizations for 16h, in the presence or absence of 100ng/mL LPS. The only functionalization that is affecting cell viability is the PVA-NH_2. In this case few viable cells could be observed. LPS has no effect on viability. Scale bar: 100μm.

Figure S3. Trypan Blue Exclusion assay. AuNPs at a concentration of 20μg/mL show levels of viability similar to the negative control. The only case when a significant reduction in cell viability takes place was when cells were exposed to 100μg/mL PVA-NH_2 AuNPs. LPS does not have an effect on cell viability. Untreated cells were used as negative control and cells that were put to -80^{o}C for 30min as positive control. Cells from different cell cultures were taken for each repetition (Error Bars: Mean±SD, **: $p<0.01$, n=3).

Figure S4. FACS Gating strategy for MDDCs population analysis. Initial gating was done in Forward (FSC) and Side Scatter (SSC) to unstained (A, A') and stained MDDCs (B, B'). Histograms (C, C') show representative frequencies of MHC-II+ MDDCs. Similar measurements were performed for the CD1c and CD83 markers.

Figure S5. MHC-II expression in the presence of OVA. The expression of MHC-II was measured at 30, 60 and 120min upon OVA incubation (Error bars: Mean±SD, n=3).

Figure S6. CD83 expression in the presence of OVA. The expression of CD83 was measured at 30, 60 and 120min upon OVA incubation (Error bars: Mean±SD, n=3).

Figure S7. Polymer mediated cytokine secretion. (A) None of the polymers tested showed significant increase of TNF-α production at 20 and 100μg/ml. Untreated cells were used as negative control and cells exposed to 100ng/ml LPS as positive control. Each experiment was repeated 3 times and cell supernatants from different cell cultures were used (Error bars: Mean±SD, *p<0.05, n=3). (B) No significant amounts

of IL-1β were produced for the tested polymers at 20 and 100μg/ml. Untreated cells were used as negative control and cells exposed to 100ng/ml LPS as positive control. Each experiment was repeated three times and cell supernatants from different cell cultures were used (Error bars: Mean±SD, n=3).

Figure S8. Correlation of AuNPs uptake and cell mediated cytokine secretion. The numbers of cell-associated AuNPs of all the tested types were plotted against the released cytokine concentration. The findings, expressed as Pearson's r correlation coefficients indicate that there is no correlation ($p>0.05$) observed between the two variables (all the different tested AuNPs types versus the amounts of produced cytokines).

REFERENCES

[1] Li Y et al., Optimizing the use of commercial LAL assays for the analysis of endotoxin contamination in metal colloids and metal oxide nanoparticles. Nanotoxicology 2014, Early online:1-12

[2] Neun and Dobrovolskaia (2011) Detection and quantitative evaluation of endotoxin contamination in nanoparticle formulations by LAL-based assays *Methods Mol Biol* 697:121-30

[3] Smulderss et al (2012) Contamination of nanoparticles by endotoxin: evaluation of different test methods. *Particle and Fibre Toxicology* 9:41

[4]http://www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ucm314718.htm#_Toc315937935

[5] Gao J, Lai Y, Zhao Y Exploring and exploiting the synergy of non-covalent interactions on the surface of gold nanoparticles for fluorescent turn-on sensing of bacterial lipopolysaccharide. *Nanoscale* 2013 5(17):8242-8

[6] Lan M, Wu J, Liu W, Zhang W, Ge J, Zhang H Copolythiophene-derived colorimetric and fluorometric sensor for visually supersensitive determination of lipopolysaccharide. *J Am Chem Soc* 2012 134(15):6685-94