ADVANCED MATERIALS

Supporting Information

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# Melanin-like Hydrogels Derived from Gallic Macromers

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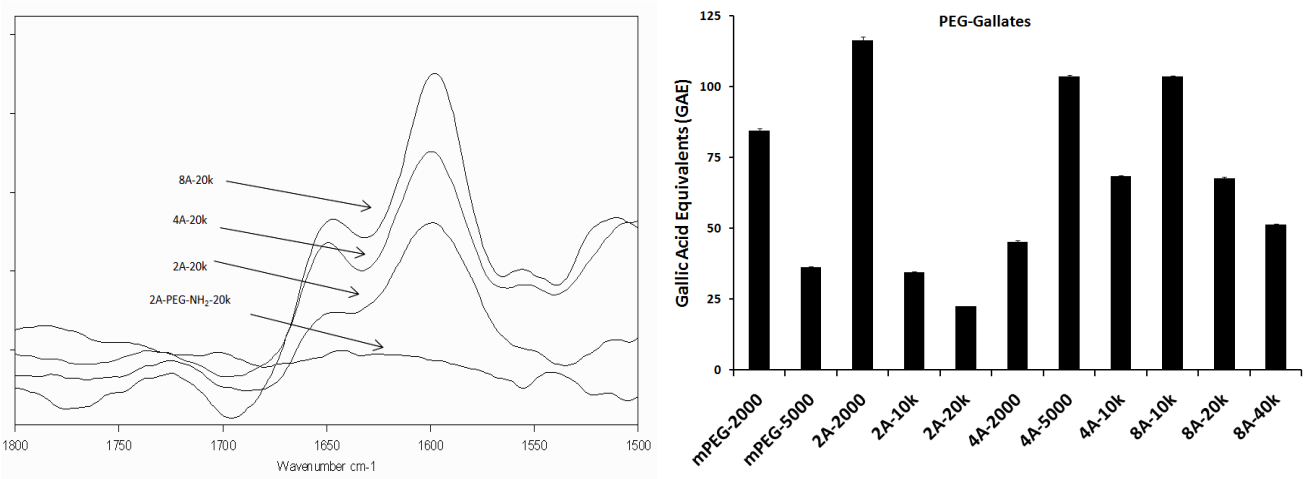
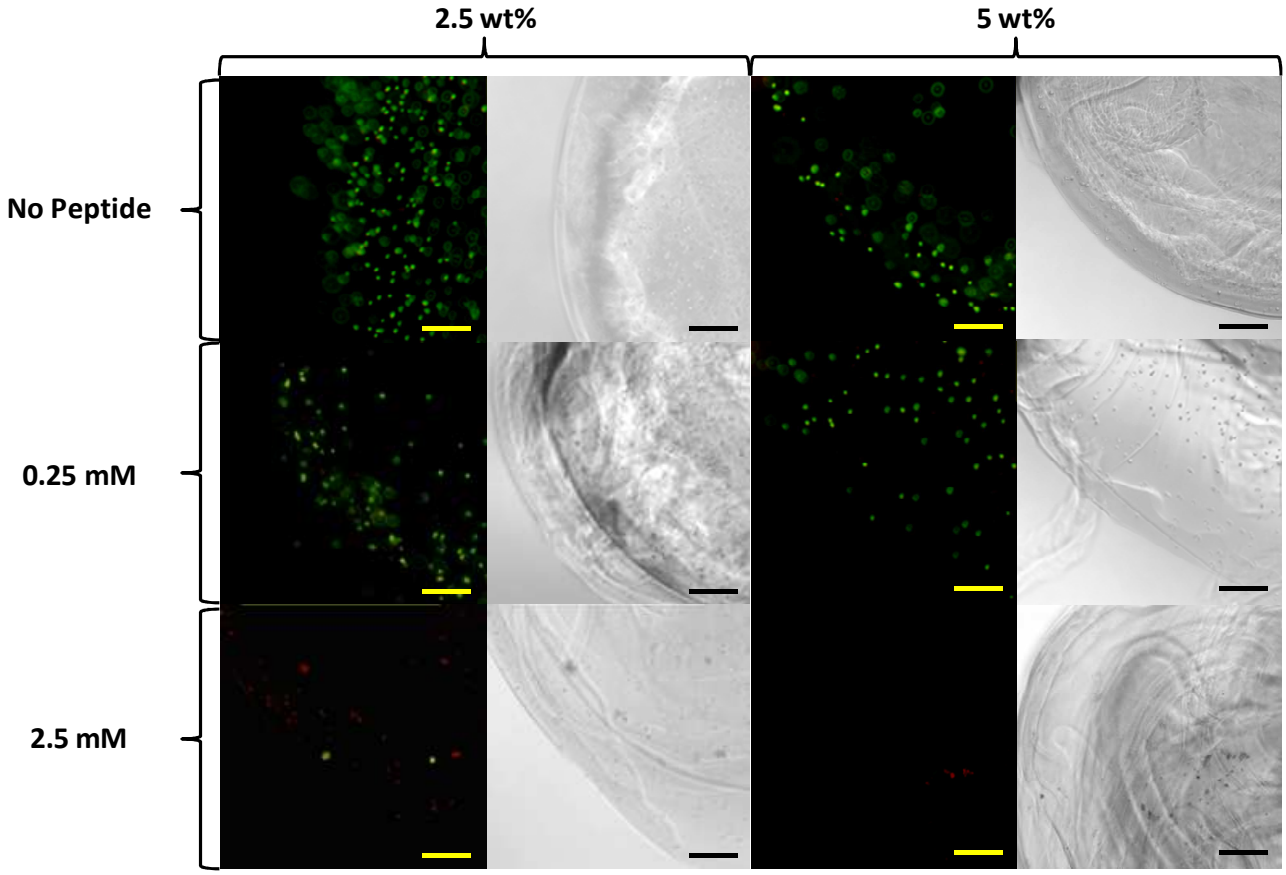
## Supplemental Information




**Supplemental Figure 1.** (**Left**) FT-IR spectra for bi-, tetra-, and octafunctional 20 kDa PEG-gallate macromers versus bifunctional PEG-$NH_2$. (**Right**) The antioxidant capacity of PEG-gallate macromers was quantified using the Folin-Ciocalteu assay. The results are expressed as gallic acid equivalents. Data points = mean + standard deviation (2A=2 arm, 4A=4arm, 8A=8arm, n=8).




**Supplemental Figure 2.** LIVE/DEAD® stained human mesenchymal stem cells encapsulated in hydrogel droplets for 72 h. The addition of DOPA-GRGDS to the macromer solutions causes a decrease in cell viability.

*FT-IR*

Infrared spectroscopy was used to confirm amide coupling in dry samples using a Bruker Alpha-E (Bruker Optics Inc, Billerica MA) spectrometer in single reflection ATR mode at a resolution of 4 cm.. Infrared absorbance spectra for a 2arm PEG-$NH_2$, and bifunctional, tetrafunctional, and octafunctional PEG-gallates were collected. The spectra for gallate substituted and unmodified PEG-$NH_2$ only differed between 1500-1700 $cm^{-1}$(Supplemental Figure ). The spectra for PEG-gallates all contain amide I and II peaks at 1598 $cm^{-1}$ and 1655 $cm^{-1}$. PEG-$NH_2$ does not absorb at these frequencies. The peak absorbance of gallate groups increases with PEG branching. This is reflected in the relative increase in amide peak absorbance as PEG arm number increases. A relative increase in peak absorbance for aromatic C=C stretching is also observed at 1313 $cm^{-1}$.

*Folin-Ciocalteu Assay*

The Folin-Ciocalteu assay is commonly used to quantify the total phenolic content of wine and other botanical extracts. In this work it was used to confirm the substitution of gallate groups on PEG-$NH_2$. A 100 µg/mL gallic acid solution was prepared and serial diluted as a standard. In microplate wells, 60 µL of 1 mg/mL aqueous PEG-gallate or gallic acid was mixed with 10µL of assay reagent for 8 minutes. Afterward, 180 µL of 0.36M aq. $Na_2CO_3$ was added. The samples were vortexed in the dark at RT for 1 h before reading absorbance at 750 nm. The assay results confirmed that the antioxidant power of PEG-gallates was a function of branching molecular weight (Supplemental Figure ). The analysis of 2 kDa 4-arm macromers was limited by their tendency to form a blue precipitate during the assay. Total antioxidant power was also consistent with PEG arm length. For example, both the 10 kDa/8-arm and 5 kDa/4-arm macromers theoretically have average arm lengths of 1.25 kDa. Their average antioxidant capacities were both approximately 103.6 GAE. A fluorescamine assay was also performed on the macromers (results not shown) to determine the amount of primary amines remaining after synthesis. No amines were detected on PEG-gallates but were easily detected on PEG-amines. There was no detectable anti-oxidant activity measured when PEG-amines were analyzed using the Folin assay.

*Electron Paramagnetic Resonance*

Electron paramagnetic resonance (EPR) measurements were carried out using quartz tubes on a Bruker EMX spectrometer operating at operating at 9.66 GHz, at temperature of 293 K, and modulation frequency of 100 kHz. Sepia melanin (Sigma-Aldrich) was analyzed as a dry powder directly from the manufacturer (microwave power = 2 mW). Hydrogels made from 8-arm 10 kDa PEG-gallate macromers were first soaked in 3 mM aq. zinc acetate then crushed with a mortar and pestle before adding to sample tubes. Hydrogels spectra were recorded at a microwave power of 20 mW.

*Zeolite Microcontact Printing*

Zeolite L nanocrystals were synthesized approximately 800 nm in diameter and 250 nm in height[53] and loaded with *N*,*N*′-bis(2,6-dimethylphenyl)perylene-3,4,9,10-tetracarboxylic diimide (DXP, a fluorescent dye molecule)[54] for visualization. Zeolites were functionalized with 3-aminopropyltriethoxysilane (APTES) using a standard procedure[55,56] or with arginine–glycine–aspartic acid (RGD) sequences[57]. SAMs of RGD–zeolite L on glass substrates were prepared according to methods described for other types of zeolites.[58,59] Soft lithography was used to

prepare a patterned zeolite L monolayer on thin gel films. A patterned PDMS elastomeric stamp was used to create patterned self-assembled monolayers (SAMs) of zeolite L crystals on thin gel film. The stripe-patterned (50 µm) PDMS stamp was pressed on a zeolite L monolayer on glass for about 20 s and peeled off quickly. The peeled striped patterned stamps picked up the corresponding zeolite L crystals from the glass surfaces. PDMS was patterned with the zeolites as a negative replica of the substrate. Subsequently, the DXP loaded zeolite L crystals of the stamp were imprinted on the thin hydrogel surface. The gel film was washed with PBS x 3 and kept in PBS at 4 $^{o}$C. For bilateral printing, thin gel films were prepared on glass slides. The gels were removed from glass carefully in PBS in after the initial printing and flipped over for secondary printing. Final micropatterned films were washed in PBS 3x and kept in PBS at 4$^{o}$C until cell seeding.

HeLa cells were seeded on the patterned gels at an initial cell density of $1x10^{6}/cm^{3}$ with a 16 h incubation period (37$^{o}$C, 5% $CO_2$) in 1 mL of culture medium. Subsequently, cells were washed with PBS, fixed with 4% paraformaldehyde solution and stained with blue fluorescence dye 4',6-diamidino-2-phenylindole (DAPI).