

Exploring *in vitro* biological cellular responses of Pegylated β -cyclodextrins

Juliana Rincón-López¹, Miguelina Martínez-Aguilera¹, Patricia Guadarrama¹, Karla Juárez-Moreno^{2,*}, Yareli Rojas-Aguirre^{1,*}

¹ Laboratorio de Materiales Supramoleculares (SupraMatLab), Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Circuito Exterior S/N, Ciudad Universitaria, 04510, Mexico City

² Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, (CFATA-UNAM), Blvd. Juriquilla #3001 Col. Jurica La Mesa CP 76230, Querétaro, Qto., Mexico

*Correspondence: yareli.rojas@materiales.unam.mx (Y.R-A), kjuarez@fata.unam.mx (K. J-M)

Table S1. Effect of β CDPEGs, β CD, and PEGs on the cell cycle of MC3T3-E1 osteoblasts. Results are presented as mean values \pm SD of triplicate experiments.

| Compound | Parameter | Relative cell population (%) | | | | | |
|---------------------------------|------------|------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | control | 25 μ g/mL | 50 μ g/mL | 100 μ g/mL | 250 μ g/mL | 500 μ g/mL |
| <i>βCDPEG2</i> | Sub G0/G1 | 3.7 \pm 0.1 | 3.7 \pm 0.4 | 3.7 \pm 0.2 | 3.8 \pm 0.4 | 3.4 \pm 0.3 | 3.8 \pm 0.3 |
| | G0/G1 | 47.4 \pm 2.1 | 47.1 \pm 2.3 | 44.9 \pm 4.2 | 47.4 \pm 2.2 | 47.1 \pm 2.6 | 43.8 \pm 2.2 |
| | S | 18.4 \pm 0.6 | 16.5 \pm 4.0 | 15.5 \pm 3.8 | 15.9 \pm 0.8 | 18.0 \pm 5.7 | 23.6 \pm 3.7 |
| | G2/M | 36.1 \pm 2.3 | 36.4 \pm 1.4 | 34.0 \pm 3.5 | 37.4 \pm 0.8 | 30.6 \pm 1.6 | 27.9 \pm 1.3 |
| | *Viability | 100.0 \pm 9.1 | 97.3 \pm 3.9 | 100.0 \pm 1.2 | 76.4 \pm 14.2 | 85.7 \pm 4.0 | 77.5 \pm 9.5 |
| <i>βCDPEG5</i> | Sub G0/G1 | 3.7 \pm 0.1 | 3.6 \pm 1.5 | 4.2 \pm 0.4 | 3.7 \pm 0.7 | 4.2 \pm 1.4 | 4.0 \pm 0.2 |
| | G0/G1 | 47.4 \pm 2.1 | 45.5 \pm 2.9 | 54.7 \pm 4.9 | 56.5 \pm 2.4 | 57.7 \pm 1.8 | 56.4 \pm 1.6 |
| | S | 18.6 \pm 0.8 | 19.8 \pm 1.9 | 22.5 \pm 1.2 | 28.1 \pm 1.3 | 26.5 \pm 2.8 | 27.0 \pm 2.6 |
| | G2/M | 36.1 \pm 2.3 | 28.2 \pm 0.8 | 18.6 \pm 0.8 | 17.2 \pm 5.4 | 16.6 \pm 4.0 | 12.0 \pm 1.2 |
| | Viability | 100.0 \pm 9.1 | 77.4 \pm 14.9 | 59.5 \pm 15.2 | 51.1 \pm 14.4 | 55.3 \pm 14.4 | 53.9 \pm 10.4 |
| <i>PEG2</i> | Sub G0/G1 | 3.7 \pm 0.1 | 2.8 \pm .4 | 3.7 \pm 0.3 | 4.1 \pm 0.1 | 3.4 \pm 0.5 | 3.9 \pm 0.2 |
| | G0/G1 | 47.4 \pm 2.1 | 46.4 \pm 3.4 | 5.2 \pm 1.0 | 50.2 \pm 1.5 | 49.7 \pm 3.5 | 47.7 \pm 6.9 |
| | S | 18.6 \pm 0.8 | 23.5 \pm 3.0 | 25.5 \pm 3.0 | 26.9 \pm 2.4 | 24.0 \pm 3.3 | 24.0 \pm 1.8 |
| | G2/M | 36.1 \pm 2.3 | 11.6 \pm 0.8 | 13.5 \pm 1.1 | 12.9 \pm 1.1 | 13.1 \pm 1.5 | 12.5 \pm 2.1 |
| | *Viability | 100.0 \pm 9.1 | 68.3 \pm 9.3 | 55.3 \pm 5.1 | 52.8 \pm 5.6 | 49.8 \pm 1.2 | 53.4 \pm 2.5 |
| <i>PEG5</i> | Sub G0/G1 | 3.7 \pm 0.1 | 3.9 \pm 0.4 | 3.8 \pm 0.6 | 3.4 \pm 1.2 | 3.9 \pm 0.6 | 4.1 \pm 0.5 |
| | G0/G1 | 47.4 \pm 2.1 | 46.8 \pm 3.4 | 50.5 \pm 1.4 | 54.9 \pm 4.0 | 52.4 \pm 2.7 | 53.7 \pm 3.2 |
| | S | 18.6 \pm 0.8 | 21.1 \pm 0.8 | 23.4 \pm 3.2 | 22.8 \pm 5.3 | 23.3 \pm 3.7 | 24.9 \pm 4.0 |
| | G2/M | 36.1 \pm 2.3 | 18.2 \pm 5.5 | 17.2 \pm 5.2 | 10.4 \pm 0.8 | 13.7 \pm 2.3 | 13.7 \pm 5.3 |

| | | | | | | | |
|--|------------|-----------|-----------|-----------|-----------|-----------|----------|
| | *Viability | 100.0±9.1 | 54.6±10.8 | 41.9±12.5 | 46.1±12.8 | 49.4±9.2 | 50.6±4.2 |
| <i>β</i> CD | Sub G0/G1 | 3.7±0.1 | 3.9±0.1 | 4.0±0.1 | 5.5±2.1 | 5.2±3.2 | 5.9±1.6 |
| | G0/G1 | 47.4±2.1 | 53.8±2.2 | 53.9±10.3 | 60.6±2.5 | 59.5±7.2 | 59.9±9.2 |
| | S | 18.6±0.8 | 21.6±8.3 | 24.3±3.8 | 24.6±3.8 | 21.8±2.9 | 18.9±5.7 |
| | G2/M | 36.1±2.3 | 7.8±4.0 | 7.8±2.4 | 8.8±4.8 | 6.4±3.5 | 6.3±3.5 |
| | *Viability | 100.0±9.1 | 77.3±10.4 | 54.0±3.7 | 59.2±8.0 | 37.0±28.2 | 70.3±4.2 |
| *% Cell viability values obtained from the assay reported in Section 2.2.1. have been included to facilitate their comparison with the % relative cell population. | | | | | | | |

Table S2. Effect of β CDPEGs, β CD, and PEGs on the cell cycle of MDCK cells. Results are presented as mean values \pm SD of triplicate experiments.

| Compound | Parameter | Relative cell population (%) | | | | | |
|----------------|------------|------------------------------|------------|-----------|----------|-----------|-----------|
| | | control | 25μg/mL | 50μg/mL | 100μg/mL | 250μg/mL | 500μg/mL |
| β CDPEG2 | Sub G0/G1 | 4.1±0.5 | 3.7±0.3 | 3.6±0.5 | 3.6±1.0 | 3.7±0.1 | 3.8±0.3 |
| | G0/G1 | 44.6±6.4 | 39.6±1.0 | 39.5±5.6 | 38.4±0.7 | 36.5±2.2 | 40.9±2.3 |
| | S | 13.0±5.4 | 11.9±4.3 | 10.7±1.1 | 10.9±1.7 | 7.2±3.3 | 7.4±2.5 |
| | G2/M | 31.7±0.2 | 28.9±1.8 | 30.8±1.8 | 27.7±1.0 | 29.1±5.2 | 30.5±3.2 |
| | *Viability | 100.0±13.0 | 100.0±11.7 | 100.0±7.2 | 94.8±8.1 | 100.0±2.5 | 100.0±7.7 |
| β CDPEG5 | Sub G0/G1 | 4.1±0.5 | 3.6±1.5 | 4.2±0.4 | 3.7±0.7 | 4.2±1.4 | 4.0±0.2 |
| | G0/G1 | 44.6±6.4 | 43.1±2.5 | 42.8±2.8 | 45.8±5.9 | 46.5±2.2 | 50.2±8.0 |
| | S | 13.0±5.4 | 24.5±3.4 | 27.8±4.3 | 27.9±0.9 | 31.1±1.0 | 30.2±2.7 |
| | G2/M | 31.7±0.2 | 11.9±4.3 | 10.7±1.1 | 10.9±1.7 | 7.2±3.3 | 7.4±2.5 |
| | *Viability | 100.0±13.0 | 91.8±8.8 | 83.9±1.9 | 82.9±4.5 | 70.8±5.7 | 72.1±2.2 |
| PEG2 | Sub G0/G1 | 4.1±0.5 | 3.5±1.0 | 4.0±0.9 | 5.5±1.2 | 4.6±0.3 | 4.2±0.1 |
| | G0/G1 | 44.6±6.4 | 43.6±4.1 | 45.2±5.5 | 46.8±1.1 | 45.0±1.9 | 45.3±4.3 |
| | S | 13.0±5.4 | 32.4±3.3 | 29.1±2.2 | 28.1±5.3 | 27.5±3.4 | 29.1±4.2 |
| | G2/M | 31.7±0.2 | 11.6±0.8 | 13.5±1.1 | 11.2±0.8 | 11.6±1.1 | 9.7±1.0 |
| | *Viability | 100.0±13.0 | 78.9±3.5 | 69.2±6.8 | 64.3±2.3 | 58.6±20.3 | 74.0±3.9 |
| PEG5 | Sub G0/G1 | 4.1±0.5 | 3.3±0.4 | 3.5±0.3 | 3.6±0.5 | 3.3±0.2 | 3.5±0.6 |
| | G0/G1 | 44.6±6.4 | 45.4±3.1 | 47.9±10.4 | 56.3±1.2 | 47.3±7.4 | 46.4±7.6 |
| | S | 13.0±5.4 | 26.0±3.4 | 28.9±1.4 | 25.0±0.9 | 26.8±2.9 | 25.4±2.8 |
| | G2/M | 31.7±0.2 | 13.1±2.3 | 11.5±1.7 | 11.7±0.6 | 11.6±1.1 | 16.7±3.4 |

| | | | | | | | |
|-----|------------|------------|-----------|----------|----------|-----------|----------|
| | *Viability | 100.0±13.0 | 79.5±5.4 | 76.9±2.5 | 71.8±7.1 | 71.1±10.7 | 81.4±5.6 |
| | Sub G0/G1 | 4.1±0.5 | 2.8±0.9 | 3.7±0.3 | 4.9±1.1 | 4.1±1.1 | 3.5±0.7 |
| | G0/G1 | 44.6±6.4 | 42.0±8.9 | 36.0±7.4 | 41.8±3.7 | 41.8±3.2 | 45.1±5.0 |
| βCD | S | 13.0±5.4 | 13.3±2.2 | 38.6±2.4 | 35.2±1.2 | 35.6±3.1 | 37.8±4.0 |
| | G2/M | 31.7±0.2 | 37.8±12.4 | 17.1±4.1 | 9.4±1.3 | 2.7±0.5 | 7.2±2.6 |
| | *Viability | 100.0±13.0 | 95.1±5.8 | 97.6±9.4 | 73.7±6.4 | 79.0±2.7 | 76.1±2.8 |

*% Cell viability values obtained from the assay reported in Section 2.3.1. have been included to facilitate their comparison with the % relative cell population.