

**Effects of matrix viscoelasticity on cell-matrix interaction, actin cytoskeleton organization, and apoptosis for osteosarcoma MG-63 cells**

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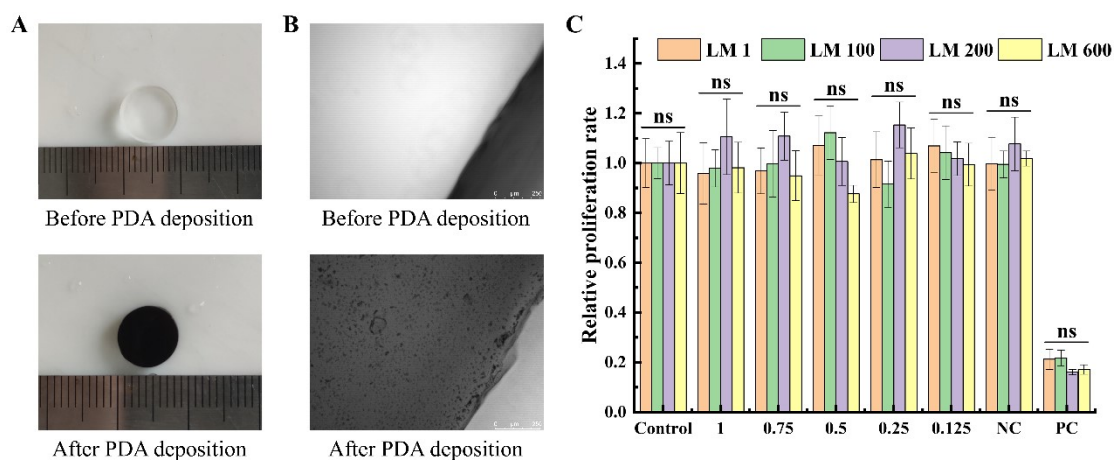
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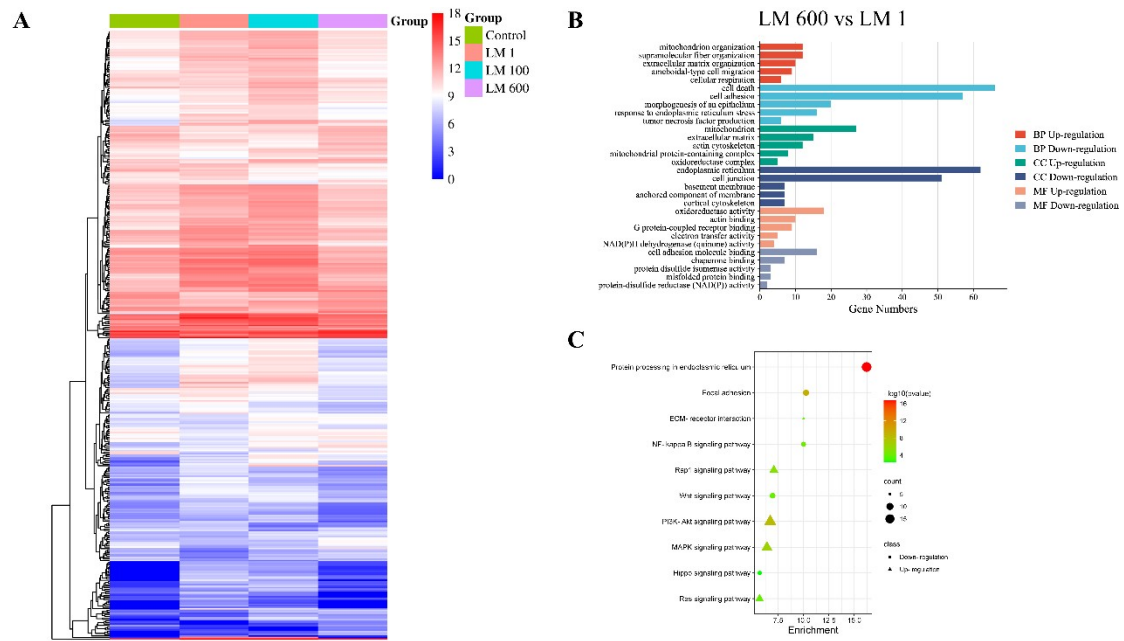
**Formula of PAM matrixes with different elasticity**

Loss Modulus	40% AM	2% BIS	H <sub>2</sub> O	5% Alg	TEMED	10% APS
LM 1	1 ml	250 $\mu$ l	3.7 ml	0 ml	2 $\mu$ l	50 $\mu$ l
LM 100	1 ml	200 $\mu$ l	1.8 ml	2 ml	2 $\mu$ l	50 $\mu$ l
LM 200	1.1 ml	100 $\mu$ l	0.8 ml	3 ml	2 $\mu$ l	50 $\mu$ l
LM 600	1.6 ml	20 $\mu$ l	0 ml	3.4 ml	2 $\mu$ l	50 $\mu$ l

SI Table. 1. Preparation scheme of viscoelastic matrix with controlled elastic and viscous. The preparation could be completed by adding all the raw materials in turn, mixing well and setting still.



SI Fig. 1. Supplementary characterization of viscoelastic matrices. (A) The Change of matrix before and after deposition of polydopamine. (B) The changes of polydopamine before and after deposition under inverted microscope, the blank area in the lower right corner of the picture is aseptic and acellular fresh medium as blank control. (C) The extracts of different viscoelastic matrices had good cytocompatibility, that was, there was no obvious cytotoxicity.



SI Fig. 2. A supplement to mRNA sequencing analysis of the effect of matrix viscoelasticity on MG-63 cells. (A) Heat map analysis showed that a total of 404 genes showed differences among different viscoelastic matrix groups. (B) GO analysis showed that the mitochondrial related processes of MG-63 cells on the high viscosity matrix were upregulated and the endoplasmic reticulum stress-related processes continued to be downregulated, while the cell-matrix interaction related processes included more upregulation and downregulation, compared with the soft elastic matrix. (C) KEGG analysis showed that the upregulation of Ras, Rap1, PI3K-Akt, and MAPK signaling pathways and the downregulation of Hippo, Wnt, and NF-kappaB signaling pathways and protein processing in the endoplasmic reticulum in MG-63 cells on the high viscosity matrix compared to soft elastic matrix. This was in support of our conclusion that activation of the mitochondrial pathway on highly viscosity matrix was the primary cause of apoptosis of MG-63 cells, rather than endoplasmic reticulum stress pathway.