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2	Received 16 August 2016, Revised 23 September 2016, Accepted 12
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5	A simple fabricated thickness-based stiffness gradient for cell studies
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21	Abstract
22	In this work, we developed a simple method to fabricate a thickness-based continuous
23	stiffness gradient for biological studies. It was made by glass slides, polydimethylsiloxane
24	(PDMS) pre-polymer, spacer and clips only, without any sophisticated equipment. It is easy
25	to fabricate in any general biological and pharmaceutical laboratories. The stiffness gradient
26	was characterized in terms of apparent Young's modulus by atomic force microscopy (AFM)
27	and the Young's modulus along the gradient was found to be 8.5 to 120 kPa, which is within
28	the physiological relevant range. HeLa-C3 cells were cultured on the gradient to study their
29	morphological behavior according to the substrate stiffness. Furthermore, the drug efficiency
30	of etoposide, an anti-cancer drug, was studied along the substrate stiffness gradient. It was
31	found that HeLa-C3 cells cultured on the soft region of the gradient (8.5-11 kPa) are more
32	sensitive to etoposide. We believe the proposed device could promote cell investigations and
33	drug screenings on a substrate with comparable stiffness to the native tissue.

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