Patterned graphene for alignment and myogenic differentiation of human mesenchymal stem cells

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Human mesenchymal stem cells (hMSCs) have attracted a lot of attention for their potential use in tissue repair. Such strategies rely on controlled differentiation of hMSC's into specific lineage; however the control and induction of the skeletal myogenic differentiation still poses a challenge. Graphene have been shown to enhance the cardiac myogenic differentiation of embryonic stem cells [1] as well as guide muscle progenitor cells into preferred orientation of myotube development [2].

Here we show the long range alignment of fully confluent layers of hMSCs by patterned graphene giving rise to an enhancement of myogenic differentiation. The initial attachment of cells show an immediate preference to the graphene coated stripes causing the cells to elongate in the predefined direction. hMSCs were seeded onto patterned graphene and control substrates and live phase contrast imaging showed preferential attachment and formation of focal adhesion on the graphene stripes. Figure 1 shows an image sequence clearly revealing how the cells spread along the prepatterned graphene stripes. After 3 weeks of culture cells were stained with DAPI, Rhodamine Phalloidin and Cellmask Deep Red to visualize cell nucleus, f-actin and membrane, respectively. Fluorescence imaging was carried out over several centimeters to confirm the long range alignment of the cells. Image analysis of more than 300 images per sample of the actin fibers and cell mask reveals the alignment and directionality is maintained even as the cells reach confluence. Figure 2 shows the cells commitment to alignment on patterned graphene stripes peaks at 20μm width/spacing and no preferential direction is found on fully coated or control coverslips.

Real time polymerase chain reaction showed upregulation of myogenic markers including early commitment markers of myoblast differentiation protein-1 (MyoD) and desmin and late phase marker of myosin heavy chain-2 (MHC), when induced with myogenic media. The combination of graphene patterning and myogenic induction media enhances the myogenic differentiation, which we envisage to play a significant role in future stem cell based therapies.

References:

[1] Lee, T.-J. et al. Biochemical and Biophysical Research Communications 452, 174–180 (2014).
[2] Bajaj, P. et al. Adv. Healthcare Mater. 3, 995–1000 (2014).

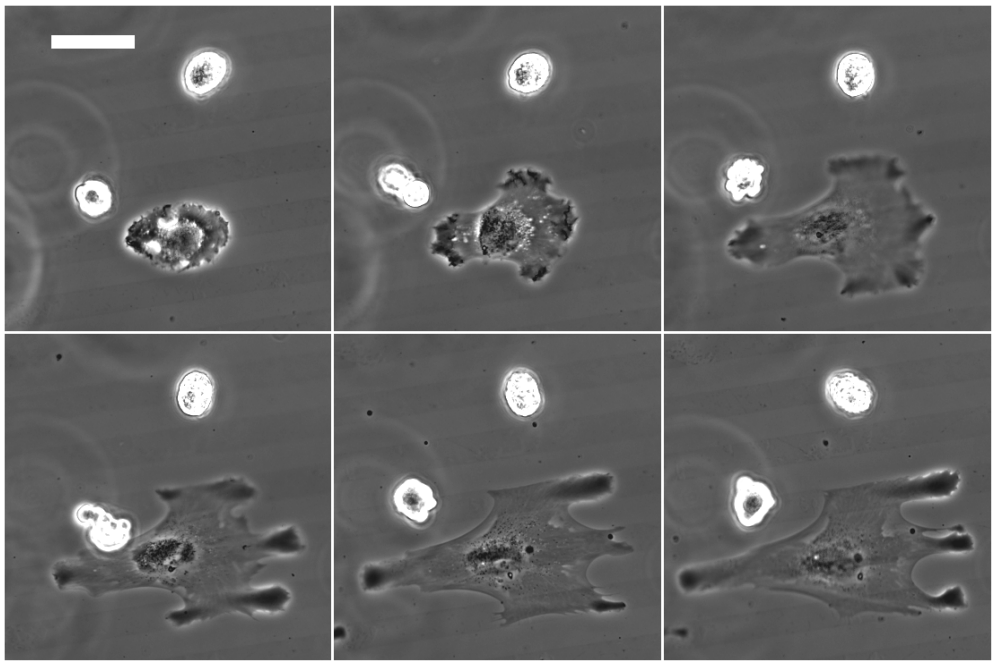

Figure 1: Time-lapse of 3 hours imaging from initial attachment on the top left frame with 30 min interval to the bottom right, showing the cells preferential attachment to the graphene stripes. Scale bar is 50µm.

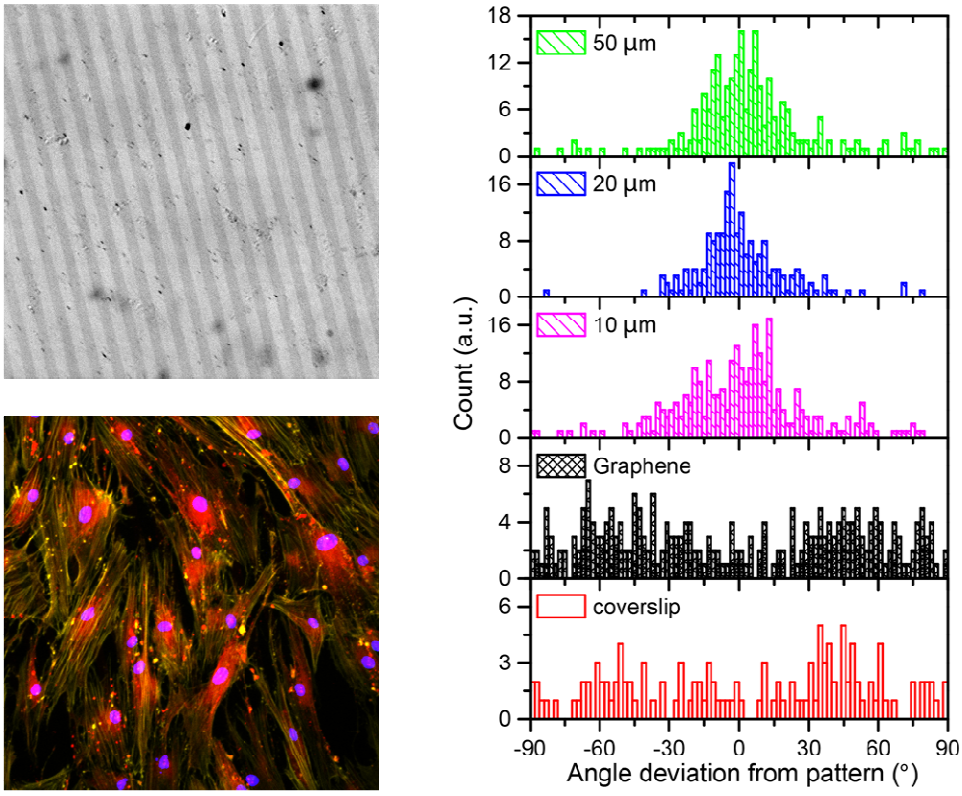

Figure 2: **Top left** Graphene stripes of 20µm width and spacing with hMSCs grown for 21days. **Bottom left** Fluorescence imaging visualizing nucleus, actin polymers and cell membrane. **Right** Actin fiber alignment to the graphene direction on 50µm, 20µm and 10µm graphene stripes as well as fully coated and bare coverslip.