Effects of Peptoid Nanosheets on Stem Cell Culture

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Introduction: Nanomaterials have attracted great attention in the fields of drug delivery and regenerative medicine.[1] Unlike nanoparticles and nanotubes, few studies have been conducted with regard to nanosheets. It is unclear how these novel nanostructures interact with cells including mesenchymal stem cells (MSCs). In response, we are studying the interaction "peptoid" nanosheets with MSCs.

Peptoid nanosheets are bilayer membranes formed from a amphiphilic N-substituted glycine peptide-mimetic sequence (Figure 1). The sequence is designed with alternating hydrophobic and ionizable side chains[2] (Figure 2A). Nanosheets are 3 nanometers in thickness and tens to hundreds of microns in length. In our observations, these nanosheets often extend to their full lengths in solution despite their very thin cross-section, indicating a high intrinsic structural stiffness (Figure 2B).

The physical properties of the stem cell niche are critical for determining stem cell differentiation and fate. For example, a stiff extracellular matrix (ECM) will direct mesenchymal stem towards osteogenic differentiation. Therefore we have investigated how peptoid nanosheets may be able to influence MSC behaviour in the present study.

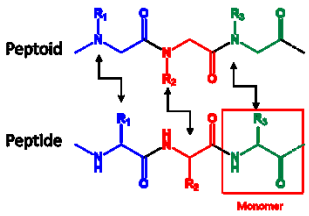

Figure 1: Structure peptoid vs peptide

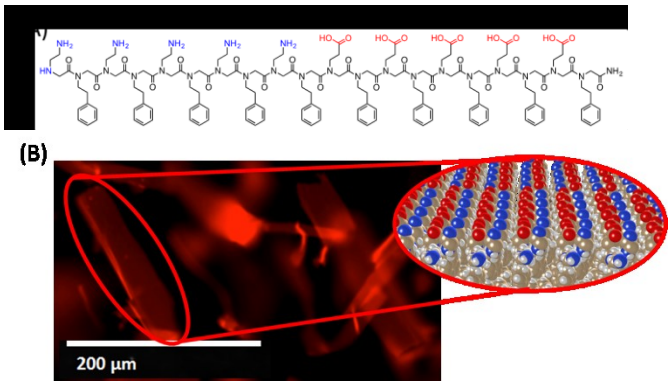

Figure 2: (A) Peptoid nanosheets sequence, (B) Microscopy nanosheets picture

Methods: Nanosheets quantity and size were studied using microscopy and image analysis. Their stability in cell culture conditions was also assessed. The nanosheets/MSCs interaction was explored using optical (Figure 3) and confocal microscopy. The effect on cell viability and differentiation was also studied. We hypothesis that the stiffness of peptoid nanosheets might enhance the MSCs' commitment to the osteogenic lineage. So osteogenic genes and proteins were monitored.

Results: We were able to increase the distribution of nanosheets towards larger sizes by increasing the time used for sheet assembly. Also, nanosheets remained stable in cells living conditions for 3 weeks. The cell/nanosheets interaction study suggests that nanosheets interact physically with the MSCs (i.e. no cell membrane penetration). Cells incubated with nanosheets displayed an increase in their metabolic activity (quantified by Alamar Blue assay) and an overexpression of some osteogenic genes and proteins.

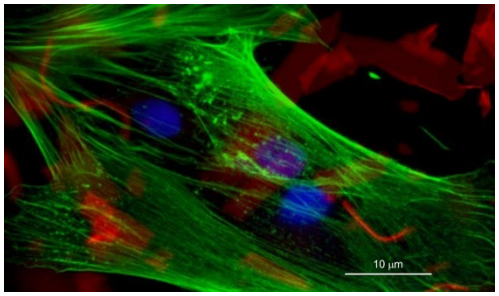

Figure 3: Fluorescence microscopy image of MSCs cultured on multiwell plate (PS plastic) with nanosheets added at "20 µM" (blue (DAPI): nucleus; green (rhodamine): actin, Nile Red: nanosheets)

Conclusions: This work is laying the foundation for future studies using nanosheets to influence cell behaviour. Further work is being conducted to improve and control the effect of nanosheets on MSC but also on other type of cells.

References:

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(2) K.T. Nam, S.A. Shelb, P.H. Choi, A.B. Marciel, R. Chen, T. Tan, T.K. Chu, R.A. Mesh, B.C. Lee, M.D. Connolly, C.Kisielowski, R.N. Zuckermann, Nature Materials, 2010, 9, 454-460.

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