High content imaging based mapping of stem cell phenotypes on polymeric biomaterials

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Statement of Purpose: One of the emerging strategies for the restitution of dysfunctional tissues, particularly following resection of cancerous loading bearing tissues such as bone, involves the implantation of biomaterial scaffolds populated with stem cells (e.g. mesenchymal stem cells (MSC)). Thus, the role of biomaterials in promoting stem cell differentiation and regeneration while suppressing possible cancerous transformation needs to be rationally tailored. This study investigates the possibility of developing high content imaging based mapping methods for identifying the phenotypic state of stem cells (normal stem cells, differentiated, abnormal stem cells) in the context of various extracellular stimuli. We demonstrate the use of the imaging based mapping tool to screen biomaterials and identify candidate biomaterials with enhanced tissue regeneration potential even under pathological situations.

Methods: In this study, we first investigated the fates of MSCs following distinct lineage differentiation pathways (osteogenic vs adipogenic), and metal carcinogen-induced cancerous transformation. Multi-photon imaging and quantitative analysis of cell nuclear structure and organization followed by data mining efforts, including feature dimension reduction and classification, were utilized to evaluate relationships between cell nuclear organization and MSCs differentiation or transformation in the context of combinatorially designed biomaterial substrates.
Tyrosine-derived polycarbonates were copolymerized in various molar ratios with (i) poly(ethylene glycol) (PEG), and (ii) negatively charged monomers (DT), as previously described[1]. A subset of polymethacrylates were also selected based their osteogenic induction capabilities. Human mesenchymal stem cells (MSCs) were cultured on the aforementioned biomaterial- coated substrates in a carcinogenic environment (basal medium containing metal carcinogen, nickel sulfate) or in osteogenic or adipogenic media at 37°C. The abnormal transformation of MSCs was characterized in terms of telomerase mRNA expression, stemness markers, cytokine secretion, and changes in nuclear descriptors. A new high-resolution 2-photon fluorescent imaging-based method was developed for the multi-functional characterization of MSC responses based on: 1) cell nuclear staining with Hoechst 33342 dye, 2) Fluorescent In-Situ Hybridization using Qdot-conjugated telomerase mRNA probe, 3) morphometrics of a nuclear matrix protein reporter, nuclear mitotic apparatus (NuMA)). This high-resolution imaging allowed the quantification of a large pool of nuclear descriptors, reporting nuclear organization and morphometric features, via a series of image analysis procedures. Individual nuclear descriptors were analyzed statistically utilizing ANOVA with Tukey's post-hoc test. Feature dimension reduction and classification were performed using Matlab dimension reduction toolbox.

Results: Nuclear descriptors obtained following feature dimension reduction and classification process could be used to effectively identify/discern different lineages of MSCs, or lineage committed MSCs/transformed MSC from normal MSCs, as shown in figure 1. Moreover, we found that biomaterials differentially regulated the transformation of MSC in the presence of the metal carcinogen. We found that biomaterials differentially regulated the abnormal transformation of hMSC. The polycarbonates containing 8%PEG promoted MSC transformation, while polycarbonates containing 10% anionic carboxylate DT, or a polymethacrylate, poly (10%EHA-65%NIPAAM), alleviated transformation, as demonstrated by data mining on cell nuclear descriptors. Nuclear descriptors, a combined pool of subcellular features, can thus be applied to potentially classify the degree of transformation and differentiation of MSCs in response to changes in biomaterial compositions.

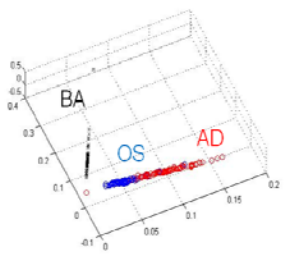

Fig 1: "Integrated" nuclear signatures after data mining on a pool of cell nuclear descriptors on osteogenic, adipogenic cells and normal MSCs cultured on basal medium.

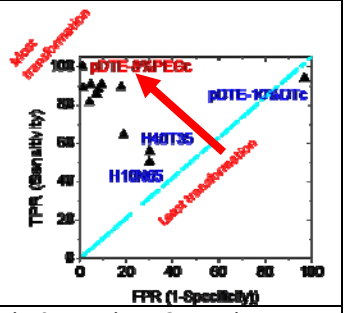

Fig 2 Receiver Operating Characteristic (ROC) curve Representation of volunrability of MSCs under transformation induction when cultured on biomaterials.

Conclusions: The subcellular features (e.g. cell nuclear morphology, nuclear protein localization, structure and organization, etc.) were deployed to identify different fates of mesenchymal stem cells using high content imaging tool. Thus, the high content imaging based mapping tool can potentially be developed further to identify and design improved synthetic biomaterials for tissue regeneration and cancer therapy applications.

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References:

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