Combinatorial Biomaterials as an Engineered Niche for Control of Stem Cell Transformation/Regeneration

Er Liu[1], Hak-Joon Sung[2],Hira Rizvi[1], Hiral Patel[1], Matthew L. Becker[4], Joachim Kohn[3], and Prabhas V. Moghe[1,2].
Department of [1] Biomedical Engineering, [2] Chemical & Biochemical Engineering, [3] Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854 [4] Polymers Division, Biomaterials Group, National Institute of Science and Technology, Gaithersburg, MD 20899

Statement of Purpose: This study investigates the possibility of engineering a stem cell niche for inhibition of stem cell transformation, while promoting stem cell growth necessary for regenerative therapies against cancer. Biomaterials scaffold incorporated with stem cells have been proposed for tissue regeneration therapies. However, recent studies demonstrate that mesenchymal stem cells (MSCs) have potential to become malignant due to abnormal signaling from interactions with cancerous microenvironments[1]. It is therefore desirable to design biomaterials that prevent abnormal MSCs transformation while preserving favorable performance. In this study, we developed an in vitro carcinogenic model inducing aberrant MSC transformation by metal carcinogen— nickel sulfate, and applied multi-photon imaging-based quantitative analysis of cell nuclear organization to evaluate relationships between cell nuclear organization and MSCs transformation in the context of combinatorially designed biomaterial substrates. We propose quantitative cell profiling methods for identifying biomaterials with controlled tissue regeneration potential in cancerous microenvironment.

Methods: Tyrosine-derived polycarbonates were copolymerized in various molar ratios with (i) lipophilic monomers, (ii) hydrophilic monomers, poly(ethylene glycol) (PEG), and (iii) negatively charged monomers (DT), as previously described[2]. Human mesenchymal stem cells (MSCs) were cultured on the aforementioned biomaterials coated substrate in a carcinogenic environment (basal medium containing 36uM metal carcinogen nickel sulfate) at 37°C. The abnormal transformation of MSCs was characterized by in terms of cell growth, the expression of telomerase mRNA and stemness markers, and changes in nuclear descriptors. A new high-resolution imaging-based method was developed for the multi-functional characterization of MSC responses: 1) cell nuclei were counter stained with Hoechst 33342 or Topro-3 dye, 2) Fluorescent In-Situ Hybridization was performed using Qdot-conjugated telomerase mRNA probe (Em=590nm), 3) a nuclear matrix protein, nuclear mitotic apparatus (NuMA) was stained via Immunocytochemistry, and 4) imaging was performed using Leica TCS SP2 multiphoton microscopy (Leica Microsystems Inc. Exton, PA). 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 (Image Pro Plus, Silver Spring, MD). Individual nuclear descriptors were analyzed statistically utilizing ANOVA with Tukey's post-hoc test (SPSS Inc. Chicago, IL).

Results: The treatment of metal carcinogen induced the abnormal transformation of MSCs, as indicated from by an increase in cell proliferation, telomerase mRNA expression (Figure 1), and TopoisomeraseII alpha (TopoII-α) expression. When the metal carcinogen was added to MSCs, cultured on biomaterials, we found that biomaterials differentially regulated the abnormal transformation of hMSC (Figure 2). The polycarbonates containing negative charge DT, as opposed to PEG, both promoted MSC growth and reduce abnormal transformation. Nuclear descriptors were highly sensitive to detect biomaterials type-dependent changes in the abnormal transformation of MSCs. Certain nuclear descriptors showed strong correlations with markers of abnormal transformation. For example, nuclear roundness showed an inverse correlation to telomerase mRNA expression (Figure 3). Such nuclear descriptors can potentially be applied to predict the degree of transformation and differentiation of MSCs in response to changes in biomaterial compositions.

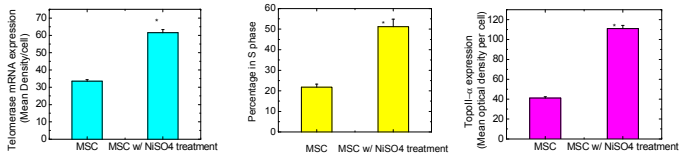

Figure 1. Comparison of Telomerase mRNA expression (left), proliferation (middle) and TopoisomeraseII-alpha (right) of MSCs cultured in metal carcinogenic environment with basal maintenance medium. *P <0.05: vs. MSCs cultured in basal medium.

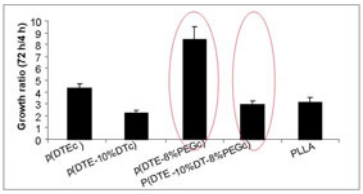

Figure 2. Differential growth behavior of MSCs in the context of various biomaterial substrates under metal carcinogen treatment.

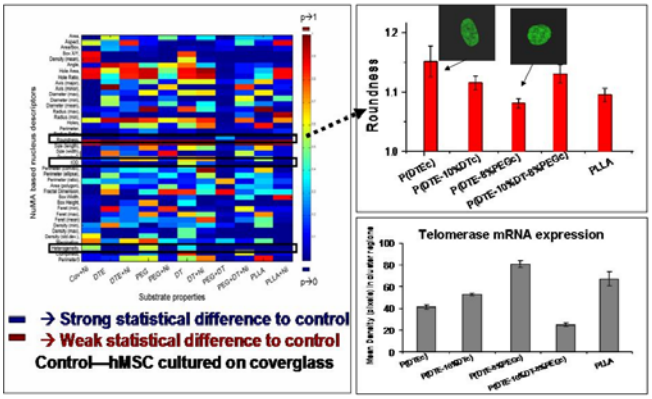

Figure 3. Left: A "heatmap" representation of statistical differences of each nuclear descriptor among different culturing conditions; Right: changes of a nuclear descriptor—roundness(upper right) and Telomerase mRNA expression (lower right) under various culture conditions under metal carcinogen treatment.

Conclusions: An in vitro system to transform MSCs was developed. Under the nickel sulfate treatment, p(DTE-8%PEG_{1k}c) increased hMSC transformation, whereas the addition of 10%DT decreased the transformation, A high-content imaging –based nuclear feature analysis enabled us to monitor the transformation of stem cells in the context of biomaterials. Our results suggested a new concept to design biomaterials for the regeneration of damaged tissues from the surgical resection of cancer. **Acknowledgments**: NIH P41 EB001046

References:

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2. Bourke, SL. Adv Drug Deliv Rev 2003(55):447-466