Investigating the Melanoma Extracellular Matrix Environment

Michael B. Fenn1, Gouchang Ye[1], Aysan Abedinpoor[1], Sudipta Seal[2,3], Soumen Das[2], Vipuil Kishore[1]
[1]Department of Biomedical Engineering, Florida Institute of Technology, Melbourne, FL 32901.
[2]NanoScience Technology Center, [3]Materials Science and Engineering, University of Central Florida, Orlando, FL 32816

Introduction: The tumor microenvironment is known to play an important role in tumor development and metastasis. Much attention has been given to the role of extracellular matrix (ECM) stiffness and composition in regards to breast cancer progression and metastasis, with important findings directing the development of new therapies and diagnostics. Yet, relatively few studies have evaluated the role of the ECM in terms of melanoma growth and metastatic behavior, much less therapeutic efficacy. This is of particular importance as the incidence of many cancers has declined in the U.S., while the incidence of melanoma has continued to rise more rapidly than any other cancer[1]. Signaling cues from the ECM have been shown to regulate growth and differentiation of malignant cells different from that of non-malignant cells in breast cancer, with factors such as matrix stiffness correlating with dramatic changes in cell morphology, migration and aggressive growth[2]. We are investigating the effect of ECM stiffness on melanoma cell proliferation and phenotype in 3D cell culture using tissue engineering techniques to fabricate substrates with defined stiffness. Investigating the role of the ECM on melanoma tumors using 3D culture will provide a better understanding of tumor formation, metastasis and treatment susceptibility.

Materials and Methods: A375 cells, obtained from ATCC, were cultured in DMEM with 10% FBS at 37°C and 5% CO_2. Uncompressed collagen gels were synthesized by mixing acid soluble monomeric collagen solution with 10x PBS and 0.1 N NaOH (8:1:1 ratio; pH 7.4) and gelled at 37°C for 1 hour. A subset of gels were subjected to unconfined compression under a load of 10 g for 5 min to form plastically compressed collagen gels (PCGs). Subsequently, a set of uncompressed gels and a set of the PCGs were then crosslinked (0.625% genipin in 90% ethanol for 24 hours) to form crosslinked gels (Xgels) and crosslinked-PC collagen (XPC). Cells were then seeded on the substrates as well as on tissue culture-treated plastic (TCTP) at $1x10^4$ cells/cm^2. Proliferation was assessed via Alamarblue® Cell Viability Assay for each of the four cell-seeded substrates at 1, 3, 5 and 7 days. In order to assess phenotype, cells were fixed with 3.7% formaldehyde at each time point, stained with AlexFluor-488 Phalloidin and DAPI, and subsequently imaged with a Zeiss Axiovert fluorescence microscope.

Results and Discussion: Chemical crosslinking using genipin and physical densification via plastic compression are apt methods for modulating collagen matrix stiffness in a controlled manner, and have been widely used for tissue engineering applications[3]. The four types of collagen substrates were fabricated such that the range of stiffness represents that found in native tissues (~100Pa-1MPa) and which was compared to TCTP (~1GPa). Cell morphology is assessed in Figure 1 (A.) and (B.) showing the actin cytoskeleton stained green and nuclei stained blue. As seen in Figure 1(A.) the cells on the gel exhibit a much more rounded morphology, whereas in Figure 1(B.) the cells on the XPC substrate show a significantly higher degree of spreading, visible stress fibers and cell-cell interaction. Increased spreading of cells on the stiffer XPC substrates is indicative of a more mesenchymal-like state, increased focal adhesion organization, and more aggressive growth, as has been seen in malignant breast cells on stiffer substrates[2]. Cell proliferation is quantified in Figure 1(C.), showing increased proliferation with increasingly stiff substrates. Again, the collagen gel exhibiting the lowest cell number after 7 days when compared to XPCs that exhibited the cell number ($p < 0.05$) of all collagen substrates at day 7, and similar to that of TCTP. **Conclusion:** These findings imply that the role of ECM stiffness, and thus ECM structure and composition, is important in melanoma tumor progression, and that more in-depth understanding of these fundamental interactions is warranted. Further studies will focus on quantifying tumorigenic markers, metastatic potential, and drug-induced apoptosis susceptibility in relation to the ECM, looking at various combinations of different ECM proteins and a wider range of stiffness for multiple types of melanoma.

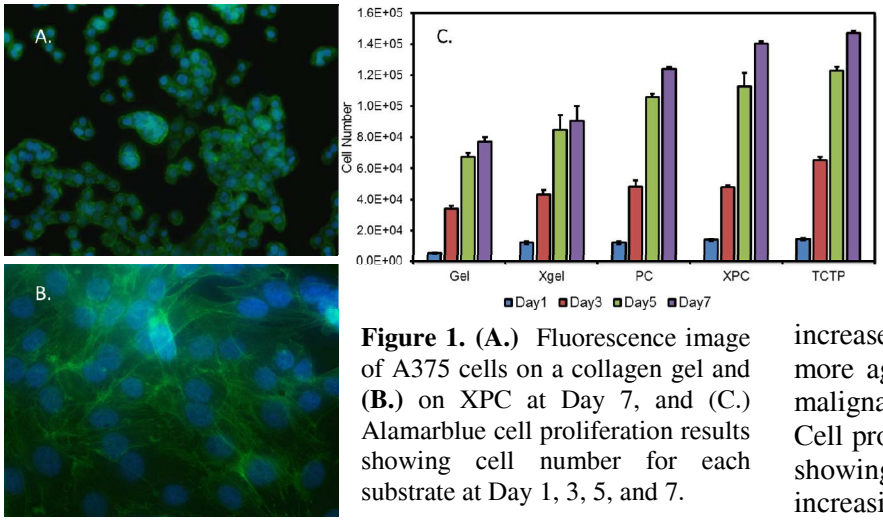

Figure 1. (A.) Fluorescence image of A375 cells on a collagen gel and **(B.)** on XPC at Day 7, and (C.) Alamarblue cell proliferation results showing cell number for each substrate at Day 1, 3, 5, and 7.

References: 1.) Mayer, J. E., (2014) *J. Am. Ac. Derm.*,*71*(4), 599-e1. 2.) Butcher, D. T., (2009) *Nat. Rev. Cancer*, 9(2), 108-122. 3.) Brown, R., (2005) *Adv. Func. Mat.* 15(11), 1762-1770