Design of Bone Microenvironment Mimicking Antibiotic-based Hydrogels for Generation of Three Dimensional Tumor Models of Dormancy and Relapse

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Abstract: Prostate cancer cells are known to undergo prolonged periods of dormancy in bone microenvironment. Disseminated tumor cells shed from the primary prostate disease even before it is detected and removed. Upon arriving at the bone they remain dormant in the bone microenvironment for extended periods of time before relapsing to cause metastasis. The exact mechanism triggering the relapse still remains unknown. We designed novel antibiotic-based hydrogel (Amikagel) to mimic the bone microenvironment and studied the response of prostate cancer cells on them. Antibiotic and cross linker gave rise to a new hydrogel (Amikagel) by simple mixing at the room temperature. The mechanical properties of Amikagels were tunable to match the mechanical stiffness of trabecular regions in the long bones (site of metastasis). Amikagels prepared were extensively characterized for their chemo-mechanical properties. Culture of cancer cells on Amikagels gave rise to three dimensional tumor microenvironments (3DTMs) with interesting properties such as nerosis, hypoxia and dormancy. Total dormancy was detected in epithelial prostate cancer cell line 22Rv1 which reversed upon transfer to trabecular region mimicking Amikagels.

Methods: Different quantitative weight ratios of antibiotic and the cross-linker were dissolved in Nanopure® water, mixed and incubated at 40°C for 7.5 h in an oven to obtain Amikagels of different compositions. Three different Amikagels (AM1, AM2 and AM3) were generated during the procedure. They were washed to get rid of remaining Antibiotic from the gel. To generate 3DTMs, liquid-overlay cultures were set up by either plating 100,000 cancer cells alone or by first plating 50,000 feeder fibroblast/stromal cells and then adding 50,000 cancer cells upon them. Cell culture media was refreshed every day. 3DTMs were collected on day 7 and analyzed via Scanning electron microscopy, cell cycle analysis, H&E staining and actin staining etc. 3DTMs were also transferred to weaker Amikagels (AM2) that mimicked trabecular region of bone after 7 days.

Results: Non-adhesive amikagel AM3 supported co-cultures of various cancer cells with stromal/stellate cells resulting in the formation of single spheroidal 3DTM per well. Scanning electron microscopy images of 3DTMs clearly showed the presence of extracellular matrix around the 3DTM (Figure 1a). Cell cycle analysis on prostate epithelial cancer 22Rv1 3DTMs generated on amikagels revealed a total arrest of cells in G0/G1 phase of cell cycle indicating cellular quiescence (Figure 1b). Chemotherapeutic insult with docetaxel and mitoxantrone resulted in minimal cell death even at very high doses of 100 μM (Figure 1c). Once the dormant 3DTM was transferred to weaker Amikagel (AM2/AM1), cells were seen to leave the dormant 3DTM. Shed cells also formed microcolonies in the gel as seen in (Figure 1d).

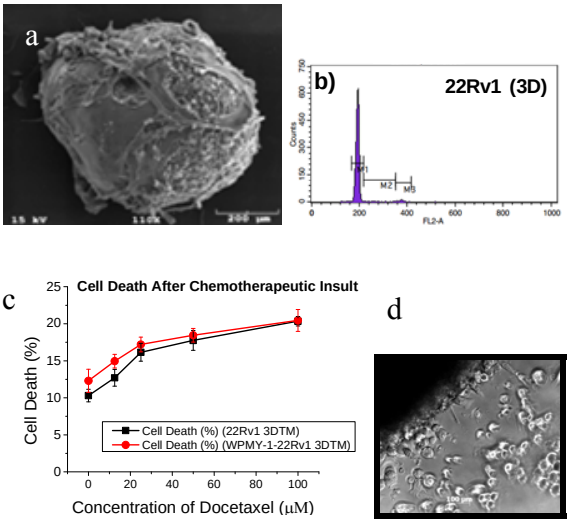

Figure 1. a) Scanning electron microscopic image of NIH3T3-22RV1 spheroid. b) Flow cytometry-PI staining revealed cell cycle arrest in G0/G1 phase of cell cycle. c) Cell death(%) studied by MTT assay after docetaxel treatments for 96 hours on 3DTMs. d) Cells spreading out of 3DTM when transferred to AM2 gel.

Conclusions: Antibiotic and cross-linker gave rise to a novel hydrogel with tunable chemo-mechanical properties. High-throughput 3DTM culture across multiple cell lines is possible on Amikagel platform. 3DTMs cultured on AM3 Amikagel exhibited interesting cellular properties such as cellular dormancy as shown in 22Rv1 3DTM.Dormant 22Rv1 3DTMs generated on these gels were found to be highly resistant to traditional chemotherapy. Escape from cellular dormancy was seen upon transferring the dormant 3DTM to trabecular bone like hydrogel. Presence of microcolonies was also noticed on the trabecular bone like hydrogel after initial spreading from the dormant spheroid.

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