Modified Layered Agarose Gels for the Comet Assay to Evaluate Laser DNA Damage

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Statement of Purpose: We have developed a laser micropatterning technique to precisely control the position of multiple cell types in culture to investigate cell-cell interactions. The technique employs laser guidance that radially attracts a cell into the center of the weakly focused beam and axially guides the cell to a surface. Proving the method to be effective is significant; however, it is equally vital to show that cells are viable following the treatment. Although previous investigations have been conducted to assess viability following alternate forms of laser manipulation of cells and tissue, it must be noted that these investigations have demonstrated effects of laser trapping that employs a tightly focused laser beam with less intensity to manipulate cells. The change in focus of the beam creates a different range of motion for the cell to undergo in patterning, which generates a need to identify the viability effects of laser guidance as an independent situation. Based on previous work, it is unlikely that the damage due to a weakly focused beam is a photothermal effect but rather has effects stemming from mechanical stress and electrical stimulation, especially for electrically responsive cells types. [Mohanty] Previous research in our lab, has shown no significant difference between irradiated cells and non irradiated cells in a near proximity to one another. [unpublished data] However, repairable DNA damage could be occurring as a result of laser irradiation. The Comet assay has been used to test the DNA damage experienced by individual cells. However, to assess specific individual cells that have been subjected to a potential DNA damaging agent, such as laser irradiation, modifications were necessary.

Methods: The conventional Comet assay uses a microscope slide to hold a layered gel sandwich composed of an agarose gel, a second gel layer with embedded cells that have been subjected to a DNA damaging source, and a third gel layer. Following gel solidification, the cells are lysed, unwound, and electrophoresed to pull the broken DNA strands away from the nucleoid. When imaged this creates the appearance of a comet wherein the individual cells are represented by a head (nucleoid) and tail (broken strands). [Singh] The main limitations of using the standard Comet assay to evaluate specific individual cells are geometric compatibility and tracking specific cells. Geometric constraints and specific cell tracking forced modification of the original slide format to a gridded coverslip attached to a Poly Dimethyl Siloxane (PDMS) membrane. This modification allowed specific cells to be manipulated by the laser without diffraction of the laser beam by the gel and allowed the cells to be recognized via the grid after laser interaction. To recreate the effect of the cell suspension in agarose, cells were patterned on the first gel layer, the second gel layer was added on top of the cell media layer, and cells were repositioned from slight shift due to motion. Following patterning, the gel was removed from heat to allow solidification and a third layer of gel was added to create an agarose sandwich. [See Figure 1]

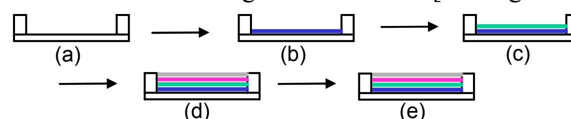

Figure 1. (a) Modified chamber composed of a PDMS membrane with a slide affixed to the base (b) 1% LMPA for base layer covered with PDMS microholed stencil (c) Cell media layer (d) Second layer of gel to blend with cell media layer (e) Final 1% LMPA layer.

The slick agarose texture inhibited cell patterning along the base layer so the surface was textured using a PDMS stencil with microholes prior to gel solidification. When the base solidified, the PDMS stencil was removed and cells were added in media suspension prior to adding gel to prevent diffraction of the laser beam by solidified agarose. The second gel layer was added after laser prepositioning of cells and maintained as a liquid to allow laser repositioning of cells. Following pattern completion, gels were cooled to allow solidification and a third gel layer was added to complete the agarose sandwich.

Results/Discussion: Experiments confirm that positive and negative controls generate damaged and undamaged samples [See Figure 2] proving that the modifications of the COMET assay produce results comparable to prior investigations [Collins]. Cells have also been patterned to reveal the damage due to laser micropatterning.

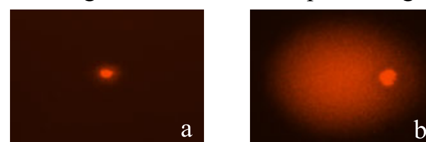

Figure 2. Images of neurons after the comet assay showing a) negative control exposed to no laser irradiation, b) positive control depicting damage following 5 minutes of UV irradiation

Conclusions: To complete assessment of laser DNA damage, the modified Comet assay should be applied for increasing ranges of exposure time and laser intensities. The modifications made to the Comet assay allow not only the evaluation of DNA damage in laser micropatterned cells but also the evaluation of any specific individual cells to be tracked for DNA damage.

References:

[1] Mohanty SK. Radiat Res. 2002; 157:378-385
[2] Singh NP. Exp Cell Res. 1988; 175:184-191
[3] Collins AR. Mol Biotechnol. 2004; 26:249-261