NMR-based Metabolomics Study of Kidney (HEK 293T) Cells Cultured on SAM Coated Indium Tin Oxide (ITO) Glass Substrates

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Abstract

The use of glass substrates to enhance cellular adhesion exemplifies the principles of green and sustainable chemistry in practice. Upcycling waste glass into a valuable resource mitigates environmental pollution, promotes innovation, and conserves resources. The Human embryonic kidney293T (HEK293T) cells have been used in organoid formation and specialized applications within organoid systems; however, they often suffer from loose adherence, which limits their applicability. To improve the HEK 293T cellular adhesion and proliferation and to provide insights into the pathways involved in adhesion, HEK 293T cells were cultured on tissue culture plastic and glass substrates with surfaces modified with various self-assembled monolayers (SAMs). The ITO-MPS SAM-coated glass substrate gave the most promising improved results. This study provides the 1D and 2D NMR spectroscopy metabolomics analysis of HEK293T cells cultured on ITO-MPS substrate. The ITO-MPS substrate was characterized by FTIR, XPS, SEM, and Contact Angle Measurement. The proliferation of the cells was measured using the MTT Assay. These findings provide valuable insights into optimizing substrate conditions and highlight novel metabolomic changes associated with improved HEK 293T cellular adhesion. As researchers persist in studying the potential of glass substrates, we may unveil novel applications and prospects that transform diverse fields. Eventually this circular economy concept will facilitate a more sustainable future in which waste is transformed into a valuable resource.

Concept

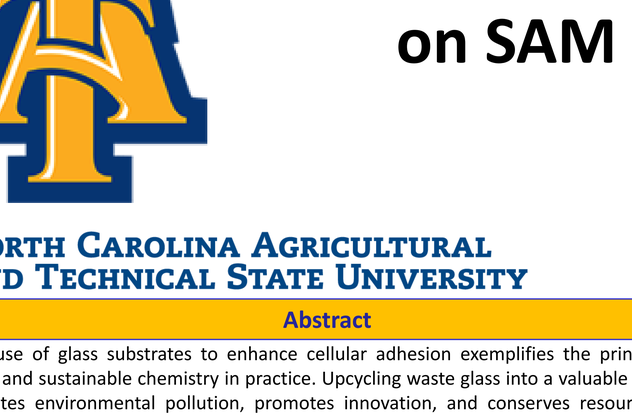


Fig 1. a) Scheme for ITO-MPS SAM Preparation on glass slides. b) Sample Collection for Spectroscopic Analysis of HEK 292T cells cultured on ITO-MPS SAM Substrate.

Applications

- ❑ Biomedical research
- ❑ Tissue engineering
- ❑ Regenerative medicine
- ❑ Surface Chemistry

Relation to Green Chemistry

This is a new method that uses the modification of the surface qualities of glass to create conditions that promote cell adhesion and proliferation, significantly impacting biomedical research, tissue engineering, and regenerative medicine. Glass is among the most used materials worldwide, and the production process yields substantial waste, mainly as cullet, which consists of broken and recycled glass. Nonetheless, glass waste frequently ends up in landfills or incinerators despite its recyclability, exacerbating pollution and environmental deterioration. In cellular adhesion, we create self-assembled monolayers on glass substrates and employ them in research to enhance cell adherence and proliferation, optimizing the use of the glass. This practically demonstrates the principles of green and sustainable chemistry. Transforming discarded glass into a valuable resource reduces environmental pollution, fosters creativity, and preserves resources.

Results/ Discussion

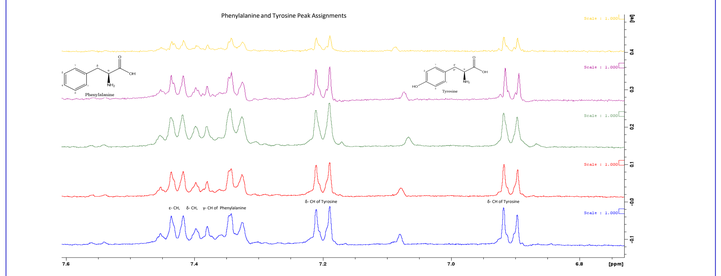


Fig 2. 1D ^{1}H NMR spectrum showing the changes in the concentration of Histidine metabolite in HEK 293T cell-media sample over a period of five days.

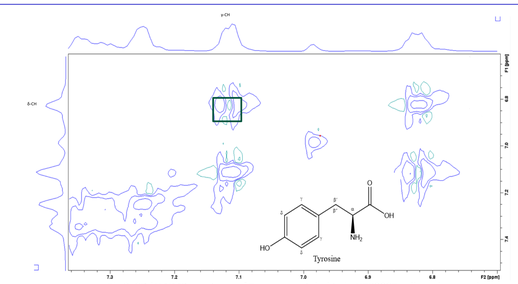


Fig 3. 2D ^{1}H NMR spectrum of Tyrosine metabolite in HEK293T cell-media sample.

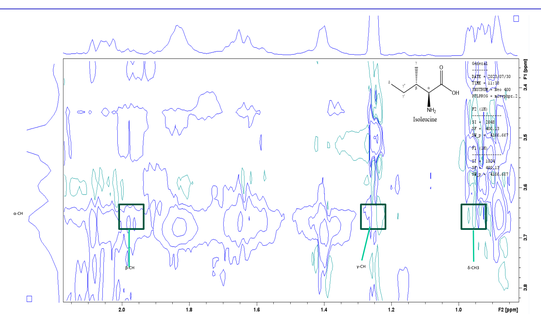


Fig 4. 2D ^{1}H NMR spectrum of isoleucine metabolite in HEK293T cell-media sample.

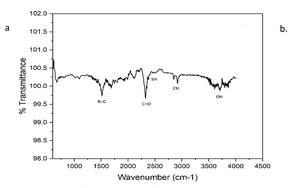


Fig 5. FTIR images of ITO-MPS as confirmatory SAM test results.

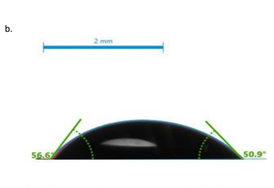
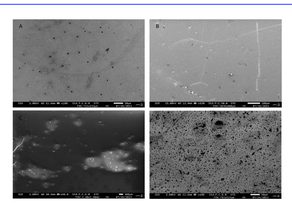


Fig 6. Contact angle measurement of ITO-MPS SAM



The Scanning Electron Microscope (SEM) images show the different substrates used for the MTT proliferation assay (Fig 7).

Fig 7. SEM images of a. ITO-MPS b. ITO c. ITO-APTES and D. ITO-ODT.

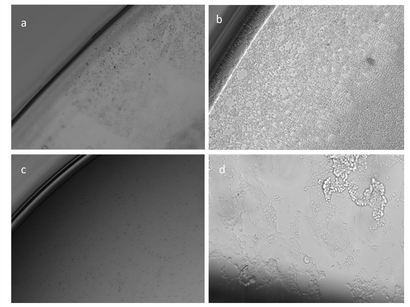
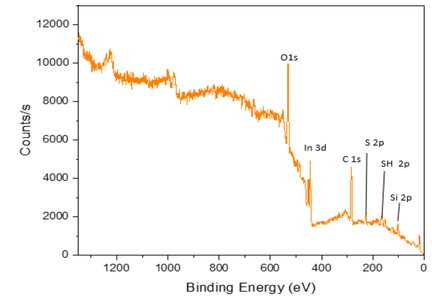


Fig 8. Confocal Microscope images of HEK 293T cells on a. ITO b. ITO-MPS SAM c. MPS and d. TCP substrates

The X-ray photoelectron spectroscopy (XPS) result (Fig 9).



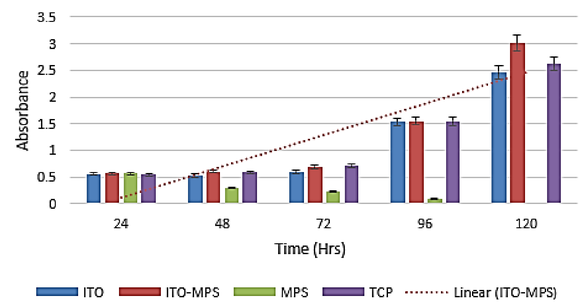


Fig 10. MTT assay showing proliferation of HEK 293T cells on various substrates.

Conclusion

❑The MTT assay showed that the ITO-MPS glass coated substrate best supports HEK 293T proliferation. NMR based metabolomics study is underway, however we have observed the presence of Histidine, Isoleucine, and Tyrosine metabolites in the sample. This provides foundational information about the cell line before proceeding to utilize it for our nephrotoxicity microfluidic studies. This also proves that glass can be utilized in the improvement of cell adhesion and proliferation studies, when modified with self assembled monolayers. This is a significant contribution to green chemistry as glass waste can be remediated into something valuable.

Future directions

❑Further analysis on the NMR-based metabolomics results to identify the metabolites that are biomarkers of kidney diseases..

❑ Raman spectroscopy analysis.

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