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Design verification and enhanced risk mitigation for cytocompatibility evaluation of cell delivery devices

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Abstract

The impact of medical device design on the delivery of cell-based therapies requires further investigation. Here we present the results of an in vitro panel of tests designed to evaluate several parameters of device compatibility with cell-based therapies, which we define as device cytocompatibility are presented. Parameters included: cell recovery, immune activation, cell viability, and cell metabolic activity. The results of these assays suggest device cytocompatibility may need to be added to standard lot release testing to ensure safe and effective delivery of therapeutic cells.

Introduction

Device cytocompatibility in the delivery of cell-based therapies is not currently a component of device lot release testing or the regulatory approval processes. In order to more fully understand the potential impact a device may have on the therapeutic cells it delivers, a panel of in vitro tests was developed. Human embryonic cells transfected with Toll-like receptors (TLR2 or TLR4) and a human monocytic cell line (U937) were selected for these device cytocompatibility studies (1-2). TLR cells evaluate cell surface receptor modulation and consequent changes in downstream signaling pathways that could occur after cells are delivered (3-7). Human monocyte cells examine potential toxicity of substances released from the device (8-12). Taken together, data from these tests represent alterations that arise in therapeutic cells when delivered with a device.

Objectives

To develop tests to verify the cytocompatibility of cell delivery devices using TLR and monocytic cell(s). Cytocompatibility parameters investigated include:

1. Immune activation: Ensure that cell delivery through a device avoids extracting substances that could trigger an immune response.
2. Cell recovery: Ensure that cells do not adhere to the device in an amount such that they compromise the intended dose for therapy.
3. Cell viability: Ensure that cells are intact and not entering apoptosis following delivery through the device.
4. Cell activity: Ensure that cells maintain their metabolic activity.

Methods

Devices were exposed to complete cell culture media for exaggerated periods of time (>24 hours) under static conditions (37°C, 5% CO_2) to extract potential mediators of cytocompatibility. Another group of devices were exposed to media for a shorter period of time, simulating IFU use of the device for cell delivery. Control samples were incubated similarly. The device and control samples were then exposed to the cells and assayed for cytocompatibility. TLR response was measured using a reporter enzyme system. Cell recovery was measured via haemocytometer count. Cell viability via fluorescence activated cell sorting (FACS) utilized two apoptotic dyes. Cell metabolic activity was determined using a PrestoBlue® spectrophotometric assay.

Methods

Figure 1. Example cell delivery devices, accessories, and cell processing supplies tested for cytocompatibility.

Results

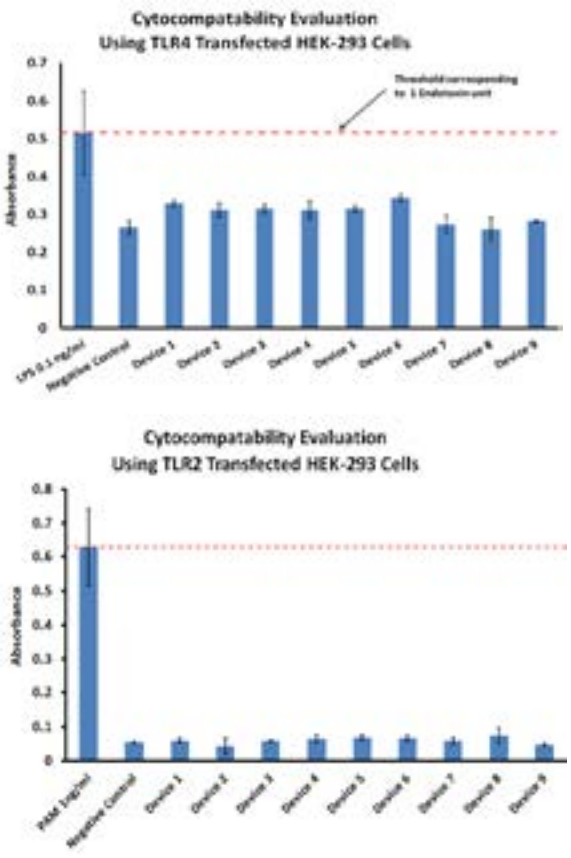

Figure 2. Cytocompatibility evaluation of selected devices using Toll-like receptor-expressing HEK-293 cell lines. Quantitation of an NF-kB-inducible secreted alkaline phosphatase reporter gene was performed following explosure.

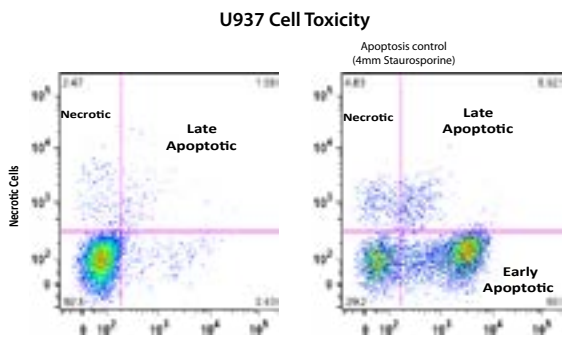

Figure 3. Cytocompatibility evaluation of devices using a FACS-based apoptosis assay that can distinguish dead and dying cells from the surviving cell population.

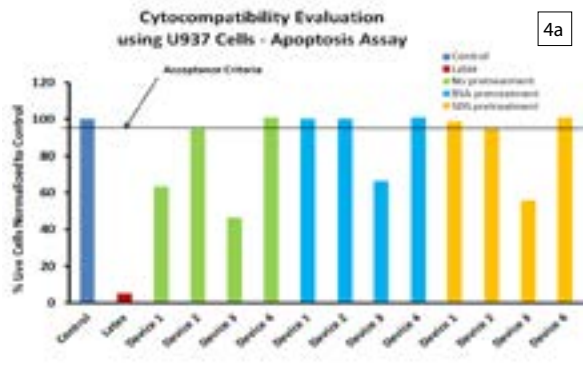

Figure 4: Evaluation of viability and metabolic activity of U937 cells after exposure to extracts from exaggerated media contact with an example delivery catheter. 4a) Cell viability, it can be seen that 4 devices failed this test. 4b) Metabolic activity, it can be seen that 3 devices failed this test.

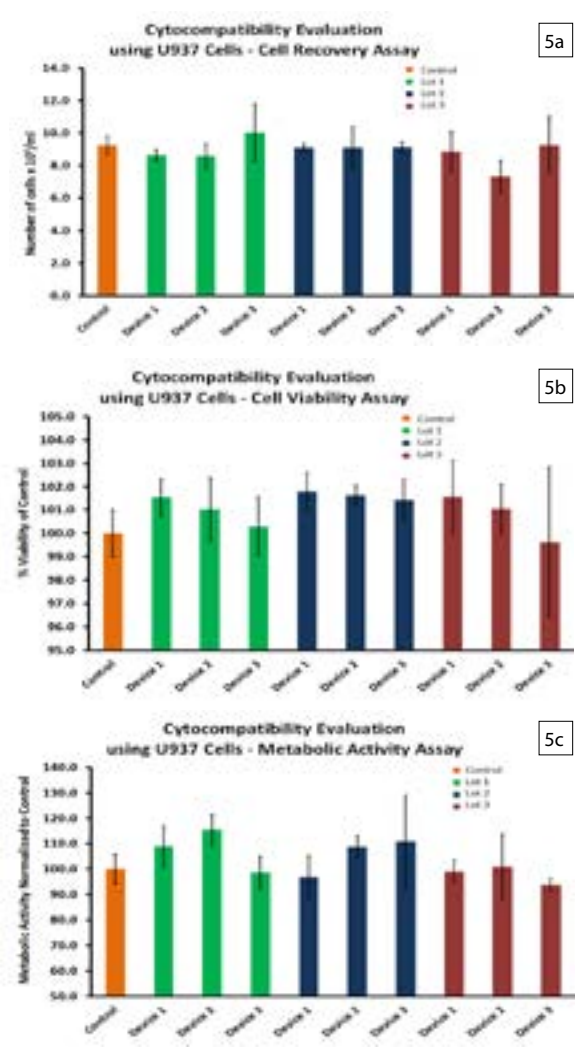

Figure 5: Evaluation of of U937 cells after exposure to extracts from IFU contact with an example infusion needle. 5a) Cell Recovery 5b) Viability and 5c) Metabolic activity.

Conclusions

- Tests using human cell lines were developed to assess cytocompatibility for a wide variety of cell delivery devices.
- Cytocompatibility testing may be used as part of design verification or for lot release testing.
- Manufacturing method, processing aides, materials, device and component level design can influence cytocompatibility results.

References

1. HEK-Blue hTLR4 cells technical reference. InvivoGen. Version #12I12-MM.
2. HEK-Blue hTLR2 cells technical reference. InvivoGen. Version #12C15-MM O'Neill et al. Nat. Imm. 7:353-364, 207.
3. Huang et al. J. Clin. Micro. 47(11):3427-3434, 2009.
4. Chen et al. Biomaterials. 31:8172-8180, 2010.
5. Shokouhi et al. 31:5759-5771, 2010.
6. Shoenfelt et al. J. Leu. Bio. 86:303-312, 2009.
7. Heng et al. Cell Trans. 18:1111-1121, 2009.
8. Polaschegg et al. Sem. In Dial. 22(5):524-531, 2009.
9. Arora et al. Artif. Org. 28(11):1002-1015.
10. Minafra et al. Leuk. Res. 35:226-236, 2011.
11. Liu et al. Hepa. Pan. Dis. Int. 4:84-89, 2005.
12. Hajighasemi et al. Res. J. Bio. Sci. 7:209-214, 2012.

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