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RESEARCH ARTICLE

Assessing the suitability of DVD discs for cell growth and differentiation

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Abstract: ***Background***: In vitro experiments have demonstrated that cell alignment by underlying topographical cues influences crucial biological processes like differentiation and functional maturation.Optical media, such as CD-R, DVD-R, and optical grating, provide good surfaces for many cell types to adhere to and develop.The physical dimensions of the grooves in these optical media allowed for confluent cell culture with maximum cell cell contact. ***AIM:*** The aim of this study is to assess the suitability of DVD discs for cell growth and differentiation. ***MATERIALS AND METHODS***: For culturing osteoblast cells, the DVDs were processed with 70% ethanol for one hour and then autoclaved at 121 C for 15 minutes. Micrographs of data-written and data-unwritten DVD images were scanned. Measurements of the water contact angle on DVD surfaces that had been exposed to oxygen plasma and air at various times were measured. ***RESULTS AND DISCUSSION***: It was observed that in light microscopy, Tissue Culture Plates (TCP) have a random orientation, whereas DVD discs have an anisotropically oriented architecture. In fluorescent microscopy, in TCP, the cells have a random orientation, whereas DVD discs have an isotropically aligned architecture.Commercial optical media are designed for spectroscopy and data storage, thus it is crucial to prepare them so that they are compatible with cell culture. ***CONCLUSION:*** This study concludes that DVDs can be used for these investigations and applications as scalable cell alignment substrates with micro- and nano-topography and is suitable for cell growth and differentiation.

Keywords: DVD, cell growth, optical media, osteoblast cells, proliferation, groves .

INTRODUCTION

The corresponding extracellular matrix (ECM) in the basement membrane provides various topographical aspects to cells in several organs.The basement membrane is made up of elements of the extracellular matrix (ECM), including glycosaminoglycans, fibrous proteins like fibronectin and collagen, growth factors and cytokines bound to ECM fibres, hyaluronic acid, laminin, etc. These elements exhibit distinctive nanometer-scale features such as pores, fibres, and ridges.These ECM molecules are arranged in a way that provides morphological and differentiation cues to the cells that are resting on top of them(Lechleitner *et al.*, 2008). A good example is the heart in vivo, which is a highly anisotropic organ and in which the parallel arrangement of the collagen fibres causes the cardiomyocytes to align. (Balda and Matter, 2003) Presenting topographical features to cells in vitro can also have an impact on cellular morphology and differentiation abilities, much as these topographical cues influence biological processes in vivo. ECM molecules can be micropatterned into line geometries to align cells in vitro or grooves and ridges can be made on surfaces to do the same thing.

When using a substrate to cultivate living cells, the surface characteristics are crucial. The interconnectors between cells and the extracellular matrix (ECM) or the surface of a cell growth support are represented by integrins and other cell-adhesion molecules. They start signalling processes that define the cellular phenotype and differentiation state.The effectiveness of cell attachment, development, and ultimately differentiation is influenced by the micro- and nano-roughness, polarity, and surface charge distribution of a substrate.

Important biological processes including differentiation and functional maturation have been shown to be impacted by cell alignment by underlying topographical signals in vitro. However, the high cost and specialised infrastructure needed to make these substrates now prevent their frequent usage as cell culture substrates with micro- or nano-topographies, such as grooves. (Khang *et al.*, 2008) Different cell types can connect to and grow successfully on optical medium, such as CD-R, DVD-R, and optical grating.Confluent cell cultures with maximum cell-cell interaction were made possible by the physical dimensions of the grooves in these optical media, and this cell alignment had an impact on the morphology and differentiation of heart (H9C2), skeletal muscle (C2C12), and neuronal (PC12) cell lines. For the purpose of cultivating distinct cell types, the optical media is accessible to a variety of chemical changes using fibronectin, laminin, and gelatin.(Zutter, 2007) These readily available, low-cost optical media can be used on an industrial scale as scalable substrates for research or drug safety screening applications.

According to a prior study, easily available commercial optical media like CD-R, DVD-R, and optical gratings provide a source of nano/micro-grooved substrates that meet the needs of aligning cells in an affordable way. Seven 24-well plates' worth of micro-grooved cell culture inserts can be created from a standard-size CD-R or optical grating, which costs between $1 and $5 USD. Cell culture has been demonstrated to be supported by the optical media's polycarbonate (CD-Rs and DVD-Rs) and polyester (optical gratings) components.The dimensions are within the range that has been documented for in vitro cell alignment. (Anene-Nzelu *et al.*, 2013) Previous studies have provided an optical detection system for single biological cells that uses a normal DVD pickup head.The detection of various particles and cells (yeast) suspended on a platinum mirror was observed and a remarkable sensitivity was discovered(Kostner and Vellekoop, 2008). Although some types of optical media have been utilised in the past to shape other polymers for cell culture ,direct cell culturing and alignment on these optical media have not been documented.The purpose of this study was to discover the typical surface qualities that are best for cell adhesion and proliferation as well as the acceptability of these biomaterials as a support for cell proliferation.

MATERIALS AND METHODS

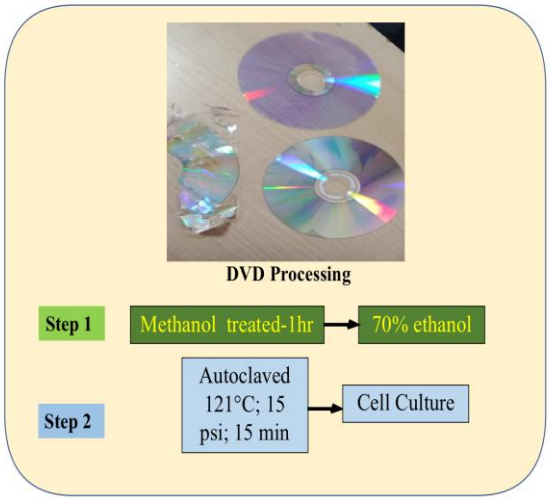

Figure 1: Flow diagram of the method employed use DVD discs for culturing osteoblast cells

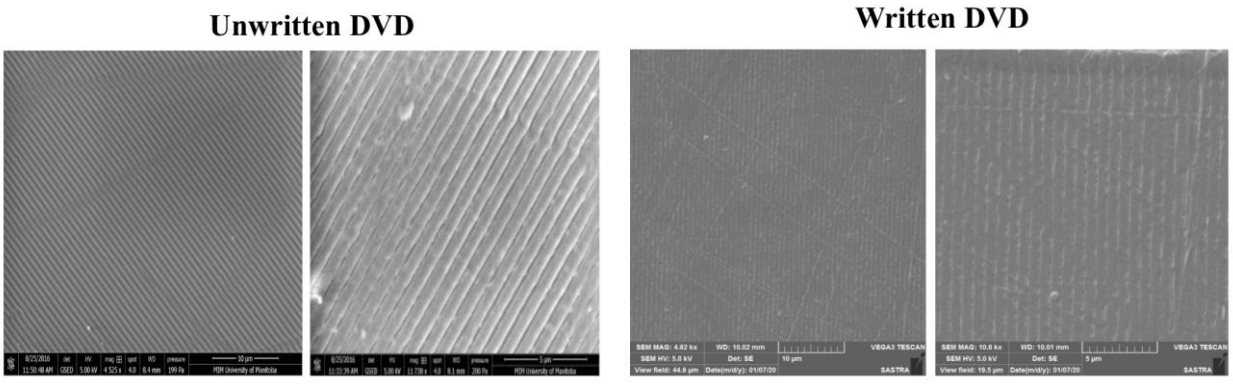

Figure 2: Scanning micrographs of unwritten and data written DVD images. Writing data onto the DVD disrupts the nanostructured channels

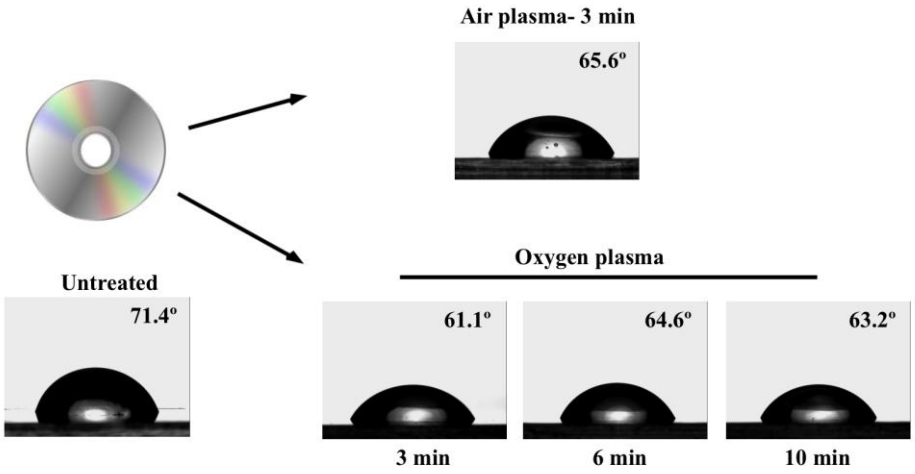

Figure 3: Water contact angle measurements of DVD surfaces treated with air and oxygen plasma for different time points

RESULTS

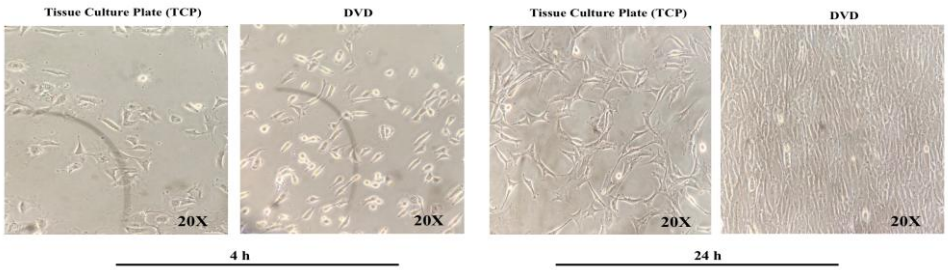

Figure 4: Light microscopic images of human osteoblastic cells grown on normal tissue culture plate and DVD discs. The cells show a random orientation in TCP, while anisotropically aligned morphology is observed in DVD discs.

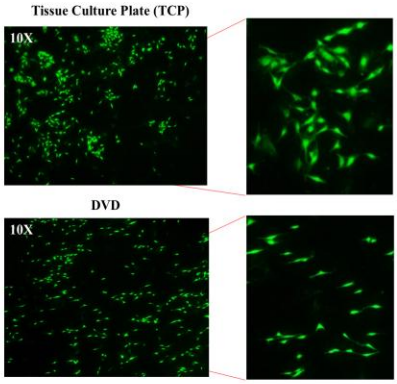

Figure 4: fluorescent microscopic images of human osteoblastic cells grown on normal tissue culture plate and DVD discs. The cells show a random orientation in TCP, while an isotropically aligned morphology is observed in DVD discs.

DISCUSSION

In this study, in light microscopic images of human osteoblast cells grown on normal tissue culture plates and DVD discs, it was observed that Tissue Culture Plates (TCP) have a random orientation, whereas DVD discs have an anisotropically oriented architecture. In fluorescent microscopy, in Tissue Culture Plates (TCP) , the cells have a random orientation, whereas DVD discs have an isotropically aligned architecture.

Commercial optical media are designed for spectroscopy and data storage, thus it is crucial to prepare them so that they are compatible with cell culture. In order to expose the micro-groove polymeric substrate, any metal coatings and organic dyes must be removed from the optical media. In a previous study, a metal stamper is used to manufacture the spiral pre-groove seen in the CD/DVD. The pre-groove aids in directing the laser beam during data writing and reading. This pre-groove

has been used by us for cell culture and alignment (Kearns et al., 2012).

In a previous study, it was observed that while there was no clear differentiation in growth between the flat and grooved surfaces for CD-R and DVD-R, the grooved surface of the optical grating greatly inhibited cell proliferation(Aubin et al., 2010). The effects of cell alignment on cell development have been documented in conflicting ways in earlier investigations. Researchers found no actual difference in fibroblast proliferation between aligned and unaligned cells on electrospun polymeric fibres in various trials(Lee et al., 2005). However, other researchers have discovered a considerable decrease in the proliferation of cells grown on micro-grooved surfaces and electrospun polymers that have been aligned, reportedly as a result of a restriction on cell spreading during cell alignment (Bettinger et al., 2008). Cells grown on the flat control substrates seemed to have no particular orientation, but those grown on the grooved surfaces exhibited a predominance of spindle-shaped morphology and were orientated in the groove direction.

Another study found that the outcome was consistent for cells grown on CD-R, DVD-R, and PET gratings, confirming the idea that topography was a key factor in alignment.Another study found that MHC 1 and MHC 4 were much more upregulated on DVD-R than CD-R (Bettinger et al., 2008; Ricotti et al., 2012). This could be because of the groove dimensions, as prior research showed that nano groove dimensions were more effective at promoting myoblast development than microgroove dimensions. In a different study, it was found that cells grow into bipolar morphologies on gratings and multipolar morphologies on flat surfaces. We found that the groove width affected how topographical signals affected polarity selection. On the DVD-R, there were more bipolar cells than there were on the CD- R's cells (Cecchini et al., 2007).

CONCLUSION

In this study, it can be shown how optical media such as DVDs can be used for these investigations and applications as scalable cell alignment substrates with micro- and nano-topography. DVD discs have a vast surface area, can cultivate a variety of cell types, are inexpensive, and are optically transparent, making them ideal for imaging-based tests. In the future, we can see optical media being further utilized to quickly broaden the scope of research or commercial applications.

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