Medical Grade Collagen in Cell Culture Applications

Saoirse Murray O'Neill[1], Richard Ellson[1], Beatriz Lopes[2], Fiona O'Neill[1,2], Paula Bourke[2], Liam O'Neill[1] and Bryan Hoadley[1]
1. TheraDep Inc., Palo Alto, CA, USA | 2. School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland

OVERVIEW

TheraDep is introducing medical grade collagen coated microplates for cell culture applications. This bovine material leverages decades of surgical applications and will facilitate both research use and the rapid translation of cell culture research and cellular therapies into medical device applications. Combining the benefits of structurally intact triple helix and medical grade purity may also provide enhanced benefits *in vitro*.

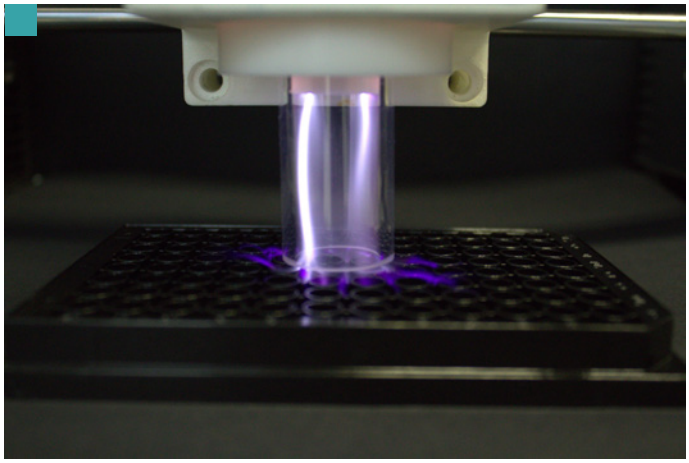

Figure 1. BioDep plasma process for rapid coating of microplates with proteins

INTRODUCTION

Medical grade collagen requires high levels of purity and can be supplied with the native triple helix structure intact[1,2]. This source has been used in over 40 million medical procedures to date. Incorporating this collagen into cell culture trials should allow for rapid and reproducible translation into medical device development.

Additionally, the high quality of the collagen may impact cellular responses *in vitro* and lead to improved cell attachment, proliferation and consistency with benefits in the research arena.

This study will investigate this hypothesis.

MATERIALS AND METHODS

Medical grade collagen was supplied by dsm-firmenich (Exton, PA, USA). Polystyrene microplates from two separate vendors were coated using the BioDep plasma coating process[3,4] using the solubilised medical grade collagen. Commercial rat tail collagen coated 96 well polystyrene microplates were purchased from both commercial vendors (Vendor A and Vendor B).

Coated plates were compared using various assays (MTS, Resazurin, crystal violet) to monitor cell mass and cell metabolic rates over time. Chemical structure was probed using atomic force microscopy (AFM).

RESULTS

Structural studies performed to date are consistent with the retention of the triple helix structure in the coated substrates. AFM studies of protein layers applied under identical conditions show considerably higher roughness of the medical grade collagen than either of the rodent collagens. This can be attributed to the retention of the three dimensional protein structure in the medical grade material. DSC studies also suggest that the triple helix is retained in the medical grade collagen.

Collagen	Roughness (nm)
Medical Grade	20.2
Vendor A	3.6
Vendor B	7.5

Table 1. Roughness of various collagen layers

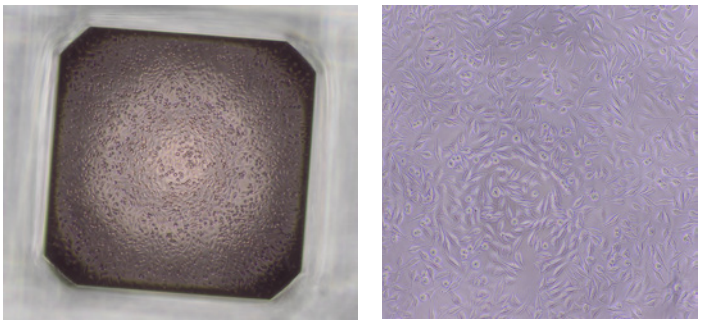

Figure 2. L929 cells growing on medical grade collagen inside a coated 1536 well plate (left) and 96 well plate (right)

Preliminary cell studies using monocytes showed increased proliferation on the medical grade collagen.

Further studies conducted using HUVEC cells showed similar results, with the medical grade materials providing higher levels of cell mass when seeded with either 5,000 or 10,000 cells.

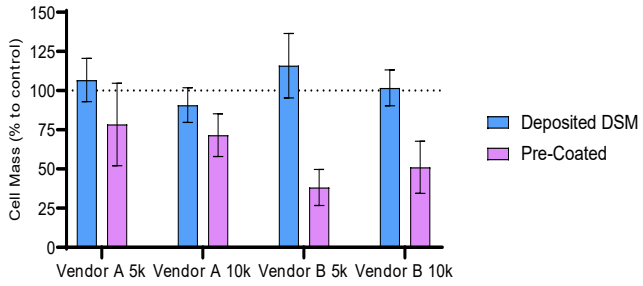

Figure 3. Crystal violet assay conducted at 48 hours using HUVEC cells suggesting improved cell mass and increased consistency on medical grade collagen

Metabolism assays with HUVEC cells showed significant variation in the commercially produced microplates, indicating high well to well variability. However, the combination of the BioDep process and high quality medical grade collagen produced more repeatable measurements indicating higher well to well consistency.

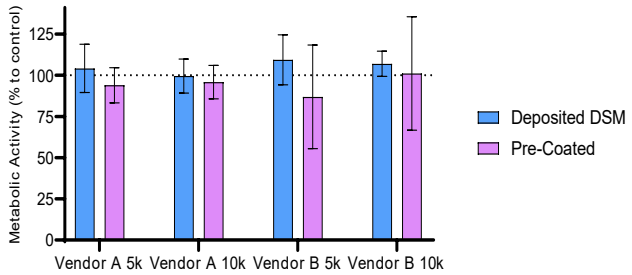

Figure 4. Metabolic (Resazurin) assay conducted at 48 hours using HUVEC cells showing differences in consistency

CONCLUSIONS:

- Medical grade collagen was effectively applied to the microplates using the BioDep process.
- The collagen enhanced cell proliferation consistency across multiple cell lines.
- The combination of medical grade collagen and the BioDep process reduced well to well variability.

REFERENCES:

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