

## EFFECT OF EXTRACELLULAR MATRICES ON CULTURED HUMAN RENAL PROXIMAL AND DISTAL TUBULAR CELLS

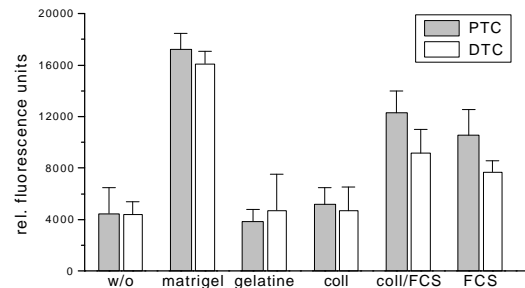
[Patrick C. Baer](#), & Helmut Geiger

*Medical Clinic, Nephrology, [J.W.Goethe University](#), Frankfurt/M; Germany*

**INTRODUCTION:** Chronic renal failure is a basic problem which is currently far away from a satisfactory medical treatment. With the demonstration of experimental success with animal renal cells [1], the aim of this project is to develop functional human kidney equivalents for the replacement of tubular function during chronic renal failure. Therefore, the effects of extracellular matrices (ECM) on the attachment and proliferation of primary human renal cells were examined in order to use tubular cell monolayers cultured in precoated hollow fibers as bioartificial renal tubular devices.

**METHODS:** Human renal proximal and distal tubular epithelial cells (PTC / DTC) have been isolated immunomagnetically and cultured in medium 199 with 10% FCS [2-4]. 96-well-plates were precoated with gelatin (1%), matrigel (50µg/ml), collagen IV (50µg/ml), collagen IV+FCS, FCS, or PBS (as a control) for 1 hour. The liquids were removed and washed three times with PBS. 2000 cells were seeded and cultured for 96 hours. A rapid non-radioactive fluorescence assay (DAPI, 4,6-diamino-2-phenylindole) for the measurement of both cell number and proliferation was used [5]. Data are represented as relative fluorescence intensity (means  $\pm$  SD, n=6). Alterations in per cent are calculated versus PBS-“precoated” wells.

**RESULTS:** The data from the DAPI-assay are summarized in Fig.1. The highest cell numbers were observed after matrigel-precoating (PTC +285%, DTC +167%). Gelatin- and collagen IV-precoating resulted in no significant higher cell numbers after 96 hours of culture, whereas a significant increase by precoating with collagenIV+FCS and FCS alone was observed (PTC: +198% / +137%, DTC: +109% / +76%,  $p < 0.01$  vs. PBS). This effect was significant higher in proximal cell cultures compared to distal cell cultures ( $p < 0.01$ ).



*Fig 1: Effects of different matrices on total cell number of PTC and DTC. Data are represented as relative fluorescence intensity (means  $\pm$  SD, n=6).*

**DISCUSSION & CONCLUSIONS:** In the development of a bioartificial renal tubular device it is necessary to evaluate the optimal environment for cell attachment, spreading, proliferation and differentiation. Our data indicate the positive effects of precoating with matrigel or FCS. As in older studies with tubular cell lines (MDCK) no positive effects of collagen IV or gelatin alone could be demonstrated [6]. The described effects may be due to growth factors involved in matrigel / FCS. Further studies have to be performed to characterize the effects of growth factor and the influence of ECM-precoating on cell differentiation. Nevertheless, this study is the first step in the development of a bioartificial renal tubular device with seeded human tubular cells.

**REFERENCES:** <sup>1</sup> Humes HD, Buffington DA, MacKay S, et al (1999) *Nature Biotech* **17**:451-455. <sup>2</sup> Baer PC, Nockher WA, Haase W, et al (1997) *Kidney Int* **52**:1321-1331. <sup>3</sup> Baer PC, Tunn UW, Nunez G, et al (1999) *Exp Nephrol* **7**:306-313. <sup>4</sup> Baer PC, Scherberich JE, Bereiter-Hahn J, et al (2000) *Transplant* **69**(11): 2456-2462. <sup>5</sup> Blaheta RA, Franz M, Auth MKH, et al (1991) *J Immunol Meth* **142**:199-206. <sup>6</sup> Kanai N, Fujita Y, Kakuta T et al (1999) *Artif Org* **23**:114-118.