

2.3.2 Evaluation of Cell Growth Using the MTT Assay

Growth curves were determined to ensure that cells used in experiments were within the exponential growth phase. Cell proliferation was assessed by monitoring the conversion of MTT to formazan. The reduction of MTT is catalysed by mitochondrial dehydrogenase enzymes and is therefore a measure for cell viability (Fig 2.1) (Mosmann, 1983).

Briefly, cells (100 μ l/well) were seeded at seeding densities of 1×10^5 , 1×10^4 or 1×10^3 cells/ml into 96 well microtitre plates and allowed to adhere for 24 h. Cell viability was assessed on a daily basis by adding 20 μ l of filter sterilised MTT (5 mg/ml in PBS) to a single row of six wells. Following a 5 h incubation period with MTT, media was removed with a needle and syringe and the blue formazan crystals trapped in cells dissolved in sterile DMSO (100 μ l) by incubating at 37 °C for 30 min. The absorbance at 550 nm was measured with a plate reader. Only the inner rows of the microtitre plate were used for these studies to minimise cell growth variations due to different medium evaporation rates at the periphery. The growth curve was constructed by plotting absorbance (blanked with DMSO) against time. The growth curves for DU145 cells are shown in Fig. 2.2.

2.3.3 Evaluation of Cytotoxicity Using the MTT Assay

The MTT assay (as described above) was also used to assess the *in vitro* cytotoxicity of polymers and polyplexes used in this study (see Chapter 4) (Sgouras & Duncan, 1990). In brief, COS7 and MCF7 cells (100 μ l; 1×10^5 cells/ml) were seeded into 96 well microtitre plates as before and left to adhere for 24 h. The next day, the medium was removed from the wells and replaced with filter sterilised complete medium containing polymer or polyplex. The plates were then incubated with polymer solutions for either 6 h or 24 h. In the case of the 6 h incubation; medium was removed after 6 h and replaced with culture medium only and further incubated to the total time of 24 h. Chitosan derivatives and PEI were applied onto the cells in DMEM at concentrations ranging from 20 to 10^4 μ g/ml (100 μ l/well) and cell viability compared to cells treated with DMEM only. In the case of the 24 h incubation media containing polymer was removed at 24 h and replaced with complete media. MTT (20 μ l of 5 mg/ml in PBS) was added to each well of the plates for both incubation times. Plates were incubated for a further 5 h. Then the medium was removed and DMSO (100 μ l) added before a further incubation of 30 min at 37 °C.