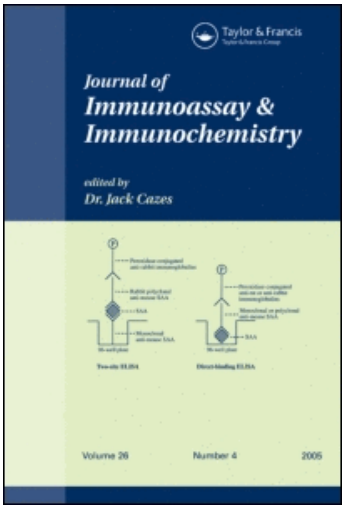


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# Transfection of Human Peripheral Blood Mononuclear Cells Using Immunoporation

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# Transfection of Human Peripheral Blood Mononuclear Cells Using Immunoporation

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**Abstract:** Immunoporation has been found to be able to efficiently transfect a wide range of human cultured cell lines. This report shows that peripheral blood mononuclear cells can also be efficiently transfected using immunoporation. The immunoporation of the cells with fluorescent TMR-Dextran, using Immunofect MG beads, indicates that transient holes of 5.4 nm in diameter or larger are formed during immunoporation. The efficiencies of transfection of lymphocytes transfected with vectors coding for EGFP and *lac*Z were found to be within the range of 15–30% with high levels of cell viability of more than 90%. In addition, it was observed that mononuclear cells stimulated with PHA expressed transfected reporter genes with a higher efficiency. In conclusion, these results demonstrate that immunoporation using Immunofect MG beads can be used for the efficient transfection of primary lymphocytes with DNA or other macromolecules.

**Keywords:** Immunoporation, Transfection, Lymphocytes, Mononuclear cells, Gene therapy

## INTRODUCTION

Previous work from this laboratory has demonstrated that human cultured cell lines in suspension can be efficiently transfected using immunoporation with high viability.[1–3] This is in contrast to lipofection, which is generally not as efficient for the transfection of suspension cells and electroporation which tends to kill most of the cells, either immediately, or as a result of apoptosis following electroporation.[4,5]

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The unique feature of immunoporation is that transfection is achieved by antibody-coated beads binding to and being sheared off of the cell membrane, thus creating transient holes, so that individual cells from mixed populations can be targeted for transfection, solely depending on their immunological identities.[1–3] In addition, this method appears to be well tolerated by cells in terms of their viability and it allows transfection of cells with various macromolecules, including DNA, proteins, and polysaccharides. Based on the fact that primary and tissue culture cells have different morphological characteristics and physical properties, a study has been carried out to examine whether immunoporation can also be used successfully as a transfection method for primary human cells.

## EXPERIMENTAL

### Isolation of Lymphocytes from Peripheral Blood and Cell Culture

Peripheral blood was obtained from healthy volunteers; Lymphoprep™ was used to isolate human mononuclear (PBM) cells according to the manufacturer's instructions. After isolation, the cells were washed twice with RPMI 1640 (Invitrogen, UK) containing 1% FCS before counting. Cells were then washed with PBS (1x) and cultured in flasks containing RPMI with 10% FCS. PBM cells were stimulated with PHA by culture in the presence of 10 μg/mL phytohaemagglutinin (PHA) for 24 h.[6]

### Reporter Molecules Used for Transfection Studies

Plasmid pEGFP-C1 (4.7 kb) (Clontech, UK) encodes an enhanced green fluorescent protein that is a variant of the *Aequorea victoria* green fluorescent protein gene (GFP). Plasmid pCMV-SPORT β-gal (8.7 kb) (Invitrogen Ltd, UK) contains the *E. coli* β-gal gene together with a CMV promoter. The plasmid DNA vectors were purified using MaxiPrep kits from Clontech (Clontech, UK). TMR Dextran (mol. Wt. 70,000 daltons) was purchased from Molecular Probes Ltd, UK. The diameter of TMR-Dextran was calculated to be 5.4 nm based on the assumption that it is a spheroidal molecule.

### Analysis of Cell Viability

Before and after immunoporation, cell viability was determined using Trypan Blue exclusion.[6] For this, cells were stained with 0.1% Trypan blue in PBS and the number of live and dead cells were determined by bright field microscopy.

## Transfection Procedure

Immunoporation was carried out using an immunoporation kit (IPBio Sciences Ltd Maidstone UK). Mononuclear cells ($4 \times 10^5$) were washed once with PBS, suspended in a microcentrifuge tube containing 0.5 mL of Immunoporation medium, together with varying numbers of Immunofect MG beads, and either 0.3 μg of plasmid DNA or 1.0 μg polysaccharide or protein. After mixing for 20 min at 20 rpm in a horizontal position on a TMX Dynal Mixer (Dynal, UK), the numbers of beads bound to the cells were examined using bright field microscopy. Subsequent to this, samples were put in a vertical position on the mixer and mixing was continued for 6 h at 40 rpm, at room temperature (21–23°C).[2,3] Expression of GFP and β-gal was detectable after 24 h with maximum expression between 48 h and 72 h. For these experiments, cells were analysed by either flow cytometry or enzyme assay 72 h post transfection. The uptake of fluorescently labelled macromolecules was studied immediately after incubation using fluorescence microscopy and flow cytometry.[2]



## Analysis of pEGFP-C1 Transfected Cells by Flow Cytometry

The fluorescence intensities of the cells were analysed by flow cytometry using a FACS Calibur (Becton-Dickinson). The argon laser was tuned at 488 nm and measurement of the fluorescent cells was carried out using a 525 nm band-pass filter. Prior to this analysis, the viability of cells was determined using Trypan Blue; the viability in most cases was in excess of 90%. To set the parameters for the flow cytometric analysis, non-transfected cells were cultured and analysed at the same time as the transfected cells. During flow cytometry, dead cells were gated (R1) and excluded from the statistical analysis.[2] Raw data obtained from the flow cytometer were converted into Windows format using WinMDI.2.8.[2]

## Analysis of Transfected Cells Expressing β-Galactosidase

For each enzyme assay $10^5$ of the transfected cells were used. The cells were washed once with PBS, the pellet was suspended at 150 μL of 0.25 M Tris-HCl (pH 7.5), and the samples were vortexed and sonicated for 20 sec. Afterwards, 150 μL of 2x Assay Buffer (200 mM phosphate buffer, pH 7.3, 2 mM $MgCl_2$, 100 mM 2-mercaptoethanol, and 1.33 mg/mL ONPG) was added to each sample and gentle mixed. The samples were incubated overnight (16 h) at 37°C. The reaction was stopped by the addition of 150 μL of 0.25 M $Na_2CO_3$ and the absorbance of each assay was read at 420 nm.

## RESULTS AND DISCUSSION

### Binding of Immunofect MG Beads to Human Peripheral Blood Mononuclear Cells

#### Effect of Using Different Ratios of Beads to Cells on the Binding of Immunofect MG Beads to PBM Cells

Firstly, the effect of different ratios of beads to cells on the binding of beads to cells was examined by incubating the cells ($4 \times 10^5$) in 0.5 mL of immunoporation medium at 20 rpm for 20 min at room temperature. As shown in Fig. 1, the binding of beads to cells depends on the number of beads, when using a 10 : 1 ratio of beads to cells a significant number of cells did not bind any beads. Using a 20:1 ratio of beads to cells in the same volume resulted in more than 60% of the cells binding three or more beads within 20 minutes. Increasing the ratio of beads to cells to 40:1 did not cause any significant increase in binding. Analysis of the cells after mixing with the beads for 6 h at 40 rpm showed that most of the cells were bead free and that the bead bound cells had no more than two beads attached to them. This is an indication that after mixing most of beads are being sheared off of the membrane during mixing. The conclusions from these experiments are that the optimum ratio for binding beads to cells is achieved using a ratio of beads to cells of at least 20:1.

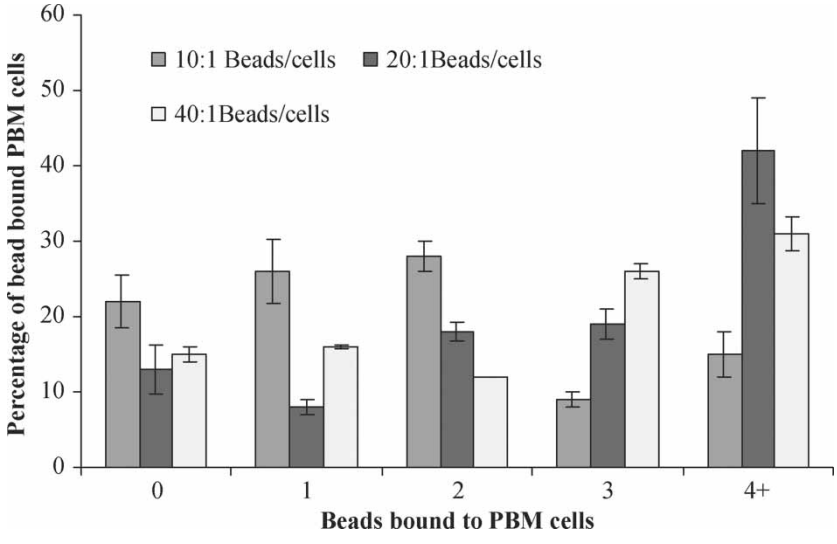


***Figure 1.*** The effect of the ratio of beads to cells on the binding of Immunofect MG beads to PBM cells after mixing for 20 min at 20 rpm. The experiment was performed twice, where $n = 6$.

*Table 1.* Effect on viability of PBM cells after mixing with Immunofect MG beads for 6 h, at 40 rpm and room temperature (20–22°C). The counting was carried out immediately after immunoporation and 24 h after the end of incubation using Trypan Blue exclusion. The experiment was performed twice where $n = 6$

| Ratio of beads to cells | After 6 h incubation | 24 h post transfection |
|---|---|---|
| No beads | 97 ± 3.2 | 96 ± 2.3 |
| 10:1beads | 98 ± 1.8 | 95 ± 2.2 |
| 20 : 1 beads | 96 ± 2.1 | 93 ± 0.9 |
| 40 : 1 beads | 89 ± 1.3 | 83 ± 1.0 |



### Viability of PBM Cells After Mixing with Immunofect MG Beads and Subsequent Culture

It has been reported that some transfection techniques such as electroporation can cause either immediate cell death or apoptosis.[4] In order to examine the effects of immunoporation on cell growth of the transfected cells, viability counts were performed every 24 h post transfection over a culture period of 24–48 h. As it can be seen from Table 1, the viability of cells transfected in the presence of Immunofect MG beads at a ratio of beads to cells of 10:1 or 20:1 did not appear to have any significant effect on cell viability, while using a ratio of 40:1 beads to cells viability appeared to cause a slight decrease in viability to about 90%. Upon further culture for 24 h the viability of the cells remained at the same levels, although there was an indication of slightly higher cell death after longer culture time when ratios of beads to cells of 40:1 were used.

Based on these results and the previous set of data it can be concluded that the optimum ratio of beads to cells for cell transfection is 20:1 since it allows efficient bead binding while not affecting the cell viability.

## Transfection of PBM Cells by Immunoporation Using Immunofect MG Beads

### TMR-Dextran Transfection of PBM Cells

The results shown in Table 2 clearly indicate that lymphocytes can be transfected with TMR-dextran using Immunofect MG beads at a ratio of beads to cells of 20:1 by mixing the cells and beads for 6 h at 40 rpm in the presence of TMR-dextran under standard immunoporation conditions. Analyses of the transfected cells were performed using flow cytometry and fluorescent microscopy. These results indicate that the efficiency of transfection using immunoporation is in the range of 20–30% (Table 2).

***Table 2.*** Transfection efficiency of PBM cells after immunoporation with TMR-dextran using Immunofect MG beads for 6 h mixing at 40 rpm at room temperature (20–22°C). The experiment was performed twice where $n = 6$. The percentage of fluorescent cells was determined using flow cytometry (counts of $10^4$ cells/sample)

| Samples | Total (%) fluorescent cells |
|---|---|
| Cells + beads + TMR dextran | $31 \pm 4.8^a$ |
| Cells + beads | $3.4 \pm 1.1$ |
| Cells + TMR dextran | $2.9 \pm 0.9$ |

[a]Background fluorescence determined by control samples was deducted from this percentage.



Fig. 2 shows typical light microscopy images of the transfected cells. Calculations indicate that the mean diameter of the TMR-dextran molecules with a molecular weight of 70,000 Daltons is 5.4 nm and so these results suggest that the transient holes formed in the cells are at least 5.4 nm in diameter.

## Transfection of PBM Cells with pEGFP-C1 DNA

PBM cells were transfected by immunoporation of the cells with Immunofect MG beads in the presence of 0.2 μg of pEGFP-C1 DNA (4.76 kb), cultured for 48 h and analysed by flow cytometry. The results obtained show that a transfection efficiency of 15% (Table 3) for PBM cells can be obtained using exactly the same immunoporation conditions as has been developed for cultured cell lines.[1,3]

## Immunoporation of Normal and PHA-Stimulated PBM Cells Using pCMV-SPORT-βgal DNA

In order to perform a more quantitative analysis of the efficiency of immunoporation, PBM cells were transfected by Immunoporation in the presence of 0.3 μg of pSPORT.CMV.βgal DNA (8.2 kb) and cultured for 48 h after which time the cells were analysed for the presence of β-galactosidase using an enzyme assay (Table 4). Stimulation of cells with PHA for 24 h before Immunoporation resulted in a significantly higher level of expression of β-galactosidase (Table 4), indicating that stimulation of lymphocytes with PHA may increase the efficiency of transfection using Immunofect MG beads.

Previously immunoporation has been demonstrated to be an effective method for transfecting a range of different human cell lines growing as either suspension or adherent cells.[1,3]The human cell lines that have been

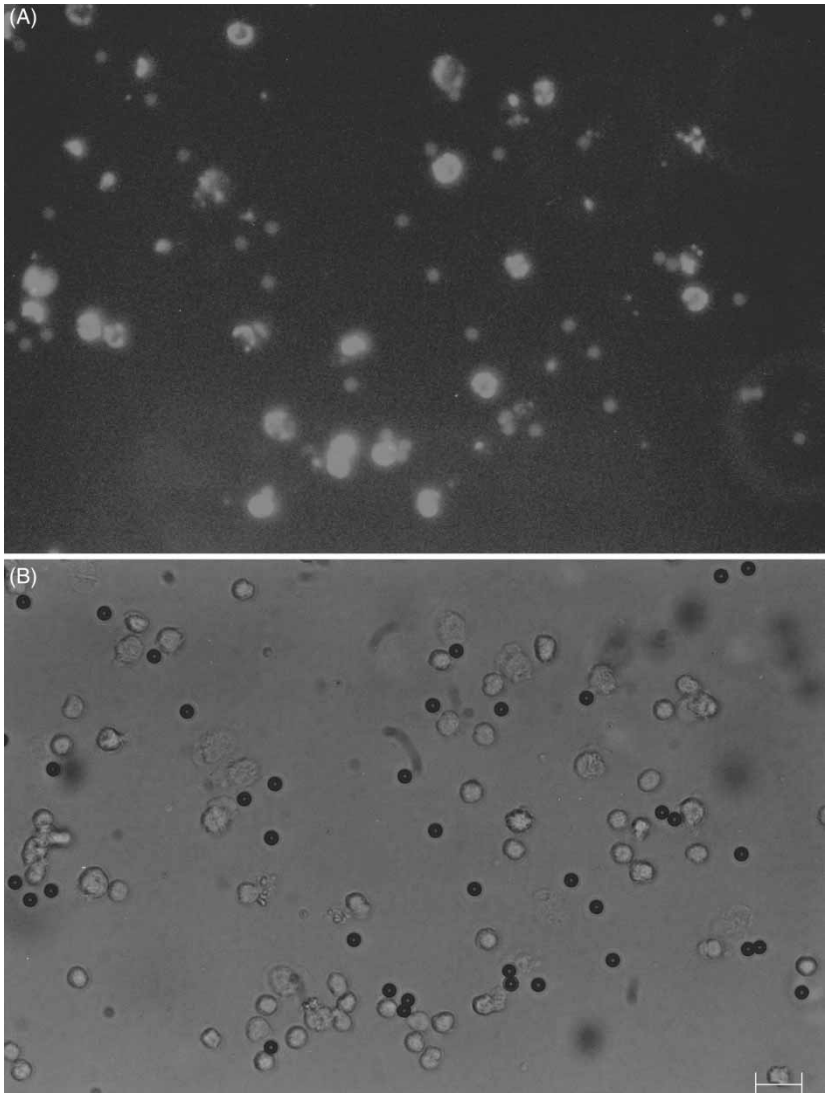


***Figure 2.*** Human blood mononuclear cells, isolated from a healthy male donor using density gradient centrifugation, and transfected with TMR-Dextran using Immunofect MG beads. Analysis was performed immediately after incubation using A) Fluorescent and B) Bright Field microscopy. The scale bar equals 20 μm.

transfected include some of the more difficult cell lines such as HL60 cells. This work describes the efficient use of immunoporation for the transfection of PBM cells using Immunofect MG beads.

The efficiency of transfection observed in these experiments varied from about 10–30% with average viabilities in excess of 90%. These levels of

***Table 3.*** Transfection efficiency of PBM cells after immunoporation with pEGFP-C1 vector using Immunofect MG beads for 6 h, mixing at 40 rpm at room temperature (20–22°C). The experiment was performed twice where n = 6. The percentage of fluorescent cells was determined using flow cytometry (counts of $10^4$ cells/sample)

| Samples | Transfection efficiency (%) |
|---|---|
| Cells + beads + DNA | $15 \pm 3.5^a$ |
| Cells + DNA | $1.2 \pm 0.4$ |
| Cells | $2.2 \pm 0.7$ |

[a]Background fluorescence determined by control samples was deducted from this percentage.



***Table 4.*** Transfection efficiency of PBM cells after immunoporation with pCMV-SPORT-$\beta$gal vector using Immunofect MG beads for 6 h, 40 rpm at room temperature (20–22°C). The experiment was performed twice where $n = 6$

| | nmol ONP/$10^5$cells | |
|---|---|---|
| Samples | Normal PBM cells | PHA-stimulated cells |
| Cells + beads + DNA | $182.6 \pm 24.3$ | $291.3 \pm 11.8$ |
| Cells + DNA | $9 \pm 1.1$ | $8 \pm 2.1$ |
| Cells (blank) | $1.1 \pm 0.03$ | $1.4 \pm 0.09$ |

transfection are less than those previously obtained using human cultured cell lines,[1,2] but still significantly better than those obtained by other transfection methods. However, it should be noted that the technique used for these experiments was based very closely on that developed for cultured cell lines and so it may still be possible to improve the transfection efficiency and yield of the cells by further optimising the conditions for immunoporation of primary cells.

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## REFERENCES

1. Bildirici, L.; Smith, P.; Tzavelas, C.; Horefti, E.; Rickwood, D. Transfection of cells by immunoporation. Nature **2000**, *405*, 298.
2. Tzavelas, C.; Smith, P.; Horefti, E.; Rickwood, D. Effect of osmolarity and presence of serum on the efficiency of cell transfection using immunoporation. Analyt. Cell. Pathol. **2001**, *22*, 223–227.
3. Tzavelas, C.; Bildirici, L.; Rickwood, D. Factors that influence the effectiveness of immunoporation for cell transfection. Anal Biochem. **2004**, inpress.
4. Hofmann, F.; Ohnimus, H.; Scheller, C.; Strupp, W.; Zimmermann, U.; Jassoy, C. Electric field pulses can induce apoptosis. J. Membrane Biol. **1999**, *15*, 103–109.
5. Shimokawa, T.; Okumura, K.; Ra, C. DNA induces apoptosis in electroporated human promonocytic cell line U937. Biochem. Biophys. Res. Comm. **2000**, *270*, 94–99.
6. Freshney, R. *Animal Cell Culture, A Practical Approach*; IRL Press: Oxford, UK, 1996.

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