

OPTIMIZATION OF SOX-TRIO GENE TRANSFECTION IN PRIMARY CHONDROCYTES CULTURE USING LIPOFECTAMINE™ 3000

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The primary cells are hard to transfect with a DNA fragment larger than two kbp. The difficulty of the DNA fragment to diffuse into the cytoplasm may be due to the complex environment and viscoelastic properties of the cytosol. The use of cartilage transcription factor, SOX9 alone is not enough to facilitate in vitro chondrogenesis. To improve cartilage formation, this study designed a polycistronic plasmid, SOX-trio plasmid vector (VectorBuilder Inc., US) using a combination of SOX5, SOX6 and SOX9 genes. The mCherry is used as the fluorescent marker. Before evaluating the potential SOX-trio effects in chondrocytes, this study aimed to optimise and achieve an acceptable transfection efficiency for primary chondrocytes which is more than 30%. The optimisation is challenging because the size of the SOX-trio plasmid is more than ten kbp which is 5-time bigger than the recommended vector size. The topology (linear or supercoiled) and the size of the plasmid DNA vector influence the transfection efficiency. Transient transfection is most efficient with supercoiled plasmid DNA. Lipofectamine™ 3000 transfection reagent (Thermo Fisher Scientific, US) was used in this study. It is one of the most popular transfection reagents kit used and developed to improve the transfection efficiency for difficult-to-transfect cells. The methodology follows the manufacturer's protocol with some modifications. The transfection procedure was carried out using SOX-trio/DNA (2.5 ng/μL and 5.0 ng/μL) and Lipofectamine reagents with different ratios or volumes. The cells were cultured and transfected using DNA-to-lipofectamine ratio of 1:1, 2:1 and 2:2. The transfected cells were observed using fluorescent microscopy (Cytell™ Cell Imaging System, GE Healthcare, UK). Successful transfection is indicated by the presence of the red fluorescence protein expression of mCherry in the cells. The transfection efficiency was calculated and recorded as a percentage. The average transfection efficiency in this study was 54.22%. It is interesting to note that the highest transfection efficiency, i.e. 60%, can be achieved using 5.0 ng/μL SOX-trio/DNA and a double-volume Lipofectamine. It is assumed that the increased of Lipofectamine is needed to compensate for the size of the SOX-trio plasmid. Based on the findings, the 2:2 SOX-trio: Lipofectamine ratio exhibited better transfection efficiency than the 1:1 and 2:1 ratio. It is hoped that the use of SOX-trio can enhance chondrogenesis in vitro.

Keywords: SOX-trio, lipofectamine, transfection, articular cartilage, tissue engineering

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