ABSTRACT

Introduction
The cellular membrane constitutes an effective barrier that protects the complex, yet highly ordered, intracellular compartment of the cell. Passage of molecules across this barrier is highly regulated and highly restricted, with molecular size and amphilicity being the most significant criteria. Cell penetrating peptides (CPPs) are a class of small cationic peptides that are able to defy the rules of membrane passage and gain access to the intracellular environment. MPG is one member of this class of cell penetrating peptides capable of translocating the impervious hydrophilic cell membrane to deliver non-covalently attached cargo. In this study MPG was used to deliver FAM-labelled siRNA into hMADS cells.

Method
Confluent and differentiated hMADS cells were transfected for 4h with 30nM siRNA-MPG-complex (SMC) and the controls were overlaid with free siRNA (SO) and cell only (CO) which contained MPG buffer for 4h and then analysed after 24h, 48h and 72h incubation post-transfection for internalization of the siRNA complex using Zeiss Axioimager for fluorescence detection and localisation. The pre-confluent cells were transfected with 15 nM of SMC for 4h and incubated for 24h and 48h post-transfection under standard cell culture conditions.

Result
There was no noticeable adverse morphological variation between the siRNA-MPG complex transfected groups and the 'free siRNA' and the 'cell only' overlaid groups in the pre-confluent, confluent and differentiated stage following above mentioned transfection procedure. There was also no observed mortality associated with transfection. The differentiation of the cells into adipocyte phenotype was observed normally in all the groups following induction with adipocyte differentiation cocktail. Bright fluorescence speckles were exclusively detected in the MPG-mediated siRNA delivery of pre-confluent, confluent and differentiating hMADS cells in the cytoplasm as well as in the nucleus 24h and 48h post-transfection. The efficiency of delivery 24h after transfection in the confluent cells was about 90%, in cells differentiating 3 days toward adipocytes about 80%, and 50-60% of the cells showed internalised siRNA-MPG complex even 48h and 72h post-transfection.

Discussion
MPG is non–viral and non electroporation-based transfection method that efficiently delivered double stranded siRNA into cytoplasm and nuclei of hMADS cells during proliferation and adipocyte differentiation. That MPG mediated nuclear localization of siRNA in this study supports the observation of Divita and co-workers (Crombez et al., 2007b; Simeoni et al., 2005). The pattern of fluorescence was punctuated in distribution, which was in line with the report of Veldhoen and colleagues (Veldhoen et al., 2006b). In variance with Zaragosi et al (2007) MPG as non-electroporation and n based transfection agent efficiently delivered siRNA into hMADS cells.

Conclusion
In this study we could show that MPG efficiently delivered double-stranded siRNA into human mesenchymal stem cells in proliferating, confluent and differentiating stages. The cell viability was not adversely affected while differentiation of hMADS cells proceeded normally. These results demonstrated that MPG is a very effective and robust non-viral based transfection agent, easy to apply to non-dividing adherent cells during proliferation and differentiation, without necessity of detaching. The siRNA could be still detected up to 6 days after transfection. Thus MPG is a valuable tool for transient gene and microRNA silencing *in vitro*.