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Avoiding lentiviral transduction culture induced MSC senescence

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Dear Editor:

We read with interest the recent article by Böcker and colleagues in *JCMM* [1]. As noted by the authors, mesenchymal stem cells (MSC) enjoy considerable therapeutic potential in regenerative medicine, cell therapy for select genetic diseases and as immunomodulators [2, 3]. Expanding on a prior study that explored the genetic modification of MSC using HIV-1 derived lentiviral vectors, the authors describe a strategy that minimizes the replicative senescence seen in MSC during subculture necessary for propagation, transduction or selection [4]. The work nicely demonstrates that proviral overexpression of human telomerase reverse transcriptase (hTERT) after stable modification with a late generation lentiviral vector can produce clonal populations of MSC capable for differentiation without premature replicative exhaustion. Clearly, the efficient and stable genetic modification of MSC without compromise to the proliferative potential during extended propagation and selection represents a significant step in reaping the full rewards of their clinical potential. However, we would like to echo the authors' caution, that the generation of such clonal cell populations for therapeutic purposes is time intensive and the issue of cell cycle acceleration and osteogenic bias in differentiation described by the authors may indeed denote other, less desirable, genetic changes. In addition, there remain fundamental concerns over the extended *in vitro* manipulation necessary after the proposed transduction with hTERT vector.

We would like to advance an alternative, and more simple, approach to the problem. Conventional *ex vivo* culture for propagation and transduction is usually performed in adherence, at considerable expense in personnel and reagents. Adherent propagation during vector exposure and for clonal selection carries the antecedent risk of microbial contamination, entails bovine and/or equine serum exposure, and amplifies the risk of inducing MSC senescence. We have recently reported the use of lentiviral vectors to shorten *ex vivo* exposure duration and manipulation during transduction of haematopoietic stem cells [5, 6]. Based on that experience we explored the rapid transduction of murine MSC with vesicular-stomatitis G protein (VSV-G) pseudotyped third-generation HIV-1 derived lentivector in suspension rather than during adherent subculture. Experiments revealed that 1° mMSC exposed to vector in suspension for 1 hr demonstrate improved gene transfer rates (Fig. 1A). Reflecting in part the greater particle density achieved during vector exposure, or alternatively the increased cell surface area available in suspension, these gains in efficiency as well as savings in reagents and vector will be even more pronounced once large, clinically relevant, cell numbers are considered. Gene transfer efficiency to non-adherent MSC is maintained in previously cryopreserved cells (liquid nitrogen storage, MSC >fifth passage) transduced immediately upon thawing. Further, across a range of multiplicities of infection, gene transfer to cells in suspension is significantly improved in the absence of serum – 2.4-fold on average (range: 1.6–3) – when compared to transduction in 10% foetal bovine serum (Fig. 1B). Importantly, in all conditions tested, cells stably express the transgene during subsequent expansion culture and maintain adipogenic as well as osteogenic differentiation properties (others not tested).

In conclusion, the transduction of MSC in suspension and under serum-free conditions permits rapid, durable and efficient gene marking of previously cryopreserved, defined, cell populations while avoiding the implicit disadvantages of conventional adherent propagation for transduction and selection. Brief vector exposure in suspension reduces the expenditure for tissue culture supplies, certified serum and growth factors. This strategy shortens the time at risk for contamination during transduction and will minimize the culture-induced loss of replicative capacity without requiring the generation of clonally derived, hTERT-modified cells [1]. Unlike the approach taken by Böcker and colleagues, the protocol should therefore be well suited for scale-up to high-throughput, good manufacturing practice, standards and can be applied to the polyclonal populations currently explored for clinical cell therapy.

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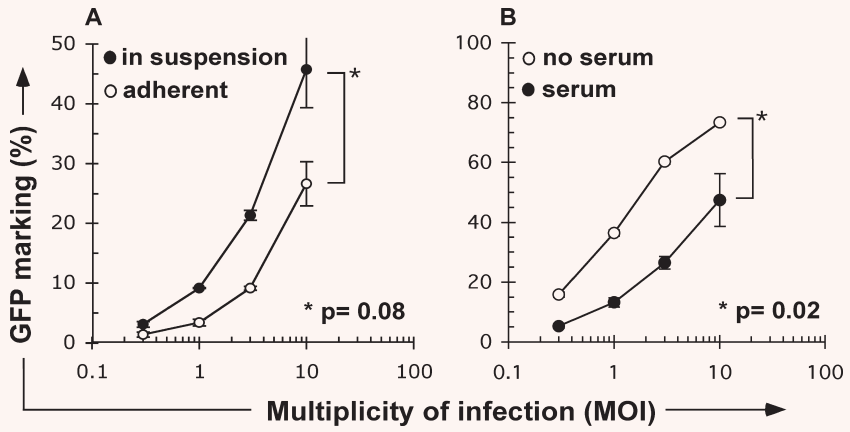

Fig. 1 (**A**) Lentiviral transduction of non-adherent murine mesenchymal stem cells (MSC). MSC were exposed to VSV-G pseudotyped green fluorescent protein (GFP) expressing lentivirus for 1 hr at the indicated multiplicities of infection (MOI), alternatively in adherence after plating 16–24 hrs earlier (open symbols), or in suspension (closed symbols). (**B**) Gene transfer to mMSC is improved under serum free conditions. MSC were thawed from liquid nitrogen, washed twice in phosphate buffered saline (PBS), resuspended in media, and immediately exposed to lentivector at the indicated MOI. All transductions were performed over 1 hr in 1ml final volume, in the presence of protamine sulphate at 4 μg/ml. At the end of transduction cells were washed twice in PBS and plated in Iscoves based MSC expansion medium, as described by Peister [7]. Retrovirus vector was produced as previously described [6]. For transduction in suspension, cells were trypsinized from log-phase expansion culture, or thawed from cryopreservation (5% dimethyl sulfoxide [DMSO]) in liquid nitrogen and washed in PBS. Averages from multiple repeat determinations are shown. Data were analysed using the paired two-tailed Student's t-test. *P*-values of less than 0.05 were considered significant.

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