Letters From Our Readers

To: Editor, The Angle Orthodontist


In the current version of this paper, on the basis of the data shown in Table 2, the authors concluded that a single local injection of mesenchymal stem cells (MSCs) into the rat mid-palatal suture increases new bone formation (1.21-fold) by increasing the number of osteoblasts (1.13-fold) and leading to new vessel formation (1.42-fold). The findings of this pre-clinical study may support the clinical use of MSCs after maxillary expansion (as stated by the authors) but, although they suggest it, they do not demonstrate that this approach reduces the relapse rate and achieves more stable results.

The data in Table 2 show a 1.059-fold increase in the area of new bone formation in the suture, a 1.20-fold increase in the number of osteoblasts, and 1.71-fold increase in new vessel formation in the MSC-treated group: the fold-changes in Table 2 are therefore different from those described in the Results. Moreover, the reported statistical significance ($P < 0.05$) of the small difference in the area of new bone formation between the MSC-treated and control rats (23370 $\mu m^2$) is surprising and, in relation to the reported statistical significance ($P < 0.05$) of the increase in new vessel formation in the MSC-treated group, the fact that the mean value is equal to the standard deviation raises doubts about the appropriateness of the statistical analysis.

On the whole, the effect of a single local administration of MSCs on bone regeneration seems to be limited in comparison with the results of other osteoanabolic treatments (vitamin E, ED-71 vitamin D analogue, resveratrol, TGF-$\beta$, and direct electrical current stimulation) described by the same group and by Sawada and Shimizu. Moreover, greater amounts of new bone have been found in sutures undergoing intermittent loading and unloading.

In the protocol described, MSCs were delivered into the interpremaxillary suture (there is no indication of the exact site) 24 hours after expansion started, but something in the procedure or endogenous micro-environment slightly affected osteogenic differentiation (a 1.059-fold increase in the area of new bone formation). DAPI staining revealed cell concentration in the area to which the MSCs were applied, and co-localisation with Phk67$^+$ MSCs. In line with the rapid progression of bone growth, apoptosis was also observed in the area of proliferating cells. Unfortunately, the paper of Ekizer et al. does not provide any data concerning apoptosis, but it has been reported that DAPI also stains apoptotic cells.

The results raise some doubts concerning the phenotype and function of the selected MSC population, which has only been characterised by means of 3 expression markers (CD29, CD90 and CD45). The authors describe the in vitro osteogenic differentiation revealed by analysing collagen type 1, osteocalcin and von Kossa staining, but there are no data concerning the 2D population dynamics of the MSCs and differentiated cells, ALP activity, or osteogenic marker gene expression.

Finally, the information concerning enforced in vitro differentiation does not necessarily indicate differentiation in an in vivo 3D micro-environment. A critical variable in stem cell-based therapy is the number of cells used for implantation (10$^7$-10$^8$) in a suitable 3D graft, but the treatment used by Eziker et al. involved 10$^6$ MSCs/100 $\mu L$ floating in a vehicle medium (sterile saline or PBS), without the presence of platelet-rich plasma, which has been shown to be able to regenerate functional bone in alveolar deficiencies.

We think that sound results are crucial in stem cell research and particularly important in order to avoid “dark stem cell therapy” in orthodontic patients. In the absence of elucidations from Eziker et al., the following questions remain open:

1. Were the MSCs insufficient or stressed, or was their osteogenic capacity lost or impaired?
2. How do the physical and chemical changes that take place in the suture during maxillary expansion affect the osteogenic induction of MSCs?
3. Is the site of MSC transplantation (suture vs masticatory muscle area) important?
4. As rodents are not considered to be very suitable models for studying the long-term effects of stem cells, are they really appropriate for investigating the role of stem cells during maxillary expansion or the tendency towards relapse?
5. Looking to the future, can orthodontics combine animal testing with the advantages of suitable organs-on-chips and 3D and gene microarray platforms \(^\text{12}\) in order to reveal the role of stem cells during maxillary expansion?

6. Is stem cell mechanical memory involved in the tendency toward relapse? \(^\text{13}\)

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REFERENCES


