Editorial

Callus-derived cells: are they mesenchymal stem cells?

In this issue, Zaki and Ismail compared two- and four-week old fracture site calluses to iliac crest bone marrow of New Zealand rabbits as the source of plastic-adherent cells, and found that two- and four-week old calluses were potential sources of plastic-adherent cells. In this study, all cells were cultured in 10% fetal bovine serum (FBS) containing low glucoce Dulbecco's modified Eagles medium (DMEM).[1] It is well known that bone marrow cells, which are cultured in 10% FBS containing DMEM, will grow as fibroblastic plastic-adherent cells that are called mesenchymal stem cells (MSCs). However, to be sure, a panel of positive and negative markers are needed to prove the MSCs.[2, 3]

In fracture, bone healing process begins with proliferation and differentiation of mesenchymal stem cells into cartilage cells (chondroblasts and chondrocytes) that lead to soft callus formation, which further develops into hard (bony callus), and finally undergoes remodeling. These processes resemble the endochondral bone formation, in which the cartilage undergoes serial processes subsequently, i.e. chondrocyte proliferation, hypertrophy, maturation, degeneration and calcification. In every process, various transcription and growth factors are involved, such as the transcription factors sox and runx, together with fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), Indian Hedgehog (Ihh), and transforming growth factor alpha (TGF-α), which are needed for the commitment of MSCs to differentiate into chondrocytes.[4] Therefore, insufficient levels of certain transcription and growth factors that are needed in those processes may cause impairment in the healing process, which may cause non-union.

Zaki and Ismail highlighted the possible use of mesenchymal stem cells to enhance the healing of non-union fracture, and the possible use of two- and four-week calluses as potential sources of stem cells.[1] However, in non-union cases, usually there is impairment in signaling of the healing process. Therefore, using callus-derived cells that are taken from the non-union cases without addition of growth factors, whose level is impaired, may not be helpful.

In this study, the morphology of bone marrow derived cells were fibroblastic, while callus-derived cells were rounded.[1] Therefore, it is highly probable that the callus-derived cells are mostly non MSCs, though they are plastic-adherent. It is highly probable that the two week-callus (soft callus) derived cells were mostly chondrogenic progenitors, while the four week-callus (soft and hard callus) derived cells were a mixture of chondrogenic and osteogenic progenitors. Therefore, upon culture, the chondrogenic and osteogenic progenitors might differentiate further into chondrocytes and osteocytes respectively. When the progenitors were fully differentiated, their proliferation capacity could be highly reduced, as fully differentiated cells would loose their self renewal capacity.

Moreover, this study only compared the results of primary cultures. Therefore, it is advisable to do subcultures and prolonged cultures of callus-derived cells. Comparing the proliferation of primary cultures to subsequent subcultures might give insight to the property of the cells, whether they are mostly stem cells, or progenitors. If the cells are progenitors, proliferation of subcultures are highly probable to be inferior compared to the primary culture. Further, prolonged cultures of callus-derived cells can be used to see whether the cells form micromasses in case they are chondrogenic progenitors, or undergo calcification, in case they are osteogenic progenitors. A study have shown spontaneous osteogenic differentiation in prolonged cultures.[5] Chondrogenic micromass can be easily detected by only observing the culture,[6] and calcification can be detected by akizarin red staining.

Finally, full characterization of the cells are highly needed to know the exact nature of the cells, and whether they can be really used in non-union long bone fractures or other cases of regenerative medicine.

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