Stem Cells 101: Letter to the Editor

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Dear Editor:

I read with some interest the editorial by Dr. Scott Rodeo entitled "Stem Cells 101."[8] After reading this editorial, I must profoundly apologize to the entire orthopaedic community for calling the cells isolated from bone marrow mesenchymal *stem cells* (MSCs).[2] The mistake that I made, and which was compounded by Dr. Rodeo, is that in cell culture, a variety of highly powerful, inductive agents can take cells of mesenchymal origin (eg, we can do this with adult chondrocytes[10]) and drive them down a variety of phenotypic lineages. Thus, originally in the 1980s, when my colleagues and I isolated and expanded these human and animal marrow cells in culture, we could cause these cells to differentiate into bone, cartilage, fat, muscle, and so on. Given these observations and the dogma of the day "that what we saw in cell culture is what happens in vivo," I mistakenly assumed that these cells would likewise differentiate into mesenchymal cell types in the body. This was not correct, and some years later, I properly apologized to my colleagues and changed the name to medicinal signaling cells, keeping the MSC nomenclature.[3] This change of name reflects the fact that these cells do, indeed, function naturally at sites of tissue damage and provide a cornucopia of bioactive factors that assist the injured tissue in managing its own regenerative and repair capabilities. MSCs arise from perivascular locations[1] and are not resident in connective tissue or the stroma surrounding organs; thus, calling them "stromal cells" is quite inappropriate. Indeed, MSCs arise at sites of broken or inflamed blood vessels and function as natural sentinels guarding the internal aspects of the body from invasion by a variety of pathogens. These MSCs are quite different from tissue-specific committed progenitors that can differentiate, which are discussed below.

The entire analytical characterization of MSCs by putting them into cell culture or cloning them and then looking for cell surface markers completely misses the point that these cells do not differentiate in vivo. These cells are medicinal; they provide molecular cues to assist in the management of sites of injury by virtue of their paracrine capabilities. Not only do MSCs not differentiate, but also they exit the injury sites of activity very soon after arriving. Their initial activities are to stimulate the repair, regeneration, and immunomodulation of sites of tissue damage.

The difficulty I have with Dr. Rodeo's editorial piece is in the title: "Stem Cells 101." He, like many lay individuals, wants to put cells in vivo and to have these cells form new and vital replacement tissue. This does not happen, but putting MSCs at sites of damage or injury has the potential to stimulate the surrounding tissue to fix itself. Fat grafting or putting microfragmented fat preparations (eg, Lipogems) at sites of injury can bring about a therapeutic effect. This positive effect is medicinal since none of these cells engraft or differentiate; they do not contain *stem cells*.

Dr. Rodeo very correctly discusses MSC preparations from a variety of sources and clearly states that these preparations are heterogeneous. Indeed, single-cell RNAseq analysis of such MSC preparations from a variety of tissues indicates that they have most of their transcripts in common and about 5% to 9% of their transcripts are unique to the tissue of origin of the MSCs.[6,7] With RNAseq technology and appropriate software, experimentalists have documented that the MSC preparations from marrow or from fat are heterogeneous, with up to 10 to 12 separate groupings of cells within cell culture preparation.[6,7] Some of such subsets could be identified on the basis of RNA transcripts as committed progenitors in the case of both marrow- and fat-derived MSCs. Which cell or combination of cells in these heterogeneous preparations is responsible for the observed therapeutic effects is currently unknown. It may well be that the mixture of cells itself is the therapeutic, and that by purifying one cell population from this mixture, the therapeutic activity could itself be lost.

Again, it is my fault that I was so persuasive in convincing the orthopaedic community that stem cells could be isolated and used for therapeutic purposes. Again, I profoundly apologize for this mistake. For sure, *there are no stem cells* that I am aware of in any adult tissue. The hemopoietic stem cell is not a stem cell; it is a committed progenitor that can only produce a wide range of very different blood cells. Because of the number of different blood cells with uniquely different properties that originate from hemopoietic stem cells, the stem cell name is still favored by many. This cell is not a stem cell; this cell cannot form muscle or neural tissue. This cell is a committed progenitor cell. Every single tissue of the body has such a committed progenitor in residence that is used to regenerate small injuries and to provide differentiated cells for the normal turnover of that tissue. The keratinocyte stem cell is, likewise, a committed progenitor that only produces keratinocytes. Bone marrow clearly contains osteochondral committed progenitors,[5] which are necessary for normal bone turnover and are available for fracture repair. Since fracture repair can require a cartilaginous bridge between fragments, these cells, by necessity, must have the capacity for both bone and cartilage differentiation.

In the early days of the development of MSC technology, we used small 3-mm calcium phosphate ceramic cubes filled with cells implanted subcutaneously in rodents as a test indicator of the quality of the MSC preparation.[4] In "very good" bone marrow–derived MSC preparations, we could observe the pores of the ceramic filled with newly formed bone, and in some cases where vasculature was excluded from the internal pores, we saw plugs of cartilage that later endochondrally transformed into bone. These observations are now understood in terms of the osteochondral committed progenitors that are naturally found in bone marrow. In this regard, it is important to also recognize that fat-derived MSCs, when tested in vitro or in

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cubes, have very poor osteogenic capabilities but have enhanced adipogenic capacities because of the committed adipogenic progenitors. The studies Dr. Rodeo cited by Dr. Muschler are a perfect example of the culture and cloning technique documenting the heterogeneity of marrow MSC preparations, with some clones showing commitment to osteogenic differentiation.

Again, in summary, if one assumes that there are no stem cells available in adults, it simplifies the analysis of the available data. Importantly, committed progenitors reside in highly specialized sites where, in the cases that have been described, these committed progenitors are situated in close contact with perivascular–mesenchymal stem cells, and both of these cells are in contact with blood vessels.[9] The associated perivascular–mesenchymal stem cells uniquely provide molecules for these committed progenitors and molecularly assist when progenitors are active or when they are resting. The exact chemistry of this interaction has not been elucidated.

Again, my profound apologies to sports medicine, to the orthopaedic industry, and to my esteemed colleague, Dr. Rodeo, for painting the wrong picture of MSCs over 30 years ago. The correct picture has been amply outlined in several of my publications referenced to herein.

Arnold I. Caplan, PhD
Cleveland, Ohio, USA

Address correspondence to Arnold I. Caplan, PhD (email: arnold.caplan@case.edu)

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REFERENCES

1. Caplan AI. All MSCs are pericytes? *Cell Stem Cell*. 2008;3(3):229-230. doi:10.1016/j.stem.2008.08.008
2. Caplan AI. Mesenchymal stem cells. *J Orthop Res*. 1991;9(5):641-650. doi:10.1002/jor.1100090504
3. Caplan AI. What's in a name?. *Tissue Eng Part A*. 2010;16(8):2415-2417. doi:10.1089/ten.TEA.2010.0216
4. Dennis JE, Haynesworth SE, Young RG, Caplan AI. Osteogenesis in marrow-derived mesenchymal cell porous ceramic composites transplanted subcutaneously: effect of fibronectin and laminin on cell retention and rate of osteogenic expression. *Cell Transplant*. 1992;1(1):23-32. doi:10.1177/096368979200100106
5. Han J, Ishii M, Bringas P Jr, Maas RL, Maxson RE Jr, Chai Y. Concerted action of Msx1 and Msx2 in regulating cranial neural crest cell differentiation during frontal bone development. *Mech Dev*. 2007;124(9-10):729-745. doi:10.1016/j.mod.2007.06.006
6. Ménard C, Dulong J, Roulois D, et al. Integrated transcriptomic, phenotypic, and functional study reveals tissue-specific immune properties of mesenchymal stromal cells. *Stem Cells*. 2020;38(1):146-159. doi:10.1002/stem.3077
7. Merrick D, Sakers A, Irgebay Z, et al. Identification of a mesenchymal progenitor cell hierarchy in adipose tissue. *Science*. 2019;364(6438):eaav2501. doi:10.1126/science.aav2501
8. Rodeo S. Stem cells 101. *Am J Sports Med*. 2021;49(6):1417-1420. doi:10.1177/03635465211011082
9. Somoza R, Correa D, Caplan A. Roles for mesenchymal stem cells as medicinal signaling cells. *Nat Protoc*. 2016;11(1). Accessed September 4, 2021. https://www.stemcell.com/media/files/wallchart/WA10021-Roles_for_MSCs_as_Medicinal_Signaling_Cells.pdf
10. Tallheden T, Dennis JE, Lennon DP, Sjögren-Jansson E, Caplan AI, Lindahl A. Phenotypic plasticity of human articular chondrocytes. *J Bone Joint Surg Am*. 2003;85(suppl 2):93-100. doi:10.2106/00004623-200300002-00012

Stem Cells 101: Response

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Author's Response:

I thank Dr. Caplan for his thoughtful and expert insight. As a recognized thought leader and authority in this area, readers of the journal will benefit from his comments. In fact, this is the very essence of what the *American Journal of Sports Medicine* stands for—a forum to stimulate scientific exchange and discourse, and no area of sports medicine has as many outstanding questions as the whole area of "orthobiologics" and cell therapy.

In reading Dr. Caplan's letter, I believe that we are very much in agreement on all of the fundamental points raised. Herein I will briefly touch on the major points raised by Dr. Caplan to provide further insight into our current understanding of these important points:

1. Transplanted cells do not differentiate.

I agree that transplanted cells likely do not differentiate in vivo. It is clear that what happens in cell culture does not simulate what happens in vivo. My general points about the use of cell surface markers to begin to describe cell populations apply to connective tissue progenitor cells *that are culture-expanded*, trying to describe what happens with freshly harvested cells (from marrow, adipose, etc) that contain connective tissue progenitors (CTPs), defined by the ability to form colony-forming units (CFU) in culture. This description does *not* imply that the behavior in vivo after injection into a patient simulates what occurs in cell culture. The reason for describing the use of cell surface markers to characterize the cells (International Society for Cell and Gene Therapy [ISCT] criteria) is simply as a method to begin to characterize and describe cell formulations. Such differentiation and expression of cell surface markers likely do not happen in vivo. We clearly need methods to rigorously characterize transplanted cells (whether based on cell surface markers, cell morphology, secretory profiles, etc) as indicators of ultimate biologic activity. The ISCT criteria are just one way to start this