We are confident by nature.

However, when it comes to biologies in arthroscopy, we still have a lot to learn. Most of the literature on biologies, tissue-engineering, growth factors, and stem cells shows that there is great variability that must be accounted for. And some published related research results are promising.1-5

In the current issue, readers will find a “must read” systematic review of the literature. We are grateful to our contributors Ahmad, Wardale, Brooks, Henson, Noorani, and Rushton, of the Orthopaedic Research Unit at Cambridge University in England for "Exploring the Application of Stem Cells in Tendon Repair and Regeneration."6 A limitation of their review is that the articles included range from Levels of evidence II to IV. The strength of the review is the clear writing, the excellent quality of the methods, and the clear, thought-provoking conclusions.

In addition, your letters to the editor are heating up,7-12 and our focus is the letter from F. Soler, R. Soler, Peirau, and Orozco of Barcelona,7 expressing their concern that there may be confusion about the recent article “Rapid Isolation of Human Stem Cells (Connective Progenitor Cells) From the Distal Femur During Arthroscopic Knee Surgery” by Beitzel et al.13 Soler et al. are “... afraid that this reading matter may lead some others to misunderstand it, and it is important to clarify some concepts ....” They assert that “The healing potential of cellular therapy is based on the quality of the cellular product and on the dose... cultivation from mononucleated cells (in a laboratory, a laborious process) is considered necessary if the aim is to obtain a significant dose of progenitor cells... the application of a weak number of progenitor cells... is not efficient... So it is important to make the difference, in scientific publications as well as in the medical practice, between products that actually are self-graft and other products that belong to the ‘advanced therapies’ and that, at least in European Union, are considered as drugs... ‘drugs’ are the centrifuged bone marrow products that some apply by joint infusion with therapeutic intentions. This change in the place and function of bone marrow requires a regulated experimentation that proves its safety before its clinical application.”

Your Editors are trying to understand if these regulations are similar to regulatory concerns in the United States between autologous grafting and homologous tissue engineering, or are there really clinically relevant differences?

The authors wrote in reply to Soler’s letter, saying, “In our opinion, ... different research groups use (1) multiple methods of cell procurement and (2) different differentiation media for the determination of the final cell number. It was never the intention of our study and it is difficult to directly compare amounts of isolated cells between previously published studies, because of variable isolation methods, culture time until CFU formation and specifically the use of various differentiation media exist, resulting in diverse outcome determinations. Additionally, within the specific literature, no overall agreement on the optimal isolation method, passage time, differentiation medium, and appropriate number of MSCs exist. Therefore, scientists and surgeons can chose out of a plethora of methods with only low levels of evidence available to prove their therapeutic effect. There is a wide discussion on which additional factors are needed to improve the effects of MSC application with the aim of improvement of the healing environment in orthopaedic surgery.”8

These discussions are important because, in addition to the methods cited above, Mazzocca et al. have previously extracted stem cells from the humerus during rotator cuff repair, in order to produce connective tissue progenitor cells and, in addition, found that bone marrow–derived stem cells differentiate into tendon-like cells when tagged with insulin.14,15

We are fascinated by the possibility that, some day, cells extracted from the humeral head could be used to enhance...
arthroscopic shoulder rotator cuff repair, and cells extracted from the distal femur could be used to enhance arthroscopic knee anterior cruciate ligament reconstruction.

The possibilities are endless.

And let’s not forget that McCullough, Frisbie, Rodkey, Kisiday, Werpy, Kawcak, and Steadman\(^\text{14}\) evaluated intra-articular injection of bone marrow-derived mesenchymal stem cells to augment healing of microfractured chondral defects compared with microfracture alone in horses.

Although there were no significant clinical or histologic differences between groups, there was some suggestion that bone marrow–derived mesenchymal stem cells did enhance cartilage repair quality.

In addition, Saw, Anz, Merican, Tay, Ragavanaidu, Lee, and McGuire reported “Articular Cartilage Regeneration With Autoologous Peripheral Blood Progenitor Cells and Hyaluronnic Acid After Arthroscopic Subchondral Drilling: A Report of 5 Cases With Histology.”\(^\text{15}\) Patients with knee cartilage lesions had arthroscopic subchondral drilling followed by postoperative intra-articular injection of peripheral blood progenitor (stem) cells. Second-look arthroscopic biopsy and histologic examination revealed articular hyaline cartilage regeneration, and postoperative radiographs even suggested joint space restoration.

As we have previously opined, “To be honest, the idea that articular hyaline cartilage regeneration and joint space restoration could be possible as a result of a single-stage arthroscopic procedure followed by injections seems too good to be true... At the end of the day... we believe many will agree that stem cells have vast potential. In the future, we are prepared for inevitable steps backward, but today we are optimists who advance two steps forward.”\(^\text{16}\)

To borrow a Lincolnesque construction: We are confident of nature, by nature, and for nature.

Some published research results are promising relevant to stem cells, and the vast magnitude of biologic variability is the obstacle researchers currently address.

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REFERENCES


We welcome the comments by Soler et al. and believe that these comments may further assist the reader in understanding the different methods currently being evaluated in translational research for concentrating bone marrow aspirates to obtain mesenchymal stem cells.

We recognize the "period of adaptation" often required prior to the "incorporation of therapeutic innovations" and are aware of the lessons learned in the "early stages of orthopaedic surgery." In prior publications regarding our approach to obtaining bone marrow, we have discussed the multiphase process of development and implementation of a biologic option suitable for use in the operating room, highlighting the thorough basic science investigation needed before consideration of use in human subjects. We encourage our approach to biological research and the context in which our results were presented and discussed were commended in a recent editorial in the Journal of Arthroscopy and Related Research. We are encouraged that Dr. Soler and his colleagues understand the importance of exercising caution when conducting and reporting cell-based research in orthopaedic medicine. Having recognized our mutual appreciation for a mindful approach to this type of research, we would like to specifically address a few of their concerns.

Soler et al. states that it would be "a mistake to compare the therapeutic potential of cellular products derived from centrifuged bone marrow with suspensions of stem cells properly typified." We agree and are encouraged that Dr. Soler and colleagues share this viewpoint. We want to emphasize that such a statement was never made in our study and any thoughts thereof were unintended. The purpose of our study was to evaluate a novel method for harvesting and concentrating bone marrow with an emphasis on increasing the amount of connective tissue progenitors (CTPs) contained within the aspirate. The study protocol was laboratory based thereby prohibiting conclusions regarding therapeutic use of these cells. This is stated as a limitation in the discussion section of our manuscript: "These data represent empirical evidence of CTP (connective tissue progenitor cell) concentration only and do not allow valid conclusions regarding sufficient amounts of CTPs for clinical purposes." Solar et al. states concern over "the quality of the cellular product." We recognize that there are efforts among researchers to determine adequate cell number to optimize healing. Comparisons of these data can be challenging due to subtle differences in the methodologies used by investigators. Variations including different methods of cell procurement, differentiation media, and methods of counting introduce an additional element of variability that limits direct comparisons between studies.

We are confused with regard to "the quality of the cellular product." In our study, isolated cells were plated and found to adhere to tissue culture plastic and form colonies. Consistent with our previous publications, a group of aspirates underwent fluorescence-activating cell sorting and were found to express markers of mesenchymal stem cells (data not shown).

Soler et al. also states concern over the volume of bone marrow aspirated in our study, specifically volumes exceeding 2 mL typically do not contain progenitor cells. CTPs are believed to adhere to the microstructure of the trabecular bone. Musshter et al. recommended dividing the process into repetitive aspirations of smaller volumes (2 mL) from different locations (e.g., turning the aspiration needle 45°). While valid, the goal of our approach was to minimize morbidity by using a technique that did not require additional surgical sites or impede, interrupt, or extend the surgical process. Solar et al. describes their approach to harvesting bone marrow through small aspirations of 1 mL, which are repeated to obtain up to 100-mL of marrow. While this technique may have some merit, we do not feel it is appropriate for our use. In our experience, repetitive aspirations at a rate of 1 mL would substantially extend the time needed to complete the index procedure while increasing the amount of anesthesia required.

Soler et al. is concerned about the potential for the "weak number of progenitor cells in the 'lethargic' state" to become "lost in the fight for oxygen and nourishment with the rest of the cells." As previously discussed, the minimal number of cells for optimal healing is unknown. While we are unsure what the term "lethargic" is referring to with regards to the cells in our study, we presume that the authors are referring to cells in a diminished state with respect to viability. In our study, cell viability was tested for each aspirate and consistently found to be greater than 98%.

The purpose of our paper was to demonstrate a novel surgical technique for harvesting bone marrow that does not involve long and complicated laboratory isolation methods associated with currently
To the Editor:

The incorporation of therapeutic innovations in the health system requires a period of adaptation. At first, there may be some confusion about the concepts, as happened in the early stages of orthopaedic surgery, regulated osteosynthesis, and even arthroscopy. If we go back to the origins of medicine, the same could be said about anesthesia or vaccines.

Cellular therapy is at the stage of moving from laboratory to clinical application. This is the perfect time for errors to arise, along with the risk of perpetuating them. For instance, it is a mistake to compare the therapeutic potential of cellular products derived from centrifuged bone marrow with suspensions of stem cells properly cultured after a selection and growing process. The recent article "Rapid isolation of human stem cells (connective progenitor cells) from the distal femur during arthroscopic knee surgery" by Beitzel et al. refers to the results of an excellent practice in vitro, as an experienced reader in cellular therapy will understand. However, we are afraid that some of the wording may cause other readers to misunderstand it, and it is important to clarify some concepts.

The article suggests the possibility of "rapid isolation" of progenitor cells by a device that makes an aspirated 30 mL of bone marrow in 60 seconds and that, with no need to perform a standard laboratory procedure such as the Ficoll-Hypaque technique, one can obtain a product rich in progenitor cells with the ability to help bone regeneration.

The healing potential of cellular therapy is based on the quality of the cellular product and on the dose. The scientific community invests huge effort and money in developing laboratory procedures to isolate progenitor cells from bone marrow in the stem cells in lethargic state and grow them, achieving this intake on cycle and replication without differentiation. In this way one obtains a significant dose of progenitor cells properly cyted after a selection and growing process.

Afterward, through an essential laboratory process such as the Ficoll-Hypaque technique, the fraction of mononucleated cells where the mesenchymal progenitor cells are present at the very low rate of 1:10,000 to 1:1,000,000 is removed. That is why a cultivation procedure from mononucleated cells is considered necessary if the aim is to obtain a significant dose of progenitor cells.

What we are saying does not contradict the possible therapeutic potential of the product described in the article, but we understand that the application of a weak number of progenitor cells in the "iathargic" state is not efficient enough because many of these cells will be lost in the fight for oxygen and nourishment with the rest of the cells.

So, it is important to establish the difference, in scientific publications as well as in medical practice, between products that actually are self-grafting products and other products that belong to the "advanced therapies" category and that, at least in the European Union, are considered drugs. By the way, also considered "drugs" are the centrifuged bone marrow products that some clinicians apply by joint infusion with therapeutic intentions. This change in the place and function of bone marrow requires a regulated experimentation "that proves its safety before its clinical application."

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References