Abstract: Bone fracture non-unions are often the result of high-impact injuries and are characterized by an inability to heal due to a reduced blood supply at the fracture location. Sufferers are often incapable of regaining the proper strength and function to carry out normal activities. Treatment options were limited mostly to medications and casting until research discovered the uses of adult stem cells. Human mesenchymal adipose derived stem cells (HMADSCs) and human bone marrow mesenchymal stem cells (hBM-MSCs) have been the basis of most non-union fracture research. By creating and managing inflammatory responses, promoting superior callus formation, and shortening fracture recovery period, adult mesenchymal stem cell injections heal fracture non-unions and normal fractures better than other conventional treatment methods during animal experimental studies.

In the modern world, most broken bones can be expected to heal without serious problems if they are professionally diagnosed, properly realigned, and expertly stabilized. If the patient is generally healthy and well nourished and the blood supply to the area is adequate, new bone tissue almost always forms to reconnect the broken pieces of bone. Occasionally a “delayed union” may take place in which the fracture eventually heals over a longer period of time than expected and, in rare instances, a broken bone fails to heal at all. When a broken bone does not heal, even after an extended period of time, it is called a “non-union”. A fracture non-union is a serious medical condition that can be difficult to treat (AAOS 2013). The most probable cause of a fracture non-union is lack of stability at the fracture site coupled with a lack of an adequate blood supply. The risk of having a fracture non-union is highest in people who smoke or use tobacco in any form, in the elderly, in people who are anemic, in people with diabetes, in people taking anti-inflammatory drugs such as aspirin and ibuprofen, and in people with a current infection. The lack of an adequate blood supply to a bone may be due to the nature of the affected bone itself or it may be the result of severe trauma to the fracture site. The bones of the toes, for example, are very stable and this, coupled with the fact that they also have an excellent blood supply, is one of the reasons that toe fractures are likely to heal with minimal treatment or without treatment at all. The femur and the scaphoid, both commonly broken bones, possess a limited blood supply that is often disrupted when a fracture occurs. Fractures in these bones may take a little longer to heal. The blood supply to the tibia is considered to be moderate but when fractured, there may be difficulties with healing because the shaft of the bone is so close to the overlying skin and muscle, which can easily be destroyed when there is trauma to the leg. Trauma to the surrounding soft tissues can destroy the blood supply to the marrow cavity of the tibia. Rarely it may happen that bone fractures occur in people with no apparent risks for nonunion. These people may have access to proper treatment and appear to be healthy, but the fracture simply does not heal, even after surgical intervention. It is this last group that is most problematic (Shoji et al., 2013, AAOS 2013).

One of the signs that a fracture non-union has occurred is that pain is felt for a long time, months or even years, after the initial pain of the fracture has disappeared. Imaging studies, including X-rays, CT scans, and MRI scans, are used to diagnose a fracture non-union. The diagnosis of a non-union may be made based on an observable gap between bone ends at the fracture site, the lack of observable healing progress over many months, or an unacceptable length of time a patient feels pain associated with the fracture. Along with imaging studies, blood tests may be used to determine if the patient has an infection or another medical condition that might be slowing the repair process, such as diabetes or anemia. Traditional treatment for nonunion fractures may involve the use of electromagnetic and ultrasonic pulsed waves to the bone and surgical intervention. Surgical treatment options include bone grafting, bone substitute grafting, and internal or external fixation (AAOS 2013). Surgery is both invasive and expensive and carries a significant risk of infection. One possible, less invasive, solution to the problem of fracture non-unions that has shown significant promise, involves...
the injection of stem cells into the non-union site. The limitations placed on embryonic stem cell research combined with extremely limited funding in this area, led to a renewal of interest in human bone marrow mesenchymal stem cells and stem cells derived from adipose tissue. Human bone marrow mesenchymal stem cells (hBM-MSC) are a form of adult stem cells that can be extracted non-invasively from an infant’s umbilical cord or placenta and adult tissues such as bone marrow and peripheral blood. Much like embryonic stem cells, adult stem cells have received their share of criticism, presumably because the term “adult” implies that they are being removed from a living human. hBM-MSCs are collected from bone marrow via needle biopsy, while donors are under general anesthesia, and from the umbilical cord after birth. When hBM-MSCs are taken from peripheral blood, they are removed through a catheter into a machine that filters out the stem cells and circulates the blood back into the donor. An average extraction from any of these three cell sources contains only about one human bone marrow mesenchymal stem cell for every 34,000 cells processed. Although this number is very low, it takes only a few weeks to grow a sufficient number of cells for injection. Research collected from many animal-model organisms injected with hBM-MSCs has shown that the cells have the ability to treat or cure many common diseases including red blood cell disorders such as sickle cell anemia, many cancers including lymphoma and leukemia, and even autoimmune disorders like multiple sclerosis and Crohn’s disease. In addition, there are now many studies that show the ability of hBM-MSC’s to regenerate bone damaged by fractures and to repair torn cartilage (cancer.org 2012, NSCF 2011, Prentice 2004, Wexler et al., 2003).

One form of “adult” stem cell that is free from controversy or ethical concerns is human multipotent adipose derived stem cells (hMADSCs). These cells were discovered in 2002 by researchers at UCLA and they have emerged as the leading stem cell used in current research. They possess the same multipotency as bone marrow stem cells but they are much more plentiful and less invasive to extract. The removal of hMADSCs is done via liposuction, which has already been established as a cosmetic procedure that enjoys worldwide acceptance. The ability to extract a very large number of cells at once means that the extracted cells do not need to be grown in vitro for very long. This saves a great deal of time, making the use of adipose derived stem cells a more sensible solution for clinical usage. There are roughly 500 times more mesenchymal stem cells extracted from one gram of adipose tissue than can be extracted from a gram of bone marrow. While HAMADSCs have shown the ability to treat cardiac muscle repair following heart attack, and circulatory damage resulting from diabetes in research animals, these adipose derived cells have been most successful in repairing both bone and cartilage following serious injuries (NSCF 2011, Zuk, 2010).

The differentiation process for both hBM-MSCs and HAMADSCs into new bone requires only a few steps, but when examined on a molecular level the process is quite complex. Stem cells are isolated by centrifugation and cultured with growth factors, inorganic salts, vitamins, and amino acids. Proliferation of the stem cells generally takes between two and three weeks. Following proliferation, the second step involves commitment of the stem cells to the osteoblast lineage. This is accomplished by the introduction of bone morphogenetic proteins to the culture medium to direct the differentiation of the stem cells. Fifteen different bone morphogenetic proteins (BMPs) have been isolated and they can be added to the culture medium as needed, causing the stem cells to differentiate into the appropriate cell lineage. The third step requires lineage progression into mature osteoblasts. This progression is accomplished using a medium that contains bone morphogenetic proteins that are specific for this purpose. The culture medium is replaced with fresh medium every 48-72 hours for a week. Once mature osteoblasts have been formed, they are frozen in a special freezing medium until they are needed for implantation. Freezing mediums slow the freezing process of the stem cells to reduce the crystallization rate. When needed, the differentiated cells are quickly thawed in a warm water bath where they can stay for up to 48 hours. The cells are treated with phosphate buffered saline (PBS) that adjusts them to the proper pH and osmolarity for implantation. They are injected into a specific body location and they are attracted to the fracture site by chemotaxis. The fourth and final step requires the transformation of the osteoblasts into osteocytes. Osteocytes are formed when the osteoblasts become embedded in the bone-forming matrix. New bone formation and the healing process in general are important in several types of bone injuries including fractures (Burastero et al., 2010, Caplan & Bruder 2001, Violini et al., 2009).
In natural bone healing, the damaged bone endings bleed immediately following a fracture and bleeding continues until a clot forms. This is followed by acute local inflammation, which is caused by the migration of histamine-secreting mast cells into the epithelium. Inflammation is important in the promotion of healing because it attracts neutrophils, macrophages, and mesenchymal cells to the site of the injury. Acute inflammation is followed by the “repair” stage. Since the vascular tissue surrounding the injured bone has been destroyed or partially damaged, fibroblasts begin laying down stroma, which allows the bone to become re-vascularized. Immediately following an established vascular system, a collagen matrix begins to form around the bone, which quickly becomes a callus. The callus bridges the cut ends of the bone encouraging the bone parts to fuse. The callus is generally very weak for 4 to 6 weeks and can easily re-fracture if stress is placed upon it. This is the reason a cast is required for most fractures. The final step for bone healing is termed the remodeling stage. During this stage the bone regains its form and strength. It takes from three to six months following a fracture for a bone to regain optimal strength following a fracture. Typically, the elderly require longer recovery periods. As many as ten percent of all bone fractures may require surgery in order to achieve proper healing (Hannouche et al., 2001, McKibbin, 1978, Kalfas 2001).

When fracture healing does not proceed along expected lines and healing is delayed, stem cell injection into the fracture site may address the limitations of natural bone healing. hBM-MSCs have been found to regulate and reduce the inflammatory response in non-union fracture sites where the fracture site may require more inflammation to establish a good blood supply or less inflammation to promote proper healing. In animal studies where fractures were induced in mouse tibias, the injection of hBM-MSCs promoted more callus volume and a stronger bone matrix than standard healing while simultaneously shortening the recovery time. In studies where fracture non-unions were induced in rat femurs, injection with hBM-MSCs resulted in the production of new bone with a significant ability to take on more torque and withstand more force than naturally healed bone. Biomechanical testing on non-union fractures in rat femurs that had been injected with HMADSCs also showed greater strength when compared with naturally healed bone. Femurs injected with HMADSCs showed the ability to withstand nearly triple the amount of stress placed on the bone and exhibited nearly quadruple the amount of percent energy failure, which is the ability to absorb mechanical energy until breakage occurs. These results strongly indicate the ability of stem cell injections to speed and enhance the recovery process of fracture non-union healing (Granero-Molto et al., 2009, Undale et al., 2011 Shoji et al., 2011).

Human bone marrow mesenchymal stem cells and adipose derived mesenchymal stem cells have shaped the field of regenerative bone repair for the future. Sometime in the near future healing time for many tendon, ligament, bone, and cartilage surgeries will be drastically reduced by the injection of adult stem cells. These cells will be able to effectively increase the vasculature at a fracture location, form osteoblasts around a callus, and allow the callus to become stronger, heal faster, and withstand greater forces than naturally healed bone. Best of all, everything will be accomplished with a significantly shortened recovery period.

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Literature Cited:


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