Mesenchymal stem and progenitor cells for cartilage repair

Nazish Ahmed · William L. Stanford · Rita A. Kandel

Introduction

Treatment of damaged articular cartilage is problematic due in part to the avascularity of the tissue. Regenerative medicine and tissue engineering offer new approaches for the repair or replacement of damaged or diseased tissue. There is now proof-of-concept to support cell-based regeneration of cartilage, but one of the major issues limiting its use clinically is the availability of a cell source that will form sufficient amounts of tissue comparable to in vivo cartilage both in composition and in mechanical properties. One possible option is to use stem and progenitor cells. This perspective will describe the promise and limitations of the use of stem cells for cartilage repair.

Background

Articular cartilage is a unique tissue whose functions are the distribution of applied load to the underlying subchondral bone and to provide, along with the synovial fluid, a low friction interface between the contacting surfaces of the joint

N. Ahmed · W. L. Stanford · R. A. Kandel
CIHR-Bioengineering of Skeletal Tissues Team,
Mt. Sinai Hospital, University of Toronto,
Toronto, Canada

W. L. Stanford · R. A. Kandel
Institute of Biomaterials and Biomedical Engineering,
University of Toronto,
Toronto, Canada

R. A. Kandel (✉)
Department of Pathology and Laboratory Medicine,
Mount Sinai Hospital,
600 University Avenue, Suite 6-500,
M5X 1G5 Toronto, Ontario, Canada
e-mail: rkandel@mtsinai.on.ca

[1]. Adult articular cartilage has a limited ability to spontaneously heal when damaged by trauma or disease. A variety of reasons for this lack of reparative response have been postulated, including the absence of a fibrin clot, the inability of chondrocytes to migrate into the site of injury because of their dense extracellular matrix, and the avascular nature of cartilage that prevents the influx of progenitor reparative cells [2, 3]. However, other factors may also influence the repair process, as chondrocytes are able to reconstitute at least part of their extracellular matrix in vitro as well as in vivo when supplied with the appropriate treatment [4, 5]. The morbidity, pain, and limitation of movement that arise as a result of joint disease are significant problems for the patient and the health care system. Currently, replacement of an articulating joint with a synthetic prosthesis represents the optimal treatment for end-stage joint disease. However, it has limitations, as apparently successful implants have failure rates of up to 20% after 10–20 years. So, there has been a great interest in developing biological treatments for joint repair, which would also allow for early intervention. One of these potential approaches is tissue engineering, which utilizes cells and biomaterials to regenerate tissues. Autologous chondrocyte implantation has provided proof-of-concept for this type of treatment [6]; however, identifying a source that can supply enough cells to form sufficient amounts of cartilage that approximates the quality of in vivo tissue in terms of composition and mechanical properties has been problematic. One exciting option is the use of stem and progenitor cells.

Stem cells

Stem cells have the unique capacity to generate progeny with the same developmental potential (self renew) or

differentiate into the lineages of the stem cell's tissue origins. Stem cells are generally classified as pluripotent [embryonic stem cells (ESC)] or multipotent [somatic stem cells (SSC)]. ESC are derived from preimplantation embryos (morulae or the inner cell mass of the early blastocyst) and when grown under appropriate conditions can be induced to differentiate into cells of all three germ layers (ectoderm, mesoderm, and endoderm). ESC can be readily grown as undifferentiated cells under defined conditions, providing an unlimited supply of pluripotent stem cells. Whereas chondrocyte differentiation from ESC has been reported by several groups [7–9], the culture conditions to generate pure populations of chondrocytes suitable for tissue engineering are far from being defined. Our understanding of the full potential of these cells is limited, as we are still in the early stages of understanding their biology; several issues have already been identified that will have to be addressed before they can be considered for routine use in a clinical setting. Some examples of these are the ethical concerns regarding the use of such cells, the controversy of utilizing allogeneic cells for cartilage tissue engineering, and the ability of residual implanted undifferentiated ESC to form teratomas, an unacceptable complication for a treatment designed for a non-life-threatening disease.

Alternative sources of cells are SSC that repair or maintain the tissue in which they reside. The differentiation capacity of these cells is limited to the lineages of their tissue origin; hence, SSC are considered multipotent rather than pluripotent, which is a characteristic of ESC. Interestingly, SSC have been isolated from the amniotic fluid that appear to have very broad differentiation capacity without forming teratomas, a characteristic of ESC [10, 11]. However, their potential role in cartilage tissue engineering has not yet been defined. One type of SSC that has the capacity to differentiate into chondrocytes is the mesenchymal stem cell (MSC). Bone-marrow-derived adherent cell cultures presumably containing MSC have cells capable of differentiating into osteoblasts, adipocytes [12] and myocytes (muscle), and tenocytes (tendon), as well as supporting fibroblastic cells (marrow stroma) [13]. Whereas this differentiation capacity is presumed to originate from single stem cells, these cultures are very heterogeneous, and the appropriate experiments are only now being performed by several groups, including our own, to test whether a single stem cell is capable of differentiating into each of these lineages. The International Society for Cellular Therapy recently published two position statements on the nomenclature and definition of MSC [14, 15]. In accordance to the nomenclature, for the remainder of this commentary, we use the term mesenchymal progenitor cells, or MPC. MPC were first discovered in bone marrow by Friedenstein et al. in the 1970s, and were termed colony-forming unit fibroblasts (CFU-F), adopting the hematopoietic nomenclature referring to clonogenic progenitor cells [16]. When these cells are injected into fetuses or newborn mice, they engraft in many organs and undergo site-specific differentiation [17]. MPC are highly proliferative, although the differentiation capacity of bone-marrow-derived MPC diminishes with passage in culture. Interestingly, these cells may have potential uses other than supplying cells for tissue regeneration, as they also secrete growth factors and cytokines and can modulate the immune response [18]. As MPC can also be used for gene therapy, it is easy to envisage their use in cellular therapeutics [19]. There is no cell-surface antigen that definitively identifies MPC from mature cells formed in mesenchymal cultures; therefore, their existence is usually defined functionally [20]. In culture, MPCs are characterized by their ability to form colonies (CFU-F) and, as a population, to differentiate into multiple lineages [20]. MPC capable of forming colonies (CFU-F) comprise 0.001–0.01% of nucleated cells in bone marrow; hence, there is a great deal of interest in identifying cell markers that could be used to identify and enrich for these cells, especially those with true multilineage potential. MPC are present in a variety of tissues and can be obtained from bone marrow, adipose tissue (abdominal and infrapatellar fat), synovial membrane, blood, and umbilical cord to use for cartilage repair [20]. The ideal source of these cells remains controversial, as some investigators report that MPC derived from synovium have more chondrogenic potential than cells derived from bone marrow [21], whereas bone-marrow-derived MPC appear more chondrogenic than adipose-tissue-derived cells [22, 23]. It may also be that the original location of the MPC plays a role in determining their differentiation potential, as MPC derived from adipose-type synovium have a higher chondrogenic potential than those derived from subcutaneous fat [24], and skeletal-muscle-derived MPC have relatively low potential for chondrogenesis [25]. We know little about what regulates progression of stem cells into committed progenitor cells (unipotent) with the acquisition of lineage-specific properties, although this is clearly influenced by the microenvironment (niche) in which they are located. Clearly, a series of gene-expression changes occurs, and this is an area of active investigation. For example, a study using expression microarrays identified 52 genes that are upregulated during the transition from MPC to chondrocytes, however of these, three are also upregulated during differentiation to osteocytes [20]. Also, the question of whether plasticity (or transdifferentiation) or fusion with differentiated cells is involved in the acquisition of lineage-specific characteristics by MPC has not been resolved. An in vitro study by Song and Tuan showed that MPC-derived chondrocytes (as well as osteoblasts and adipocytes) can be manipulated to lose their phenotype and

then induced to redifferentiate into a cell type different from their original phenotype, which demonstrates the plasticity of these cells [26]. Yet fusion of bone-marrow cells with differentiated cells has also been demonstrated [27]. Perhaps both mechanisms are at play, and a specific pathway is activated depending on the situation.

Chondroprogenitor cells (MPC-like) have been isolated from articular cartilage and reported to represent as high as 12% of chondrocytes, although these numbers were determined in cells obtained from osteoarthritic cartilage [28]. Interestingly, Dowthwaite et al. demonstrated that in normal cartilage, these cells are present only in the superficial zone [29], suggesting they may not originate in cartilage but may have migrated there from the synovial fluid. This would explain why chondroprogenitors are present in such high numbers in diseased tissues, as it has been shown that MPC will migrate to areas of tissue damage [30]. Nevertheless, it is not clear why there is a lack of articular cartilage regeneration even in the apparent presence of MPC. Alsalameh et al. have proposed that "inappropriate programming" of the MPC may be responsible for this lack of repair [31].

MPC and cartilage repair

For many years, autogenous MPC have been indirectly utilized clinically, e.g., in microfracture and perichondral grafts, for focal cartilage repair. However, their direct use in chondral repair is recent. The main advantages of MPC for regenerative medicine and tissue engineering applications are their easy access, potential for cell-number expansion, ability to readily differentiate into the cells of interest, lack of immunogenicity, and limited capability to form tumors. There are several factors limiting the use of MPC. For example, their proliferation and differentiation potential can deteriorate with age, gender (female-derived cells appear less robust), and under certain conditions with disease. In fact, we have demonstrated that defective MPC self-renewal is a mechanism underlying at least some types of age-related osteoporosis [32]. Another critical issue is that MPC can differentiate to hypertrophic chondrocytes and form cartilage that can mineralize. Although methods to prevent this process have been identified, such as treating the cells with parathyroid hormone-related protein (PTHrP), it is not known whether this will be effective when the cells are implanted in vivo [33]. Differentiation to hypertrophic chondrocytes is not entirely unexpected, as MPC from bone marrow are known to generate fibrocartilage—as has been shown to occur after joint-surface microfracture, for example—and do not appear to be a good source of MPC [34]. Nevertheless, MPC have been used to repair focal cartilage defects in animal models and the results have been promising, even when allogeneic cells have been used [35, 36]. There was one human study in which MPC were resuspended in a collagen gel and used to repair osteoarthritic cartilage. Although there was no significant improvement clinically compared with knees that did not receive MPC, the repair tissue appeared more robust, confirming the potential of these cells [37] even in the face of degenerative disease. How these cells will perform in an inflammatory setting such as rheumatoid arthritis is unknown.

Future of stem cells in regenerative medicine

Articular cartilage, even though composed of one cell type, is a complex tissue, and its repair or replacement is a challenge. The widespread clinical application of stem cells in these settings will require further understanding of the biology of MPC and delineation of the pathways that regulate lineage-specific differentiation. Given the major advances that have occurred over the past few years, it should not be long until we know the full potential of these cells.

References

1. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthr Cartil 2002; 10: 432–463.
2. Hunziker EB. Articular cartilage repair: problems and perspectives. Biorheology 2000; 37: 163–164.
3. Kinner B, Capito RM, Spector M. Regeneration of articular cartilage. Adv Biochem Eng Biotechnol 2005; 94: 91–123.
4. Boyle J, Luan B, Cruz TF, et al. Characterization of proteoglycan accumulation during formation of cartilagenous tissue in vitro. Osteoarthr Cartil 1995; 3: 117–125.
5. Chuma H, Mizuta H, Kudo S, et al. One day exposure to FGF-2 was sufficient for the regenerative repair of full-thickness defects of articular cartilage in rabbits. Osteoarthr Cartil 2004; 12: 834–842.
6. Brittberg M, Lindahl A, Nilsson A, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994; 331: 889–895.
7. zur Nieden NI, Kempka G, Rancourt DE, et al. Induction of chondro-, osteo- and adipogenesis in embryonic stem cells by bone morphogenetic protein-2: effect of cofactors on differentiating lineages. BMC Dev Biol 2005; 5: 1.
8. Vats A, Bielby RC, Tolley N, et al. Chondrogenic differentiation of human embryonic stem cells: the effect of the micro-environment. Tissue Eng 2006; 12: 1687–1697.
9. Kramer J, Hargus G, Rohwedel J. Derivation and characterization of chondrocytes from embryonic stem cells in vitro. Methods Mol Biol 2006; 330: 171–190.
10. De Coppi P, Bartsch G Jr, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 2007; 25: 100–106.

11. Kim J, Lee Y, Kim H, et al. Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. Cell Prolif 2007; 40: 75–90.

12. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–147.

13. Caplan AI. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopedics. Tissue Eng 2005; 11: 1198–1211.

14. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315–317.

15. Horwitz EM, Le Blanc K, Dominici M, et al. Clarification of the nomenclature for MSC: the international society for cellular therapy position statement. Cytotherapy 2005; 7: 393–395.

16. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 1976; 4: 267–274.

17. Liechty KW, MacKenzie TC, Shaaban AF, et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 2000; 6: 1282–1286.

18. Otto WR, Rao J. Tomorrow's skeleton staff: mesenchymal stem cells and the repair of bone and cartilage. Cell Prolif 2004; 37: 97–110.

19. Leo AJ, Grande DA. Mesenchymal stem cells in tissue engineering. Cells Tissues Organs 2006; 183: 112–122.

20. Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med 2004; 8: 301–316.

21. Sakaguchi Y, Sekiya I, Yagishita K, et al. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 2005; 52: 2521–2529.

22. Im GI, Shin YW, Lee KB. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? Osteoarthr Cartil 2005; 13: 845–853.

23. Huang JI, Kazmi N, Durbhakula MM, et al. Chondrogenic potential of progenitor cells derived from human bone marrow and adipose tissue: a patient-matched comparison. J Orthop Res 2005; 23: 1383–1389.

24. Mochizuki T, Muneta T, Sakaguchi Y, et al. Higher chondrogenic potential of fibrous synovium- and adipose synovium-derived cells compared with subcutaneous fat-derived cells: distinguishing properties of mesenchymal stem cells in humans. Arthritis Rheum 2006; 54: 843–853.

25. Yoshimura H, Muneta T, Nimura A, et al. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell Tissue Res 2007; 327: 449–462.

26. Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. FASEB J 2004; 18: 980–982.

27. Terada N, Hamazaki T, Oka M, et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002; 416: 542–545.

28. Fickert S, Fiedler J, Brenner RE. Identification of subpopulations with characteristics of mesenchymal progenitor cells from human osteoarthritic cartilage using triple staining for cell surface markers. Arthritis Res Ther 2004; 6: R422–R432.

29. Dowthwaite GP, Bishop JC, Redman SN, et al. The surface of articular cartilage contains a progenitor cell population. J Cell Sci 2004; 117: 889–897.

30. Le Blanc K. Mesenchymal stromal cells: tissue repair and immune modulation. Cytotherapy 2006; 8: 559–561.

31. Alsalameh S, Amin R, Gemba T, et al. Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. Arthritis Rheum 2004; 50: 1522–1532.

32. Bonyadi M, Waldman SD, Liu D, et al. Mesenchymal progenitor self-renewal deficiency leads to age-dependent osteoporosis in Sca-1/Ly-6A null mice. Proc Natl Acad Sci USA 2003; 100: 5840–5845.

33. Kafienah W, Mistry S, Dickinson SC, et al. Three-dimensional cartilage tissue engineering using adult stem cells from osteoarthritis patients. Arthritis Rheum 2007; 56: 177–187.

34. Robinson D, Nevo Z. Articular cartilage chondrocytes are more advantageous for generating hyaline-like cartilage than mesenchymal cells isolated from microfracture repairs. Cell Tissue Bank 2001; 2: 23–30.

35. Huang JI, Durbhakula MM, Angele P, et al. Lunate arthroplasty with autologous mesenchymal stem cells in a rabbit model. J Bone Joint Surg Am 2006; 88: 744–752.

36. Shao X, Goh JC, Hutmacher DW, et al. Repair of large articular osteochondral defects using hybrid scaffolds and bone marrow-derived mesenchymal stem cells in a rabbit model. Tissue Eng 2006; 12: 1539–1551.

37. Wakitani S, Imoto K, Yamamoto T, et al. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 2002; 10: 199–206.