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Engineering stem cells for treatment of osteochondral defects

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Osteochondral defects due to degenerative or inflammatory arthritis represent one of the major causes of disabilities in the world, leading to annual health care costs in the order of US $95 billion in the United States alone. The pain associated with osteochondral defects leads to secondary effects, such as impaired joint mobility or function, decreasing work productivity, loss of quality of life—and in severe cases, potentially, depression-induced tertiary effects. Unlike other tissues in the body, cartilage cannot regenerate by itself. Therefore, there is an urgent need for effective approaches to repair osteochondral defects in order to prevent progression of small defects to chronic disease and to restore larger defects and limit related disabilities. Cartilage repair is one of the most challenging areas in tissue engineering because of its complex and unique structure and exposure to high pressure and motion. The avascular and aneuronal nature of cartilage limits the ability to deliver signaling molecules, growth factors, or cellular components for tissue repair. In addition, mature chondrocytes are not able to migrate to sites of injury and the attraction of other cells (e.g., from the bone marrow or synovium) to cartilage defects is hindered by the complex and tight extracellular cartilage structure. Currently employed clinically applicable osteochondral defect repair strategies are as follows:

1) Palliative methods (arthroscopic debridement):

arthroscopic removal of areas of loose, mechanically redundant cartilage and inflamed synovial tissue, mostly used for relief of minor pain symptoms without potential for actual tissue repair.

2) Intrinsic repair enhancement (microfracture):

Tiny fractures are created in the bone underneath a cartilage defect via a minimally invasive surgery. The microfractures allow migration of blood and bone marrow cells (including stem cells) into the defect, which repair the defect by generating fibrocartilage. Suitable for relatively small defects (≤1 cm). The resultant fibrocartilage is less durable compared to the original hyaline cartilage, often leading to returning symptoms and need for new/additional interventions after 1–2 years.

3) Whole tissue transplantation (osteochondral autograft):

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Harvest of one or more osteochondral autograft cylinders from a healthy non-weight-bearing area of a joint; the cylinders are then autografted into an osteochondral defect of a weight-bearing area of the joint. Main advantages of this method are a single surgery approach and transplantation of live hyaline cartilage. Challenges include donor site morbidity and limitation to single defects with a size of 1–2.5 cm.

4) Cell-based tissue repair [autologous chondrocyte implantation (ACI)]:

The ACI technique was introduced by Brittberg [1] in 1994 and is based on a two-step surgery. During a first surgery, cartilage tissue is collected from a non- weight-bearing area of the joint. Chondrocytes are extracted from this tissue sample and are expanded via cell cultures for 4–5 weeks. In a second surgery, the target defect is cleared from dead tissue, a periosteal flap is sutured over the defect and approximately 5–10 million chondrocytes are implanted into the defect, under the periosteal flap. This method has the advantage of producing hyaline-like cartilage and is suitable for repair of larger defects. Disadvantages are the need for two surgeries and potential complications of chondrocyte apoptosis/necrosis or hypertrophy of the cell implant beyond the periosteal graft.

5) Cell-scaffold–based repair [matrix-associated chondrocyte implants (MACI) and matrix-associated stem cell implants (MASI)]:

Autologous chondrocytes, harvested from autologous cartilage samples as described above, or autologous stem cells, harvested from a bone marrow aspiration or biopsy, are seeded in scaffold as an advanced approach to hold implanted cells in cartilage defects. The scaffold can be enriched with growth factors to support stem cell differentiation and/or cell engraftment. A variety of approaches for MACI and MASI are currently being investigated, based on different cell sources and different scaffolds. Advantages and disadvantages of MACI and MASI will be subsequently discussed.

All of the clinically available techniques described above can provide transient or permanent, partial or complete relief of pain related to osteochondral defects. However, only MASI have the potential to truly regenerate hyaline cartilage without creating defects (and potential secondary morbidity) at other sites of the joint. A variety of MASI approaches are currently being investigated with the goal to engineer durable cartilage tissue that provides smooth joint resurfacing, is resistant to high weight load and shearing stress, and has low friction properties. Three main variables affect MASI engraftment outcomes: (1) cell type and source, (2) scaffold composition and architecture, and (3) integrated growth factors and/or cytokines.

Cell type and source for MACI and MASI The ideal cell source for MACI/MASI should allow easy and inexpensive cell harvesting, expansion and maintenance; involve minimal donor morbidity; and have a low risk of immune responses or transmission of other diseases. Autologous chondrocytes were the first cell type applied for matrix-associated cell implants. However, preclinical and clinical applications revealed limited long-term survival and limited tissue regeneration capacities of these cells. In addition, the surgical procedure needed to harvest chondrocytes from cartilage tissue samples is associated with high costs, secondary morbidity, limited cell yield, and low cell quality, especially in elderly patients.

Therefore, stem cells were introduced as an alternative cell source for cartilage regeneration. The most frequently used adult stem cells for MASI to date are bone marrow–derived stem cells (BMSC), harvested from bone marrow aspirations or bone marrow biopsies, and adipose-derived stem cells (ADSC), typically harvested via a liposuction. Both BMSC and ADSC as the source for MASI have the advantages over autologous chondrocytes of requiring one less knee surgery, resulting in less donor-site morbidity, and being more cost effective, while yielding equal or better long-term out- comes [2]. BMSC have shown superior osteochondral differentiation capacities over ADSC, while ADSC pro- vide a more easily accessible and more abundant source for stem cells.

Other recently explored stem cell sources include synovial- and periosteal-derived stem cells. Harvesting synovial-derived stem cells results in minimal donor site morbidity because of the high regeneration potential of the synovial membrane, but cell yield is still limited compared to BMSC and ADSC. A periosteal membrane, harvested, e.g., from the proximal tibia, can be used to secure MASI. It contains progenitor cells, which can support the osteochondral repair. However, all adult stem cells share the limitation of invasive harvesting procedures, limited yield, and donor-dependent quality.

Alternative cell sources include amniotic fluid–derived stem cells (AFSC), umbilical cord blood–derived stem cells (UBSC), and stem cells harvested from the human fetal membrane or placenta. Due to their immaturity, these cells demonstrate a higher proliferation and chondrogenic differentiation capacity compared to adult stem cells. However, the source of these cells (amniotic fluid, umbilical cord blood, or placenta) is very limited at this time. Embryonic stem (ES) cells are a very appealing source for cartilage regeneration due to their virtually unlimited proliferation and differentiation capacity. However, the use of ES cells is ethically highly controversial. In addition, ES cells have a high potential for genetic instability and tumorigenicity. Nevertheless, investigators have shown the capacity of ES to regenerate hyaline cartilage [3]. A less ethically controversial cell source with presumed similar cartilage regeneration potential is induced pluripotent stem (iPS) cells. iPS cells are generated by reprogramming somatic cells to the pluripotent stage. A classic method for reprogramming these cells involves viral transduction, which would be difficult to translate to clinical applications. However, new techniques for generating integration-free iPS cells are under development. The capacity and limitations of iPS cells to form cartilage need to be further investigated before conclusions about their utility can be drawn.

Scaffold design Scaffolds represent biocompatible, biodegradable materials that support the engraftment process of transplanted stem cells. Initial scaffold preparations based on fibrin or agarose were mostly designed to retain transplanted cells at the implantation site. Recently designed scaffolds have a more sophisticated, three- dimensional architecture and integrated growth factors and/or cytokines. The following variables are currently being investigated with regards to stem cell engraftment outcomes [4–6]: (1) biocompatibility: the scaffold material should be able to integrate into the target tissue and support the proliferation and differentiation of the transplanted stem cells; (2) mechanical property: the scaffold should resist local mechanical forces; (3) pore size: the pore size of the scaffold should allow cell aggregation and differentiation; (4) structure and geometry: the 3D architecture of the scaffold can affect the proliferation and differentiation of the transplanted

cells; (5) biodegradation property: the composition of the scaffold can be tailored towards fast or slow biodegradation, which affects the integration of stem cells into adjacent tissue; (6) biochemical integration ability: long-term integration and availability of growth factors or cytokines facilitate stem cell differentiation.

Growth factors and/or cytokines Growth factors and/or cytokines can be used to differentiate stem cells in vitro, before MASI, or they can be integrated into scaffolds to support stem cell differentiation in vivo, after MASI. A more recent approach to enhance the long-term availability of growth factors and cytokines in vivo is to genetically modify the transplanted stem cells or co-transplanted supporting cells to produce the needed differentiation factors.

The role of imaging A major barrier for long-term success of MACI and MASI is our current inability to recognize complications of the engraftment process in a timely manner in vivo. To date, a large proportion of transplanted stem cells and chondrocytes undergo apoptosis and/or are cleared from the transplantation site by host macrophages or other immune cells [7]. MR imaging is currently the only noninvasive diagnostic test capable of depicting cartilage defects in vivo. We can determine a successful repair of cartilage defects with high resolution MR imaging approaches months or years after MACI/MASI. We can draw conclusions about the composition of the regenerated cartilage (hyaline versus fibrocartilage) based on advanced imaging techniques (e.g., T1 rho sequences, T2* maps), and we can diagnose hypertrophy or tumor formation of the stem cell transplants. A better understanding of the MR signal characteristics of successful versus unsuccessful stem cell transplants within days or weeks after MASI/MACI could guide the development of more successful tissue regeneration strategies. Novel, cellular MR imaging approaches may provide this information by direct in vivo visualization of the presence, engraftment, or loss of the transplanted cells [8]. Our group and others have established labeling techniques for stem cells with iron oxide nanoparticles, which allowed us to define signal characteristics of successful versus unsuccessful MASI, based on distinct MR signal characteristics of nanoparticle-labeled viable versus apoptotic stem cell transplants [9, 10]. We expect that these cellular imaging techniques may spur translational molecular imaging research and stem cell imaging applications. Cellular imaging techniques with clinically applicable cell markers (such as ferumoxytol) can be readily translated to clinical applications and may be useful to guide treatment decisions [11]. For example, patients with apoptotic and/or lost MASI, as diagnosed by cellular MR imaging, could be directed to repeated or alternative treatment options, while patients with successful transplants could be spared from invasive follow-up studies. These cellular imaging techniques could be also utilized to study the effect of different cell types, scaffolds, growth factors, and immune response modifiers on MASI engraftment outcomes, which could in turn inform the development of more successful MASI approaches. Since clinical trials of new combination therapies are expensive and take years to complete, the impact of such imaging techniques could be immense. Additional developments include the possibility to noninvasively depict the host immune response to MASI by prelabeling bone marrow macrophages before MASI with intravenously administered iron oxide nanoparticles. This prelabeling of bone marrow macrophages with iron oxide nanoparticles allows us to track the migration of the labeled macrophages into MASI with MRI [12]. We expect that these novel imaging techniques will

be able to guide and tailor improved and individualized MASI approaches, and ultimately, improve successful joint regeneration and long-term outcomes.

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