New frontiers in Regenerative medicine

Stem cells and Gene therapy for cartilage repair of the joint:

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Osteoarthritis (OA) is a common chronic degenerative joint disease, contributed by multiple factors that include aging, obesity, injury, trauma, joint congenital abnormalities and joint deformity. It primarily occurs after middle age and predominantly affects women. Most common presentation include joint pain and stiffness and impaired mobility, while pathological changes include cartilage destruction, subchondral cysts and sclerosis and synovial hyperplasia.

Being avascular and aneural, the regenerative potential of hyaline cartilage is poor. While medical management and physical therapy can provide symptomatic improvement especially in the early stages, they do not essentially alter, slow down or halt the disease process.

Continued research towards making true an old dream to rebuild "spare parts" to replace injured or diseased tissues led to advancements in regenerative medicine. Autologous chondrocyte transplantation has been used quite successfully in repairing damaged cartilage. However, the cultured chondrocytes have exhibited dedifferentiation and decreased chondrocyte-specific gene expression, raising concerns of senescence and poor outcomes. This redirected interests to consider mesenchymal stem cells (MSCs) for cartilage repair.

Cultured MSCs

Mesenchymal Stem cells (MSCs) have the potential of self-renewal and directional differentiation, which are essential steps to repair cartilage tissue and suppress chondrocyte secretion of inflammatory factors. They also exhibit homing properties, which make them ideal seed cells for gradual OA treatment, and express enzymes and secrete growth factors, cytokines and chemokines that nourish cartilage by activating cellular and angiogenesis pathways. In addition, MSCs also have immunomodulatory function, which can suppress T cell proliferation and activation, proliferation and antibody secretion of B cells.

MSCs can be isolated from various sources: bone marrow, adipose tissue, tendon, periodontal tissue, , umbilical cord blood and Wharton's jelly. However, bone marrow still remains the most common source of MSC harvest. Being the progenitors of the mesodermal cells, MSCs have the potential for multilineage differentiation into muscle, tendon, ligament, fat, bone, and cartilage, dermis and other connective tissues.

The international society for cell therapy (ISCT) defines MSCs with three criteria: (1) plastic-adherence, (2) expression of surface markers CD105, CD73 and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface markers and (3) must be able to differentiate into osteoblasts, adipocytes and chondroblasts in vitro.

They can be delivered in the form of intra-articular injections or as MSC-laden scaffolds. The ease of injectable delivery of MSCs in clinical practice provides an added advantage of avoiding surgery and its adverse effects. However, each patient should be assessed individually on a case-to-case basis to decide on the suitability of this treatment option and the best mode of MSC therapy.

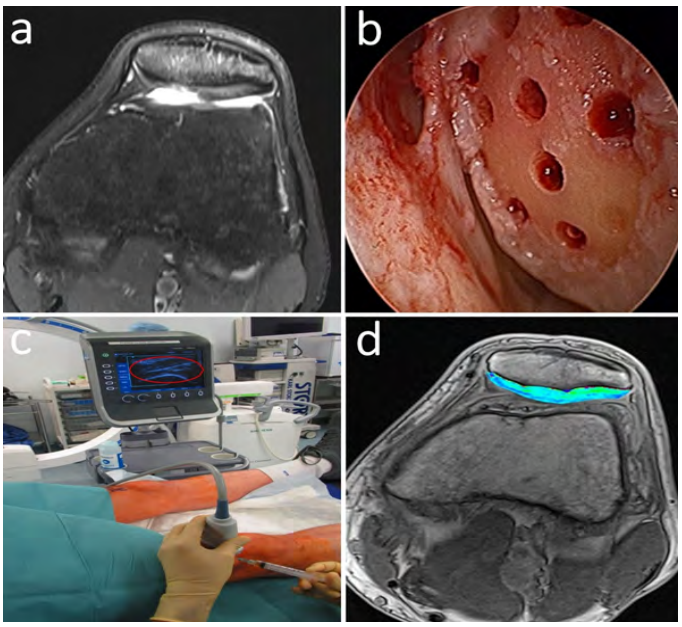

Figure 1:
Cultured MSCs being used by the author for treatment of a large patellar chondral lesion (a). In the first stage the lesion is debrided and microfractured (b) and MSCs are harvested for culture. In the second stage, the cultured MSCs are injected under ultrasound guidance into the knee joint (c). Follow-up dGEMRIC MRI at 18 months shows hyaline-like cartilage

Concerns regarding use of MSCs:

1. While both methods have shown clinical improvement in patients with OA, further investigation is needed on the type of MSCs, the concentration of cells, the number of injections needed and the stage of osteoarthritis to address, to achieve optimal response.
2. It is also to be noted that autologous MSCs are best avoided for genetic disorders due to their genetic influence. Allogeneic MSCs are an option in these patients.
3. The quality of MSCs might be too low in older patients.
4. Though multiple safety studies have proven the safety of MSCs, the theoretical risk of uncontrolled cell division and disease transmission remain a concern.

Large quantitative studies with safety assessment would be needed rule this out.

Mesenchymal cell induced chondrogenesis (MCIC, Shetty-Kim technique)

One important limitation of autologous chondrocyte implantation and cultured MSCs is they are two-stage procedures, composed of an initial harvesting, in vitro culture that is expensive and subsequent transplantation into the joint. Traditional microfracture does not have this limitation but relies on a small volume of the bone marrow emerging from subchondral bone.

A technique that uses a large volume of bone marrow concentrate in addition to microfracture would logically lead to better results.

The molecular structural component applied to the treated lesion is also important. Improved outcomes have been shown with use of bio-scaffold after microfracture procedure whether combined with BMAC or not. Collagen based scaffolds enhance the fixation of the graft. Hyaluronic acid, a major component of articular cartilage has the ability to drive chondrotypic regeneration. MCIC is a hybridization of these techniques, delivering chondrogenic cells with a conductive biological matrix onto a surface prepared for regeneration. This single-staged, arthroscopic procedure is cost-effective, has low morbidity, and is a logical evolution of cartilage repair surgical techniques. Our comparative study of 42 patients who underwent Total Knee Replacement(TKA) vs 52 patients who underwent MCIC for Kellgren-Lawrence grade IV osteoarthritis revealed equivalent patient satisfaction in both the groups.

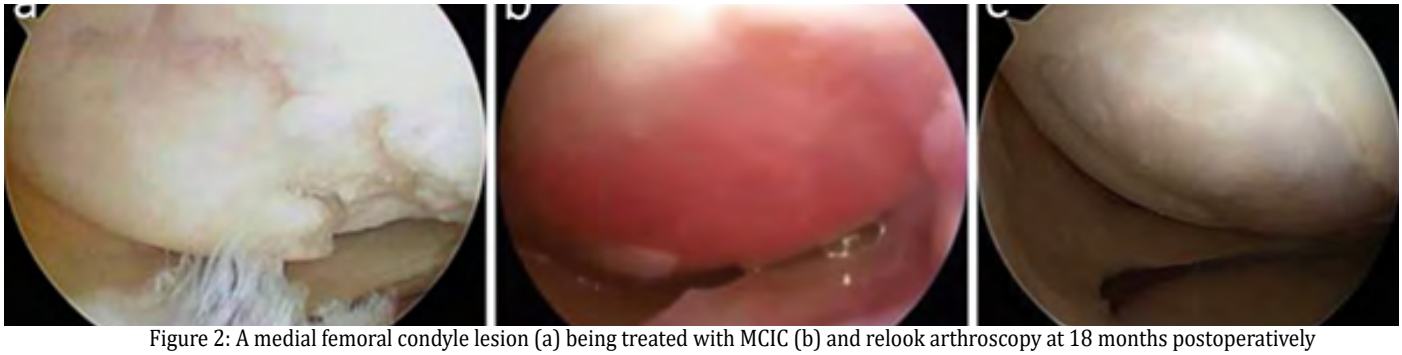

Figure 2: A medial femoral condyle lesion (a) being treated with MCIC (b) and relook arthroscopy at 18 months postoperatively

Gene therapy

The history of gene therapy dates to the 1970s when Roblin and Friedmann reported on gene therapy for human genetic disease. The year 1984 saw the designing of a retrovirus vector that could be used to insert foreign genes into chromosomes. The first clinical application of this technique in the US took place in 1990 to correct the genetic defect of ADA-SCID, a severe immune system deficiency. Gene therapy has been in clinical application since 1990, initially for severe combined immunodeficiency and later in the treatment of conditions of joints including rheumatoid arthritis and OA.

The technique consists of transfer of a therapeutic gene into chromosomes of injured tissue to cause synthesis of a therapeutic protein. The goal is to either increase expression of a good gene or inhibit the expression of a bad gene.

OA, being a localised pathology, is well suited for local, intra-articular gene therapy. The two most common targets are synovial tissue and the articular cartilage.

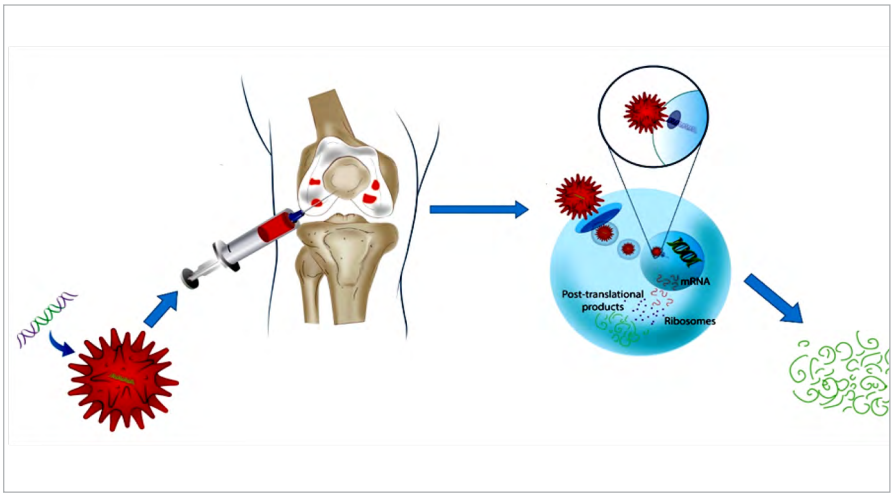

Figure 3: Principles of gene therapy in knee osteoarthritis

Interleukin-1 (IL-1) has a major role in the pathophysiology of OA. Hence gene transfer of its receptor antagonist (IL-1Ra) has shown promising future towards cure from OA. This is an anticatabolic approach. Similarly, stimulation of IGF-1 has been attempted in a proanabolic approach. Approaches combining both inhibition of cartilage breakdown and promotion of cartilage repair (eg., IGF-I/IL-1RA) have also produced attractive results.

Vectors

The gene is inserted into the cell with the help of a vector, which can be viral or non-viral. Retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV) and herpes simplex virus (HSV) are some of the viral vectors. They are more efficient in transferring genes than non-viral agents. However, they also carry risk of inflammatory response, immunogenicity and mutagenesis. The problems with viral gene delivery can be solved by ex vivo gene delivery.

The nonviral approach uses various nonionic, physical (electromagnetic) and biochemical modes for gene transfer. These vectors are not immunogenic or mutagenic. However, they are less efficient than viral vectors.

Dual expression vectors harbour two genes and provide an opportunity to carry both proanabolic and anticatabolic genes to more effectively limit inflammation, apoptosis and also enhance matrix synthesis, joint lubrication and healing of chondral defects.

Gene transfer can be performed in two ways:

In, 'in vivo' gene therapy, the vector–transgene construct is delivered through an intra-articular injection. This method is used for infecting the synovial cells which are more easily accessible than the chondrocytes which are embedded in dense extracellular matrix.

In, 'ex vivo' gene therapy, gene transfer is performed on cells that are harvested, cultured and are later reimplanted. This technique can be used to directly address the damaged tissue such as the cartilage. Chondrocytes or stem cells or progenitor cells are isolated, cultured, transduced using viral or transfected using non-viral approaches, and reimplanted at the site of the lesion. This technique has high transduction efficiency, precise targeting of the cells of interest and the ability to evaluate cells after transduction.

Concerns and future directions

Safety, stability, cost-effectiveness are still concerns with gene therapy. Slowing down the progression of OA and delaying the need for a joint replacement for at least a decade can impact the patients' quality of life significantly and hence can be considered a success of therapy.

Combining gene therapy techniques with tissue engineering principles, and cell-based regenerative techniques (bone marrow-derived stem cells, adipose-derived stem cells, stromal vascular fraction) and also 3D bioprinting technology could result in new and effective therapies to enhance healing of the osteochondral unit.

We hope that this article will provide a realistic image of the huge potential, promise and challenges facing the fantastic field of regenerative medicine in its quest to cure disease and prolong life.

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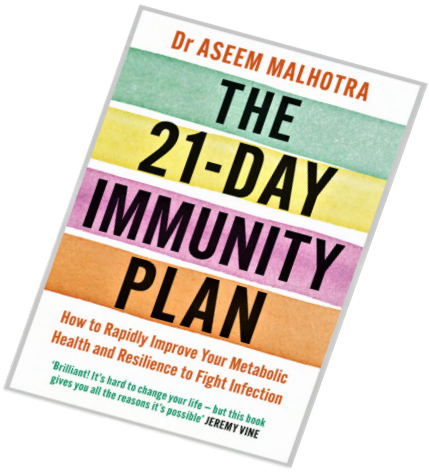

BOOK REVIEW

THE 21-DAY IMMUNITY PLAN

The 21-Day Immunity Plan' book is written by Dr Aseem Malhotra who is a leading NHS-trained cardiologist and a pioneer of lifestyle medicine. This new book offers a "simple, evidence-based" plan to help improve metabolic health and normal immune function and reduce the risk of damaging effects from Covid-19.

The good news is that in just 21 days we can prevent many of the underlying risk factors that exacerbate how infections, including Covid-19, affect us and improve our ability to recover from them. Arguing for the huge benefits to global health of these highly effective lifestyle changes, he shows how just 21 days can help us to start the journey to lead a healthier and longer life.

Dr Aseem Malhotra has been championing an anti-obesity drive as a means to combat the severe effects of COVID-19. He has been at the forefront of citing the health conditions which make us vulnerable to the worst effects of Covid-19.

Obesity, Type 2 diabetes and heart disease are high among them - and are all indicators of poor metabolic health. Giving us the evidence-based science behind the plan, Dr Malhotra shares how simple changes to our diet as well as daily exercise and stress relief can have remarkable results in improving our markers for metabolic health, as well as helping to put Type 2 diabetes into remission, reducing risk factors for heart disease, decreasing weight and enhance vitality.

'The 21-Day Immunity Plan' is an essential read for all of us who are conscious about our well being and I would whole heartedly commend this book.

Prof J S Bamrah CBE

Prof Bamrah is a senior consultant psychiatrist at the Greater Manchester Mental Health Trust and an Honorary Reader at the University of Manchester. He is a member of the Synergi Collaborative Centre's Advisory Board, a national initiative to reframe, rethink and transform the realities of ethnic inequalities in severe mental illness. He is Deputy Chairman of the Board of Science, BMA. In 2020 he was appointed member of an Advisory Board member of ICMR-Centre for Innovation and Bio-Design (CIBioD) by the Govt. of India.