Osteoarthritis: from upcoming treatments to treatments yet to come

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Abstract: Osteoarthritis affects hundreds of millions of people worldwide, and its prevalence is constantly increasing. While there is currently no treatment that can alter the course of the disease, promising therapeutic strategies and novel targets are being investigated. Innovative cell therapies are already reaching clinical trials, and recent progress in our understanding of the disease is opening new routes for gene therapy. In the long term, the development of new biofabrication tools, such as 3D bioprinting, may pave the way for personalized mini-joint models that could be used to screen drugs and to personalize treatments. This review provides an overview of the most promising therapeutic approaches in the field of osteoarthritis, from upcoming treatments to those that are yet to be discovered.

Keywords: osteoarthritis, cell therapy, encapsulation, therapeutic target, gene therapy, bioprinting, 3D models

1. Introduction

Osteoarthritis (OA) is a degenerative and inflammatory disease that affects all the joint tissues (synovial membrane, bone, cartilage, meniscus, ligament, tendon, etc.) and impacts a considerable part of our aging population.[1] OA has a complex pathogenesis involving multiple cellular and molecular players. Because of the lack of any etiological treatment that can stop or slow down the changes in the joint tissues, the current management of OA patients is symptomatic and mainly based on the use of analgesics and anti-inflammatory drugs. Recently, thanks to our better understanding of this disease, new therapeutic avenues have appeared (Figure 1), some of which have been evaluated in clinical trials. Among them, cell therapy using mesenchymal stem/stromal cells (MSCs) is certainly the most advanced strategy. Several clinical trials in which MSCs were intra-articularly injected have reported promising results. In parallel, the identification of new therapeutic targets involving certain signaling pathways, immune cells, or the autophagy/senescence process, have given rise to new hope. Lastly, the advent of biofabrication processes, especially 3D bioprinting, will allow us to manufacture organoids or mini-joints for large-scale screening of OA drug candidates in the not-too-distant future.

2. Cell therapy: today and tomorrow

MSCs have biological properties (e.g., proliferation, differentiation) that make them promising candidates for cell therapy, especially in skeletal tissues. They can be isolated easily from different tissues such as bone marrow, adipose tissue, synovial fluid or even cord blood in clinically relevant numbers. Other than their ability to regenerate tissues, MSCs can also interact with the immune system by the secretion – direct or mediated by extracellular vesicles – of various immunomodulating and anti-inflammatory molecules. Because of these secretion properties, the status of MSCs has been elevated from cells that are simply able to differentiate and regenerate damaged tissues, to cells that can act as factories to produce therapeutically useful molecules. Thus, for more than 15 years, they have been contemplated as a potential treatment for OA by intra-articular (IA) injection. Initially tested in various preclinical models of inflammatory or post-traumatic OA in rodents and large animals (e.g. sheep, dogs,

horses), MSCs quickly demonstrated their anti-OA potential. Based on these preclinical results, various clinical trials using MSCs (from bone marrow or adipose tissue in particular) were initiated with IA injection of autologous MSCs and then, more recently, allogeneic MSCs. In fact, MSCs appear to have a certain immune privilege given their low expression of major histocompatibility complex molecules and T-cell costimulatory molecules, which allows them to be implanted in an allogeneic context. Nevertheless, there have been some reports of MSC graft rejection, particularly in the long term.[2] Of note, although a history of IA injection of MSC for OA, there does not appear to be any major contraindications to MSC use in these patients. While randomized controlled trials with a large number of patients are still needed, on the whole, the first clinical trials (see [3] for a review) showed an analgesic effect and better joint function following MSC injection.[4] However, this clinical effect is rarely visible beyond a few months and is only very occasionally associated with remodeling of the cartilage tissue. Several explanations have been put forward to justify this relative effectiveness. First, it is known that the IA injection of MSCs by a needle induces shear forces on the cell surface that may compromise their viability. Moreover, MSCs have a propensity to migrate away from the injection site,[5] making it difficult to restrict their production of therapeutic molecules to a certain location. Lastly, it is now known that MSCs are a very heterogeneous population of cells, whose secretion capacities vary and depend on poorly identified factors (e.g. donor age, purification/amplification techniques).[6] To get around this limitation, MSCs are now available from cell banks, allowing the injection of cells specifically prepared and validated for therapeutic use. Furthermore, recent studies suggest that certain MSC metabolic or signal pathways (PPAR β/δ,[7] HIF1-α,[8] autophagy/senescence[9]) could be modulated by agonists or antagonists to stimulate their anti-OA properties. All these avenues could help MSCs join the daily therapeutic arsenal used by clinicians more quickly.

To prevent MSC death and leakage away from the injection site, it has been suggested to encapsulate them within injectable and cryoprotective biomaterials. This strategy could improve cell survival inside the joint and optimize their therapeutic effects. Encapsulation is a process that aims to embed molecules or cells in three-dimensional structures to protect them from their environment. In the pharmaceutical industry, the encapsulation of active molecules limits their biodegradation and controls their release rate. Similarly, cellular microencapsulation, which refers to cell

encapsulation in particles between 1 µm and 1 mm in size, aims to protect cells from the rest of the body, while allowing for exchanges with the microenvironment, thus ensuring nutrition, cell communication, and elimination of metabolic waste.

The main challenge when selecting the manufacturing process for microparticle preparation resides in the need to maintain cell viability and functions. Natural polymers (e.g., alginate, gelatin) in hydrogel form have been widely used to encapsulate cells because of their biocompatibility and stability in vivo. Their crosslinked network forms a permeable structure that limits interactions between the cells and their external environment, while still allowing the diffusion of the molecules needed for cell survival and communication. The mechanical properties of hydrogels also help to protect the cells from the shearing forces that occur during injection. The main microencapsulation methods consist of suspending cells in an aqueous polymer solution, which is further dispersed in droplets by extrusion through a nozzle before gelification ($CaCl_2$ bath for alginate, photopolymerization for methacrylate polymers). Other techniques such as the use of electrospray (i.e., electric gradient), micromolding or microfluidic-assisted emulsion processes [10] result in microparticles of homogeneous size and shape, in a manner that is reproducible and can be automated.

Encapsulation of human bone marrow-derived MSCs in alginate microbeads does not interfere with their capacity to secrete anti-OA factors when they are in an inflammatory environment.[11,12] In a recent study with a post-traumatic OA model in rats, Xing et al. showed that the injection of human cord blood MSCs combined with gelatin microgels led to a reduction in cartilage damage.[13] However, the long-term therapeutic effect of this cell encapsulation strategy on the disease progression still remains to be demonstrated.

Recently, the delivery of extracellular vesicles as an alternative to cell therapy has been explored. [14] These natural vectors contain various bioactive molecules, and could be intra-articularly injected to exert their therapeutic effects. The possibility of loading these vesicles with specific anti-OA factors opens the way for the development of novel therapeutic strategies and personalized medicine [15].

3. Past, current and future therapeutic targets

3.1. Inflammation

For several years, inflammation has been the target of OA therapies, especially by the targeting of pro-inflammatory cytokines such IL-1β, TNF-α and IL-6. However, up to now, these agents, even the most recently developed ones like the anti-IL-1β Lutikizumab, have not been effective in OA, despite the presence of synovitis.[16] Anti-TNF-α antibodies (e.g., Etanercept, Adalimumab) also showed no effect on pain and only limited effect on cartilage structure.[17] Finally, the anti-IL-6 strategies that were promising during preclinical studies of experimental OA in mice[18] ended up not being effective in clinical trials. In fact, tocilizumab was not superior to placebo at reducing pain in patients with hand OA.[19]

During OA, the installation of an inflammatory process concomitant with the production of enzymes that degrade the extracellular matrix (e.g., MMPs, ADAMTS) has led some to suggest that cartilage matrix degradation products may have an important role in sustaining OA-associated inflammation. It has now been demonstrated that fragments of collagen[20] or fibronectin[21] can contribute to supporting OA synovitis, by directly stimulating synovial cells. Hence, these fragments may be potential targets for the development of anti-inflammatory molecules. In addition to matrix fragments, calcium-containing crystals (e.g., dihydrate calcium phosphate, basic calcium phosphate) are now considered as important players in OA pathogenesis.[22] These calcium crystals can directly stimulate chondrocytes and synoviocytes, and also contribute to matrix degradation and the persistence of inflammation.[23] Besides these calcium crystals, urate crystals also emerge as a contributor of OA pathogenesis .[24] A better understanding of the molecular mechanisms underpinning the effect of these various crystals should allow us to identify new therapeutic targets for OA in the near future.

The synovitis observed at different stages of OA progression, sometimes very early before the appearance of cartilage degradation, is characterized by a predominant macrophage component, which has been associated with disease severity. Recently, two subtypes of OA have been defined based on the phenotype of the synovial macrophages. “Inflammatory” OA, is characterized by highly proliferative macrophages, very similar to those observed in rheumatoid arthritis, that infiltrate the synovium. Conversely, “classic” OA is characterized by the presence of macrophages presenting a phenotype that supports cartilage remodeling.[25] The targeting of pro-inflammatory cytokines expressed by macrophages and chondrocytes, such as IL-36α, may help to reinstate tissue homeostasis, especially by regulating the signaling associated with TGFBR2.[26]

Recent studies have also highlighted that the recruitment of immune cells into the OA synovium may be related to the presence of a rare chondrocyte population characterized by the expression of IL-1 and TNF receptors, and of chemokines that attract monocytes/macrophages.[27] Their pharmacological targeting could help limit the deleterious intercellular communication that is responsible for chronic synovitis. Thus, a stratification of OA patients that would take into account both the histopathological diversity of the synovial membrane and the various chondrocyte phenotypes, could lead to the development of new personalized treatments targeting synovial inflammation.

3.2. FGF18

The first investigations of the use of recombinant human fibroblast growth factor-18 (FGF-18) (Sprifermin) in vitro and in vivo in mouse models have shown very promising results, with an unprecedented significant effect on cartilage restructuring.[28] In humans, the FORWARD phase 2 study evaluated the effects of three IA injections 1 week apart, every 6 or 12 months. The clinical outcomes at 2 years were disappointing: no significant improvement in pain, function or mobility was found in the treated patients. Conversely, there were significant but modest improvement in the tibiofemoral cartilage thickness on MRI (0.02 to 0.03 mm) compared to a loss of 0.02 mm in the control group. At the 3- and 5-year follow-up visits, the overall clinical parameters had still not improved significantly, while the structural modification of cartilage thickness was maintained less in the 100 µg group.[29,30] The overall tolerance to these IA injections was good, with minor local inflammatory reaction at the injection site observed for 13–23% of the patients, and 5-11% of them developing sprifermin antibodies. While the observed structural effect is an important advance, pain management and the reinstatement of joint functions over time are expected signs of clinical effectiveness.

3.3. Anti-NGF

The neutralization of NGF (nerve growth factor) by monoclonal antibodies inhibits the nociception transmitted by small type C nerve fibers. Three different

antibodies (Tanezumab, Fulranumab, Fasinumab) have been studied for knee and hip OA with spectacular pain relief observed but the appearance of rapidly progressive OA.[31] The investigation of lower doses administered subcutaneously, showed a lower, but still significant, effect on pain and functions.[32] Unfortunately, 2% to 3% of trial participants still experienced rapidly progressive OA. A more recent dose-ranging study with two monthly infusions was done in 74 patients.[33] At 16 weeks, the effectiveness in reducing pain during walking was significant, with a maximal efficacy observed at 2 weeks for the lower doses (pain −30%) and at 4–6 weeks for the higher doses (pain −40 to −50 %). Several adverse events were observed dose-dependently: headaches (7–11%), upper respiratory infections (3–9%), joint pain (1–9%), extremity pain (1–12%), oedema (0–11%), paresthesia / hypoesthesia (1–8%). Given that the risk/benefit ratio still needs to be defined over a longer period of time, the relevance of this type of treatment in the therapeutic arsenal continues to be debated.

3.4. Autophagy/Senescence

Given that the development of OA is closely linked to aging, mechanisms that are known to be altered during aging have recently been investigated in OA. Among these mechanisms, autophagy and senescence are of utmost interest, with autophagy being reduced while senescence is increased in OA. The suppression of autophagy, as observed in OA chondrocytes, has been shown to promote OA development.[34] Restoring autophagy using compounds such as spermidine or rapamycin has already been proven to mitigate OA.[35] Similarly, the clearance of senescent cells in OA preclinical models was shown to reduce disease severity.[36] This last discovery highlighted the therapeutic potential of senolytic compounds able to inhibit the survival mechanisms of senescent cells, and thereby promote their elimination by apoptosis. Among these senolytics, UBX0101, has been tested in phase I and II clinical trials (NCT03513016, NCT04229225, NCT04129944, and NCT04349956). However, despite promising effects in reducing pain in phase I (NCT 03513016),[37] the phase II clinical trial (NCT04129944) did not demonstrate the superiority of a single UBX0101 injection (0.5 mg, 2 mg, and 4 mg) over placebo.

The strong links between autophagy, senescence, aging and OA also suggest that anti-aging molecules or pharmacological agents (e.g., klotho, GDF11) could represent new therapeutic targets in OA.

3.5. Epigenetics

Several epigenetic regulators appear to be involved in the pathogenesis of OA. Epigenetics is a field of research that analyzes the changes in gene expression or cell phenotype occuring without modification of the DNA sequence. The main mechanisms of epigenetic regulation involve chemical modifications of the DNA (methylation and hydroxymethylation of cytosine-guanine dinucleotides), post-translational histone modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, deamination and proline isomerization) and regulatory non-coding RNA (lncRNA, siRNA, miRNA). Epigenetic profiling of articular chondrocytes has revealed the existence of an activating sequence that is present in billions of people with a risk locus (GDF5-UQCC1) that affects knee shape and is involved in OA progression. These epigenetic modifications can also suppress the expression of protective genes in OA such as PPARγ, further highlighting their potential role in the disease progression.[38] Some epigenetic regulators themselves can be perturbed in OA. Thus, the overexpression of TET1, responsible for the deposit of hydroxymethylated cytosines, activates multiple pathways involved in OA.[39] Conversely, deficiency of the methyltransferase DOT1L, an enzyme involved in histone methylation, increases the susceptibility of mice to developing OA.[40] Lastly, a role for the CLOCK protein in stabilizing the heterochromatin, which contributes to the cartilage regeneration and the attenuation of age-related articular degeneration in mice, has been recently highlighted.[41] Together, these results show the importance of epigenetic regulations in OA.

3.6. Metabolism

Our knowledge of the relationship between metabolism and OA has increased in recent years. For example, OA chondrocytes present higher cholesterol levels due to the expression of the cholesterol receptor RORα, which increases the absorption of

cholesterol, the regulation of cholesterol hydroxylases (CH25H and CYP7B1), and thereby increases the production of oxysterol metabolites.[42] OA-related inflammation leads to several metabolic changes at the origin of joint degradation. It shifts the chondrocytes energy metabolism toward glycolysis and lactate production by lactate dehydrogenase (LDHA) instead of using the classic oxidative phosphorylation pathway. This generates reactive oxygen species (ROS) triggering an oxidative stress that promotes cartilage catabolism.[43] It has recently been reported that ANP32A, of which expression is reduced in OA cartilage, can, when over-expressed, protect cartilage from oxidative stress and thereby prevent the development of OA.[44]

3.7. Gene therapy

The modulation of new therapeutic targets (e.g., klotho, TET1, CLOCK, RORα, LDHA, ANP32A) involved in the pathophysiology of OA by gene therapy represents a promising avenue of research. Gene therapy consists of using a vector (viral or non-viral) to bring genes into cells and tissues to treat a disease. The viral vectors include RNA viruses (retrovirus and lentivirus) and DNA viruses (adenovirus and adeno-associated viruses (AAV)). RNA viruses are characterized by their ability to integrate the genome. On one hand, this allows for long-term expression of the transgene, but on the other, it can lead to serious side effects like oncogene activation. Lentiviruses, which are now also available in non-integrative forms, are the only RNA viruses capable of infecting non-proliferating cells, which makes them attractive candidates for targeting chondrocytes in vivo. DNA viruses (adenovirus and AAV) remain primarily in episomal form, and are therefore safer. They are currently being used in two on-going clinical trials that evaluate the overexpression of IFN-β and IL-1Ra (NCT02727764 and NCT02790723, respectively) in OA. However, the immunogenicity of adenovirus vectors and the pre-existence of humoral immunity for certain AAV serotypes may limit their clinical use. The use of non-viral vectors (organic and non-organic), which are not limited by pre-existing immunity and are easier to produce in large quantities, is also considered. However, although currently being used for *ex vivo* cellular modifications, their in vivo transduction effectiveness appears modest relative to viral vectors.

Two gene therapy strategies are currently in preclinical and clinical development for OA. The first (NCT03383471) is an *ex vivo* gene therapy that consists in modifying and amplifying cells *in vitro*, followed by their intraarticular injection. This *ex vivo*

strategy is applied by Invossa™ to over-express a growth factor – TGF-β1 – in irradiated allogenic chondrocytes, which are then mixed with non-modified chondrocytes before IA injection.[45] The second approach consists in a more conventional *in vivo* gene therapy by local or systemic injection of viral vectors containing the transgene of interest. In general, OA gene therapy aims to reduce inflammation by overexpressing transgenes such as IL-1Ra or soluble TNF receptor,[46] to inhibit the destruction of cartilage matrix (TIMP) or to activate matrix synthesis (e.g., TGF-β, IGF-1, PRG4, SOX9).[47] Among the transgene candidates for OA gene therapy are some of the previously described epigenetic targets. For example, the overexpression of CLOCK by lentiviral vectors may contribute to cartilage regeneration and attenuate the age-related articular degeneration in mice.[41] Gene therapy combining the overexpression of anti-aging molecules such as the protein Klotho and TGFβR2, prevents the progression of OA by reducing the immune response and contributing to cartilage homeostasis.[48] In the near future, gene therapy could thus become a strategy of choice to regulate the intra-articular expression of new therapeutic targets in OA.

4. 3D Bioprinting of mini-joints: future OA joint models?

While no treatment is currently able to stop the development of OA, new tissue engineering techniques have been introduced that can help us better understand this disease and find innovative ways of treating it. Among these techniques, 3D bioprinting, which consists of printing objects that contain biological material (i.e., cells, proteins) in three-dimensions, promises unprecedent manufacturing of custom biological tissues.[49] Over the past 20 years, various bioprinting tools (e.g. extrusion, inkjet, laser-assisted) have been developed and are now available commercially.[50] Tey allow cells, materials and biological factors to be organized in space to better mimic the architecture of living tissues. High resolution, automatization, reproducibility, and the easy conversion of medical imaging results into 3D models, are among the advantages of using bioprinting technologies to advance new therapeutic solutions. The bioprinting of implantable, and potentially personalized, tissues is being studied for the treatment of several diseases.[51] In the context of OA, the recourse to bioprinted implants would require extensive surgical procedures, which are themselves accompanied by important secondary risks, making them relatively unlikely. However, bioprinting allows us to

consider the development of new in vitro models to understand biological mechanisms, screen therapeutic agents, and reduce animal testing.

Up to now, little work has been done regarding the bioprinting of cartilage tissue and osteochondral units.[52] The ultimate goal would be to reproduce the joint architecture and all of the local interactions (i.e., cell-cell and cell-material interactions) that govern the development of OA. Recreating the different layers of cartilage alone requires the spatial organization of chondrocytes with distinct phenotypes, the development of gradients of extracellular matrix components, and the controlled orientation of fibers in space. To this bioprinted cartilage, one must add structures of calcified cartilage, subchondral bone or even synovial membrane, each with specific compositions and architectures, participating in the structural complexity of a joint. Beyond tissue shape and architecture, the challenge is to reproduce its biomechanical properties and biological functions. To provide printed osteochondral units with the mechanical strength of native osteochondral tissue, while reproducing its architecture, researchers have printed fibers from rigid thermoplastic materials combined with extrusion of viscoelastic materials.[53–55] These controlled architectures, sometimes cultured under mechanical stimulation to mimic joint loading, also make it possible to guide the local differentiation of stem cells to obtain the desired cell phenotypes.[56]

Considerable work still needs to be done to optimize and validate the materials, printing methods and architectures. However, the development of bioprinted joint tissues could offer new platforms to study the complex interactions between synovial membrane, subchondral bone and cartilage. These models, possibly combined with other emerging technologies (e.g., microfluidics, high-throughput analysis), provide the possibility of new discoveries for treating OA, for example, by using patient's cells to generate personalized OA tissue model.

5. Conclusion

Despite flourishing research, the advances in our understanding of OA pathophysiological mechanisms have still not resulted in new efficient treatments that address the causes of the disease. It is now well recognized that OA is a multifaceted disease with several endotypes related to specific pathological origins (post-traumatic, metabolic, age-related, etc.). The existence of several types of OA means that a more

personalized medicine should be developed, based on the identification of specific therapeutic targets for each OA subtype. The recent discovery of new therapeutic targets, and the development of innovative models, will help accelerate the research and development into effective and personalized treatments of OA.

Acknowledgements

The authors are grateful to the Agence Nationale de la Recherche (ANR) for funding the projects JCJC KLOTHOA (ANR-18-CE14-0024-01; CV) and PRC PPAROA (ANR-18-CE18-0010-01; JG). They are also grateful to the Foundation for Research in Rheumatology for funding the project FOREUM-EULAR SEN-OA (CV), to the INSERM for funding the cross-disciplinary program AGEMED 2.0 (JG), the region of the Pays de la Loire RFI BIOREGATE for funding the projects GENOA (CV) and MONOMER (CLV), the Agence de la Biomédecine (18GREFFE012; JG), the Fondation de l'Avenir pour la Recherche Médicale Appliquée (AP-RM-18-005; CLV) and the Société Française de Rhumatologie (SFR 4015; CV and SFR 3637; JG). Lastly, the authors are especially grateful to the Fondation pour la Recherche Médicale (ARF201809007012; VD and ARF202004011786; MAB), the Nantes Excellence Trajectory program (NExT Junior Talent 2018; VD) and the Marie-Sklodowska Curie Actions (BABHY-CART project, GAP-846477; VD) for their financial support. The illustrations were made using the image bank "Servier Medical Art".

Conflict of interest disclosure: Conflicts of interest: none

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