Therapeutic strategies for the effective management of OA and cartilage defects.

From osteoarthritis treatments to future regenerative therapies for cartilage

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Osteoarthritis (OA) is associated with cartilage degeneration and an accompanying inflammatory syndrome of the synovium in addition to alteration of the subchondral bone. The molecular and cellular events involved in OA have only partially been elucidated. This review provides a global view of the physiopathology of OA, as well as non-pharmacological and pharmacological treatments for the disorder. An update on surgical treatments and their indications is given with an orientation towards the management of OA and cartilage repair by cell-based regenerative therapies. These promising biological technologies will, potentially, play a major role in the treatment of cartilage-associated diseases.

Osteoarthritis (OA) is a public health concern particularly in modern society and is the leading osteoarticular pathology of developed countries. In the United States, OA is the primary reason for medical consultation in persons older than 60 years of age and affects at least 30% of this subpopulation [1]. Population ageing will probably worsen the socio-economic impact of such pathologies. The growing epidemic of obesity is also an exacerbating factor, with an indisputable role in knee OA [2].

The current view is that OA is a complex syndrome that is, in fact, the ultimate outcome of various factors affecting the joint [3]. Once established, OA is characterised by a decrease in articular cartilage (AC) thickness, subchondral bone sclerosis (bone thickening), formation of osteophytes (bone outgrowth on the joint margin) and modification of the synovial fluid composition (Fig. 1). Several joints might be affected by OA but the sites most commonly affected are knees, hips, fingers and the lumbar and cervical spine. Given that many questions, particularly those concerning the physiopathology of OA, remain unanswered, it is not surprising that treatments, either pharmacological or surgical, only partially address the clinical issue.

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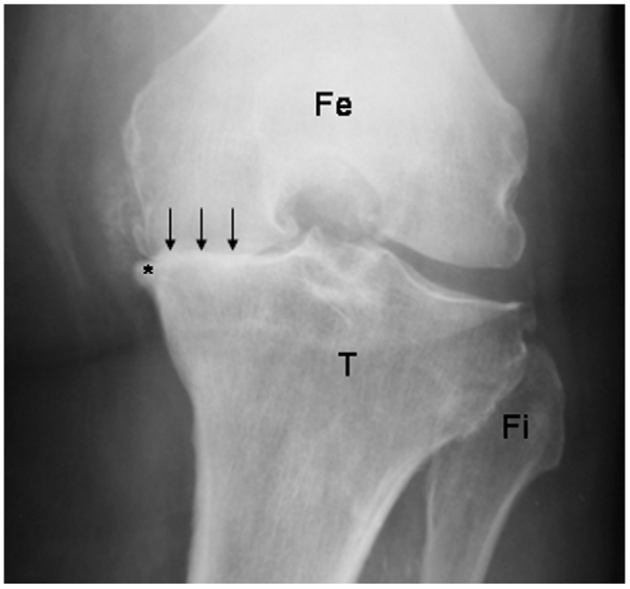


FIGURE 1

X-ray radiographic observation of an osteoarthritic knee (standing anterioposterior view). Characteristic features of advanced osteoarthritis of the medial tibiofemoral joint are shown. Note the joint space narrowing (→) and the formation of osteophytes (*). Femur (Fe); Tibia (T) and fibula (Fi).

For many years, various treatments likely to slow down the OA degenerative process have been assessed in preclinical studies, particularly the Disease-Modifying OsteoArthritis Drugs (DMOADs). In parallel, advances in the field of cell therapy and tissue engineering also deserve to be given major attention.

This review provides a global view of the physiopathology of OA, as well as the non-pharmacological and pharmacological treatments of this debilitating osteoarticular disease.

The joint and the articular cartilage

A diarthrodial joint is a complex structure comprising various connective tissues including AC, synovial membrane, subchondral bone, ligaments and sometimes menisci. All of these structures contribute to joint function and performance. In particular, AC possesses a chemical composition that enables the execution of repetitive loading cycles and a physical structure that allows for essentially frictionless motion.

AC is a slick, white tissue that covers joint surfaces. AC is composed of an extracellular matrix (ECM) produced by chondrocytes, and is characterised by the absence of blood vessels and nerves. Being avascular, cartilage has a low oxygen tension, ranging from 1 to 7%. Chondrocytes are developmentally adapted to these hypoxic conditions by having an enhanced anaerobic glycolysis. Contrary to other mesenchymal tissues (liver, heart, brain, kidney and so on) yet in common with bone, the properties of cartilage are mainly related to its ECM rather than to its cells [4]. Nevertheless, articular chondrocytes play a central role in the equilibrium between ECM synthesis (anabolism) and degradation (catabolism).

AC is organised into four layers according to the type and orientation of collagen fibres, the amount of proteoglycans (PG) and water, as well as the shape and activity of chondrocytes (Fig. 2):

- The outer surface area can be divided into two zones, is in contact with the synovial fluid and provides an essentially frictionless surface. The superficial zone is acellular and contains types I, II and III collagen fibres and low amount of PG. The deepest zone contains ellipsoidal chondrocytes which synthesise lubricin and types I, II and III collagen fibres.
- The transitional area is made up of types II, VI, IX and XI collagen fibres that intersect obliquely in a poorly organised network. This network is less dense and hydrated than that of the outer articular surface. The network of type VI collagen is essentially concentrated around the chondrocytes in the pericellular area [5]. The role of this type VI collagen is not yet clear but certain elements suggest that it interacts with fibres of type II collagen and create a mechanical interface between the chondrocyte and the ECM [6]. The chondrocytes have a round morphology.
- The deep area of the AC contains types II, IX and XI collagen fibres directed perpendicular to the joint surface. Chondrocytes form radial columns are aligned along the collagen fibres.
- The calcified area is in contact with the subchondral bone. In this area, cartilage contains a limited number of hypertrophic chondrocytes that synthesise type X collagen. Calcification takes place on collagen fibres, which anchor cartilage to the subchondral bone.

This histological organisation confers the cartilage to its biomechanical properties. The orientation of the collagen fibres decreases shear and compression constraints respectively on the surface and the deep area of the AC.

Physiopathology of osteoarthritis

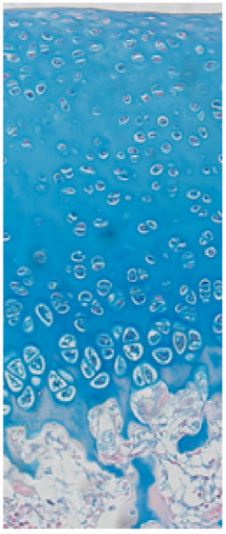
The first physiopathological hypothesis concerning OA was primarily mechanical, based on an age-associated degenerative process of AC. Young joints may, however, also exhibit some clinically benign but erosive lesions of the cartilage, characterised by a slow evolution. The frequency and precocity of these lesions contrast with the slow degenerative process classically described during OA development in elderly patients. This recent consideration makes the physiopathology of OA more complex than a simplistic age-dependent degenerative process of AC and can no longer be regarded as a single disease. It should be seen as a group and perhaps the term osteoarthritic diseases would be more suitable [7]. OA is, therefore, today considered to be a degenerative osteoarticular disease with multiple affected targets including AC, synovium and subchondral bone.

Articular cartilage impairments

During OA degenerative processes, major modifications of AC are observed at the tissue, cellular and molecular levels.

Tissue and cellular levels

Compared with the slick appearance of healthy cartilage, osteoarthritic cartilage surface is rough [3]. The osteoarthritic chondrocyte is obviously activated and exhibits a capacity to divide into clusters. Interestingly, it has also been reported that type IIA



	Collagens types	Proteoglycans content	Water content	Cell density and shape	Collagen fibers orientation
Surface area (superficial and deepest)	I, II, III			+ Circle	Parallel to the joint surface
Transitional area	II, IX, XI			++ Circle	Randomly organized
Deep area	II, IX, XI			+ Stack	Perpendicular to the joint surface
Calcified area	X			+ Hypertrophic	Entanglement
Subchondral bone	I and III				

Drug Discovery Today

FIGURE 2

Histological organisation of articular cartilage. Articular cartilage is organised in four zones according to the type and orientation of collagen fibres, amount of proteoglycan and water as well as shape and activity of chondrocytes. A histological section of articular cartilage of the knee stained with Alcian Blue and photographed under a light microscope is shown. +: Moderate cell density, ++: high cell density.

collagen, a splice variant of mature collagen type II mainly expressed during embryologic chondrogenesis, was re-expressed by adult articular chondrocytes in OA cartilage [8]. This data support the hypothesis that OA chondrocytes reverse their phenotype towards a chondroprogenitor phenotype, thereby highlighting the recapitulation of embryonic genes at the adult stage in the pathophysiology of OA [9]. The levels of PG and collagen synthesis are largely increased, at least during the early stage of the disease [10]. It is usually acknowledged that chondrocytes, at this stage, attempt to counterbalance the upregulated catabolic processes. This supraphysiological metabolism precedes the first symptomatic evidence of OA. After these early compensating mechanisms, caspase-mediated chondrocyte apoptosis increases and could therefore contribute to the late mechanisms of cartilage degeneration [11]. With respect to their role in OA, caspases are considered the ultimate messengers of a multiple-step signalling cascade with a variety of upstream activators, notably interleukin-1 (IL-1) and nitric oxide. Inhibition of chondrocyte apoptosis through the caspase signalling pathway could thus be a promising therapeutic target for the management of OA [12].

Molecular level

The destruction of AC and the loss of its biomechanical properties are largely related to the alteration of ECM, particularly the loss of aggrecan. This process results from an imbalance between degradation and synthesis of the matrix components, despite the compensatory activity of chondrocytes (Fig. 3). This point highlights the pivotal role of chondrocytes in the physiopathology of OA.

Among the cartilaginous anabolic factors, Insulin Growth Factor-1 (IGF-1), Transforming Growth Factor-beta (TGF-β), Bone Morphogenetic Proteins (BMPs) and Fibroblast Growth Factors (FGFs) have been extensively described. Interestingly, the level of expression of these factors declines with ageing and advanced OA [13].

An increase in catabolic enzymes responsible for ECM degradation has been reported during OA, predominantly matrix metalloproteinases (MMP-2, -7, -8, -9, -13, -14), A disintegrin and metalloproteinase with thrombospondin repeats-1 (ADAMTS-1) and aggrecanases 1 and 2 (ADAMTS-4 and -5 respectively). IL-1β and Tumor Necrosis Factor (TNF) have been largely implicated in the increased synthesis of catabolic enzymes by osteoarthritic chondrocytes [14]. Of interest, it has also been reported that a deficit in Tissue Inhibitors of Metalloproteinases (TIMPs) could also play a pivotal role in the excessive ECM degradation [15].

Several lines of evidence also highlight the role of adipokines in OA [16,17]. Adipokines (leptin, adiponectin and resistin) are proteins produced by white adipose tissue. They are essential regulators of immune and inflammatory responses. All three adipokines have been detected in synovial fluid from OA-affected joints. Fat tissue is, therefore, an active organ that greatly contributes to inflammatory and degenerative processes during OA.

Recently, a role has also been suggested for Wnt/β-catenin and Smad ubiquination-related factor 2 (Smurf2) in chondrocyte function and apoptosis [18,19]. Whether the control of these signalling pathways could lead to the development of new therapeutic intervention strategies in OA deserves consideration.

Synovium and subchondral bone alterations

Whilst studies of OA mainly focus on the comprehension of catabolic disorders described in cartilage, a pivotal role for synovium and/or subchondral bone has been recently described. Inflammation of the synovium (synovitis) has often been asso-

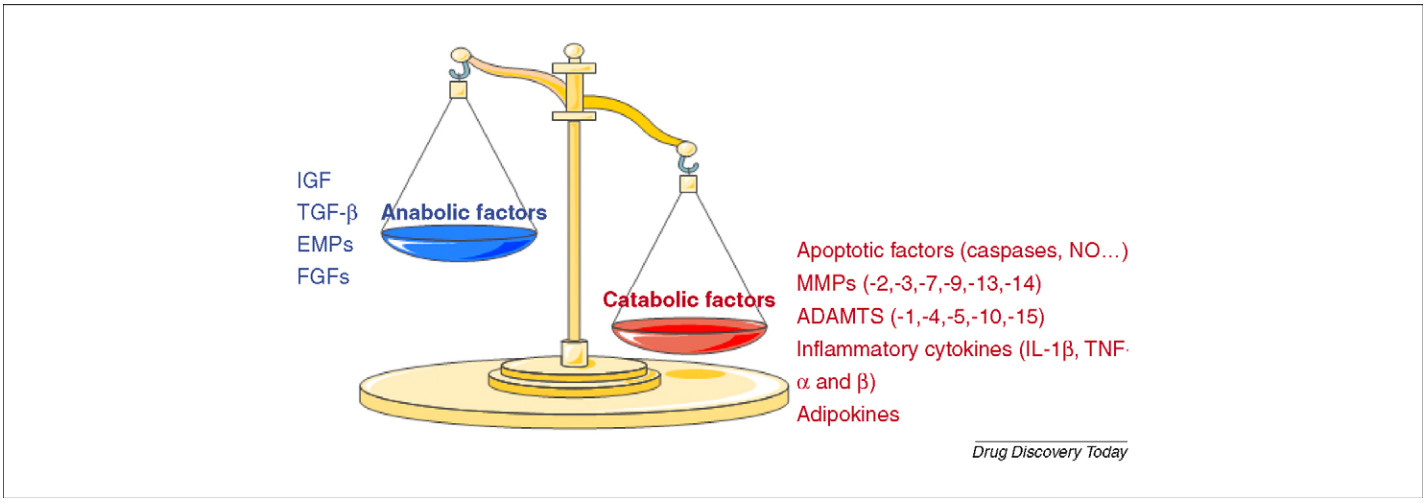


FIGURE 3

Imbalance between anabolic and catabolic factors in the physiopathology of osteoarthritis. This imbalance contributes to the alteration of the biomechanical properties of articular cartilage related to the destruction of its ECM. IGF: Insulin-like Growth Factors, TGF-β: Transforming Growth Factor-β, BMPs: Bone Morphogenetic Proteins, FGFs: Fibroblast Growth Factors, NO: Nitric Oxide, MMPs: Matrix Metalloproteinases, ADAMTS: A Disintegrin And Metalloproteinase with ThromboSpondin repeats, IL-1β: Interleukin-1β, TNF: Tumor Necrosis Factor.

ciated with advanced OA [20]. Synovitis leads to an overexpression of pro-inflammatory cytokines (IL-1β, TNF-α and -β) that in turn contribute to the subsequent catabolic degenerative processes of AC [21]. These cytokines also stimulate the production of nitric oxide by upregulating the expression of iNOS (inducible Nitric Oxide Synthase) and other pro-inflammatory cytokines, such as IL-6, LIF (Leukemia Inhibitory Factor), IL-17, IL-18 and chemokines [22].

The subchondral bone also exhibits noticeable alterations at an early stage of OA with a decrease in osteoblast activity that induces a thinning of the adjacent trabecular bone [3]. At later stages, an excessive bone remodelling is observed in the areas where AC has degraded, which unfortunately results in sclerosis and necrosis of the subchondral bone. This excessive bone remodelling has been suggested to increase the production of cytokines by osteoclasts and could induce the loss or damage of cartilage [23,24]. In addition, the leakage of synovial fluid towards the medullar spaces of the subchondral bone affects the bone marrow mesenchymal stem cells (MSCs), thereby contributing to the formation of osteophytes and cartilage nodules. These deteriorations of the subchondral bone are responsible for joint pain and are largely involved in the progression of OA [25].

Viewed together, these recent advances in the understanding of OA physiopathology clearly indicate that OA is a multi-target disease that affects AC, synovium and subchondral bone. The chronic evolution of OA could consequently be explained by the existence of a vicious circle comprising these three structures.

Risk factors for osteoarthritis

Among the risk factors for OA, it is necessary to distinguish between intrinsic risk factors (age, genetic polymorphisms, sex and hormonal status) and extrinsic risk factors (cartilaginous defects, obesity, microtraumatisms, joint misalignment, hyperlaxity and tabagism) [26]. Among the intrinsic risk factors, it is clear that age plays a major role in OA. A large body of evidence indicates that the major components of ECM, type II collagen and PGs undergo alterations in content, composition and structural organisation during ageing. There is also an accumulation of advanced glycation end-products, which enhances collagen cross-linking and contributes to the increase in cartilage stiffness observed with ageing [27]. Their effects are mediated through their direct binding to a specific receptor RAGE (Receptor of Advanced Glycation Endproducts) expressed by chondrocytes. Genetic polymorphisms are also crucial to OA, particularly when they affect genes encoding proteins involved in cartilage biology and ECM structure. Thus, polymorphisms of genes encoding type IX collagen, IGF-1 and vitamin D receptor have been correlated with an increased risk of OA [28]. Among the extrinsic risk factors, cartilaginous defects and obesity are probably the most significant ones. Owing to its poor capacity for spontaneous repair, when AC is damaged, it hardly heals. The traumatic loss of cartilaginous tissue therefore greatly contributes to the subsequent development of osteoarthritic lesions. The deleterious role for obesity in OA is also well established [29] and a prevailing hypothesis is that an increased load on the joint surface because of a large body weight leads to cartilage wear. The most significant link between OA and obesity has been reported for the knee joint (a BMI increase by 1 kg/m^2 above 27 accounts for an additional 15% increase in risk) [16]. Nevertheless, OA in non-load bearing joints such as metatarsophalangeal joints is also associated with obesity. These data suggest that systemic factors, including adipokines, may be involved in the high prevalence of OA among obese individuals [16,17].

More in-depth research is currently being conducted to evaluate the real impact of polymorphisms, as well as other risk factors, and could end up highlighting the multifactorial nature of OA. Such a multifactorial nature is likely to complicate epidemiological analyses and thereby hamper the development of future treatments.

Treatments for osteoarthritis

The optimal management of OA patients requires a critical combination of both non-pharmacological and pharmacological therapies. Patients who cannot obtain adequate pain relief and functional joint improvement should be considered for the

ultimate OA treatment: the prosthetic replacement of the affected joint.

Non-pharmacological therapies

Non-pharmacological therapies are currently still considered the first intention treatment in OA by the American College of Rheumatology (ACR), EULAR and OARSI guidelines [30–32]. These non-pharmacological treatments are, however, mainly suitable for patients suffering from knee and hip OA. Among the multitude of non-pharmacological modalities, the most widely proposed include weight reduction, education and self-management, referral to a physical therapist, aerobics, muscle strengthening, walking aids, thermal modalities, transcutaneous electrical nerve stimulation and acupuncture.

Pharmacological therapies

For many osteoarthritic patients, the non-pharmacological therapies are not sufficient to produce sustained reduction in the pain and restoration of joint function. Various pharmacological treatments have, therefore, been developed including both the fast- and slow-acting drug families. Some of these drugs are still in development and could be promising for therapeutic intervention, primarily in advanced OA.

The fast-acting drug family

The fast-acting drug family is mainly used for pain relief and includes acetaminophen, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), Cyclooxygenase-2 (Cox-2) inhibitors, glucocorticoids and opioids.

Acetaminophen (otherwise known as paracetamol) significantly reduces pain and increases the quality of life of osteoarthritic patients. The doses of acetaminophen (up to 4 g/day) can, however, trigger adverse hepatic events in patients with hepatic insufficiency [33]. Although OA does not involve systemic inflammation, typical anti-inflammatory compounds such as NSAIDs and Cox-2 inhibitors are largely used as analgesic treatments [34]. They exhibit some adverse effects, however, such as gastrointestinal, renal and cardiovascular toxicity [35]. Today, the association of NSAIDs with gastrointestinal protectors, particularly the proton pump inhibitors, leads to an improved gastrointestinal tolerance. NSAIDs are therefore widely used with adapted doses and are restricted to short-term treatments. For many years, intra-articular injections of glucocorticoids have been successfully administered to prevent pain [26]. They provide, however, only short-term efficacy [36] and exhibit adverse metabolic events. Consequently, the ACR recommended limiting intra-articular glucocorticoid injections to every three or four months. Opioids are considered in the treatment of OA as a final resort when pain is not controlled or for patients with intolerance to other pharmacological treatments [35]. They too, however, exhibit a wide range of adverse effects such as gastrointestinal (nausea, vomiting and constipation), alteration in the cognitive function, dependence and respiratory depression.

The slow-acting drug family

The slow-acting drug family is dedicated to the prevention of pain as well as the slowing down of the cartilage destruction. Several drugs are available, including glucosamine, chondroitin sulfate, *S*-adenosyl methionine, avocado/soybean unsaponifiables and hyaluronic acid (HA). Glucosamine and chondroitin sulfate belong to the large family of dietary supplements. Glucosamine is a natural precursor of GAGs that stimulates GAG production by chondrocytes, as well as the synthesis of collagen [37]. The glucosamine found in dietary supplements is usually extracted from the shells of prawns and other crustaceans, or made from maize starch. Positive effects of the oral administration of synthetic glucosamine in OA patients have been demonstrated by a significant reduction in the rate of joint space narrowing [38]. Nevertheless, a direct effect of glucosamine on the prevention of AC erosion has not yet been demonstrated to date [39]. Chondroitin sulfate is one of the major components of cartilaginous ECM. It can be extracted from cartilage of various origins (shark, cow, pig, fish and bird) by chemical treatment. Oral administration of chondroitin sulfate has been reported to decrease the activity of catabolic enzymes in osteoarthritic cartilage and to stimulate the synthesis of GAGs and collagens [40]. The GAIT (Glucosamine/chondroitin Arthritis Intervention Trial) did not, however, demonstrate any effect on pain by comparison with the placebo, when chondroitin sulfate was administered either alone or in association with glucosamine [41]. *S*-adenosyl methionine (SAM) is a small non-protein metabolite, namely a coenzyme, involved in methyl group transfers between enzymes. Endogenous SAM has been described to exert an antidepressant effect in humans. *In vitro*, SAM increases the synthesis of GAGs in articular chondrocytes, which could suggest that it may be able to aid in the repair of damaged cartilage through this mechanism [42]. Oral administration of SAM induces a significant decrease in pain and an improvement in joint function comparable to that of NSAIDs. In the absence of long-term follow-up, however, it remains difficult to rule out the possibility that the effectiveness of SAM may be related to its antidepressant effect [43]. The evidence for symptomatic efficacy of avocado/soybean unsaponifiable in patients with OA hip or knee available is not clearly established. However, of four studies three studies showed some evidence of efficacy for relief of pain in OA hip and knee [32]. HA is a polysaccharide ubiquitously found in ECMs. It can be extracted from mammalian cartilaginous tissues or produced by bacterial fermentation. The therapeutic concept of visco-supplementation suggests that the intra-articular injections of HA can help restore the viscoelastic and tribologic properties of the synovial fluid. In addition, HA has been proposed as a chondroprotective compound, since it is able to stimulate the production of TIMPs in chondrocytes [44]. Intra-articular injection of HA decreases the symptoms of OA with significant improvements in pain and functional outcomes [45]. This effect appears from 2 to 5 weeks after injection and can persist for up to 12 months. Visco-supplementation is not, however, indicated for patients with advanced OA, or for patients with an articular misalignment [46]. The rare adverse effects of intra-articular HA injection include pain and infection at the injection site, inflammatory responses and hypersensitivity due to excipient components.

Future treatments

To address further the clinical outcome of OA prevention and treatment, several new pharmacological compounds are under intense investigation. On the one hand, novel analgesic and anti-inflammatory drugs able to decrease pain but with reduced

TABLE 1

Analgesics and anti-inflammatory drugs in preclinical development

Targets	Drugs	Clinical status in OA	*Company*
COX/LOX inhibitor	Licofelone	Phase III beginning in 2008	*Merckle*
CINODs[a]	Naproxcinod	Phase III (results in 2009)	*Nicox*
	SD-6010	Phase II–III (results in 2011)	*Pfizer*
NSAIDs	IDEA-033	Phase I	*Idea Therapeutics*
Phospholipase inhibitor	Efipladib	Phase I	*Wyeth*
TRPV1[b]	ALGRX-4975 (Adlea) Injectable capsaicin	Phase III (results in 2010)	*Adolor*
Serotonin–norepinephrine reuptake inhibitor	Zucapsaicin	Phase I	*Winston laboratories*
Bradykinin B_2 receptor antagonist	Icatibant	Preclinical study stopped	*Aventis*
Unknown	SFPP	Phase I	*Mitsubishi Pharmaceuticals*
	MK-0686	Phase I	*Merck*
	Bicifadine	Phase I	*XLT Biopharmaceuticals*

[a] Cyclooxygenase-Inhibiting Nitric Oxide Donators.
[b] Transient receptor potential vanilloid subfamily 1 receptor agonist.

gastrointestinal and cardiac adverse events are in clinical study. On the other hand, a new class of compounds has been developed, the Disease-Modifying OsteoArthritis Drugs (DMOADs), which may slow down the degenerative process of OA. Finally, the use of growth factors that could have chondroprotective effects is also being contemplated.

Analgesic and anti-inflammatory drugs

Numerous pharmaceutical companies are testing new drugs, especially COX/LOX inhibitors (Cyclooxygenase/Lipooxygenase) and CINODs (Cyclooxygenase-inhibiting nitric oxide donors) [47,48]. The results of a phase III study with the naproxcinod (Nicox), the first CINOD, showed an effective anti-inflammatory activity with no detrimental effects on blood pressure and good gastrointestinal tolerability and safety. Table 1 indicates the different drugs currently in development with an update on their clinical status.

Disease-Modifying OsteoArthritis Drugs (DMOADs)

These drugs aim at slowing down the inevitable OA-associated cartilage degeneration by affecting various targets such as catabolic enzymes or cytokine-activated signalling cascades [48,49]. Table 2 illustrates the different drugs currently in development. Studies have been conducted to identify small molecular weight compounds that selectively inhibit the catabolic activity of enzymes from the MMP family . Several investigators have, however, reported some adverse events related to the musculoskeletal system (prinomastat, marimastat, BMS-27591 and PG-116800) with MMP inhibitors during the course of clinical trials in oncology and cardiology [50]. Many anti-cytokines are also under development. The anakinra (KINERET®, Amgen), an IL-1 receptor antagonist, is indicated for the treatment of rheumatoid arthritis. In OA, this anti-cytokine showed no significant effect on gonarthrosis symptoms [51]. This antagonist also has some drawbacks, primarily its high cost and the necessity of intra-articular injection. The interest of this type of anti-cytokine for the OA therapeutic arsenal is, thus, still difficult to delineate. In parallel, some studies are also being performed to decipher whether humanised monoclonal antibodies (adalimumab HUMIRA®, Abbott and infliximab REMICADE®, Schering Plough) that blunt the biological activity of TNF-α may be of therapeutic interest in OA. These monoclonal antibodies are well known to block the inflammatory processes in rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease. In OA, only one study was performed and unfortunately efficacy was not demonstrated [52]. Finally, great attention has also been paid to synthetic inhibitors of various signalling pathways implicated in the physiopathology of OA, such as MAP kinases [48,49]. With respect to the alteration of the subchondral bone in OA [53], bone anti-resorptive agents bisphosphonates calcitonin or licofelone have ultimately been proposed, but with disappointing results [54–56]. In addition, the pivotal role of RANKL (receptor activator of NF-κB ligand) and osteoprotegerin in bone resorption could also be potential targets and future clinical trials will hopefully be able to provide answers to the efficiency of these treatments [57]. A potential association of anti-resorptive compounds with specific chondroprotective drugs could be of interest in OA and deserves further consideration.

Growth factors

The administration of growth factors, such as basic-Fibroblast Growth Factor (FGF), BMPs (particularly BMP-2 and -7) and TGF-β, is also being considered as a potential therapeutic strategy. The *in vitro* effects of growth factors on chondrocyte function could make them useful for the prevention of cartilage degradation [58]. Among them, FGF-18 stimulates the repair of damaged cartilage in progressive OA in rats [59]. Nevertheless, the direct and repetitive injection of growth factors into the joints does not appear feasible for the management of chronic diseases such as OA. Much attention is therefore being paid to the development of drug delivery systems enabling the sustained delivery of growth factors.

Surgical treatments for osteoarthritis

Surgical treatments are generally considered the ultimate procedure when drug therapy has failed to relieve pain and/or to restore an adapted joint function. All these techniques are mainly dedicated to highly degenerative and advanced OA. Three procedures are currently used: osteotomy, arthrodesis and arthroplasty.

TABLE 2

Update in Disease-Modifying OsteoArthritis Drugs (DMOADs)

Targets	Drugs	Clinical status in OA	*Company*
MMPs	CPA-926	Preclinical study	*Kureha*
	PD-0200347		*Pfizer*
	VX-765	Preclinical study stopped	*Vertex*
	ONO-4817		*Pfizer*
	CP-544439	Preclinical study	*Pfizer*
	S-3536	Preclinical study stopped	*Shionogri*
	PG-530742	Unknown	*Procter and Gamble*
	BAY12-9566	Preclinical study stopped	*Bayer*
	RO32-3555 (cipemastat)		*Roche*
	RS-130-830		*Roche*
	Doxycycline	Studies stopped	–
	Minocycline		–
ADAMTS-5	ST109	Unknown	*BMS*[g]
	ST154		*BMS*[g]
Cathepsin K	SB-357114		*GSK*[h]
	SB-462795		*GSK*[h]
ICE[a]	Pralnacasan	Preclinical study	*Sanofi-Aventis*
p38 MAP-kinase[b]	SB 242235	Preclinical study	*GSK*[h]
NF-κB[c]	NF-κB decoy oligonucleotide		*AnGes MG*
iNOS[d]	*N*-iminoethyl-L-lysine		–
iNOS activity	PD-0200347		*Pfizer*
Caspase	Isatin sulfonamide		*GSK*[h]
MEK-1/2[e]	PD 198306		*Pfizer*
PPARγ[f] **(agonist)**	Pioglitazone		*Takeda*
Bone resorption	Bisphosphonates (risedronate)	Phase III in progress	*Procter and Gamble*
	Calcitonin	Phase III beginning in 2008	*Novartis*
	Ranelate strontium	?	–
Anabolism	FGF-18	Preclinical study	–

[a] IL-1 converting enzyme (=caspase-1).
[b] Mitogen Activated Protein.
[c] Nuclear Factor-KappaB.
[d] Inducible Nitric Oxide Synthase.
[e] MAP Erk Kinase.
[f] Peroxisome proliferator activated receptor gamma.
[g] Bristol Myers Squibb.
[h] Glaxo Smith Kline.

Osteotomy

Osteotomy is indicated in OA patients with joint misalignment, such as a valgus or varus knee. It is a surgical procedure that involves the removal of bone. A wedge of bone located near the damaged joint is removed to cause a realignment of the varus/valgus deformity. This realignment reduces mechanical stress on the affected compartment by redistributing load to healthy cartilage. The clinical outcome depends on the angulation of joint axis correction [60]. This procedure remains, however, associated with adverse events like haemorrhage, inflammatory reactions and nerve damage.

Arthroplasty

Total joint replacement or arthroplasty is reserved for the most severe and recalcitrant forms of OA or when other treatments have failed. Several human joints are routinely replaced, such as the hip and knee. Technology has advanced such that other joints can be replaced, including the shoulder and wrist. Total and partial knee replacements, which are now considered relatively routine surgery, have a 95% success rate at 20 years and are associated with an effective improvement in health-related quality of life [61]. There are more than 300,000 total knee replacements in the United States each year and a projection model predicted a 673% increase in primary knee arthroplasty to a total of 3.48 million procedures in 2030 [62]. Among all these surgeries, approximately 80% are unilateral, meaning only one knee is replaced, and 20% are bilateral. Recent advances in surgical technology have enabled total knee replacements to be performed as a minimally invasive surgical procedure, conducted under local anaesthesia that requires only a small incision in the centre of the knee. Physical therapy generally begins two days following surgery. Patients generally rely on walking aids for the first few weeks and are back to normal health in a few months. Nevertheless, the overall complication rate of 5.5% includes infection that continues to dominate the literature concerning complications after total knee replacement. Deep vein thrombosis and poor wound healing have also been described. Moreover, the revision rate after five or more years is 2% [63].

Arthrodesis

Arthrodesis, also known as artificial ankylosis or syndesis, is the artificial induction of joint ossification between two bones. Arthrodesis, however, is limited to a certain number of joints within the body. Most arthrodesis surgery is performed in either the wrist or hands, or the foot or ankle. Historically, knee and hip arthrodesis was also performed as a pain-relieving procedure. Given the great success achieved in hip and knee arthroplasty, however, arthrodesis of these large joints has fallen out of favour as a primary procedure and is now only used as a last resort in some cases of failed arthroplasties.

Regenerative therapies for articular cartilage defects

As previously described, cartilaginous defects constitute one of the major extrinsic risk factors for OA. The incidence of cartilaginous defects is estimated at 63% in the United States population of 31,516 arthroscopies [64]. Over the past 20 years, a great deal of attention has, therefore, been paid to therapeutic procedures for the early treatment of cartilaginous defects. Early treatments of cartilaginous defects could indeed be crucial to slowing down the chronic development of OA. Current procedures include washing, shaving and debridement, stem cell stimulation-based procedures (Pridie drilling and microfracture) and chondrogenic explant grafts (allo and autografts) [65]. The major challenges in regenerative medicine for cartilage are the restoration of a biomechanically competent ECM and the integration of this newly synthesised matrix within the resident tissue. To address this specific issue, Autologous Chondrocyte Implantation (ACI) was developed and has paved the way for cell therapy and biomaterial-assisted cartilage engineering.

Washing, shaving and debridement

Endoarticular washing consists of irrigating the joint with a physiological salt solution. Although washing has shown beneficial effects on pain, it remains an experimental approach. Elimination of inflammatory waste by this technique could explain the analgesic effect [66]. Thus, some studies have shown a positive effect for up to one year [67], whilst others observed no decrease in pain [68]. Arthroscopic shaving consists in decreasing friction by removing fibrillated cartilage with rapid shaving [69]. Today this method is fairly controversial and is therefore used less and less. Debridement combines washing, meniscectomy, ablation of foreign bodies and osteophytectomy. The long-term follow-up of patients undergoing such treatment has indicated that debridement leads to an aggravation of OA [70].

Stem cell stimulation-based procedures

These procedures aim at improving the poor spontaneous repair of cartilaginous lesions by taking advantage of the presence of reparative stem cells in the subchondral bone marrow [71]. Two techniques have been developed: Pridie drilling and microfracture.

Pridie drilling consists in perforating AC [72]. During this procedure the cartilage and bone sustain a trauma with ensuing therapeutic bleeding from the subchondral bone space. The benefit of this procedure is related to the fact that the blood clot triggers the spontaneous formation of a cartilage-like fibrous tissue. This procedure is disadvantageous in that it is largely invasive and has a longer recovery period and a higher probability of complications. Moreover, this technique leads to the formation of a fibro-cartilaginous matrix that remains transitory and does not possess the biomechanical properties of the native cartilage. Whilst effective at preventing further bone damage, the newly formed fibro-cartilaginous tissue is very poor at handling compressive force and has a very limited load bearing capacity. As a consequence, Pridie drilling is associated with excruciating amounts of pain largely because of the loss of smooth articulation and probably leading to bony crepitus. Microfracture is derived from Pridie drilling and consists in creating multiple small perforations in the cartilage defects (4 mm in depth). The size-reduced perforations can be performed via a mini-invasive procedure [60] and have less of an impact on joint function [73]. Microfracture is mainly indicated for the treatment of young patients and athletes where it has been shown to be efficient [73]. Nevertheless, the newly formed fibro-cartilaginous tissue is, as described above, poorly competent from a mechanical point of view. Long-term results of Pridie drilling and microfracture procedures need further careful consideration.

Allo and autografts

The principle of cartilage grafting procedures is to fill cartilage defects with healthy cartilage generally derived from human cadavers (allografts) or from the patients themselves (autografts).

Allografts

Although allografts have been used for several decades to treat AC defects, grafts derived from human cadavers induce immunological reactions [74]. In addition, it has also been reported that allografts lead to an increased risk of viral and NCTA (NonConventional Transmissible Agents) transmission.

Autografts

The first generation of osteochondral autografts consisted of harvesting a single and large patch of healthy osteochondral tissue (single graft) [75]. Unfortunately this procedure has several major drawbacks, such as a prominent morbidity of the donor site and an unsuitable geometry of the collection specimen [76]. As a consequence, a second generation of osteochondral autografts (multiple grafts or mosaicplasty) has been developed and is still largely used today. This second generation of multiple osteochondral autografts was first developed by Hangody *et al.* [77]. Mosaicplasty is a one-step procedure that consists of collecting several small cylindrical grafts in a low-weight bearing area of the joint and transferring the explants to the defect. Currently, mosaicplasty is restricted to subjects under the age of 50 years who exhibit small lesions located at the femoral condyles (lower than 4 cm^2 requiring fewer than 6 grafts), without mirror lesions and misalignment of the knee. Despite the promising clinical results obtained [78], this technique presents some major disadvantages, such as the difficulty to treat large lesions (>4 cm^2) and the instability of the graft. In addition, there is some uncertainty concerning the long-term outcome of the graft because of the discrepancy in the mechanical properties between the donor and the recipient sites [60].

Despite their numerous disadvantages, microfracture and mosaicplasty are largely considered the method of choice for the treatment of cartilage defects and, therefore, occupy a strategic place in orthopaedic surgical therapy.

Cell-based surgical therapy: Autologous Chondrocyte Implantation

Autologous Chondrocyte Implantation (ACI) is based on the grafting of isolated cells with chondrogenic properties within the cartilage defect. Brittberg *et al*. were the first to publish clinical results in humans with this technique [79]. The technique consists of three steps: cartilage collection, isolation and *in vitro* expansion of chondrocytes in monolayer culture, and implantation of the cultured chondrocytes in the lesion under a periosteal flap. Today, this technique is largely used in the United States where the FDA (Food and Drug Administration) delivered in 1997 the first agreement for CARTICEL® (Genzyme Corporation, Cambridge, MA), a commercial process for the production of autologous chondrocytes for transplantation. Today, in addition to CARTICEL®, several new products are being developed and tested, such as ChondroCelect® (Tigenix, Leuven, Belgium) or Hyalograft-C® (Fidia Advanced Biopolymers, Abano Terme, Italy). CARTICEL® is indicated for the repair of symptomatic cartilage defects in femoral condyles (medial, lateral or trochlea), caused by acute or repetitive traumatisms, in patients who have had an inadequate response to a prior repair procedure (e.g. debridement, microfracture and mosaicplasty). In 2005, the French National Authority for Health (HAS) evaluated the ACI technique [80]. Clinical studies have shown an encouraging improvement in clinical signs of OA. ACI-derived techniques, like the MACI technique (Matrix guided Autologous Chondrocytes Implantation), have subsequently undergone further developments. In the MACI, the periosteal flap is replaced by a membrane composed of a mixture of type II and I collagens stabilised on the defect by fibrin glue [81]. These cell-based surgical therapies for cartilage defects have led to encouraging results but also remain disappointing, particularly because the recovery of articular chondrocytes leads to damage at the donor collection site [60]. In addition, chondrocytes lose expression of the main chondrocytic markers during their *in vitro* expansion in monolayer culture, and this process of dedifferentiation leads to the formation of a fibrocartilage, biomechanically inferior to the original hyaline cartilage [82]. Another limitation is related to the use of a periosteal flap or a membrane to retain transplanted cells within the defects, which is not totally impervious and sometimes leads to hypertrophy [83] or uncontrolled calcification [60]. To overcome these limits, much attention has been paid to the development of three-dimensional scaffolds for the transfer and maintenance of cells in the recipient site. In addition, the increase in minimally invasive surgery has pushed researchers towards the development of injectable cartilage tissue engineering systems [84,85].

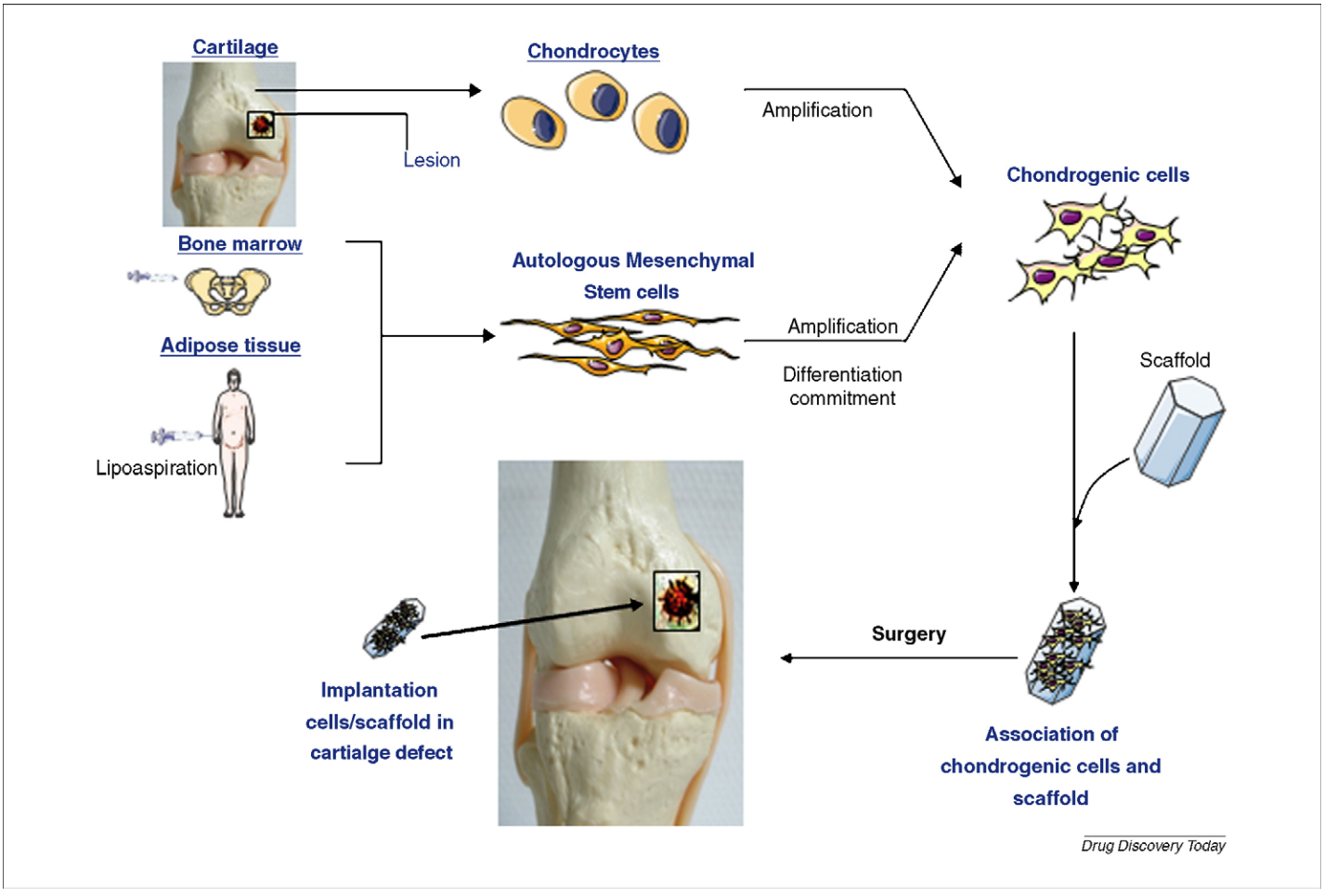


FIGURE 4

Strategy for cartilage tissue engineering. Autologous reparative cells are isolated from cartilage or derived from bone marrow or adipose tissue. Cells are then amplified and committed towards the chondrogenic lineage by exposure to various morphogenetic factors and finally seeded onto a scaffold. Hybrid constructs can finally be implanted into the defect.

Cartilage tissue engineering

Tissue engineering associates the principles and methods of engineering and life sciences with the development of biological substitutes that restore, maintain or improve tissue function [86]. Tissue engineering involves seeding a biocompatible scaffold with appropriate cells. The biomaterial scaffold can be loaded with signalling molecules (morphogens) that promote cell differentiation and maturation into the desired tissue. Two tissue engineering approaches have been developed. One consists of generating functional tissue *in vitro* then implanting the construct into the joint. In the other approach, the construct is cultured briefly, implanted when still immature and allowed to mature *in vivo* within its intended environment [87]. With respect to its biological features and its poor capability for endogenous repair, much attention has been paid to the development of tissue engineering applied to the repair of cartilage [88]. The theoretical scheme of cartilage tissue engineering is illustrated in Fig. 4 and involved a scaffold, cells and morphogens.

Scaffolds

Many scaffolds have been investigated for cartilage tissue engineering. They can be classified according to their nature (protein, polysaccharide, synthetic or natural), their shape (massive, porous massive, foams, viscous liquids and hydrogels) or their chemical formulation. The ideal scaffolds must exhibit the following essential properties. They should be biocompatible to prevent inflammatory and immunological responses, constitute a three-dimensional environment favourable to the maintenance of a differentiated chondrocyte phenotype. They should also be permeable to allow for the diffusion of molecules and nutrients. They should be adhesive to allow for the fixation of cells in the lesion, and bioactive to enable homogeneous and controlled release of growth factors. Finally, they should be injectable to enable mini-invasive surgery, and biodegradable to enable long-term integration into host tissues. The principal scaffolds used in cartilage tissue engineering are cited in Table 3. Because of their structure and properties, hydrogels are probably the most promising candidates, given the assumption that cartilage tissue engineering may become successful not only *in vitro* or *ex vivo* [85] but also in clinical situations. Hydrogels are composed of chains of synthetic or natural absorbent macromolecules. Cross-linking agents (glutaraldehyde, irradiation, pH or temperature) lead to chemical modifications resulting in the formation of a reticulated hydrogel [89]. The macromolecular network contains a high proportion of water which reproduces the characteristics of the three-dimensional environment of the cartilaginous ECM [90]. The porosity of hydrogels can be adjusted by the modification of the network density [84]. The fact that they can be injected is another advantage of hydrogels, enabling minimally invasive surgery [91], thereby reducing morbidity and the hospitalisation period. These injectable scaffolds must also be able to increase in volume, to acquire the desired shape once implanted. Preclinical studies to evaluate the mechanical properties of hydrogels are underway [85].

TABLE 3

Scaffolds developed in cartilage tissue engineering

Scaffolds	Hydrogel available
Proteic scaffolds	
Collagen	Yes
Gelatin	No
Fibrin	Yes
Laminin (MATRIGEL®)	No
Polysaccharidic scaffolds	
Agarose	Yes
Alginate	Yes
Cellulose	Yes
Chitosan	Yes
Hyaluronic acid	Yes
Artificial scaffolds	
Carbon fibre	No
Calcium phosphate	No
Dacron®[a]	No
Polybutyric acid	No
Polyestherurethane	No
Polyethylmethacrylate	Yes
Polyglycolic acid (PGLA)	Yes
Polylactic acid (PLA)	Yes
Teflon®[b]	No

[a] Terephtalate polyethylene.
[b] Polytetrafluoroethylene.

Cells

Several sources of cells have been considered for cartilage tissue engineering, including chondrocytes of various origins (articular, nasal and costal) [92] and MSCs isolated from bone marrow, periosteum, perichondrium or adipose tissue [93].

A recent study [94] compared different chondrocyte origins and suggested that nasal chondrocytes could be the most appropriate cell source for cartilage tissue engineering. Whether these data obtained in a rabbit preclinical study can be extrapolated to human remains to be demonstrated. The key limitations to the use of chondrocytes, besides their origin, are their phenotypic instability observed during the course of their expansion in monolayer culture. This phenotypic instability, called 'dedifferentiation' is characterised by a decreased expression of type II collagen, increased expression of type I collagen and a shift of cellular morphology from a rounded shape to the typical fusiform shape of fibroblasts [82]. This process of dedifferentiation may, however, be reversible when dedifferentiated chondrocytes are cultured in a three-dimensional environment [95]. Considering their chondrogenic potential, MSC could constitute an alternative source of reparative cells for cartilage tissue engineering [87,96]. The term 'mesenchymal stem cell' originally refers to adult stem cells from bone marrow (BMMSC). These BMMSC are characterised by an extensive capacity to proliferate whilst retaining their multipotentiality and ability to generate different connective tissue lineages (osteoblasts, chondrocytes, adipocytes, cardiomyocytes and so on) [97]. More recently, it has been demonstrated that MSC can also be reproducibly isolated from human adipose tissue (ATSC). Whereas the chondrogenic potential of ATSC is probably not as effective as BMMSC [98], these cells have the ability to differentiate along the chondrocyte lineage [99]. These ATSC have the advantages that they can be harvested with a low morbidity of

the donor site. Moreover, once digested and adipocytes removed, adipose tissue contains a high proportion of MSC (1–5%) compared to bone marrow (0.01–1%) [100]. MSC are easily amplified in monolayer culture and can undergo a differentiation process towards the chondrogenic lineage under appropriate conditions. The optimal conditions to differentiate MSC towards chondrocytes still have to be clearly established. Many pivotal parameters have been demonstrated to influence this process, such as growth factors, tri-dimensional culture and oxygen tension. MSC also have therapeutic potential as a result of their immunosuppressive properties. It has been demonstrated that BMMSC and ATSC are well tolerated and decrease the host response to the graft in the context of allogenic transplantations [101].

Culture conditions and morphogens

Culture conditions and morphogens (growth factors, oxygen tension and mechanical constraints) are essential parameters to take into account in the development of tissue engineering.

As previously described, three-dimensional culture enables preservation of the differentiated phenotype of chondrocytes [102]. Moreover, dedifferentiated chondrocytes recover their phenotype when they are placed in three-dimensional culture [95]. The molecular mechanisms governing the processes of dedifferentiation and re-differentiation are only partially understood, but a key role for integrins has been proposed [103]. Bioreactors constitute mechanically active and controllable culture systems. The ideal bioreactor must provide the tissue with mechanical stimulation similar to the *in vivo* conditions and increase ECM synthesis, nutrition and oxygenation of the tissue [104]. Physiological load exerted in the joint is essential to the development and the regeneration of normal AC [105]. Mechanical stimuli impact the behaviour of chondrocytes *in vivo* and *in vitro* [106]. Nevertheless, the consensus from *in vitro* mechanical loading experiments is that static compression stimulates depletion of PG and damage to the collagen network and decreases the synthesis of cartilage matrix proteins, whereas dynamic compression increases matrix synthetic activity [107]. The choice of the ideal parameters of stimulation is still under evaluation. Among the morphogens, growth factors are largely used to maintain chondrocytic phenotype or to differentiate MSC towards a chondrocytic phenotype. Many growth factors are involved in chondrocyte maturation and formation of cartilage [108]. These factors include the TGF-β family (Transforming Growth Factors), BMPs (Bone Morphogenetic Protein), CDMP (Cartilage Derived Morphogenetic Protein), FGFs (Fibroblast Growth Factors) and IGF-1 (Insulin-like Growth Factor-1). Another morphogen that has been considered as a potential tool for cartilage tissue engineering is hypoxia. Indeed, AC is a non-vascular tissue and chondrocytes are, therefore, immersed in a hypoxic environment (between 1 and 5% O_2) [109]. Hypoxia is involved in the differentiation of chondrocytes [109] and MSC [110] through the HIF (Hypoxia Inducible Factor) pathway [111]. It has also been suggested that hypoxia could be a major factor for the prevention of chondrocyte terminal differentiation and cartilage mineralisation [109].

Gene therapy

Whereas the majority of research is directed towards the development of growth factor delivery systems, gene therapy that uses cells for the *in situ* production of therapeutic proteins is considered with interest [112]. In the context of cartilage tissue engineering, this type of therapy aims at stimulating the expression of genes involved in the processes of tissue regeneration. Genes coding for various members of the TGF superfamily (TGF-β, BMPs), IGF-1, Sox family (-5, -6, -9), FGF-3 and SMADs could be potential candidates [113,114]. However, the clinical use of gene therapy is still in its infancy and will require further *in vitro* and *in vivo* evaluation before becoming part of the therapeutic arsenal in osteoarticular diseases.

Conclusion

The increasing knowledge regarding the pathogenesis of OA, particularly the role of cytokines, growth factors and signalling molecules, has provided new perspectives for cartilage repair and treatment of OA. The huge number of aetiological factors means that a multidisciplinary approach is necessary for the successful management of this disease. Regenerative therapies for the articular surface alone may not necessarily lead to pain relief and improvement of joint function, because other tissues including bone, muscles, tendons, ligaments and the synovial membrane are also involved in the pathogenic processes. The expanding repertoire of potentially therapeutic options offers the possibility to combine pharmacological treatments and tissue engineering towards regenerative medicine and thus to improve OA treatment and optimise cartilage repair. An inevitable pre-requisite for choosing the proper strategy and achieving the highest therapeutic benefit is, however, the ability to define the stage and pathogenetic background of the disease, which requires very sensitive diagnostic methods. Prevention of OA will be a key issue in the quest to decrease OA incidence in our ageing societies. The main challenge in tissue engineering is to find a compromise between the benefits to the patients, regulatory agencies, costs, coverage by health insurance and the role of pharmaceutical companies.

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