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GENERAL ORTHOPAEDICS

Advanced therapies in orthopaedics

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- Advanced therapies are expected to play a crucial role in supporting repair after injury, halting the degeneration of musculoskeletal tissue to enable and promote physical activity.
- Despite advancements, the progress in developing advanced therapies in orthopaedics lags behind specialties like oncology, since innovative regenerative treatment strategies fall short of their expectations in musculoskeletal clinical trials.
- Researchers should focus on understanding the mechanism of action behind the investigated target before conducting clinical trials.
- Strategic research networks are needed that not only enhance scientific exchange among like-minded researchers but need to include early on commercial views, companies and venture perspectives, regulatory insights and reimbursement perspectives. Only in such collaborations essential roadblocks towards clinical trials and go-to-patients be overcome.

Keywords: advanced therapies; biomaterials; cell therapy; degeneration; gene therapy; orthobiologics

Introduction

The history of orthopaedics has been dominated by surgical innovation. Outcomes and complication rates in common procedures such as total joint arthroplasty are continuously improving (1). Surgical strategies and techniques are evolving towards minimally invasive procedures with less tissue damage (2). Ongoing innovation is also happening on the implant side. Examples of new innovative inventions are intramedullary nails with magnetically driven motor units enabling non-invasive transcutaneous distraction osteogenesis (3) or portable surgical sewing devices that may shift paradigms in surgical repair of tendon and ligament tissue (4). Also within the traditional implant market, some new approaches can be observed such as dynamic fixation principles realized by biphasic fixation plating (5) or angle stable nail fixations (6). However, most of these strategies are based on continuous improvements and rather seldom revolutionize medical treatment in orthopaedics and trauma surgery.

Today, regenerative therapy approaches – although initially introduced in orthopaedics – lag today behind

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other medical fields such as oncology. In 2024, 283 U.S. Food and Drug Administration (FDA)-approved and 207 European Medicines Agency (EMA)-approved drugs were listed for cancer therapy. Many of them are classified as biologics (7). In contrast, advanced biologic therapeutics in orthopaedics, such as cell therapies, have so far mostly failed to reach primary endpoints and get regulatory approval. Many do not even enter this phase and never manage their way to the stage of being tested in approval trials. Among other reasons, primary endpoints in orthopaedic trials, such as patient-related outcome measures, are often significantly influenced by placebo effects that overshadow the drug-induced effects and destroy statistical efficacy (8). Powering studies enough to show relevant drug-induced effects remains a challenge in musculoskeletal research. Patient training on correct and non-biased reporting, such as those offered by companies like Analgesics Solutions (https://www.wcgclinical.com/analgesic-solutions/), might also be an appropriate approach to mitigate placebo effects.

Detailed knowledge about the pathophysiology of the disease, the mechanism of action for biological drugs and biomaterials and the regulatory requirements for advanced biological products are necessary to design a targeted drug that has a chance to show clinically relevant efficacy in a randomized, placebo-controlled trial (RCT). Furthermore, successful implementation of advanced therapeutics into orthopaedic practice will mandate advanced diagnostic tools to identify early disease stages and also a deep knowledge of the market dynamics.

This review aims to provide a brief overview of promising advanced therapy agents and targets designed to treat musculoskeletal diseases in an ever-evolving field.

Indications for advanced therapies in orthopaedics

In musculoskeletal tissues, the ability to regenerate after an injury seems to correlate with blood supply. Articular cartilage is naturally avascular, and ligament and tendon tissues have comparably low vessel densities, while bone tissue benefits from uninterrupted blood supply. All highly prevalent diseases in orthopaedics involve some kind of regeneration failure due to overload, injury, scarring, denervation and subsequent degeneration. Advanced therapies in orthopaedics intend to restore the regeneration potential of musculoskeletal tissues to avoid, reduce or augment surgical procedures.

Promising developments in advanced therapies for the highly prevalent disease complexes cartilage degeneration, tendon and ligament degeneration and impaired bone healing will be discussed in the following sections.

Cartilage regeneration

With an estimated prevalence of 40% in individuals over 70 years of age and an increasing global burden (9), osteoarthritis (OA) can be considered one of the most relevant musculoskeletal diseases of the 21st century. The disease term ‘osteoarthritis’ resulted in a total of 628 hits on ClincialTrials.gov for completed phase-II or phase-III trials (accessed April 9, 2024, (10)). However, there is not a single regenerative FDA-approved therapy on the market to date (11). An ideal drug for OA should address all or most of the known aspects of the pathophysiology of OA, such as low-grade inflammation, macrophage dysfunction, pathological angiogenesis, subchondral sclerosis, abnormal osteophytic bone formation, hypertrophy and apoptosis of chondrocytes, cartilage matrix degradation, deterioration of chondrocyte metabolism and synovial inflammation (12).

Newer findings from a large cohort study suggest that OA is a family of joint diseases, in which not all known aspects of the pathophysiology are present in every patient (13). Advanced biomarker-assisted sub-classification of OA patients may revolutionize clinical trial designs soon and already do so in some cohorts. For example, using the presence of an inflammatory subtype of OA as an inclusion criterion for a trial investigating an anti-inflammatory drug could greatly increase the chance of reaching the primary endpoint.

Meanwhile, about one out of 300 of all citizens in Europe received replacement surgery for a knee or hip joint in 2021 (mean of all OECD member countries, (14)). The revenue of the global joint replacement product market has grown to USD21 billion in 2023 (15). Reproducing preclinical results of advanced therapeutics in human trials still seems to be an unsolved problem in OA research. One major aspect of the problem is the routine use of animal models mimicking injury-related post-traumatic OA (ptOA) in preclinical trials, while the later following human trials recruit patients with spontaneous primary osteoarthritis. Furthermore, therapy in animal models is often initiated at the time of injury, corresponding to the early stage of degeneration, while the corresponding human trial is then recruiting patients with end-stage disease.

The Osteoarthritis Research Society International (OARSI) aims to connect OA researchers worldwide and facilitate the development of advanced therapies. One major target is the early identification of at-risk patients by establishing biomarkers for early OA (13). We further understand that immediate changes inside the joint after a critical joint injury might pave the way for ptOA which seems to become irreversible at some point (16). However, whether there is a critical threshold for irreversible joint damage is unknown. Advanced therapeutics should focus both on early intervention after injuries with an increased risk for ptOA, such as ACL ruptures or intraarticular fractures, as well as addressing cartilage regeneration in end-stage primary OA.

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If patients with end-stage disease are the target cohort and an intraarticular route of administration is chosen, appropriate preclinical animal models like the Dunkin Hartley guinea pig (17) should be chosen to get a preclinical proof-of-concept.

Tendon and ligament regeneration

Hypovascular tendon and ligament tissues provide suboptimal conditions for adequate healing. Anatomically challenged tendons such as that of the supraspinatus muscle in the shoulder or the Achilles tendon in the lower limb are often affected by overload-induced chronic inflammation and consecutive degeneration, ultimately leading to partial or complete rupture of the tendon. Transcriptomic analysis suggests that chronically diseased tendon cells undergo complex mechanically induced changes ultimately leading to processes resembling endochondral ossification (18). Chronic inflammation, pathological neoangiogenesis and endochondral ossification are similar key pathways in the pathogenesis of both tendinopathy and OA.

Surgical reconstruction of degenerated and healthy ruptured tendons and ligaments such as the anterior cruciate ligament, rotator cuff or Achilles tendon can result in treatment failure and revision surgery, preventing the return to prior physical activities (19, 20, 21).

Researchers who develop advanced therapies for tendinopathies have to respect the unique biology of the tendon as well as the often-affected interface between tendon and bone tissue, called enthesis (22). Research networks such as the TEndon Regeneration NETwork (TENET), can help promote these and support the establishment of new therapies. The TENET aims to build a multidisciplinary platform to facilitate the development of advanced therapies for tendon regeneration.

Bone healing and regeneration

When adequate surgical or conservative therapy is initiated early and maintained for a sufficient period, complete regeneration occurs, reducing the need for additional advanced therapies. Unlike avascular cartilage, bone benefits from its rich vascularization, facilitating optimal conditions for regeneration following injury. However, various complicating factors such as infection, soft tissue damage, incorrect fixation, compromised healing capacity due to comorbidities, and unknown variables can lead to impaired bone healing, affecting up to 10% of all fractures, with the highest incidence observed in the tibia and clavicle (23). Currently, there is no established biomarker for predicting which patients may require advanced regenerative therapies due to an elevated risk of impaired healing. Reinke *et al.* have recently identified terminally differentiated CD8+ TEMRA cells as independent predictors of impaired bone healing (24). Their finding not only emphasises the significant influence of individual adaptive immunity on endogenous regeneration but also suggests that CD8+ TEMRA may be a promising prognostic marker for predicting healing outcomes prior to any surgical intervention. At the same time, it may provide a starting point for early and targeted intervention strategies (25).

While clinical intervention to date detects failed bone regeneration at later stages, when delayed or non-union occurs, evidence suggests that regeneration failure could be determined in the very early phase of fracture healing (26). Traumatology lacks advanced diagnostics so far. The AO fracture monitor is a novel advanced *in vivo* load measuring device that can quantify the healing process continuously when applied to metal fixation plates (27).

Muscle regeneration

Muscle tissue is rich in blood vessels. However, major damage to a muscle can result in the formation of afunctional fibrous scar tissue and fatty muscle degeneration. Furthermore, low muscle quality in older patients significantly impairs outcomes and treatment efficacy in high-frequency surgeries knee or hip arthroplasty or spine surgery (28, 29). Moreover, any surgical intervention itself creates iatrogenic muscle damage that accumulates in case of multiple revision surgeries (30). Regenerative biological therapies that improve muscle tissue healing may revolutionize rehabilitation and regain function after orthopaedic surgeries soon.

Promising advanced therapies in orthopaedics

Detailed overviews of clinical trials investigating advanced therapeutics for OA (11), tendon regeneration (31), muscle regeneration (32), and biomaterials and biologics for bone regeneration (33, 34) have been performed elsewhere and shall be referenced here.

Preclinical studies investigating innovative small molecules, cell-based therapies, gene therapeutics or smart biomaterials are published rapidly, with only low translation rates into human applications. This review aims to give a concise summary of promising therapeutic approaches that have, in our opinion, the potential to get approval within the next years.

The global market revenue of orthobiologics is estimated to grow from 6.2 billion USD in 2022 to 10.3 billion USD in 2031 (35), also reflects the increase in therapies evolving from new and previously underdeveloped fields.

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Small molecule targeted therapies

In 2024, numerous preclinical trials investigating various small molecules for the treatment of high-impact musculoskeletal diseases have been conducted, with low rates of translation into human trials (11).

In 2020, a phase-II trial (NCT01919164) investigating the use of recombinant human fibroblast growth factor 18 (rhFGF-18) to treat knee OA was completed. While not reaching the primary endpoint, intra-articular administration of the substance twice a year significantly improved cartilage thickness at 5-year follow-up (36). Despite the official trial failure, subgroup analyses suggest that anabolic cartilage treatment with rhFGF-18 may be a promising target for end-stage rather than early-stage OA. However, one has always to bear in mind that complex musculoskeletal disorders exhibit multiple deranged pathways, that ideally would have to be addressed via multiple mechanisms.

Gene therapies

As all degenerative orthopaedic disorders are chronic processes, continuous long-term restoration of healthy physiology with gene therapy appears to be a sophisticated treatment approach. The global market for gene therapies is estimated to grow from 9.0 billion USD in 2023 to 23.9 billion USD in 2028 (37). However, most 36 FDA-approved gene therapy drugs target monogenetic congenital orphan diseases or cancer (38). In preclinical research, recombinant adeno-associated virus (AAV) vectors that induce overexpression of a target protein have been used to promote regeneration in osteochondral lesions, bone, muscle, and tendon and ligament tissue for more than 15 years (39). However, up to date, not a single gene therapy is in clinical use for a highly prevalent musculoskeletal disease in 2024. Huge costs, time-consuming production, carcinogenicity and immune responses to carrier vectors are major roadblocks in the translation of promising drug targets into clinical trials (40, 41). Transferring the success of gene therapies into orthopaedics will be challenging because, in contrast to orphan diseases and cancer, common musculoskeletal diseases are usually highly prevalent, localized and non-lethal. To increase the chances of regulatory approval, the cost-efficiency needs to be improved and safety profiles established.

Cell-based therapies

Isolation and characterization of bone marrow-derived mesenchymal-like stromal cells (bMSCs) have been described since the 1970s (42). The increased popularity of pluripotent stem cell treatments in the 2000s led to an uncontrolled boom with the rapidly spreading use of different 'stem cell products', while clear evidence was lacking. While musculoskeletal indications exhibited good safety profiles and the largest problem was in many of them proof of efficacy, the FDA then observed increasing numbers of serious adverse events, for example in patients suffering from macular degeneration and being treated with uncontrolled cell applications and reacted with stricter regulatory guidelines (43, 44). To date, detailed preclinical proof-of-concept evidence is mandatory to get approval for an in-human trial with any cell-based product. Despite numerous experimental and clinical studies, the broad application of stromal cell therapeutics is not yet emerging (45).

To ensure comparability between cell experiments, the term MSC should only be used for cells that fulfil at least the minimal criteria of MSCs, published by the International Society of Cellular Therapy (ISCT) (46). MSCs act as potent pro-regenerative immunomodulators via the reduction of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β, the elevation of anti-inflammatory interleukin 10 (IL-10) and suppression of pro-inflammatory T cells (47). More than 200 clinical trials investigating therapeutic applications of MSCs have been conducted since 2010 in the USA (48). Of these trials, the majority (25%) investigated MSCs for the treatment of musculoskeletal disorders, and 71% used bMSCs (48). MSC-like adipose tissue-derived stem cells (ADSCs) have been proposed as a promising alternative to bMSCs for musculoskeletal disorders because of higher cell density (5000 vs 100–1000 cells per gram) and minimal donor-side morbidity (49). However, products that use enzymatic digestion to isolate ADSCs are classified as advanced medicinal products by regulatory authorities in many countries.

The term stromal vascular fraction (SVF) refers to autologous processed fat tissue products that contain ADSCs, pericytes, haemopoietic-lineage cells and endothelial cells (50). SVF can be obtained via enzymatic or mechanical processing (51). While SVF is used autologously, also allogeneic cell products are in clinical trial phases. One of these, is for example, NVD-X3 3M$^{\text{ALLO-REG}}$ (Novadip Biosciences, Belgium), which is a matrix-augmented, ADSC-based allogeneic off-the-shelf product that recently entered a phase-I/II trial to test its efficacy in augmenting the surgical fixation of distal radius fractures (NCT05987033).

For the treatment of OA, multiple clinical trials have been conducted and an impressive variety of MSC products have been used. However, no product has yet been shown beneficial in the long term. A recent multicentre RCT with 480 patients could not show superiority in pain and function scores at 1-year follow-up when comparing intraarticularly administered bone marrow aspirate concentrate (BMAC), SVF, and human umbilical cord tissue MSCs to corticosteroid injections (52). In summary, evidence could not show a clear beneficial effect of MSC treatment for musculoskeletal indications so far (48, 53, 54), but many are in the pipeline and clinical trial designs have been adapted to better be able to address the specifics of cell therapeutic effects and also new pathomechanisms are explored.

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Newer findings suggest that dysfunctional resident protective synovial macrophages (55) and overactive monocyte-derived pro-inflammatory macrophages in the synovium are essentially involved in the pathogenesis of inflammatory joint diseases (56). Allocetra-OTS, an off-the-shelf cell-based therapeutic approach for targeted macrophage reprogramming, has been successfully tested in a human phase-I trial in sepsis (57). A first-in-human trial investigating the product for the treatment of end-stage knee OA is being developed.

In phase-I/IIa trial, Winkler *et al.* showed that placental-derived mesenchymal stromal-like cells (PLX-PAD, PLURI Biotech, Haifa, Israel) were able to increase muscle strength after hip arthroplasty when injected locally (58).

Advanced biomaterials

Biomaterials are xenogeneic, allogeneic, autologous or synthetically produced products that support or replace injured or diseased tissue. To restore physiological architecture, an optimal biomaterial should provide a transient or permanent regenerative matrix to facilitate the ingrowth of surrounding regenerative cells. Newer findings suggest that scaffolds should be viscoelastic rather than stiff to facilitate adequate cell ingrowth in soft tissue (59).

Various biomaterials for bone grafting have been tested for more than 100 years with limited success (60). Novel advanced biomaterials, mostly in preclinical stages, incorporate regenerative cells and growth factors into engineered scaffolds (61). In 2018, Petersen *et al.* described a cell- and growth factor-free collagen scaffold that supports physiological bone formation via endochondral ossification by providing an organized pore network (62).

Autologous chondrocyte implantation (ACI) also used as matrix-associated ACI (MACI), a cell-based approach (in the case of MACI using an advanced biomaterial), has shown promising results in the treatment of cartilage defects in clinical trials and also in the clinical routine (63). In Germany, MACI is the only advanced, cell-based biomaterial and ACI is the only ATMP in the orthopaedic space with regulatory approval.

However, unwanted hypertrophy and final endochondral ossification of transplanted chondrocytes still impair treatment success. To improve the long-term efficacy of chondro-anabolic treatments, an effective suppression of endochondral ossification during the regeneration process must be ensured (64). Manipulation and modification of advanced cell–scaffold–matrix complexes through therapeutical mRNA or gene therapy could overcome this problem while creating new safety concerns and roadblocks in regulatory approval. A recent preclinical study also suggested that CO_2 laser engraving of transplanted cartilage matrix tissue can facilitate cell ingrowth and incorporation of the transplant (65).

The Alliance for Advanced Therapies in Orthopaedics

To connect and support experts in the field of musculoskeletal diseases, the Alliance for Advanced Therapies in Orthopaedics (ATiO) was founded in 2021. The ATiO uses its exclusive network of world-renowned researchers, clinicians, business, pharma and regulatory experts to enable and accelerate the development of new innovative therapies for musculoskeletal diseases and injuries. The goal is to fuse the in most cases compartmentalized knowledge about advanced product development and their fast translation to patients. One of the core missions of the ATiO is to analyze, develop and increase quality standards for advanced diagnostics and therapies to reach a sustainable growth of the field.

Future perspectives digest

Cell-engineering

Recent advances in our understanding of genome editing enable novel treatment approaches. Targeted *ex vivo* modification of regenerative cells like MSCs, chondrocytes or T cells via coding RNAs (mRNA), non-coding RNAs (siRNA, miRNA) or next-generation RNA-guided CRISPR interference (CRISPRi) editing have been tested in OA animal models and are promising platform technologies for the future of musculoskeletal research (66). Furthermore, MSCs secrete membrane-coated extracellular vesicles (EVs) that contain pro-regenerative nucleic acids, lipids or proteins (67). Wang *et al.* recently showed that xenogeneic ADSC-derived extracellular vesicles can improve tendon–bone interface healing while reducing fatty muscle infiltration after a surgically induced tendon rupture in rabbits (68). Indirect treatment with stem cell-derived EVs may be a promising alternative to direct stem cell treatment.

Therapeutic mRNA

De La Vega *et al.* recently described the direct application of lipid particle-encapsulated *in vitro* transcribed (IVT) mRNA encoding BMP-2 in a mouse model for critical-size bone defects. The mRNA therapy was able to promote endochondral ossification *in vivo* (69). Zhang *et al.* successfully combined IVT mRNA encoding Runx2 and VEGF to synergistically improve bone healing in a rat model of mandibular bone defects (70). The safety, increased bioavailability of the desired protein via local production and the fact that multiple targets can easily be combined in one product qualify therapeutic mRNAs as a promising approach for musculoskeletal indications.

Roadblocks in the translation of therapeutic mRNA products into orthopaedic practice are the short half-

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life, potentially undesired immune responses and the molecular instability of the product (71).

Rapid clearance of mRNA formulations might be compensated by molecular stabilization or sustained release concepts in the future.

Conclusion

Medical research in the 21st century will be dominated by advanced therapies. However, the footprint of advanced therapies in orthopaedics is still small today. That illustrates a huge potential to raise, but at the same time clear strategies are needed to address the increasing demand for innovative regenerative treatments.

We believe that the future is bright but essential roadblocks for advanced therapeutic developments need to be addressed early and adequately. Frequently those are lack of clear mode of action, shortcomings in trial design, inadequate health technology assessments and also in some cases lack of financial support for high-quality studies, which are extremely cost-intensive. An early involvement of all relevant stakeholders as realized in the scientific network of the ATiO will help to overcome such challenges.

ICMJE Conflict of Interest Statement
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

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