Intra-articular biomaterials-assisted delivery to treat temporomandibular joint disorders

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Abstract
The temporomandibular joint disorder, also known as myofascial pain syndrome, is considered one of the prevalent chronic pain diseases caused by muscle inflammation and cartilage degradation in head and neck, and thus influences even biopsychosocial conditions in a lifetime. There are several current treatment methodologies relieving inflammation and preventing degradation of the joint complex. One of the promising non-surgical treatment methods is an intra-articular injection of drugs such as corticosteroids, analgesics, and anti-depressants. However, the side effects of drugs due to frequent injections and over-doses, including dizziness, dry mouth, and possible drug dependency are considered limitations. Thus, the delivery of therapeutic molecules through the use of nano/microparticles is currently considered as a promising strategy primarily due to the controlled release. This review highlights the nano/microparticle systems for effective intra-articular therapeutics delivery to prevent cartilage degradation and protect subchondral bone in a temporomandibular joint.

Keywords
Temporomandibular joint, therapeutics delivery, nano/microparticles, intra-articular injection, temporomandibular joint disorder, myofascial pain syndrome

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Introduction
The temporomandibular joint (TMJ) is a bilateral joint (synovial articulation) between the condyle (head of mandible) and part of skull (upper temporal bone). In addition to the above two skeletal structures, TMJ additionally consists of lateral pterygoid muscle, articular disk, surrounding capsule, and three ligaments (sphenomandibular, temporomandibular, and stylomandibular ligament) from front to back in a sagittal plane. The surrounding capsule consists of a dense fibrous membrane and is filled with synovial fluid, providing nutrition for the avascular central area of the disk, lubricating during opening and closing of mouth, and providing a space for therapeutic molecules used to cure diseases.1-3 Because of the biconcave shape of the articular disk, the central area of TMJ lacks blood and nerve innervation, inflammatory-associated pain or swelling is
from the peripheral region which is full of blood vessels and nerves.

Temporomandibular joint disorder (TMD), also known as myofascial pain syndrome (MPS), is a general term to describe pain and dysfunction of the masticatory actions. The cause of the onset of TMD is poorly understood but considered from multiple reasons of psychosocial, habitually occlusal, mentally, genetic, or hormonal factors. Although TMD is not life-threatening, it can be critical to the quality of life because it becomes prevalent even in young generations, chronic, irreversible, and usually difficult to manage, leading to increasing number of TMD patients and undesirable effects on patients’ professional and social lives. The most common symptom of TMD is acute or chronic pain, followed by restricted mandibular movement and noises during jaw movement, leading to progressive TMJ degeneration and limitation of talking, chewing, and other basic daily mouth activities. Interestingly, unlike other degenerative joint diseases such as arthritis which are more common in the elderly, TMD even affects up to one-third of adolescents and young adults.

TMD is generally classified into three categories according to the symptoms: myofascial pain, internal derangement of the joint with/without reduction, and inflammatory joint disease. Conventional treatment aims at decreasing hypertonic muscles and increasing TMJ mobility. Based on this, current clinical treatments (Figure 1) are divided into four groups: noninvasive, minimally invasive, invasive, and alloplastic replacement. The first stage of clinical treatment for TMD is noninvasive therapy which includes physical treatment using low-level laser or ultrasonic device, behavioral interventions often with occlusal splint, or pharmacologic approaches (i.e. taking analgesics, anti-depressants, muscle relaxant, or non-steroidal anti-inflammatory drugs (NSAIDs)). However, above conventional noninvasive treatment is often limited to regenerate severe or chronic TMD and systemic side effects such as gastrointestinal toxicity, cardiovascular risk, and anaphylaxis, disorders to the other tissues can be raised especially from oral administration in a dose-dependent manner.

Second, minimally invasive therapies include the injections and arthrocentesis where a needle is used to flush and drain the joint space for removing inflammatory mediators and enhancing lubrication. The intra-articular injection is considered as a more promising method to deliver drugs or biomolecules to the target site, leading to a reduction of the
risk of the systemic side effects. Currently, there are several injectable drugs such as NSAIDs, corticosteroid (glucocorticoids), triamcinolone acetonide, and dexamethasone), platelet-rich plasma (PRP) from blood and hyaluronic acid (HA) which relieve pain and improve maximal incisal opening. However, the side effects of injected (bio) molecules, including dizziness, dry mouth, and possible drug dependency are considered limitations of the procedures. Thus, biomaterial-based carriers have been developed mainly to deliver the drugs controllably into the specific anatomical area.

Surgical treatments of invasive intervention or alloplastic replacement are also considered when advanced or chronically degenerative TMD is present and mouth opening is extremely limited. They are quite effective in cleaning up inflammatory granular tissues and their degenerative cytokines, but they need high demand of cost and time. In case of alloplastic replacement, unpredictable outcomes are sometimes produced from uncontrollable inflammation and immune responses after replacement surgery using the artificial disk. Thus, minimally invasive intra-articular injection is highlighted as a promising general treatment even in severe TMD patients due to their high efficiency of regeneration and relatively easy procedure according to the development of stem cell biomaterial–based tissue regeneration.

There are many successful in vitro or in vivo studies on intra-articular injections to TMD patients using nano/micro biomaterials, (stem) cells, drugs, or their composite (Table 1). However, the clinical outcomes of intra-articular injections to TMD patients are by far still controversial because reports have revealed diverse outcomes from “therapeutic” to “degenerating.” In addition, like other inflammatory disease in the joint area (i.e. osteoarthritis), no agents are available to reverse the ongoing TMD to a healthy state. Current clinical therapies using intra-articular injections are only effective in pain relief at an early stage of disease but fail to alleviate severe or chronic pain lacking regenerative potential. To overcome the limitations of current intra-articular injections, nano and microscale biomaterials with or without (stem) cells or curable molecules (RNA, proteins, drugs, and macromolecules) have been introduced. They can effectively relieve pain and symptoms for long time, reduce systemic side effects of injections and tissue damages, control the release of drug and macromolecules delivered, and in turn increase regeneration process. This review discusses current therapeutic strategies of using injectable biomaterial-based delivery carriers for the treatment of TMD.

**Delivery vehicles of therapeutic molecules**

Delivering drugs through oral administration to the targeted site takes long (1–5 h) and leads to the decrease of drug efficiency due to their gastrointestinal adsorption and systemic circulation. Corticosteroid injection has a palliative (not curative) effect, is only used in severe acute pain, and generally for limited doses only. The effect of steroid on the disk is widely reported to irritate. Therefore, intra-articular injection of (therapeutic) biomaterials (i.e. HA and corticosteroids) has been widely used in clinics because high drug concentrations are possibly delivered with minimal side effects compared with systemic administration. However, rapid clearance of the injected biomolecules requires repeated injections, which causes complications like infection, fibrous tissue formation, and consequent joint damage. Such obstacles are possibly tackled through the use of nano and microparticles that can deliver therapeutic molecules, such as anti-inflammatory drugs, steroids and genes, and release them in a sustained manner. In addition, nano and microparticles interact with cells at the intra and extracellular space depending on their size (Figure 2). Thus, another strategy to enhancing regeneration of TMD is through regulating the interaction between (newly conjugated or residual) cells and injected intra-articular biomaterial.

**Nanoparticle-based drug delivery system**

Nanoparticles have been widely used in biomedical applications due to their unique properties such as large surface-to-mass ratio, quantum properties, and ability to load and deliver small-sized biomolecules (proteins and drugs). Nanoparticles are solid or colloidal particles with sizes ranging from tens to hundreds of nanometers. Particle size is a typical factor that determines toxicity, delivery capability, and in vivo distribution. Nanoparticles readily enter into cells by an endocytosis mechanism. Thus, they can deliver small-sized biomolecules intracellularly to control cell fate. Also, degraded products (i.e. ions) of nanoparticles can sometimes be therapeutic, exerting co-delivery functions with drugs (i.e. ion-drug).

Biodegradable synthetic (poly (lactic-co-glycolic acid) (PLGA) and poly lactic acid (PLA) or natural polymers (chitosan and gelatin) are mainly developed into nanoparticles. For example, drugs for antinociceptive or anti-inflammatory effects were incorporated into biodegradable synthetic nanoparticles for controlled delivery, which revealed high antinociceptive activity and healing process. HAS has also been delivered for anti-inflammatory action. HA has the binding ability to CD44 receptor expressed on the surface of chondrocytes which are the major target cell source for regeneration of TMJ. When HA moieties were coated on the surface of the biodegradable polymer while incorporating therapeutic drugs, the targeted delivery to chondrocytes was successful to elicit therapeutic effects. A direct use of protein moiety to target cell type was also made with interleukin-1 receptor antagonist (IL-1Ra), which targeted synoviocyte cells in TMJ capsule. The nanoparticles increased the retention time of IL-1Ra in rat joint over 14 days and inhibited IL-1-mediated inflammatory signaling through IL-1Ra and IL-1 receptor
Table 1. Summary of studies regarding intra-articular drug delivery through biomaterials.

| No. | Year | Materials                                                                 | Type            | Drug or cell | Test model | Outcome                                                                 | References                  |
|-----|------|----------------------------------------------------------------------------|-----------------|--------------|------------|-------------------------------------------------------------------------|------------------------------|
| 1   | 2012 | Gelatin                                                                   | Microsphere     | Ibuprofen    | Rat TMJ    | Nociceptive response reduction and sustained drug release              | Kramer et al.25             |
| 2   | 2009 | PLGA + superparamagnetic iron oxide nanoparticles                         | Microsphere     | Dexamethasone| Mice knee  | Sustained release and inflammation reduction                            | Butoescu et al.26           |
| 3   | 2009 | –                                                                          | –               | TGF-β        | Rabbit TMJ | Potential benefit in protecting articular cartilage                     | Man et al.27                |
| 4   | 2009 | Hyaluronic acid                                                           | Hydrogel        | –            | TMJ patient| Inflammation reduction and maintenance of improvements over a 6-month follow-up period | Manfredini et al.28         |
| 5   | 2009 | PLGA + superparamagnetic iron oxide nanoparticles                         | Microsphere     | Dexamethasone| Mice knee  | Pronounced retention of magnetic particles and internalization efficiency. | Butoescu et al.19           |
| 6   | 2010 | –                                                                          | Microsphere     | Tenoxicam    | Human patient TMJ | Reduced TMJ pain and no complications                              | Aktas et al.29              |
| 7   | 2010 | PLGA, PLA, and hyaluronic acid                                            | Nanoparticles   | FITC–dextran | Rat knee  | No inflammation                                                         | Zille et al.30              |
| 8   | 2010 | PLGA                                                                      | Microparticles  | –            | Rat TMJ    | Biocompatible and suitable for drug delivery                           | Mountziaris et al.31        |
| 9   | 2011 | Hyaluronic acid                                                           | Hydrogel        | –            | Rat TMJ    | Inhibiting the progression of osteoarthritic changes                   | El-Hakim and Elyamani32      |
| 10  | 2011 | PLGA                                                                      | Microspheres    | Lornoxicam   | Rabbit/rat knee | Good efficiency in synovial fluid and prolonged retention              | Zhang et al.33              |
| 11  | 2011 | PLGA                                                                      | Microspheres    | Methylprednisolone | Rat knee | Inflammatory response and prolonged retention                           | Panusa et al.34             |
| 12  | 2011 | PC; DOPE; cholesterol:stearylamine                                          | Nanoparticles   | Chondroitin sulfate | In vitro | Good interaction with collagen                                           | Zamescu et al.35           |
| 13  | 2011 | Chitosan and hyaluronic acid                                               | Nanoparticle    | –            | In vitro  | Safe and effective non-viral vector for gene delivery                   | Lu et al.36                 |
| 14  | 2012 | –                                                                          | –               | Monosodium iodoacetate | Rat TMJ | Successfully induced OA in TMJ by using MIA and evaluated structure of the TMJ | Wang et al.37               |
| 15  | 2012 | Genipin-cross-linked chitosan                                              | Microsphere     | Flurbiprofen | Rat knee  | Prolonged release and biocompatible                                     | Kawadkar et al.38           |
| 16  | 2012 | Tetraethylene glycol methacrylate and cyclohexyl methacrylate              | Nanoparticles   | Interleukin-IRa | Rat knee | No inflammatory response and prolonged retention                       | Whitmire et al.39           |
| 17  | 2012 | PLGA                                                                      | Microsphere     | siRNA-PEI    | Rat TMJ    | Sustain drug release and increased meal duration                       | Mountziaris et al.40        |
| 18  | 2012 | –                                                                          | –               | Corticosteroid and triamcinolone hexacetonide | Human patient TMJ | Delivered in the absence of imaging guidance                           | Stoll et al.41              |
| 19  | 2013 | –                                                                          | –               | Simvastatin  | Rat TMJ    | Inhibit inflammatory response                                           | George et al.42             |
| No. | Year | Materials | Type | Drug or cell | Test model | Outcome | References |
|-----|------|-----------|------|-------------|------------|---------|------------|
| 20  | 2013 | Sulforaphane and PLGA | Microsphere | – | Rat knee | The significant delay in the progression of OA with the use of SFN-PLGA | Ko et al.43 |
| 21  | 2013 | PLGA-cross-linked PEG | Microsphere | Brucine | Sheep knee | Decreased degradation rate and slight inflammation | Bédouet et al.44 |
| 22  | 2014 | PEG | Hydrogel/ microsphere | Ibuprofen | Sheep shoulder | Sustain release for several months | Bédouet et al.45 |
| 23  | 2014 | – | – | BSP/BDP | Rabbit TMJ | BSP/BDP 0.1–0.3 ml/0.7–1.5 mg range is optimal dose for TMJ OA | Kostina and Valamina46 |
| 24  | 2014 | – | – | Alendronate | Rat TMJ | Early treatment of alendronate blocked the up-regulation of matrix metalloproteinase (MMP)-13 expression in the chondrocytes | Chen et al.47 |
| 25  | 2014 | Poly(acrylamide) | Hydrogel | – | Human patient knee | Bio-stable, mechanical cleaning should be performed immediately | Tonbul et al.48 |
| 26  | 2015 | Hyaluronic acid | Hydrogel | – | Rat knee | Pain release and decreased mechanical hyperalgesia | Ikeuchi et al.49 |
| 27  | 2015 | – | Steroid | p-Dexamethasone | Rat TMJ | Inflammation reduction and reduced bone density | Knudsen et al.50 |
| 28  | 2015 | – | – | Triamcinolone acetonide | Knee patient | A clinically relevant improvement in pain relief in patients | Bodick et al.51 |
| 29  | 2016 | Chitosan/b-glycerophosphate/hyaluronic acid | Hydrogel | – | Rabbit TMJ | Controllable drug release | Talaat et al.52 |
| 30  | 2016 | Chitosan | Microsphere | Lornoxicam | Rat knee | Anti-inflammatory effect and biocompatible | Abd-Allah et al.23 |
| 31  | 2016 | PCL | Microsphere | Etoricoxib | Rat knee | A better drug-retention capacity | Arunkumar et al.53 |
| 32  | 2016 | – | – | Synovial mesenchymal stem cell | Mouse knee | Improved articular cartilage regeneration | Mak et al.54 |
| 33  | 2017 | – | – | Adipose mesenchymal stem cell | Human patient knee | Significant improvements in pain levels and function compared with baseline | Pers et al.55 |

PLGA: poly(lactic-co-glycolic acid); PLA: poly lactic acid; PC: phosphatidylcholine; DOPE: dioleylphosphatidylethanolamine; PEG: polyethylene glycol; PCL: polycaprolactone; TMJ: temporomandibular joint; TGF-β: transforming growth factor beta; FITC: fluorescein isothiocyanate; OA: osteoarthritis; MIA: monosodium iodoacetate; siRNA: small interfering RNA; PEI: poly(ethylenimine); SFN: sulforaphane; BSP: betamethasone sodium phosphate; BMP: betamethasone dipropionate.
biding. Furthermore, the non-viral gene was delivered via chitosan/HA nanoparticles (100–250 nm), successfully transferring an exogenous gene (~20% for 5 days) into primary chondrocytes for protecting degradation of the cartilage.36 Collectively, the nanoparticle-based delivery system seems to be suitable for delivering various types of biomolecules (i.e. drugs and genes), releasing them in a controlled manner and even targeting specific cell sources (chondrocytes or synovioocytes) to regenerate TMD.

Recently, mechanical stress or inflammatory reaction in articular tissue is reported to increase reactive oxygen species (ROS) level, leading to cartilage destruction in TMJ.65,66 Therefore, the control of ROS generation during inflammation is highlighted as one of the strategies to prevent cartilage and bone degradation in TMJ. Antioxidant biomolecules or antioxidant nanoparticles have recently been suggested as potential options for such purposes.67,68 However, the underlying mechanism is still not clearly understood and further researches regarding the roles of antioxidant and how to combine this with drug delivery strategies aforementioned remain. Moreover, there are some challenges for the clinical uses of nanoparticles developed so far, such as biocompatibility for Food and Drug Administration (FDA) approval and limited physiochemical properties associated with agglomeration, which need further improvement.69–71

**Microparticle-based drug delivery system**

Microparticles have three-dimensional (3D) spherical shapes with sizes of ten to hundreds of micrometers that are suitable to deliver large-sized drugs or biomolecules. In particular, microparticles can be tuned to deliver cells within macro/micro-porous inner structure or onto the surface, enabling co-delivery of cells and biomolecules (or drugs).72,73 Because of micron-scale, when microparticles contact with cells, they interact mainly extracellularly, unlike the intracellular interaction of nanoparticles. Therefore, intra-articular injected microparticles contact directly with the joint cavity and avoid from the macrophage engulf and removal.74,75 This allows microparticles to retain in target regions while releasing therapeutic molecules to target cells (i.e. synovial lining cells or chondrocyte). One exemplar study showed that intra-articular injection of dexamethasone-loaded poly (d, l-lactide) (PDLA) microspheres (size range, 40–110 µm) in healthy rabbits exhibited no drug detection in the serum over 24 h, suggesting most of the drug successfully localized in the synovial cavity.76 Small (1–20 µm) biodegradable microspheres presented faster degradation in rabbit joints than large microparticles (35–105 µm), suggesting the importance of microparticle size for TMD applications.77

Microparticles are a good candidate due to their high loading capacity of biomolecules and controllable release benefited from different morphologies (bulk, porous, and hollow, etc.) or functionalization. Typical composition of microparticles varies from synthetic (polycaprolactone (PCL), poly(l-lactide) (PLLA), PLGA, poly(propylene sulfide) (PPS), and (polyphosphazene based)56 to natural polymers (collagen, gelatin, alginate, HA, and chitosan).78 Common drugs loaded in microparticles are steroidal (i.e. methylprednisolone and dexamethasone) and NSAIDs (i.e. ibuprofen, flurbiprofen, lornoxicam, and brucine). Besides, anti-inflammatory small interfering RNA (siRNA) was also used with PLGA microspheres functionalized with poly(ethyleneimine) (PEI), and the system

**Figure 2.** Nano and microparticles have the potential to deliver various biomolecules and drugs and act intracellularly or extracellularly depending on the particle size.
demonstrated a controllable release of siRNA, consequently reducing inflammation in TMJ.40,43

Compared with solid microparticles, hydrogel microparticles are often preferred because they can provide extracellular matrix (ECM) mimicking native tissues.79 Alginate-based hydrogel microparticles were first introduced due to their good biocompatibility and easy process to fabricate.90,83 Other biopolymers such as PLGA, HA, and PLLA were also developed into hydrogel microparticles. Bédouet et al.44 designed nondegradable or degradable polyethylene glycol (PEG) hydrogel depending on the type of cross-linker to evaluate degradability and the consequent inflammatory response when delivered to the shoulder joint. After 4 weeks’ implantation in sheep shoulder joint, histological analysis presented degraded PEG hydrogel microparticles cross-linked by (PLGA–tri ethylene glycol (TEG)–PLGA) dimethacrylate indicated less inflammatory cells around compared with nondegradable hydrogel control. The covalent bond between drug and polymer can sustain the releases. Degradable PLGA–PEG (40–100μm) hydrogel microparticles were functionalized with a methacrylic derivative of ibuprofen drug using a degradable cross-linker oligo(ethylene-glycol) methacrylate and poly(PLGA–PEG) dimethacrylate. Ibuprofen loaded in PEG hydrogel microparticles by a covalent bond showed reduced burst release and more sustained release up to months, resulting in the enhanced preservation of the anti-inflammatory response and cyclooxygenase-inhibition.45,82

Furthermore, microparticles have the potential to deliver or tune stem cells due to their large surface area and tailored 3D architecture where cells adhere and differentiate into specific cell lineage. The microparticles also release biomolecules while the cell-produced ECM can mimic host environment for less anti-inflammatory and enhanced healing process.73,83 Therefore, microparticles as cell carriers have been used also in TMD area, which described in the following section.

However, some challenges still exist for the clinical applications of microparticles. For instance, large diameter gauge for injection can induce pain during procedures, and limited amounts of microparticles for small articular space can restrict therapeutic functionality.11,84 Also, the in vivo biocompatibility of microparticles associated with their derivatives/byproducts and the possible fragmentation makes it difficult to get the FDA approval.19,53,85

**Intra-articular stem cell delivery**

Stem cells self-renew and differentiate to target cells, and produce ECMs that are favorable for regenerative process.86 Therefore, stem cell–based therapies have the potential to bring substantial benefit to patients suffering a wide range of diseases and injuries.87 Many studies have tried for different tissues including heart, bone, tendon, and neuron, and some stem cell therapies were FDA-approved for clinical uses.88 Given the potential of this strategy, a lot of effort has also been dedicated to TMD through intra-articular delivery with or without biomaterials.

The majority of TMJ structures derive from mesenchymal cells during morphogenesis. Mesenchymal stem cells (MSCs) maintain physiologically for tissue remodeling/turnover and, upon injury or disease, differentiate into target tissue including cartilage, bone, ligaments, and musculature to start tissue repair/regeneration. MSCs isolated from synovial fluid in TMJ have markers typically including CD44, CD73, CD90, and CD 105 but not the hematopoietic stem cells markers such as CD34 and CD45.89 Among the stem cell sources, MSCs from TMJ, knee joints, bone marrow, adipose tissue, and dental pulp has been widely used for intra-articular injections.54,55 More recently, MSCs differentiated from induced pluripotent stem cells (iPSCs) were suggested as a candidate cell source due to the noninvasive cell collection (i.e. from skin) and unlimited self-renewal capacity of iPSCs.90 Of course, synovium-derived MSCs displayed superiority in proliferation and differentiation to chondrocytes compared with MSCs from other tissues, supporting the beneficial use of these cells for TMJ repair and regeneration.91,92 Cell density also matters in the tissue regeneration of TMJ. An unsatisfactory tissue maturation was made using a low number of pre-differentiated MSCs (5 × 10⁶ cells/mL) in immune-deficient mice, whereas tissue maturation and osteochondral integration were made using high number of pre-differentiated MSCs (20 × 10⁶ cells/mL).93,94 At present, clinical study to optimize MSCs number for TMJ regeneration is under investigation, while intra-articular injection for treatment of osteoarthritis of the knee was recently made clinically. The low-dose (1.0 × 10⁷ cells), mid-dose (5.0 × 10⁶), and high-dose (1.0 × 10⁷) groups, the high-dose of adipose tissue derived MSCs into the osteoarthritic knee, improved function with regeneration of hyaline-like articular cartilage, and reduced pain of the knee joint without causing adverse events.95

However, acute donor-cell death within a short period after cell delivery remains a critical hurdle for clinical translation. Biomaterials including micro/nanoparticles offer a potential vehicle for an effective delivery and maintaining cells close to implanted tissue while driving cellular fate into a target lineage (Figure 3).96-98 Recently, the in vivo differentiation or survival of stem cell grafts has been proposed by the controlled release of biomolecules conjugated to injected micro/nanoparticles; growth factors (i.e. betamethasone dipropionate (BMP)-2, basic fibroblast growth factor (bFGF), or transforming growth factor beta (TGF-β)) and drugs (i.e. dexamethasone, corticosteroid, or other single chemicals).99 In another approach, pre-differentiated MSCs into certain cell lineages (chondrocyte or osteoblast) by in vitro culture with micro/nanoparticles were utilized for the advanced MSCs delivery strategy due to the enhanced biological interaction of pre-differentiated MSCs to a target tissue. As to the type of other carriers
such as 3D scaffolds and their use as stem cell delivery for TMD, readers are recommended to a reference.7

For intra-articular delivery of stem cells, typical hydrogel-based microparticles were initially utilized, which allow injection after gelation. However, the cell-loading capacity to conventional hydrogels is limited because incorporation of stem cells increases viscosity and thereby leads to decrease of injectability of mixture and stem cells delivered. To enhance loading capacity of stem cells, in situ forming hydrogels have been developed and evaluated to encapsulate stem cells more.

In situ forming hydrogels were revealed to encapsulate stem cells more and even homogeneously due to their solution state when injected, which become gel in body temperature (~37°C) or normal pH conditions (~7).100 Poly(N-isopropylacrylamide) (PNIPAM), polyelectrolytes, chitosan, polyphosphazenes, polycarbonates, polycyanooacrylates, polyoxyethyl ethers, poly(ethylene glycol)-b-poly(l-alanine), polypeptides, and their composite hydrogels have been developed.101,102 When the thermo-sensitive in situ forming gels can deliver biomolecules for chondrocyte targeting, the effects are synergized. For example, chitosan-based thermo-sensitive hydrogel incorporated with HA and beta-glycerophosphate was used for intra-articular injectable vehicles to the TMJ in a rabbit model. The hydrogel could release therapeutic biomolecules (HA and beta-glycerophosphate) with improved stability.52 Besides, on demand gelation from external stimuli, including magnetism or heating over body temperature (~40°C), is another promising tool to improve stem cells delivery/differentiation potential from hydrogel-based microparticles.103 These findings highlight that the intra-articular injected degradable hydrogel microparticles stay in synovial cavity up to a few months while delivering biomolecules to the inflamed joint and providing ECM structure for TMJ regeneration, not scavenged by macrophages.

In particular, the hydrogels can be designed to have chemical (ECM composition; for example, collagen type I and glycosaminoglycans (GAGs)) or physical properties (e.g. stiffness and stress relaxation) that can mimic the TMJ tissues.58,104 Among them, the static stiffness value has been a key factor in hydrogels until the emergence of stress relaxation, a time-dependent stress change.105 This stress relaxation mimics the viscoelastic responses of ECM in the target tissue, determining cellular mechanotransduction and consequent ECM-integrin networks remodeling. Recently, the stress relaxation effects were revealed on chondrocytes behaviors; the chondrocytes in rapidly relaxing hydrogels could produce cartilage ECM significantly, whereas those in slowly relaxing hydrogels suffered limited volume expansion due to persistent elastic stresses from the surrounding matrix, leading to a degeneration of cartilage.106

Taken some of the recent key findings related to tunable physical/chemical properties, in situ forming hydrogels with TMJ mimic stiffness and stress relaxation may be promising for future stem cell delivery and regeneration of TMD.
With the advance in nanotechnology, clinicians experience the significant therapeutic potential of stem cells through nanoparticle delivery, and at the same time faces the need to reduce harmful side effects related to nanoparticles.\cite{84,107} Nanoparticles are biomedical materials developed to host and deliver therapeutic and diagnostic molecules, ranging from drugs, peptides, proteins, genetic molecules, ions, or their combination, intracellularly or extracellularly.\cite{62,107} Especially, when nanoparticles are co-delivered with stem cells through an intra-articular injection, they internalize by endocytosis and the loaded therapeutic molecules intracellularly functioning are efficiently delivered to specific cellular components such as mitochondria and nucleus, possibly accelerating their carrier performance and the biological fate for TMJ regeneration.\cite{108} Nanoparticles can also be incorporated into hydrogel-based microparticles that support and deliver stem cells. Such a combinatory approach remains as a promising future study for the therapeutic treatment of TMD through biomaterial-based intra-articular delivery.

**Concluding remarks**

TMD is considered one of the complex joint diseases caused by inflammation and irreversible degeneration, degrading the quality of life associated with malfunctions in chewing and opening mouth. Intra-articular injection, albeit highlighted as a minimally invasive clinical treatment, still suffers low regenerative potential of single injection or complications from the repeated injection. With the development of injectable biomaterials (nano/microparticles and hydrogels), the intra-articular injection holds great promise for therapeutic roles in TMD with the effective and controlled delivery of drugs and stem cells. More in vitro and animal studies to optimize the compositions and formulations of injections will gain clinical acceptance in the near future.

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