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Conditioning and Scaffolding of Chondrocytes: Smart Steps Towards Osteoarthritis Gene Therapy

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1. Introduction

Osteoarthritis (OA), the most common form of arthritis, is a chronic degenerative joint disease that remains a leading cause of chronic disability in the aged population and a financial burden on healthcare resources. It affects approximately 15% of the world's human population over 60 years of age (Wolf & Pfleger, 2003) and has even higher prevalence in dogs (Johnston, 1997), cats (Godfrey, 2005) and horses (Trumble et al., 2001). It is established that OA in dogs and horses has a model character for the human disease since it parallels the human form of OA in all aspects (Innes & Clegg, 2010).

The pathogenesis of OA is characterized by an imbalance in the network of anabolic and catabolic processes through complex interactions of mechanical and biochemical forces (M.B. Goldring, 2001; Sandell & Aigner, 2001). This imbalance leads, inevitably, to progressive articular cartilage destruction, osteophyte formation, subchondral bone remodelling and chronic inflammation (M.B. Goldring & S.R. Goldring, 2007; Martel-Pelletier & Pelletier, 2007). These events are further manifested by a loss of both tissue architecture and joint functionality, painful limited movement, disability and an inferior quality of life (Abramson & Yazici, 2006; Buckwalter et al., 2006; Pelletier et al., 2001). Despite major progress over the last few years, we still have a lot more to understand about the aetiology, pathogenesis and progression of OA.

A complete therapy for OA still remains elusive as manifold efforts made in this direction failed to provide a successful long-term remedy. The therapeutic strategies for OA have been predominantly directed to (i) alleviate symptomatic pain by suppressing the inflammatory process, (ii) reducing the cartilage degenerative process or (iii) enhancing cartilage regeneration. However, none has so far been applied to achieve all the three objectives.

Non-pharmacologic and pharmacologic treatments have been employed for early OA while surgical interventions for partial or total joint replacement are often indicated in advanced

OA when the symptoms cannot be controlled by non-invasive means. The long-term use of pharmacologics is commonly concomitant with side-effects while surgery contributes little to reduce the process of joint destruction and inflammation (Katz et al., 2010; Wei et al., 1998).

Articular cartilage destruction is a key pathological characteristic of OA and rheumatoid arthritis (RA). Therefore, attempts have been made to repair the OA-related cartilage defects. In this context, autologous chondrocyte transplantation (ACT) offers a practical solution (Brittberg et al., 1994). Evidence suggests that damaged cartilage plays a pivotal role in disease progression and severity (Buckwalter et al., 2006). ACT attempts to build hyaline repair tissue, delays total or partial joint replacement, helps to temporarily relieve pain and improves the joint function (Minas et al., 2010; Peterson et al., 2002). A further developed alternative technique is the matrix associated ACT, where the cultivated cells are seeded on a tissue-engineered (biomaterial) scaffold and the cell–scaffold complex is then implanted into the defect. The ACT/scaffold coupling paved the way for the use of functional tissue substitutes in the treatment of cartilage defects (Tuli et al., 2003). However, inflammatory mediators in the arthritic joint could negatively affect the implanted cells, potentiating the need to suppress inflammation (Hennerbichler et al., 2008).

Gene therapy represents another promising approach for OA treatment (Bandara et al., 1992; Evans et al., 2004; Evans et al., 2009). This concept utilizes viral or non-viral vectors to deliver genetic information encoding biological agents into the target tissue for their local expression. It appears that viral strategies provide high transfection efficiency and many additional assets for a clinical development (Mease et al., 2010) but immunogenicity and possible mutagenicity are their main drawbacks. Non-viral strategies, on the other hand, have a fascinating preclinical development in arthritis. They are safer but less efficient. Of major concern is the regulated expression of the therapeutic genes. Ideally, gene therapy outperforms the systemic intake of medicines by only affecting the transfected joint. Normally, drugs can be applied in a controlled dosage, while transgene expression is switched by intrinsic, normally immutable, regulatory elements. In other words, the expression of the therapeutic candidate gene should be limited to the site of OA occurrence and only applied in the presence of inflammation or other significant markers of OA arising in the affected joint. Moreover, the gene expression should ideally be self-limiting (=conditioned) and not constitutive (Geurts et al., 2007; Rachakonda et al., 2008a).

The information presented above provides evidence for a limited success of several efforts towards achieving effective therapeutic strategies for OA. This is mainly because they are associated with one or the other limitation. Nonetheless, it is tempting to speculate that gene therapy (coupled with tissue-engineering) has great potential for improvement. It is therefore very likely that it becomes a leading force for future OA treatment if suitably improved. This chapter will provide extended information on this topic and discuss as well as suggest some novel ways to improve the current gene therapy procedures for their safe application.

2. Non-pharmacological and pharmacological therapies

Pain is the presenting symptom in OA. Thus, the therapeutic efforts for OA specifically aim at the pain relief through the combination of non-pharmacological and pharmacological interventions. Non-pharmacological approaches include exercise therapy, weight loss, social support, self-management and awareness of patients to OA (Clouet et al., 2009). These

approaches help partially prevent the disease, reduce pain and may delay the degenerative process in early-stage OA but do not offer a long-term remedy. Pharmacological therapy improves the quality of life of OA patients also through alleviating pain. Acetaminophen treatment followed by non-steroidal anti-inflammatory drugs (NSAIDs) inhibits the activation of immune cells while NSAIDs suppress the inflammatory process by intervening with the prostaglandin synthesis. Long-term usage of NSAIDs is often accompanied by adverse side-effects such as gastrointestinal, renal or cardiovascular disorders, as most of these compounds inhibit cyclooxygenases (COX)-1 and -2 indiscriminatorily (Hotz-Behofsits et al., 2003; Scarpignato & Hunt, 2010; Wolfe et al., 1999).

Selective COX-2 inhibitors reduce pain through prostaglandin blockade, but owing to their harmful effects on cardiovascular events they were recalled from the market (Park et al., 2006). Later on, the application of some disease-modifying OA drugs (DMOADs) was being enhanced by advances in imaging and biomarkers that serve as validated surrogate endpoints for key clinical outcomes (Abramson & Yazici, 2006). However, the clinical development program for DMOADs is complicated due to the slowly progressive nature of OA.

Intra-articular injections of corticosteroids (Habib et al., 2010) or hyaluronic acid (Liao et al., 2005) exert pain reducing effects that last for a couple of weeks but do not significantly improve the physical function of the joint. Nutritional supplements such as chondrotin sulphate and glucosamine are asymptomatic slow-acting safe drugs for the management of OA, but their efficacy in OA is questionable (Dougados, 2006) just like avocado, soybean, unsaponifiables and Vitamin E (Clouet et al., 2009).

3. Surgical treatment

Surgical intervention offers another option for OA therapy after medications have failed to restore joint function. Arthroscopic lavage and debridement of the arthritic joint is only indicated in cases of superimposed structural lesions in the affected joint such as meniscal tear (Katz et al., 2010). Osteotomy is performed by trimming the joint resulting in a spread of the mechanical load which relieves the defect area (Parker et al., 2011). Partial or total joint replacements are the last resorts in orthopaedic surgery for OA. But these procedures are more prone to fail in young and middle-aged patients (Wei et al., 1998). In addition, the evidence supporting the use of various surgical approaches is limited mainly by the poor study design and relatively small sample size (Katz et al., 2010).

4. Concept of gene therapy in OA

The term gene therapy is commonly understood to mean the use of molecular methods to replace defective or absent genes or to counteract those that are over-expressed. The key technologies needed for gene therapy are the methods by which genes are isolated (cloned), manipulated (engineered), and transferred (delivered) into host cells. Since its inception, the field of gene therapy in medicine has received much attention, but still remains a great challenge for routine clinical applications to treat diseases including OA and RA (Gibbons & Hyrich, 2009).

4.1 *In vivo* gene delivery in the arthritic joint
OA is well-suited for local, intra-articular gene delivery because it affects a limited number of joints and lacks obvious extra-articular manifestations (Bandara et al., 1992). Thus, the

delivery of locally expressed gene products may have therapeutic benefits for localized areas of cartilage damage with minimal systemic side-effects. In this approach genes encoding biological agents are introduced directly into the intra-articular tissues through viral and non-viral vectors. Despite its simplicity, the *in vivo* route has a number of disadvantages that limit its application. For example, targeting the appropriate cells may be difficult (Evans et al., 2004). In spite of that there are many published reports in which *in vivo* gene delivery has been the main subject (see Table 1).

Vector	Transgene	Animal model	Reference
Adenovirus	IL-1Ra	Dog ACLT	Pelletier et al., 1997
Plasmid	IL-1Ra	Rabbit meniscectomy	Fernandes et al., 1999
Adenovirus	IL-1Ra	Horse osteochondral defects	Frisbie et al., 2002
rAAV	bFGF	Rabbit osteochondral defects	Cucchiarini & Madry, 2005
Plasmid	BMP-2	Rabbit cartilage defects	Di Cesare et al., 2006
Plasmid	HSP-70	Rat MIA injection	Grossin et al., 2006
Adenovirus	TSP-1	Rat ACLT	Hsieh et al., 2010

Table 1. *In vivo* gene delivery in experimentally induced OA in animal models. Abbreviations: IL-1Ra=interleukin-1 receptor antagonist; ACLT=anterior cruciate ligament transection; rAAV=recombinant adeno-associated virus; bFGF=basic fibroblast growth factor; BMP-2= bone morphogenic protein-2; HSP-70=heat shock protein-70; MIA=mono-iodoacetate; TSP-1=thrombospondin-1.

4.2 *Ex vivo* gene delivery in arthritic joint

An efficient targeting of specific types of cells *in vivo* by using vectors is presently not possible. Therefore, most applications requiring selective cell transduction involve the removal of cells from the body and their genetic manipulation *in vitro* before re-implantation. This is known as *ex vivo* gene delivery. The logic behind such an approach is mainly to augment the expression of a therapeutic gene of interest or an inhibition of the expression of disease-associated genes (Jorgensen & Apparailly, 2010). Through *ex vivo* gene transfer the amount of genetic material introduced in a target cell can be controlled and expression levels monitored before application. A selection of transfection methods used in routine lab and clinical studies is listed in Table 2 and a comparison of *in vivo* and *ex vivo* gene transfer methods is presented in Figure 1.

4.3 Selective anti-catabolic gene products

The pro-inflammatory cytokines interleukin-1 beta (IL-1β) and tumour necrosis factor alpha (TNFα) secreted by chondrocytes, synovial cells and invading immune cells, are important mediators of matrix degeneration and cell apoptosis in OA (M.B. Goldring, 2001). Therefore, inhibitors of these cytokines may counteract inflammatory destruction of cartilage. Besides cytokines, matrix metalloproteinases (MMPs) and aggrecanases such as ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin-like motif-4) and -5 (ADAMTS-5) secreted by the chondrocytes degrade a surplus of matrix in OA (Heinegård & Saxne, 2011). In order to diminish the lysis of cartilage and thus to reduce OA progression, tissue inhibitors of metalloproteinases (TIMPs) can be over-expressed through gene therapy (Celiker et al., 2002; van der Laan et al., 2003).

Target cell	Transgene	Vector	Animal model	Reference
Chondrocytes	LacZ-neo	Retrovirus	Rabbit	Kang et al., 1997
	BMP-7	Adenovirus	Horse	Hidaka et al., 2003
	IGF-1	Plasmid	Rabbit	Madry et al., 2005
	bFGF	rAAV	Rabbit	Yokoo et al., 2005
	bFGF	Plasmid	Rabbit	Kaul et al., 2006
Synovial cells	LacZ-neo	Retrovirus	Rabbit	Bandara et al., 1992
	IL-1Ra IL-10	Retrovirus	Rabbit	Zhang et al., 2004
Perichondrium-derived stem cells	BMP-1 IGF-1	Adenovirus	Rat	Gelse et al., 2003
Bone-marrow-derived stem cells	CDMP1	Plasmid	Rabbit	Katayama et al., 2004
Fibroblasts	TGF-β	Retrovirus	Rabbit	Lee et al., 2001
Muscle-derived cells	LacZ	Retrovirus	Rabbit	Adachi et al., 2002

Table 2. *Ex vivo* gene delivery to various cell types in animal models for cartilage damage. Abbreviations: LacZ-neo=β-galaktosidase-neomycin phosphotransferase; BMP-7=bone morphogenic protein-7; IGF-1=insulin-like growth factor-1; bFGF=basic fibroblast growth factor; rAAV=recombinant adeno-associated virus; IL-1Ra=interleukin-1 receptor antagonist; IL-10=interleukin-10; CDMP1=cartilage-derived morphogenic protein 1; TGF-β=transforming growth factor β.
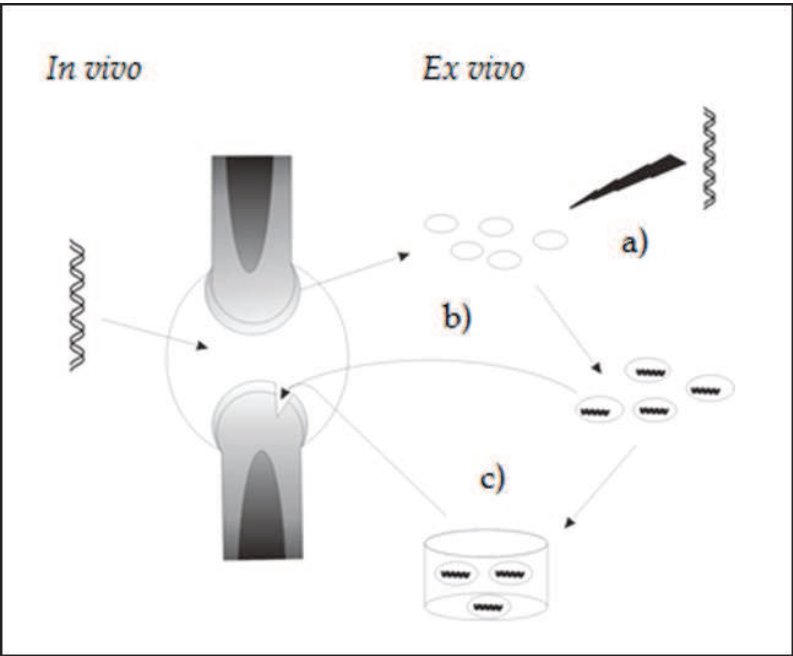

Fig. 1. *In vivo* and *ex vivo* gene transfers to the arthritic joint. *In vivo* gene delivery involves a direct injection of the transgenic vector, in the form of a naked DNA or coupled with a virus vehicle into the joint. In an *ex vivo* approach, gene transfer includes an intermediate step of transfection of the explanted, allogeneic cells (chondrocytes/synoviocytes) with the transgenic vector (a). The transfected cells can be injected directly into the joint (synoviocytes) or to the cartilage defect (chondrocytes) (b) or be embedded into a scaffold followed by the reimplantation into the cartilage lesion (c).

4.4 Selective anabolic gene products

While inhibition of catabolic pathways will diminish OA damage, it will not enhance repair of already degraded matrix. Therefore, gene products with anabolic functions are of special interest. These gene products comprise anabolic enzymes and growth factors that promote matrix synthesis and morphogenic mediators for chondrogenic differentiation. Matrix synthesis can be enhanced by expressing enzymes required for the synthesis of precursors of matrix constituents (J.N. Gouze et al., 2004). An overview of genes employed for OA gene therapy is provided in Table 3.

Candidate transgene	Effect	Reference
Anti-catabolic		
IL-1Ra	Blocks IL-1R on articular cells	Evans & Robbins, 1994
sIL-1R	Neutralizes secreted IL-1	Ghivizzani et al., 1998
sTNFR	Neutralizes secreted TNFα	Ghivizzani et al., 1998
IL-4	Down-regulates IL-1 and TNFα expression	Geurts et al., 2007; Rachakonda et al., 2008a; Woods et al., 2001
IL-10	Down-regulates IL-1 and TNFα expression	Amos et al., 2006; Müller et al., 2008; Zhang et al., 2004
IL-13	Anti-inflammatory action	Nabbe et al., 2005
TIMPs	Matrix proteinase inhibitor	Celiker et al., 2002; van der Laan et al., 2003
Anabolic		
IGF-1	Chondrocyte growth factor	Madry et al., 2002
TGF-β	Chondrocyte growth factors	Lafeber et al., 1997; Watson et al., 2010
BMPs	Chondrocyte growth factors	Hidaka et al., 2003
bFGF	Chondrocyte growth factor	Madry et al., 2004
SOX-9	Transcription factor	Cucchiarini et al., 2007
GFAT	Enhances matrix synthesis	J.N. Gouze et al., 2004
GlcAT-I	Enhances matrix synthesis	Venkatesan et al., 2004

Table 3. Examples of candidate transgenes for OA gene therapy. Abbreviations: IL-1Ra=Interleukin-1 receptor antagonist; sIL-1R=soluble IL-1 receptor; sTNFR=soluble tumour necrosis factor-α receptor; IL-4, -10, -13=interleukin-4,-10,-13; TIMPs=tissue inhibitors of metalloproteinases; IGF-1=insulin like growth factor 1; TGF-β=transforming growth factor β; BMPs=bone morphogenic proteins; bFGF = basic fibroblast growth factor; SOX-9=sex-determining region Y box 9; GFAT=glutamine fructose-6 phosphate aminotransferase; GlcAT-I= β1,3-glucuronosyltransferase-I.

4.5 Viral gene transfer

Viruses provide a natural system for horizontal gene transfer into a variety of cells. Several virus types (given below) infect mammalian cells and are adopted as vehicles for gene transfer. Retroviral transfection systems derived from Moloney murine leukaemia oncoretrovirus and others require dividing cells for infection and integration and have therefore been used mainly in *ex vivo* transfection (Evans et al., 2005) and occasionally *in vivo* (Ghivizzani et al., 1997). Retroviruses insert parts of their genome non-specifically into the

host cell DNA, thus enabling stable transfection of the cells but carry the risk of insertional mutagenesis.

Lentiviruses, derived from the human immunodeficiency virus, are able to infect resting-state cells with high efficiency, show low immunogenicity and can integrate transgenes into the cell DNA. To avoid possible insertion mutagenesis, non-integrating lentiviruses have been developed which maintain the high transduction efficiency (Philpott & Thrasher, 2007) and can be used for *in vivo* and *ex vivo* transduction of articular cells (E. Gouze et al., 2007).

Adenoviruses readily transduce dividing and non-dividing cells. The integration frequency of adenoviral genome into host DNA is very low (Harui et al., 1999), and engineered replication deficient adenoviruses enhance biosafety. Many adenoviruses, however, elicit immune responses from the patient, which excludes them from *in vivo* approaches. To circumvent their immunogenicity, either adenovirus strains which normally infect different host species are used, or immunogenic viral proteins are deleted from the constructs (Evans et al., 2001). Adeno-associated viruses have low immunogenicity, are small in size and can carry only small gene constructs. They possibly penetrate the cartilage matrix and could therefore transduce chondrocytes *in vivo* (Madry et al., 2003).

In general, viral gene transfer is highly efficient. Synovial cells can be transduced *in vivo*, and chondrocytes *ex vivo* with high transduction rates. Drawbacks of viral systems are biosafety issues, the need of extra cell lines for virus generation and propagation, and special plasmids for viral delivery. Therefore, several non-viral methods are used parallel to viral approaches (Capito & Spector, 2007; Thomas et al., 2003).

4.6 Non-viral gene transfer

Non-viral gene transfer delivers plasmid DNA into the target cells with selected chemical and physical methods. In general, non-viral methods are less elaborate than the generation of transduction viruses. All non-viral methods achieve transient transfection (Welter et al., 2004).

The most common method uses non-ionic or cationic lipids to form DNA-lipid particles. These particles are taken up by the cell and transcribed after transfer into the nucleus. Several compounds are available, which transfect cells with variable efficiencies (Madry et al., 2000). Many protocols for *ex vivo* gene transfer with lipofection are established despite of the drawback of relatively low transfection efficiency. The cytotoxicity of most transfection agents causes limited damage to target cells and thus this method is not suitable for *in vivo* use. Intra-articular injection of naked DNA plasmids without addition of liposomal agents is also feasible for synoviocyte transfection (Sant et al., 1998). On the other hand, electroporation achieves high transfection rate and does not need cytotoxic chemicals (Welter et al., 2004). By applying intra-articular DNA injection and external electric pulses to knee joints, *in vivo* transfection of articular chondrocytes can be achieved (Grossin et al., 2003). Binding plasmid DNA to magnetic microparticles allows the complexes to be forced into cells by strong directional magnetic fields and does not require cytotoxic transfection reagents. This approach has been used on chondrocytes in monolayer (Plank et al., 2003), but its feasibility *in vivo* has not been tested yet. A selection of viral and non-viral gene transfer procedures are compiled in Table 4.

Gene transfer method	Efficiency	Reference
Viral		
Retrovirus	Low transduction efficiency *in vivo*, integrating	Evans et al., 2005
Lentivirus	High transduction efficiency of dividing and non-dividing cells, integrating	E. Gouze et al., 2002
Adenovirus	Immunogenic, high transduction efficiency	Ghivizzani et al., 1998
AAV	Moderate transduction efficiency *in vivo*, only small ORFs as transgenes	Jorgensen & Apparailly, 2010
Non-viral		
Lipofection	Cytotoxic, varying transfection efficiency, low cost	Madry et al., 2005
Electroporation	Efficient, high equipment costs	Welter et al., 2004
Matrix bound DNA	Long lasting, low transfection efficiency	Capito & Spector, 2007

Table 4. Comparison of gene transfer methods for articular gene therapy. Abbreviations: AAV=adeno-associated viruses; ORFs=open reading frames.

5. Tissue-engineered scaffolds for ACT

In the context of OA, repair of cartilage surface defects through biological regeneration and transplantation of various tissues or cells have been investigated (Goehring et al., 2010; Stoop, 2008). The technology of scaffold-free ACT pioneered by Brittberg and colleagues has been widely applied to repair small cartilage lesions. Herein, a biopsy is taken from non-weight-bearing regions of cartilage; the chondrocytes are enzymatically extracted, expanded in monolayer culture, and then injected beneath a periosteal flap sutured over the cartilage lesion (Brittberg et al., 1994). This strategy has been used to treat thousands of patients worldwide and offers a transient but practical solution for cartilage repair.

Porous 3-dimensional scaffolds are increasingly used to facilitate cellular attachment and at the same time provide superior mechanical properties. This technique is particularly useful given the lack of cell retention when cell suspensions are directly transplanted at the cartilage defect site. It also reduces potential donor site morbidity associated with procedures that utilize a periosteal flap to increase cellular retention (Brittberg et al., 1994). A wide range of materials have been produced to serve as scaffolds for cartilage repair. Generally they can be classified into three categories: natural, synthetic and biosynthetic materials (Goehring et al., 2010; Langer & Tirrell, 2004; Stoop, 2008). In the following paragraphs, we will highlight some of the important scaffolds from these categories.

5.1 Natural scaffold materials

Natural materials are composed of native biocompounds which mimick the natural surface for cell adhesion and maintain the required physiological environment. In addition, these materials (i) are non-toxic, (ii) follow a physiological biodegrading mechanism and (iii) are used to produce scaffolds for cartilage tissue-engineering (Hunziker, 2002; Stoop, 2008).

Collagen-based biomaterials are widely used in clinical practice and have been employed as carriers for chondrocytes (Wakitani et al., 1998) and mesenchymal stem cells (MSCs) (Im et al., 2001). Collagen type I hydrogels resemble hyaline cartilage. They are biodegradable, elicit no inflammation and can be metabolized by MSCs through the action of endogenous collagenases. Rat-tail extracted, collagen type I has been recently developed to construct scaffolds termed cartilage regeneration system (CaReS; Amedrix, Esslingen, Germany). It has been reported that patellofemoral transplantation of CaReS scaffolds for two years showed a significant improvement in cartilage repair in patients (Andereya et al., 2007) resulting in complete defect filling with superior quality repair tissue (Welsch et al., 2010).

Both fibrinogen and its polymer fibrin have been shown to play major roles in healing osteochondral defects (Shapiro et al., 1993), but owing to the exceedingly high concentrations and protein densities, the glue impeded rather than facilitated cell invasion, thus limiting its use (Brittberg et al., 1994). Hyaluronic acid is a non-sulphated glycosaminoglycan (GAG) that makes up a large part of cartilage extracellular matrix. In its unmodified form, it has a high biocompatibility and plays an important role in determining the biophysical microenvironment for chondrocyte growth and proliferation (Poole et al., 1990).

Cell-seeded agarose hydrogels enhance the matrix elaboration upon dynamic deformational loading (Häuselmann et al., 1994). Despite its suitability for implantation, the non-degradable nature of the gel limits its application in tissue-engineering. Alginate, a naturally derived polysaccharide gel, has been successfully shown to support cell retention and the chondrocytic phenotype by maintaining cell shape through encapsulation (Guo et al., 1989; Häuselmann et al., 1994; Rai et al., 2009). On the other hand, its inferior biomechanical properties as well as concerns over its immunogenicity have raised biocompatibility issues (Kulseng et al., 1999). Alginates are easy to use and suitable for the repair of small cartilage defects but fragile during surgery.

5.2 Synthetic scaffold materials

Many synthetic scaffolds commonly used in cartilage tissue-engineering are fabricated using poly(α-hydroxy acid) polymers such as poly-L-lactide, polyglycolic acid and their copolymers poly-DL-lactide-co-glycolide and poly-ϵ-caprolactone (Li et al., 2006; Nöth et al., 2002). They offer optimal fibre diameter, pore size, degradation time and reproducibility in production. The major advantages associated with the use of synthetic polymers are their design flexibility and elimination of disease transmission. Disadvantages of some synthetic polymers are the potential increase in local pH resulting from acidic degradation products, excessive inflammatory responses and poor clearance and chronic inflammation associated with these high molecular weight polymers (Stoop, 2008).

5.3 Biosynthetic scaffold materials

In recent years, a wide array of novel biosynthetic materials has been developed based on natural materials such as silk or cellulose and synthetic materials such as poly(1,8-octanediol citrate) or poly(ether ester) copolymer scaffolds. In addition, numerous attempts have been sought to optimize scaffold properties by combining several different materials. For example, scaffolds have been produced from gelatine, hyaluronic acid and chondroitin-6-sulphate and mixed with fibrin glue or polylactic-co-glycolic acid. To support bone as well as cartilage formation, hydroxyapatite was combined with chitosan to produce scaffolds

that allow the treatment of osteochondral defects. Conversely, most of these developments show promise for future clinical application, so far they have mainly been investigated using *in vitro* systems or at the most, in animal models. Translation of these developments to the clinical and commercial setting will take longer (Stoop, 2008).

6. Obstacles and challenges in clinical application of gene therapy

Gene therapy for OA has been described extensively for the last twenty years and a large body of impressive preclinical safety and efficacy information has been documented (Bandara et al., 1992; Trippel et al., 2004). Despite this fact, only a few clinical trials of this therapeutic strategy have been conducted mainly due to scientific, technological, financial and sociological hurdles (Evans et al., 2011). A single phase II study has been reported recently and this slow pace indicates the unlikelihood of gene therapy to become clinically available in the near future (Mease et al., 2010). One of the most serious reason for the current slow progress in gene therapy research in OA (and RA) lies in the widespread public scepticism with anything relating to genes. The term gene is simply not understood by the average citizen and any genetic intervention in research has a priori a negative connotation. Fitting with this general attitude, regulatory bodies demand extremely hard safety precautions with any genetic intervention with patients which leads to an explosion of cost and duration for the development of these very promising therapies.

Nonetheless, this should not disappoint gene therapy researchers. Despite its slow progress, gene therapy promises to fulfil unmet needs in the treatment of OA and allied joint disorders and therefore has a bright future in the long run. It will eventually offer a focussed, local and perhaps personalized therapy avenue which may well cover the non-responders to the conventional treatment of joint disorders which in the case of RA amount to a substantial percentage (Gibbons & Hyrich, 2009). Furthermore, by applying local gene therapy it will certainly be possible to avoid the widely observed side-effects that occur with the present therapeutics.

Another leading challenge facing gene therapy today is the improvement of current vectors and this area continues to be another limiting factor in gene therapy applications. Even in the field of viral vectors, attempts have been made to combine their application with immunomodulating agents (Ikeda et al., 2000), still obvious problems exist with this approach (Zhou et al., 2004). Similarly, in the area of non-viral gene transfer, researchers are generating new transfection methods/strategies with a number of new products becoming commercially available every year (Donkuru et al., 2010; Haag et al., 2009; Madeira et al., 2011). Yet, an ideal agent that satisfies the requirements for application with the relevant target cells for OA gene therapy is not in sight.

Although the tissue-engineering scaffolds described above are becoming increasingly popular due to the high standards of cellular attachment and mechanical stability, their role in a pro-inflammatory environment in arthritic condition raises some doubts and has not been completely evaluated. An effective therapy of OA in the context of inflammation, however, may be achieved through genetic modification of suitable target cells present in the scaffolds to deliver therapeutic transgenes to the site of disease. Below we elaborate on the choice of cells that can be utilized in this approach and on the question which are the best vehicles for gene delivery mechanism as well as the type of scaffolds for engineering cartilage defects.

7. Controlled expression from "Smart Transplants"

As we all know that the conventional gene therapy has many potential limitations and therefore the need of customized gene therapy arises. The customized form of gene therapy could be defined as a therapy in which autologous cells are genetically modified by species-specific genes under the control of endogenous and disease-responsive elements and made clinically applicable by seeding in the scaffolds. As these scaffolds harbour cells that deliver therapeutic proteins in a fine-tuned manner to the defect site based on the severity of inflammation in the joint, we termed them smart transplants. Each component of smart transplants has been summarized below and is depicted in Figure 2.
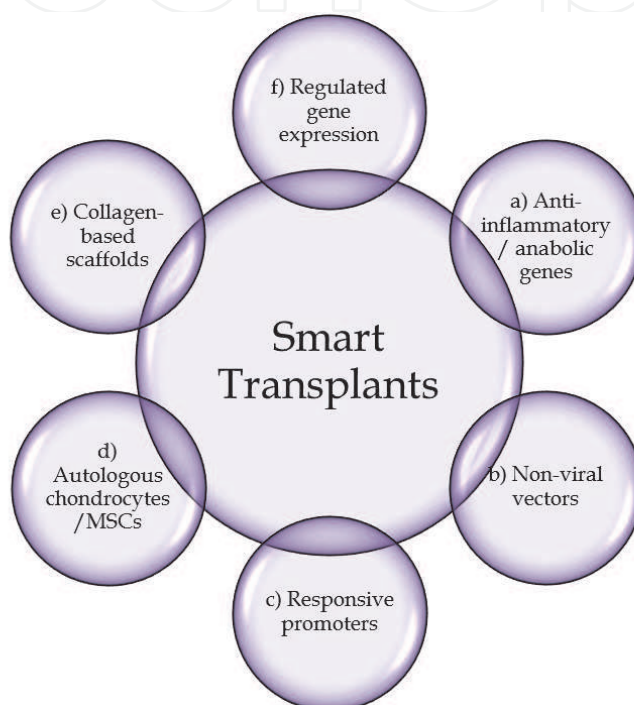
Fig. 2. A diagrammatic sketch showing the components of the smart transplant approach favoured by our research unit for future clinical application in veterinary as well as human medicine. Through recombinant DNA technology, (a) anti-inflammatory (IL-4) and/or anabolic (IGF-1) genes (a) are cloned with non-viral vectors (b) and endogenous responsive elements like COX-2 (c). Then the gene/vector cassette is introduced into autologous chondrocytes or MSCs (d) through an efficient transfection approach. The conditioned cells are finally seeded into collagen scaffolds (e) and finally implanted into the defected joint. Here it should secure a fine-tuned and regulated expression (f) of therapeutic proteins at the time when they are needed, i.e. when inflammatory mediators dominate the surrounding milieu.

7.1 Selection of genes for OA gene therapy

Though various biological factors have been independently identified as necessary for reducing inflammation or promoting regeneration in the diseased joint, the most promising therapeutic agents are those that modulate the activities of the pro-inflammatory cytokines IL-1β and TNFα (Fukui et al., 2003; M.B. Goldring, 1999; Martel-Pelletier et al., 1999). Several (anti-inflammatory) agents have been tested that suppress the production of pro-

inflammatory mediators (Fernandes et al., 2002). Among these IL-4 (Woods et al., 2001), IL-10 (Amos et al., 2006), and IL-13 (Jovanovic et al., 1998) are of utmost significance in the context of OA. IL-4 compared to IL-10, has a higher potential to inhibit IL-1β (Rachakonda et al., 2008a, 2008b) and only IL-4 (not IL-10) can induce the production of IL-1Ra (Relic et al., 2001). Further, IL-4 can antagonize the effects of TNFα by inducing down-regulation and shedding of both forms of TNFα receptors while IL-13 failed to produce such effects (Manna & Aggarwal 1998) and unlike IL-4, it does not appear to directly regulate the growth of T_H2-type cells (Chomarat & Banchereau, 1998).

OA – particularly in its later stages – is characterized by inflammation and catabolism going side by side (M.B. Goldring & S.R. Goldring, 2007; Pelletier et al., 2001). Therefore, simultaneous expression of both anti-inflammatory and regenerative (anabolic) genes should be taken into consideration. One of the main anabolic mediators, which naturally aids in the protection of cartilage from regular wear and tear, is insulin-like growth factor-1 (IGF-1). IGF-1 is known to maintain homeostasis in articular cartilage by stimulating the synthesis of cartilage matrix proteins noted by an increased production of aggrecan and type II collagen (Manning et al., 2010). IL-4 and IGF-1 would offer a more complete therapy to combat the different aspects of the disease. We consider the dual expression of both proteins and surmise that an effective therapy should comprise the expression of both anti-inflammatory cytokines and cartilage anabolic factors to counteract the effects of catabolic mediators.

7.2 Selection of non-viral vectors

The most worrisome weakness of gene therapy is that many of the immunological defence systems which normally tackle wild-type infections are activated against the vectors and new transgene products might be recognized as foreign. In the past, great advances have been made to create new systems for the efficient production of gene-deleted less immunogenic vectors. These include the improvements such as expansion of the repertoire of vector tropisms and the evasion of pre-existing immune responses through the development of alternative viral serotypes (Hill et al., 1999; Thomas et al., 2003). Non-viral gene delivery is potentially safer than virus-mediated delivery with the exception of a few promising applications, such as vaccines. However, non-viral systems are, at the present, limited by their relatively low transfer efficiency (Thomas et al., 2003). Nevertheless, we prefer the use of non-viral vectors because of their higher safety level.

There is still a tremendous amount of work to be done in gene therapy research. We have encountered many obstacles so far, and will probably encounter more, but these obstacles are not insurmountable. By continuing to identify and address potential hurdles and by maintaining a strong focus on improving vectors and delivery protocols, gene therapy will eventually play a significant role in the treatment of severe inflammatory joint diseases.

7.3 Selection of suitable target cells

The most obvious source for cells that can regenerate cartilage is the tissue itself. Chondrocytes are the cells which reside within and retain and remodel this tissue (M.B. Goldring, 2006). They represent a homogenous population with limited (cell) number and tend to rapidly de-differentiate upon expansion *in vitro* with a complete loss of phenotype. Adequate tissue repair strategies may require specific cellular targeting to the site of injury as retention and engraftment of transplanted cells are inadequate. Besides clinically applied

tissue-specific chondrocytes, undifferentiated bone-marrow origin MSCs are of special interest as promising candidates due to multiple advantages over the conventional chondrocytes (Pittenger et al., 1999). These advantages include, for instance, the trophic production of bioactive factors to initiate endogenous regenerative activities in OA (Nöth et al., 2008), their anti-inflammatory and immunosuppressive properties (Jorgensen& Apparailly, 2010) and their capacity to differentiate into chondrocytes.

7.4 Regulation of gene expression

Most gene therapy protocols involve constitutive delivery of therapeutic genes due to the transient nature of expression. In such instances, efficient gene transfer, expression, or stability have encountered limitations in preclinical and clinical applications mainly due to pleiotropic effects of the inducer, low-level basal expression and toxicity of the inducing agents. However, a non-toxic regulation of transgene expression may offer an effective means to control the expression levels of proteins with a narrow therapeutic index such as cytokines and hormones. Obviously, it would be most desirable to control the expression of therapeutic proteins in a disease-dependent manner. This would add considerably to the safety level of the gene therapy application. Incidentally, constitutive over-expression of transgenes typically would lead to detrimental effects under disease conditions. Therefore, regulation of gene expression is warranted. We have previously designed and reported a self-limiting promoter construct that expresses an anti-inflammatory gene only in the presence of inflammation (Patent: PCT/EP 2008/061408 published as US-2010-0255572-A1). The use of this construct for the expression of anti-inflammatory genes allows the production of a therapeutic gene product that is controlled by the severity of the disease. The effectiveness of this promoter construct for combating inflammation makes it a suitable candidate for the development of a new local gene therapy strategy for the treatment of OA (Rachakonda et al., 2008a). Furthermore, the ability to effectively control gene expression should also facilitate gene therapy studies because it will permit the expression of therapeutic genes and subsequently proteins to be regulated within the host. Thus, the application of inflammation-regulated therapeutic gene expression in arthritis conditions increases the efficiency of gene therapy by self-limiting the transgene.

Such elegant approaches have been adopted by applying disease-regulated promoters in a number of published reports (Cui et al., 2006; Godbey & Atala, 2003; Meynier de Salinelles et al., 2002; Miagkov et al., 2002; Rygg et al., 2001; Uhlar et al., 1997; Varley et al., 1995). Elements of the IL-1 and IL-6 promoter or of the COX-2 promoter, which are activated under inflammatory conditions, should up-regulate the transcription of transgenes such as IL-4 during acute OA and decrease expression rates after cessation of inflammation (Geurts et al., 2007; Rachakonda et al., 2008a). These self-regulating approaches may lead to safer stable transplants, which smartly react to the OA status of the joint.

7.5 Selection of suitable scaffolds

The selection of suitable scaffolds represents an important step to restore the damaged cartilage. Therefore, the selection of a suitable matrix composition may stabilize the transplant and support the regenerative process. Furthermore, the delivery of therapeutic genes will be more effective in a matrix that provides suitable protection, since the target cell population must often be exposed to factors throughout the entire course of repair, or at least for an extended period of time. An increased retention at

treatment sites could also enable regenerative tissue-forming cells to migrate to the area of injury and to proliferate and differentiate. In addition, the materials should be biodegradable and remodel as the new cartilage forms and replaces the original construct. These matrix materials could be combined with other types of carriers, which release active factors into the environment as they disperse or are degraded without providing any matrix function.

In this regard, the matrix should be non-toxic, non-adhesive and non-stimulatory for inflammatory cells such as lymphocytes, macrophages and neutrophils. Furthermore, any matrix material needs to be non-immunogenic since immunological attack to this material (then serving as an antigen) would be detrimental to tissue regeneration. The topography and material properties of natural scaffolds should also support the differentiation of MSCs. Finally, the scaffolds should be easy to handle during surgery thereby allowing the fixation of the transplant into the implant site with ease. From our experience rat tail collagen-based scaffolds have a number of advantages along these lines.

8. Preliminary data on "Smart Transplants"

It has been shown previously that chondrocytes in scaffolds are susceptible to inflammatory mediators (Kuroki et al., 2005; Rai et al., 2008). This scenario indirectly raises a question on the validity of ACT into cartilage lesions surrounded by progressive inflammation (Hennerbichler et al., 2008). In order to address this problem, we validated the inducible expression of IL-4 in chondrocytes (Rachakonda et al., 2008a, 2008b) and in a chondrocyte-based, 3-dimensional inflammation model (Rai et al., 2011). The main objective was to examine whether IL-4 produced within scaffolds can down-regulate inflammation and recoup extracellular matrix synthesis in the face of inflammation. We believe that this was the first study of its type to assess the use of cytokine-therapy devoid of viral vectors in a 3-dimensional *in vitro* inflammation model.

Mature canine chondrocytes were conditioned through transient transfection using pcDNA3.1.IL-4 (constitutive) or pCOX-2.IL-4 (cytokine-responsive) plasmids. Conditioned cells were seeded in alginate microspheres and rat-tail collagen type I matrix (CaReS) to generate two types of tissue-engineered scaffolds, alginate beads and CaReS-matrices containing engineered chondrocytes. Inflammation was induced in the packed chondrocytes through addition of recombinant IL-1β plus TNFα into the culture medium. Harvested cells and culture media were analysed by various assays to monitor the anti-inflammatory and regulatory (anabolic) properties of IL-4 (Rai et al., 2011).

The data obtained proved that IL-4 was expressed at sufficient levels to effectively down-regulate inflammation in both types of scaffolds. This indicated that both scaffolds containing conditioned chondrocytes allowed unrestricted diffusion of cytokines in and out of the cells and through the matrix network into the surrounding culture medium. It was shown that IL-4 was able to successfully down-regulate several pro-inflammatory cytokines, matrix degrading enzymes and various catabolic end products such as nitric oxide and prostaglandin. Further, the biochemical assessment of the levels of collagen and sulphated GAG also indicated the anabolic net effect of IL-4 on chondrocytes.

One of the important characteristics of our approach is the ability of the pCOX-2.IL-4 construct to deliver the therapeutic gene (in this case IL-4) only upon stimulation with exogenous IL-1β and TNFα. Thereby the expression of IL-4 is controlled through the

severity of inflammation as defined by the presence of pro-inflammatory cytokines (Figure 3).
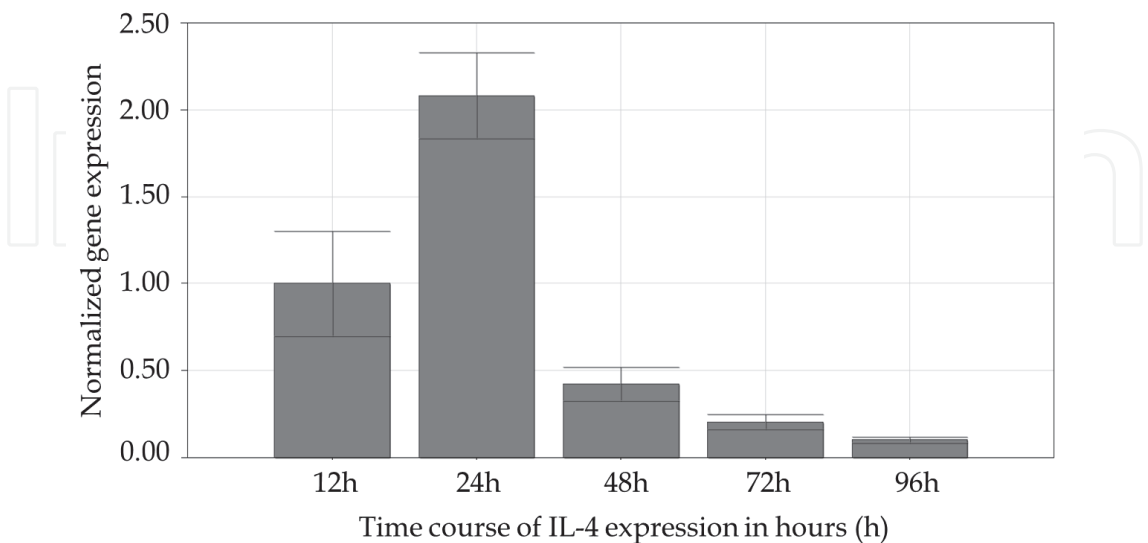

Fig. 3. Control of IL-4 expression through inflammatory mediators via the COX-2 promoter. **Experimental setup:** Conditioned chondrocytes (transfected with COX-2.IL-4) in scaffolds (CaReS) were stimulated by a 96 h exogenous treatment with IL-1β and TNFα. Thereafter the cells were re-isolated from the scaffolds and the expression levels of IL-4 assessed by qRT-PCR over time. The results show that after inflammatory induction IL-4 reaches peak levels at 24 hours followed by downregulation. **Interpretation:** Since endogenous pro-inflammatory mediators IL-1β and TNFα are both reduced under the influence of the regulatory action of IL-4 (data not shown, compare Manning et al., 2010 & Rai et al., 2011), the induction of the inflammation sensitive COX-2 promoter will also be attenuated. Since the IL-4 gene is transcribed under the control of this promoter, its own expression will eventually fade out. **Take home:** In smart transplants therapeutic genes under the control of the COX-2 promoter will only be expressed when inflammatory mediators are present close to the defective joint area. Thus, local therapy occurs only when it is needed – it is simply smart.

Since chondrocytes were conditioned by transfection prior to the generation of scaffolds, we coined a new term for this type of approach: ACCT (autologous conditioned cell therapy). Thus, therapeutic expression only occurs in a clearly defined condition, i.e. when the "conditioned" cells present in the scaffold "sense" inflammation in their surroundings.

We have also worked on the dual expression of IL-4 and IGF-1 in chondrocytes (Manning et al., 2010) because this approach potentially offers a more complete therapy to combat the different aspects of OA. The use of multiple genes could better alleviate the signs and symptoms characteristic for the disease process. Extension of this strategy to other suitable genes or combination of genes could in the future provide a better outlook to effectively heal OA. Ideally, if the cytokine-responsive matrices described above do work in the patient as they do *in vitro*, a promising strategy for the treatment of OA may emerge in the future.

Without any doubt, future therapy trials will have to occur to validate this novel approach.

9. Summary and future perspectives

OA affects a huge population of elderly. Recent progress in the understanding of OA pathophysiology has facilitated the development of therapeutic strategies aimed specifically at effectively retarding the disease process. Unfortunately most therapeutic efforts appear to have been directed either to temporal pain relief, or to the repair of OA-related cartilage lesions. As a final resort total joint replacement through surgery will be necessary. Gene therapy still has many pitfalls. The use of viral vectors and the unrestricted expression of transgenes are still subject to unanswered safety questions posed by the regulatory authorities. Nevertheless, it is tempting to speculate that an effective stimulation of cartilage regeneration paralleled by an inhibition of inflammation must be the key objectives of new therapeutic approaches. The negative impact of gene therapy can be overcome if it is suitably improved in each of its components. The first and foremost component of gene therapy is the vector. Viral vectors can be replaced by non-viral vectors regardless of which therapeutic genes they orchestrate and what expression levels may be achieved. Further, the promoter placed upstream of the therapeutic gene should be disease-dependent. In OA it should be inflammation dependent – like a COX-2 promoter that is always up-regulated when IL-1β and TNFα are up in the joint. Because IL-1β and TNFα drive OA progression and cartilage degradation, they become an important target in OA. Thus, genes like IL-4 and IGF-1 not only can coup with these pro-inflammatory cytokines, they also help to rebuild the normal cartilage tissue architecture.

For the delivery of suitable therapeutic genes in a non-viral approach, the selection of target cells is very important. Nature has provided the cartilage with only one cell type i.e. chondrocytes. In our opinion they are the best choice for transplantation in cartilage-related problems. But owing to their rapid differentiation into fibroblasts and lower cell number from a limited area of cartilage, stem cells may offer an important alternative in the future. Cell transplantation will require assembly for their implantation in the joint for which a wide-array of scaffolds is available. Whenever conditioned cells are involved that produce therapeutic proteins the best scaffolds are those that provide free diffusion of biological agents in and out.

To this end, we have established the applicability and usefulness of cell-seeded scaffolds *in vitro*. We cloned IL-4 downstream of a COX-2 promoter and transfected chondrocytes with the COX-2.IL-4 construct. The chondrocytes were then seeded into collagen type I scaffolds. To test the regulated expression of IL-4, a model of inflammatory arthritis was simulated by adding IL-1β and TNFα to the culture medium overlaying chondrocytes that were previously transfected (conditioned). Through the nuclear factor kappa B (NFκB) pathway IL-1β and TNFα up-regulated COX-2 which drove the expression of downstream IL-4. IL-4 in turn down-regulated multiple pro-inflammatory cytokines (e.g. IL-1β and TNFα), enzyme mediators and their catabolites and up-regulated the matrix molecules. Once the expression of IL-1β and TNFα dropped, COX-2 stopped the expression of IL-4 through a negative feedback loop mechanism (compare Figure 3). In this way the cells which were conditioned and incorporated within the scaffold, delivered the therapeutic effects of proteins in a coordinated and controlled manner. Our preliminary data form *in vitro* experiments are promising in many respects. It is realized that future therapy trials will have to reveal, whether ACCT with scaffolds containing conditioned cells will satisfy expectations as an effective approach.

The future of gene therapy as a viable medical application can be summarized in the words phrased by French Anderson in 1998: "Despite our present lack of knowledge, gene therapy

will almost certainly revolutionize the practice of medicine over the next 25 years. In every field of medicine, the ability to give the patient therapeutic genes offers extraordinary opportunities to treat, cure and ultimately prevent a vast range of diseases that now plague mankind"(Anderson, 1998). Impressive progress has been made thus far, but nevertheless it seems we need the remaining 12 years to see where we stand.

10. References

Abramson, S.B. & Yazici, Y. (2006). Biologics in Development for Rheumatoid Arthritis: Relevance to Osteoarthritis. *Advanced Drug Delivery Reviews*, Vol.58, No.2, (May 2006), pp. 212-225, ISSN 0169-409X

Adachi, N., Sato, K., Usas, A., Fu, F.H., Ochi, M., Han, C.W., Niyibizi, C. & Huard, J. (2002). Muscle-derived, Cell-based *Ex Vivo* Gene Therapy for Treatment of Full-thickness Articular Cartilage Defects. *Journal of Rheumatology*, Vol.29, No.9, (September 2002), pp. 1920-1930, ISSN 0315-162X

Amos, N., Lauder, S., Evans, A., Feldmann, M. & Bondeson, J. (2006). Adenoviral Gene Transfer into Osteoarthritis Synovial Cells using the Endogenous Inhibitor IκBα Reveals that most, but not all, Inflammatory and Destructive Mediators are NFκB Dependent. *Rheumatology*, Vol.45, No.10, (October 2006), pp. 1201-1209, ISSN 1462-0324

Andereya, S., Maus, U., Gavenis, K., Müller-Rath, R., Miltner, O., Mumme, T. & Schneider, U. (2007). First Clinical Experiences with a Novel 3D Collagen Gel (CaReS) for the Treatment of Focal Cartilage Defects in the Knee. *Zeitschrift für Orthopädie und Ihrer Grenzgebiete,* Vol.144, No.3, (May-June 2007), pp. 272-280, ISSN 0044-3220

Anderson, W.F. (1998). Human Gene Therapy. *Nature,* Vol.392, No.S6679, (April 1998), pp. 25-30, ISSN 1476-4687

Bandara, G., Robbins, P.D., Georgescu, H.I., Mueller, G.M., Glorioso, J.C. & Evans, C.H. (1992). Gene Transfer to Synoviocytes: Prospects for Gene Treatment of Arthritis. *DNA & Cell Biology,* Vol.11, No.3, (April 1992), pp. 227-231, ISSN 1044-5498

Brittberg, M., Lindahl, A., Nilsson, A., Ohlsson, C., Isaksson, O. & Peterson, L. (1994). Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. *New England Journal of Medicine*, Vol.331, No.14, (October 1994), pp. 889-895, ISSN 0028-4793

Buckwalter, J.A., Martin, J.A. & Brown, T.D. (2006). Perspectives on Chondrocyte Mechanobiology and Osteoarthritis. *Biorheology*, Vol.43, No.3-4, (September 2006), pp. 603-609, ISSN 0006-355X

Capito, R.M. & Spector, M. (2007). Collagen Scaffolds for Non-viral IGF-1 Gene Delivery in Articular Cartilage Tissue-engineering. *Gene Therapy,* Vol.14, No.9, (May 2007), pp. 721-732, ISSN 0969-7128

Celiker, M.Y., Ramamurthy, N., Xu, J.W., Wang, M., Jiang, Y., Greenwald, R. & Shi, Y.E. (2002). Inhibition of Adjuvant-induced Arthritis by Systemic Tissue Inhibitor of Metalloproteinases-4 Gene Delivery. *Arthritis & Rheumatism*, Vol.46, No.12, (December 2002), pp. 3361-3368, ISSN 0004-3591

Chomarat, P. & Banchereau, J. (1998). Interleukin-4 and Interleukin-13: Their Similarities and Discrepancies. *International Reviews of Immunology,* Vol.17, No.1-4, (January 1998), pp. 1-52, ISSN 0883-0185

Clouet, J., Vinatier, C., Merceron, C., Pot-vaucel, M., Maugars, Y., Weiss, P., Grimandi, G. & Guicheux, J. (2009). From Osteoarthritis Treatments to Future Regenerative Therapies for Cartilage. *Drug Discovery Today*, Vol.14, No.19-20, (October 2009), pp. 913-925, ISSN 1359-6446

Cucchiarini, M. & Madry, H. (2005). Gene Therapy for Cartilage Defects. *Journal of Gene Medicine*, Vol.7, No.12, (December 2005), pp. 1495-1509, ISSN 1099-498X

Cucchiarini, M., Thurn, T., Weimer, A., Kohn, D., Terwilliger, E.F. & Madry, H. (2007). Restoration of the Extracellular Matrix in Human Osteoarthritic Articular Cartilage by Over-expression of the Transcription Factor SOX-9. *Arthritis & Rheumatism*, Vol.56, No.1, (January 2007), pp. 158-167, ISSN 0004-3591

Cui, X., Yang, S.C., Sharma, S., Heuze-Vourc'h, N. & Dubinett, S.M. (2006). IL-4 Regulates COX-2 and PGE2 Production in Human Non-small Cell Lung Cancer. *Biochemical & Biophysical Research Communications,* Vol.343, No.4, (May 2006), pp. 995-1001, ISSN 0006-291X

Di Cesare, P.E., Frenkel, S.R., Carlson, C.S., Fang, C. & Liu, C. (2006). Regional Gene Therapy for Full-thickness Articular Cartilage Lesions using Naked DNA with a Collagen Matrix. *Journal of Orthopaedic Research*, Vol.24, No.5, (May 2006), pp. 1118-1127, ISSN 0736-0266

Donkuru, M., Badea, I., Wettig, S., Verrall, R., Elsabahy, M. & Foldvari, M. (2010). Advancing Nonviral Gene Delivery: Lipid- and Surfactant-based Nanoparticle Design Strategies. *Nanomedicine*, Vol.5, No.7, (September 2010), pp. 1103-1127, ISSN 1743-5889

Dougados, M. (2006). Symptomatic Slow-acting Drugs for Osteoarthritis: What are the Facts? *Joint Bone Spine*, Vol.73, No.6, (December 2006), pp. 606-609, ISSN 1297-319X

Evans, C.H. & Robbins, P.D. (1994). The Interleukin-1 Receptor Antagonist and its Delivery by Gene Transfer. *Receptor*, Vol.4, No.1, (Spring 1994), pp. 9-15, ISSN 1052-8040

Evans, C.H., Ghivizzani, S.C., Oligino, T.A. & Robbins, P.D. (2001). Future of Adenoviruses in the Gene Therapy of Arthritis. *Arthritis Research*, Vol.3, No.3, (n.d. 2001), pp. 142-146, ISSN 1465-9905

Evans, C.H., Gouze, J.N., Gouze, E., Robbins, P.D. & Ghivizzani, S.C. (2004). Osteoarthritis Gene Therapy. *Gene Therapy*, Vol.11, No.4, (February 2004), pp. 379-389, ISSN 0969-7128

Evans, C.H., Robbins, P.D., Ghivizzani, S.C., Wasko, M.C., Tomaino, M.M., Kang, R., Muzzonigro, T.A., Vogt, M., Elder, E.M., Whiteside, T.L., Watkins, S.C. & Herndon, J.H. (2005). Gene Transfer to Human Joints: Progress toward a Gene Therapy of Arthritis. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.102, No.24, (June 2005), pp. 8698-8703, ISSN 0027-8424

Evans, C.H., Ghivizzani, S.C. & Robbins, P.D. (2009). Gene Therapy of the Rheumatic Diseases: 1998 to 2008. *Arthritis Research & Therapy*, Vol.11, No.1, (January 2009), pp. 209, ISSN 1478-6354

Evans, C.H., Ghivizzani, S.C. & Robbins, P.D. (2011). Getting Arthritis Gene Therapy into the Clinic. *Nature Reviews Rheumatology*, Vol.7, No.4, (April 2011), pp. 244-249, ISSN 1759-4790

Fernandes, J.C., Tardif, G., Martel-Pelletier, J., Lascau-Coman, V., Dupuis, M., Moldovan, F., Sheppard, M., Krishnan, B.R. & Pelletier, J.P. (1999). *In Vivo* Transfer of Interleukin-1 Receptor Antagonist Gene in Osteoarthritic Rabbit Knee Joints. Prevention of Osteoarthritis Progression. *American Journal of Pathology*, Vol.154, No.4, (April 1999), pp. 1159-1169, ISSN 0002-9440

Fernandes, J.C., Martel-Pelletier, J. & Pelletier, J.P. (2002). The Role of Cytokines in Osteoarthritis Pathophysiology. *Biorheology,* Vol.39, No.1-2, (January 2002), pp. 237-246, ISSN 0006-355X

Frisbie, D.D., Ghivizzani, S.C., Robbins, P.D., Evans, C.H. & McIlwraith, C.W. (2002). Treatment of Experimental Equine Osteoarthritis by *In Vivo* Delivery of the Equine Interleukin-1 Receptor Antagonist Gene. *Gene Therapy*, Vol.9, No.1, (January 2002), pp. 12-20, ISSN 0969-7128

Fukui, N., Zhu, Y., Maloney, W.J., Clohisy, J. & Sandell, L.J. (2003). Stimulation of BMP-2 Expression by Pro-inflammatory Cytokines IL-1 and TNFα in Normal and Osteoarthritic Chondrocytes. *The Journal of Bone & Joint Surgery (America),* Vol.85, No.3, (August 2003), pp. 59-66, ISSN 1535-1386

Gelse, K., von der Mark, K., Aigner, T., Park, J. & Schneider, H. (2003). Articular Cartilage Repair by Gene Therapy using Growth Factor–producing Mesenchymal Cells. *Arthritis & Rheumatism*, Vol.48, No.2, (February 2003), pp. 430-441, ISSN 0004-3591

Geurts, J., Arntz, O.J., Bennink, M.B., Joosten, L.A., van den Berg, W.B. & van de Loo, F.A. (2007). Application of a Disease-regulated Promoter is a Safer Mode of Local IL-4 Gene Therapy for Arthritis. *Gene Therapy*, Vol.14, No.23, (December 2007), pp.1632-1638, ISSN 0969-7128

Ghivizzani, S.C., Lechman, E.R., Tio, C., Mulé, K.M., Chada, S., McCormack, J.E., Evans, C.H. & Robbins, P.D. (1997). Direct Retrovirus-mediated Gene Transfer to the Synovium of the Rabbit Knee: Implications for Arthritis Gene Therapy. *Gene Therapy*, Vol.4, No.9, (September 1997), pp. 977-982, ISSN 0969-7128

Ghivizzani, S.C., Lechman, E.R., Kang, R., Tio, C., Kolls, J., Evans, C.H. & Robbins, P.D.(1998). Direct Adenovirus-mediated Gene Transfer of Interleukin-1 and Tumour Necrosis Factor-alpha Soluble Receptors to Rabbit Knees with Experimental Arthritis hasLocal and Distal Anti-arthritic Effects. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.95, No.8, (April 1998), pp. 4613-4618, ISSN 0027-8424

Gibbons, L.J. & Hyrich, K.L. (2009). Biologic Therapy for Rheumatoid Arthritis: Clinical Efficacy and Predictors of Response. *BioDrugs,* Vol.23, No.2, (April 2009), pp. 111-124, ISSN 1173-8804

Godbey, W.T. & Atala, A. (2003). Directed Apoptosis in COX-2-overexpressing Cancer Cells through Expression-targeted Gene Delivery. *Gene Therapy,* Vol.10, No.17, (August 2003), pp. 1519-1527, ISSN 0969-7128

Godfrey, D.R. (2005). Osteoarthritis in Cats: ARetrospective Radiological Study. *The Journal of Small Animal Practice,*Vol.46, No.9, (September 2005), pp. 425-429, ISSN 0022-4510

Goehring, A.R., Lübke, C., Kaps, C., Häupl, T., Pruss, A., Perka, C., Sittinger, M. & Ringe, J. (2010). Tissue-engineered Cartilage of Porcine and Human Origin as In Vitro Test System in Arthritis Research. *Biotechnology Progress*, Vol.26, No.4, (July-August 2010), pp. 1116-1125, ISSN 1520-6033

Goldring, M.B. (1999). The Role of Cytokines as Inflammatory Mediators in Osteoarthritis: Lessons from Animal Models. *Connective Tissue Research*, Vol.40, No.1, (January 1999), pp. 1-11, ISSN 0300-8207

Goldring, M.B. (2001). Anticytokine Therapy for Osteoarthritis. *Expert Opinion on Biological Therapy*, Vol.1, No.5, (September 2001), pp. 817-829, ISSN 1471-2598

Goldring, M.B. (2006). Update on the Biology of the Chondrocyte and New Approaches to Treating Cartilage Diseases. *Best Practice & Research: Clinical Rheumatology*, Vol.20, No.5, (October 2006), pp. 1003-1025, ISSN 1521-6942

Goldring, M.B. & Goldring, S.R.(2007). Osteoarthritis. *Journal of Cellular Physiology*, Vol.21, No.3, (December 2007), pp. 626-634, ISSN 0021-9541

Gouze, E., Pawliuk, R., Pilapil, C., Gouze, J.N., Fleet, C., Palmer, G.D., Evans, C.H., Leboulch, P. & Ghivizzani, S.C. (2002). *In Vivo* Gene Delivery to Synovium by Lentiviral Vectors. *Molecular Therapy*, Vol.5, No.4, (April 2002), pp. 397-404, ISSN 1525-0016

Gouze, E., Gouze, J.N., Palmer, G.D., Pilapil, C., Evans, C.H. & Ghivizzani S.C. (2007). Transgene Persistence and Cell Turnover in the Diarthrodial Joint: Implications for Gene Therapy of Chronic Joint Diseases. *Molecular Therapy*, Vol.15, No.6, (June 2007), pp. 1114-1120, ISSN 1525-0016

Gouze, J.N., Gouze, E., Palmer, G.D., Kaneto, H., Ghivizzani, S.C., Grodzinsky, A.J. & Evans, C.H. (2004). Adenovirus-mediated Gene Transfer of Glutamine: Fructose-6-phosphate Amidotransferase Antagonizes the Effects of Interleukin-1β on Rat Chondrocytes. *Osteoarthritis & Cartilage*, Vol.12, No.3, (March 2004), pp. 217-224, ISSN 1063-4584

Grossin, L., Cournil-Henrionnet, C., Mir, L.M., Liagre, B., Dumas, D., Etienne, S., Guingamp, C., Netter, P. & Gillet, P. (2003). Direct Gene Transfer into Rat Articular Cartilage by *In Vivo* Electroporation. *The FASEB Journal*, Vol.17, No.8, (May 2003), pp. 829-835, ISSN 0892-6638

Grossin, L., Cournil-Henrionnet, C., Pinzano, A., Gaborit, N., Dumas, D., Etienne, S., Stoltz, J.F., Terlain, B., Netter, P., Mir, L.M. & Gillet, P. (2006). Gene Transfer with HSP 70 in Rat Chondrocytes Confers Cytoprotection *In Vitro* and during Experimental Osteoarthritis. *The FASEB Journal*, Vol.20, No.1, (January 2006), pp. 65-75, ISSN 0892 6638

Guo, J., Jourdian, G. & Maccallum, D.K. (1989). Culture and Growth Characteristic of Chondrocytes Encapsulated in Alginate Beads. *Connective Tissue Research*, Vol.19, No.2-4, (n.d. 1989), pp. 277-285, ISSN 1607-8438

Haag, J., Voigt, R., Soeder, S. & Aigner, T. (2009). Efficient Non-viral Transfection of Primary Human Adult Chondrocytes in a High-throughput Format. *Osteoarthritis & Cartilage*, Vol. 17, No.6, (June 2009), pp. 813-817, ISSN 1063-4584

Habib, G.S., Saliba, W. & Nashashibi, M. (2010). Local Effects of Intra-articular Corticosteroids. *Clinical Rheumatology*, Vol.29, No.4, (April 2010), pp. 347-356, ISSN 0770-3198

Harui, A., Suzuki, S., Kochanek, S. & Mitani, K. (1999). Frequency and Stability of Chromosomal Integration of Adenovirus Vectors. *Journal of Virology*, Vol.73, No.7, (July 1999), pp. 6141-6146, ISSN 0022-538X

Häuselmann, H.J., Fernandes, R.J., Mok, S.S., Schmid, T.M., Block, J.A., Aydelotte, M.B., Kuettner, K.E., Eugene, J.M. & Thonar, E.J. (1994). Phenotypic Stability of Bovine Articular Chondrocytes after Long-term Culture in Alginate Beads. *Journal of Cell Science*, Vol.107, No.1, (January 1994), pp. 17-27, ISSN 1477-9137

Heinegård, D. & Saxne, T. (2011). The Role of the Cartilage Matrix in Osteoarthritis. *Nature Reviews Rheumatology*, Vol.7, No.1, (January 2011), pp. 50-56, ISSN 1759-4790

Hennerbichler, A., Rosenberger, R., Arora, R., Hennerbichler, D. (2008). Biochemical, Biomechanical and Histological Properties of Osteoarthritic Porcine Knee Cartilage: Implications for Osteochondral Transplanation. *Archives of Orthopaedeic & Trauma Surgery*, Vol.128, No.1, (January 2008), pp. 61-70, ISSN 0936-8051

Hidaka, C., Goodrich, L.R., Chen, C.T., Warren, R.F., Crystal, R.G. & Nixon, A.J. (2003). Acceleration of Cartilage Repair by Genetically Modified Chondrocytes Over-expressing Bone Morphogenetic Protein-7. *Journal of Orthopaedic Research*, Vol.21, No.4, (July 2003), pp. 573-583, ISSN 0736-0266

Hill, C.L., Bieniasz, P.D. & McClure, M.O. (1999). Properties of Human Foamy Virus Relevant to its Development as a Vector for Gene Therapy. *The Journal of General Virology*, Vol.80, No.8, (August 1999), pp. 2003-2009, ISSN 0022-1317

Hotz-Behofsits, C.M., Walley, M.J, Simpson, R. & Bjarnason, I.T. (2003). COX-1, COX-2 and the Topical Effect in NSAID-induced Enteropathy. *Inflammopharmacology*, Vol.11, No.4 (n.d. 2003), pp. 363-370, ISSN 0925-4692

Hsieh, J.L., Shen, P.C., Shiau, A.L., Jou, I.M., Lee, C.H., Wang, C.R., Teo, M.L. & Wu, C.L. (2010). Intra-articular Gene Transfer of Thrombospondin-1 Suppresses the Disease Progression of Experimental Osteoarthritis. *Journal of Orthopaedic Research*, Vol.28, No.10, (October 2010), pp. 1300-1306, ISSN 0736-0266

Hunziker, E.B. (2002). Articular Cartilage Repair: Basic Science and Clinical Progress. A Review of the Current Status and Prospects. *Osteoarthritis & Cartilage*, Vol.10, No.6, (June 2002), pp. 432-463, ISSN 1063-4584.

Ikeda, K., Wakimoto, H., Ichikawa, T., Jhung, S., Hochberg, F.H., Louis, D.N. & Chiocca, E.A. (2000). Complement Depletion Facilitates the Infection of Multiple Brain Tumors by an Intravascular, Replication-Conditional Herpes Simplex Virus Mutant. *Journal of Virology*, Vol.74, No.10, (May 2000), pp. 4765-4775, ISSN 0022-538X

Im, G.I., Kim, D.Y., Shin, J.H., Hyun, C.W.& Cho, W.H. (2001). Repair of Cartilage Defect in the Rabbit with Cultured Mesenchymal Stem Cells from Bone-marrow. *The Journal of Bone & Joint Surgery (British)*, Vol.83, No.2, (March 2001), pp. 289-294, ISSN 0301-620X

Innes, J.F. & Clegg, P. (2010). Comparative Rheumatology: What can be Learnt from Naturally Occurring Musculoskeletal Disorders in Domestic Animals? *Rheumatology*,Vol.49, No.6, (June 2010), pp. 1030-1039, ISSN 1462-0324

Johnston, S.A. (1997). Osteoarthritis. Joint Anatomy, Physiology, and Pathobiology. *The Veterinary Clinics of North America: Small Animal Practice,* Vol.27, No.4, (July 1997), pp. 699-723, ISSN 0195-5616

Jorgensen, C. & Apparailly, F. (2010). Prospects for Gene Therapy in Inflammatory Arthritis. *Best Practice & Research: Clinical Rheumatology*, Vol.24, No.4, (August 2010), pp. 541-552, ISSN 1521-6942

Jovanovic, D., Pelletier, J.P., Alaaeddine, N., Mineau, F., Geng, C., Ranger, P. & Martel-Pelletier, J. (1998). Effect of IL-13 on Cytokines, Cytokine Receptors and Inhibitors on Human Osteoarthritis Synovium and Synovial Fibroblasts. *Osteoarthritis & Cartilage*, Vol.6, No.1, (January 1998), pp. 40-49, ISSN 1063-4584

Kang, R., Marui, T., Ghivizzani, S.C., Nita, I.M., Georgescu, H.I., Suh, J.K., Robbins, P.D. & Evans, C.H. (1997). *Ex Vivo* Gene Transfer to Chondrocytes in Full-thickness Articular Cartilage Defects: A Feasibility Study. *Osteoarthritis & Cartilage*, Vol.5, No.2, (March 1997), pp. 139-143, ISSN 1063-4584

Katayama, R., Wakitani, S., Tsumaki, N., Morita, Y., Matsushita, I., Gejo, R. & Kimura, T. (2004). Repair of Articular Cartilage Defects in Rabbits using CDMP1 Gene Transfected Autologous Mesenchymal Cells Derived from Bone Marrow. *Rheumatology*, Vol.43, No.8, (August 2004), pp. 980-985, ISSN 1462-0324

Katz, J.N., Brandon, E.E. & Gomoll, A.H.(2010). Surgical Management of Osteoarthritis. *Arthritis Care & Research*, Vol.62, No.9, (September 2010), pp. 1220-1228, ISSN 2151-464X

Kaul, G., Cucchiarini, M., Arntzen, D., Zurakowski, D., Menger, M.D., Kohn, D., Trippel, S.B. & Madry, H. (2006). Local Stimulation of Articular Cartilage Repair by Transplantationof Encapsulated Chondrocytes Over-expressing Human Fibroblast Growth Factor-2 (FGF-2) *In Vivo. Journal of Gene Medicine*, Vol.8, No.1, (January 2006), pp. 100-111, ISSN 1099-498X

Kulseng, B., Skjak-Braek, G., Ryan, L., Andersson, A., King, A., Faxvaag, A. & Espevik, T. (1999). Transplantation of Alginate Microcapsules: Generation of Antibodies against Alginates and Encapsulated Porcine Islet-like Cell Clusters. *Transplantation*, Vol.67, No.7, (April 1999), pp. 978-984, ISSN 1534-0608

Kuroki, K., Stoker, A.M. & Cook, J.L. (2005). Effects of Proinflammatory Cytokines on Canine Articular Chondrocytes in a Three-dimensional Culture. *American Journal of Veterinary Research*, Vol.66, No.7, (July 2005), pp. 187-196, ISSN 0002-9645

Lafeber, F.P., van Roy, H.L., van der Kraan, P.M., van den Berg, W.B. & Bijlsma, J.W. (1997). Transforming Growth Factor-beta Predominantly Stimulates Phenotypically Changed Chondrocytes in Osteoarthritic Human Cartilage. *The Journal of Rheumatology*, Vol.24, No.3, (March 1997), pp. 536-542, ISSN 0315-162X

Langer, R. & Tirrell, D.A. (2004). Designing Materials for Biology and Medicine. *Nature,* Vol.428, No.6982, (April 2004), pp. 487-492, ISSN 1476-4687

Lee, K.H., Song, S.U., Hwang, T.S., Yi, Y., Oh, I.S., Lee, J.Y., Choi, K.B. & Kim, S.J. (2001). Regeneration of Hyaline Cartilage by Cell-mediated Gene Therapy using

Transforming Growth Factor-β1-producing Fibroblasts. *Human Gene Therapy*, Vol.12, No.14, (September 2001), pp. 1805-1813, ISSN 1043-0342

Li, W.J., Jiang Y.J. & Tuan, R.S. (2006). Chondrocyte Phenotype in Engineered Fibrous Matrix is Regulated by Fiber Size. *Tissue Engineering,* Vol.12, No.7, (July 2006), pp. 1775-1785, ISSN 2152-4955

Liao, Y.H., Jones, S.A., Forbes, B., Martin, G.P. & Brown, M.B. (2005). Hyaluronan: Pharmaceutical Characterization and Drug Delivery. *Drug Delivery*, Vol.12, No.6, (November-December 2005), pp. 327-342, ISSN 1071-7544

Madeira, C., Ribeiro, S.C., Pinheiro, I.S., Martins, S.A., Andrade, P.Z., da Silva, C.L. & Cabral, J.M. (2011). Gene delivery to human bone marrow mesenchymal stem cells by microporation. *Journal of Biotechnology*, Vol.151, No.1, (January 2011), pp. 130-136, ISSN 0168-1656

Madry, H. & Trippel, S.B. (2000). Efficient Lipid-mediated Gene Transfer to Articular Chondrocytes. *Gene Therapy*, Vol.7, No.4, (February 2000), pp. 286-291, ISSN 0969-7128

Madry, H., Padera, R., Seidel, J., Langer, R., Freed, L.E., Trippel, S.B. & Vunjak-Novakovic, G. (2002). Gene Transfer of a Human Insulin-like Growth Factor 1 cDNA Enhances Tissue-engineering of Cartilage. *Human Gene Therapy*, Vol.13, No.13, (September 2002), pp. 1621-1630, ISSN 1043-0342

Madry, H., Cucchiarini, M., Terwilliger, E.F. & Trippel, S.B. (2003). Recombinant Adeno-associated Virus Vectors Efficiently and Persistently Transduce Chondrocytes in Normal and Osteoarthritic Human Articular Cartilage. *Human Gene Therapy*, Vol.14, No.4, (March 2003), pp. 393-402, ISSN 1043-0342

Madry, H., Emkey, G., Zurakowski, D. & Trippel, S.B. (2004). Over-expression of Human Fibroblast Growth Factor-2 Stimulates Cell Proliferation in an *Ex Vivo* Model of Articular Chondrocyte Transplantation. *The Journal of Gene Medicine*, Vol.6, No.2, (February 2004), pp. 238-245, ISSN 1099-498X

Madry, H., Kaul, G., Cucchiarini, M., Stein, U., Zurakowski, D., Remberger, K., Menger, M.D., Kohn, D. & Trippel, S.B. (2005). Enhanced Repair of Articular Cartilage Defects *In Vivo* by Transplanted Chondrocytes Over-expressing Insulin-like Growth Factor-1 (IGF-1). *Gene Therapy*, Vol.12, No.15, (August 2005), pp. 1171-1179, ISSN 0969-7128

Manna, S.K. & Aggarwal, B.B. (1998). Interleukin-4 Down-regulates both Forms of TumourNecrosis Factor Receptor and Receptor-mediated Apoptosis, NF-kappa B, AP-1, and c-Jun N-terminal Kinase. Comparison with Interleukin-13. *Journal of Biological Chemistry,* Vol.273, No.50, (December 1998), pp. 33333-33341, ISSN 1083-351X

Manning, K., Rachakonda, P.S., Rai, M.F. & Schmidt, M.F. (2010). Co-expression of Insulin-like Growth Factor-1 and Interleukin-4 in an *In Vitro* Inflammatory Model. *Cytokine*, Vol.50, No.3, (June 2010), pp. 297-305, ISSN 1043-4666

Martel-Pelletier, J., Alaaeddine, N. & Pelletier, J.P. (1999). Cytokines and their Role in the Pathophysiology of Osteoarthritis. *Frontiersin Bioscie*nce, Vol.4, No.D, (October 1999), pp. 694-703, ISSN 1093-9946

Martel-Pelletier, J. & Pelletier, J.P. (2007). Inflammatory Factors Involved in Osteoarthritis, In: *Osteoarthritis, Inflammation & Degradation: A Continuum*, J. Buckwalter, M. Lotz & J.F. Stolz, (Eds.), 3-13, IOS Press, ISBN 978-1-58603-773-4, Amsterdam, Netherlands

Mease, P.J., Wei, N., Fudman, E.J., Kivitz, A.J., Schechtman, J., Trapp, R.G., Hobbs, K.F., Greenwald, M., Hou, A., Bookbinder, S.A., Graham, G.E., Wiesenhutter, C.W., Willis, L., Ruderman, E.M., Forstot, J.Z., Maricic, M.J., Dao, K.H., Pritchard, C.H., Fiske, D.N., Burch, F.X., Prupas, H.M.,Anklesaria, P. & Heald, A.E. (2010). Safety, Tolerability, and Clinical Outcomes after Intra-articular Injection of a Recombinant Adeno-associated Vector Containing a Tumour Necrosis Factor Antagonist Gene: Results of a Phase 1/2 Study. *The Journal of Rheumatology*, Vol.37, No.4, (April 2010) pp. 692-703, ISSN 1499-2752

Meynier de Salinelles, V., Berenbaum, F., Jacques, C., Salvat, C., Olivier, J.L., Béréziat, G., Raymondjean, M. & Massaad, C. (2002). Design of a Chimeric Promoter induced by Pro-inflammatory Mediators in Articular Chondrocytes. *FEBS Letters*, Vol.518, No.1-3, (May 2002), pp. 67-71, ISSN 0014-5793

Miagkov, A.V., Varley, A.W., Munford, R.S. & Makarov, S.S. (2002). Endogenous Regulation of a Therapeutic Transgene Restores Homeostasis in Arthritic Joints. *Journal of Clinical Investigation*, Vol.109, No.9, (May 2002), pp. 1223-1229, ISSN 0021-9738

Minas, T., Gomoll, A.H., Solhpour, S., Rosenberger, R., Probst, C., Bryant, T. (2010). Autologous Chondrocyte Implantation for Joint Preservation in Patients with Early Osteoarthritis. *Clinical Orthopaedic & Related Research*,Vol.468, No.1, (January 2010), pp. 147-157, ISSN 0009-921X

Müller, R.D., John, T., Kohl, B., Oberholzer, A., Gust, T., Hostmann, A., Hellmuth, M., Laface, D., Hutchins, B., Laube, G., Veh, R.W., Tschoeke, S.K., Ertel, W. & Schulze-Tanzil, G. (2008). IL-10 Over-expression Differentially Affects Cartilage Matrix Gene Expression in Response to TNF-alpha in Human Articular Chondrocytes*In Vitro*. *Cytokine*, Vol.44, No.3, (December 2008), pp. 377-385, ISSN 1043-4666

Nabbe, K.C., van Lent, P.L., Holthuysen, A.E., Sloëtjes, A.W., Koch, A.E., Radstake, T.R. & van den Berg, W.B. (2005). Local IL-13 Gene Transfer Prior to Immune-complex Arthritis Inhibits Chondrocyte Death and Matrix-metalloproteinase-mediated Cartilage Matrix Degradation Despite Enhanced Joint Inflammation. *Arthritis Research & Therapy*, Vol.7, No.2, (January 2005), pp. R392-R401, ISSN 1478-6362

Nöth, U., Tuli, R., Osyczka, A.M., Danielson, K.G. & Tuan, R.S. (2002).*In Vitro*Engineered Cartilage Constructs Produced by Press-coating Biodegradable Polymer with Human Mesenchymal Stem Cells. *Tissue Engineering*, Vol.8, No.1, (n.d. 2002), pp.131-144, ISSN 2152-4955

Nöth, U., Steinert, A.F. & Tuan, R.S. (2008). Technology Insight: Adult Mesenchymal Stem Cells for Osteoarthritis Therapy. *Nature Clinical Practice: Rheumatology*, Vol.4, No.7, (July 2008), pp. 371-380, ISSN 1745-8382

Park, J.Y., Pillinger, M.H. & Abramson, S.B. (2006). Prostaglandin E2 Synthesis and Secretion: The Role of PGE2 Synthases. *Clinical Immunology*, Vol.119, No.3, (June 2006), pp. 229-240, ISSN 1521-6616

Parker, D.A., Beatty, K.T., Giuffre, B., Scholes, C.J.&Coolican, M.R. (2011). Articular Cartilage Changes in Patients with Osteoarthritis after Osteotomy. *The American Journal of Sports Medicine,* Vol.39, No.5, (May 2011), ISSN 0363-5465

Pelletier, J.P., Caron, J.P., Evans, C., Robbins, P.D., Georgescu, H.I., Jovanovic, D., Fernandes, J.C. & Martel-Pelletier, J. (1997). *In Vivo* Suppression of Early Experimental Osteoarthritis by Interleukin-1 Receptor Antagonist using Gene Therapy. *Arthritis & Rheumatism*, Vol.40S, No.6, (June 1997), pp. 1012-1019, ISSN 0004-3591

Pelletier, J.P., Martel-Pelletier, J. & Abramson, S.B. (2001). Osteoarthritis, an Inflammatory Disease: Potential Implication for the Selection of New Therapeutic Targets. *Arthritis & Rheumatism*, Vol.44, No.6, (June 2001), pp. 1237-1247, ISSN 00043591

Peterson, L., Brittberg, M., Kiviranta, I., Akerlund, E.L.& Lindahl, A. (2002). Autologous Chondrocyte Transplantation. Biomechanics and Long-term Durability. *The American Journal of Sports Medicine,* Vol.30, No.1,(January-February 2002), pp. 2-12, ISSN 0363-5465

Philpott, N.J. & Thrasher, A.J. (2007). Use of Non-integrating Lentiviral Vectors for Gene Therapy. *Human Gene Therapy*, Vol.18, No.6, (June 2007), pp. 483-489, ISSN 1043-0342

Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S. & Marshak, D.R. (1999). Multilineage Potential of Adult Human Mesenchymal Stem Cells. *Science,* Vol.284, No.5411, (April 1999), pp. 143-147, ISSN 1095-9203

Plank, C., Schillinger, U., Scherer, F., Bergemann, C., Rémy, J.S., Krötz, F., Anton, M.,Lausier, J. & Rosenecker, J. (2003). The Magnetofection Method: Using Magnetic Force to Enhance Gene Delivery. *Biological Chemistry,*Vol.384, No.5, (May 2003), pp. 737-747, ISSN 0021-9258.

Poole, C.A., Honda, T., Skinner, S.J., Schofield, J.R., Hyde, K.F. & Shinkai, H. (1990). Chondrons from Articular Cartilage (II): Analysis of the Glycosaminoglycans in the Cellular Microenvironment of Isolated Canine Chondrons. *Connective Tissue Research,* Vol.24, No.3-4, (n.d. 1990), pp. 319-330, ISSN 1607-8438

Rachakonda, P.S., Rai, M.F. & Schmidt, M.F. (2008a). Application of Inflammation Responsive Promoter for an *In Vitro* Arthritis Model. *Arthritis & Rheumatism*,Vol. 58, No. 7, (July 2008), pp. 2088-2097, ISSN 0004-3591

Rachakonda, P.S., Rai, M.F., Manning, K. & Schmidt, M.F. (2008b). Expression of Canine Interleukin-4 in Canine Chondrocytes Inhibits Inflammatory Cascade through STAT6. *Cytokine*, Vol.44, No.1, (October 2008), pp. 179-184, ISSN 1043-4666

Rai, M.F., Rachakonda, P.S., Manning, K., Vorwerk, B., Brunnberg, L., Kohn, B. & Schmidt M.F. (2008). Quantification of Cytokines and Inflammatory Mediators in a Three-dimensional Model of Inflammatory Arthritis. *Cytokine,* Vol.42, No.1, (April 2008), pp. 8–17,ISSN 1043-4666

Rai, M.F., Rachakonda, P.S., Manning, K., Palissa, C., Sittinger, M., Ringe, J. & Schmidt, M.F. (2009). Molecular and Phenotypic Modulation of Primary and Immortalized Canine Chondrocytes in Different Culture Systems. *Research in Veterinary Science,* Vol.87, No.3, (December 2009), pp. 399-407,ISSN 0034-5288

Rai, M.F., Graeve, T., Twardziok, S. & Schmidt, M.F. (2011). Evidence for Regulated Interleukin-4 Expression in Chondrocyte-scaffolds Under *In Vitro* Inflammatory Conditions. (submitted)

Relic, B., Guicheux, J., Mezin, F., Lubberts, E., Togninalli, D., Garcia, I., van den Berg, W.B. & Guerne, P.A. (2001). IL-4 and IL-13, but not IL-10, Protect Human Synoviocytes from Apoptosis. *The Journal of Immunology,* Vol.166, No.4, (February 2001), pp. 2775-2782, ISSN 0022-1767

Rygg, M., Uhlar, C.M., Thorn, C., Jensen, L.E., Gaughan, D.J., Varley, A.W., Munford, R.S., Göke, R., Chen, Y. & Whitehead, A.S. (2001). *In Vitro* Evaluation of an Enhanced Human Serum Amyloid A (SAA2) Promoter-regulated Soluble TNF Receptor Fusion Protein for Anti-inflammatory Gene Therapy. *Scandinavian Journal of Immunology,* Vol.53, No.6, (June 2001), pp. 588-595, ISSN 1365-3083

Sandell, L.J. & Aigner, T. (2001). Articular Cartilage and Changes in Arthritis. An Introduction: Cell Biology of Osteoarthritis. *Arthritis Research*, Vol.3, No.2, (January 2001), pp.107-113, ISSN 1465-9905

Sant, S.M., Suarez, T.M., Moalli, M.R., Wu, B.Y., Blaivas, M., Laing, T.J. & Roessler, B.J. (1998). Molecular Lysis of Synovial Lining Cells by *In Vivo* Herpes Simplex Virus-thymidine Kinase Gene Transfer. *Human Gene Therapy*, Vol.9, No.18, (December 1998), pp. 2735-2743, ISSN 1043-0342

Scarpignato, C. & Hunt, R.H. (2010). Nonsteroidal Antiinflammatory Drug-related Injury to the Gastrointestinal Tract: Clinical Picture, Pathogenesis, and Prevention. *Gastroenterology Clinics of North America,* Vol.39, No.3, (September 2010), pp. 433-464, ISSN 0889-8553

Shapiro, F., Koide, S. & Glimcher, M.J. (1993). Cell Origin and Differentiation in the Repair of Full-thickness Defects of Articular Cartilage. *The Journal of Bone & Joint Surgery (America),* Vol.75, No.4, (April 1993), pp. 532-553, ISSN 1535-1386

Stoop, R. (2008). Smart Biomaterials for Tissue-engineering. *Injury,* Vol.39, No.S1, (April 2008), pp. S77-S87, ISSN 0020-1383

Thomas, C.E., Ehrhardt, A. & Kay, M.A. (2003).Progress and Problems with the Use of Viral Vectors for Gene Therapy. *Nature Review Genetics,* Vol.4, No.5, (May 2003), pp. 346-358, ISSN 1471-0064

Trippel, S.B., Ghivizzani, S.C. & Nixon, A.J. (2004).Gene-based Approaches for the Repair of Articular Cartilage. *Gene Therapy,*Vol.11, No.4, (February 2004), pp. 351-359, ISSN 1476-5462

Trumble, T.N., Trotter, G.W., Oxford, J.R., McIlwraith, C.W., Cammarata, S., Goodnight, JL., Billinghurst, R.C. & Frisbie, D.D. (2001). Synovial Fluid Gelatinase Concentrations and Matrix Metalloproteinase and Cytokine Expression in Naturally Occurring Joint Disease in Horses. *American Journal of Veterinary Research,*Vol.62, No.9, (September 2001), pp. 1467-1477, ISSN 0002-9645

Tuli, R., Li, W.J. & Tuan, R.S. (2003). Current State of Cartilage Tissue-engineering. *Arthritis Research & Therapy,* Vol.5, No.5, (August 2003), pp. 235-238, ISSN 1478-6354

Uhlar, C.M., Grehan, S., Steel, D.M., Steinkasserer, A. & Whitehead, A.S. (1997). Use of the Acute Phase Serum Amyloid A2 (SAA2) Gene Promoter in the Analysis of Pro- and Anti-inflammatory Mediators: Differential Kinetics of SAA2 Promoter Induction by

IL-1 beta and TNF-alpha Compared to IL-6. *Journal of Immunological Methods,* Vol.203, No.2, (April 1997), pp. 123-130, ISSN 0022-1759

van der Laan, W.H., Quax, P.H., Seemayer, C.A., Huisman, L.G., Pieterman, E.J., Grimbergen, J.M., Verheijen, J.H., Breedveld, F.C., Gay, R.E., Gay, S., Huizinga, T.W. & Pap, T. (2003). Cartilage Degradation and Invasion by Rheumatoid Synovial Fibroblasts is Inhibited by Gene Transfer of TIMP-1 and TIMP-3. *Gene Therapy*, Vol.10, No.3, (February 2003), pp. 234-242, ISSN 0969-7128

Varley, A.W., Coulthard, M.G., Meidell, R.S., Gerard, R.D. & Munford, R.S. (1995). Inflammation-induced Recombinant Protein Expression *In Vivo* Using Promoters from Acute-phase Protein Genes. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.92, No.12, (June 1995), pp. 5346-5350, ISSN 0027-8424

Venkatesan, N., Barré, L., Benani, A., Netter, P., Magdalou, J., Fournel-Gigleux, S. & Ouzzine, M. (2004). Stimulation of Proteoglycan Synthesis by Glucuronosyltransferase-I Gene Delivery: A Strategy to Promote Cartilage Repair. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.101, No.52, (December 2004), pp. 18087-18092, ISSN 0027-8424

Wakitani, S., Goto, T., Young, R.G., Mansour, J.M., Goldberg, V.M. & Caplan, A.I. (1998). Repair of Large Full-thickness Articular Cartilage Defects with Allograft Articular Chondrocytes Embedded in a Collagen Gel.*Tissue Engineering,* Vol.4, No.4, (Winter 1998), pp. 429-444, ISSN 2152-4955

Watson, R.S., Gouze, E., Levings, P.P., Bush, M.L., Kay, J.D., Jorgensen, M.S., Dacanay, E.A., Reith, J.W., Wright, T.W. & Ghivizzani, S.C. (2010). Gene Delivery of TGF-β1 Induces Arthrofibrosis and Chondrometaplasia of Synovium *In Vivo. Laboratory Investigation*, Vol.90, No.11, (November 2010), pp. 1615-1627, ISSN 0023-6837

Wei, L., de Bri, E., Lundberg, A. & Svensson, O. (1998). Mechanical Load and Primary Guinea Pig Osteoarthrosis. *Acta OrthopaedicaScandinavica,*Vol.69, No.4, (August 1998), pp. 351-357, ISSN 0001-6470

Welsch, G.H., Mamisch, T.C., Zak, L., Blanke, M., Olk, A., Marlovits, S. & Trattnig, S. (2010). Evaluation of Cartilage Repair Tissue after Matrix-associated Autologous Chondrocyte Transplantation Using a Hyaluronic-based or a Collagen-based Scaffold with Morphological MOCART Scoring and Biochemical T2Mapping: Preliminary Results. *The American Journal of Sports Medicine,* Vol.38, No.5, (May 2010), pp. 934-942, ISSN 0363-5465

Welter, J.F., Solchaga, L.A. & Stewart, M.C. (2004). High-efficiency Non-viral Transfection of Primary Chondrocytes. *Methods in Molecular Medicine*, Vol.100, No.X, (July 2004), pp. 129-146, ISSN 1543-1894

Wolf, A.D. & Pfleger, B. (2003). Burden of Major Musculoskeletal Conditions. *Bulletin of the World Health Organization*, Vol.81, No.9, (November 2003), pp. 646-656, ISSN 0042-9686

Wolfe, M.M., Lichtenstein, D.R. & Singh, G. (1999). Gastrointestinal Toxicity of Nonsteroidal Antiinflammatory Drugs. *The New England Journal of Medicine*, Vol.340, No.24, (June 1999), pp. 1888-1899, ISSN 0028-4793

Woods, J.M., Katschke, K.J., Volin, M.V., Ruth, J.H., Woodruff, D.C., Amin, M.A., Connors, M.A., Kurata, H., Arai, K., Haines, G.K., Kumar, P. & Koch, A.E. (2001). IL-4

Adenoviral Gene Therapy Reduces Inflammation, Pro-inflammatory Cytokines, Vascularization, and Bony Destruction in Rat Adjuvant-induced Arthritis. *The Journal of Immunology,* Vol.166, No.2, (January 2001), pp. 1214-1222, ISSN 0022-1767

Yokoo, N., Saito, T., Uesugi, M., Kobayashi, N., Xin, K.Q., Okuda, K., Mizukami, H., Ozawa, K. & Koshino, T. (2005). Repair of Articular Cartilage Defect by Autologous Transplantation of Basic Fibroblast Growth Factor Gene–transduced Chondrocytes with Adeno-associated Virus Vector. *Arthritis & Rheumatism,* Vol.52, No.1, (January 2005), pp. 164-170, ISSN 0004-3591

Zhang, X., Mao, Z. & Yu, C. (2004). Suppression of Early Experimental Osteoarthritis by Gene Transfer of Interleukin-1 Receptor Antagonist and Interleukin-10. *Journal of Orthopaedic Research*, Vol.22, No.4, (July 2004), pp. 742-750, ISSN 0736-0266

Zhou, H.S., Liu, D.P. & Liang, C.C. (2004) Challenges and Strategies: The Immune Responses in Gene Therapy. *Medicinal Research Reviews,* Vol.24, No.6, (November 2004), pp. 748-761, ISSN 1098-1128

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The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.

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