Review

Over-production of therapeutic growth factors for articular cartilage regeneration by protein production platforms and protein packaging cell lines

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Abstract: This review article focuses on the current state-of-the-art in the area of cellular and molecular biotechnology for over-production of clinically relevant therapeutic and anabolic growth factors. We discuss how the currently available tools and emerging technologies can be used for the regenerative treatment of osteoarthritis (OA). Transfected protein packaging cell lines such as GP-293 cells may be used as "cellular factories" for large-scale production of therapeutic proteins and pro-anabolic growth factors, particularly in the context of cartilage regeneration. However, when irradiated with gamma or x-rays, these cells lose their capacity for replication, which actually makes them safe for use as a live cell component of intra-articular injections. This innovation is already here, in the form of TissueGene-C, a new biological drug which consists of normal allogeneic primary chondrocytes combined with transduced GP2-293 cells that overexpress the growth factor transforming growth factor β1 (TGF-β1). TissueGene-C has revolutionized the concept of cell therapy, allowing drug companies to develop live cells as biological drug delivery systems for direct intra-articular injection of growth factors whose half-lives are in the order of minutes. Therefore, in this paper, we discuss the potential for new innovations in regenerative medicine for degenerative diseases of synovial joints using mammalian protein production platforms, specifically protein packaging cell lines, for over-producing growth factors for cartilage tissue regeneration and give recent examples. Mammalian protein production platforms that incorporate protein packaging eukaryotic cell lines are superior to prokaryotic bacterial expression systems and are likely to have a significant impact on the development of new humanized biological growth factor therapies for treating focal cartilage defects and more generally for the treatment of degenerative joint diseases such as OA, especially when injected directly into the joint.

Keywords: osteoarthritis; articular cartilage; degeneration; regeneration; therapeutic protein; growth factor; protein production platform; protein packaging cell line; transforming growth factor β (TGF-β); GP2-293 cells; TissueGene-C

 doi:10.20944/preprints202007.0674.v1

1. Introduction

Growth factors (GFs) are evolutionary-conserved proteins that enhance the growth, proliferation, migration, survival, and differentiation of a variety of cell types [1–3]. They have the capacity to regulate the specialized function and phenotype of cells, whether they are added directly to cells or co-cultured with cells that have been engineered to over-express them [4]. GFs can stimulate proliferation in many cell types but there are a number of cell types, including mature neurons, that are postmitotic and cannot re-enter the cell cycle. Therefore, precursors and progenitors of more specialized cells can be stimulated with GFs to stimulate proliferation and differentiation [5]. GFs and their receptors can be grouped into 'families,' based upon shared features of amino acid sequence, and into 'superfamilies,' based upon shared structural folds [6–8]. Many GF families display significant evolutionary conservation in sequence; for example, homologs of the fibroblast growth factor (FGF), epidermal growth factor (EGF), and transforming growth factor β (TGF-β) families can be found across the animal kingdom, playing important roles in growth, tissue remodeling and repair [9,10]. However, higher vertebrates have larger GF families than invertebrates. For example, there are currently 22 members of the FGF gene family in the human genome, but only one in *Drosophila melanogaster* and *Caenorhabditis elegans* [11].

GFs are relatively small and stable polypeptides that are secreted by cells in the body [12]. GFs are present in the extracellular matrix (ECM) as secreted or membrane-bound proteins [13]. GFs can regulate a variety of cellular behaviors including growth, migration, differentiation, apoptosis, and survival, in both positive and negative manners, in the context of homeostasis and neoplasia [14–16]. GFs produced by stem cells have an array of functions during development, and play important roles in the maintenance of tissue homeostasis and wound healing in the adult skin [17] and in other connective tissues such as articular cartilage [18]. IGF-I and basic FGF have been shown to augment articular cartilage repair *in vivo* [18].

The transforming growth factor-β (TGF-β) superfamily is encoded by 33 genes and includes TGF-β, bone morphogenetic proteins (BMPs) and activins [19–22]. Recent evidence suggests that TGFs, BMPs and activins have important roles in regulating immune responses in the context of infection, inflammation and cancer [23–25]. TGF-β is the prototype member of the TGF-β family of growth and differentiation factors[26]. It is the best-studied factor among the TGF-β family proteins, with its diversity of roles in the control of cell proliferation and differentiation, wound healing and immunoregulation, and key roles in pathology, for example, in skeletal diseases, fibrosis, and cancer [26]. In the synovial joint TGF-β is a pleiotropic cytokine that is important for regulation of tissue homeostasis, degeneration and regeneration [27–30]. Its action on articular cartilage is particularly dependent upon the context in which it acts, eliciting seemingly opposite effects under different experimental conditions; it may counteract pathological changes in a young healthy joint, altering its signaling during ageing and may be an active participant in pathology in OA joints [30]. In the context of the present review, promotion of TGF-β activity in articular cartilage and inhibition of TGF-β activity in subchondral bone may provide new avenues of treatment for OA [31].

GFs can be produced by genetic engineering in the research laboratory setting, and exploited using biotechnology platforms for further applications and used in various clinical, therapeutic and regenerative contexts [32,33]. In this paper we focus on GFs for cartilage regeneration. We review the current state-of-the-art in the area of cellular and molecular biotechnology for over-production of clinically relevant therapeutic proteins. We propose that transfected and irradiated protein packaging eukaryotic cell lines may be used as "cellular factories" for over-production of therapeutic proteins and pro-anabolic growth factors, particularly in the context of regenerative medicine for treating focal cartilage defects and degenerative diseases of the joints, such as osteoarthritis (OA). These cellular tools may be used to produce cocktails and combinations of GFs for intra-articular injection in concentrated format or even injected in live cells form directly into synovial joints, combined with allogeneic primary cells (i.e. chondrocytes), chondroprogenitors or mesenchymal stem cells (MSCs). In the future, it is conceivable to combine different clones of live genetically engineered cells as viable protein factories producing different combinations of growth factors or pro-inflammatory cytokine antagonists for intra-articular injections.

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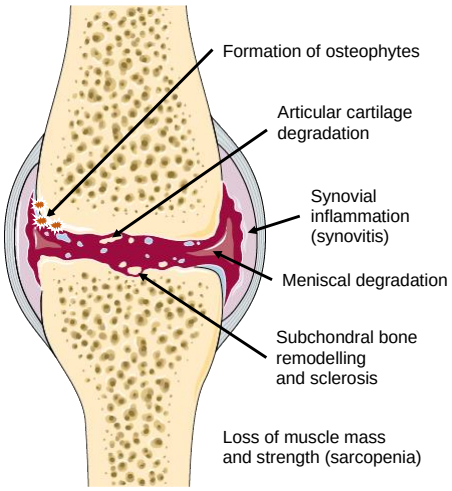
2. Osteoarthritis (OA)

OA is a progressive and degenerative condition that causes load-bearing synovial joints to become painful and stiff [34]. According to the World Health Organization (WHO), OA is the most common type of arthritis affecting millions of people worldwide [1]. Although the main symptoms of OA are joint pain and stiffness, some patients also experience swelling (effusion), tenderness and a grating or crackling sound when moving the affected joint. OA can occur in any joint, but the disorder most commonly affects joints in knees, hips, hands and the spine. While OA is related to ageing, it is, along with many other forms of chronic disease, also associated with a variety of both modifiable and non- modifiable risk factors, including: obesity [35], sedentarism [36] and lack of physical exercise [37], genetic predisposition, bone deformities or reduced bone mineral density, occupational injuries, repeated stress and trauma in sport, certain metabolic and endocrine diseases and, importantly, the female gender, especially after menopause. . In terms of disease initiation, it is thought that there is a long and asymptomatic "molecular phase", which is followed many years later by radiographic changes and the appearance of symptoms [38]. In addition to aging, obesity, gender and genetics, inciting risk factors for OA may include previous joint trauma or repetitive injuries or the presence of metabolic and endocrine disease [39]. There are biomechanical [40], inflammatory [41] and metabolic [42] factors that have been shown to play key roles in the initiation and progression of the disease. The major risk factors for OA are summarized in Figure 1.



Risk factors for the development of OA
Ageing - The risk of osteoarthritis increases with age
Sex and the female gender - Women are more likely to develop OA especially after the age of 50 and the onset of the menopause
Obesity - Excess body weight adds additional stress to weight-bearing joints, such as your hips and knees. Excess white fat produces inflammatory cytokines that can cause further degradation and inflammation in and around the joints
Joint injuries - Sports and injuries can increase the risk of OA. Even injuries that have occurred many years ago and seemingly healed can increase your risk of developing OA
Repeated stress on the joint – Related to occupations and sport that place repetitive stress on a joint
Genetics - Some people inherit a tendency to develop OA and there are genes associated with the development of OA in the knees, hips, hands and the spine
Bone deformities - Some people are born with malformed joints with defective cartilage
Metabolic and endocrine - These may include diabetes, alkaptonuria hemophilia and hemochromatosis

Figure 1. Major structural changes that occur in the joint and risk factors for the development of osteoarthritis (OA).

[1] https://www.who.int/medicines/areas/priority_medicines/Ch6_12Osteo.pdf

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3. Growth Factors and OA

GFs are important for the synthesis and maintenance of articular cartilage *in vivo* and *in vitro* [18,43–45]. The use of bioactive GFs is under consideration as a potential therapy to enhance healing of chondral injuries and modify the arthritic disease process [46,47]. The most important growth factors that are relevant to cartilage homeostasis are summarized in Table 1.

Growth factor	Function	References
Platelet-derived growth factor (PDGF)	Regulates the secretion and synthesis of collagen	[47–49]
Epidermal growth factor (EGF)	Stimulates cellular proliferation, endothelial chemotaxis and angiogenesis	[50,51]
Vascular endothelial growth factor (VEGF)	Increases angiogenesis and vascular permeability	[52]
Transforming growth factor-β (TGF-β)	Stimulates the proliferation of undifferentiated mesenchymal stromal cells (MSCs), stimulates chemotaxis of endothelial cells and angiogenesis	[53]
Basic fibroblast growth factor (bFGF)	Promotes the growth and differentiation of chondrocytes and osteoblasts stimulates mitogenesis of mesenchymal cells, chondrocytes and osteoblasts	[54,55]
Connective tissue growth factor (CTGF)	Contributes to joint homeostasis and OA severity by controlling the matrix sequestration and activation of latent TGF-β	[56,57]

Table 1. Major growth factors involved in cartilage homeostasis, the development of osteoarthritis (OA) and applications in cartilage and bone repair and regeneration. Some of the growth factors listed have negative as well as positive impacts on joint tissues [58].

4. Mammalian Protein Production Platforms

Mammalian cell lines derived from human, mouse and hamster tissues (Figure 2) are excellent hosts for the production of complex recombinant proteins that require extensive folding, the assembly of multiple subunits and posttranslational modifications including N-glycosylation and many others. Over the past 20 years the industrial demand for recombinant therapeutic proteins has significantly increased [59,60]. Mammalian protein production platforms and protein packaging cell lines have been extensively used to produce recombinant proteins [61,62]. For these reasons, such mammalian cells are widely used by the pharmaceutical and biotechnology industries for the large-scale production of recombinant proteins [63–65], which may include diagnostic and therapeutic proteins, peptides, antibodies and antibody fragments [66]. Different mammalian cell platforms are used according to the quantity and quality of the desired product required and the platforms can be scaled according to yield requirements [67]. The most commonly used mammalian cell lines found in the research and industrial therapeutic protein production settings are Chinese hamster ovary cells (CHO) [68,69] and human embryonic kidney 293 cells (HEK-293) [70]. Some of these expression systems are transient, whereas others are stable.

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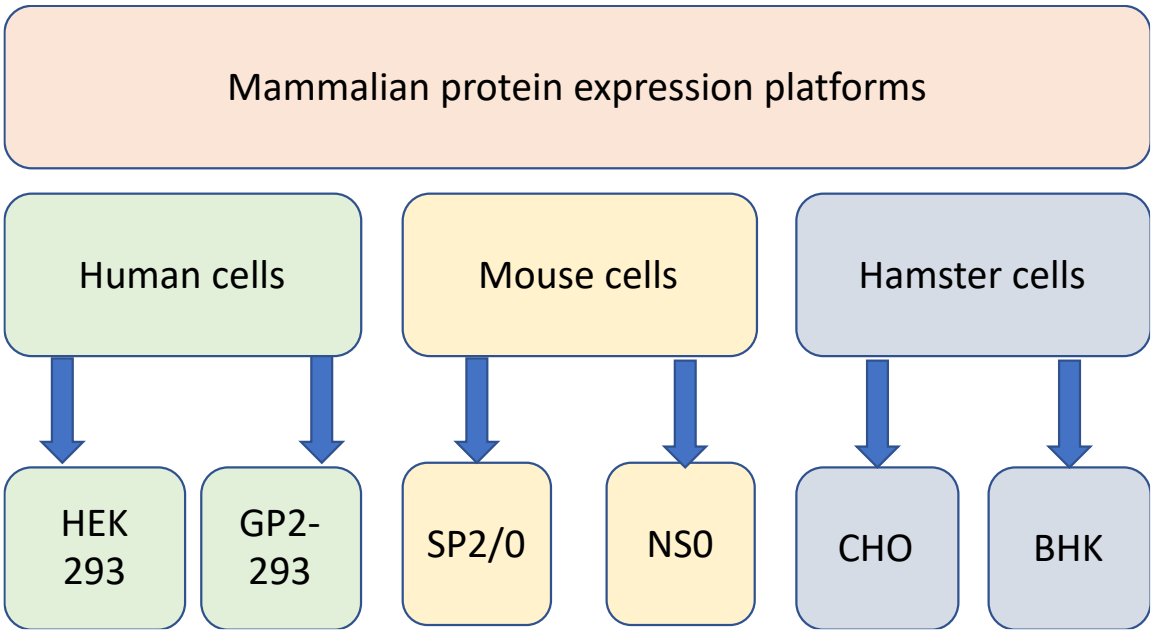

Figure 2. Mammalian protein production platforms using human, mouse and hamster cell lines.

4.1. Transient Expression Systems for Recombinant Proteins

Transient expression platforms for the production of mammalian proteins often use human HEK-293 or hamster CHO cells [71,72]. HEK-293 is a cell line derived from human embryonic kidney cells grown in tissue culture and is widely used in cell biology and biotechnology [73]. The cells were derived from human embryonic kidney but the phenotypic origin of the cells is thought to be neuronal [2]. CHO cells are an epithelial cell line derived from the ovary of the Chinese hamster [69]. CHO cells are used in diverse biological and medical research applications [74]. CHO cells are also used commercially for the production of clinically relevant therapeutic proteins. Glycoengineering of CHO cells is a thriving area of research focusing on enhanced glycosylation capabilities for highly glycosylated proteins [74]. However, many of these expression systems are "transient", meaning that they can only be manipulated acutely to drive over-expression of a desired protein for a given period of time. Therefore, they enable the rapid production of milligram to gram quantities of protein on a flexible scale within a few weeks. Detailed description of the workflow involved in the development of such tools is beyond the scope of this review article but in essence it involves the transfer of the gene of interest into an expression vector, over production and purification of the recombinant protein, quantification of the protein, determination of protein integrity, and specific functional studies, if necessary [75]. However, there are industrial applications that require sustained and stable production, packaging and secretion of proteins.

4.2. Stable CHO Cell Line Development

It is possible to develop stable cell lines that continuously produce a target protein of therapeutic value. Establishing highly productive clonal cell lines with constant productivity over 2–3 months of continuous culture is extremely challenging, but possible and has already been achieved [76]. Transfected CHO DG44 cells are often used as a model for this purpose. They are cultivated under several rounds of methotrexate selection [77]. Monoclonal CHO-derived cell lines may be generated by subcloning pools of the most productive cells, and clone stability is confirmed. For example, stable cell lines have been designed to produce recombinant monoclonal anti-tumor necrosis factor α (TNF-α) antibody [78]. Once the clone has been established, the cells may be frozen and archived or shipped and distributed to other locations and banked at multiple sites.

[2] https://www.hek293.com/

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4.3. Mammalian Protein Production Platforms for Large Scale Production of Therapeutic Proteins

Mammalian protein production platforms have important advantages as eukaryotic expression systems and are currently being employed as indispensable "cellular factories" for the large-scale production of humanized therapeutic antibodies and proteins [79]. Mammalian cell expression systems can now support the large-scale production of proteins, especially of those of clinical relevance and human origin [80]. Over the last few decades these platforms have gradually evolved and found new applications in biology, biotechnology and medicine. Protein production platforms have had a profound impact in many areas of basic and applied research, and an increasing number of biological drugs and vaccine antigens are now recombinant mammalian proteins made using these tools [81]. Recombinant proteins and a vast array of monoclonal antibodies are now produced in mammalian cell lines instead of bacterial expression systems (i.e. *Escherichia coli*) to ensure that proper protein folding and post-translational modifications, which are essential for full biological activity, are properly introduced in the most appropriate eukaryotic and "mammalian" context. Mammalian cell expression systems are the dominant tools for producing complex biotherapeutic proteins [82]. As already mentioned, various mammalian expression systems are also being used for protein and glycoprotein production and recent cellular engineering strategies have been developed to increase glycoprotein productivity [83], an important feature that bacterial expression systems do not possess.

5. Viral and Non-Viral Gene Therapy for OA

Most of the early experimental progress in the area of gene therapy for OA was made with gene transfer to the synovium, a tissue that is particularly amenable to genetic modification by a variety of gene vectors, using both *in vivo* and *ex vivo* protocols [84]. However, despite the importance of targeting inflammatory pathways in the synovium to treat the synovitis associated with OA, the main research priority has been regenerative therapy for joints the focus has specifically been on cartilage regeneration and this is also where most of the gene therapy has been focused [85–88], The focus so far has been upon the transfer of genes whose products enhance synthesis of the cartilage ECM, or inhibit its breakdown, although there is certainly room for finding novel and alternative targets, which may include cytoprotective factors and molecular chaperones. There are also numerous possibilities and opportunities for targeting multiple catabolic and anabolic pathways, and, upstream regulators of key catabolic switches in chondrocytes and synoviocytes.

Early work on non-viral gene delivery used cationic liposomes and non-liposomal lipid formulations for cartilage regeneration. Goomer et al., proposed tested von-viral *in vivo* gene therapy for articular cartilage and tendon repair [89]. They were successfully able to transfect TGF-β1, parathyroid hormone related protein (PTHrP) and another marker gene into primary perichondrium and articular chondrocyte *in situ* with efficiencies of over 70%. They also demonstrated the efficacy of expression in a rabbit model of osteochondral defect repair a canine model of intrasynovial flexor tendon injury and repair [89].

Madry et al. used isolated lapine chondrocytes transfected with an expression plasmid vector carrying the *P. pyralis* luciferase gene by a lipid-mediated gene transfer method using the reagent FuGENE 6 [90]. In their pioneering work they proposed that lipid-mediated gene transfer to primary chondrocytes within a gel suspension delivery system directly into osteochondral defects and the sustained expression of the transgene *in vivo* may facilitate cartilage repair and may provide alternative treatments for articular cartilage defects [90].

The same team of researchers pushed the boundaries even further by demonstrating that therapeutic growth factor gene delivery (with cDNA encoding the human insulin-like growth factor I (IGF-I) as an exemplar) using encapsulated and transplanted genetically modified chondrocytes may be applicable to sites of focal articular cartilage damage [91]. They also tested gene transfer of human fibroblast growth factor 2 (FGF-2) via transplantation of encapsulated genetically modified articular chondrocytes to show that overexpression of FGF-2 enhances the repair of cartilage defects via stimulation of chondrogenesis [92].

Developing this technology also allowed these authors to look at co-overexpression of IGF-I/FGF-2 for early repair of cartilage defects *in vivo* and testing the prospect of providing protection

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for healthy neighboring cartilage tissue [93]. This also raises the possibility of achieving synergistic analgesic effects by combining different genes in a combination therapy approach, as used in the treatment of cancer. The data provided by this team demonstrate that combined delivery of genes encoding multiple therapeutic growth factors to cartilage defects may have clinical value for promoting cartilage repair *in vivo*. The protective effect of combined IGF-I/FGF-2 co-overexpression on the neighboring articular cartilage is certainly interesting in the context of protecting healthy cartilage from damage, for example in the high-impact sport context.

Recombinant adenovirus associated vectors may be used to directly transfer candidate gene sequences in human articular chondrocytes *in situ*, providing a potent tool to modulate the structure of OA cartilage. Although very few preclinical animal studies in OA models have been performed thus far, equine models of OA have been proposed for proof of concept studies in translational models [94]. Several gene therapy clinical trials have also been carried out in patients with end-stage knee OA based on the intraarticular injection of human juvenile allogeneic chondrocytes overexpressing a cDNA encoding TGF-β1 via retroviral vectors [95].

Targeting the synovium is another approach and it has already been tried but unfortunately, it has not been possible to build a convincing clinical case for targeting interleukin-1 (IL-1β) as a key mediator of cartilage loss in OA, as the clinical trials conducted by AbbVie for targeting IL-1β have produced generally disappointing results. Nevertheless, the therapeutic effects of IL-1β receptor antagonist (IL-1Ra) gene transfer have been confirmed in three different experimental models of OA [84] and it is likely that targeting IL-1β may still be a viable solution for targeting the more inflammatory phenotypes of human OA [96]. Gene therapy may be combined with cell therapy for developing innovative new treatments for OA.

6. Cell Therapy for OA

A detailed discussion of cell and stem cell therapy for OA is beyond the scope of this review. The readers are referred to a series of excellent research and review articles that cover this topic [97,98] including several comprehensive reviews from our own group [99] that discuss the potential for using primary chondrocytes [100] adipose, bone marrow and synovial mesenchymal stromal cells (MSCs) [101–103], menstrual blood-derived stem cells [104] and induced pluripotent stem cells (iPSCs) [105]. The most important point to make is that MSCs derived from patients with advanced OA exhibit attenuated chondrogenic activity, suggesting that these cells may be poor candidates for cell-based therapies for OA [106].

7. Therapeutic Growth Factors for the Treatment of OA

As outlined in the previous section, a number of GFs have been proposed as novel biological agents for cartilage regeneration [107]. GFs represent a broad range of biologically active agents that are capable of activating and stimulating the growth and repair of damaged tissues as well as protecting cells from premature death [108]. They therefore offer a very promising avenue for both treatment and further study, especially in the context of OA. As previously stated, cartilage degradation and subsequent OA is more common in people aged over 50 years, but people of any age have a significantly increased risk of cartilage and joint damage that may lead to post-traumatic osteoarthritis after sports or other joint injuries. GF treatments offer potential benefits to prevent OA (especially at the earliest stages of disease pathogenesis) later in life, as well as being an immediate consideration after sports injury, when the prevention of further damage is a priority.

8. GP2-293 Protein Packaging Cells in TissueGene-C

TissueGene-C, developed by Kolon is a revolutionary cell and gene therapy for the treatment of knee OA. TissueGene-C is undoubtedly the current state-of-the-art cell and gene therapy platform for the treatment of OA. In this product transfected and irradiated protein packaging cell lines are used as “cellular factories” for production of therapeutic TGF-β1. TissueGene-C is actually a unique combination of cell and gene therapy targeting knee OA through a single intra-articular injection of joint-derived chondrocytes, irradiated GP2-293 cells (derived from HEK293 cells and, most importantly, the biological GFs that they acutely overproduce to possibly promote anabolic repair

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and regeneration in the diseased joint" as a future possibility in the treatment for OA. The general concept for TissueGene-C is presented in Figures 3 and 4.

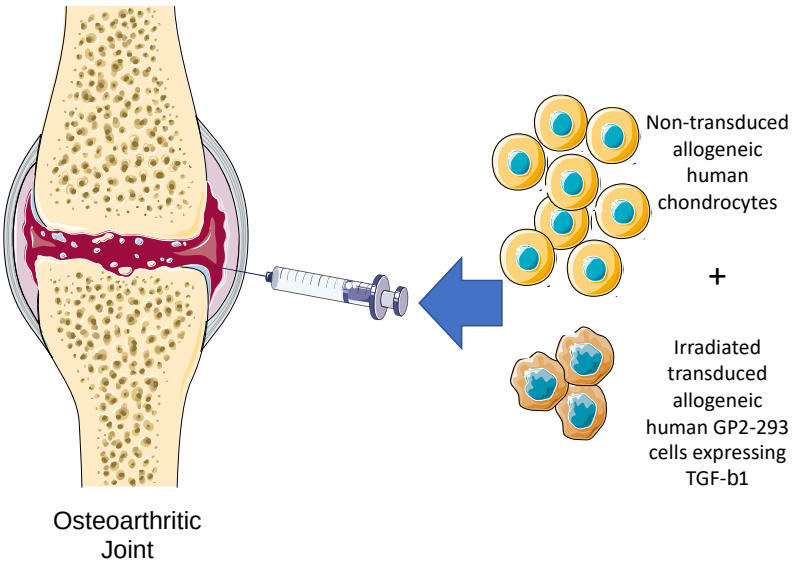

Figure 3. The intra-articular injection concept for TissueGene-C, a novel cell and gene therapy targeting knee OA through a single intra-articular injection of non-transduced allogeneic human chondrocytes, irradiated transduced allogeneic human GP2-293 cells that produce TGF-β1, the biological growth factor that promotes anabolic repair and regeneration.

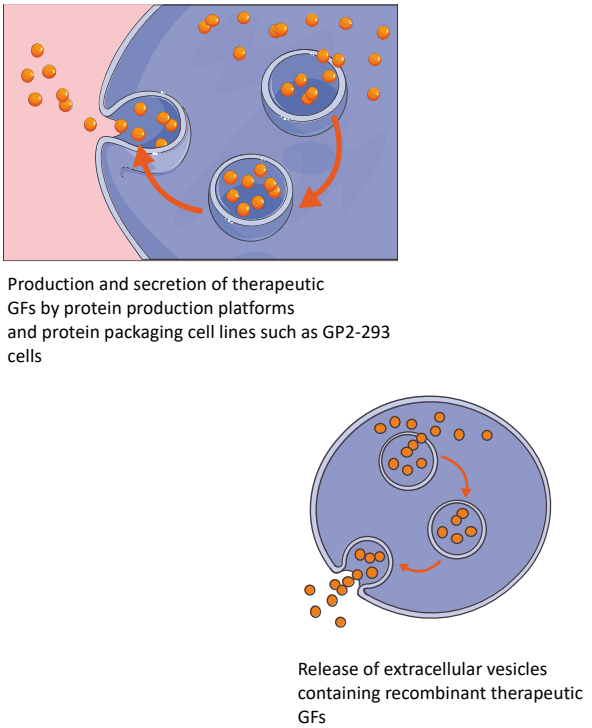

Figure 4. Production and secretion of GFs by GP2-293 cells in TissueGene-C.

The human GP2-293 cell line is one of the key components of TissueGene-C. These cells carry out the vital function of over-producing the crucially important TGF-β1. The GP2-293 cells have been used throughout the whole developmental process from the first production of the Master Cell Bank (MCB) to the next step, which is the development of the working cell bank and the final product

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formulation. As mentioned earlier, GP2-293 is a HEK 293-based retroviral packaging cell line used for large-scale protein production and packaging. It is a cellular platform for over-production of therapeutically relevant human proteins. This is the first time that such a human protein production platform has been employed in the context of OA treatment and cartilage regeneration. Effectively these cells are a protein producing tool and "cellular factory". Native patient derived chondrocytes simply do not have the capacity to over-produce TGF-β1 in sufficiently high quantities for effective cellular therapy and regenerative applications. Transduced and irradiated GP2-293 cells may be transformed cells but since they have lost their capacity for proliferation, they cannot proliferate. Therefore, the GP2-293 cells in TissueGene-C cannot survive and proliferate in the joint. These cells will simply carry out their transient function as radiation inactivated transfection models, protein packaging tools and "cellular factories" for over-production of therapeutic TGF-β1. Therefore, the cells cannot survive for more than a very short period after being injected into the joint. Furthermore, no drug-related serious side effects were identified from those subject to clinical trials[3]. After the cells carry out their TGF-β1 production duties, they will die and their remains will be cleared by joint resident macrophages through the process of phagocytosis (Figure 5).

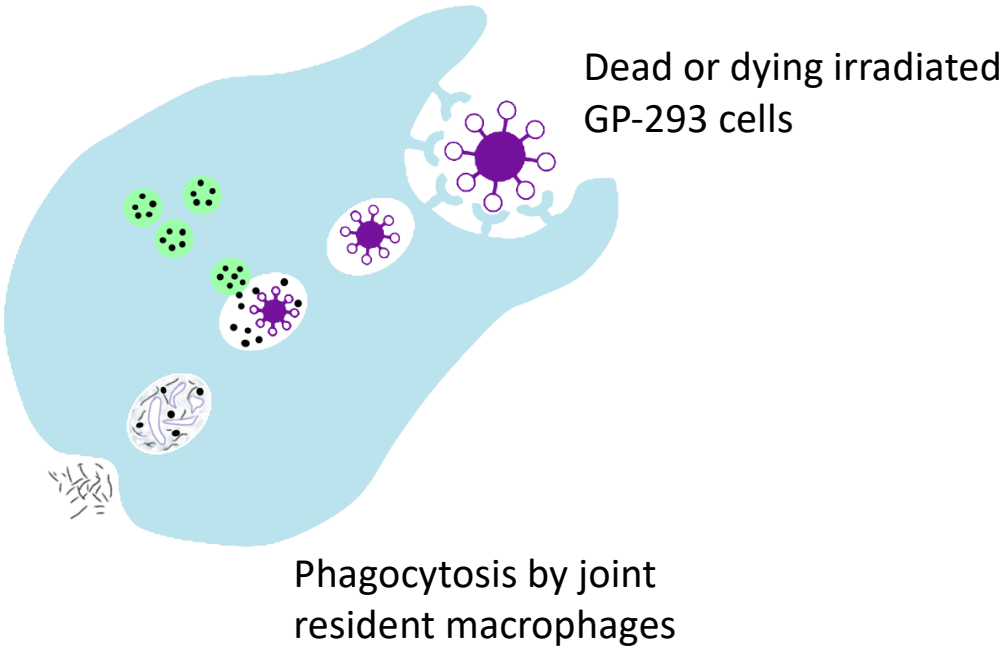

Figure 5. Phagocytosis and destruction of dead GP2-293 and their cellular debris by joint resident macrophages.

The scientific basis for the use of mammalian cell transfection models is clear in the development of TissueGene-C. There is a well-established literature on the use of HEK-293 cells and their GP2-293 derivatives as transfection and cell culture models for protein production. The efficacy and safety of HEK-293 cells and their GP2-293 derivatives in regenerative medicine has not been extensively

[3] http://www.businesskorea.co.kr/news/articleView.html?idxno=32318

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reviewed but the prospects for future use of transfection tools in regenerative medicine and cell therapy is very positive, especially since native and untransformed cells do not have the appropriate regenerative capacity.

9. Conclusions

The concept of using gene therapy for cartilage repair originates from the idea of transferring genes encoding therapeutic growth factors into the joint to promote tissue repair and regeneration [109–112]. Delivering genes into a degenerate synovial joint, even temporarily, could induce changes in the inflammatory micro-environment. Whether the genes are targeted to the synovial space, the synovium or articular cartilage, the spatially defined delivery of therapeutic molecules to sites of cartilage damage could facilitate endogenous tissue repair and regeneration [109]. However, gene expression levels may be low, inefficient or overwhelmed by the inflammatory and catabolic micro-environment. Genetically engineered cells can provide the "factories" for the transcription of genes encoding therapeutic growth factors. Conventionally, cells have been used to over-produce proteins and then the over-produced proteins have been used in purified and concentrated form experimentally in therapeutic applications. However, it is also possible to use live cells to deliver a therapeutic protein in a continuous but time-limited manner. The ability to deliver large quantities of a therapeutic growth factor in a time-limited manner is important, especially for TGF-β, because sustained production of this growth factor can drive osteophyte formation and synovial fibrosis in OA joints [113–117]. Although TGF-β acts as a protective, load-induced factor in a young healthy young joint, its sustained presence can function as a deleterious factor in an OA joint [118,119]. The protective function of TGF-β is decreased in aged cartilage compared to young and this is one of the reasons for using TGF-β as a therapeutic factor. Cell factories, such as GP-293 cells have the capacity to over-produce large quantities of this growth factor and if they have been previously irradiated and rendered incapable of replication, they then gradually decline in activity and die off within the synovial joint, thus avoiding any possibility of long-term concerns about osteophyte formation and synovial fibrosis. Importantly, irradiation of the cells also renders them incapable of replication and survival beyond a few days in the synovial space.

Therapeutic strategies that combine cell and gene therapy are now a reality with significant potential for clinical development in orthopedics and rheumatology [120,121]. However, cell and gene therapy already rely on protein production platforms and protein packaging cell lines as the indispensable tools for over-production of the desired therapeutic proteins. Future cell and gene therapy strategies for treating OA and promoting cartilage and intervertebral disc (IVD) regeneration may exploit the potential for using mammalian protein production platforms and irradiated and transfected protein packaging cell lines for over-production of therapeutic proteins and growth factors, individually or in combination, within the synovial joint or even in the IVD [122,123]. Further developments in this area may include combinations of cell clones that over-produce several growth factors and cell and gene therapies that may be used to target other joint tissues and the IVD in the spine [123] (Figure 6).

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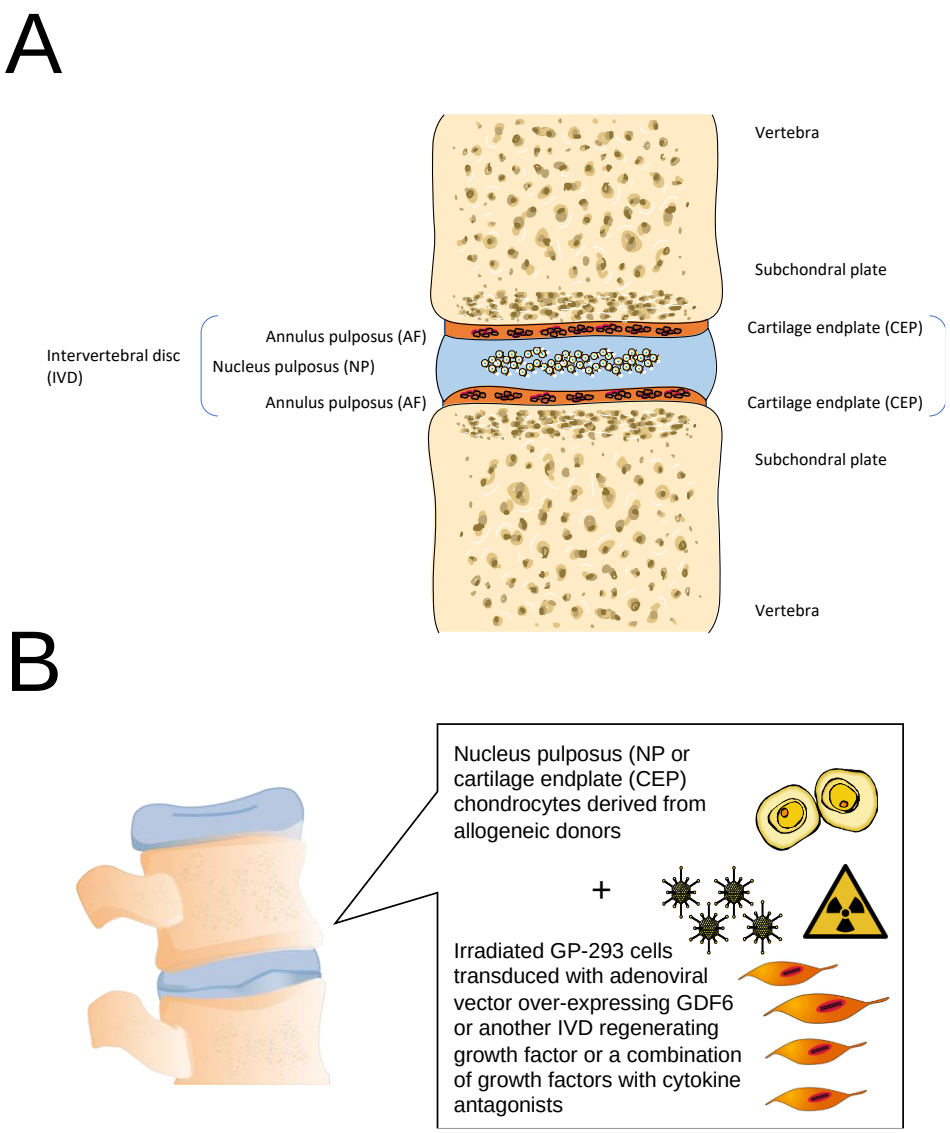

Figure 6. A. structure of the healthy intervertebral disc (IVD). B. General concept for cell and gene therapy targeting the nucleus pulposus (NP) and the cartilage endplate (CEP) in the IVD.

Most of the emerging biological agents in the current drug development pipelines are produced using mammalian protein production platforms[124]. Protein production tools are essential for large-scale production of therapeutic proteins and growth factors. These tools may be used to generate functional native and mutant proteins with appropriate folding, assembly and posttranslational modifications. In most cases these platforms have been used to produce therapeutic proteins that are then purified, characterized and incorporated into products and derivatives. However, there is potential for using protein packaging cells in new therapies that include live cells for transient release of large concentrating of growth factors with short half-lives directly into the joint or the spine. The concept for Tissue Gene-C has been reviewed in this paper as the current state of the art for the treatment of knee OA [122]. A study published in July 2020 investigated the efficacy and mechanism of action of Tissue Gene-C in a rat model of OA. Using the monosodium-iodoacetate (MIA) model of OA. We demonstrated that Tissue Gene-C provides pain relief and cartilage structural improvement in the MIA OA model over 56 days. In parallel with these long-term effects, cytokine profiles obtained on day 4 revealed increased expression of interleukin-10 (IL-10), an anti-inflammatory cytokine, in

the synovial lavage fluid. Moreover, the increased levels of TGF-β1 and IL-10 stimulated by Tissue Gene-C induced the expression of arginase 1, a marker of M2 macrophages, and decreased the expression of CD86, a marker of M1 macrophages. These novel results suggest that Tissue Gene-C exerts a beneficial effect on OA by inducing a M2 macrophage-dominant micro-environment [125].

The stark realization that many primary, aged and senescent chondrocytes and MSCs possess feeble regenerative properties means that future regenerative medicine and tissue engineering strategies for the joints and the spine could use primary allogeneic cells or stem cells combined with mammalian protein production platforms to drive the production of therapeutic proteins and pro-anabolic growth factors [122,123]. We now have the capacity to manipulate the replicative potential of the cellular component of protein production platforms by x-ray or gamma irradiation and make them replication incompetent. This will allow us to inject them directly into the joint in order to drive GF production in controllable way. Advances in the field of molecular and cellular biotechnology and the development of new and more robust and customizable protein production platforms are likely to have a positive impact on the treatment of arthritic diseases, tissue engineering and regenerative treatments for the musculoskeletal system.

Author Contributions: Conceptualization, A.M., H.C. and P.M-V. writing—original draft preparation, A.M. and P.M-V.; writing—review and editing, A.M,. H.C., J.S. and P.M-V.

Funding: A.M. has received funding from the following sources: The European Commission Framework 7 programme (EU FP7; HEALTH.2012.2.4.5-2, project number 305815; Novel Diagnostics and Biomarkers for Early Identification of Chronic Inflammatory Joint Diseases). The Innovative Medicines Initiative Joint Undertaking under grant agreement No. 115770, resources of which are composed of financial contribution from the European Union's Seventh Framework programme (FP7/2007-2013) and EFPIA companies' in-kind contribution. A.M. also wishes to acknowledge funding from the European Commission through a Marie Curie Intra-European Fellowship for Career Development grant (project number 625746; acronym: CHONDRION; FP7-PEOPLE-2013-IEF). A.M. also wishes to acknowledge financial support from the European Structural and Social Funds (ES Struktūrinės Paramos) through the Research Council of Lithuania (Lietuvos Mokslo Taryba) according to the activity 'Improvement of researchers' qualification by implementing world-class R&D projects' of Measure No. 09.3.3-LMT-K-712 (grant application code: 09.3.3-LMT-K-712-01-0157, agreement No. DOTSUT-215) and the new funding programme: Attracting Foreign Researchers for Research Implementation (2018-2022).

Acknowledgments: We would like to acknowledge members of our research teams and collaborators for their support and encouragement.

Conflicts of Interest: The authors except A.M and P.M-V. are current or past employees of Kolon Life Science, Inc. developing TissueGene-C. This does not alter our adherence to all journal's policies on sharing data and materials. A.M and P.M-V. declare no financial conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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