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# Matrix-based gene delivery for tissue repair

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## Abstract

Scaffolds for tissue repair must provide structural and biochemical cues to initiate the complex cascade of events that lead to proper tissue formation. Incorporating genes into these scaffolds is an attractive alternative to protein delivery since gene delivery can be tunable to any DNA sequence and genes utilize the cells' machinery to continuously produce therapeutic proteins, leading to longer lasting transgene expression and activation of autocrine and paracrine signaling that are not activated with bulk protein delivery. In this review, we discuss the importance of scaffold design and the impact of its design parameters (e.g. material, architecture, vector incorporation, biochemical cue presentation) on transgene expression and tissue repair.

## Introduction

In the design of scaffolds for tissue repair, biochemical, biophysical, and cell-cell signals must be intricately orchestrated to guide the formation of healthy tissue at sites of injury or disease. Ideally, the manner in which these signals are incorporated allows for necessary changes during tissue growth. For example, the biochemical signals that contribute to the start of morphogenesis (tissue growth) are very often detrimental if they are present at the final stages of growth which, in many cases, cause pathological conditions. Thus, the biochemical signals (e.g. peptides, proteins, small molecules) must be introduced such that their activity can be regulated. Proteins are the most common bioactive signal introduced into scaffolds for tissue repair. Although delivery mechanisms have been designed to control release rates of one or multiple proteins, protein stability and cost are still major limitations. For example, the biological half-life of platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are less than 2 [1], 3 [2], and 30 minutes [3], respectively, when injected intravenously. Thus, to achieve therapeutic success, proteins often require large doses and multiple injections [4–6]. Gene delivery has been used as an alternative to protein and protein fragment delivery [7], and it holds the advantage that a universal delivery strategy can be designed for any DNA sequence. A universal delivery strategy is not possible for growth factor delivery since the tertiary and quaternary structures are different for each protein and immobilization or other processing conditions affect each protein differently. Furthermore, the secretion of a protein by a transfected cell may be present for a longer duration. This increased residence time eliminates the need for repeated injections [8] and stimulates autocrine and paracrine



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signaling in tissue formation, which cannot be induced by delivery of the protein to the bulk media [9]. One major limitation of gene delivery is that the cargo is not immediately available as a bioactive signal, whereas proteins can begin their biochemical activation of targeted cells and commence tissue repair immediately after implantation. To this end, successful gene delivery and transfection depends on a series of critical steps, which take several hours to days to commence in vivo, with transgene expression peaking in the order of days after injection for naked plasmid, minicircles [10,11] and polyplexes [12]. Figure 1 details the steps that must occur for gene transfer to take place from the point of view of scaffolds for tissue repair. Figure 2 summarizes the major design characteristics for matrix based gene delivery for tissue repair. In this review, we explore the use of genes as bioactive signals to guide tissue repair from the point of view of scaffold design.

## Vector Design

The two main types of vectors used for gene transfer in the context of tissue repair are plasmid DNA or modified viruses. The major design characteristics for vector design are the attenuation of the immune response, the promoters used to drive expression (Figure 3A), and the therapeutic protein expressed. Table 1 details these major design characteristics for vector design and points the reader to further reading on the subject.

## Delivery of the Vector

Although naked DNA has shown success in the delivery of genes in vivo for tissue repair, the field has moved towards the use of packaged DNA (nDNA), either in synthetic particles or viruses. This review does not intend to focus on delivery vector design, however, the most commonly used delivery vectors used in the context of tissue repair are mentioned in Table 1. The reader is referred to the following recent review articles that focus on this topic [13–15].

## Design of the Matrix

Although the primary focus to enhance transgene expression in vivo has been the design of the delivery vector, the matrix itself can provide alternative approaches to enhance transfection efficiency as well as promote tissue formation. Gene transfer from a matrix offers a three-dimensional distribution of complexes for more controlled, localized transfection as compared to a bolus delivery that may result in an unfavorable systemic delivery or unintended delivery to neighboring organs and tissues. In addition, delivery from a matrix can maintain the level of the vector over time, providing repeated opportunities for transfection/transduction and extending transgene expression as compared to bolus delivery. Incorporation of polyplexes into hydrogels scaffolds have shown sustained expression compared to soluble polyplexes (35 days compared to 7 days) [12]. Below, we review how the matrix has been designed to modulate transgene expression and guide tissue formation (Figure 2).

### Controlled Release of nDNA

Controlled release strategies are often described as an important design parameter for matrix mediated gene transfer, with the belief that sustained release of the transfection vector achieves prolonged transgene expression over burst-released vectors. The hypothesis is that maintaining the level of the vector in the local microenvironment constant (since vector is continuously released) provides repeated opportunities for transfection/transduction resulting in sustained transgene expression. Prolonged transgene expression of a single protein is desired in situation where the tissue takes time to mature. For example, the sustained release of VEGF is necessary to promote the formation of mature vasculature [16].

To achieve controlled release, the nucleic acid is encapsulated within the scaffold during scaffold fabrication, and the release rate is controlled through modulating the degradation rate of the scaffold. In this approach, typically the scaffold degradation rate is not dependent on cellular action but rather it is chemically mediated through processes such as hydrolysis. Additionally, the scaffolds may be highly porous to allow for cellular infiltration within the scaffold such that as the DNA is released, it can reach the infiltrating cells. Poly(lactide-co-glycolide) (PLGA) scaffolds were some of the first to be used for matrix mediated gene delivery [17] and can be designed to release plasmid DNA in hours, weeks or months in vitro and have resulted in sustained transgene expression for up to 105 days [18]. Although controlled release is often cited as a desired quality to ensure long lasting expression, recent data suggest that release rate in vitro does not lead to corresponding differences of transgene expression in vivo. The Shea lab designed PLGA scaffolds with vastly different DNA release rates in vitro and showed they all achieved the same level and duration of transgene expression in vivo [19]. This suggests that in vitro release kinetics for nDNA do not correlate well with in vivo release or that sustained release of DNA is not the reason sustained transgene expression is observed. Hydrogels have also been designed to achieve controlled release. Oxidized alginate hydrogels loaded with DNA/PEI nDNA were shown to achieve sustained release in vitro and achieve enhanced revascularization in vivo [20].

## Controlling Cellular Infiltration into nDNA loaded scaffolds

An alternative approach to controlled release is to design scaffolds that allow cell mediated degradation and cellular infiltration within the bulk of the scaffold. These approaches involve the use of hydrogels either naturally crosslinked (e.g. collagen [21], fibrin [22,23], gelatin [24]) or synthetically crosslinked with protease degradable peptides (e.g. PEG [25], hyaluronic acid [26,27]). The hypothesis in this case is that cells uptake the DNA as they infiltrate the scaffold and thus the transgene expression can be sustained or increased with time. This hypothesis has been proven to be true in vitro with cells embedded in DNA loaded MMP-degradable PEG [28,29] or hyaluronic acid [30] hydrogels, showing sustained transgene expression when the hydrogels were designed to enhance the cellular migration rate. The incorporation of nDNA into protease degradable scaffolds can result in aggregation either due to the interaction of nDNA with the gel precursor solutions such as in the case with fibrin or hyaluronic acid [26], or the interaction of nDNA particles with themselves as in the case for high nDNA concentrations [28]. To prevent such aggregation, a caged nanoparticle encapsulation (CnE) approach has been designed, where the nDNA are generated under dilute conditions and lyophilized in the presence of sucrose and agarose (Figure 3C). The sucrose is used as a cryo-protectant while the agarose functions as an inert polymer that prevents nDNA from interacting with the gel precursor solution and itself. This approach has been shown to result in active and non-aggregated polyplexes [26,31], resulting in transgene expression in vivo in a subcutaneous model (Figure 3C). Since synthetically crosslinked hydrogels have been shown to result in poor cellular infiltration in vivo in areas of low protease expression [27], micron sized pores have been introduced into PEG [32] and HA [27] hydrogels to enhance cellular infiltration and angiogenesis. Lentiviral vectors encoding for VEGF encapsulated in porous PEG hydrogels demonstrated blood vessel formation and lectin-positive cells at 2 and 4 weeks, while significant collagen deposition was observed by 4 weeks when compared to encapsulated lentivirus encoding for luciferase [32].

To achieve further control over transgene expression of encapsulated nDNA and prevent premature release of the nDNA, nDNA has been covalently immobilized to the scaffold backbone. In this case, the release rate and transfection efficiency are either related to the degradation rate of the scaffold (to allow nDNA release and internalization by infiltrating cells surrounding the implant [33]) or the degradation rate of the tether between the nDNA

and the scaffold (which can control release rate or target a particular cell population) [34,35].

### Surface Associated nDNA

A complementary or stand-alone approach to control both release and cellular infiltration involves associating the DNA to the scaffold surface through nonspecific adsorption. The hypothesis in this case is that the loosely associated DNA can achieve sufficient nDNA retention to avoid premature release, allowing transgene expression to promote tissue repair soon after implantation and the embedded DNA (if present) can prolong this expression or express a different gene. Moreover, since nDNA are adsorbed following scaffold formation, it avoids the harsh processing conditions that that may occur during scaffold synthesis and can avoid polyplex aggregation [36]. In vitro, surface associated DNA polyplexes result in enhanced transgene expression compared to embedded polyplexes [37]. Surface associated DNA was the most widely used approach to deliver DNA in vivo from scaffolds this past year. Effective gene transfer and tissue formation was demonstrated with collagen/gelatin meshes or sponges [38–40], silk fibroin scaffolds [41], PLGA multichannel bridges [42], electrospun fibers [43] and collagen/chitosan scaffolds [44] [45].These studies achieved regeneration of critical size defects in animal models and yielded similar results compared to the delivery of recombinant protein.

### Biochemical Cues

Since gene transfer efficiency is correlated with cellular process such as proliferation rate, cellular infiltration rate into the scaffold, and actin/microtubule polymerization or de-polymerization, the scaffold itself can be engineered to enhance transgene expression. Integrin cell adhesion to the scaffold can be engineered to achieve enhanced cell migration and proliferation. Alginate hydrogels conjugated with various RGD densities for siRNA-mediated knockdown of eGFP demonstrated that increasing RGD density resulted in significantly higher knockdown of the targeted protein [46]. Moreover, RGD gradients and presentation (homogeneous vs. clustered) in different scaffolds have been used to influence transfection [30,47]. Hydrogel stiffness can also be used to modulate migration and gene delivery rates; stiffer gels result in slower release rates of encapsulated polyplexes and decreased cell populations, spreading, and transfection [30] (Figure 3B). ECM proteins have also shown to have a significant impact on gene transfer with different ECM molecules enhancing or inhibiting gene transfer in vitro [48,49]. Although the mechanism of the ECM mediated enhancement is not completely understood, RhoGTPases have been shown to play a significant role [48]. The co-delivery of proteins from the scaffold can be used to modulate the proliferative state of the infiltrating cells. Delivery of plasmid encoding for BMP-2 with along with recombinant bFGF encapsulated in PLG microspheres in vivo demonstrated significantly enhanced gene expression and increased blood vessel density compared to pDNA alone [50].

## Future directions and Conclusion

Current tissue engineering approaches to help guide wound healing and tissue repair primarily focus on developing scaffolds to deliver bioactive signals to aid these events. In this report, we aimed to elucidate the complexity of designing gene-loaded scaffolds for tissue engineering. Although this review focused primarily on gene delivery, it is important to note that a successful scaffold may not necessarily be successful based solely on the delivery of proteins or genes, but rather a combination of both. A dual delivery hydrogel system of proteins and genes can utilize the transient expression of protein delivery, and achieve sustained expression through encapsulated or immobilized plasmids. Moreover, studies on gene incorporation, scaffold material, architecture, and presentation of

biochemical cues highlight the importance of how cells experience the local microenvironment and their effect on gene transfer. It is paramount to also investigate strategies to prime cells for transfection which may include providing proliferative cues, ECM components, integrins, and better mimicking the heterogeneity of the cellular microenvironment by incorporating growth factors or plasmids in a gradient or spatially-patterned scaffold. Modulating the scaffold composition in layers may allow future investigations on delivering multiple proteins, genes, or a combination with more control over design parameters (e.g. number of polymeric layers, amount of nucleic acid deposition). As a result, careful consideration of these parameters must be taken to create a successful gene loaded scaffold for regenerative medicine and tissue repair.

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## Highlights

- We present a detailed description of gene loaded scaffold design for tissue repair.
- Infiltrating cells are transfected through released DNA or matrix residing DNA.
- Surface associated of DNA via nonspecific adsorption is a popular current technique.
- Biochemical cues may be used to “prime” cells for transgene expression.

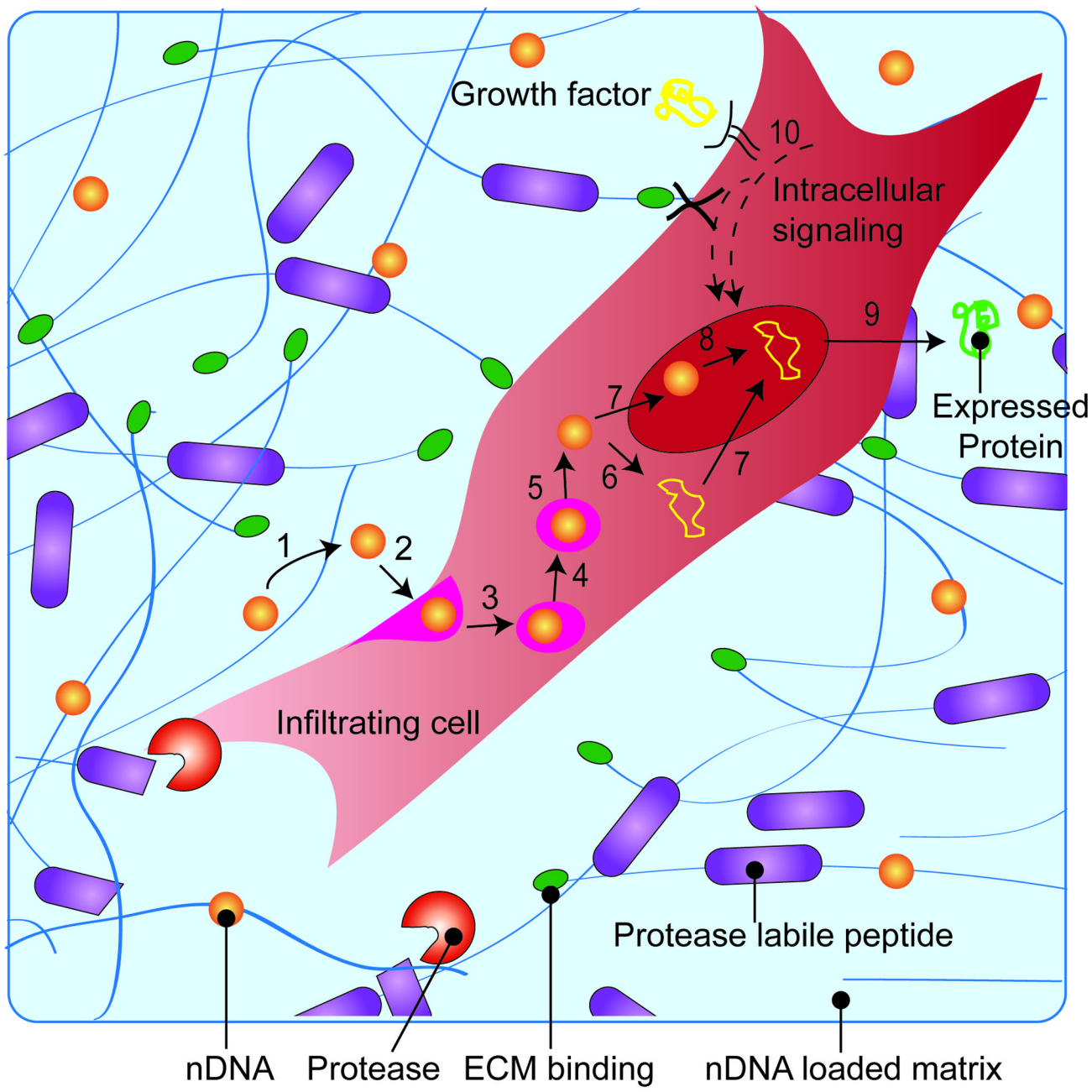


**Figure 1.**

Schematic overview of protein expression. For gene delivery, nDNA (**1**) is released from the scaffold through either hydrolysis or cellular migration (**2**) and internalized into the endosome (**3**). The endosome matures changing its oxidative and acidity resulting in endosomal escape of nDNA (**4**–**5**). nDNA can enter the nucleus (**7**) to be unpacked (**8**) or be de-coupled in the cytosol (**6**) for nuclear entry (**7**), where transcription and translation occurs (**9**) for protein expression. Growth factors or other bioactive signals can be used to induce intracellular signaling pathways that prime cells for transfection (**10**).

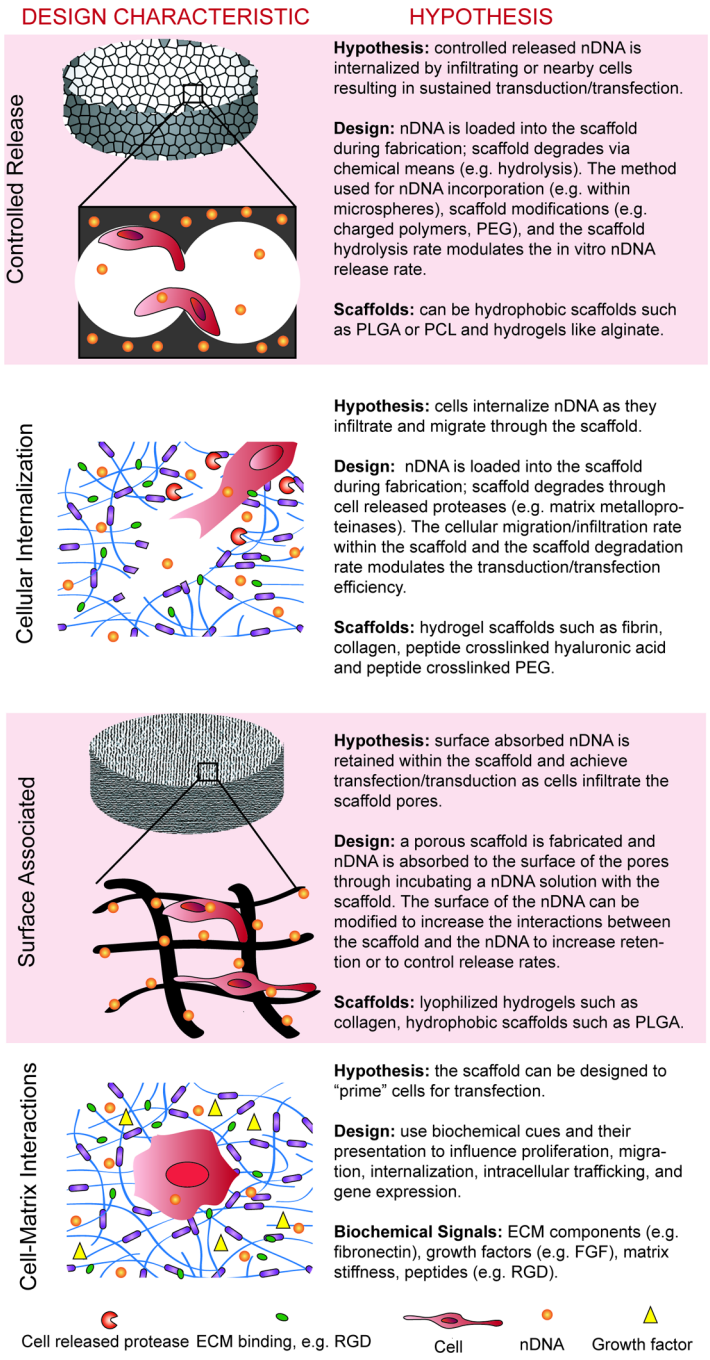


**Figure 2.**

The design of scaffolds for tissue repair that use genes as a bioactive signal goes beyond incorporating the nDNA into the scaffold. See text for corresponding references.

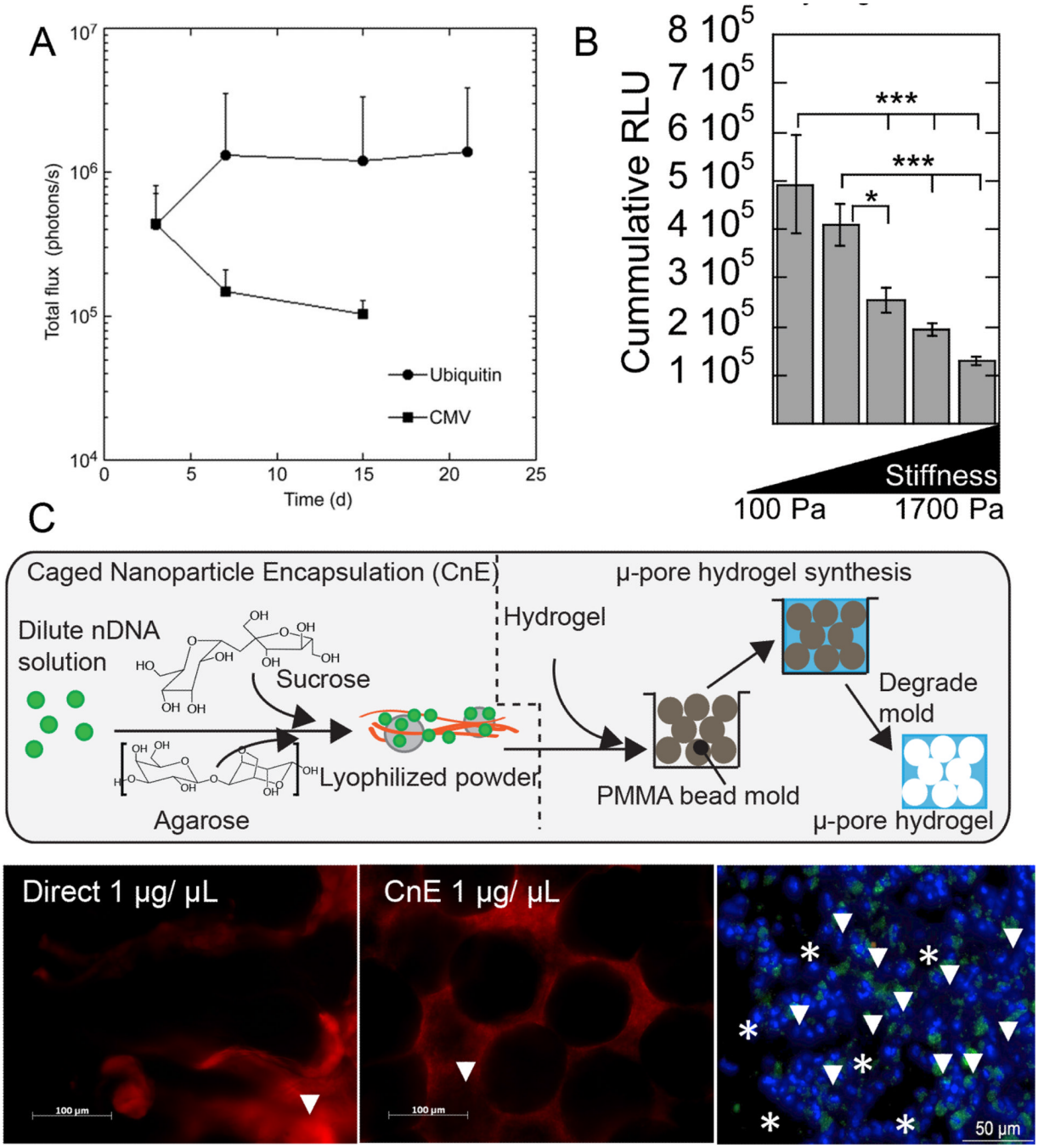


**Figure 3.**

Novel approaches to investigate the effect of design parameters on transgene expression. Different plasmid promoters (UbC vs. CMV) showed to have an effect on in vivo transgene expression (**A**), while stiffness had an inverse correlation with in vitro transgene expression in hyaluronic acid hydrogels (**B**). To decrease nDNA aggregation at higher nDNA concentrations, a caged nanoparticle encapsulation (CnE) technique was developed and applied to porous hydrogels for in vivo transfection (arrows show transfected cells, **C**).

## Table 1

Vector and carrier design characteristics.

| Consideration | Type | Details | References |
|---|---|---|---|
| **Immune Response** | CpG Motifs | • Mammalian DNA has less CpG motifs and most motifs are methylated whereas bacterial pDNA has more CpG motifs that are unmethylated<br>• Unmethylated CpG motifs induces pro-inflammatory cytokines (e.g. IL-6, IL-12, TNF-α, IFN-γ)<br>• Inverse correlation with number of CpG motifs and transfection | [51–53] |
| | Minicircle DNA | • Minicircles are supercoiled DNA molecules.<br>• Small in size, lack bacterial origin of replication and antibiotic resistance gene<br>• Decreased number of CpG sequences (better evasion of immune system<br>• Significantly increases transgene expression *in vitro* and *in vivo*. Transfection observable at day 1 compared to day 4 for regular plasmid | [10,54,55] |
| | Viral Capsid | • Directed evolution of adeno-associated virus (AAV) and impact on transduction | [56] |
| | | • Pro-inflammatory cytokine concentrations return to baseline by 4 weeks post-implantation of hydrogel with adenovirus | [41] |
| **Promoters** | Cytomegalovirus (CMV) | • Most widely used viral promoter.<br>• Robust expression, but short-lived due to silencing *in vivo* | [57] |
| | Ubiquitin C (UbC) | • Mammalian promoter results in sustained *in vivo* transgene expression | [19] |
| | Tissue-Specific | • Lower expression intensity, but longer transgene expression *in vivo* | [58] |
| **Bioactive Signal** | Growth Factors | • Spinal cord/nerves: BDNF, NGF, NT-3, FGF<br>• Bone: BMP-2<br>• Skin: FGF, KGF, SDF1α<br>• Cartilage: TGF-β1, TGF-β3<br>• Wound healing: PDGF, VEGF | [59] |
| | Transcription Factors | • HIF-1α lacking the oxygen-sensitive degradation domain (HIF-1αΔODD) upregulated expression of VEGF and resulted in mature vessels *in vivo* | [33] |
| | siRNA | • Induces gene silencing via mRNA degradation in cytosol<br>• Higher cargo loading required for silencing as compared to gene expression | [60] |
| **Carriers** | Polyplex | • Nucleic acids complexed with cationic polymers (e.g. linear polyethylene imine (LPEI))<br>• N/P ratio is crucial (High N/P results in higher transfection but is toxic, low N/P results in lower transfection but less toxic) | [30,60,61] |

| Consideration | Type | Details | References |
|---|---|---|---|
| | | • Size range: 20–500nm | |
| | Lipoplex | • Nucleic acids complexed with cationic lipids (e.g. 1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), 3β-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol))<br>• Hydrophobic units of lipid determine: size, shape, and stability<br>• Size range:60–200nm | [14,61] |
| | Viral | • Lentiviruses, adenoviruses, and adeno-associated viruses can infect non-dividing and dividing cells<br>• Lentiviral insert size is 7–8kb, has long duration of gene expression with risk of insertional mutagenesis<br>• Adenoviral insert size is ~30kb, has short duration of expression (does not integrate in host genome), and has risk of insertional response<br>• AAV insert size is 4.5kb, has long duration of expression with risk of insertional mutagenesis | [15,62] |
| | Inorganic Particles | • Nano-hydroxyapatite (<200nm) particles are used to adsorb viral vectors/DNA | [21,63] |