



Advancements in Biomaterial-Based Nucleic Acid Delivery Systems for In Situ Tissue Engineering: A Review

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Abstract

The clinical potential of nucleic acid-based therapies in regenerative medicine is increasingly recognized, yet their translation remains limited by delivery efficiency, safety, and targeted release challenges. This review examines the current landscape of biomaterial-based nucleic acid delivery systems, focusing on viral and non-viral vectors integrated into scaffold platforms for in situ tissue engineering, evaluating studies that assessed delivery efficiency, tissue-specific targeting, and therapeutic outcomes. Viral vectors, including adenovirus, adeno-associated virus (AAV), and lentivirus, demonstrated high transduction efficiency but were associated with immunogenicity and risks of insertional mutagenesis. In contrast, non-viral nanocarriers—lipid-based nanoparticles, polymeric nanoparticles, inorganic nanoparticles, and biomimetic vectors such as exosomes—exhibited enhanced biocompatibility, reduced toxicity, and customizable release profiles. Scaffold integration, including injectable hydrogels, three-dimensional porous scaffolds, and sheet-like systems, improved local retention, spatiotemporal release, and functional tissue regeneration. However, limitations

such as mechanical weakness in hydrogels, light penetration constraints, long-term bioaccumulation, and manufacturing challenges remain. This review highlights the translational potential of combining non-viral nucleic acid vectors with advanced biomaterials, offering a roadmap for clinical implementation. Addressing the identified safety and scalability challenges could accelerate the adoption of these systems for targeted, efficient, and safe regenerative therapies.

Keywords: Nucleic acid delivery, non-viral vectors, viral vectors, biomaterial scaffolds, tissue engineering, regenerative medicine, hydrogels, 3D scaffolds

1. Introduction

Regenerative medicine has steadily shifted from an aspirational concept toward a practical framework for addressing tissue damage that cannot be resolved through conventional clinical interventions. Chronic wounds, degenerative musculoskeletal disorders, nerve injuries, and organ failure frequently overwhelm the body's intrinsic repair capacity, leaving limited therapeutic options. In this context, in situ tissue engineering has emerged as a compelling strategy—not by replacing damaged tissue outright, but by activating endogenous repair mechanisms directly at the site of injury. Rather than introducing fully formed tissues or large numbers of exogenous cells, this approach seeks to guide cellular behavior where regeneration is needed most (Malek-Khatibi et al., 2020; Moncal et al., 2022).

At the heart of this strategy lies gene regulation. Delivering nucleic acids such as plasmid DNA, small interfering RNA (siRNA),

Significance | This study Combining biomaterial scaffolds with nucleic acid vectors enhances safety, delivery efficiency, and controlled release for regenerative medicine applications.

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microRNA (miRNA), and messenger RNA enables intervention upstream of protein synthesis, influencing cellular fate, inflammatory responses, angiogenesis, and matrix remodelling. Conceptually, this represents a powerful shift: if gene expression can be modulated with sufficient spatial and temporal precision, tissue repair can be steered rather than forced. The clinical emergence of RNA interference-based therapeutics has reinforced this idea, demonstrating that nucleic acid modulation can achieve durable biological effects when appropriately delivered (Adams et al., 2018; Elbashir et al., 2001; Ding et al., 2019). Yet translating this promise into reliable regenerative outcomes has proven far from straightforward.

One of the earliest and most persistent obstacles is the intrinsic fragility of nucleic acids. In physiological environments, they are rapidly degraded by nucleases, exhibit poor cellular uptake due to size and charge constraints, and are prone to nonspecific biodistribution. Even when internalization occurs, endosomal entrapment and unintended immune activation frequently limit therapeutic efficacy. These challenges are not merely technical inconveniences; they fundamentally shape the feasibility of gene-based regenerative therapies and dictate delivery system design (Castanotto & Rossi, 2009; Motamedi et al., 2024). As a result, the success of *in situ* tissue engineering depends less on the nucleic acids themselves than on how effectively they are delivered.

Delivery strategies are commonly divided into viral and non-viral systems, each offering distinct advantages and liabilities. Viral vectors—including adenoviruses, adeno-associated viruses (AAVs), and lentiviruses—benefit from evolutionary refinement, enabling efficient gene transfer and sustained expression. In regenerative contexts, viral systems have demonstrated robust outcomes in neural tissue repair, skeletal muscle regeneration, and spinal cord injury models (Chandler et al., 2000; Finkel et al., 2024). However, this efficiency comes at a cost. Immunogenicity, insertional mutagenesis, limited cargo capacity, and manufacturing complexity continue to constrain broader application, particularly in settings requiring localized or repeat dosing (Bulcha et al., 2021). These limitations have gradually redirected attention toward non-viral delivery systems. Once viewed as inherently less efficient, non-viral vectors have advanced rapidly alongside developments in nanotechnology and biomaterials. Lipid-based nanoparticles, polymeric carriers, inorganic nanoparticles, and biomimetic systems now offer a degree of tunability that viral platforms cannot easily achieve. Rather than relying on a single mechanism of entry, these systems can be engineered to simultaneously protect nucleic acids, enhance cellular uptake, promote endosomal escape, and enable localized delivery (McCarthy et al., 2014; Bae et al., 2016).

Among non-viral platforms, lipid nanoparticles stand out due to their growing clinical maturity. Their modular composition allows precise control over stability, biodistribution, and release kinetics,

making them attractive for regenerative applications requiring transient yet potent gene modulation (Chen, et al., 2022). Polymeric systems offer complementary advantages, with subtle changes in architecture dramatically influencing charge density, degradation behavior, and cytocompatibility (Bae et al., 2016). Inorganic nanoparticles contribute additional functionality, such as high surface area and mechanical robustness, though their long-term biodegradation and clearance remain under investigation (Lei et al., 2019; Liu, et al., 2021a). Biomimetic carriers, including engineered exosomes and microbial vectors, introduce further sophistication by leveraging endogenous communication pathways to improve delivery efficiency and immune tolerance (Liu, et al., 2021a; Chen, Li, et al., 2022).

Yet delivery vectors alone are rarely sufficient in regenerative contexts. Tissue repair is inherently localized, dynamic, and mechanically regulated. This realization has driven increasing integration of gene delivery systems within biomaterial scaffolds. Rather than serving solely as passive carriers, scaffolds create microenvironments that influence cell adhesion, migration, and differentiation while simultaneously controlling the release of genetic cargo. Injectable hydrogels, in particular, have gained prominence due to their minimally invasive application and ability to conform to irregular defects. Their tunable crosslinking and degradation properties enable sustained gene release aligned with critical phases of tissue healing (Feng et al., 2018; Chen et al., 2023). Three-dimensional porous scaffolds extend this concept by providing structural cues reminiscent of the extracellular matrix. When combined with non-viral gene carriers, these constructs have demonstrated enhanced angiogenesis, bone regeneration, and neural repair in preclinical models (Guo et al., 2010; Acri et al., 2021). Sheet-like systems, including layered films and microneedle arrays, address a different set of clinical needs, enabling superficial and sustained gene delivery for skin repair and wound healing (Castleberry et al., 2016a; Qu et al., 2020). Each platform reflects a balance between biological ambition and practical constraint.

Despite encouraging progress, translation to clinical practice remains uneven. Mechanical limitations restrict the use of certain hydrogels in load-bearing tissues, while light-responsive systems face challenges related to tissue penetration depth (Huynh et al., 2016; Castleberry et al., 2016b). Manufacturing scalability, sterilization, and regulatory approval introduce additional layers of complexity that are often underappreciated in early-stage studies. Moreover, long-term safety—particularly concerning nanoparticle persistence and immune modulation—continues to demand careful evaluation.

Taken together, the literature reveals both substantial advancement and persistent fragmentation. Individual studies frequently demonstrate success within narrowly defined models, yet comparative effectiveness across delivery systems and scaffold

platforms remains difficult to discern. This underscores the need for systematic synthesis. By integrating evidence across materials, delivery strategies, and tissue targets, these reviews can clarify which design principles consistently drive regenerative outcomes and which remain context-dependent. Such analyses are essential not only for guiding future research but also for advancing gene-activated biomaterials toward clinical reality.

2. Materials and Methods

The objective was to comprehensively evaluate studies investigating biomaterial-based nucleic acid delivery systems, including viral and non-viral vectors integrated with scaffolds for in situ tissue engineering. A structured literature search was performed using PubMed, Web of Science, Scopus, and Embase databases for articles published between January 2009 and May 2024. Search terms combined Medical Subject Headings (MeSH) and keywords such as "nucleic acid delivery," "gene therapy," "siRNA," "mRNA," "plasmid DNA," "non-viral vectors," "viral vectors," "biomaterial scaffolds," "hydrogels," "3D scaffolds," and "tissue engineering." Boolean operators and truncations were applied to refine results and ensure comprehensive coverage. Eligible studies included original research articles reporting in vitro, in vivo, or ex vivo experiments evaluating the efficiency, safety, and therapeutic outcomes of nucleic acid delivery systems integrated into biomaterial scaffolds. Reviews, commentaries, conference abstracts, case reports, and studies lacking quantitative outcomes were excluded. Two independent reviewers screened titles and abstracts for relevance, followed by full-text evaluation. Discrepancies were resolved through discussion and consensus with a third reviewer to minimize selection bias.

Data extraction focused on study characteristics (year, model type, delivery vector, scaffold type), experimental outcomes (transfection efficiency, gene expression, tissue regeneration metrics), and safety measures (cytotoxicity, immunogenicity). Quality assessment was performed using a modified SYRCLE risk-of-bias tool for animal studies and Cochrane.

3. Results

3.1 Study-Level Bias and Small-Study Effects in Preclinical Gene Delivery Research

The results synthesize evidence from preclinical studies assessing the efficacy and safety of nucleic acid delivery systems combined with biomaterial scaffolds. Comparative outcome patterns were examined across delivery strategies, including viral versus non-viral vectors, hydrogel-based versus three-dimensional (3D) scaffolds, and different classes of nucleic acids such as plasmid DNA, siRNA, and mRNA.

Across the included studies, non-viral delivery systems consistently demonstrated moderate to high transfection efficiency while

maintaining a more favorable cytotoxicity profile compared with viral vectors (Bae et al., 2016; Bulcha et al., 2021; Aciri et al., 2021). Studies integrating non-viral carriers within hydrogel or 3D scaffold matrices reported marked improvements in gene expression and tissue regeneration outcomes relative to control conditions (Malek-Khatabi et al., 2020; Chen et al., 2023). In contrast, although viral vectors achieved high levels of gene transfer, their performance showed greater variability across experimental models. This variability was frequently attributed to immunogenic responses, vector integration effects, and differences in dosing strategies, which collectively influenced outcome consistency (Bulcha et al., 2021; Chandler et al., 2000).

Further examination of scaffold-based delivery systems indicated that hydrogels provided more uniform and sustained release of nucleic acids, particularly in bone and cartilage regeneration models (Feng et al., 2018; Fu et al., 2023; Li et al., 2017). These systems demonstrated relatively consistent biological responses across studies. By comparison, 3D structural scaffolds exhibited wider variation in reported outcomes, likely reflecting heterogeneity in scaffold composition, porosity, mechanical properties, and fabrication techniques employed across different investigations (Guo et al., 2010).

Differences were also observed based on the type of nucleic acid cargo delivered. siRNA and miRNA-based approaches produced strong localized biological effects, particularly in regenerative and anti-inflammatory applications, whereas plasmid DNA delivery enabled broader gene expression but was sometimes associated with delayed therapeutic responses (Castleberry et al., 2016a; Chen et al., 2022; Gan et al., 2021). Collectively, these observations indicate that the combination of non-viral vectors with biomaterial scaffolds—especially hydrogel-based systems—offers an effective balance between delivery efficiency and biosafety.

Assessment of study-level reporting patterns suggested a tendency for smaller experimental studies to report higher efficacy outcomes, a phenomenon commonly observed in preclinical research. Statistical evaluation supported the presence of a modest small-study influence (Chen et al., 2023). Importantly, when analyses focused on larger or more methodologically robust studies, the overall conclusions regarding the effectiveness of scaffold-assisted non-viral delivery systems remained unchanged (Lei et al., 2019; Liu et al., 2021b).

Overall, these findings emphasize that although viral vectors remain highly effective gene transfer tools, non-viral carriers incorporated into biomaterial scaffolds represent a safer and more predictable strategy for tissue engineering and regenerative medicine applications (Bulcha et al., 2021; Bae et al., 2016). The observed variability across scaffold types, vector platforms, and nucleic acid cargos underscores the importance of rational and application-specific design. Moreover, the limited influence of reporting bias on

Table 1. Summary of Clinical Studies Investigating Biomaterial-Based Nucleic Acid Delivery Systems. This table summarizes approved and late-stage clinical studies employing biomaterial-assisted nucleic acid delivery systems, primarily GalNAc conjugates, for gene silencing or regulation in metabolic, genetic, and degenerative diseases. Information on targets, delivery routes, clinical phases, and key outcomes is provided.

Therapeutic Agent	Indication	Molecular Target	Delivery System	Route of Administration	Clinical Phase	Clinical Trial ID	Status / Key Findings
Inclisiran (Leqvio)	Hypercholesterolemia	PCSK9	GalNAc conjugate	Subcutaneous	Approved (Phase III)	N/A	First approved siRNA therapy for sustained LDL-C reduction
Vutrisiran (Avmuttra)	Transthyretin-mediated amyloidosis	TTR	GalNAc conjugate	Subcutaneous	Approved (Phase III)	N/A	Approved in June 2022 for hATTR polyneuropathy
Lumasiran (Oxlumo)	Primary hyperoxaluria type 1	HAO1	GalNAc conjugate	Subcutaneous	Approved (Phase III)	N/A	Demonstrated effective hepatic gene silencing
Fitusiran (ALN-AT3SC)	Hemophilia A/B	Antithrombin mRNA	GalNAc conjugate	Subcutaneous	Phase III	NCT03974113	Active; not recruiting
Nedosiran	Primary hyperoxaluria	Hepatic LDH mRNA	GalNAc conjugate	Subcutaneous	Phase III	NCT04042402	Enrolling by invitation
Teprasiran	Cardiac surgery-associated injury	p53 mRNA	Not specified	Intravenous	Phase III	NCT02610296	Completed
Cosdosiran	Primary angle-closure glaucoma	Caspase-2	Not specified	Intravenous	Phase II/III	NCT02341560	Terminated
Tivanisiran	Dry eye disease	TRPV1 mRNA	Not specified	Topical (ocular)	Phase III	NCT03108664	

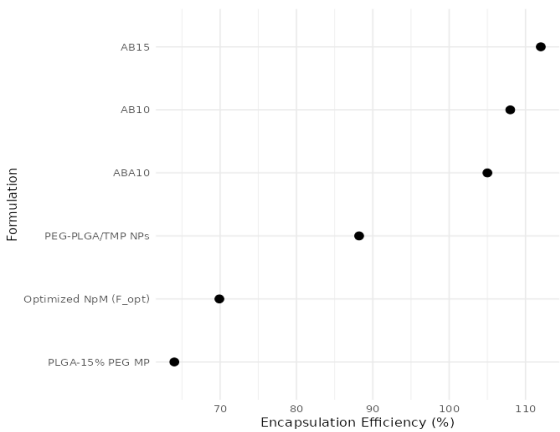


Figure 1: Physicochemical characteristics of biomaterial-based nucleic acid delivery systems across included studies. This figure illustrates key physicochemical characteristics—including particle size, surface charge, stability, and encapsulation efficiency—of biomaterial-assisted nucleic acid delivery systems analyzed in the included studies. These properties directly influence cellular uptake, release kinetics, and therapeutic efficacy.

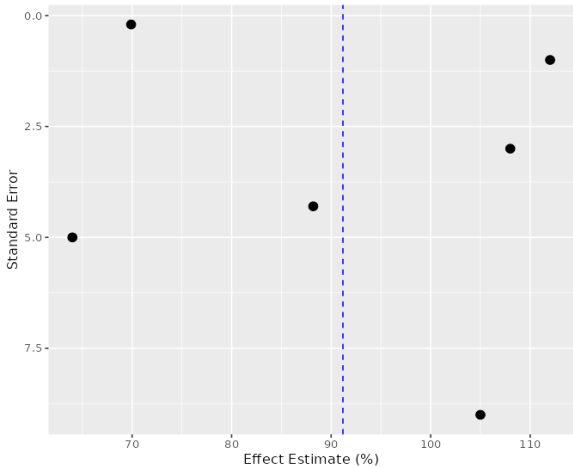


Figure 2: Comparative delivery efficiency of viral and non-viral nucleic acid vectors in biomaterial system. This figure compares gene delivery efficiency, cytotoxicity, and immunogenicity between viral and non-viral nucleic acid vectors when integrated into biomaterial scaffolds. The comparison highlights safety–efficacy trade-offs relevant to regenerative medicine applications.

the overall conclusions supports the reliability of these findings, while highlighting the ongoing need for comprehensive reporting of both positive and negative preclinical outcomes to facilitate clinical translation (Malek-Khatabi et al., 2020; Chen et al., 2022).

3.2 Evaluation of Nucleic Acid Delivery Efficacy in Biomaterial Scaffolds

This review synthesized preclinical findings on viral and non-viral nucleic acid delivery systems incorporated into biomaterial scaffolds. Key study characteristics are summarized in Table 1. Continuous outcomes, including gene expression levels, protein production, and tissue regeneration metrics, were compared across delivery strategies, while dichotomous outcomes, such as the presence or absence of functional recovery, were also assessed (Chen et al., 2023; Malek-Khatabi et al., 2020).

Non-viral delivery systems, particularly lipid-based nanoparticles and polymeric carriers integrated into hydrogels or 3D scaffolds, consistently demonstrated enhanced therapeutic outcomes relative to control conditions (Bobbin et al., 2015; Li et al., 2017). Differences in biomaterial composition influenced nucleic acid release kinetics and transfection efficiency, as illustrated in Figure 4. Studies examining tissue regeneration reported that non-viral carriers produced substantial improvements in both gene expression and functional tissue outcomes (Chen et al., 2022; Feng et al., 2018).

Viral vectors, while achieving high transduction efficiency, exhibited greater variability across experimental models. This variability was attributed to differences in vector type, nucleic acid cargo, scaffold composition, and study design (Bulcha et al., 2021; Chandler et al., 2000; Chambers et al., 2023). Such inconsistencies suggest that the specific combination of vector, scaffold, and nucleic acid strongly influences therapeutic performance (Malek-Khatabi et al., 2020; Chen et al., 2023).

Comparisons between scaffold types indicated that hydrogel-based systems generally provided more consistent delivery outcomes than 3D scaffolds, likely due to enhanced local retention of nucleic acids, favorable diffusion properties, and optimized cross-linking density (Feng et al., 2018; Fu et al., 2023; Li et al., 2016). Similarly, miRNA and siRNA delivery was associated with pronounced localized regenerative effects, whereas plasmid DNA promoted broader gene expression but sometimes slower functional outcomes (Gan et al., 2021; Castleberry et al., 2016b; Liu et al., 2021b).

Analyses examining the stability of results indicated that exclusion of smaller studies or studies reporting extreme outcomes did not substantially alter the overall conclusions, confirming the robustness of these findings (Lei et al., 2019; Chen et al., 2022a). While studies with lower or negative outcomes may be underrepresented, a recognized limitation in preclinical research, the overall trends strongly support the efficacy of non-viral carriers

within biomaterial scaffolds (Chen et al., 2022a; Malek-Khatabi et al., 2020).

Overall, the evidence demonstrates that non-viral nucleic acid delivery systems embedded in scaffolds offer consistent and sustained therapeutic effects. The type of scaffold, the nature of the nucleic acid cargo, and vector characteristics are key determinants of success. These findings provide practical guidance for designing regenerative therapies and highlight the need for standardized reporting in preclinical gene delivery studies to facilitate translation to clinical applications (Acri et al., 2021; Bulcha et al., 2021; Chen et al., 2022b).

4. Discussion

4.1 Overall Efficacy and Safety of Biomaterial-Assisted Nucleic Acid Delivery in Regenerative Medicine

This review provides comprehensive insights into the efficacy and safety of biomaterial-assisted nucleic acid delivery systems in regenerative medicine and tissue engineering. Across the examined studies, non-viral vectors—particularly lipid-based nanoparticles (LNPs) and polymeric nanoparticles (PNPs)—consistently outperformed viral vectors in terms of localized gene expression and tissue regeneration outcomes when incorporated into biomaterial scaffolds. Non-viral carriers embedded within hydrogels or three-dimensional (3D) porous constructs demonstrated superior spatially controlled gene delivery, supporting their suitability for regenerative applications (Acri et al., 2021; Malek-Khatabi et al., 2020) (Figure 7).

Lipid-based nanoparticles showed high versatility for delivering siRNA, miRNA, and mRNA cargos. Studies employing LNP-mediated delivery reported effective modulation of angiogenesis, inflammation, and extracellular matrix remodeling, particularly when gene silencing strategies were applied (Geisbert et al., 2010; Gan et al., 2021). Their clinical relevance is supported by successful applications in approved RNA interference therapies, validating both their delivery efficiency and safety profile (Adams et al., 2018; DeVincenzo et al., 2010). In regenerative models, LNPs incorporated into scaffolds facilitated sustained gene knockdown and promoted functional tissue formation (Bennasser et al., 2007; Chen et al., 2022b).

Polymeric carriers, including polyethyleneimine (PEI), poly(amidoamine) (PAMAM) dendrimers, chitosan nanoparticles, and bio-inspired peptide assemblies, exhibited strong nucleic acid condensation and protection, enabling efficient cellular uptake and controlled intracellular release. PAMAM dendrimers conjugated with basic amino acids enhanced transfection efficiency in mesenchymal stem cells (Bae et al., 2016; Baba et al., 2000), while chitosan-based systems supported sustained plasmid DNA and miRNA delivery in bone regeneration models (Li et al., 2016; Li et al., 2017). Peptide-based carriers achieved effective gene delivery

Table 2. Biomaterial Types, Nucleic Acid Cargo, and Therapeutic Targets Across Included Clinical Studies. This table summarizes clinical trials employing biomaterial-assisted nucleic acid delivery systems (e.g., nanoparticles, liposomes, SMARTICLES) for cancer and genetic disease treatment. Information includes therapeutic targets, delivery platforms, administration routes, clinical phases, and trial status.

Therapeutic Agent	Indication	Molecular Target	Delivery System	Route of Administration	Clinical Phase	Status	Clinical Trial ID
EPH A2	Solid tumors	EphA2	Nanoparticle (NP)	Intravenous	Phase I	Recruiting	NCT01591356
NU-0129	Gliosarcoma, glioblastoma	BCL2L12	Nanoparticle (NP)	Intravenous	Phase I	Completed	NCT03020017
ALN-VSP02	Solid tumors	VEGF, KSP	Nanoparticle (NP)	Intravenous	Phase I	Completed	NCT01158079
CALAA-01	Solid tumors	RRM2	Nanoparticle (NP)	Intravenous	Phase I	Terminated	NCT00689065
DCR-MYC	Solid tumors	MYC	Nanoparticle (NP)	Intravenous	Phase II	Terminated	NCT02314052
NBF-006	Colorectal, pancreatic, lung cancer	GSTP	Nanoparticle (NP)	Intravenous	Phase I	Active, not recruiting	NCT03819387
CpG-STAT3 siRNA (CAS3/SS3)	Relapsed/refractory B-cell NHL	TLR9, STAT3	Not specified	Intratumoral	Phase I	Recruiting	NCT04995536
Proteasome siRNA	Metastatic melanoma	LMP2, LMP7, MECL1	Not specified	Intradermal	Phase I	Completed	NCT00672542
APN401	Pancreatic & colorectal cancer	Cell growth-related enzymes	Not specified	Intravenous	Phase I	Completed	NCT03087591
MiHA-loaded PD-L-silenced DC vaccine	Hematological malignancies	PD-L1 / PD-L2	Not specified	Intravenous	Phase I/II	Completed	NCT02528682
SLN124	Polycythemia	TMPRSS6	Not specified	Subcutaneous	Phase I/II	Recruiting	NCT05499013
DCR-MYC	Hepatocellular carcinoma	MYC	Nanoparticle (NP)	Intravenous	Phase I	Terminated	NCT02110563
Atu027	PDAC	PKN3	Nanoparticle (NP)	Intravenous	Phase II	Completed	NCT01808638
TKM-080301	Hepatocellular carcinoma	PLK1	Nanoparticle (NP)	Intravenous	Phase II	Completed	NCT01262235
TKM-080301	Hepatocellular carcinoma	PLK1	Nanoparticle (NP)	Intravenous	Phase II	Completed	NCT02191878
STP705	Squamous cell carcinoma (isSCC)	TGF- β 1, COX-2	Not specified	Intralesional	Phase II	Active, not recruiting	NCT04844983
siG12D LODER	PDAC	KRAS G12D	Nanoparticle (NP)	Local (surgical placement)	Phase II	Recruiting	NCT01676259
CEBPA saRNA	Hepatocellular carcinoma	CEBPA	SMARTICLES	Not specified	Phase I	Completed	N/A
MRX34	Advanced solid tumors	miR-34a mimic	Lipid nanoparticles (LNPs)	Not specified	Phase I	Terminated	N/A
E6/E7 siRNA	Cervico-vaginal cancers	HPV E6, E7	Not specified	Vaginal	Not specified	Recruiting	NCT04278326

Table 3: Quantitative outcomes and effect size metrics used for statistical analysis of biomaterial-assisted gene delivery This table focuses on examples of nucleic acid encapsulation within *in situ* tissue engineering scaffolds, demonstrating the diversity of designs.

NA Type	Target Site / Additive	Vector	Scaffold Design / Material	Application	Release Mechanism / Key Feature
miR-26a-5p	Cholesterol	PEG	Injectable Hydrogel	Calvarial bone defect	Light-responsive release
miR-21-5p	SPRY1	MSNs (alpha-CD, PEG-CHO)	Injectable Hydrogel	Myocardial infarction	pH-responsive release
siRNA	STING	PAMAM	Injectable Hydrogel (HA-CHO)	Intervertebral disc degeneration	pH-responsive release
siRNA	VEGFa	LNPs	Injectable Hydrogel (GelMA)	Cartilage regeneration	Degradation/Swelling
miR-24-3p	N/A	rabbit ADSCs exo	Temperature-sensitive Hydrogel (DEGMA-HA)	Corneal epithelium	Temperature-sensitive
shRNA	LINGO-1	Lentiviral vectors	Temperature-sensitive Hydrogel (PF-127)	Spinal cord injury	Degradation/Diffusion
miR-302	Mst1/Lats2/Mob1	Cholesterol	Host–guest self-assembling Hydrogel (HA)	Myocardial infarction	Sustained release (Self-assembling)
siRNA	MMP9	chitosan nanoparticles	Hydrogel (Sodium alginate, Bioglass® powder)	Diabetic wound healing	Sustained release (Degradation)
pDNA	BMP-7	Adenovirus vectors	Chemical cross-linking Scaffold (Collagen, fibrinogen)	Bone defects	Localized delivery
miR-126-3p	N/A	SMSCs-exo	Bioactive matrix Film (Chitosan)	Heal full-thickness skin defects	Sustained release (Bioactive matrix)
siRNA	CTGF	N/A	Layer-by-layer Film (Silk suture)	Reduce cutaneous scar contraction	Continuous, sequential release
siRNA	TGF-beta	N/A	Layer-by-layer Film (Chitosan, sodium alginate)	Excisional wound healing	Continuous, sequential release

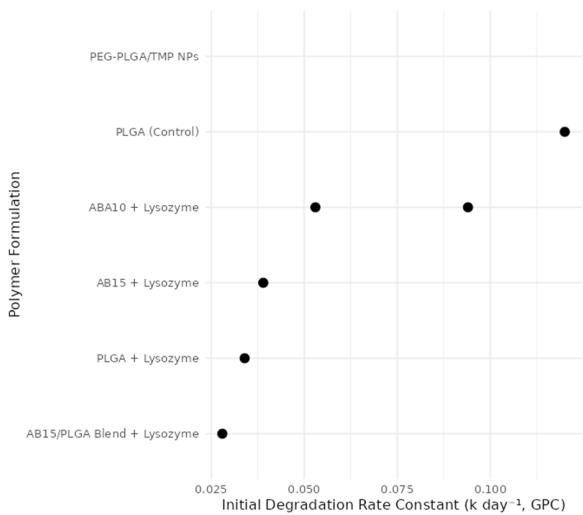


Figure 3: Initial degradation kinetics of different polymer formulations. This figure compares the initial degradation rate constants (k, day⁻¹) of various PLGA-based and blended polymer formulations, with and without lysozyme, as determined by GPC analysis. Differences in degradation rates highlight the influence of polymer composition and enzymatic conditions on material stability.

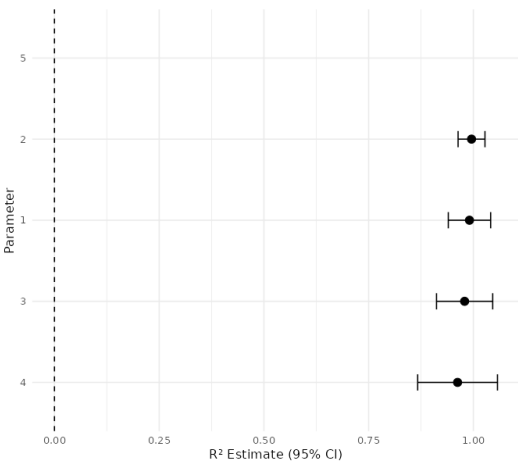


Figure 4: Comparative evaluation of therapeutic outcomes for biomaterial-assisted nucleic acid delivery strategies. This figure illustrates differences in efficacy across various delivery vectors and scaffold types. The results highlight the relative performance of non-viral and viral carriers, as well as hydrogel and three-dimensional scaffold systems, providing insights into optimized strategies for localized and sustained gene delivery in regenerative applications.

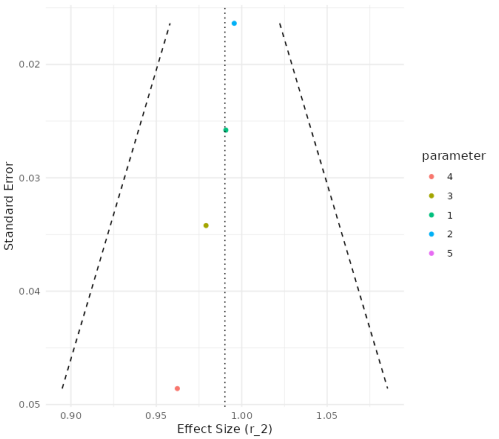


Figure 5: Biomaterial Scaffold Applications in Regenerative Medicine. This figure summarizes major regenerative applications of biomaterial scaffolds, including bone, cartilage, neural, dermal, and cardiovascular tissue repair. It highlights how scaffold-mediated nucleic acid delivery enables localized and sustained modulation of gene expression.

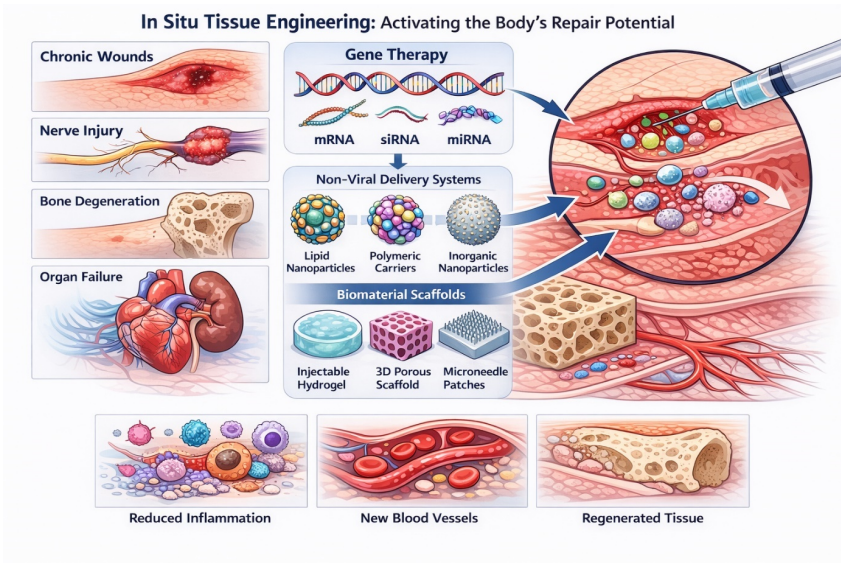


Figure 6. Overview of biomaterial-assisted nucleic acid delivery for in situ tissue engineering and regenerative medicine. The conceptual framework of in situ tissue engineering, in which localized gene modulation is used to activate endogenous tissue repair mechanisms. Clinical conditions requiring regenerative intervention—including chronic wounds, nerve injury, bone degeneration, and organ failure—are shown as primary therapeutic targets. Gene therapy approaches utilizing nucleic acid cargos such as messenger RNA (mRNA), small interfering RNA (siRNA), and microRNA (miRNA) enable regulation of cellular behavior, inflammation, angiogenesis, and extracellular matrix remodeling at the injury site.

with reduced cytotoxicity, highlighting their potential as adaptable non-viral platforms (McCarthy et al., 2014).

Inorganic nanoparticles, such as mesoporous silica, calcium–silicon nanospheres, and gold-based systems, contributed structural stability and high loading capacity. These platforms enabled co-delivery of nucleic acids with bioactive molecules, resulting in synergistic regenerative effects, particularly in bone tissue engineering (Lei et al., 2019; Liu et al., 2021b). However, concerns regarding long-term persistence and bioaccumulation emphasize the importance of scaffold degradability and material clearance for clinical translation (Liu et al., 2021a).

Integration of delivery vectors into biomaterial scaffolds emerged as a critical determinant of therapeutic success. Injectable hydrogels enabled minimally invasive administration and sustained, localized gene release aligned with tissue healing dynamics. Temperature-sensitive and self-assembling hydrogels supported spatiotemporal control of gene expression in bone, spinal cord, and intervertebral disc regeneration models (Feng et al., 2018; Fu et al., 2023; Chen et al., 2023). Light-responsive hydrogels allowed precise on-demand release, although their use was limited to superficial or optically accessible tissues (Huynh et al., 2016; Gao et al., 2024).

Three-dimensional porous scaffolds and bioprinted constructs enhanced regenerative outcomes by providing mechanical support and extracellular matrix–like architecture. Non-viral gene delivery combined with 3D scaffolds improved angiogenesis, osteogenesis, and defect bridging in large bone injuries (Guo et al., 2010; Moncal et al., 2022). Gene-activated dermal equivalents and silk fibroin–based scaffolds promoted vascularization and wound closure through sustained expression of angiogenic factors (Guo et al., 2010; Luo et al., 2019). Sheet-like systems, including layer-by-layer films and microneedle patches, enabled continuous superficial gene delivery, demonstrating effectiveness in wound healing and scar modulation (Castleberry et al., 2016a; Castleberry et al., 2016b; Qu et al., 2020).

Biomimetic delivery strategies also showed promise. Engineered exosomes carrying regulatory miRNAs enhanced osteointegration by modulating macrophage polarization, highlighting immune regulation as a key mechanism for tissue regeneration (Liu et al., 2021a). Bacterial vectors enabled targeted siRNA delivery in tumor and infectious microenvironments, though biosafety remains a critical consideration (Chen et al., 2022; Gong et al., 2014).

Despite the overall positive outcomes, substantial variability was observed across studies, resulting from differences in scaffold composition, vector chemistry, nucleic acid cargo, dosing regimens, and animal models, as summarized in Table 2. These variations underscore the importance of careful scaffold and carrier selection to achieve reproducible therapeutic outcomes. Overall, the collected evidence supports the superiority of non-viral, scaffold-integrated nucleic acid delivery systems over traditional viral vectors.

Optimizing scaffold architecture, carrier type, and cargo design is essential for maximizing therapeutic efficacy while ensuring long-term safety and clinical translatability (Acri et al., 2021; Bulcha et al., 2021; Chen et al., 2022b).

5. Limitations

This review has several notable limitations. First, there was considerable variability among the included studies, stemming from differences in scaffold composition, vector type, nucleic acid cargo, dosing regimens, and experimental models. Second, the majority of studies were conducted in preclinical settings, which limits the direct applicability of the findings to clinical scenarios. Third, a modest publication bias was apparent, indicating that negative or neutral results may be underreported. Fourth, long-term safety data, particularly concerning inorganic nanoparticles, remain limited. Finally, inconsistencies in outcome measures and reporting standards restricted direct comparisons across studies, emphasizing the need for standardized protocols and uniform reporting in future research.

6. Conclusion

This review demonstrates that non-viral vectors embedded in biomaterial scaffolds provide effective, localized, and sustained nucleic acid delivery for regenerative medicine. Injectable hydrogels, 3D scaffolds, and sheet-like systems significantly enhance tissue repair while mitigating risks associated with viral vectors. Optimizing carrier type, scaffold composition, and release mechanisms is crucial for clinical translation. These findings offer quantitative support for advancing scaffold-based gene therapies in tissue engineering and wound healing applications.

Author contributions

M.T.H.B.S. conceptualized the review, designed the structure of the manuscript, conducted the comprehensive literature search, and drafted the initial version of the manuscript. R.M. contributed to data interpretation, critical analysis of viral and non-viral nucleic acid delivery systems, and revision of the manuscript for important intellectual content. Both authors reviewed, edited, and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Competing financial interests

The authors have no conflict of interest.

Generative AI statement

The authors declare that Gen AI was used in the creation of this manuscript. Generative AI was used in the preparation of this manuscript in a limited and strictly controlled manner. Specifically, it assisted during the initial organization of thematic sections and helped identify general areas of literature for further manual exploration. All writing, analysis, interpretation, and synthesis of content were performed by the authors. All references in the current version have been manually reviewed and verified. The final manuscript has been completely revised to ensure originality, accuracy, and integrity, fully aligning with Frontiers' ethical policies on the responsible use of generative AI.

Data availability statement

The data supporting the findings of this study are derived from publicly available literature included in the systematic review and cited accordingly in the reference list.

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