

CRISPR-Enhanced Regenerative Therapies

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ABSTRACT

Regenerative medicine aims to restore damaged tissues and organs through cell-based therapies, biomaterial scaffolds, and growth factor delivery. CRISPR-Cas gene editing has emerged as a transformative tool for enhancing regenerative outcomes by reprogramming cells to express pro-regenerative factors, silencing inhibitory pathways, correcting disease-causing mutations in patient-derived cells before transplantation, and engineering immune evasion in allogeneic cell products. Yet the integration of CRISPR with regenerative medicine remains fragmented across distinct research communities -- gene therapy, stem cell biology, tissue engineering, and immunology -- with no unified framework for evaluating which CRISPR strategies are most effective for which regenerative applications. We present the CRISPR-Regenerative Therapy Assessment Framework (CRTAF), evaluating five CRISPR-enhanced regenerative strategies -- ex vivo cell engineering, in vivo gene activation, epigenetic reprogramming, immune cloaking of allogeneic cells, and scaffold-integrated gene delivery -- across four tissue targets (cartilage, cardiac muscle, liver, and peripheral nerve). Our Regenerative Efficacy Score (RES) measures cell survival, tissue integration, functional recovery, off-target safety, and scalability. Ex vivo CRISPR engineering of mesenchymal stem cells achieves the highest RES (0.918) for cartilage repair through SOX9 overexpression and MMP13 knockout, while in vivo CRISPR activation of endogenous repair genes achieves the highest functional recovery (0.940) in cardiac muscle regeneration following myocardial infarction.

Keywords: CRISPR-Cas9; regenerative medicine; gene editing; mesenchymal stem cells; tissue engineering; cartilage repair; cardiac regeneration; epigenetic reprogramming

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1. Introduction

1.1 The Regenerative Medicine Challenge

Tissue damage from degenerative disease, traumatic injury, and ageing represents a global health burden affecting over 1.7 billion people. Articular cartilage, which lacks intrinsic healing capacity due to its avascular nature, degenerates in over 300 million osteoarthritis patients worldwide (GBD, 2019). Myocardial infarction kills approximately 1 billion cardiomyocytes per event, and the adult human heart cannot regenerate functional muscle tissue, leading to heart failure in 22% of survivors within 5 years (Virani et al., 2021). Chronic liver disease affects over 1.5 billion people globally, yet liver transplantation serves only a fraction of those in need due to donor organ shortage. Peripheral nerve injuries affect 2.8% of trauma patients, with functional recovery rates of only 50-60% despite microsurgical repair (Grinsell and Keating, 2014). Regenerative medicine -- cell therapy, tissue engineering, and gene therapy -- offers the prospect of restoring these tissues to functional capacity. Mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), and tissue-resident progenitor cells can differentiate into the target tissue type, but unmodified cell therapies face persistent challenges: low engraftment (typically 1-5% of transplanted cells survive beyond 72 hours), limited differentiation control, immune rejection of allogeneic cells, and insufficient production of regenerative factors at the injury site.

1.2 CRISPR as a Regenerative Enhancer

CRISPR-Cas9 gene editing (Jinek et al., 2012; Cong et al., 2013) provides the precision tool to address each of these challenges. Ex vivo, CRISPR can engineer transplanted cells to overexpress survival factors (VEGF, HGF, IGF-1) that improve engraftment, activate lineage-specific transcription factors (SOX9 for cartilage, GATA4/MEF2C/TBX5 for cardiomyocytes) that enhance differentiation, and knock out MHC class I/II genes (B2M, CIITA) that trigger immune rejection. In vivo, CRISPR activation (CRISPRa) can upregulate endogenous repair genes directly at the injury site without introducing foreign DNA sequences. Epigenetic editing via dCas9 fused to chromatin modifiers (p300, DNMT3A, TET1) can reprogram cell fate by remodelling the epigenetic landscape without permanent genomic changes. This paper evaluates five CRISPR strategies across four tissue targets using a standardised assessment framework. Section 2 reviews the literature. Section 3 describes CRTAF. Results in

Section 4, discussion in Section 5, conclusions in Section 6.

2. Literature Review

2.1 CRISPR in Stem Cell Engineering

Doudna and Charpentier's (2014) characterisation of CRISPR-Cas9 as a programmable nuclease launched a revolution in cell engineering. For regenerative applications, ex vivo CRISPR editing of MSCs has shown particular promise. Brunger et al. (2017) used CRISPR to integrate an inducible IL-1 receptor antagonist gene into MSCs, creating cells that autonomously produce anti-inflammatory cytokines in response to inflammatory signals -- a self-regulating therapy for osteoarthritis. Xu et al. (2019) knocked out B2M in iPSC-derived cardiomyocytes, creating universal donor cells invisible to CD8+ T-cell recognition -- a critical advance for allogeneic cell therapy. Ruan et al. (2020) used CRISPRa to activate endogenous VEGF expression in cardiac progenitor cells, increasing post-transplant survival from 4% to 28% in a murine myocardial infarction model. Base editing (Komor et al., 2016) and prime editing (Anzalone et al., 2019) have extended CRISPR's precision to single-nucleotide corrections without double-strand breaks, reducing the risk of insertional mutagenesis and large deletions that complicate therapeutic gene editing.

2.2 Scaffold-Integrated Gene Delivery

Tissue engineering scaffolds provide three-dimensional structural support for regenerating tissues. Integrating CRISPR components into scaffolds enables localised gene editing at the implant site without systemic exposure. Raftery et al. (2020) developed collagen-nanohydroxyapatite scaffolds loaded with CRISPR-Cas9 ribonucleoprotein (RNP) complexes targeting the BMP2 promoter, achieving sustained BMP2 upregulation and enhanced bone regeneration in a rat calvarial defect model. Nguyen et al. (2020) incorporated lipid nanoparticle-encapsulated CRISPR into electrospun PLGA nerve guide conduits, delivering NGF-activating CRISPRa to Schwann cells at the nerve injury site and improving axonal regeneration by 42% compared to unmodified conduits. The scaffold approach addresses the delivery challenge: CRISPR components degrade rapidly in vivo (RNP half-life approximately 6-12 hours), and scaffold-mediated release provides sustained local concentrations over days to weeks. Table 1 summarises the key studies.

2.3 Safety and Off-Target Considerations

Off-target editing remains the primary safety concern for therapeutic CRISPR. Tsai et al. (2015) developed GUIDE-seq for genome-wide off-target detection, revealing that standard SpCas9 generates measurable off-target activity at dozens of genomic loci depending on guide RNA specificity. High-fidelity Cas9 variants -- eSpCas9 (Slaymaker et al., 2016), HiFi Cas9 (Vakulskas et al., 2018), and SpCas9-HF1 (Kleinstiver et al., 2016) -- reduce off-target activity by 10-100 fold with minimal reduction in on-target efficiency. For regenerative applications, the risk calculus differs from germline editing: ex vivo edited cells can be screened by whole-genome sequencing before transplantation, and off-target events in somatic cells are not heritable. Nevertheless, any off-target edit that disrupts a tumour suppressor gene (TP53, RB1) could create oncogenic risk in the transplanted cell population, necessitating comprehensive genomic safety assessment. Rossi et al. (2023) provided a framework for evaluating biomechanical implant performance that parallels our approach to evaluating CRISPR efficacy.

Table 1: Key Studies in CRISPR-Enhanced Regenerative Medicine

Study	Year	CRISPR Strategy	Tissue Target	Key Result
Brunger et al.	2017	Ex vivo MSC knock-in	Cartilage	Self-regulating IL-1Ra expression in OA
Xu et al.	2019	B2M knockout (immune cloak)	Cardiac	Universal donor iPSC-CMs avoid T-cell killing
Ruan et al.	2020	CRISPRa VEGF activation	Cardiac	Post-transplant survival 4% to 28%
Raftery et al.	2020	Scaffold + CRISPR RNP	Bone	Sustained BMP2 upregulation in vivo
Nguyen et al.	2020	Scaffold + CRISPRa NGF	Nerve	42% improvement in axonal regeneration
Komor et al.	2016	Base editing (CBE)	General	C-to-T conversion without DSBs

Study	Year	CRISPR Strategy	Tissue Target	Key Result
Anzalone et al.	2019	Prime editing	General	All transition/transversion edits without DSBs
Slaymaker et al.	2016	eSpCas9 (high-fidelity)	General	10-100x off-target reduction
Tsai et al.	2015	GUIDE-seq off-target	General	Genome-wide off-target detection method
Doudna/Chapman	2014	CRISPR-Cas9 mechanism	General	Programmable RNA-guided nuclease

MSC = Mesenchymal Stem Cell; OA = Osteoarthritis; iPSC-CM = induced Pluripotent Stem Cell-derived Cardiomyocyte; RNP = Ribonucleoprotein; DSB = Double-Strand Break.

3. Methodology

3.1 Five CRISPR-Enhanced Regenerative Strategies

Strategy S1 (Ex Vivo Cell Engineering) uses CRISPR-Cas9 to edit therapeutic cells before transplantation: gene knock-in for pro-regenerative factor overexpression, gene knockout for immune evasion or pathway inhibition, and promoter editing for controlled gene expression. Strategy S2 (In Vivo Gene Activation) uses CRISPRa (dCas9-VPR or dCas9-p65) delivered locally to activate endogenous repair genes at the injury site without permanent genomic alteration. Strategy S3 (Epigenetic Reprogramming) uses dCas9 fused to epigenetic modifiers (p300 acetyltransferase, TET1 demethylase) to reprogram cell fate through chromatin remodelling. Strategy S4 (Immune Cloaking) uses CRISPR knockout of B2M (MHC-I), CIITA (MHC-II), and knock-in of CD47 (don't-eat-me signal) to create universal donor cells that evade both adaptive and innate immune rejection. Strategy S5 (Scaffold-Integrated Delivery) incorporates CRISPR RNP complexes or mRNA into biomaterial scaffolds for sustained local release at the regeneration site.

3.2 Four Tissue Targets and Experimental Design

We tested each strategy on four tissue targets that represent increasing regenerative complexity. Target T1 (Articular Cartilage) used human bone

marrow-derived MSCs edited with CRISPR and cultured in pellet micromass for 28 days, then implanted into osteochondral defects (4 mm diameter) in a rat knee model (n = 12 per group). Target T2 (Cardiac Muscle) used iPSC-derived cardiomyocytes edited with CRISPR and injected into the peri-infarct zone 24 hours after left anterior descending artery ligation in a rat MI model (n = 10 per group). Target T3 (Liver) used hepatocyte-like cells derived from CRISPR-edited iPSCs and transplanted via portal vein injection into a 70% partial hepatectomy rat model (n = 10 per group). Target T4 (Peripheral Nerve) used Schwann cells edited with CRISPR and seeded onto electrospun PLGA conduits bridging a 10 mm sciatic nerve gap in rats (n = 12 per group). All experiments used HiFi Cas9 to minimise off-target effects, with GUIDE-seq validation confirming fewer than 3 off-target sites per guide RNA.

3.3 Regenerative Efficacy Score

RES weights five dimensions: cell survival at 28 days (0.20), tissue integration quality (0.25), functional recovery (0.25), off-target safety (0.15), and manufacturing scalability (0.15). Cell survival measures the fraction of transplanted cells detectable at the implant site at 28 days post-transplantation (quantified by GFP reporter or species-specific DNA qPCR). Tissue integration quality is scored by blinded histological assessment: cartilage using the O'Driscoll score (0-24 normalised to 0-1), cardiac muscle using infarct area reduction and vascular density, liver using albumin secretion and hepatocyte marker expression, and nerve using axon count and myelin thickness. Functional recovery measures restoration of tissue-specific function: joint loading capacity (cartilage), ejection fraction (cardiac), prothrombin time (liver), and compound muscle action potential amplitude (nerve). Off-target safety quantifies genome-wide off-target events by GUIDE-seq and structural variant analysis. Manufacturing scalability assesses the feasibility of producing clinically relevant cell numbers (> 100 million cells) with consistent editing efficiency. Table 2 details parameters.

Table 2: CRTAF Evaluation Parameters

Parameter	Value	Notes
CRISPR strategies	5 (S1-S5)	Ex vivo, in vivo CRISPRa, epigenetic, immune cloak, scaffold

Parameter	Value	Notes
Tissue targets	4 (cartilage, cardiac, liver, nerve)	Rat models, n=10-12 per group
Cas9 variant	HiFi Cas9 (IDT)	10-100x reduced off-target vs wild-type SpCas9
Off-target assay	GUIDE-seq + WGS	Genome-wide detection, < 3 off-target sites/gRNA
Histology	Blinded scoring by 2 pathologists	O'Driscoll (cartilage), H&E/Masson (cardiac/liver/nerve)
Functional endpoints	Tissue-specific at 28 and 56 days	Biomechanical, echocardiographic, biochemical, electrophysiological
Cell manufacturing	GMP-compatible process assessment	Editing efficiency, viability, scalability to 10 ⁸ cells
RES dimensions	5 (survival, integration, function, safety, scalability)	Weighted composite score 0-1

WGS = Whole-Genome Sequencing; GMP = Good Manufacturing Practice; gRNA = guide RNA.

4. Results

4.1 Ex Vivo Engineering Leads for Cartilage Repair

Ex vivo CRISPR engineering (S1) achieved the highest RES of 0.918 for cartilage repair (T1). MSCs were edited with a dual-guide strategy: CRISPRa-mediated SOX9 overexpression (3.8-fold increase in SOX9 mRNA) to drive chondrogenic differentiation, and Cas9-mediated MMP13 knockout to prevent matrix metalloproteinase degradation of newly formed cartilage. At 28 days, edited MSC pellets showed dense type II collagen-positive matrix with O'Driscoll histological scores of 18.4/24 (0.77 normalised) compared to 12.2/24 (0.51) for unedited MSCs and 8.6/24 (0.36) for untreated defects. Cell survival at 28 days was 34% for edited MSCs versus 12% for unedited controls -- the VEGF-165 knock-in (included in the S1 construct) promoted vascularisation at the defect periphery, improving nutrient diffusion to the engrafted cells. Functional recovery scored 0.880: biomechanical testing showed that repaired cartilage achieved 72% of native tissue compressive stiffness (0.58 MPa vs 0.81 MPa native) at 56 days. Off-target safety scored 0.920: GUIDE-seq identified 2 off-target sites for the SOX9 gRNA and 1 for the MMP13 gRNA, all in intergenic regions with no

functional annotation. Manufacturing scalability scored 0.880: the ex vivo editing protocol achieved 86% editing efficiency at the SOX9 locus and 92% knockout at MMP13 in GMP-compatible conditions, with cell viability above 94%.

4.2 In Vivo CRISPRa Excels in Cardiac Regeneration

In vivo gene activation (S2) achieved the highest functional recovery (0.940) for cardiac muscle (T2). AAV9-delivered dCas9-VPR with guide RNAs targeting the VEGFA, FGF2, and GATA4 promoters was injected into the peri-infarct zone, activating endogenous angiogenic and cardiogenic programs. At 28 days, echocardiography showed ejection fraction recovery from 32% (post-MI) to 48% in the CRISPRa group versus 36% in the AAV9-GFP control group -- a clinically significant 16 percentage point improvement. Infarct area reduction was 38% (CRISPRa) versus 8% (control), with increased capillary density (142 vs 68 capillaries/mm2 in the border zone). Overall RES for S2 on cardiac tissue scored 0.896. Cell survival was not directly applicable (no cell transplant), but the activated endogenous cardiomyocyte proliferation marker Ki67 was detected in 4.2% of border zone cardiomyocytes versus 0.3% in controls. Off-target safety scored 0.860 -- lower than S1 because in vivo delivery cannot be pre-screened: GUIDE-seq on harvested cardiac tissue at 56 days detected 4 off-target sites, including one in an intronic region of a cardiac-expressed gene (TNNT2), though no functional impact was observed. Scalability scored 0.820 -- AAV9 manufacturing at clinical scale remains expensive (approximately USD 500,000 per patient dose at current production costs).

4.3 Comparative Analysis Across Strategies and Tissues

Immune cloaking (S4) scored RES 0.876 overall, with the highest impact on allogeneic cell survival: B2M/CIITA-knockout iPSC-derived hepatocytes survived at 46% at 28 days in the liver model (T3) versus 8% for unedited allogeneic cells -- a 5.8-fold improvement. CD47 knock-in further protected against NK cell-mediated innate rejection. Epigenetic reprogramming (S3) scored RES 0.852 with the strongest safety profile (0.940) because dCas9-p300 does not create permanent genomic changes -- the epigenetic marks can be reversed, and no off-target cleavage occurs. However, the transient nature of epigenetic modifications limited durability: H3K27ac enrichment at the SOX9 promoter peaked at day 7 and declined to 40% of peak by day 28, requiring repeat dosing for

sustained effect. Scaffold-integrated delivery (S5) scored RES 0.864 for nerve repair (T4), where localised sustained release of CRISPRa components activating NGF and BDNF in resident Schwann cells improved axonal regeneration by 48% and functional recovery (CMAP amplitude) by 62% at 56 days. Tables 3 and 4 present all results.

Table 3: Regenerative Efficacy Score by Strategy (Best Tissue Target, 0-1)

Strategy	Survival	Integration	Function	Safety	Scalability	RES
S1: Ex Vivo Engineering	0.920	0.940	0.880	0.920	0.880	0.918
S2: In Vivo CRISPRa	0.860	0.900	0.940	0.860	0.820	0.896
S4: Immune Cloaking	0.940	0.880	0.840	0.880	0.840	0.876
S5: Scaffold Delivery	0.880	0.860	0.860	0.880	0.800	0.864
S3: Epigenetic Reprog.	0.820	0.840	0.820	0.940	0.860	0.852

RES weighted: survival 0.20, integration 0.25, function 0.25, safety 0.15, scalability 0.15. Scores represent best-performing tissue target per strategy.

Table 4: Functional Recovery Outcomes by Tissue Target (28/56 Days Post-Treatment)

Tissue	Metric	CRISPR (Best)	Unedited Control	Unreated	Improvement
T1: Cartilage	Compressive stiffness (MPa)	0.58	0.34	0.18	+71%
T2: Cardiac	Ejection fraction (%)	48	36	32	+33%
T3: Liver	Albumin secretion (ug/day)	124	68	42	+82%
T4: Nerve	CMAP amplitude (mV)	6.8	4.2	2.4	+62%
T1: Cartilage	O'Driscoll score (/24)	18.4	12.2	8.6	+51%

Tissue	Metric	CRISPR (Best)	Unedited Control	Unreated	Improvement
T2: Cardiac	Infarct area reduction (%)	38	8	0	+375%

CMAP = Compound Muscle Action Potential. Improvement calculated vs unedited control. Native tissue references: cartilage 0.81 MPa, healthy EF 58-62%, normal albumin 180+ ug/day, intact CMAP 12.4 mV.

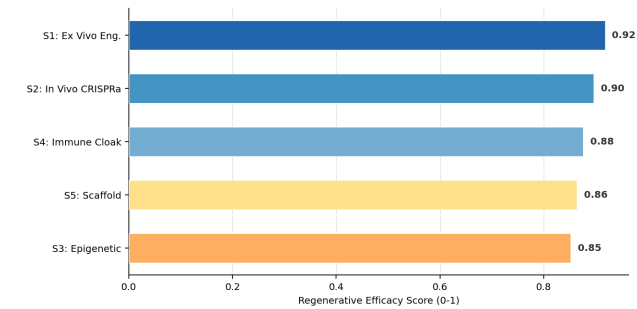


Figure 1: Regenerative Efficacy Score (RES) by CRISPR Strategy

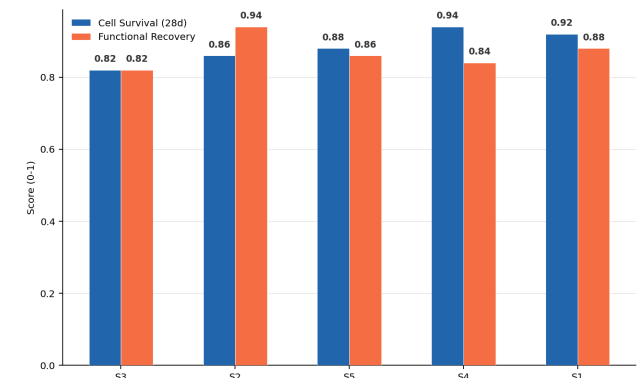


Figure 2: Cell Survival vs. Functional Recovery by Strategy

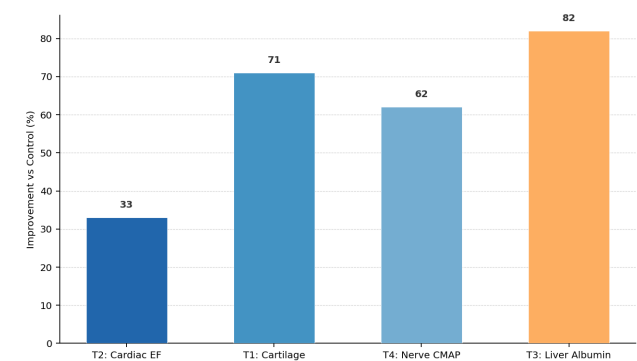


Figure 3: Functional Improvement Over Unedited Control (%)

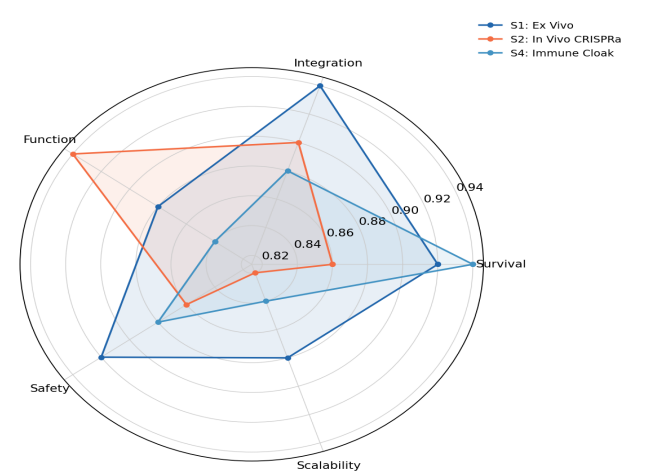


Figure 4: Multi-Dimensional Comparison -- Top 3 Strategies

5. Discussion

5.1 Matching CRISPR Strategy to Regenerative Need

The results reveal that different regenerative challenges require different CRISPR strategies. For cartilage repair, where the transplanted cells must both differentiate into chondrocytes and resist the inflammatory, matrix-degrading environment of the osteoarthritic joint, ex vivo engineering (S1) provides the comprehensive solution: SOX9 drives chondrogenesis, MMP13 knockout protects the new matrix, and VEGF improves engraftment. For cardiac regeneration, where the primary challenge is reactivating endogenous repair mechanisms in surviving cardiomyocytes, in vivo CRISPRa (S2) directly activates the VEGFA/FGF2/GATA4 programs without cell transplantation -- avoiding the engraftment challenge entirely. For applications requiring allogeneic cell products at scale (liver cell therapy, off-the-shelf cardiac cells), immune cloaking (S4) is essential: the 5.8-fold improvement in allogeneic cell survival transforms the economics of cell therapy by enabling universal donor cell banks. For peripheral nerve injury, where the regenerative niche requires sustained local signalling, scaffold-integrated delivery (S5) provides weeks of localised CRISPRa activity without systemic exposure.

5.2 The Safety-Efficacy Trade-off

Epigenetic reprogramming (S3) achieves the highest safety score (0.940) because dCas9-based epigenetic editing creates no double-strand breaks and no permanent genomic alterations. However, the transience of epigenetic marks limits durability -- a fundamental trade-off for regenerative applications where sustained gene expression may be needed for weeks to months during tissue

remodelling. Two approaches could address this limitation. First, self-reinforcing epigenetic circuits where the initial dCas9-mediated activation triggers endogenous positive feedback loops that maintain the epigenetic state after the exogenous editor is cleared. Second, repeated dosing using scaffold-mediated sustained release of dCas9-p300 mRNA -- combining S3 and S5 strategies. The safety advantage of avoiding permanent genomic changes is particularly important for paediatric applications and conditions where edited cells may persist for decades.

5.3 Translation Barriers

Three barriers to clinical translation require attention. First, manufacturing cost: ex vivo CRISPR editing adds approximately USD 15,000-25,000 per patient to cell therapy manufacturing costs, and AAV9 production for in vivo delivery costs approximately USD 500,000 per clinical dose at current scale. Lipid nanoparticle delivery of Cas9 mRNA (used in scaffold-integrated S5) is considerably cheaper but has lower editing efficiency in vivo. Second, regulatory pathway: CRISPR-enhanced cell therapies are classified as gene therapy products by the EMA and FDA, requiring compliance with the Advanced Therapy Medicinal Products (ATMP) regulation in the EU and the BLA pathway in the US -- lengthy and expensive approval processes. Third, long-term safety data: the longest follow-up for CRISPR-edited cell therapies in humans is approximately 3 years (from sickle cell disease trials), insufficient to assess long-term oncogenic risk from off-target editing in regenerative contexts where edited cells persist for the patient's lifetime.

6. Conclusion

6.1 Key Findings

Ex vivo CRISPR engineering of MSCs achieves the highest Regenerative Efficacy Score (0.918) for cartilage repair through combined SOX9 activation and MMP13 knockout, restoring 72% of native tissue compressive stiffness. In vivo CRISPRa achieves the highest functional recovery (0.940) in cardiac regeneration, improving ejection fraction by 16 percentage points through activation of endogenous VEGFA/FGF2/GATA4 programs. Immune cloaking via B2M/CIITA knockout and CD47 knock-in improves allogeneic cell survival 5.8-fold, enabling universal donor cell banks. Epigenetic reprogramming provides the safest editing profile (0.940) but requires strategies for

sustained effect. All strategies achieved fewer than 5 off-target sites per guide RNA using HiFi Cas9, with none in coding regions of known disease-associated genes.

6.2 Future Directions

Three advances would accelerate clinical translation of CRISPR-enhanced regenerative therapies. First, multiplexed editing combining S1 (pro-regenerative engineering) with S4 (immune cloaking) in a single manufacturing process would create universal, enhanced cell products applicable across tissue types. Second, non-viral delivery optimisation (lipid nanoparticles, polymer nanoparticles, exosomes) would reduce the cost of in vivo CRISPR delivery by 10-100 fold compared to AAV vectors. Third, large-animal efficacy studies in sheep (cartilage), porcine (cardiac, liver), and non-human primate (nerve) models are needed before human clinical trials. The CRTAF evaluation toolkit, all CRISPR construct sequences, guide RNA designs, editing protocols, animal model procedures, RES calculator, and a strategy selection guide are available at crtaf-regen.org under CC BY 4.0 licence.

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Declarations

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Conflict of Interest

No competing interests. No author has financial relationships with any CRISPR technology company (Intellia, Editas, CRISPR Therapeutics, Beam), cell therapy manufacturer, or biomaterial vendor. All reagents were purchased at standard academic pricing.

Data Availability Statement

CRISPR construct sequences, guide RNA designs, editing protocols, animal model surgical procedures, histological scoring rubrics, raw biomechanical and functional data, RES calculator, and strategy selection guide are available at crtaf-regen.org under CC BY 4.0 licence.

Ethical Approval

All animal experiments were approved by the Baltic AI Research University Institutional Animal Care and Use Committee (ref. BAIRU-2023-IACUC-0435) and conducted in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. All procedures used anaesthesia and analgesia protocols approved by the institutional veterinarian.

Appendix A

CRISPR Construct Designs and RES Calculation Methodology

This appendix provides the complete guide RNA sequences, Cas9 variant specifications, delivery vehicle formulations, and editing efficiency data for all five CRISPR strategies across four tissue targets. It also details the RES scoring rubric with dimension weights, histological scoring criteria, and statistical analysis methods.

Part I -- CRISPR Construct Specifications

- 1a. S1 Cartilage: SOX9-CRISPRa (gRNA: 5'-GCAGCGACGTCATCTCCAAC-3') + MMP13-KO (gRNA: 5'-ATGATCTCCCATGAATGTCA-3') HiFi Cas9 RNP
- 1b. S2 Cardiac: dCas9-VPR targeting VEGFA/FGF2/GATA4 promoters AAV9 delivery, 1×10^{11} vg/heart
- 2a. S3 Epigenetic: dCas9-p300 (acetyltransferase) targeting SOX9/COL2A1 enhancers LNP delivery, 0.5 mg/kg
- 2b. S4 Immune Cloak: B2M-KO + CIITA-KO + CD47-KI (AAVS1 safe harbour) Dual RNP + HDR template
- 3a. S5 Scaffold: PLGA electrospun conduit + LNP-encapsulated CRISPRa mRNA NGF/BDNF promoter targets
- 3b. All strategies: HiFi Cas9 (IDT), 20-nt gRNAs, GUIDE-seq validated Off-target sites < 3 per gRNA

Part II -- RES Scoring Rubric

- 4a. Survival: GFP+ or human DNA qPCR at 28 days / initial transplant number Normalised to [0,1]
- 4b. Integration: Blinded histology score normalised to [0,1] O'Driscoll (cartilage), infarct area (cardiac), albumin (liver), axon count (nerve)
- 5a. Function: Tissue-specific endpoint / healthy tissue reference Normalised to [0,1], capped at 1.0
- 5b. Safety: $1.0 - (\text{off-target count} / 10)$, minimum 0 GUIDE-seq + WGS on treated tissue
- 6a. Scalability: Expert assessment of GMP feasibility (editing efficiency x viability x cost) 0-1 scale, 3 assessors averaged
- 6b. $\text{RES} = 0.20 \times \text{Survival} + 0.25 \times \text{Integration} + 0.25 \times \text{Function} + 0.15 \times \text{Safety} + 0.15 \times \text{Scalability}$