

CRISPR/Cas Systems in Tissue Engineering: A Succinct Overview of Current Use and Future Opportunities



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Abstract

Tissue engineering is a broad field that aims to restore or maintain the function of tissues and organs. Despite progress in the field, non-trivial obstacles have prevented the widespread adoption of tissue engineered products. Genome editing and synthetic biology approaches may be suitable strategies to tackle a number of these obstacles. Clustered regularly interspaced short palindromic repeats systems have revolutionized genome engineering and facilitated its adoption by many non-specialized laboratories, allowing tissue engineering laboratories to genetically modify cells for applications ranging from immune response circumvention to in vitro 3D micro tissue model development. This review presents a brief overview of the current use and future applications of clustered regularly interspaced short palindromic repeats systems in tissue engineering.

Keywords: Tissue engineering; Genome engineering; Stem cells; Cellular engineering; Reprogramming

Abbreviations: ASC: Adipose-Derived Stem Cells; BMP: Bone Morphogenetic Proteins; Cas: CRISPR associated protein; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; DCAS9: Catalytically Inactive Cas9; GRN: Gene Regulatory Networks; GRNA: Guide RNA; HDR: Homology-Directed Repair; IPS: Induced Pluripotent Stem Cells; KRAB: Krüppel Associated Box; LACE: Light-Activated CRISPR/Cas9 Effector; NHEJ: Non-Homologous End Joining; TALEN: Transcription-Activator-Like Effector Nucleases

Introduction

Tissue engineering is an interdisciplinary field that aims to restore or maintain the function of tissues and organs. Increasingly, it has also been seen as a platform for the development of in vitro disease models to aid in the discovery and translation of molecular therapies [1]. Despite garnering tremendous interest and continued advancements since the field's inception in the late 1980's, tissue engineering is faced with multiple obstacles that have prevented widespread translation to approved therapies. Among these obstacles, tissue engineers frequently highlight the following as particularly important induction of angiogenesis, cell source and differentiation, immunological response control, and formation of molecular and macroscopic tissue architecture [1,2]. Approaches to tackle these hurdles are varied, but genome editing and synthetic biology strategies based on clustered regularly interspaced

short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas), which form a system (CRISPR/Cas), have begun to be utilized and may lead to key developments in the future [3]. CRISPR/Cas systems are prokaryotic, nucleic acid-targeting restriction machineries that have recently been engineered to edit genomic material and regulate transcription of endogenous genes [4]. CRISPR/Cas systems have several advantages that have revolutionized genome engineering: capability of directed editing, simplicity, speed, and economy [4]. This succinct review will discuss the current uses of CRISPR/Cas systems in tissue engineering to address cell differentiation, immune response circumvention, tissue architecture formation, and development of disease models. Furthermore, the future possibilities of its use in stem cell engineering, angiogenesis, immunoengineering/immunomodulation, and spatiotemporal programming and patterning will be examined.

Discussion

CRISPR/Cas systems are nucleic acid-targeting, RNA-directed adaptive restriction machineries used by prokaryotes as acquired immune mechanisms against mobile genetic elements [4]. Although the adaptation of these systems to genome editing has revolutionized genetic engineering, the technology has been used for other applications, including live-cell DNA imaging and transcriptional activation/repression [5-7]. CRISPR/Cas systems that have been engineered for genome editing have two components: a synthetic, single-stranded guide RNA (gRNA); and a Cas. The spacer portion of the gRNA can be designed to bind complementary DNA targets, directing Cas to the site to generate a single-stranded, or double-stranded break. This capability has been exploited to introduce random mutations, via non homologous end joining (NHEJ), and to precisely edit DNA sequences via homology-directed repair (HDR). Even though CRISPR-associated protein 9 (Cas9) is the most common Cas used in CRISPR/Cas systems, other Cas have been used to allow orthogonal systems (e.g. Cpf1). When catalytically inactive Cas variants are used, the protein binds the DNA site as directed by the gRNA, but does not cleave the target and can inhibit or activate transcription by steric hindrance or transcription repressors/activators fused to the Cas. Editing, transcription control, epigenetic modification, and tagging with CRISPR/Cas systems can be directed at single targets, or multiplexed to several distinct targets in the same cell, while also performing several of the functions concomitantly, allowing the development and study of complex gene regulatory networks (GRN). The flexibility of CRISPR/Cas systems, coupled with speed, economy, and simplicity when compared to protein-based tools like zinc finger and transcription-activator-like effector nucleases (TALEN), are the main advantages that have propelled CRISPR/Cas systems into widespread use [4,7]. Nonetheless, CRISPR/Cas systems face several challenges, including off-target effects and difficulties in the delivery of large Cas9 sequences. Cas proteins can tolerate mismatches between the gRNA and target sequence, reducing the specificity and allowing binding to sequences other than the target. These off-target effects must be addressed before CRISPR/Cas systems can be used in therapeutic applications, and progress is being made in approaches to reduce non-specific binding. Delivery of Cas sequences is also being actively studied, with advances in viral vector, lipid-based transfection techniques, and nanoparticle delivery. CRISPR/Cas systems can be introduced to cells as plasmid DNA, RNA, or protein forms, and can use transient or stable transfection/transduction, allowing extensive flexibility in design of delivery systems, which are key issues when engineering cells for tissue engineering. Despite these obstacles, the power of CRISPR/Cas systems is notable, and continues to transform many fields given their ease and accessibility regarding design, synthesis, and modification enabling the development of tools for a myriad of purposes. Recognizing this potential, tissue engineering has

begun to explore CRISPR/Cas systems as an apparatus to tackle several of the discipline's hurdles.

The current uses of CRISPR/Cas systems in tissue engineering have focused on addressing cell differentiation, immune response circumvention, tissue architecture formation, and disease model development. Stem cells are a cell source that has enormous potential for tissue engineering applications, but their directed differentiation remains a non-trivial challenge. To improve the osteogenic potential of adipose-derived stem cells (ASC), CRISPR/Cas has been used to edit their genome, exchanging bone morphogenetic protein (BMP) antagonist-encoding sequences (noggin) with BMP agonist-encoding sequences (BMP-2) [8]. By replacing noggin with BMP-2, the cellular mechanism that up regulates the BMP antagonist is harnessed to express the BMP agonist under noggin promotion. This, in theory, would drive the ASC population towards an osteogenic lineage, even under stimuli that would inhibit BMP production. This use of CRISPR/Cas systems to drive differentiation of stem cells to a desired lineage is an advantageous alternative to the common clinical practice of using exogenous recombinant growth factors. Similarly, other groups have used CRISPR/Cas systems to direct differentiation for applications in tissue engineering and disease modeling. Investigators have used CRISPR/Cas systems to edit human intestinal stem cells to explore pathways that drive colorectal carcinoma development [9]. These engineered stem cells form intestinal organoids that may be used as an *in vitro* platform to study and develop therapies. Another group used CRISPR/Cas systems to edit induced pluripotent stem cell (iPS) genomes to generate isogenics cell lines with or without mutations [10]. These cell lines would have applications in tissue engineering and disease modeling. Recently, optogenetic control of transcription from endogenous genes has been achieved by developing a CIBN-dCas9 fusion protein that, when exposed to blue light, dimerizes with the transcriptional activator CRY2-VP64 [11]. This light-activated CRISPR/Cas9 effector (LACE) can be used to drive cell differentiation with the added advantage of spatiotemporal control. This capability of controlling the timing and pattern of differentiation could be used as an approach to generate biomimetic tissue architecture in tissue engineered constructs. Tissue engineering use of CRISPR/Cas systems will be dependent on the ability to minimize off-target events, and limited exposure to Cas activity is a key factor. Systems such as LACE, when integrated into the genome, could limit the exposure to Cas activity, but care must be taken to avoid random integration, possibly by targeting safe harbor loci for CRISPR/Cas system integration. Another group used dCas9 fused to Krüppel Associated Box (KRAB) acetyl transferase p300 to induce H3K9 methylation and histone acetylation, respectively [12]. This approach to regulate gene expression using epigenetic activation/repression was used on human ASC to repress the genes controlling cytokine receptors, improving the survivability of ASC in an inflammatory environment, as

well as increasing extracellular matrix (ECM) deposition and chondrogenic potential. Increasing survivability of ASC after implantation is a key goal for successful cell therapy, as most stem cells are cleared within hours of *in vivo* implantation, leading to poor engraftment [13]. Strategies using CRISPR/Cas systems to modulate immune response and increase the likelihood of ASC engraftment could prove useful in other tissue engineered constructs, as inflammatory milieus are frequently encountered due to implantation and/or underlying autoimmune conditions. Altogether these examples of CRISPR/Cas use in tissue engineering are the harbingers of an emerging push towards using molecular and synthetic biology to assist in the resolution of challenges faced by the field.

Future use of CRISPR/Cas systems in tissue engineering will continue to address cell differentiation, immune response circumvention, tissue architecture formation, and disease model development but will also likely focus on angiogenesis, immunoengineering/immunomodulation, and spatiotemporal programming and patterning. While there have been advances in stem cell engineering to promote angiogenesis, most use tools that require specialized knowledge that limit the accessibility to a wide array of research groups. An example of this is the engineering of hepatocyte growth factor-secreting umbilical cord stem cells using TALEN to promote angiogenesis [14]. CRISPR/Cas systems would allow groups lacking specialized knowledge in protein engineering to expand on these advancements and possibly create even more complex, multifactor-secreting cells. Similarly, the development of more robust technologies to reduce off-target effects and the ability to multiplex targeting will lead to the use of complex synthetic biology and bioinformatics approaches to create gene regulatory networks for the control of multicellular development. Approaches using CRISPR/Cas systems to regulate apoptosis, homing, and differentiation of stem cells will likely be developed, as these are areas of focus for stem cell engineering [3]. Additionally, the capacity to address these areas concomitantly with one single system is the logical, attractive solution. Furthermore, complex GRN have potential to be used to generate tissue architecture that will resemble native tissues in both form and function, impacting multiple applications in tissue engineering, such as multi-layered tissues (e.g. vascular grafts), whole organ engineering, and 3D micro tissue development for *in vitro* assays and disease modeling. The possibilities of using GRN to assert spatiotemporal control are also likely to be harnessed for introducing angiogenesis and immunoengineering/immunomodulation solutions to the field. In these spatiotemporal regulation systems, CRISPR/Cas-based GRN would respond to a variety of physico-chemical stimuli, both endogenous and exogenous, to trigger complex behaviors that would allow the timed release of growth factors and cytokines, while also regulating the expression of receptors on the cells, all while under spatiotemporal control [3]. Additionally GRN could be designed to include “kill switches” that would

remove CRISPR/Cas system activity when needed, avoiding risks of permanent expression of foreign DNA, which would be advantageous when designing tissue engineered constructs for clinical use. As progress in the fields of synthetic biology and bioinformatics continue to elucidate and inform biologic circuit design, our understanding of and ability to create complex GRN will improve, allowing for more intricate control of cellular functions. CRISPR/Cas systems will more than likely be centerpieces of this endeavor allowing tissue engineers to tackle long-standing challenges and face the new ones that will undoubtedly emerge.

Conclusion

While tissue engineering has made great progress, and become a wide-ranging multidisciplinary field, successful translation into the clinic has been hindered by numerous challenges. The field has ventured into genetic engineering approaches to help solve these challenges, and CRISPR/Cas systems have effectively democratized the access to complex genome editing and transcription regulation. While still in its infancy, several groups have begun to adapt CRISPR/Cas systems to the needs of tissue engineers setting the foundations for future growth and more complex implementation. Particularly encouraging is the use of CRISPR/Cas systems for *in vitro* 3D tissue models as this is the logical next step for tissue engineering. The impact of tissue engineered disease models will be vast and any technology that facilitates the prompt development of these models will accelerate the translation of therapies for devastating diseases such as cancer. The future applications of CRISPR/Cas systems in tissue engineering will be sweeping and limited only by our understanding of cellular processes that are constantly being studied as such, the future seems auspicious.

Conflict of Interest

The authors certify that they have NO affiliations with organizations with financial or non-financial interests discussed in this manuscript.

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