Chapter

Tuning Phage for Cartilage Regeneration

Ayariga Joseph Atia, Abugri Daniel Azumah, Bedi Deepa and Derrick Dean

Abstract

The ever-broadening scope of phage research has left behind the simplistic view of studying phages as just model systems in phage biology to a much broader application ranging from ecological management to immunity. Improved throughput technology in crystallography and structural studies has helped our understanding of these systems as supramolecular machines that possess the capacity of self-assembly. The idea of phages as self-assembling supramolecular nano-machines that are bioactive biomaterials in characteristics, tunable and easily producible have lent its utility to recent fields such as regenerative medicine and tissue engineering. Due to low metabolic activity and slow nutrient diffusion within cartilage, damage to this tissue often inevitably consist of slow and delayed regeneration and healing, the restriction of blood from reaching most part of this tissue and the resultant limitations in the availability of oxygen and other essential amino acids dictates a very slow systemic metabolic response also since transports system in this tissue have to employ less speedy forms. Cartilage regeneration therefore is a huge challenge. This chapter takes a look at the application of the phage display technology in cartilage tissue regeneration.

Keywords: self-assembling, supramolecular, bioactive biomaterials, cartilage tissue regeneration, phage display

1. Introduction

In nature, there exist remarkable structural complexities created out of self-assembly, for instance ice crystals from falling snow. In Molecular self-assembly, molecules adopt specific arrangement automatically without the direction of outside source. Phages like liquid crystals behave in such similar fashion, having the ability to self-assembly. Phages are viruses that infect bacterial cells, and also serve as most commercial vectors for recombinant DNA studies. Molecular self-assembly is a key concept in phage chemistry. The components of most phages or viruses in general have an assembly system which usually is directed through non-covalent interactions such as hydrogen bonding, hydrophobic forces, van der Waals forces, and electrostatic etc., leading to the formation of supramolecular assemblies composed of different shapes and sizes [1]. For instance, the interaction of the P22 phage tailspike protein with its capsid to form an infective phage is entirely non-covalent, however, once interaction is complete, bond reversibility is impossible [2]. Molecular self-assembly allows the construction of interesting molecular

topologies. This self-assembly system is also crucial in biological systems in the form of the formation of biomolecular condensates in living organisms, also found in oligomerization of protein subunits to form multimers of complex structures [3]. The application of this system therefore is a bottom-up approach, in which components of the phages are directed to self-assembly to achieve a programmed molecular topology, consisting of the desired shape and functional groups.

Most researches have delved into self-assembling filamentous phages, thus shed light on the pathways for their self-assembly. Filamentous bacteriophages such as the *Escherichia coli* K12-infecting Ff phages (F1, Fd or M13) replicate episomally and contain a circular single-stranded DNA packaged into long filaments. These phages are secreted into the environment without lysing their host. The knowledge of phages in general and filamentous phages in particular can played such a vital role in formulating new approaches in fabricating bioactive biomaterials [4] and providing for synergies and opportunities in phage display and tissue engineering approaches.

2. Phage as biotechnological platform for cartilage study, therapy and diagnosis

Due to a low metabolic activity and slow nutrient diffusion within cartilage, damage to this tissue often inevitably consist of slow and delayed regeneration and healing, the restriction of blood from reaching most part of this tissue and the resultant limitations in the availability of oxygen and other essential amino acids dictates a very slow systemic metabolic response since transports system in this tissue have to employ less speedy forms such as transport proteins across the thick ECM. Accidents that cause injury to the knee may sometimes rupture the articular cartilage. Most diseases associated with articular cartilage include the following; 1) osteoarthritis; a condition where the cartilage covering the bones in joints is thinned and sometimes completely worn out. This leads to exposure of the bone ends to friction and erosion which causes bone damage. Aberrant immunometabolism has also been implicated in most phenotypes of osteoarthritis [5]. 2) Rheumatoid arthritis is a chronic systemic autoimmune disease that primarily affects the lining of the synovial joints. This disease is progressive with the pathological mechanism driven via the deterioration of cartilage, bone erosion; hyperplastic synovium and systemic consequences [6]. Most symptoms of rheumatoid arthritis include arthralgia, swelling, redness, joint pain and hence limiting the range of motion [7]. 3) Some other disease/conditions related to cartilage degeneration are relapsing polychondritis [8, 9], achondroplasia [10], costochondritis [11, 12], herniation [13], chondrosarcoma [14], chondroma [15] etc. While biological factors have been well known to play crucial roles in the etiology of these diseases, therapeutic management of these conditions have proved less reliable. Below, we discuss some related works that have been done in the cell-phage research interface, and how the knowledge of both fields could synchronize to help find answers for cartilage regeneration and therapy challenges.

2.1 Using phage to regulate alignment and morphology of chondrocytes

Chondrocytes in articular cartilage have unique alignment with respect to the articular surface, this is crucial for the functional performance of the cartilage. A deeper comprehension of the chondrocytes and collagen alignment is important for a better appreciation of the load bearing and shock absorption function of this tissue. Chondrocytes are organized into four zonal layers in the articular cartilage

tissue: superficial zone, middle zone, deep zone and calcified zone. The superficial zone contains elongated and flattened chondrocytes whereas the middle zone has rounded chondrocytes as shown in **Figure 1**. The deep zone and calcified zone have hypertrophic chondrocytes. Cartilage tissue has one of the poorest proliferative capacities and loss of chondrocytes as well as abrasions to the articular surface could give rise to osteoarthritis [16–18]. In cartilage regenerative effort, the interest usually is to produce cartilage with high performance comparable to the natural tissue, and this implies supplying chondrocytes with the right physical and molecular cues to direct their proliferation, differentiation and tissue regeneration. Therefore, guiding the topological and structural organization of the scaffold in which chondrocytes are seeded as well as modulating the molecular cues functionalized to the scaffold is of crucial importance for cartilage tissue regeneration.

In a biomimetic strategy, He and his colleagues synthesized nanofibrous bio-inorganic hybrid materials by using phage as a model biological nanofiber and calcium hydroxyapatite (HAP) as a model inorganic material [19]. They induced the nanofibers self-assembly into phage-cation complex structures through electrostatic interaction between anionic phage nanofibers and the free precursor cations of the inorganic materials. Successful orientation of collagen molecules was also reported. This bioengineered phage bio-nanofibers as biotemplates oriented the nucleation of HAP, formed cluster of structures induced by calcium ions. They observed that the orientations of HAP crystals were formed along Ca^{2+} induced phage bundles, then finally, their co-assembled collagen-phage hybrid bundles induced an aligned nucleation of HAP on them [19].

This gives an excellent platform for cartilage tissue regeneration experiments since collagen fibers arrangement and alignment is a critical measure of cartilage performance. In articular cartilage, the superficial zone consists of mostly type I collagens that are aligned parallel to the articular surface to reduce friction. Hence, the use of such a novel framework for collagen orientation in cartilage regenerative effort will prove useful. The ability to determine the assembly and orientation of collagen or minerals by this co-assembly process in a bio-mimetic scaffold presents

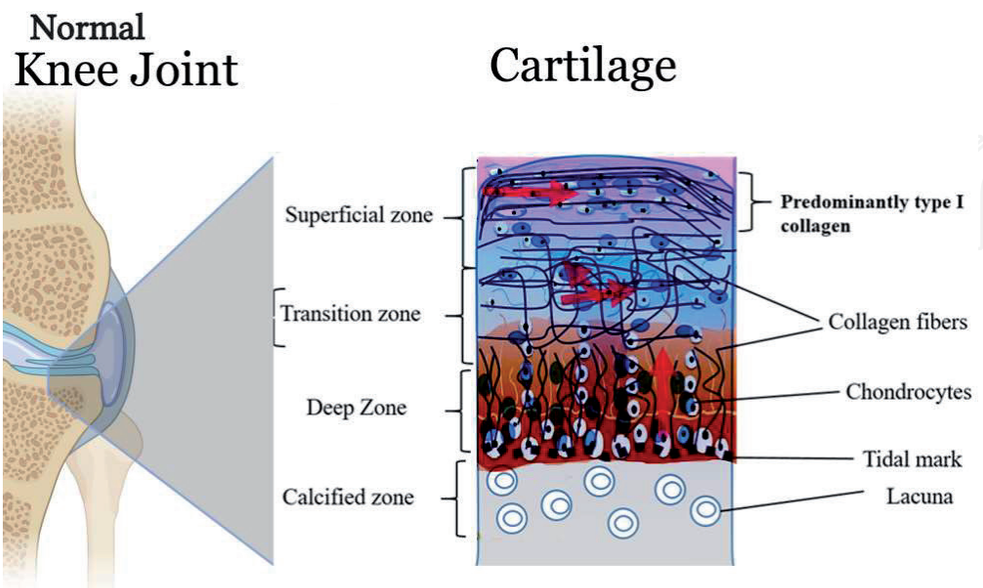

Figure 1.
Cartoon illustration of the anatomical structure of the articular cartilage, depicting the collagen fibers orientation and chondrocytes morphology. The superficial zone consists of flattened and horizontally aligned chondrocytes with also horizontally aligned collagen fibers, predominantly collagen I. the transition zone has rounded chondrocytes, with randomly aligned collagen II fibers; the deep zone has vertically aligned columns of chondrocytes with vertical collagen alignment.

an interesting process for producing excellent cartilage or bone tissue via tinkering the organization and orientation of both proteins and HAP in order to produce superior functional and mechanical properties of cartilage or bone tissue [20].

Young and his colleagues demonstrated that phage-based array chips could be used for an optically readable cell proliferation and morphology assays. They engineered M13 phages that that displayed RGD on its major coat proteins and also functionalized the growth factor, FGF2, on its minor coat proteins. Since M13 can self-assembly, they constructed from them a nanofibrous network scaffold, then grew cells on them. They monitored for biochemical cues displayed by the phage on cell proliferation and morphology. This elegant work allowed for the utility of engineered phages for sensitive monitoring of the effects of functional peptides on cell growth [21].

2.2 Phage used for chondrogenic differentiation of stem cells for cartilage engineering

The chondrogenic potential of mesenchymal stem cells (MSCs) allows for stem cell therapy of damaged cartilage possible. These stem cells can easily be obtained via biopsy from the patient then amplified in the laboratory. This has therefore made MSCs a routinely used cell types for cartilage regeneration [22, 23].

Phage display derived functional peptides has been employed for chondrogenic differentiation of MSCs [24].

TGF-β1- and collagen II-binding peptides were identified through phage display biopanning by Meng and his colleagues [25]. They discovered the peptide HSNGLPL to have high affinity to TGF-β1 receptor, the peptide was then functionalizedbypolyurethane with side propynyl groups via CuAAC click reaction to form nanofiber gel materials with high TGF-β1-binding affinity which acted as an absorbent for TGF-β1 within gels [24, 25]. Their findings demonstrated that their construct induced chondrogenic differentiation of human MSCs *in vitro* and promoted rabbit articular cartilage regeneration.

2.3 Using phage display to initiate cellular signaling

Integrins are transmembrane receptors for extracellular matrix proteins [26]; they play a crucial role in signal transduction in chondrocytes and control cellular attachment, migration, proliferation and apoptosis etc. One of the severally known signaling pathways initiated by integrinsare the Src pathway, this pathway is known to coordinate very vital cellular processes [27], downstream of which include the RHO, SMAD, AKT etc. as depicted in **Figure 2**. While the RHO pathway transduced via integrins acts to regulates actin cytoskeleton [28] leading to cell spread and migration, the AKT through ERK pathway is crucial for chondrocytes growth, proliferation and survival [27, 28]. Interestingly also, downstream of Src, upon ERK pathway activation, the transcription factor SMAD1/5/8 is known to be blocked from nuclear translocation, thereby blocking chondrocyte's hypertrophy, and bone formation processes [29–31]. This step is crucial for forming normal cartilage. While the SMAD signaling pathway will eventually lead to the inhibition of chondrocyte's hypertrophy, the RHO signaling improves cells motility and migration, whereas, the ERK pathway could signal chondrocytic differentiation of MSCs into chondrocytes for cartilage regeneration.

To influence chondrocytic differentiation of MSCs for cartilage regeneration therefore, as diagrammatically illustrated in **Figure 2**, integrin binding peptides such as RGD, IKVAV or DGEA can be genetically engineered to display on the surface of the coat protein of the phage. The displayed peptides which have high

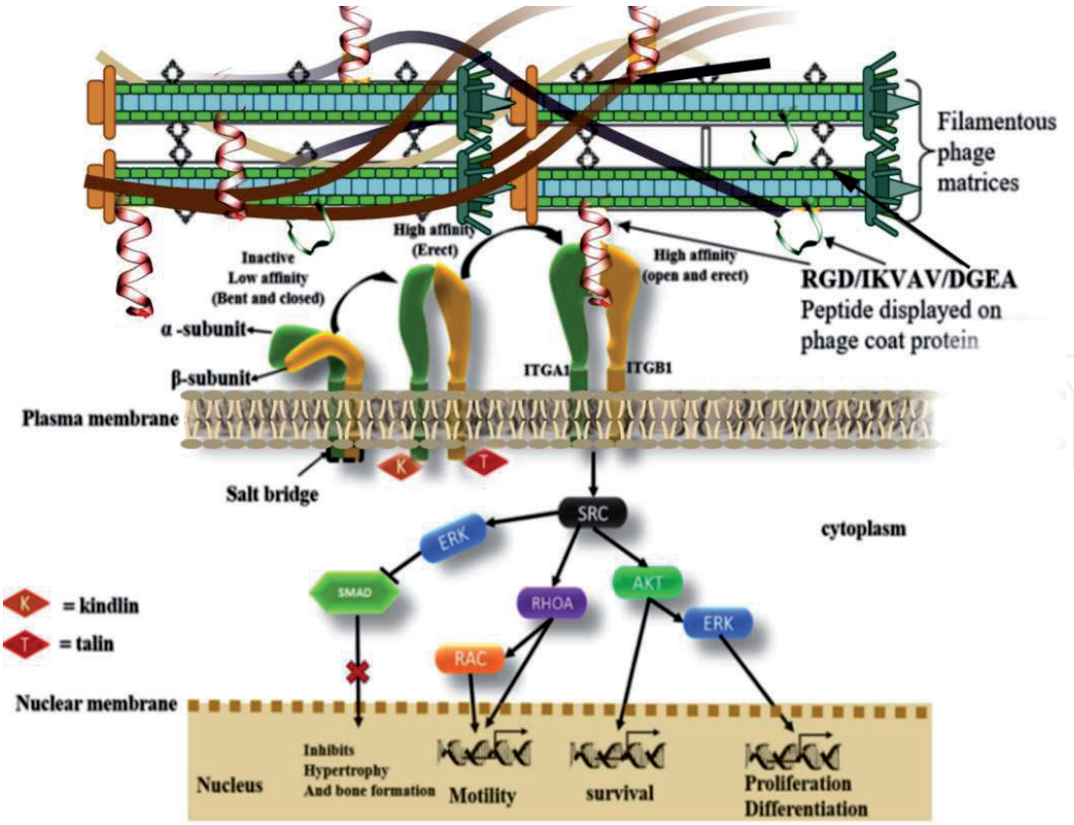

Figure 2.
Diagrammatic illustration of the possible mechanisms of phage displayed peptide on integrin mediated signaling pathway. Integrin binding peptide such as RGD, IKVAV or DGEA can be genetically engineered to display on the surface of the coat protein of the phage. The displayed peptide which have high affinity for integrin receptor will interact with integrin on the plasma membrane, the extracellular subunits of integrin then initiates several conformational changes leading to a more opened and sensitive state from the previously closed and insensitive state, enabling the bound formation between the peptide (ligand) and the receptor. Upon binding to the phage displayed peptide, the integrin receptor activates cascades of signaling including the SMAD, ERK, RHO signaling pathways via signal transduction. While the SMAD signaling pathway will eventually lead to the inhibition of chondrocyte's hypertrophy, the RHO signaling improves cells motility and migration, whereas, the ERK pathway could signal chondrocytic differentiation of prechondrocytes into chondrocytes for cartilage regeneration.

affinity for integrin receptor will interact with integrin on the plasma membrane of the stem cells. This will allow for direct activation of the integrin receptors by the displayed peptide. The process of activating integrin receptor occurs in sequential conformational changes. First, the extracellular subunits of integrin initiate several conformational changes leading to a more opened and sensitive state from the previously closed and insensitive state, the sensitive state allows for bond formation between the peptide and the receptor. Upon binding to the phage displayed peptide, the integrin receptor activates cascades of signaling including the SMAD, AKT, and RHO signaling pathways which could signal chondrocytic differentiation of MSCs into chondrocytes for cartilage regeneration.

2.4 Bio-responsive materials with optimal mechanical and degradation characteristics

The development of scaffolds for cartilage tissue regeneration must include mechanical properties since loading conditions have substantial effect on this tissue. Hence, the optimal mechanical properties of scaffold for cartilage regeneration usually are expected to produce better cartilage tissue formation that must suit the functional role of load bearing. For this reason, it is imperative to carefully regulate the mechanical as well as the degradation properties of the scaffolds used

in cartilage engineering. In an ideal case, the biomaterial should eventually be remodeled and replaced by the chondrocytes and the chondrocytes' secreted ECM and studies along these lines have been conducted [32, 33]. The development and application of 'smart' bioresponsive materials that can respond to biological cues or to pathological abnormalities are of great interest to both researchers and clinicians, and this is more so important especially in the case of cartilage tissue regeneration and osteoarthritis therapy in which precise administration of therapeutics with minimal invasiveness is key. A good review on this topic is covered by Yu and his colleagues [33].

In a related study, osteogenic differentiation of mouse preosteoblasts induced by collagen-derived DGEA-peptide on nanofibrous phage tissue matrices was carried out by Yoo and his colleagues [34]. They constructed genetically engineered M13 phage with DGEA-peptide displayed in high density on the major coat proteins and studied the effects of the DGEA-peptides on preosteoblast morphologies. Their results demonstrated that preosteoblasts grown on DGEA-incorporated phage matrices exhibited significant outgrown morphology with early bone cell marker protein expression. In the cartilage tissue, since it is nonvascular, cell to cell communication is slow and most signaling is via ECM embedded proteins, physical cues, peptides, etc. in the tissue extracellular matrices and such play vital role in controlling chondrocyte's growth, proliferation, and ECM molecules deposition and remodeling of cartilage ECM. A replicate study in which the peptide KRTGQYKL is displayed on M13 and prechondogenic cells are grown on such matrices will be of interesting discovery since this peptide is known to induce chondrogenesis [35].

2.5 Assessment of normal cartilage and degenerative cartilage using phage display derived functional peptides

The cartilage is an avascular tissue that expresses high levels of hyaluoronic acid (HA) via the hyaluoronic acid synthase. Classic histochemical analysis of HA are usually performed using Alcian blue or using the HA-specific probe, known as HA-binding protein (HABP), however since HABP is a complex of aggrecans and link proteinsderived from bovine cartilage, published data seems to indicate discrepancies [36]. Zymolik and Mummert via phage display identified a novel HA binding peptide for which they coined pep-1 which demonstrated excellent staining for dermis, however, they recorded sensitivity of pep-1 conformation changes in HA [22]. The pep-1 peptide therefore could serve as HA expression probe for *in situ* detection of hyaluronans since normal cartilage tissue formation is governed by high expression levels of HA, hence assessment of cartilage tissue could be probed via pep-1 too.

The activation of hyaluronan synthase leads to the production and deposition of HA on the ECM of the cartilage for repair and remodeling. It has been shown to modulate inflammation and fibroplasia during wound repair. Tolg et al. using phage display identified another peptide, P15-1 (STMMSRSHKTRSHHV), by biopanning through 7-to 15mer phage display libraries. This 15mer peptide showed similarity to the receptor for hyaluronan mediated motility (RHAMM) binding sequences, and was demonstrated to show high affinity to HA and keenly mimicked the functional properties of RHAMN. In an *in vivo* experiment, P15-1 significantly reduced wound macrophage number, fibroblast number, and blood vessel density compared to negative control peptides in rat wounds and promoted scarless wound healing. They showed that P15-1 blocks RHAMM-regulated focal adhesion kinase pathways in fibroblasts and attenuated fibrotic repair by blocking hyaluronan oligosaccharide signaling [37]. Since the avascular articular cartilage

must deal with frictional forces, scar formation is unwanted; therefore the ability to ensure scarless healing is of paramount importance.

Another important molecule of the characteristically thick ECM of cartilage tissue is decorin. It is known to bind to aggrecan to increase its adhesion with other aggrecan molecules and with collagen II fibrils, thereby enhancing the assembly and structural integrity of the aggrecan network in cartilage ECM. At the cellular level, decorin functions to increase the retention of aggrecan in the newly formed matrix of chondrocytes. Also, this molecule increases the adhesion between aggrecan and aggrecan molecules and between aggrecan molecules and collagen II fibrils [38]. It has been shown to inhibit TGF-β and hence prevent tissue fibrosis and promote tissue regeneration. Jarvinen and Ruoslahti genetically displayed a wound-homing CAR peptide (CARSKNKDC) on the decorin surface to form a recombinant CAR-decorin. After intravenous injection of CAR-decorin, these complexes selectively accumulated in the wound sites, and promoted wound healing, without scar formation in a mice wound model [39], this displayed peptide therefore can be employed for cartilage wound healing process, since osteoarthritis is characterized by a persistent deterioration of the cartilage tissue or basically and an non-healing wound.

2.6 Phage used for targeted cartilage tissue drug delivery

By phage biopanning, Pi and his colleagues discovered the chondrocyte-homing peptide, DWRVIIPPRPSA (CAP). They also chemically conjugated the peptide with polyethyleneimine (PEI) to construct a non-viral gene vector [40]. The CAP-functionalized PEI vectors showed specificity for cartilage tissue and gene transfection efficiency in the knee joints was demonstrated to be excellent, and can be employed for cartilage therapy. In another study, they employed the same construct to deliver siRNA into the cartilage of the knee joints to silence the expression of Hif-2α [41]. Hif-2α, which is one of the molecules that triggers cartilage degradation in osteoarthritis (OA), was therefore downregulated and cartilage degeneration and synovium inflammation in the knee joints were alleviated. In both cases, they showed that the use of the cartilage specific and chondrocyte-homing peptide identified by phage display could make therapy of degenerate cartilage feasible.

2.7 Using phage display for diagnosis and imaging

The development of osteoarthritis or rheumatoid arthritis is noted to be highly linked with MMP13 expression, a collagenase that degrades collagen and biglycans [42, 43]. Sun-Jun and his colleagues' utilized phage display to map out the substrate specificity of this enzyme, their screening revealed that MMP13 targeted with specificity to peptide substrates that have proline at the P3 position and lipophilic amino acids at P1′. They observed that a change in proline via site-directed mutagenesis made these substrates less sensitive to collagenase 3 [44]. Integrins are transmembrane heterodimeric proteins that play a role as mechanotransducers; they also mediate a number of other signaling cascades and triggers endocytosis [45] and or pinocytosis [46] that mediate cellular internalization. Chondrocytes plasma membranes have surface integrins subunits [47]. A fluorophore that is therefore bound to a ligand that interacts with integrins can be internalized. Hart and his coworkers demonstrated using bacteriophage Fd that displayed the cyclic integrin-binding peptide sequence GGCRGDMFGC on the major coat protein subunits. This led to the internalization of the phage by cells, thus demonstrating that the integrin-binding peptides displayed on the phage could target cells expression integrin on its surface for internalization [48], and this could be exploited to have the phage coat protein also functionalized to a fluorophore that serve for

immunofluorescent imaging. Same process can also be exploited for the possible introduction of siRNA or preloaded drugs into cells for therapy.

2.8 Deploring CRISPR with phage technology for cartilage regeneration

CRISPR technology has proven to be a highly efficient and specific target genome editing technology for eukaryotes; and has been demonstrated as an excellent technology for specific genes silencing, genes knockouts, or knockdowns applications. Therefore this technology can be employed to silence specific gene products in cartilage tissues that amplify the deterioration of cartilage during rheumatoid arthritis or osteoarthritis. For instance, high MMP13 [49, 50], RUNX2 [51], VEGF [52] etc. expressions in cartilage tissue usually are pointers to cartilage degeneration and abnormality [53] and hence can be silenced through this CRISPR technology. The bottleneck remains nonetheless on homing CRISPR to cartilage tissue. Shefah and his team showed that P22 phage served as a robust supramolecular protein cage that could be utilized for cell type-specific delivery of encapsulated cargos [54]. They genetically fused Cas9 to a truncated form of the P22 phage scaffold protein, thereby packaging Cas9 and a single-guide RNA (sgRNA) inside the P22 capsid. Since the sgRNA is tunable, specifying which gene to target therefore is achievable. Homing such a delivery vehicle to cartilage tissue can be achieved via molecular engineering process. The chondrocyte-homing peptide, DWRVIIPPRPSA as discovered by Pi and his colleagues [40] could be chemically functionalized to the engineered P22 phage capsid construct (P22-Cas9: sgRNA complex) using polyethyleneimine. On the other hand, a genetic engineering approach in which the P22 phage tailspike protein is tinkered to contain this chondrocyte homing peptide (DWRVIIPPRPSA) especially at the C-terminus can then be assembled onto the P22 phage capsid construct encapsulating the Cas9-sgRNA. The P22 tailspike protein is well known for its tolerance to several physiological and environmental conditions such as protease, heat and detergents [55]. It is biocompatible and posse no harm to the human body. The non-covalent but irreversible binding of the phage's tailspike to its capsid will lead to the production of a phage construct with capsid loaded with the right gene regulatory factor(s) that has the capacity for cartilage specific targeting.

2.9 Using phage to develop biosensors for cartilage wound progression

Cartilage defect in knee such as in the case of rheumatoid arthritis or osteoarthritis or even in the event of joint injury can lead to matrix metalloproteinase expression enhancement and degenerative events [42, 43, 56, 57]. Inflammation is also known to be associated with joint symptoms and progression of osteoarthritis. The molecular markers of inflammation can be assessed in joint fluids and tissues from patients [58] using phage display technology. Phage based biosensors can be employed to sense the degeneration and extent of wound and even early detection of the degenerative event. These biosensors could reflect the effects of medical treatment. For instance, phage library can be screened against the MMP13 upregulation in osteoarthritis to select highly selective and affinity-binding phages to MMP13. Similar studies as done by Sun-Jun and his colleagues' as mentioned earlier utilized phage display to map out specificity to MMP13 [44]. These selective phages can detect MMP13 in the injured or degenerative joints and thus can be employed for sensor designs and constructs. Several phage-based biosensors have been constructed for detection of pathogens, antigens, secreted proteins in various disease states [59–61]. For instance, Singh and Amit used immobilized engineered tail spike proteins derived from the P22 bacteriophage onto gold surfaces using

thiol-chemistry to analytical detect *Salmonella* with the sensitivity of 10^3 CFU/mL [62]. This technique has also been employed to successfully detect *E. coli* O157:H7, methicillin-resistant *S. aureus* [63], *S. aureus* [64], and hepatitis B virus [65]. Similarly, landscape phage has been successfully used as a molecular recognition interface to detect *Bacillus anthracic* spores [66], *Salmonella* [62, 67] and even in the detection of prostate serum antigen [68].

3. Conclusions

Even though there exist copious discoveries on the genetic factors as well as the molecular mechanisms surrounding cartilage degeneration, the efficacious treatment modalities remain elusive. Phage display provides an advantageous platform to study, diagnose and treat cartilage related diseases, since this provide a nano scale molecular mechanism that have the benefits of possessing higher tissue penetration, high specificities to cartilage, tunable, and hence can be leveraged for cartilage therapy, diagnoses, imaging and research application. By far, majority of phages used for display are biocompatible, and hence can serve as ideal drug delivery systems with minimal to no side effects to the human body upon administration, and should attain tremendous efficacy. The ability to fine tune drug loaded phages by functionalizing homing peptides to the phage particle offer a special pharmacokinetic characteristic, since it provides for regulated and targeted distribution of the payload, and ensure safety. Nonetheless, the use of phages for cartilage therapy still remains an obscured subject, and many obstacles should necessarily be surmounted. First, the choice of the right phage display libraries through phage biopanning is a critical step that will ensure the generation of the right ligand peptides for display. Secondly, there must be a concerted effort to direct cartilage studies to understand more cartilage targeting peptides and the specific genetic and molecular mechanisms that should be reversed in degenerated cartilage therapy process. The specific receptor target moieties, chondrocyte-ECM dynamic relationships, the biology of cartilage tissue ECM remodeling and ECM molecules secretion, deposition and recycling must all be understood *in vivo* to ensure enhanced application of phage technology for human cartilage regeneration.

Abbreviations

AKT	Ak strain transforming
CFU	Colony forming unit.
CRISPR	clustered regularly interspaced short palindromic repeats.
ECM	extracellular matrix.
ERK	extracellular-signal-regulated kinase.
FGF2	fibroblast growth factors 2 (basic).
HA	hyaluoronic acid.
HABP	hyaluoronic acid binding protein.
HAP	hydroxyapatite.
Hif-2α	Hypoxia Inducible Factor-2 alpha.
MMPs	matrix metalloproteinases.
MSCs	Mesenchymal stem cells.
PEI	polyethyleneimine.
RHAMM	receptor for hyaluronan mediated motility.
RHO	Ras homologous.
RUNX2	Runt-related transcription factor 2.

sgRNA	single-guide RNA.
siRNA	Small interfering RNA.
SMAD	small Mothers against decapentaplegic.
Src	sarcoma, a tyrosine kinase protein encoded by the *SRC* gene.
TGF-β1	Transforming growth factor beta 1.
VEGF	vascular endothelial growth factor.

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