Frontiers in Bioscience Elite

*Front. Biosci. (Elite Ed)* **2026**; 18(2): 44545
https://doi.org/10.31083/FBE44545

*Review*

# Biotechnologically Derived Materials as Drug Delivery Systems for Tissue Regeneration

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Academic Editor: Adem Gharsallaoui
Submitted: 4 July 2025 Revised: 17 August 2025 Accepted: 16 September 2025 Published: 1 June 2026

**Abstract**

Regenerative medicine is an evolving field that seeks to restore or replace damaged tissues and organs through the activation of endogenous repair pathways or the application of engineered therapeutic strategies. Within this paradigm, drug delivery systems (DDSs) serve as essential mediators for localized, sustained, and controlled release of bioactive agents that stimulate and support tissue regeneration. The advent of biotechnology has catalyzed the development of innovative DDSs based on biologically derived materials, offering improved biocompatibility, biodegradability, and functional versatility. This review presents a critical analysis of recent advances in the design and application of biotechnologically derived materials, such as recombinant collagen, elastin, and silk, as well as microbial biosynthesized polysaccharides, including bacterial cellulose, hyaluronic acid, and alginate, as drug delivery platforms in regenerative medicine. Thus, a systematic approach was adopted based on recent peer-reviewed studies to evaluate the physicochemical properties and biofunctional characteristics of these materials. The results indicate that recombinant proteins offer tunable mechanical and biochemical properties, exhibit tunable mechanical moduli ranging from ~0.5 to 50 kPa, mimicking native extracellular matrix components; meanwhile, microbial polysaccharides demonstrate high water retention (above 90%), structural flexibility, and bioadhesive potential, making these polysaccharides highly suitable for soft tissue engineering. These materials also enable encapsulation of growth factors, nucleic acids, and small-molecule drugs, facilitating spatiotemporal release and degradation half-lives between 1 and 6 weeks, aligned with tissue-specific repair processes. Therefore, biotechnologically derived DDSs represent a promising frontier for regenerative medicine, merging the precision of recombinant engineering with the scalability of microbial fermentation.

**Keywords:** regenerative medicine; drug delivery systems; recombinant proteins; microbial biosynthesis; tissue engineering; biopolymers; controlled release; scaffold integration

## 1. Introduction

Regenerative medicine is an emerging and highly interdisciplinary field that focuses on restoring the structure and function of damaged or diseased tissues and organs by stimulating the body's own repair mechanisms or by integrating biological substitutes [1]. Regenerative medicine has evolved significantly over the past two decades to include a wide range of strategies like cell-based therapies, engineered scaffolds, gene editing, and the controlled delivery of therapeutic agents to damaged sites [1]. The clinical promise of regenerative medicine lies in its ability to provide long-term, curative treatments for conditions that are currently managed with palliative approaches, such as chronic wounds, musculoskeletal injuries, cardiovascular diseases, and neurodegenerative disorders [2]. At the basis of many regenerative strategies lies the need for precise, localized, and sustained delivery of bioactive molecules that can guide cellular behavior and orchestrate the complex processes involved in tissue repair [3]. These molecules—ranging from small-molecule drugs and peptides to proteins, genes, and even living cells—must be delivered in a temporally and spatially controlled manner to achieve therapeutic efficacy without systemic toxicity. This is particularly important given the inherently dynamic and multifactorial nature of tissue regeneration, which involves sequential stages of inflammation, cell recruitment, proliferation, extracellular matrix remodeling, and functional integration [4]. In this context, drug delivery systems (DDS) play an essential role as mediators that enable the fine-tuned release of regenerative cues. Traditional DDS, often based on synthetic polymers or inert carriers, have demonstrated the potential to improve drug pharmacokinetics and minimize off-target effects. However, their limited biocompatibility, lack of biological signaling, and unpredictable degradation profiles have restricted their effectiveness in tissue regeneration applications [5]. Moreover, the mechanical and struc-

tural mismatch between many synthetic DDS and the native extracellular matrix (ECM) can lead to poor integration with host tissue [5]. Then, the research on this field shifted toward the development of biologically inspired and biofunctional delivery systems that not only serve as passive carriers but actively participate in the healing process [5]. Biotechnology has emerged as a powerful enabler of this new generation of drug delivery platforms. Through the use of recombinant DNA technology, microbial biosynthesis, and metabolic engineering, researchers can design and produce advanced biomaterials with high reproducibility, tailorability, and functionality. These biotechnologically derived materials offer unique advantages in terms of bioactivity, mechanical tunability, immunomodulation, and compatibility with complex biological environments [6]. One of the most promising approaches involves the use of recombinant protein-based materials, which closely mimic natural ECM components while allowing for molecular customization. Proteins such as collagen, elastin-like polypeptides (ELPs), and silk fibroin have been extensively studied for their role in tissue structure, biomechanics, and cellular signaling [7]. By expressing these proteins in microbial or eukaryotic systems, it is possible to generate variants with precisely defined amino acid sequences, mechanical properties, degradation kinetics, and functional motifs. For instance, recombinant collagen can be modified to incorporate integrin-binding domains (e.g., Arginine–Glycine–Aspartic Acid (RGD) sequences), protease-cleavable linkers for controlled degradation, or even therapeutic fusion proteins for dual-functionality [8]. ELPs can be engineered to exhibit thermo-responsive behavior, enabling the creation of injectable gels that solidify at body temperature and serve as *in situ* depots for drug release [9]. Similarly, recombinant silk fibroin can be processed into a range of formats—fibers, sponges, films, and nanoparticles—each with unique loading and release characteristics [10]. In parallel, microbial biosynthesis and fermentation technologies have unlocked access to a suite of polysaccharide-based biomaterials that are highly attractive for regenerative DDS. These include bacterial cellulose (BC), hyaluronic acid (HA), and alginate, each of which exhibits a combination of structural versatility, biocompatibility, and functional adaptability. Bacterial cellulose, synthesized by strains such as *Komagataeibacter xylinus*, forms an ultrapure, nanofibrillar hydrogel with high tensile strength, remarkable water retention capacity, and a morphology similar to native collagen networks [11]. Its three-dimensional architecture supports cell adhesion and proliferation, making it ideal for wound healing and soft tissue regeneration [11]. Hyaluronic acid, a naturally occurring glycosaminoglycan, plays a central role in cell migration, inflammation, angiogenesis, and ECM remodeling. Microbial production of HA, especially through *Streptococcus* species, has enabled its large-scale and contamination-free synthesis [12]. HA can be chemically modified to tune its viscosity, degradation rate, and bioactivity, and it serves as an excellent matrix for delivering growth factors, stem cells, and genes. Its viscoelastic properties make it particularly suitable for applications in cartilage repair and ophthalmology [13]. Alginate, a linear polysaccharide composed of mannuronic and guluronic acid residues, is widely used in biomedical applications due to its mild gelation with divalent cations (e.g., $Ca^{2+}$). Produced by microbial fermentation or extracted from brown algae, alginate hydrogels can encapsulate a variety of drugs and biologics while protecting them from degradation [14]. Its low immunogenicity and ease of functionalization enable its use in injectable formulations, microbeads, and multilayered scaffolds tailored for specific regenerative applications [14]. These biotechnologically derived materials can be further engineered to exhibit stimuli-responsive behavior, releasing their payloads in response to environmental cues such as pH changes, enzymatic activity, redox potential, or mechanical stress [15]. For example, pH-responsive hydrogels typically exploit the acidic microenvironments of inflamed or ischemic tissues, where extracellular pH falls from physiological 7.4 to ~6.5–6.8, and in more severe hypoxic or tumor-like regions to as low as 6.2–6.5. Moreover, the biotechnological production of these materials also aligns with principles of sustainability and scalability, as microbial fermentation and recombinant expression systems permit a controlled, large-scale manufacturing with minimal reliance on animal-derived components. This not only ensures batch-to-batch reproducibility and regulatory compliance but also reduces the risk of immunogenicity and contamination, facilitating translation into clinical practice [16]. The aim of this review is to provide a detailed and critical overview of biotechnologically derived materials—specifically those based on recombinant proteins and microbial polysaccharides—as platforms for drug delivery in tissue regeneration. Their characteristics and their biological performance in relevant regenerative models will be examined. Fig. 1 visually recaps the scope of the review.

## 2. Hydrogels From Genetically Engineered Sustainable Proteins

Advancements in synthetic biology have revolutionized the design of protein-based polymers (PBPs), offering new possibilities for regenerative medicine through the creation of biomimetic hydrogels with precise structural and functional properties. These engineered proteins—designed from natural or synthetic amino acid sequences—provide several key advantages over conventional synthetic polymers. They are inherently biocompatible, enzymatically degradable, and produce non-toxic metabolites, making them particularly well-suited for clinical use in tissue repair and regeneration [17,18]. Additionally, PBPs can be processed under mild, aqueous conditions, preserving bioactivity and supporting the encapsulation of cells, growth factors, and therapeutic agents [19]. Genetic en-

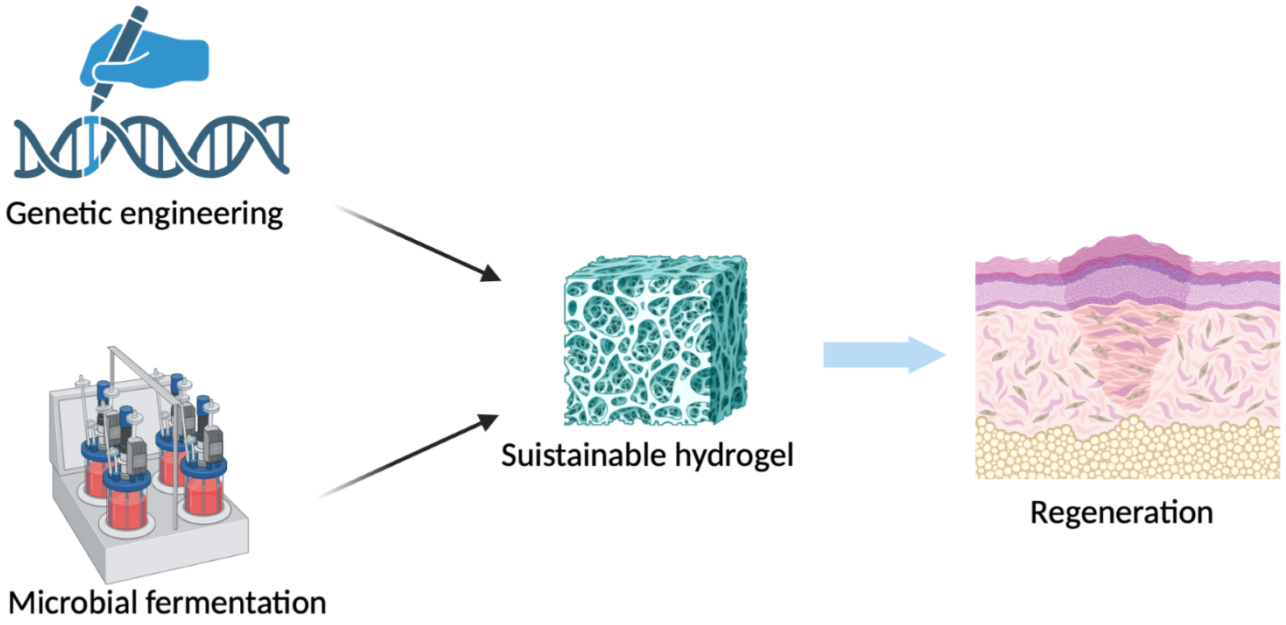


**Fig. 1. Scope of the review.** Use of Biotechnologically derived materials for tissue regeneration. Created in BioRender Sepe, F (2026) https://BioRender.com/uzysmmx.

gineering allows for meticulous control over the amino acid sequence, molecular weight, and functional group presentation of these protein polymers. This precision enables the rational design of hydrogels with tunable mechanical properties, controlled degradation rates, and specific biological functionalities. Recombinant DNA technologies also make it possible to incorporate cell adhesion motifs, growth factor-binding domains, and enzymatically cleavable sequences directly into the protein backbone, thereby enhancing cellular integration, tissue-specific remodeling, and responsiveness to the local microenvironment [20,21]. Scalable microbial systems, such as *E. coli*, are commonly employed for the high-yield, cost-effective production of these proteins with exceptional purity and consistency [22]. Particular attention has been directed toward PBPs inspired by structural proteins like silk fibroin, elastin, and collagen—each known for their repetitive sequences and capacity for self-assembly into ordered, hierarchical structures [7]. These intrinsic features endow the resulting hydrogels with a combination of mechanical strength, elasticity, and biological activity that is essential for supporting cell viability, guiding tissue organization, and facilitating regenerative outcomes. As a platform for regenerative medicine, genetically engineered protein hydrogels enable the development of tailored microenvironments that emulate the native ECM. Their modular design supports the precise delivery of bioactive cues, modulation of immune responses, and synchronization with tissue healing phases. These features make them highly adaptable for applications ranging from skin and nerve regeneration to musculoskeletal repair and vascular tissue engineering (Fig. 2) [23]. In addition, recombinant proteins intended for regenerative applications undergo multistep purification to ensure the removal of host cell proteins (HCPs), host cell DNA (hcDNA), and other process-related impurities. Standard approaches include affinity chromatography (e.g., His-tag/Ni-NTA or antibody-based capture), ion-exchange and size-exclusion chromatography for removal of aggregates and nucleic acids, and ultrafiltration/diafiltration to reduce endotoxins and residual media components. In microbial systems such as *E. coli*, additional treatments such as enzymatic nuclease digestion (e.g., Benzonase®) are applied to degrade residual DNA, while yeast and mammalian systems rely on optimized downstream processing pipelines to limit glycosylation heterogeneity.

### *2.1 Collagen*

Collagen, the most abundant protein in the ECM of animal tissues, plays a fundamental role in maintaining structural integrity and modulating cellular activities essential for tissue homeostasis and repair [24]. Of the 29 known collagen types, types I, II, and III account for over 90% of total collagen in the human body. These proteins provide mechanical strength and structural support to various tissues, including skin, bone, cartilage, and connective tissue [25]. Collagen's structure is based on repeating Gly-X-Y amino acid sequences, where glycine occupies every third position to allow tight packing of the three $\alpha$-chains into a triple-helix configuration—critical for its biological function [26]. Owing to its biodegradability, low immunogenicity, and biocompatibility, collagen has been extensively used in biomedical applications such as wound healing, tissue scaffolds, and drug delivery systems [27]. Despite its advantages, animal-derived collagen presents

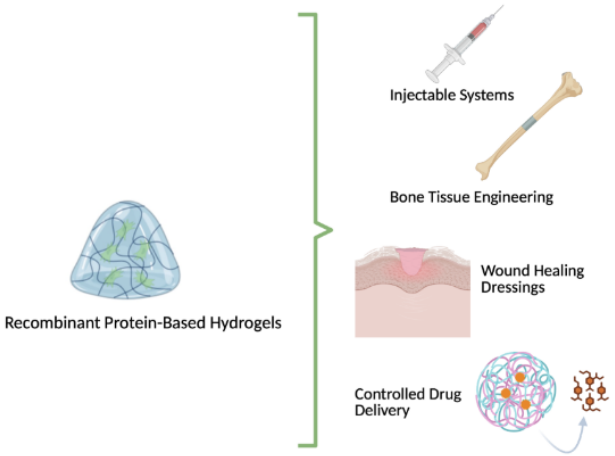


**Fig. 2. Applications of hydrogels from genetically engineered sustainable proteins.** Created in BioRender. Sepe, F(2026) https://BioRender.com/n1scx6y.

challenges including batch-to-batch variability, potential for allergic reactions, and the risk of transmitting zoonotic pathogens. To overcome these limitations, recombinant collagen has emerged as a promising and safer alternative [28]. Advanced gene expression and protein engineering techniques enable the production of recombinant collagens that mimic the structure and function of native human collagen, including essential post-translational modifications such as hydroxylation and glycosylation [29]. Quantitatively, yeast-expressed collagens typically reach ~35–45% proline hydroxylation compared to ~45–50% in native human collagen, a difference that still allows formation of stable triple helices with melting temperatures (Tm) in the range of 38–40 °C, closely approaching the ~41–42 °C observed for tissue-derived collagen. This near-native hydroxylation correlates with comparable cell-adhesion capacity (integrin binding >80% relative to native collagen) and bioactivity in angiogenesis assays. Collagen types I, II, III, and V have been successfully expressed using recombinant systems, with the capacity to form homotrimers (e.g., types I–III), heterotrimers (e.g., type XI), or hybrid structures (e.g., type IX) depending on their $\alpha$-chain composition [30]. Among these, type III collagen plays a pivotal role in tissue remodeling, especially during early stages of healing and scar modulation. It constitutes about 8–11% of the dermal ECM and declines with age, making it a valuable component for skin regeneration therapies [31,32]. Large-scale recombinant production typically employs microbial systems like *E. coli* and yeast. While bacteria offer cost-effective expression, they lack the cellular machinery for post-translational modifications. In contrast, yeast-based systems yield collagen with closer structural and biochemical resemblance to the native human form [33]. Recombinant collagen-based hydrogels are especially attractive for regenerative applications due to their structural similarity to the native ECM, excellent biocompatibility, and tunable mechanical properties [34]. These features support cell adhesion, infiltration, and nutrient diffusion—crucial parameters for promoting osteogenesis and angiogenesis in tissue repair settings. For instance, Rodríguez-Cabello *et al.* [35] functionalized a recombinant type I collagen protein (RCPhC1) with methacrylamide (RCPhC1-MA), norbornene (RCPhC1-NB), and thiol (RCPhC1-SH) groups, enabling high-resolution 3D printing via two-photon polymerization (2PP). The resulting microstructured scaffolds supported cell attachment and proliferation *in vitro*, showcasing the material's potential for fabricating anatomically precise constructs in tissue engineering [35]. In another example, Xu *et al.* [36] integrated mesenchymal stem cell-derived exosomes (MSC-EVs) into recombinant type III collagen nanoparticles. These engineered hydrogels modulated the miR-223/pKNOX1 axis to polarize macrophages toward an M2 phenotype, thereby reducing inflammation and enhancing angiogenesis in cutaneous wound repair. *In vitro* and *in vivo* studies demonstrated reduced IL-6 expression and increased levels of Ki67, CD31, and $\alpha$-SMA—markers indicative of proliferation and neovascularization—thus validating the material's regenerative efficacy [36]. Munyemana *et al.* [37] synthesized porous hybrid nanoparticles using recombinant collagen and calcium carbonate. The collagen component modulated crystal growth through electrostatic interactions, enabling controlled morphology and high drug-loading efficiency. These nanostructures demonstrated pH-sensitive drug release and excellent cytocompatibility, making them suitable candidates for regenerative therapies involving localized drug delivery [37]. Similarly, Kong *et al.* [38] developed a recombinant type III collagen (rHCIII) hydrogel for delivering human adipose-derived stem cells (hADSCs) to diabetic wounds. The hydrogel preserved cell viability and function for over three weeks and significantly accelerated tissue repair *in vivo*, improving vascularization and re-epithelialization in treated mice [38]. In regenerative medicine, implantable scaffolds play a central role in guiding host tissue regeneration by creating a bioactive microenvironment that supports ECM deposition and cell integration. Both natural polymers (e.g., collagen, hyaluronic acid) and synthetic alternatives (e.g., PLA, PGA) have been explored, but recombinant humanized collagens stand out for their reproducible structure, customizability, and reduced immunogenicity. A recent study used a photoaging skin model to evaluate the regenerative efficacy of recombinant type III collagen (rhCol III). Treatment led to increased dermal collagen content, improved elasticity, and reduced epidermal thickening, confirming its therapeutic utility in skin regeneration and rejuvenation [39]. Table 1 (Ref. [35,36,37,38,39]) recaps the applications described.

### *2.2 Elastin-like Polypeptides*

Elastin is a crucial extracellular matrix protein that imparts elasticity and resilience to dynamic tissues such

**Table 1. Applications of recombinant collagen-based devices in regenerative medicine.**

| Device | Applications in regeneration | Key features | Reference |
|---|---|---|---|
| RCPhC1-MA, RCPhC1-NB, RCPhC1-SH (functionalized recombinant type I collagen) | High-resolution 3D-printed scaffolds for tissue engineering | Tunable chemistry, sub-micrometer resolution via 2PP, supports cell proliferation | [35] |
| rhCol III + MSC-EVs hydrogel | Skin wound healing and inflammation modulation | Immunomodulation via M2 polarization, enhanced angiogenesis, promotes cell migration | [36] |
| Recombinant collagen–calcium carbonate hybrid nanoparticles | Controlled drug delivery in tissue repair | pH-responsive release, high drug loading, porous nanostructure, biocompatibility | [37] |
| rHCIII hydrogel with hADSCs | Diabetic wound healing and stem cell delivery | Prolonged cell viability, enhanced stem cell retention and regenerative outcome *in vivo* | [38] |
| rhCol III (recombinant type III collagen) | Photoaged skin regeneration | Increases collagen content and elasticity, reduces skin thickening | [39] |

RCPhC1-MA, recombinant type I collagen protein with methacrylamide; RCPhC1-NB, recombinant type I collagen protein with norbornene; RCPhC1-SH, recombinant type I collagen protein with thiol; 2PP, two-photon polymerization; rhCol III, recombinant type III collagen; MSC-EVs, mesenchymal stem cell-derived exosomes; rHCIII, recombinant type III collagen; hADSCs, human adipose-derived stem cells.

as blood vessels, lungs, and skin. Its structural architecture is composed of insoluble fibrillar networks stabilized by hydrophobic interactions, and is characterized by repetitive peptide sequences like elastin and elastin-like polypeptides (VPGG, VPGVG, and APGVGV). These properties not only contribute to the mechanical function of elastin but also support critical biological processes such as cell adhesion, proliferation, and migration—making it highly relevant for regenerative medicine applications [40]. However, the clinical translation of native elastin is hindered by several limitations: it is insoluble in aqueous media, potentially immunogenic when isolated from animal sources, and presents difficulties in processing and modification. Moreover, synthetic analogs are often expensive and inconsistent in structure [41]. A transformative advancement occurred when Urry and collaborators [42] identified VPGVG as a recurrent motif in natural elastin, leading to the development of elastin-like polypeptides (ELPs)—genetically engineered biopolymers mimicking elastin's hydrophobic domain [43]. ELPs are typically produced via recombinant techniques, predominantly in *Escherichia coli*, due to its high-yield expression and scalability, although *Pichia pastoris* has also been utilized [44]. These recombinant polymers are generally composed of repeating pentapeptide units of the sequence Val-Pro-Gly-X-Gly (VPGXG), where X is any amino acid except proline. This modularity allows ELPs to replicate key elastin functions while enabling tunable mechanical and biochemical properties [45]. One of the most compelling features of ELPs in regenerative applications is their reversible phase transition behavior in aqueous solution. ELPs are soluble below a defined transition temperature (Tt), but undergo aggregation and phase separation above it. This thermoresponsive property is programmable through the choice and arrangement of guest residues, polymer length, and overall hydrophobicity, making ELPs ideal candidates for *in situ* forming biomaterials [46]. For tissue engineering purposes, researchers have developed multiblock ELP copolymers by linking hydrophobic and hydrophilic segments (e.g., $[VPGIG]_{n1}$–$[VPGSG]_{n2}$), or appending hydrophobic terminal blocks to facilitate self-assembly and mechanical reinforcement [47]. ELPs have also been functionalized with bioactive peptide sequences or engineered into fusion constructs to enhance cellular responses such as adhesion, angiogenesis, and matrix remodeling—key factors in tissue regeneration [48]. The injectable and thermoresponsive nature of ELPs makes them particularly useful in forming depot-like structures that provide controlled, localized, and sustained release of therapeutic agents. For instance, fusion of ELP to therapeutic proteins like interferon-alpha (IFN-$\alpha$) results in constructs that preserve biological activity while dramatically prolonging systemic half-life and enhancing tissue accumulation. Hu *et al*. [49] demonstrated that an IFN-$\alpha$–ELP fusion retained its biological efficacy, exhibited enhanced stability, and enabled sustained release at the target site *in vivo*. The same team later confirmed these benefits in glioblastoma models, where the ELP-fused cytokine formed an *in situ* depot that released the therapeutic in a zero-order kinetic profile, enhanced tissue penetration, and reduced tumor recurrence [50]. These design principles can be translated to regenerative contexts where extended presence of growth factors or cytokines is critical for orchestrating tissue repair over time. In vascular tissue engineering, ELPs have been employed to improve graft biocompatibility and function. For example, a nanofiber scaffold incorporating the REDV (arginine–glutamic acid–aspartic acid–valine) sequence demonstrated superior endothelial cell adhesion and proliferation, reduced platelet aggregation, and sup-

ported smooth muscle cell contractility—essential features for small-diameter vascular grafts [51]. Similarly, combining ELPs with other structural biomaterials has yielded promising results. Feng *et al*. [52] engineered a recombinant silk-elastin copolymer expressed in *E. coli* and integrated it with a nanocellulose layer to create a bilayer skin substitute. This construct showed exceptional mechanical strength, antibacterial properties, and regenerative efficacy in skin repair models [52]. Likewise, a recombinant scaffold composed of human collagen and elastin supported long-term membrane durability and facilitated dermal regeneration, offering a viable alternative to conventional skin substitutes [53]. Furthermore, ELPs' amenability to chemical and genetic modification has enabled the development of multifunctional regenerative systems. For instance, elastin-based matrices have been tailored with pH-sensitive linkers for targeted drug release in acidic wound environments or fused with cell-penetrating peptides to enhance intracellular delivery of therapeutic cargos (e.g., Doxorubicin-Dox-) in tissue repair settings [54]. Li and Champion [55] further demonstrated how incorporating p-azidophenylalanine (pAzF) into the ELP backbone allows photo-induced crosslinking to stabilize nanovesicles, enhancing mechanical stability and control over drug release profiles. This strategy can be applied in regenerative contexts where spatiotemporal control over bioactive signaling is crucial [55]. Other ELP constructs have been designed to serve as immunomodulatory depots. For example, CpG oligodeoxynucleotides (known immune stimulants) were electrostatically bound to ELP-K12 (an ELP with 12 lysine residues) to form an injectable depot that released bioactive unmethylated cytosine-guanine (CpG) for up to three weeks. Although originally developed for immunotherapy, this sustained local immune activation concept could be leveraged in regenerative medicine to stimulate endogenous repair mechanisms and modulate inflammatory responses during wound healing [56]. ELPs can then be customized for scaffold formation, therapeutic delivery, immune modulation, and cellular microenvironment engineering—making them a powerful class of biomaterials for tissue regeneration. A summary of the discussed regenerative applications is presented in Table 2 (Ref. [49,50,51,52,53,54,55,56]).

### *2.3 Silk-like Polymers*

Silk-like polymers (SLPs) represent a prominent class of genetically engineered biomaterials with extensive applications in regenerative medicine. These polymers are composed of repeating sequences such as Gly–Ala–Gly–Ala–Gly–Ser, mimicking the structure of natural silk proteins produced by insects and spiders. Native silks, including those from *Bombyx mori* and *Nephila clavipes*, are well known for their impressive mechanical strength, elasticity, biocompatibility, and biodegradability [57,58]. Among expression systems, *Escherichia coli* is frequently used for recombinant silk production due to its cost-effectiveness, rapid growth, and ease of genetic manipulation [59]. Genetically engineered SLPs often replicate silk motifs derived from the major ampullate gland proteins, with typical repetitive sequences such as [GGAGQGGYGGLGSQGAGRGGLGGQGGAG] and [GPGGYGGPGQQGPGGYAPGQQPSGPGS] from *N. clavipes* [60]. These sequences are frequently modified to regulate crystallinity or to introduce biologically active motifs such as RGD to enhance cell adhesion. Due to their low solubility and limited flexibility, silk domains are often copolymerized with elastin-like motifs, resulting in silk-elastin-like proteins (SELPs) that combine mechanical resilience with water solubility and flexibility [61]. This configuration is advantageous for producing injectable biomaterials and drug delivery vehicles with tunable degradation profiles [62,63,64]. For instance, Florczak *et al*. [65] demonstrated that MS1 silk gels functionalized with the H2.1 peptide and loaded with doxorubicin selectively targeted Her2-positive breast cancer cells *in vivo*, significantly reducing tumor volume with minimal systemic toxicity. Notably, unloaded particles showed no adverse effects in healthy mice, and detailed histology revealed no damage to major organs [65]. Herold *et al*. [66] further developed recombinant silk particles (eADF4(C16)) chemically linked with doxorubicin via a pH-sensitive hydrazine linker. These particles exhibited minimal drug release at physiological pH, while delivering the full drug payload under acidic conditions typical of intracellular vesicles, enabling precise intracellular drug release [66]. Similarly, Mulinti *et al*. [67] developed enzyme-responsive nanospheres using a recombinant spider silk copolymer with a thrombin-sensitive linker to release vancomycin in response to *Staphylococcus aureus* infection. These nanocarriers demonstrated strong antibacterial effects and potential for managing drug-resistant infections in tissue repair [67]. Lian *et al*. [68] created a nanofibrous membrane by electrospinning recombinant silk protein blended with sodium hydrogen sulfide (NaHS). The resulting scaffold sustained hydrogen sulfide ($H_2S$) release, supported endothelial progenitor cell (EPC) function, and significantly accelerated wound healing in a murine skin injury model [68]. Further regenerative applications have focused on hybrid constructs combining silk fibroin and recombinant spidroins. For example, composite hydrogels fabricated from silk fibroin and recombinant silk proteins functionalized with fibronectin motifs, growth factors (e.g., Fibroblast Growth Factor (FGF)), and antimicrobial peptides exhibited enhanced cell adhesion, antimicrobial activity, and stimulation of dermal cell proliferation—supporting the development of bilayered skin grafts [69,70]. Other innovations include recombinant spidroins enriched with SIBLING-derived peptide tags (e.g., osteopontin and bone sialoprotein), which enhanced calcium phosphate mineralization and osteoblast adhesion—demonstrating strong potential for tendon–bone

**Table 2. Regenerative medicine applications of elastin-like polypeptides (ELPs).**

| Device | Applications in regeneration | Key features | Reference |
|---|---|---|---|
| ELP-IFN-$\alpha$ Fusion Protein | Sustained cytokine delivery for tissue repair | Extended half-life, depot formation, zero-order release kinetics, enhanced tissue accumulation | [49,50] |
| REDV-ELP Nanofibers | Vascular grafts | Promotes endothelialization, reduces platelet adhesion, supports smooth muscle contractility | [51] |
| Silk-ELP Nanocomposite with Nanocellulose Layer | Bilayer skin substitute | High mechanical strength, antibacterial activity, supports skin regeneration | [52] |
| Collagen-ELP Recombinant Scaffold | Dermal regeneration | Improved mechanical durability, biocompatibility, and wound healing performance | [53] |
| ELP-pH-Responsive Dox Conjugate with CPP | Targeted delivery in acidic wound environments | pH-triggered release, enhanced cellular uptake, genetic CPP encoding for cell penetration | [54] |
| Crosslinked ELP Nanovesicles with pAzF | Stable delivery system for bioactive molecules | UV-induced crosslinking, increased stability, controlled release, tunable size and hydrophobicity | [55] |
| ELP-K12 + CpG Depot | Immunomodulatory system for wound healing support | Electrostatic binding of CpG, sustained release (up to 3 weeks), local immune activation | [56] |

IFN-$\alpha$, interferon-alpha; REDV, arginine–glutamic acid–aspartic acid–valine; pAzF, p-azidophenylalanine; ELP, Elastin-Like Polypeptides; ELP-K12, ELP with 12 lysine residues.

interface regeneration [71]. Additionally, eADF4(C16)-based scaffolds rich in carboxylic acid residues were shown to promote calcium ion binding and mineralization. Culturing mesenchymal stem cells (MSCs) on these hydrogels resulted in elevated alkaline phosphatase activity, highlighting their osteoinductive capacity [72]. Another engineered scaffold featured a chimeric fusion protein incorporating a spider silk-inspired sequence and a hyaluronic acid-binding motif (VTK), which guided osteogenic differentiation of bone marrow-derived human MSCs, underlining its applicability for bone tissue engineering [73]. Finally, a composite construct was developed by integrating decellularized extracellular matrix (dECM) derived from equine cartilage with a silk-based hydrogel. This composite served both as a structural scaffold and a bioadhesive to promote integration with host tissue. Mechanical and biological analyses confirmed its suitability for supporting chondral regeneration under load-bearing conditions, emphasizing the value of SELP-based hydrogels in developing advanced regenerative therapies [74]. Table 3 (Ref. [65,66,67,68,69,70,71,72,73,74]) summarizes the described applications.

## 3. Hydrogels Obtained from Controlled Fermentation of Sustainable Biomaterials

Hydrogels derived from the controlled fermentation of sustainable biomaterials represent a cutting-edge approach in regenerative medicine, offering a biocompatible and functional alternative to conventional synthetic polymers. These bioengineered hydrogels not only provide essential mechanical support to damaged or developing tissues but also actively modulate the cellular microenvironment by delivering biochemical cues and bioactive molecules. This dynamic interaction allows for the precise regulation of fundamental processes such as cell proliferation, migration, and differentiation—key drivers in the repair and regeneration of complex tissues and organs [75]. A cornerstone of this technology lies in the microbial biosynthesis of polysaccharides through the fermentation of carbohydrate-rich substrates. Specific microbial strains are harnessed to produce polymers with distinct and tunable mechanical, rheological, and biological properties. These polymers are then enzymatically engineered to incorporate specific functional groups or bioactive domains that mimic native ECM components. Such functionalization enhances cell–matrix interactions and enables the localized, sustained release of growth factors and signaling molecules critical for tissue regeneration [75]. Natural polysaccharides produced via fermentation—such as xanthan gum, cellulose, and hyaluronic acid—are increasingly utilized as base materials, either alone or in composite formulations to construct hydrogels with customizable features. Their combined physicochemical and biological properties allow the creation of smart hydrogel platforms that respond to physiological stimuli and support complex regenerative processes with high spatial and temporal precision (Fig. 3) [76]. These hydrogels are particularly suited for applications in soft tissue engineering, wound heal-

**Table 3. Regenerative medicine applications of silk-like polymers (SLPs).**

| Device | Applications in regeneration | Key features | Reference |
|---|---|---|---|
| H2.1-MS1 Silk Gel Particles | Targeted delivery for breast cancer tissue regeneration post-resection | Selective cytotoxicity to Her2+ cells, low systemic toxicity, *in vivo* efficacy | [65] |
| eADF4(C16) Silk Particles with Hydrazine-Doxorubicin Linker | pH-sensitive drug delivery for tissue repair in acidic microenvironments | Controlled release, pH-responsive linker, enhanced cellular uptake | [66] |
| Thrombin-Responsive Silk Nanospheres | Infection-responsive antibiotic delivery for regenerating infected tissues | Enzyme-triggered release, effective antibacterial activity | [67] |
| Recombinant Major Ampullate Spidroin (rMaSp)/NaHS Nanofibrous Membrane | Skin wound healing and vascular tissue regeneration | Sustained $H_2S$ release, EPC support, improved healing outcomes | [68] |
| Silk Fibroin + Recombinant Spidroin Hydrogel | Skin tissue engineering, wound dressing | Enhanced adhesion, antimicrobial, growth factor delivery (a general-purpose, multifunctional platform with antimicrobial activity and growth factor delivery, suitable for wound healing applications) | [69] |
| Recombinant Spidroin with Fibronectin Motif | Burn wound repair and dermal reconstruction | Improved fibroblast attachment, scaffold bioactivity (a targeted, bioactive scaffold, optimized for fibroblast integration and dermal reconstruction, making it especially valuable for burn injuries or full-thickness skin loss) | [70] |
| Spider Silk–SIBLING Hybrid Gel | Regeneration of tendon–bone interface | Enhanced mineralization, osteoblast adhesion | [71] |
| eADF4(C16)-Based Hydrogel | Bone tissue engineering | Calcium-binding capacity, ALP upregulation in MSCs | [72] |
| Silk-Spidroin-HA Binding Domain Hydrogel | Osteogenic differentiation of hMSCs | ECM mimetic, osteoinductive signaling | [73] |
| dECM + SELP Hydrogel | Cartilage regeneration and integration with host tissue | High mechanical resistance, adhesion, tailored microenvironment | [74] |

eADF4(C16), recombinant silk particles; NaHS, sodium hydrogen sulfide; EPC, endothelial progenitor cell; MSCs, mesenchymal stem cells; ALP, alkaline phosphatase; hMSCs, human mesenchymal stem cells; ECM, extracellular matrix; HA, hyaluronic acid; dECM, decellularized extracellular matrix; SELP, silk-elastin-like protein.

ing, and the regeneration of cartilage, bone, and neural tissues. Their adaptability, biocompatibility, and ability to mimic native ECM structures make them highly effective scaffolds for promoting endogenous repair mechanisms and guiding tissue morphogenesis. As such, fermentation-derived hydrogels are rapidly becoming integral tools in the design of next-generation biomaterials for advanced regenerative therapies. However, Bacterial-derived materials may contain trace contaminants such as lipoteichoic acids, peptidoglycan fragments, or residual DNA that activate Toll-like receptors (TLR2/TLR4), triggering unwanted inflammatory responses. To reduce these effects, several strategies have been developed: advanced downstream purification (ultrafiltration, enzymatic digestion, endotoxin-removal resins) to minimize immunogenic residues; chemical modifications (e.g., esterification, methacrylation) that mask TLR recognition sites; and the use of alternative production platforms such as GRAS organisms (Bacillus subtilis) or cell-free enzymatic synthesis, which eliminate streptococcal endotoxins entirely. Additionally, maintaining HA in the high-molecular-weight form (>1000 kDa) reduces its DAMP-like activity compared to low-molecular-weight fragments. These approaches collectively enhance the biocompatibility and reduce the inflammatory response of bacterial-derived HA.

### *3.1 Bacterial Cellulose*

Bacterial cellulose (BC) is a polysaccharide macromolecule with the same chemical structure as plant-derived cellulose, yet it lacks associated plant components such as lignin, pectin, and hemicellulose that are commonly found in plant cell walls [77]. Structurally distinct due to its

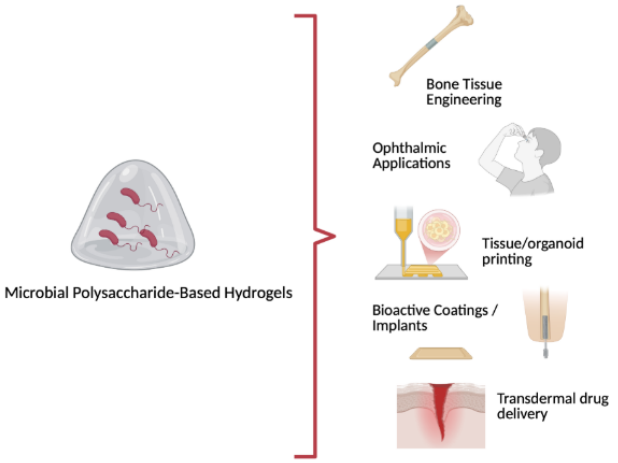


**Fig. 3. Application of hydrogels obtained from controlled fermentation of sustainable biomaterials.** Created in BioRender. Sepe, F (2026) https://BioRender.com/rg4isxk.

unique nanoscale fiber organization, BC exhibits superior mechanical strength, elevated crystallinity, and enhanced purity compared to its plant-based counterpart [78]. It is composed of $\beta$-1,4-linked D-glucopyranose units, stabilized by extensive intra- and intermolecular hydrogen bonding, and conforms to the molecular formula $(C_6H_{10}O_5)_n$ [79]. The bacterium *Acetobacter xylinum* biosynthesizes two crystalline forms—cellulose I (ribbon-like) and cellulose II (thermodynamically stable)—by extruding glucan chains as protofibrils, which aggregate into nanofibrils and self-assemble into a web-like 3D network. The abundance of hydroxyl groups confers high hydrophilicity, chemical modifiability, and excellent biodegradability to BC, making it highly suitable for regenerative medicine applications [79]. Due to its intrinsic biocompatibility, porosity, mechanical tunability, and water retention, BC is an ideal scaffold material for the controlled release of therapeutic molecules and the engineering of regenerative constructs. In bone tissue engineering, three-dimensional (3D) scaffolds that integrate biomaterials with osteogenic cells and signaling agents are gaining traction for their ability to mimic native bone architecture and promote functional repair. Cao *et al.* [80] designed a nanocomposite scaffold by incorporating oxidized BC with chitosan (CS) and nano-hydroxyapatite (nHA). This formulation showed superior mechanical strength, favorable degradation kinetics, and enhanced water absorption compared to CS/nHA-only constructs. When tested with MC3T3-E1 preosteoblasts, the scaffold promoted cell proliferation and viability. *In vivo* studies using a rat calvarial defect model further confirmed enhanced new bone formation [80]. Zhu *et al.* [81] developed a hydrogel scaffold combining BC with CS and alginate (Alg), which formed a dense, fibrous network with desirable swelling behavior and degradation rate. This composite scaffold exhibited excellent apatite nucleation, cell compatibility, and protein adsorption capacity, while also enabling controlled drug release—key features for bone and cartilage regeneration [81]. In the context of cartilage tissue engineering, Li *et al.* [82] engineered a 3D hierarchical porous scaffold composed of BC and decellularized cartilage extracellular matrix (DCECM), crosslinked using N-hydroxysuccinimide (NHS) and N-[3-(dimethylamino)propyl]-N′-ethyl carbodiimide hydrochloride (EDC). The scaffold facilitated robust adhesion and proliferation of rabbit-derived chondrocytes. *In vivo* implantation into a rabbit cartilage defect model demonstrated improved cartilage regeneration compared to native BC, attributed to its excellent elasticity, water retention, and hydrophilic properties—closely mimicking the biomechanical profile of native cartilage [82]. In ophthalmic regenerative medicine, Han *et al.* [83] fabricated a composite hydrogel using BC and poly (vinyl alcohol) (PVA) as a potential corneal stroma substitute. The BC/PVA scaffold exhibited increased optical transparency, enhanced water retention, and improved structural and surface morphology. *In vitro* cytocompatibility tests and *in vivo* rabbit implantation studies confirmed the scaffold's ability to maintain corneal architecture, stability, and transparency more effectively than BC alone [83]. Other promising developments include the formulation of BC scaffolds functionalized with quince seed mucilage, a glucuronoxylan-based hydrogel. This modification improved swelling capacity and promoted enhanced fibroblast adhesion and proliferation, making it suitable for soft tissue engineering applications [84]. BC is also under active investigation as a component in advanced drug delivery platforms for regenerative therapies. Nanoparticles made from BC (BCNPs) have been developed as biocompatible carriers for sustained therapeutic release. These nanoparticles demonstrated increased crystallinity and size with prolonged culture, and remained thermally stable up to 90 °C. *In vitro* studies using bovine serum albumin (BSA) as a model drug confirmed successful loading and sustained release, supporting their use in nanomedicine for localized tissue regeneration [85]. Further advancements include the incorporation of poorly water-soluble glucocorticoids into BC-based hydrogels via microemulsion strategies. Zahel *et al.* [86] developed multiple formulations containing hydrocortisone or dexamethasone, achieving uniform integration into the BC matrix. Transmission electron microscopy confirmed the consistent dispersion of even water-in-oil (w/o) systems. Drug permeation across synthetic membranes was efficient and tunable, and anti-inflammatory efficacy was preserved post-release—underscoring the potential of BC composites in managing local inflammation during tissue repair [86]. Table 4 (Ref. [80,81,82,83,84,85,86]) summarizes the regenerative applications of BC-based systems discussed herein.

**Table 4. Regenerative medicine applications of bacterial cellulose.**

| Device | Applications in regeneration | Key features | Reference |
|---|---|---|---|
| BC/CS/nHA Nanocomposite Scaffold | Bone tissue regeneration | Enhanced mechanical strength, controlled degradation, high water retention, supports osteoblast proliferation | [80] |
| BC/CS/Alg Hydrogel Scaffold | Bone and cartilage regeneration | Dense fibrous network, good swelling behavior, bioactivity, protein adsorption, and controlled drug release | [81] |
| BC/DCECM Composite Scaffold (NHS/EDC crosslinked) | Cartilage tissue regeneration | Hierarchical porous structure, strong chondrocyte adhesion, elasticity, shape memory, mimics native cartilage | [82] |
| BC/PVA Composite Hydrogel | Corneal stroma regeneration | High transparency, biocompatibility, water retention, stable morphology, maintains corneal integrity | [83] |
| BC/Quince Seed Mucilage Composite Scaffold | Soft tissue regeneration | Improved swelling capacity, enhanced fibroblast adhesion and proliferation | [84] |
| BC Nanoparticles (BCNPs) | Drug delivery for regenerative therapies | Biodegradable, thermally stable, capable of sustained protein release | [85] |
| BC loaded with Microemulsified Glucocorticoids | Local anti-inflammatory delivery during regeneration | Uniform drug incorporation, high and tunable drug permeation, preserved anti-inflammatory activity | [86] |

BC, bacterial cellulose; CS, chitosan; nHA, nano-hydroxyapatite; Alg, alginate; DCECM, decellularized cartilage extracellular matrix; NHS, N-hydroxysuccinimide; EDC, N-[3-(dimethylamino)propyl]-N′-ethyl carbodiimide hydrochloride; PVA, poly(vinyl alcohol).

**Table 5. Regenerative medicine applications of bacterial derived hyaluronic acid.**

| Device | Applications in regeneration | Key features | Reference |
|---|---|---|---|
| HA-based Injectable Hydrogels | Bone, cartilage, nerve, muscle, skin regeneration | Biocompatible, biodegradable; tunable viscoelasticity; supports cell proliferation and differentiation | [93] |
| Thermo-responsive HA Hydrogels | Osteoarthritis treatment, cartilage repair | Injectable liquid-to-gel transition at body temperature; minimally invasive; supports chondrocyte viability | [97] |
| HA-based Viscosupplementation Devices | Joint lubrication and osteoarthritis management | Restores synovial fluid viscoelasticity; anti-inflammatory; improves joint mobility | [98] |
| HA/Inorganic Composite Scaffolds | Bone regeneration | Combines mechanical strength with bioactivity; promotes osteogenesis and vascularization | [99] |
| Injectable HA Stem Cell Hydrogels | Myocardial regeneration | Encapsulates MSCs; promotes cell adhesion and differentiation *in situ* | [100] |
| HA-based Nerve Conduits and Scaffolds | Spinal cord injury repair | Supports axon growth and functional recovery; biocompatible scaffold | [101] |

### *3.2 Hyaluronic Acid*

Hyaluronic acid (HA) is a high molecular weight glycosaminoglycan extensively used in regenerative medicine due to its unique physicochemical and biological properties. While HA is naturally present in the ECM of vertebrate tissues, its biotechnological production via microbial fermentation—particularly from *Streptococcus equi* subsp. *zooepidemicus* and recombinant platforms such as *Lactococcus lactis*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Escherichia coli*, and *Pichia pastoris*—has become the preferred method for obtaining pharmaceutical-grade HA. This shift ensures enhanced biosafety, scalability, and product standardization while eliminating risks associated with animal-derived HA, such as immunogenicity and pathogen contamination [87,88]. Structurally, HA consists of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked via $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ glycosidic bonds. Its linear, non-sulfated configuration and ionized carboxylic groups enable strong hydration through hydrogen bonding and interaction with cations,

supporting tissue integrity and cell signaling. These characteristics confer viscoelasticity and pseudoplastic rheological behavior—essential properties for its role as a dynamic ECM component and injectable scaffold material [89]. In regenerative medicine, bacterial-origin HA has emerged as a bioactive scaffold and signaling molecule with applications across various tissue types. The biological and rheological properties of HA strongly depend on its molecular weight. Low-molecular-weight (LMW) HA is typically defined as $<$250 kDa, intermediate as 250–1000 kDa, and high-molecular-weight (HMW) HA as $>$1000 kDa. LMW HA exhibits lower viscosity and reduced viscoelastic moduli ($G' <$50 Pa), which facilitates rapid diffusion and degradation, making it suitable for applications requiring fast turnover or enhanced cell signaling. Conversely, HMW HA forms highly viscous hydrogels with storage moduli ($G'$) exceeding 200–500 Pa, improved shear-thinning behavior, and slower degradation rates (half-life ranging from 2–4 weeks *in vivo*). Then, high molecular weight (HMW) HA supports structural integrity, dampens mechanical stress, and exhibits anti-inflammatory, anti-angiogenic, and immunosuppressive properties—making it suitable for maintaining tissue homeostasis and modulating immune responses. In contrast, low molecular weight (LMW) HA penetrates tissues more efficiently and acts as a pro-regenerative agent by stimulating angiogenesis, inflammation, and cell proliferation. Intermediate-weight HA is involved in wound healing and embryonic development and demonstrates good transdermal permeability [90]. HA's regenerative effects are largely mediated by its interaction with specific cellular receptors, particularly CD44 and RHAMM (CD168), which regulate cell proliferation, motility, and differentiation. These interactions enable HA to influence a broad range of regenerative processes, including angiogenesis, inflammation resolution, ECM remodeling, and cell recruitment [91]. In tissue engineering, HA serves as a temporary ECM substitute and delivery platform. Bacterial HA-based hydrogels have been engineered for the regeneration of bone, nerve, cartilage, muscle, and skin tissues. Chemically modified HA derivatives or polyelectrolyte complexes with other biopolymers (e.g., chitosan) enhance mechanical stability, tune degradation rates, and allow for controlled drug and cell release [92]. For example, HA-based hydrogels have been loaded with stem cells, bioactive peptides, or growth factors to promote localized differentiation and tissue repair in musculoskeletal and neural contexts [93]. Advanced fabrication technologies further expand the versatility of bacterial HA. Electrohydrodynamic (EHD) techniques like electrospinning and electrospraying allow the production of HA-based fibrous matrices and microparticles with defined porosity and drug release profiles, ideal for guiding cell behavior and controlled healing [94]. In parallel, 3D bioprinting using bacterial HA-derived bioinks enables the precise construction of biomimetic scaffolds with tunable viscoelastic properties, supporting spatially organized tissue development and facilitating personalized regenerative therapies [95]. In wound healing, HA-based gels and dressings maintain a moist microenvironment, enhance epithelialization, and promote angiogenesis, with demonstrated efficacy in both acute injuries (e.g., burns) and chronic lesions (e.g., diabetic ulcers) [96]. In cartilage repair, injectable HA hydrogels—especially those modified with thermo-responsive polymers like N-isopropylacrylamide (NIPAM)—facilitate minimally invasive interventions by gelling *in situ* at body temperature and improving chondrocyte viability and matrix synthesis, thus showing significant promise in osteoarthritis management [97]. Bacterial HA also plays a central role in joint regeneration. As a physiological constituent of synovial fluid, it is a key agent in viscosupplementation therapies aimed at restoring lubrication, reducing joint friction, and stimulating cartilage regeneration. Clinical studies have reported significant improvements in pain relief and joint function following intra-articular injection of bacterial HA in osteoarthritic patients [98]. In bone tissue engineering, HA is combined with inorganic materials such as hydroxyapatite, calcium phosphates, and osteogenic factors (e.g., Bone Morphogenetic Protein BMP-2) to fabricate hybrid scaffolds that provide both mechanical support and biochemical cues for osteogenesis and neovascularization [99]. Similarly, in myocardial regeneration, injectable bacterial HA-based hydrogels loaded with mesenchymal stem cells enhance cell retention and promote *in situ* differentiation, aiding in the repair of ischemic cardiac tissue [100]. Nerve tissue repair also benefits from HA's bioactivity. HA-based conduits and hydrogels have been shown to support axonal regrowth, myelination, and functional recovery in models of spinal cord injury, owing to their permissive microenvironment and compatibility with neurotrophic agents [101]. The regenerative medicine applications of bacterial hyaluronic acid are summarized in Table 5 (Ref. [93,97,98,99,100,101]).

### *3.3 Alginate*

Alginate is a naturally occurring polysaccharide predominantly found in brown algae (Phaeophyceae), where it serves as a key structural component of cell walls, providing both mechanical stability and osmotic regulation [102]. Although bacterial production is also possible, most commercially available alginate is extracted from algal sources such as Laminaria, Macrocystis, Ascophyllum, and Sargassum. Its high viscosity and gelling capacity make alginate a valuable material, particularly in the food and pharmaceutical industries [103]. Due to its sustainable origin, biocompatibility, and biodegradability, alginate has gained considerable attention in biotechnology and biomedicine, where it is used in drug delivery systems, scaffolds for tissue regeneration, and 3D bioprinting applications [104]. Alginate is recognized as safe by the FDA, further supporting its role in regenerative medicine [105]. Traditional industrial alginate

**Table 6. Regenerative medicine applications of bacterial derived alginate.**

| Device | Applications in regeneration | Key features | Reference |
|---|---|---|---|
| Injectable alginate-hydroxyapatite hydrogel with gelatin microspheres | Bone regeneration | Enhanced mechanical stability, osteointegration, injectable formulation | [113] |
| Alginate hydrogel enriched with platelets in a chitosan–chondroitin sulfate–silk fibroin scaffold | Cartilage regeneration | Mimics native ECM, promotes type II collagen expression, supports metabolic activity | [114] |
| Biodegradable alginate–chitosan hydrogel with vitamin E | Skin tissue engineering | High porosity, improved cell proliferation, enhanced wound healing and tissue regeneration | [115] |
| Microfluidic-spun calcium alginate fibrous scaffold | Chronic wound healing | ECM-mimicking structure, excellent biocompatibility, promotes healing in chronic wounds | [116] |
| Sulfated alginate–polyurethane elastomer | Cardiovascular applications | Anticoagulant properties, improved endothelial cell adhesion, anti-inflammatory, self-healing, flexible | [117] |
| Alginate–fullerenol hydrogel | Cardiac regeneration post-MI | Injectable antioxidant system, scavenges ROS, improves cardiac function recovery | [118] |
| Alginate hydrogel with anisotropic capillary structures | Spinal cord injury repair | Oriented axonal growth, supports neuronal regeneration, non-inflammatory, promotes *in vivo* integration | [119,120] |

extraction from seaweed involves multiple steps: raw material pretreatment, alkaline solubilization, precipitation with acids or alcohols, followed by drying and milling. However, environmental concerns related to seaweed harvesting and variability in the alginate's composition—affected by seasonal and geographic factors—complicate standardization, especially in pharmaceutical contexts. As an alternative, bacterial systems such as Pseudomonas and Azotobacter species have been explored for more controlled alginate biosynthesis [106]. Among these, Azotobacter vinelandii stands out for its capacity to produce alginate with high molecular weight and rheological stability. This non-pathogenic bacterium naturally synthesizes alginate as both a carbon reserve and a nitrogenase protective barrier. The complete genome of A. vinelandii facilitates genetic modification, enabling strains that produce alginates with customized properties. For instance, oxygen tension and growth rate during fermentation significantly affect polymer molecular weight, and limiting oxygen availability can promote synthesis of highly homogeneous polymers [106,107]. Chemically, alginate is a linear anionic copolymer composed of $\beta$-D-mannuronic acid (M) and $\alpha$-L-guluronic acid (G), connected via $\beta$-(1$\rightarrow$4) bonds. These monomers form homopolymeric (MM, GG) or heteropolymeric (MG) blocks, with their distribution depending on species, growth stage, and environment. The physical and biological properties of alginate are closely linked to this structural configuration. Due to its carboxyl groups, alginate interacts electrostatically with proteins and cationic polymers, enabling development of controlled-release systems for cationic drugs [108]. In the presence of divalent cations (e.g., $Ca^{2+}$), alginate forms hydrogels at room temperature following the "egg-box" model, where G-blocks facilitate crosslinking. High G-content results in rigid gels, whereas M-rich alginate yields softer gels [109]. Gelation kinetics can be modulated by temperature, concentration, and calcium ion availability—slower gelation methods using phosphate buffers, insoluble calcium salts, or low temperature improve structural homogeneity [110,111]. These properties make alginate hydrogels ideal for bioactive molecule immobilization and for applications in cancer therapy, gene delivery, and stem cell encapsulation. Alginate has also demonstrated immunomodulatory effects, including macrophage modulation and anti-inflammatory activity, making it relevant for regenerative contexts [112]. Despite its advantages, alginate exhibits limitations such as low cell adhesion, variable degradation rates, and poor mechanical strength, restricting its use in advanced biomedical applications. These drawbacks are addressed through chemical modifications aimed at enhancing native properties or introducing new functionalities. For example, enzymatic modification using epimerase enzymes can convert $\beta$-D-mannuronic acid residues into $\alpha$-L-guluronic acid, significantly increasing the rigidity and mechanical strength of the resulting gels. This makes them crucial for bone and cartilage regeneration, as exemplified by injectable alginate-hydroxyapatite hydrogels enriched with gelatin microspheres, which have shown improved stability and osteointegration for repairing large segmental bone defects [113]. Similarly, an alginate hydrogel enriched with platelets and integrated into a 3D porous scaffold of chitosan, chondroitin sulfate, and silk fibroin effectively mimics native cartilage ECM, enhancing metabolic activity and promoting type II colla-

**Table 7. Analytical techniques commonly used to characterize biotechnologically derived biomaterials for drug delivery and regenerative applications.**

| Material | Main characterization techniques | Obtained informations |
|---|---|---|
| Recombinant Collagen | FTIR; Circular Dichroism (CD); DSC; Rheology; SEM/TEM | Triple helix confirmation; thermal stability; viscoelasticity; fibrillar morphology |
| Recombinant Elastin | FTIR; NMR; Rheology; AFM; Dynamic Light Scattering (DLS) | Secondary structure; self-assembly; nanostructural elasticity; size distribution |
| Recombinant Silk | FTIR; XRD; DSC; Rheology; Mechanical Tensile Testing | $\beta$-sheet content; crystallinity; thermal transitions; mechanical properties |
| Bacterial Cellulose | FTIR; XRD; SEM/AFM; TGA; Mechanical Tensile Testing | Degree of crystallinity; fiber morphology; thermal stability; elastic modulus |
| Hyaluronic Acid (HA) | FTIR; NMR; SEC-MALS; Rheology; Zeta Potential | Crosslinking verification; molecular weight distribution; viscoelastic moduli; surface charge |
| Alginate | FTIR; $^{1}$H NMR; Rheology; SEM; ICP-OES (per $Ca^{2+}$ crosslinking) | Crosslinking confirmation |

FTIR, Fourier Transform Infrared Spectroscopy; CD, Circular Dichroism; DSC, Differential Scanning Calorimetry; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; NMR, Nuclear Magnetic Resonance Spectroscopy; AFM, Atomic Force Microscopy; DLS, Dynamic Light Scattering; XRD, X-ray Diffraction; TGA, Thermogravimetric Analysis; SEC-MALS, Size Exclusion Chromatography – Multi-Angle Light Scattering; ICP-OES, Inductively Coupled Plasma – Optical Emission Spectroscopy.

gen expression for cartilage regeneration [114]. Oxidative modification using sodium periodate introduces reactive aldehyde groups, significantly enhancing alginate's ability to bind proteins, peptides, and bioactive molecules, while also conferring improved antimicrobial properties—a critical advantage for wound healing. For example, biodegradable alginate–chitosan hydrogels loaded with vitamin E, known for their high porosity, have demonstrated enhanced regenerative properties for skin tissue engineering [115]. Fibrous scaffolds of calcium alginate, fabricated via microfluidic spinning, offer excellent biocompatibility and an ECM-mimicking architecture, making them ideal for chronic wound healing [116]. Sulfation of alginate through the addition of sulfate groups imparts anticoagulant activity comparable to heparin, broadening its applicability in cardiovascular settings. This modification improves cell adhesion and reduces clot formation, inflammation, and undesired immune responses, as seen in alginate-based polyurethane elastomers that exhibit excellent elongation capacity, self-healing properties, and enhanced endothelial cell adhesion [117]. For cardiac regeneration following myocardial infarction, incorporating fullerenol nanoparticles into alginate hydrogels creates an injectable antioxidant system that scavenges reactive oxygen species, supporting functional cardiac recovery [118]. In neurological applications, alginate hydrogels with anisotropic capillary structures have been investigated for axonal growth and spinal cord injury repair. These scaffolds promote linear, oriented axonal regrowth and neuronal reinnervation, integrating well *in vivo* without inducing inflammatory responses, thereby positioning them as a promising strategy for post-injury spinal cord regeneration [119,120]. Table 6 (Ref. [113,114,115,116,117,118,119,120]) recaps the described applications.

## 4. Conclusions

Biotechnologically derived materials are redefining the landscape of DDS in regenerative medicine. These innovative platforms, based on recombinant proteins and microbially produced polysaccharides, offer a broad range of advantages compared to traditional synthetic carriers. Their molecular precision, biocompatibility, and the ability to integrate bioactive functionalities make them ideal candidates for guiding complex regenerative processes in a temporally and spatially controlled manner. One of the primary benefits of these systems is their biomimetics. Materials such as recombinant collagen, elastin-like polypeptides, silk fibroin, bacterial cellulose, hyaluronic acid, and alginate closely replicate the structure and biochemical cues of the native ECM. This enables enhanced cellular adhesion, migration, proliferation, and differentiation—critical processes for effective tissue repair. Additionally, the programmable nature of these biomaterials allows for the fine-tuning of mechanical properties, degradation rates, and therapeutic release profiles, offering unmatched versatility across different tissue types and clinical contexts. These materials also support stimuli-responsive drug delivery, releasing therapeutic agents in response to local environmental cues such as enzymatic activity, pH changes, or mechanical stress. This level of control ensures high therapeutic efficacy while minimizing systemic side effects. Furthermore, their compatibility with advanced fabrication technologies—including nanoparticle encapsulation, layer-by-layer assembly, and 3D bioprinting—enables the

creation of hierarchically structured systems that can deliver multiple agents in a coordinated, tissue-specific manner. Importantly, the production of these materials through microbial fermentation and recombinant expression aligns with principles of safety, reproducibility, and scalability [121,122]. The ability to manufacture these systems under defined, animal-free conditions minimizes immunogenic risks and facilitates compliance with regulatory standards, enhancing their translational potential for clinical use. The continued evolution of these systems, along with the expansion of the detection techniques used for their analysis (Table 7), is being driven by progress in material science and bioengineering, enabling the development of modular DDS that can be adapted to meet specific regenerative needs.

## Abbreviations

DDS, Drug Delivery Systems; ECM, extracellular matrix; ELPs, elastin-like polypeptides; BC, Bacterial cellulose; HA, Hyaluronic acid; SLPs, Silk-like polymers.

## Author Contributions

SY, SR, FS, GP, and UG contributed to the study conception and design. Material preparation and data collection were performed by AC and RC. The first draft of the manuscript was written by SY, SR, and FS. Supervision was provided by AC, RC, GP, and UG. GP and UG also contributed to the design of the research study, preparation of figures and tables, and literature search. All authors read and approved the final manuscript. All authors contributed to the editorial changes in the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

Not applicable.


## Acknowledgment

We would like to acknowledge Orsolina Petillo for data organization support (IRET NA, CNR).

## Funding

The work was supported by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union—NextGenerationEU, Award Number: Project code CN_00000033; Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title “National Biodiversity Future Center—NBFC”; and National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.1, Call for tender No. 1409 published on 14 September 2022 by the Italian Ministry of University and Research (MUR), funded by the European Union—Next Generation EU, (PRIN 2022 PNRR) CUP B53D23031790001, Injectable Hydrogels for treatment of periodontal disease (HYPE)—P2022RZ8WM—Grant Assignment Decree No. n. 1369 adopted on 01/09/2023 1 September 2023 by the Italian Ministry of Ministry of University and Research (MUR).


## Conflict of Interest

The authors declare no conflict of interest.

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