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# Electrospinning and 3D bioprinting for intervertebral disc tissue engineering

Andrea De Pieri[1]    |    Ann M. Byerley[1]    |    Catherine R. Musumeci[1]    |
Fatemeh Salemizadehparizi[1]    |    Maya A. Vanderhorst[1]    |    Karin Wuertz-Kozak[1,2]

[1]Department of Biomedical Engineering, Rochester Institute of Technology (RIT), Rochester, New York

[2]Schön Clinic Munich Harlaching, Spine Center, Academic Teaching Hospital and Spine Research Institute of the Paracelsus Medical University Salzburg (AU), Munich, Germany

**Correspondence**
Karin Wuertz-Kozak, Department of Biomedical Engineering, Rochester Institute of Technology (RIT), Rochester, NY 14623 USA.
Email: kwbme@rit.edu

## Abstract

Intervertebral disc (IVD) degeneration is a major cause of low back pain and represents a massive socioeconomic burden. Current conservative and surgical treatments fail to restore native tissue architecture and functionality. Tissue engineering strategies, especially those based on 3D bioprinting and electrospinning, have emerged as possible alternatives by producing cell-seeded scaffolds that replicate the structure of the IVD extracellular matrix. In this review, we provide an overview of recent advancements and limitations of 3D bioprinting and electrospinning for the treatment of IVD degeneration, focusing on future areas of research that may contribute to their clinical translation.

**KEYWORDS**

annulus fibrosus, bioink, biomaterials, endplate, extracellular matrix, MSCs, nucleus pulposus, stem cells

## 1 | INTRODUCTION

With a lifetime prevalence of >80%, low back pain (LBP) is one of the major public health problems in the world that can seriously affect the quality of life.[1] LBP not only commonly results in physical disability and psychosocial disturbance, but also has detrimental effects on the economy of our societies related to medical costs and disability benefits.[2,3] As LBP is a complex disease with numerous contributing factors (eg, physical, psychological, and hereditary factors) as well as several tissue sources, identifying its primary cause is challenging.[4] However, the degenerating intervertebral disc (IVD) is known to be one of the main origins of LBP.[5] The complex structure of the IVD comprises three areas, the nucleus pulposus (NP), the annulus fibrosus (AF), and the cartilaginous endplate (EP). The NP (with its chondrocyte-like cells) is the central area of the IVD that is mainly composed of water, proteoglycans (predominantly aggrecan) and type II collagen, as well as non-collagenous proteins. The AF (with its

fibroblast-like cells) is characterized by lamellae of type I collagen with high density, as well as a lower proteoglycan and water content compared to the NP.[6] The EP (populated by chondrocytes) allows passage of nutrients into the IVD, but also anchors the IVD to the adjacent vertebrae.[7] Several factors contribute to IVD degeneration, including aging, genetic inheritance, inadequate metabolite transport, and loading history, eventually leading to a loss of tissue hydration and functionality that can entail structural failure of the AF and subsequent herniation of the NP.[8,9] On the cellular level, IVD degeneration is characterized by an imbalance between matrix synthesis and degradation, and enhanced apoptosis and senescence, both ultimately contributing to the loss in extracellular matrix (ECM).[10] Clinical treatments, such as surgical discectomy, disc arthroplasty, and spinal fusion, are used to treat IVD degeneration and herniation. Although current treatments reduce the pain, they do not restore IVD tissue function, but can even cause further degeneration in surrounding tissues and adjacent IVDs due to changes in the biomechanics of the

spine.[11] Therefore, new methods are needed to mimic the structure of native tissue, whereby the use of biomaterials and cells (ie, tissue engineering, TE) holds the greatest promise for long-term functionality.[12,13] TE approaches are based on manufacturing of functional and biologically active scaffolds in the advanced stage of degeneration, including NP replacement, AF replacement/repair, or total IVD replacement.[14,15] A plethora of techniques have been used to fabricate 3D scaffolds for TE purposes, including freeze drying, solvent casting, phase separation, and hydrogel assembly.[16] Due to the specific structure of the IVD, two advanced techniques have emerged for the two zones of the IVD: 3D bioprinting is commonly used both for NP and AF TE, whereas electrospinning is mostly used to mimic the structure of the AF. This review will summarize the current state of the art of 3D bioprinting and electrospinning for IVD replacement and repair and will highlight areas of future investigation that may help in promoting clinical translation.

## 2 | 3D BIOPRINTING

3D bioprinting has attracted attention in TE research over the past years for its unique potential to recreate tissue architecture and assemble complex, functional living architectures.[17-27] The technique most commonly used is extrusion-based bioprinting, whereby a so-called bioink is extruded through a nozzle in a layer-by-layer fashion via mechanical pressure, thus creating 3D constructs in a pre-programmed design.[17-21] Despite the great flexibility of this technique, major limitations are related to low resolution, cell deformation induced by shear stress, and limited bioink selection.[28] Laser-assisted printing represents another 3D bioprinting technique that fabricates precise structures via a layer-by-layer technique, resulting in high printing resolutions.[29,30] The major limitation of laser bioprinting is poor cell viability in comparison to other bioprinting methods.[31] Digital light processing, a variant of stereolithography, has been used to print high resolution constructs in a layer-by-layer fashion by using UV light to cross-link photopolymerizable polymers.[17,32] Major drawbacks of this technique are the lack of biocompatible and biodegradable materials and the harsh nature of UV radiation necessary for the cross-linking.[33] Piezo-assisted bioprinting fabricates constructs through accurate ejection of cells into droplets to form highly organized patterns.[17,21] However, as this technique only works for low viscosity liquids, material selection is limited.[34]

Bioinks, which are used in each of these techniques, are printable biomaterials, typically natural and/or synthetic hydrogels and hydrogel composites, which often contain biochemical signals, living cells, and/or growth factors.[19] Thus, bioinks allow for cell encapsulation and provide relevant cues to cells that are immersed into the ink prior to printing. The establishment of scaffold-cell structures is the foundation for recreation and replacement of damaged IVDs. Over the past years, 3D bioprinting has been widely used to create the highly hydrated NP, but has also been employed for AF TE (Figure 1). However, the complex structure of the IVD, its requirements for high load bearing that can challenge biomaterial integrity, and a harsh microenvironment that can harm cell survival and functionality, makes the IVD difficult to repair or replicate.[7,35] Despite these challenges, one of the most prevalent advantages of 3D bioprinting is to fabricate an appropriate custom-made implant by using patient-derived cells which restore tissue structure and lead to faster recovery post-surgery.[11] This versatility is important when considering the differences across patients' weight, height, and lifestyle, which can change the compressive forces each disc undergoes. Micro-computed tomography (CT) images obtained prior to implantation of a printed disc have been used to analyze disc structure and to evaluate tissue regeneration after implantation.[36] Patient specific magnetic resonance imaging or CT scans with subsequent computer-aided design imaging may be used to fully understand and replicate the IVD of individual human patients.

## 3 | OPTIMIZATION OF BIOINKS FOR IVD TE

Classical bioinks consist of biocompatible materials that integrate well into the body, such as alginate and collagen. However, high mechanical loads in the spine negatively affect the functionality of these hydrogels due to a lack of mechanical strength.[19] Hence, the main challenges in IVD bioprinting are to develop bioinks that are extruded at a high resolution, recreate the complex tissue network, support cell functionality, and handle high amounts of loading seen in the spine. Table 1 summarizes the bioinks in IVD regeneration research, reporting main advantages and disadvantages.

Novel bioink formulations have been proposed to aid in the healing process via host tissue interactions as well as replace damaged tissue.[37] Thus, novel bioinks have the potential to create bioprinted IVDs with improved strength, and circumvent the hydrogel challenges, for example, via optimized composition of polymers, enhanced cross-linking or inclusion of additional support structures, such as nanofiber reinforcement.

Polymer composition is often improved by combining a mechanically tough polymer with a printable polymer, resulting in double network hydrogels that can be rapidly and effectively printed while promoting spinal integration with sufficient mechanical strength.[19,38] A prime example of this approach is the combination of polylactic acid (PLA) with gum-polyethylene glycol diacrylate (GG-PEGDA). While PLA provides biocompatibility and water retention, GG-PEGDA enables printability and supplies enhanced strength.[19,39] This pairing has shown modification abilities by varying infill patterns and densities for scaffold strength.[19] In a comparable manner, PEG can be paired with sodium alginate and 3D bioprinted. Although not yet employed for NP TE, this combination has been shown to create highly stretchable hydrogels that were found to be tougher than natural cartilage, yet with high cell viability.[26] In addition to changing polymer composition, enzymatic cross-linking shows considerable potential in creating hydrogels with improved strength while ensuring sufficient cell attachment and modulating degradation time. Interestingly, this technique has been investigated heavily for AF TE, specifically with silk fibroin (SF)[22,40-42] due to its high ultimate tensile strength, large

**FIGURE 1** State of the art of electrospinning and 3D bioprinting for the fabrication of tissue-engineered intervertebral discs
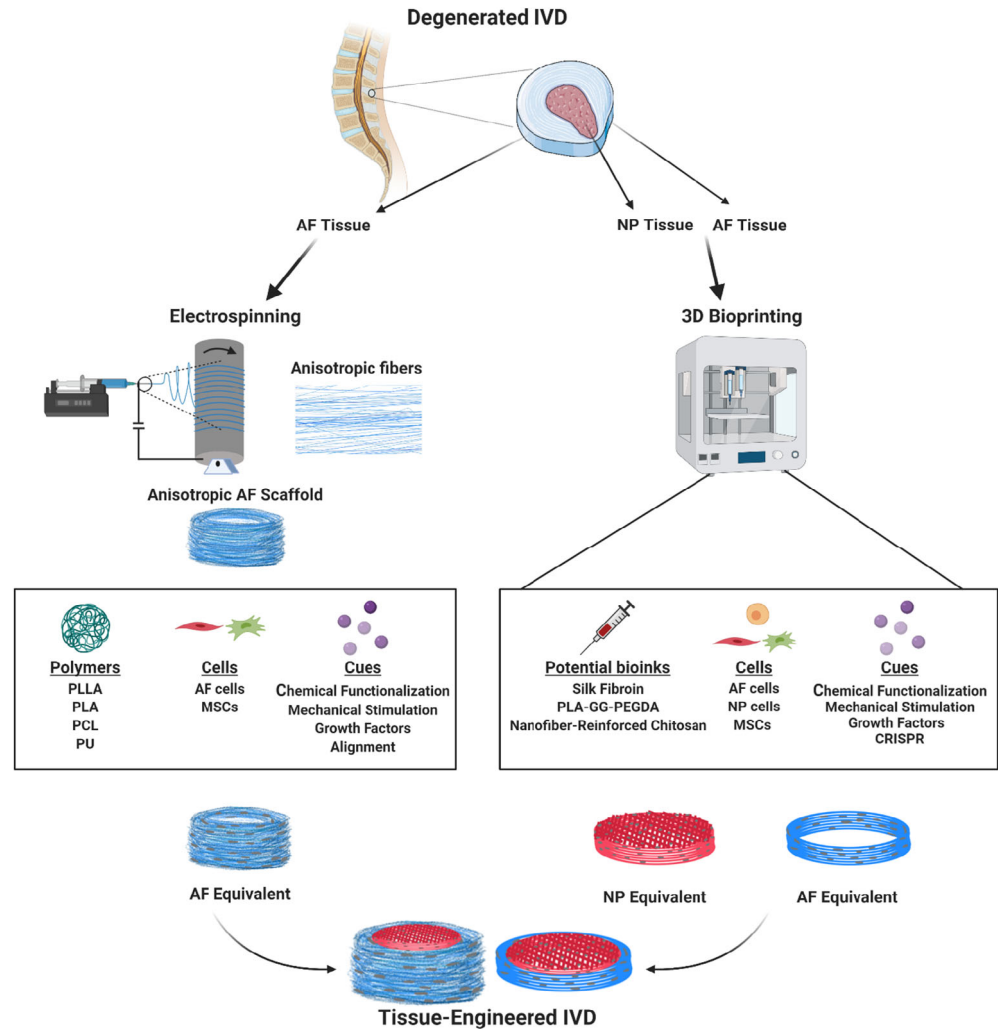


breaking strain, and high toughness.[43] To allow for cross-linking during 3D bioprinting, SF needs to be supplied with a cross linkage site for light polymerization, such as a methacrylate group.[42] This approach has been used with the addition of elastin to the cross-linkable SF.[22] Printing of this composite allowed for control of mechanical, chemical, and biological characteristics of the finalized scaffold, ensured human adipose-derived stem cell adhesion, viability, proliferation, and provided an anchorage point to the bony vertebrae.[22] Although widely tested for AF replacement, cross-linking has also shown success in providing a hydrogel template for NP cell proliferation. Cross-linking type II collagen-hyaluronic acid with a low concentration of 1-ethyl-3 carbodiimide has shown progress in creating a matrix for NP repair.[44] This technique for NP repair has been limited to the creation of hydrogels that form inside the body; however, it may be adapted to 3D bioprinting to print physical hydrogels prior to implantation. Furthermore, nanofiber-reinforced bioinks have been extensively investigated in cartilage TE.[24,25,45] Successful composite bioinks for cartilage TE include (nano-) cellulose fiber-reinforced chitosan[45] and alginate/hyaluronic acid (HA) reinforced with (nano-) cellulose fibers.[25] Embedding of nanocellulose fibers in 3D printed alginate or HA allows ECM simulation of cartilage, whereby alterations in weighted percentages of alginate or HA enable tailored

modification of cartilage viability and stability.[25] Initial studies indicate that this approach may help promote the success of IVD TE. Cellulose nanofibers have been successfully used to strengthen chitosan, with the overall goal to create 3D bioprinted AF structures with the ability to withstand compressive loads.[45] Future studies should also investigate the functionalization of bioinks with carbon fibers, silicone-carbide whiskers, alumina platelets, and cellulose nanocrystals as reinforcement structures, as these have been proven useful in enhancing and tailoring mechanical properties in complex tissue structures.[46] These strategies can be advanced for the reinforcement of load-bearing structures, such as the EPs of the IVD. Although 3D bioprinting can be a valuable tool to create EP structures, low printing resolutions may hamper their structural functionality.[47] Furthermore, it will be crucial to achieve suitable EP diffusivity to maintain cell viability, especially in the center of the engineered IVD.[48]

## 4 | OPTIMIZATION OF CELLS AND CUES FOR IVD TE

The choice of optimal cell populations and the recreation of biomimetic in vitro microenvironments are crucial for the development of

**TABLE 1** Bioinks for IVD regeneration with main advantages and disadvantages

| Bioink | Cell type | Main advantage(s) | Main disadvantage(s) | References |
|---|---|---|---|---|
| PLA with GG-PEGDA | Human BMSCs | • High biocompatibility<br>• High water retention<br>• Enhanced strength | • Limited research | 19,38,39 |
| PEG with Alginate | Human BMSCs | • Highly stretchable<br>• High cell viability | • Not yet employed for NP TE<br>• Limited research | 26 |
| SF[a] | Bovine AF cells | • High resolution<br>• Reproducibility<br>• Reliability<br>• Strength via crosslinking | • Extensive processing | 40-43 |
| Elastin with SF[a] | Human ADSCs | • Mechanical, chemical, and biological control<br>• High cell adhesion, viability and proliferation | • Limited in vivo studies | 22 |
| Collagen-II-HA[a] | Human NP cells | • Increased gel stability<br>• High cell viability | • High concentration of EDC increases toxicity to cells<br>• Limited in vivo studies<br>• Mechanical properties and gel composition negatively affected over time<br>• Reduced water uptake | 44 |
| Chitosan with (nano-) cellulose fibers | Porcine chondrocytes | • Biomechanical integrity | • Variability of porosity and pore size | 45 |
| HA with (nano-) cellulose fibers | IPSCs + human chondrocytes | • High cell viability<br>• Able to mimic complex architecture<br>• Sustained stem cell pluripotency<br>• Supported cell differentiation in 3D | • Research limited to injectable hydrogels thus far | 25 |

Abbreviations: ADSC, adipose-derived stem cell; AF, annulus fibrosus; BMSCs, bone marrow stromal cells; GG-PEGDA, gum-polyethylene glycol diacrylate; HA, hyaluronic acid; IPSCs, induced pluripotent stem cells; IVD, intervertebral disc; NP, nucleus pulposus; PEG, polyethylene glycol; PLA, polylactic acid; SF, silk fibroin; TE, tissue engineering.
[a]It indicates enzymatically cross-linked bioinks.

functional IVD substitutes. The cells native to the degenerated IVD are not an ideal cell source due to their diseased nature, although their metabolic activity and proliferation may be enhanced by use of specific growth factors.[35] Culturing degenerated human NP cells in alginate with exposure to transforming growth factor beta 3 (TGF-β3) and dexamethasone (Dex) or notochordal cell factors demonstrated that TGF-β3 and Dex lead to higher cell proliferation.[35] Although these results were obtained in cast hydrogels, these preconditioned cells may also be beneficial for 3D bioprinting of NP replacements using native cells. In addition to modifying NP cells, researchers have also attempted to use mesenchymal stem cells (MSCs) for IVD TE due to their availability and differentiation prospects.[49] However, the harsh microenvironment of the IVD (characterized by high osmolarity, high mechanical loads, low oxygen and glucose, acidic pH, and inflammation) negatively affects MSC viability and functionality,[50] thus limiting the translatability of MSC-based IVD TE into clinical practice. 3D bioprinting offers the possibility to use biologically-enhanced bioinks that can induce preconditioning or pre-differentiation of MSCs, thus likely improving their survival. Chemical functionalization, such as incorporation of growth factors (TGF-β1, growth differentiation

factor-5), either through simple mixing or conjugation, can provide pronounced cues for embedded cells.[35,51,52] More recently, CRISPR/ Cas9 genome engineering has been utilized to create MSCs with higher resistance to harsh microenvironments. This approach has been successfully employed by repressing the expression of cytokine receptors in order to minimize the detrimental effects (eg, apoptosis, ECM degradation) of the inflammatory environment on MSCs.[53,54] In the years to come, the potential of CRISPR-modulated MSCs will likely become increasingly evident, especially when considering the possibility for CRISPR multiplexing, that is, the simultaneous modulation of several targets.[55] Future research should furthermore investigate the incorporation of (eg, MSC-derived) extracellular vesicles into bioinks, which may represent an alternative to growth factor treatment and may provide an even more efficient means to protect embedded cells from apoptosis and inflammation, and enhance ECM production.[56] Furthermore, future research efforts should focus on the use of bioreactor systems for the mechanical stimulation of 3D bioprinted construct, in order to improve the maturation of IVD substitutes and increase their therapeutic effect.

# 5 | ELECTROSPINNING

Electrospinning represents a simple, versatile, and controllable technique for the production of micro-/nanofibers from polymer solutions or melts using electrostatic forces.[57,58] A typical electrospinning setup requires a syringe (containing the polymer solution) connected to a metallic needle, a syringe pump to regulate the flow rate, a high voltage power supply, and a metallic collector. Upon application of voltage between the syringe and the collector, the solution extruded through the metallic needle turns into an electrically charged jet, which is attracted toward the collector. As the solvent evaporates during the travel from the spinneret to the collector, the jet diameter significantly shrinks along its trajectory, thus resulting in the formation of a mass of fibers deposited on the metallic collector.[59,60] Diameter and reproducibility of the electrospun fibers can be affected by numerous parameters, such as solution properties (solvent, concentration, molecular weight, viscosity), process settings (flow rate, voltage, distance between the needle and collector), and environmental conditions (humidity, temperature).[61] Fiber orientation (random vs aligned) can be precisely controlled through collector rotation[62] or collector architecture.[63] One of the major advantages of electrospinning for TE is the ability to generate 3D scaffolds with tailored architectural features that simulate the nano- to micro- scale fibrillar structure of the ECM.[64,65] Given the anisotropic architecture of the AF region, electrospinning has emerged as an ideal technique to replicate the highly organized structure of the AF's ECM (Figure 1). Table 2 summarizes TE strategies based on electrospun scaffolds for IVD regeneration.

## 5.1 | Polymers of choice

Synthetic polymers, such as polycaprolactone (PCL), polylactic acid (PLLA), and polyurethane (PU), have been extensively used for IVD TE for their improved physical and mechanical properties, specifically high elastic moduli and tensile strength, compared to natural polymers (eg, collagen, gelatin, chitosan).[66] Due to their hydrophobic nature, synthetic polymers do not possess ideal water wetting behavior to allow for optimal cell attachment. Indeed, most human and animal cells prefer a surface of moderate hydrophilicity for adhesion and growth.[67] Wetting scaffolds in cell culture media is a simple technique to significantly reduce the hydrophobicity of synthetic biopolymers. Other techniques utilized to tune the surface properties of electrospun mats are plasma treatment, coatings, or chemical modifications.[68] PCL has been used to fabricate anisotropic electrospun fibers which supported proliferation and ECM deposition of AF cells, and the scaffold-cell construct matched the modulus of native inner AF after 4 weeks of culture.[69] Nevertheless, PCL is associated with low Young's modulus (15-16 MPa), which does not match the modulus of single lamella sheets of the human AF (59-136 MPa),[70] and could lead to failure of the AF in response to high mechanical loads. Composite electrospun fibers based on a blend of PCL with PLLA have been demonstrated to possess higher mechanical properties in comparison to PCL-only scaffold.[71]

However, during the biodegradation of polylactides and polyglycolides, acidic degradation products could adversely affect biocompatibility.[72] For this reason, PU can be a valid alternative, generating milder acidic conditions upon degradation.[73]

## 5.2 | Influence of architectural features

Despite mimicking the anisotropic structure of the ECM, aligned electrospun fibers do not replicate the hierarchical AF architecture necessary to support multiaxial spinal loads. The AF collagen lamellae are aligned at a ~30° angle with respect to the diagonal plane of the spine axis, but in alternate directions in each successive layer, producing an angle-ply structure.[74] Advanced fabrication strategies have focused on the development of disc-like angle ply structures (DAPS) by arranging PCL nanofiber sheets into AF lamellar patterning.[75] Interestingly, DAPS have been coupled with cell-laden hydrogels to engineer AF-NP composite to imitate the multiscale architecture of the native IVD.[76] In addition, recent research efforts have focused on the integration of DAPS with in vitro engineered cartilage tissue in order to fabricate a tissue-engineered AF-EP substitute and develop a biomimetic IVD implant suitable to use as disc replacement.[77] These strategies evidenced the possibility to combine electrospun AF equivalents with other tissue engineering strategies to develop whole IVD substitutes, increasing their therapeutic potential and clinical translatability.

Despite the great utility of electrospinning for AF TE, several limitations need to be addressed to unleash the full potential of this versatile technique. Extremely small pore sizes and high packing density of electrospun fibers produced under standard conditions constrain cellular infiltration into the mesh.[78] To overcome this limitation, sacrificial components (polymers, salt, and ice crystals) can be incorporated during the electrospinning process and subsequently removed to create void spaces within the fibrous mesh. For instance, the water-soluble polymer polyethylene oxide (PEO) has been utilized as a sacrificial fiber fraction to improve cellular colonization and ECM deposition. The abundant deposition of glycosaminoglycans (GAGs) and collagen led to an improvement of micromechanical properties of electrospun-based DAPS, as evidenced by an increase in the indentation modulus.[79] Differently from 3D bioprinting, where cells are dispersed within the bioink and precisely dispensed during the process, cell seeding onto electrospun mats is performed after the scaffold has been formed. This method could lead to non-uniform distribution of cells over a given scaffold architecture, resulting in regions of acellularity.[80] An alternative approach is represented by cell electrospinning, which generates fibrous structures with living cells embedded within.[81] For instance, myoblast cell-laden constructs with highly aligned microstructure and high cell viability were fabricated for skeletal muscle regeneration.[82] Although cell electrospinning is still in its infancy, advantages such as improved cell-to-cell/matrix interactions and the possibility to incorporate multiple cell types during the process, make this technique a fascinating tool for the fabrication of engineered tissue equivalents.

**TABLE 2** Tissue engineering strategies based on electrospun scaffolds for IVD regeneration

| Polymer | Concentration/ solvent | Fiber diameter | Orientation | Cell type | Outcome | References |
|---|---|---|---|---|---|---|
| PCL | 285 mg/mL THF/DMF | 300-750 nm | Aligned | Bovine AF cells | AF cells oriented along the aligned scaffolds and deposited ECM that contributed to construct mechanics under loading | 69 |
| PU | 200 mg/mL HFIP | 130-890 nm | Random | Bovine AF cells | Surface functionalization with ADO improved cell attachment and collagen deposition | 86 |
| PCL | 260 mg/mL Methylene chloride | 8000-12 000 nm | Aligned | Porcine chondrocytes | Composite biomimetic scaffold composed of aligned electrospun fibers (AF analogue) and cell-laden agarose gel (NP analogue) | 102 |
| PLLA | 13% wt/wt DCM | 1.5 ± 0.9 μm + TGF-β - 620 ± 170 nm | Random | Bovine AF cells | Functionalization with TGF-β1 increased GAGs and collagen deposition, with higher neo-ECM thickness | 87 |
| PCL PU | PCL-3 g in 10 g of chloroform PU-2 g in 10 g in DMF/acetone | Aligned PCL- 2.17 -1.53 μm Aligned PU- 0.84 - 0.32 μm | Random aligned | Bovine AF cells | Fiber alignment promoted the upregulation of ECM genes | 73 |
| PCL | 200 mg/mL DMF/ chloroform | 100-1200 nm | Aligned | Human BMSCs | Fabrication of 3D hierarchic multi-lamellar scaffold by combining electrospinning with FDM technique. Scaffold promoted cell attachment and alignment | 103 |
| PCL PEO | PCL-143 mg/mL in THF/DMF PEO-100 mg/ mL in 90% ethanol | — | Aligned | Bovine AF cells | Sacrificial PEO increased cell infiltration in vitro. Electrospun scaffolds were combined with cell-seeded hydrogels (as an NP replacement) to form DAPS. In vivo, DAPS were stable in the caudal spine and were infiltrated by cells from the peri-implant space | 75 |
| PLLA/PCL | 150 mg/mL (90% PLLA-10% PCL) HFIP | 300-500 nm | Aligned | Human BMSCs, Human AF and NP cells | Whole IVD constructs were stimulated with compressive loading, which resulted in the downregulation of cartilage-related markers in AF nanofibers and upregulation in NP hydrogel | 93 |
| PCL | 143 mg/mL THF / DMF | — | Aligned | Juvenile bovine BMSCs | Inclusion of zirconium (IV) oxide radiopaque nanoparticles increased the tensile modulus of the scaffold. In a in vivo model of disc replacement, the scaffold was biocompatible and supported the deposition of fibrous tissue | 104 |
| Poly(ether carbonate urethane)-urea (PECUU) | 250 mg/mL HFIP | — | Random aligned | Rabbit AF-derived stem/progenitor cells (AFSCs) | On aligned scaffolds, cells exhibited higher gene and protein of collagen-I and aggrecan | 105 |

**TABLE 2** (Continued)

| Polymer | Concentration/solvent | Fiber diameter | Orientation | Cell type | Outcome | References |
|---|---|---|---|---|---|---|
| PCL | THF | 1.41 ± 0.36 μm (random) 1.33 ± 0.40 μm (aligned) | Random aligned | — | In an ovine model of AF impairment, the aligned scaffold integrated with the surrounding tissue and homogeneously aligned collagen fibers within each lamella | 94 |
| PCL/PLLA | HFIP | PLLA: 357 nm PCL: 234 nm Blend (50:50): 468 nm | Aligned | Bovine AF cells | Scaffolds made from 50:50 and 20:80 blends of demonstrated yielded tensile properties within the range of human AF. 50:50 blends exhibited optimal structural integrity and supported desirable cellular response in vitro | 71 |
| PCL/PLLA | HFIP | — | Aligned | Bovine AF cells | Tube-like structures (6 layers) were created by rolling ±30° bilayer PCL/PLLA scaffolds. Cells remained viable over 3 weeks in culture with evidence of collagen type I deposition | 106 |
| PCL/PLLA | HFIP | PCL: 214 nm PLLA: 330 nm Blend: 671 nm | Aligned | — | After 6 months, PCL/PLLA blended scaffold underwent hydrolytic degradation, as evidenced by fibers swelling, increased crystallinity, increased stiffness and decreased molecular weight | 107 |

Abbreviations: AF, annulus fibrosus; DAPS, disc-like angle ply structures; DCM, dichloromethane; DMF, dimethylformamide; ECM, extracellular matrix; GAG, glycosaminoglycans; FDM, fused-deposit-modeling; HFIP, hexafluoroisopropanol; IVD, intervertebral disc; NP, nucleus pulposus; PCL, poly-caprolactone; PEO, polyethylene oxide; PLLA, polylactic acid; PU, polyurethane; TGF-β1, transforming growth factor beta 1; THF, tetrahydrofuran.

Another limitation of electrospinning is its use for load-bearing TE applications. Mechanical properties of electrospun scaffolds are lost over extended periods due to polymer degradation. Additive materials, such as carbon nanotubes[83] or aluminum whiskers,[84] can be added to biodegradable polymers to improve resistance and mechanical behaviors. Another strategy to reinforce electrospun fibers consists in weaving, knitting, or braiding bundles of fibers to generate solid 3D constructs.[85]

## 5.3 | Biochemical, biological, and biophysical functionalization

Biochemical, biological, and biophysical functionalization offers great potential to improve the outcome of electrospinning-based TE strategies. Chemical functionalization strategies have been utilized to increase attachment and proliferation of cells in AF TE. For example, the incorporation of anionic dihydroxyl oligomer (ADO) into PU electrospun scaffolds increased the material surface energy and decreased surface hydrophobicity, resulting in improved cellular attachment, proliferation, and collagen deposition.[86] In order to improve the functionality of electrospun fibers, scaffolds can be functionalized by encapsulating different bioactive agents, such as growth factors or cytokines, which can modulate the behavior of seeded cells. For instance, PLLA electrospun scaffolds functionalized with TGF-β1, which was added to the polymer solution prior to electrospinning, have been shown to improve GAG and collagen deposition of AF cells.[87] However, the ECM composition of AF tissue changes between the outer region, which is abundant in collagen type I with little proteoglycans, and the inner region, which is rich in collagen type II and proteoglycans.[88] Supplementation of culture media with insulin transferrin-selenium, proline, dexamethasone, and pyruvate to a multi-lamellated outer and inner AF engineered tissue was able to promote the accumulation of collagen type I in the outer region and aggrecan and collagen type II the in inner region, replicating the ECM distribution of the native tissue.[89]

Considering the beneficial effects on ECM components in maintaining and/or directing cell phenotype,[90] future strategies could incorporate decellularized ECM either within the polymer solution or post-electrospinning to improve biocompatibility, mechanical stability,

and degradation rate.[91] Given the importance of mechanical loading in disc degeneration and regeneration, bioreactor systems have been utilized to provide more physiologically relevant conditions for the development of functional IVD substitutes.[92] For example, compressive mechanical loading applied to a composite biomaterial scaffold, composed of nanofibrous strips seeded with cocultured AF cells/MSCs and an inner core of hydrogel seeded with cocultured NP cells/MSCs, enhanced the AF and NP cell differentiation and increased the IVD ECM production.[93]

## 5.4 | Preclinical studies

Considering the success of electrospun scaffolds in mimicking the organized structure of the AF, promising in vivo data both in small and large animal models have been achieved. Electrospun-based DAPS have been validated in a rat caudal spine model. Results showed that a stable fixation system improves the retention of implanted AF substitutes and the inclusion of sacrificial PEO layers interspersed throughout the angle-ply structure promoted cellular infiltration from the peri-implant space.[75] A recent study assessed the potential of a biomimetic multilayer PCL fibrous scaffold to repair AF defects in an ovine lumbar model. Results showed that electrospun PCL successfully integrated within the AF tissue, promoting cell infiltration and deposition of oriented collagen fibers within the aligned scaffold.[94] Another study focused on the implantation of an endplate-modified DAPS (eDAPS) into a goat cervical disc replacement model.[95] Results demonstrated that the eDAPS composition and structure were maintained up to 8 weeks within the disc space, developing mechanical properties that either matched or exceeded those of the native tissue. Despite these positive studies, clinical translation of electrospinning are still limited due to scalability issues, especially when aiming to create entire IVDs.[96] The fabrication of large-scale DAPS (6 mm height × 20 mm diameter) resulted in loss of cell viability and lack of matrix deposition, due to poor diffusion of nutrients throughout the constructs.[97] Optimization of culture conditions by adding channels for nutrient transports,[98] growth factors supplementation and bioreactor systems could improve the functionality of large IVD constructs.

## 6 | CONCLUSIONS

IVD degeneration represents a significant health problem worldwide. Conservative and surgical treatments do not promote long-term tissue regeneration and fail to restore native tissue function. Although still in the experimental phase, 3D bioprinting and electrospinning hold great potential for tissue-engineered IVD repair, replacement, and regeneration. Silk-fibroin, nanofiber-reinforced (chitosan) hydrogels as well as PLA, and GG-PEGDA combinations have emerged as promising bioinks for IVD TE. Functionalization of electrospun fibrous constructs,

which closely recapitulate the architecture of the AF, has yielded highly promising results as demonstrated in preclinical studies.

The combination of AF electrospinning with NP 3D bioprinting, an approach that has also been discussed in engineering of osteochondral tissues,[99] could improve the advantages of the individual methods and lead to the fabrication of biomimetic whole IVD equivalents.

Despite these advantages in 3D bioprinting and electrospinning, sub-optimal mechanical properties, high manufacturing costs, reproducibility, and scalability issues have hindered the clinical translation of these biofabrication strategies. Future research will focus on their optimization in order to design engineered constructs with optimal structural, biomechanical, and biological properties. In addition, while electrospun-based DAPS have been successfully implanted in vivo through fixation methods, the delivery of 3D printed constructs remains a challenge. Novel intravital and noninvasive in vivo 3D bioprinting technologies could overcome these limitations by injecting photosensitive cell-laden bioinks into the target tissue and bioprinting them across the tissue using near-infrared laser light.[100,101] Furthermore, cell survival and functionality within the harsh local environment has to be improved to ultimately enable their translation into clinical practice.

## CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

## AUTHOR CONTRIBUTION

Fatemeh Salemizadehparizi and Karin Wuertz-Kozak wrote the introduction chapter. Catherine R. Musumeci, Fatemeh Salemizadehparizi, Andrea De Pieri, and Karin Wuertz-Kozak wrote the 3D bioprinting chapter (including Table 1). Andrea De Pieri, Ann M. Byerley, Maya A. Vanderhorst, and Karin Wuertz-Kozak wrote the electrospinning chapter (including Table 2). Andrea De Pieri and Karin Wuertz-Kozak wrote the conclusion chapter. Andrea De Pieri, Ann M. Byerley, and Maya A. Vanderhorst designed Figure 1 with input from Karin Wuertz-Kozak and Catherine R. Musumeci.

## ORCID

*Karin Wuertz-Kozak* https://orcid.org/0000-0002-3281-4629

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