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Review Article

Nucleus pulposus replacement and regeneration/repair technologies: Present status and future prospects

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Abstract: Degenerative disc disease is implicated in the pathogenesis of many painful conditions of the back, chief among which is low back pain. Acute and/or chronic low back pain (A/CLBP) afflicts a large number of people, thus making it a major healthcare issue with concomitant cost ramifications. When conservative treatments for A/CLBP, such as bed rest, anti-inflammatory medications, and physical therapy, prove to be ineffectual, surgical options are recommended. The most popular of these is discectomy followed by fusion. Although there are many reports of good to excellent outcomes with this method, there are concerns, such as long-term adverse biomechanical consequences to adjacent functional spinal unit(s). A surgical option that has been attracting much attention recently is replacement or regeneration/repair of the nucleus pulposus, an approach that holds the prospect of not compromising either mobility or function and causing no adjacent-level injury. There is a sizeable body of literature highlighting this option, comprising *in vitro* biomechanical studies, finite element analyses, animal-model studies, and limited clinical evaluations. This work is a review of this body of literature and is organized into four parts, with the focus being on replacement technologies, regeneration/repair technologies, and detailed expositions on 14 areas for future study. This review ends with a summary of the salient points made. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B:000–000, 2012.

Key Words: intervertebral disc, spinal implant, tissue engineering

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INTRODUCTION

Over the years, degeneration of the intervertebral disc and the ensuing disease, known as degenerative disc disease (DDD), have attracted much research attention. Five aspects of the extant knowledge base are highlighted. First, magnetic resonance imaging is the "gold standard" technique used for detecting early signs of the disease,[1] although results from *ex vivo* studies show that other techniques, notably magnetic resonance spectroscopic imaging,[2] may be feasible. Second, visual characteristics of a degenerated disc include reduced height,[3] a fibrotic appearance of both the annulus fibrosus (AF) and the nucleus pulposus (NP),[3,4] and radial, rim, and/or circumferential tears in the AF.[5] Third, different grading scales/classification schemes are in use, most being qualitative (e.g., mild, moderate, and severe grades[6]) although, recently, quantitative scoring systems have been proposed.[7] Fourth, there are myriad changes in the morphology and biochemistry of the disc associated with its degeneration, among which are cell proliferation, cell death, loss of proteoglycans (PGs), and increase in fibronectin content.[8-10] It has been postulated that these changes play a role in the initiation of a cascade of events, culminating in many clinical problems. Examples of some aspects of these problems in the cervical and lumbar sections of the spine are now given. In the cervical spine, formation of osteophytes in joints adjacent to the degenerated one, hypertrophy of the ligamentum flavum, spinal stenosis, and disc herniation are said to be precursors to spondylotic myelopathy and radiculopathy, which, in turn, are responsible for conditions such as occipital headaches, radiocular pain, severe neck pain, and severe shoulder pain.[4,10,11] Degeneration of disc(s) in the lumbar spine is strongly implicated in discogenic low back pain. This condition is commonplace (e.g., in the United Kingdom, the prevalence rate is between 12 and 35%[12]) and presents major economic ramifications (e.g., associated annual total costs—the sum of direct and indirect costs—have been estimated to be ~9.2 billion Australian dollars, ~6.0 billion yen, and ~6.4 billion Euro for Australia, Japan, and The Netherlands, respectively.[13]) Fifth, there is no agreement on the etiology and pathogenesis of

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disc degeneration, with four schools of thought being popular. The first is that it is a pathological condition (e.g., progressive loss of the lamellar organization of the AF and development of cysts within its mid-substance,[14] a failure of nutrient supply to disc cells,[10,15] and deterioration of the structure and function of the elastic fiber of the AF[16] are among the factors implicated). The second is that DDD is a concomitant of aging (e.g., start of desiccation of the normally gelatinous NP in most people by age 40[17] and decrease in population of putative notochordal stem cells as an organism ages.[18]). In the third school, disc degeneration is attributed to the superimposition of changes to the disc brought about by environmental factors, such as abnormal mechanical loading on the spine, driving and associated whole-body vibration, and smoking, on changes due to aging.[19,20] The posit of the fourth school is that there is a genetic contribution, with the allele of *COL9A2* being one of the most studied genes associated with DDD in the lumbar spine.[10,19,20] A consensus is emerging that DDD is multifactorial, with several of the aforementioned features expressing simultaneously.[10,19,20]

When conservative treatments for DDD, such as analgesics, muscle relaxants, and chiropractic adjustment,[10] do not provide relief from the pain, there is a plethora of surgical methods that may be used. On the basis of popularity of use, these methods may be divided into the "gold standard," namely, discectomy followed by fusion[21,22]; those that have been used in a modest number of cases, namely, discectomy without fusion[23,24] and percutaneous nucleotomy[25]; and those that have been used in very few cases, namely, total disc replacement[26,27] and nucleus pulposus replacement (NPR) that uses a device fabricated from polymer(s).[28-32] In addition to these methods, there are others that are at various stages of research, development, and preclinical evaluation, an example being intervertebral disc transplant.[33] Furthermore, there are many vibrant research programs on methods that aim to regenerate/repair the degenerated disc rather than to replace it, examples being direct injection of a growth factor, such as osteogenic protein-1 (OP1)[34-38]; gene therapy using, for example, an adenoviral vector[36-39]; and cell therapy using, for example, seeding of autologous disc chondrocytes or adipose tissue-derived stem cells (ASCs), on a scaffold fabricated, for example, from collagen or small intestine submucosa.[36-38,40-45]

Several limitations of reviews of the literature on technologies/approaches for replacement and regeneration/repair of the NP[17,34-43,46-52] are highlighted. First, in the case of NPR technologies, the majority of these reviews have been limited to reported work on devices designed by commercial entities and fabricated using well-characterized synthetic materials (herein referred to as "commercial devices").[17,34,46-52] Second, although there are many reviews on tissue engineering (TE) approaches,[35-43] all have, with one exception,[37] focused on strategies for disc regeneration in general. Third, biomechanical aspects of NPR devices were not included in any of the reviews.[17,34-43,46-52] Thus, there is opportunity for a review that (1) is comprehensive; namely, its ambit covers work on both replacement and regeneration/repair approaches; (2) is up-to-date; namely, includes work that is at the experimental stage; and (3) includes biomechanics studies. This work is such a review. A detailed search was conducted of relevant databases (such as MEDLINE®/PubMed and PubMed Central), science subjects-specific search engines (such as SCIRUS®), and the table of contents of relevant key peer-review, archival journals (such as *Spine*, *The Spine Journal*, *Spine Arthroplasty Society (SAS) Journal*, *Journal of Biomedical Materials Research Part B: Applied Biomaterials, Biomaterials*, and *Journal of Materials Science: Materials in Medicine*) for relevant peer-reviewed articles published, over the period 1975-date, in English as well as in other languages (provided English translations were available). Furthermore, the references list of each article, obtained from this search, was manually examined to identify additional relevant and acceptable articles. (Note that, for the purposes of the present review, abstracts and presentations were not regarded as "acceptable" articles because they were not published in peer-review archival journals.) The review is organized into four parts, with these containing, in order, (1) reviews of the literature on (a) biomechanics aspects of commercial replacement devices, (b) biomechanics aspects of "notional" replacement devices, these being herein defined as those for which no information was given in the report on any relevant aspect, such as material and geometrical configuration, and (c) properties of "emergent" materials, these being those that have only been evaluated in laboratory tests for their potential for fabricating replacement devices; (2) review of the literature on TE approaches; (3) discussion of future research topics; and (4) a summary of the most salient points made in the work.

REPLACEMENT TECHNOLOGIES

Categorization of commercial devices

Over the years, there have been many reports on a large number of these devices,[28,29,31,32,53-68] but, in this review, reference is made to a sample of those devices that have received the most attention. Also, it is to be noted that the availability of commercial devices is shifting all the time, making it very difficult to be definitive about the current state of the market. There is a variety of reasons for this situation, examples being the commercial entity responsible for design of a device is no longer in existence, a decision by such an entity to discontinue work on a device, and additions to the market through granting of patents (e.g., European Patent # EP 1 231 868 B1, June 15, 2011and US Patent Publication # 20110153021, June 23, 2011). To reiterate, then, the coverage of commercial devices given in this review is intended to provide insight into the scope of work in the field over a large timescale.

From a taxonomical perspective, there are many ways in which commercial devices may be categorized. Using physical state at the time of placement of a device in the space created by removal of the degenerated NP (disc space) as the basis of categorization, these devices may be grouped into six types: *in situ* curable polymer, preformed polymer,

TABLE I. Material(s) of Fabrication and Other Salient Features of a Sample of Commercial Nucleus Pulposus Replacement Devices

Name	Type	Material(s)/Salient Features	Company
Aquarelle	Preformed polymer	A poly(vinyl alcohol) (PVA) hydrogel (80% water)	Stryker Howmedica Osteonics, Allendale, NJ
BioDisc	*In situ* curable polymer	An albumin + glutaraldehyde hydrogel that is injected directly into the disc space begins to polymerize within 0.5 min and solidifies within 2 min	CryoLife, Kennesaw, GA
Buck (Figure 1)	Knitted mechanical	Knitted Ti filaments	Buck GmbH & Co KG, Bondorf, Germany
DASCOR	*In situ* curable polymer	A polyurethane (PU) core and a PU balloon	Disc Dynamics, Eden Prairie, MN
Hydrafil	*In situ* curable polymer	Hydrophilic PVA and poly(vinyl pyrrolidone) (PVP) copolymer	Synthes USA, West Chester, PA
HydraFlex	Composite polymer	Flexible preformed hydrogel core encased in a jacket fabricated from tightly woven fibers of ultra-high-molecular-weight polyethylene	Raymedica, LLC, Minneapolis, MN
IPD	One-piece mechanical	An elastic component (elastic springs) attached to a fixation component	Dynamic Spine, Nahtomedi, MN
NeuDisc	Preformed polymer	A modified hydrolyzed poly(acrylonitrile) reinforced with a Dacron mesh	Replication Medical, Cranbury, NJ
Newcleus	One-piece mechanical	A memory-coiling polycarbonate urethane	Centerpulse Orthopaedics, Winterthur, Switzerland
NuBac® Disc Arthroplasty System (Figure 2)	Two-piece mechanical	Poly(etheretherketone) (PEEK)-on-PEEK	Pioneer Surgical Technology, Marquette, MI
NuCore® Injectable Nucleus (Figure 3)	*In situ* curable polymer	A protein polymer hydrogel, with the polymer chains having synthetic silk and elastin components	Spine Wave, Shelton, CT
Regain	One-piece mechanical	Rigid, composed of a graphite substrate with a coating of pyrolytic carbon	EBI Medical Systems, Parsippany, NJ
SINUX ANR[a]	*In situ* curable polymer	A liquid poly(methyl siloxane) polymer cures within 15 min of being injected into the disc space	Sinitec AG/DePuy Spine

[a] Awarded CE mark, which is a mandatory compliance designation required for all products placed in the market in European Economic Area countries.

composite polymer, one-piece mechanical, two-piece mechanical, and knitted mechanical (Table I).

Device descriptions and biomechanical evaluations of commercial devices

In addition to identifying the material(s) used for fabricating these devices (e.g., see Table I), it is important to highlight the change-of-state events that occur following implantation of the device in the disc space (in the case of polymeric devices). Information about these events, together with pertinent remarks in the case of mechanical devices, is given in this subsection for eight devices, followed by summaries of results of their biomechanical evaluations. The evaluations were achieved through bench, *ex vivo* (cadaveric), and animal-model tests on either the device *per se* or a construct (device implanted in the disc space).

Aquarelle. The device is implanted in the disc, with the aid of a pressurized precision trochar, while in a hydrated state.

Based on the results from a bank of tests, such as those used to evaluate biocompatibility and toxicity (via ISO 10993), cytotoxicity, sensitization, intracutaneous reactivity, and gentotoxicity, performed on constructs (device implanted in L3-L4 and L4-L5 discs of male baboons), the device was found to be biocompatible and nontoxic.[69]

Buck. The device has the same shape as that of the bovine NP and has a rough surface.

When implanted in bovine lumbar spines (L2-L3 and L4-L5 segments), the axial deformability (AD) and height of the construct were measured after quasi-static loading (increased from 100 to 1000 N) as well as after the application of a complex cycling loading cycle (axial force of 100–600 N, at 5 Hz, that created an additional bending moment ranging from 3 to 18 Nm), while the construct was being continuously rotated around its longitudinal axis at 360° min^{-1}.[63] The median value of AD was the same as that obtained immediately after implantation but, with cyclic loading, it decreased by about 33% (relative to the intact case).[63] The height change was the same as that obtained immediately after implantation but, with cyclic loading, it increased by a small amount (relative to the intact case).[63]

To assess migration/expulsion of the device, plain anterior–posterior and lateral radiographs were taken before implantation of the device in bovine lumbar spines (L2-L3 and L4-L5 segments) and after the construct was subjected to three cycles of unconstrained loading (±7.5 Nm flexion–extension, ±7.5 Nm lateral bending, and ±7.5 Nm axial rotation).[63] No extrusion of the device was seen, but there was evidence of migration (1) within the cavity created by the surgeon to allow device implantation (note, however, that when the sizes of the cavity and the device were comparable, minimal migration was seen) and (2) toward the anterior border of the disc (attributed to the facts that the device was implanted using an anterior approach and was positioned anterior to the center of the disc).[63]

In kinematics tests, using the construct and cyclic loading protocol described above, (1) the construct range of motion (ROM) was about twice as high in extension as it was in flexion, which was attributed to the lordotic tilt caused by the implantation and (2) the effects on construct ROM were marginal in lateral bending and in axial rotation (all changes are with respect to the values obtained with the intact case).[63]

DASCOR. In implanting this device, the PU balloon is injected, under pressure (delivered by a custom-built system), with the *in situ*-curable PU. After the PU cures, the expanded balloon adheres to it and conforms to the size and shape of the disc space.

In durability tests, the heights of ellipsoid-shaped specimens were measured after being subjected to a motion and loading protocol comprising ±5.5° flexion/extension (3 Hz; 10 million cycles) combined with axial compression (180–520 N; 1.5 Hz; 20 million cycles), with these being coordinated so that the peak compression force occurred during peak flexion or extension and the minimum compression force occurred when the device was in the neutral position.[70] A specimen experienced a height loss that increased progressively with increase in number of motion/loading cycles (N_l), but, at each N_l, after recovery, it returned to practically the same height as its initial height. Furthermore, the increase in compressive modulus with increase in N_l, at a given strain level, was not significant.[70]

In flexibility tests on constructs (device implanted in cadaveric T12-L1, L2-L3, and L4-L5 segments), there was no significant difference in the displacement of the neutral zone, ROM, or segmental stiffness of the construct, relative to the level for an intact case, regardless of the applied loading [five cycles of axial compressive preload of 500 N coupled with flexion/extension, lateral bending (±7.5 Nm), or axial rotation (±7.5 Nm)].[70]

From quasi-static loading tests on ellipsoid-shaped specimens, conducted in accordance with ASTM D575,[71] ASTM E111,[72] and ASTM D732,[73] the compressive strength and modulus were found to be 25.7 ± 0.7 MPa and 3.9–5.8 MPa, respectively, whereas the shear strength and modulus were found to be 8.0 ± 0.6 MPa and 1.3–2.0 MPa, respectively.[70]

From the plot of axial compressive stress-versus-number of cycles to failure (N_f) results obtained from fatigue tests on ellipsoid-shaped specimens, conducted in accordance with ASTM E466,[74] ASTM E467,[75] and ASTM E468,[76] the axial compressive strength (defined as stress at $N_f = 10$ million cycles) was estimated to be 2.94 MPa.[70]

Wear performance was obtained by subjecting ellipsoid-shaped specimens to the motion/loading protocol described above, with some key results being (1) minimal wear line scratches, minor pitting, and some deposition of wear particles were observed, especially along the most anterior and posterior edges; (2) the mean wear rate (WR) was computed to be 0.29 mg per million loading cycles; (3) at a given N_l, the wear particles were approximately spherical, spheroidal, or agglomerated globular (1–19 μm in diameter); and (4) with increase in N_l, the relative amount of globular and flake particles significantly decreased, the relative amount of spherical or spheroid particles increased, and the particles became rounder and smoother.[70]

IDN. The hydrogel used to fabricate the device comprises a silk-elastic copolymer that is produced using a DNA bacterial synthesis fermentation process. It is implanted in the disc while liquid and cures exothermically.

When the device was implanted in cadaveric "human spinal motion segments," the maximum strain of the construct, under an axial load of 1.96 N (200 g), was greater than for the native segments, although not significantly so.[77]

NeuDisc. The device is implanted in the disc space in a dehydrated state but absorbs up 90% of its weight in water and expands preferentially in the axial direction to conform to the configuration of the disc space.

In terms of flexibility, 25-mm-diameter cylindrical specimens showed virtually no radial deformability when subjected to compressive loads ranging from 25 to 370 N.[65]

When the aforementioned specimens were subjected to quasi-static loading, it was found that (1) the "apparent modulus," under confined compression, varied from 0.55 ± 0.09 MPa under a force of 100 N to 4.28 ± 0.15 MPa under 1500 N and (2) the mean value of the force supported by the specimen at a given hydration level (sometimes called the lifting force) dropped from 1400 N to zero when hydration dropped from 42 to 90%, respectively.[65]In fatigue tests, the aforementioned specimens with hydration level of 81.03% ± 0.82% were immersed in Hank's balanced salt solution with 0.1% sodium azide, at 37°C, and held between concave platens.[65] The specimens suffered no physical damage through 10 million cycles (fixed displacement between 6.8–8.0 mm and 6.4–8.0 mm; 3 Hz).[65]

When the device was implanted in cadaveric L2-L5 segments, (1) the force to failure of the construct, in compression, was 3.58 ± 1.56 kN, with endplate fracture occurring in 10 of the 12 specimens tested and device extrusion in one specimen; (2) the moment to failure under lateral bending was 25.6 ± 11.1 Nm, with failure modes seen being annulus rupture, ligament failure, device extrusion (seen in one of the 12 specimens tested), lateral facet fracture, and

endplate fracture; and (3) the moment to failure in flexion was 52.2 ± 18.3 Nm, with ligament failure seen in five of the seven specimens that gave acceptable results and no device extrusion.[65]

Newcleus. The device is delivered to the disc space with a aid of an insertion gun, after which it rolls into a spiral shape around the AF, filling the disc space.

Using the same bank of tests conducted on Aquarelle constructs, it was found that Newcleus is biocompatible with an "animal model."[49]

NuBac. Two features of the device facilitate it proper fitting in the disc space. First, each of the two pieces of the device has an oval outer surface with a large contact area. Second, the inner articulation of the device comprises a ball-and-socket design.

Results of cytotoxicity tests, chemical analysis, and histopathological evaluations, after implantation of specimens of PEEK (the material used for fabricating NuBac) in "an animal model" for 12 months, showed that the polymer is biocompatible.[78]

Results of tests conducted on PEEK specimens in environments such as 200 Mrad of gamma irradiation followed by accelerated aging (40 days in oxygen at 5 bar and 70°C) or after storage of the device in physiological saline solution, at 90°C, for 3 months showed both the material and the device to be biodurable.[78]

Under quasi-static loading, the mean axial static compressive load at failure of the device was 10.427 kN, with the failure mode being excessive plastic deformation of the top shell.[66]

When a device was subjected to axial dynamic compression loads, at 10 Hz, until failure, run-out (taken to be no fatigue failure after 10 million loading cycles) was achieved at both 80 and 90% of the mean yield load (10.427 kN).[66]

When a construct (device implanted in cadaveric L2-L3 and L4-L5 segments) was subjected to a series of loadings [flexion–extension (±7.5 Nm) combined with a 500 N compressive load; lateral bending (±7.5 Nm) combined with a 500 N compressive load; and axial rotation (±7.5 Nm) combined with a 500 N compressive load], the construct ROM, in each case, was not significantly different from that obtained in the intact disc case.[66] The same trend was found for the change in disc height at the index level.[66]

There was no expulsion of the device when the aforementioned constructs were subjected to left bending opposite to the annular window (2.5–7.5 Nm; 2 Hz; 100,000 cycles).[66] Furthermore, there was no expulsion when constructs (device implanted in cadaveric L2-L3 and L3-L4 segments) were subjected to hand bending with an annular window of 8–10 mm followed by removal of the AF and subjecting the remaining construct to lateral bending (5° bending angle; minimum of 8000 cycles).[66]

In wear tests, a device, while immersed in newborn calf serum, at 37°C ± 3°C, was subjected to a protocol comprising three phases. In the first, the device was subjected to ~0.5 million of a loading cycle that comprised a dynamic load, P (225–1024 N; Hz) coupled with a flexion–extension (rotation angle, $\theta = 15^\circ$ total; 2 Hz) such that P_{max} coincided with θ_{max}.[66] In the second phase, the test was stopped, the specimen was cleaned and weighed, the test solution was stored at −20°C for particle analysis (via laser diffraction analysis), and then changed after the analysis. The first and second phases were repeated until 10 million cycles were achieved, at the end of which the WR and the mean volumetric diameter of the wear particles (D_m) were 0.28 ± 0.07 mg per million loading cycles and 17.5 μm, respectively.[66] In the third phase, the device was rotated 90° after which the test was restarted using the same loading as in the first phase and then allowed to run for additional 10 million cycles. WR and D_m were 0.27 ± 0.09 mg per million loading cycles and 37.3 μm, respectively.[66]

In another series of wear tests, a device was subjected to the coupled-motion wear testing protocol ISO 18192,[79] which is specified for evaluating a total disc replacement device, except that, in the present tests, the device was subjected to a dynamic compressive load (224–1024 N).[66] The mean WR was 0.50 mg per million loading cycles.[66]

NuCore. During implantation, the polymer is mixed with a crosslinking agent and then injected, while in the liquid state, into the disc space. Curing occurs in a few minutes.

The same bank of tests conducted on Aquarelle constructs was used in the case of NuCore implanted in various animal models, such as guinea, rabbit, mouse, and rat. There was no evidence of cytotoxicity, irritation, and neurotoxicity.[80]

Clinical performance of commercial devices

At the moment, no definitive statements can be made on this issue because of the very limited database, which is characterized by, among other things, a dearth of information about various aspects of the few studies reported (Table II). It is to be noted that very few of these devices are currently in the latter phases of evaluation for clinical approval (such as Investigational Device Exemption trials in the US). In addition, none of these devices have been approved by the US Food and Drug Administration and only a few have received regulatory approval outside the US; for example, HydraFlex in Europe. This situation may be the consequence of the generally weak clinical record posted by these devices that, in turn, may be attributed to three key factors. The first is that, although a few clinicians have compiled sets of patient inclusion criteria (e.g., early-stage DDD and patient age between 18 and 65 years) and exclusion criteria (e.g., Schmorl's nodes on radiographs at the level(s) to be treated and body mass index > 30),[31,68] these criteria have not been validated in clinical studies. Second, several issues, such as mechanical/physical incompatibility of a NPR device with the weakened (or, even, extensively damaged) AF, depreciation of the mechanical properties of and nutrient supply to the AF following insertion of an NPR, and subsidence of the device into adjacent endplates, may exert significant adverse influence on outcomes. Third, effectiveness

TABLE II. Summary of Some Features of Clinical Studies

Device	Surgical Approach	Patient Demographics	Number of Devices Implanted	Follow-Up (months)	Level of Evidence[a]	Results	References
DASCOR	Retroperitoneal or mini-ALIF or ALPA	83 patients		24.0	4	Significant decreases in ODI and VAS scores	28
Newcleus	Microdiscectomy	5 patients (3 men; 2 women); age: 24–52 years		6.0–64.0	4	No complications; significant decreases in ODI and VAS scores	31,32
NuBac	Anterolateral, lateral, and posterior		~100	1.5–24.0	2	No complications; significant decreases in ODI and VAS scores	66
NuBac	Posterior; extreme lateral, by a RP transpsoas via anterior column	39 patients (15 men; 24 women); age: 32–45 years (mean: 38.5 years)		1.5–24.0	4	No complications; significant decreases in ODI and VAS scores	67
NuCore	Posterior	14 patients (8 men; 6 women); age: 25–52 years (mean: 37 years)	14	24.0	4	No complications; decrease in average of leg pain VAS, back-pain VAS, ODI, and SF-36 combined scores; central and posterior disc heights each ~93% of preoperative value	68
PDN[b]	Posterior		423		4	10% explanted; complications: endplate failure with extrusion and subsidence; marked decreases in ODI and Prolo scores	29,48
PDN	ALPA	8 patients			4	Complications: transient psoas neuropraxia and device migration; significant decreases in ODI and Prolo scores	56,60

ALIF, anterior lumbar interbody fusion; ALPA, anterolateral transpsoas; ODI, Oswestry Disability Index; VAS, Visual Analog Scale; SF-36, SF-36® Health Survey Patient Questionnaire.

[a] Except for the report by Bao et al.,[66] the level of evidence shown for a given study was designated by the present worker based on criteria detailed in the document, "Oxford Center for Evidence-Based Medicine 2011 Levels of Evidence" (Oxford Center for Evidence-Based Medicine, Oxford, UK; www.cebm.net/index.aspx?o=5653), and the information given in the report.

[b] A precursor of the HydraFlex device (Raymedica, LLC).

has not been demonstrated in randomized, controlled clinical trials.

Biomechanics studies involving notional devices

Two types of these studies have been published; namely, experimental and finite element analyses (FEA).

Experimental studies. In the case of sheep discs implanted with notional NPR devices [fabricated of a polymer with modulus of elasticity (E_i) of 0.2 MPa (NPR1), 8.0 MPa (NPR2), and 40.0 MPa (NPR3)], the construct was subjected to compression at 0.2 mm s^{-1} to a maximum compression of 1 mm. Four study cases were used, namely, intact (no NPR), NPR1, NPR2, and NPR3. For each case, the outer section of the AF bulged significantly outward, and, in the NPR3 case, there was significant outward bulging of the inner anterior section of the AF.[81]

A parametric study was conducted that involved implantation of a notional NPR (specimens fabricated from a blend of PVA and PVP) in cadaveric lumbar spine discs (height, H, $= 7.65–12.50$ mm).[82] The construct was axially compressed to 15% strain and then subjected to a loading with a triangular waveform, at a rate of 15% strain s^{-1}, for five cycles. It was found that E_i (50 kPa $\leq E_i \leq$ 1500 kPa) exerted less of an influence on the compressive stiffness of the construct than did either the height of the specimen (cases: disc height + 1 mm; disc height; and disc height − 1 mm) or its diameter (D) (cases: 15, 16, and 17 mm). Specifically, (1) at a given combination of strain level, H, and D, compressive stiffness increased with increase in E_i; (2) at a given combination of strain level and E_i, compressive stiffness increased significantly with increase in H; and (3) at a given combination of strain level and E_i, compressive stiffness increased significantly with increase in D.[82]

After implantation of a "prosthetic NPR model" ("model" was taken herein to mean "device") in cadaveric lumbar spines (L4-L5) and subjecting the construct to vertical compression (50 N s^{-1} to a maximum of 1.3 kN), the stress in the central zone of the endplate was noticeably (~15%) higher compared with the value when the intact motion segment was used. This points to the possibility of the creation of a stress riser following the implantation.[83]

Finite element analysis studies. An axisymmetric FE model of a human lumbar disc (height of disc = 12 mm, radius of disc = 23 mm, and radius of NP = 12 mm) was constructed.[81,84] The NP was modeled as a fluid (bulk modulus = 1.7 GPa) and the AF was modeled as a homogeneous, isotropic, elastic solid whose modulus of elasticity was determined (through matching the predictions of the model to experimentally obtained results) to be 5 MPa. The NPR material was modeled as an elastic solid, with Poisson's ratio (ν) of 0.49. The model was subjected to a compressive load of 1.5 kN uniformly distributed on its top and constrained in such a way that ensured that the conditions for axisymmetry were maintained. In a parametric study, the modulus of elasticity of the NPR (E_n) was varied from 0.5 to 100 MPa. By comparing the stress distribution in the AF in the intact case (no NPR) to that when there was a NPR, it was suggested that the appropriate value of E_n is 3 MPa.[81,84] Furthermore, with a given E_n, the AF bulged outward, as happened in the intact case.[81,84] These studies[81,84] suffered from three major limitations, namely (1) the model was of a disc in isolation, that is, supporting tissues were not included; (2) the geometry was not anatomical; and (3) the material models used for AF and NP were simplistic.

In another study involving an axisymmetric FE model of a human lumbar disc with a notional NPR device in it, the AF was modeled as an isotropic, hyperelastic material using a second-order polynomial strain energy function.[85] The NP was modeled as a homogeneous, isotropic, elastic material, with $E = 1$ MPa and $\nu = 0.4999$. The notional NPR device was considered fabricated from a PVA/PVP hydrogel that was modeled with a first-order Mooney–Rivlin strain energy function. Four key findings are highlighted.[85] First, the modulus of elasticity of the hydrogel (E_h) (10 kPa $\leq E_h \leq$ 100 MPa) exerted less of an influence on the compressive load-displacement behavior of the construct than did either the height of the device or its diameter. Second, the von Mises stress distribution in the AF in the model that contained the implanted NPR device was comparable to that in the intact model (no implanted NPR device), but, for the NP, this distribution was more uniform and of larger magnitude compared with the intact case. Third, in the load-displacement behavior, for a given load, displacement increased with increase in E_h. Fourth, at $E_h = 150$ kPa, in terms of influence of ν ($0.10 \leq \nu \leq 0.4999$), the load-displacement behavior was markedly affected by ν in the range of 0.45–0.4999, but very marginally so at $\nu < 0.45$. The main shortcoming of the study[85] is that both the cancellous and cortical bones are modeled as linear elastic (fully isotropic) solids, when, in fact, it is known that, at the simplest, they display transversely isotropic material properties.[86,87]

Characterization studies of emergent materials

A copolymer of 95 mol % *N*-vinyl-2-pyrrolidinone (NVP) and 5 mol % 2-(4-iodobenzoyl)-oxo-ethyl methacrylate (4IEMA) was found to be hydrophilic (equilibrium water content reached in about 12 h), noncytotoxic, and biocompatible; possess a swelling ratio of 4.5; have a radiopacity that would be acceptable for imaging inside the spinal column; have a compressive modulus of 1 MPa; have a complex shear modulus that is, essentially, independent of frequency, indicating that the material is in the rubbery plateau; have a phase angle (on average, between 8° and 16°) that is marginally dependent on frequency, indicating high elastic behavior; and display little hysteresis (small dissipated energy) under cyclic compression.[88]

A PVA/PVP hydrogel displayed the following properties: a tangent modulus, under unconfined compression, that varied from 0.23 MPa at 15% strain to 0.37 MPa at 25% strain; an effect of fatigue loading on compressive modulus that depended on the test strain, such that at 15%, there was a small reduction in the modulus after 10 million cycles, whereas there was no significant change up to 10 million cycles when tested at 25% strain; and a mean compressive modulus of 0.19 MPa (when specimen was confined using a silicone rubber ring) and of 12.70 MPa (when confined using a high-density polyethylene (PE) ring), a trend that is consistent with PE being stiffer.[89]

A branched copolymer comprising poly(*N*-isopropylacrylamide) (PNIPAAm) and a high content of poly(ethylene glycol) (PEG) has a molecular weight of 4600 g mol^{-1} and water content of 52% ± 2%; showed a significant increase in equilibrium water content compared with the PNIPAAm homopolymer; has a compressive modulus, at 15% strain (90-day immersion in PBS; unconfined axial compression test) of 90 ± 9 kPa; and has a relaxation time constant, over 30-day immersion in PBS, of 75 s, with the specimens recovering between 85 and 98% of their original height within 55 min after unloading.[90]

A hydrogel system obtained via photopolymerization of glycidyl methacrylate-modified PVA was characterized under dynamic torsion (applied shear strain amplitude = 0.05 rad over the range 0.1 Hz $\leq$ frequency $\leq$ 10 Hz).[91] For a given hydrogel, its complex shear modulus increased in direct proportion to the molecular weight of the modified PVA (M_w) as well as with increase in the polymer concentration before photopolymerization (%*P*) at a given M_w, but its phase shift angle was independent of both M_w and %*P*. The water content of these hydrogels was in the range of 79–95%; hydrogels obtained with PVA of M_w = 85–124 kg mol^{-1} or M_w = 124–186 kg mol^{-1} and 25% initial polymer concentration and PVA of M_w = 50–85 kg mol^{-1} at 35% initial polymer concentration gave complex shear moduli comparable to that of sheep lumbar spine NP (7–11 kPa). The difference in phase angle was significant (hydrogels: 5°–11°; natural tissue: 18°–26°).[91]

A hydrogel formulation obtained by blending 0.9 mL of 1% hyaluronic acid (HA) solution with a 0.9 mL of 7% polyethylene glycol-*g*-chitosan solution followed by quick gelation was subjected to stress relaxation (unconfined compression and 5% strain increments followed by 5-min relaxation periods to a total of 25% strain).[92] The toe modulus, linear modulus, and Poisson's ratio were each not significantly different from the corresponding value for cadaveric lumbar spine NP, but percent relaxation was (hydrogel: 20% ± 6%; cadaveric: 65% ± 11%).[92]

A crosslinked terpolymer composed of 92.1 mol % NVP, 5.9 mol % 4IEMA, and 2.0 mol % allyl methacrylate (a crosslinking agent) possessed an equilibrium water content of 55.0 ± 0.1 wt % and a diffusion coefficient (in PBS, at 37°C) of 3.2×10^{-5} cm^2 min^{-1}; displayed no cytotoxicity; found to have a modulus (under compression at a strain rate of 3×10^{-3} s^{-1}) of 1.52 ± 0.04 MPa; found to have a complex modulus (E^*) and phase angle of 2.01 ± 0.18 MPa and 6.31° ± 0.46°, respectively; complex shear modulus (G^*) and phase angle of 48.8 ± 8.1 kPa and 7.7° ± 1.3° (all obtained at 1 Hz); showed almost no creep and no permanent deformation (specimens compressed to 0.1 MPa for 3 h and followed by a force of 0 N for 1 h); and suffered a height loss of 4.7% after fatigue test (specimens subjected to 10^6 cycles of compression from 0.1 MPa to 15% strain; 5 Hz).[93]

An amidic derivative of alginate was developed to obtain a polysaccharide that possesses the requisite combination of viscosity and rigidity and then the polysaccharide was crosslinked using 1,3 diaminopropane, resulting in a hydrogel.[94] The hydrogel swelled up to 250% in volume, a value that is similar to that for a normal human lumbar NP.[95] The hydrogel displayed thixotropic behavior, with the gel–sol transition occurring at 1270 Pa (before this point, storage shear modulus (G') > viscous shear modulus (G'') and, beyond this point, $G'' > G'$). Furthermore, (1) dynamic frequency sweep test results showed that both G' and G'' increased with increasing frequency, with the former always being greater than the latter, indicating that the hydrogel is a predominantly elastic material; (2) the mean dynamic shear modulus increased with increase in frequency (ω), the same trend seen for normal lumbar NP; (3) the influence of ω on phase shift angle (δ) was not significant, whereas, for normal human lumbar NP, it became marginally more dissipative with increase in ω (i.e., δ increased noticeably with increase in ω); and (4) with δ being < 45°, this showed that the hydrogel behaved more like a solid than a fluid. In a ramp stress relaxation test with imposed shear strain, the behavior of the hydrogel was very similar to that of normal human lumbar NP. The ability of the hydrogel to maintain its consistency was demonstrated by the similarity of G' and G'' values (obtained at 37°C and ω = 10 Hz) before and after application of various dynamic stresses corresponding to different activities of daily living. Further characterization involved determination of the effect of the hydrogel on normal human chondrocyte cell viability (chondrocytes proliferated on the hydrogel) and on the production of extracellular matrix factors (the hydrogel ameliorated the synthetic activity of these factors, particularly in terms of cathepsin B, aggrecan, and type II collagen values).[94]

A three-component injectable hydrogel was formed by blending branched copolymers of PNIPAAm and PEG with poly(ethylene imine) (PEI).[96] At 37°C, this system forms a precipitated gel because of the phase transition of PNIPAAm. When used as a NPR system, aqueous glutaraldehyde would be injected into the gel core, which will crosslink PEI to itself and continue to diffuse through the gel and to crosslink it to the AF.[96] With injection of glutaraldehyde (5 or 10 or 20 wt %), each of two measures of the strength of the bond between the gel and fresh porcine skin that were determined (maximum detachment force and work of adhesion) was significantly higher than for the case when no glutaraldehyde was injected.[96]

Neat hydrogels, based on Tween® 20 trimethacrylates (T3), were synthesized from poly(oxyethylene 20 sorbitan) monolaurate as the crosslinking agent, NVP, water, and an initiator.[97] Composite hydrogels of the same composition reinforced by nanofibrillated cellulose (NFC) (fiber diameter: 2–100 nm and fiber length: >1 μm) were synthesized with different T3 concentrations. During time-sweep measurements (at 15% strain and 10 Hz), it was found that, for neat hydrogels with T3 concentrations of 4.5–15.0%, during initial stages of curing, each of the parameters monitored (storage modulus, loss modulus, and viscosity) increased rapidly with increase in time (which indicates growth of the chain size and network formation) up to about 600 s, after which steady-state values are obtained. The same trends were seen for results obtained using a T3-8 hydrogel reinforced by either 0.8 or 4.5 wt % NFC, indicating that the fibers did not interfere with the curing mechanism(s) involved. For each of the aforementioned three material properties, the value for a composite hydrogel was significantly higher than that of the neat hydrogel.[97]

Summary

Four aspects of the reviewed studies on replacement technologies are highlighted. First, in spite of the large number of commercial devices that have been reported on over the years, the literature on biomechanical studies of them is very limited. Thus, (1) the largest volume of work is on three devices (DASCOR, NeuDisc, and NuBac) but, even for these devices, only a maximum of four properties were determined in each case; (2) results show that (i) although the flexibility of DASCOR constructs was excellent, BUCK constructs displayed a marked drop in AD when a construct was subjected to cyclical loading and (ii) compared with the intact case (native NP only), implantation of Buck, DASCOR, or NuBac in cadaveric spine segments caused no significant change in ROM and there is no accompanying expulsion of the device, when the construct was subjected to dynamic loading; and (3) there is a paucity of data on wear performance, which is one of the most desirable requirements in an NPR device. Second, in the case of commercial devices, lack of data with the requisite quality precludes any firm conclusions to be reached on their clinical performance. Third, in the case of a construct comprising a notional replacement

device implanted in a cadaveric lumbar disc and subjected to dynamic compression load, the construct stiffness increases significantly with increase in either the height or the diameter of the device and the von Mises stress distribution in the AF is approximately the same as in the intact disc case. Fourth, there are many vibrant research programs on synthesis and characterization of new polymeric materials (mostly, hydrogels) that may be suitable for fabricating replacement devices.

REGENERATION/REPAIR TECHNOLOGIES: TISSUE ENGINEERING

Salient features of different approaches

Available TE approaches for regeneration/repair of degenerated discs may be grouped into three categories.

The first approach is direct (intradiscal) injection of a low-molecular-weight protein, such as OP1, transforming growth factor-β (TGF-β), bone morphogenetic protein-2 (BMP-2), growth and differentiation factor-5 (GDF-5), insulin-like growth factor-1, a cytokine, or an anabolic enzyme, into the degenerated disc.[98]

The main attraction of this method is that is straightforward. The method does, however, have a number of disadvantages. First, it may be limited to mildly degenerated discs because, in order for the method to work, there should be sufficient numbers of cells that are still healthy and are able to respond to a stimulus. Second, the beneficial effects, such as restoration of disc height[99] and decrease in ODI and VAS scores,[100] may be limited to the period over which the injected protein is still available in the disc. (For practical purposes, this is the time up to when the protein is lost by, e.g., diffusion into the disc cells.)

The method has been used in small-animal models (e.g., OP-1 in rabbits[99] and OP-1 in rats[101]), with excellent results. For example, injection of $\geq$10 ng mL^{-1} of GDF-5 into lumbar discs of balb/c mice led to significant increases in DNA content and glycosaminoglycan (GAG) accumulation in NP cells.[102] The method has also been used in a pilot clinical study, in which a "cocktail solution" comprising a mixture of agents that are known to induce the synthesis of proteoglycan (PG) (specifically, glucosamine hydrochloride, chondroitin sulfate, hypertonic dextrose, and dimethylsulfoxide) was injected into the lumbar discs of 30 patients with chronic low-back pain.[100]

The second approach is direct gene therapy, which involves using a carrier/vector to genetically modify resident disc cells *in vivo* to achieve sustained expression of beneficial genes, examples being TGF-β1, SRY (sex-determining region Y)-box 9 (Sox9), and tissue inhibitors of matrix metalloproteinases-1.[103-105] Two types of vectors have been used. The first is a nonviral vector, such as molecular complexes of the DNA[106] and a ligand and a liposome containing DNA.[107] This type of vector has several disadvantages, such as poor expression of the transferred DNA and high likelihood that the DNA introduced will be lost from the cell, although there is an emerging generation of these vectors that may not have these limitations.[108] The second is a viral vector, with an example of its use being the transformation of isolated cells from bovine or rat discs by a retroviral construct containing the gene for the human interleukin-1 receptor antagonist.[109,110] Viral vectors are efficient transporters of genetic material mainly because of the ease with which they enter the cells, taking over DNA replication and the protein expression process.[111] Their principal drawback is that they can elicit a cellular immune response, although, with some new ones, such as an adeno-associated viral vector, this response is minimal.[112]

The third approach is cell therapy, which involves three steps. First, harvest of NP cells from the patient (autologous cells). Second, cultivation/expansion of these cells in monolayer culture(s). Two variants that have been used are genetic modification of the cells (indirect therapy) and seeding of the cells in a supporting three-dimensional structure (scaffold). An alternative to the second step is direct cultivation of the extracted disc cells in a scaffold before their implantation (an example of this approach involved AF cells in a rabbit model.[113-115]) Third, implantation of the cells or the cell-seeded scaffold (as the case may be) in the degenerated disc.

There is a very large body of literature on the materials and fabrication methods for scaffolds for use in NP TE approaches. Among the biomaterials that have been used are alginate,[116] types I and II atelocollagen,[117] hyaluronan,[118] chitosan,[119] highly dense type I collagen,[120] type I collagen/GAG composite,[121] type I collagen/hyaluronan composite,[122] gelatin/chondroitin-6-sulfate/hyaluronan composite,[123] enzymatically crosslinked atelocollagen type II/aggregan/hyaluronan composite,[124] extracellular matrix-based scaffolds derived from the decellularization of porcine NP,[125] a chitosan/glycerophosphate hydrogel,[126,127] a hydrogel comprising oxidized HA and adipic acid dihydrazide,[128] and a type II collagen hydrogel stabilized with poly(ethylene glycol) ether tetrasuccinimidyl glutarate and enriched with HA.[129]

Proof-of-concept studies of cell therapy, involving small-animal models (e.g., the Sand rat[130]) and clinical trials,[131] have been reported. There are three principal challenges with cell therapy. The first is the technical difficulty of harvesting sufficient number of disc cells without causing serious damage to the already degenerated disc or accelerating its degeneration. Second, it is limited to cases where herniation has not occurred. Third, there is a possibility of compromise of the quality of the nutrient supply to the implanted cells and, hence, their survivability.

Cell therapy with mesenchymal stem cells

An alternative to using autologous cells in cell therapy that is gaining a lot of research attention is the use of either autologous or allogenic human mesenchymal stem cells (MSCs).[132-138] With MSCs, two approaches may be taken. The first involves cultivation of the cells as progenitor cells followed by their direct injection into the degenerated disc. The second involves differentiation of the progenitor cells *in vitro* to a phenotype similar to cells found within the NP (e.g., chondrocytes or chondrocyte-like cells) followed by their injection into the degenerated disc.

The principal attraction of MSCs, arguably, is that they can be obtained from many tissues, such as bone marrow, muscle, articular cartilage, and adipose.[36,38,43,132-144] To date, in the majority of work, bone marrow-derived MSCs (bMSCs) have been used.[132-138] There are three principal challenges in using these stem cells. First, the exact phenotype of NP cells and their origin are still not definitively known; in other words, it is unknown if the phenotype of the differentiated stem cells is identical or merely similar to that of the native disc cells. Second, there is some question about the stability of the newly adopted phenotype in the degenerated disc over time. Third, the ability of the disc implanted with these stem cells to carry out and sustain the mechanical loads imposed on it during activities of daily living is unknown.

Recently, attention has started to be focused on using autologous human ASCs. These cells have many advantages compared with bMSCs, notably (1) higher yields and less invasive harvesting method; (2) high plasticity and, as such, high possibility of differentiation along a multitude of lineages, such as chondrocyte, myocyte, and adipocyte lineages; and (3) ease of manipulation.[36,38,43,139-145] There are, however, many open questions about ASCs, among which are the most appropriate isolation technique, methods to avoid immunoresponse, *in vivo* functionality, optimized protocol for cell differentiation, and removal or inactivation of degeneration byproducts.[43,145]

Summary

From the review of the published work on TE approaches, two salient points are made. First, three different approaches are taken or proposed, namely, direct injection of an "active" substance, such as OP-1; direct gene therapy using, for example, an adeno-associated viral vector; and cell therapy using, for example, scaffolds seeded with either bMSCs or ASCs. Second, there are many challenges involved in the use of each of these approaches. For example, (1) the beneficial effect of a directly injected "active" substance may be limited to the time before the substance diffuses into the degenerated disc; (2) when a nonviral vector is used in direct gene therapy, there may be poor expression of the transferred DNA; and (3) the difficulty of extracting cells from a degenerated disc for use in cell therapy.

FUTURE RESEARCH AREAS

There are several such areas, with some designed to fill gaps in the current knowledge base and the purpose of others being to provide more information on matters that have received limited attention. Expositions on 14 of these areas are now given.

From a materials perspective, the preponderance of replacement devices has been fabricated (or proposed to be fabricated) using "traditional/conventional" hydrogels, herein defined as those synthesized using established methods of synthesis, such as crosslinking copolymerization and crosslinking of reactive polymer precursors. These hydrogels are beset with a number of drawbacks, such as limited control of three-dimensional structure, low toughness, and slow response to external stimuli.[146] In response to this situation, there has been a plethora of developments in the field of hydrogel engineering, among which are controlled radical polymerization (e.g., nitroxide-mediated polymerization[147]), introduction of the concept of chain crosslinking-sliding crosslinking agents,[148] double network (DN) gels,[149] nanocomposite (clay-filled) hydrogels,[150] so-called stimulus-responsive hydrogels,[151,152] hydrogels self-assembled from block and graft copolymers driven by hydrophobic interactions,[153] and hydrogels whose self-assembly is mediated by DNA recognition.[154] Thus, the first area of research should involve exploring the potential of this new generation of hydrogels for NPR devices (injectable and preformed types). In particular, attention should focus on DN gels. A DN gel comprises two mechanically weak hydrophilic networks, one being stiff and brittle [e.g., poly(2-acrylamide-2-methylpropane sulfonic acid)] and the other soft and ductile [e.g., poly(acrylamide)].[155-157] The interest in DN gels is because although they are soft and wet, they have desirable mechanical properties, notably high toughness. They possess these properties by virtue of the fact that they are biomimetic, that is, they emulate the interpenetrating three-dimensional network of passive mechanical tissues, such as bones, tendons, cartilage, and discs, whose roles are to support, transfer, and distribute body loads and to maintain functional shape. Work to be carried out in this area should include the full panoply of preclinical tests, such as characterization of a DN gel (in particular, fatigue, creep, stress relaxation, and wear properties obtained while the specimen is immersed in a biosimulating medium, such as phosphate buffered saline, at 37°C), biomechanical evaluation of the gel in cadaveric spine segments (in particular, determination of incidence of extrusion and subsidence), and evaluation of the gel in a disc in an animal spine model (including histopathology of local and remote tissues).

The focus of the second area of research should be development of protocols for evaluating the wear performance of a replacement device when implanted in a cadaveric lumbar disc. These protocols should include all the pertinent information, such as test medium, test medium temperature, type of dynamic loading, frequency of loading, method for determining WR, and minimum number of test samples. These protocols should be presented in the form of an international testing standard, such as an ASTM standard or an ISO standard. Two adjuncts to this work should be performed, namely, stipulation of the minimum acceptable WR and a rationale given for it and investigation of the influence of the wear particles produced on contiguous tissues.

For the third area of research, the role played by contact stress between a replacement device and the endplates of the vertebral bodies in a given motion segment in the eventual clinical performance of the device should be investigated. As a first step in this direction, contact stress determinations should be made in cadaveric spine segment studies, with the applied loading used in the tests being physiological (e.g., combined axial compression and flexion/extension cycle). This determination may be made using, for example, a stress transducer,[70] a tip-mounted pressure

transducer,[158] a pressure-sensitive film,[159] a resistive ink sensor,[159] or an ultrathin polymeric piezoelectric transducer.[159] The results obtained could be added to the armamentarium of those for parameters that are used to compare existing replacement devices as well as to inform future designs.

There is a large assortment of rapid prototyping/solid-free-forming/additive manufacturing methods in use for fabricating prototypes of products, examples being as stereolithography (STL), selective laser sintering, laminated object manufacturing, and 3D printing.[160,161] As the fourth area of research, one of these methods—most likely, STL—should be applied to a model of the intact spine (to obtain baseline values of the parameters to be determined) and then to one in which a replacement device is in place in a given disc.

Recently, open-source simulation platforms have been used to build models of sections of the human body. Examples are AnyBody Modelling System software (AnyBody Technology A/S, Aalborg, Denmark) used to build a rigid-body lumbar spine model[162]; Interactive Musculoskeletal Modelling used to build a musculoskeletal model comprising the elbow, the wrist, and 16 associated muscles[163]; OpenSim (https://www.simtk.org/home/lumbarspine) used to build a cervical spine model[164]; and OpenSim used to build a musculoskeletal model for the lumbar spine that comprised the pelvis, sacrum, five vertebrae, a rigid torso consisting of a lumped thoracic spine and ribcage, and the eight main muscle groups.[165] A fifth area of research should involve using such a platform, or another solid modeling tool, to build a model of the lumbar spine that includes the ligamentous architecture and other hard tissues (such as facet joints) and soft tissues (definitely, the NP) and then using this model to obtain various parameters, such as joint reactions and muscle forces for the intact case as well as for the case when implantation of a replacement device at a given disc is simulated. In this work, both principal and coupled motions should be included.

For the sixth area of research, stress analysis parameters (such as axial displacement, maximum principal strain, and von Mises stress) should be determined for all the tissues of the spine (such as NP, AF, endplate, cancellous bone, cortical bone, and facets) at all levels. This exercise should be carried out for the case of an intact spine as well as for one in which a replacement device is implanted in a disc. Given the scale of this work, it is well known that the FEA method, rather than an experimental method, is attractive. When FEA is used, it is suggested that, at the minimum, it has the following six features. One, models of both normal and degenerated discs are constructed. Two, a given model is (1) of the full spine section (say, L1-S1), rather than of spine segment(s); (2) three dimensional; and (3) anatomically correct, that is, the lordotic curvature is maintained and it comprises all the tissues, including the major muscles. (To date, muscles have been included in models in very few FEA studies, one such being that of the C1-T1 model.[166]) Three, the intact model is validated using an array of experimentally obtained displacement, stress, strain, and kinematics data. Four, in a given model, appropriate constitutive models are used for each of the tissues and synthetic material(s). For example, in the case of tissues and other relevant materials, samples of appropriate constitutive models and values of material properties commonly used in elastic and poroelastic FEA are presented in Tables III and IV, respectively. Five, in the case where replacement device implantation is simulated, all changes made in the geometries and properties of all the tissues in the model are the same as those that occur as a result of the degeneration in the disc as well as the surgical procedure used. Six, for a given model, the loadings are physiological (i.e., shear loading should be included in the suite of loadings and both quasi-static and cyclic loadings should be included).

The seventh area of future work should be the determination of the clinical effectiveness of replacement devices that are in current production or are fabricated using "emergent" material(s) (several features of some of these materials are described in a previous section in this review), once research and development efforts on these materials have reached maturity. This would involve the performance of properly designed (e.g., long-term, large number of subjects, many sites) randomized controlled clinical studies, such as a US Preventive Services Task Force Level I study or a UK National Health Service Level A study.

With human growth factors, there are concerns about the facts that they (1) have been implicated in undesired blood vessel ingrowth in discs[186] and (2) are very expensive, this being a consequence of the elaborate methods used to produce them, the very short half-life of the used protein (due to enzymatic degradation *in vivo*), and the laboriousness of the regulatory requirements for characterizing their safety and effectiveness for clinical use. Thus, in the eighth area of research, the focus should be on developing methods to identify small, long-lasting, and stable nonproteineous molecules that can stimulate BMP-2 expression and, consequently, promote anabolic metabolism of disc cells, culminating in regeneration of the matrix of the disc. These molecules should be evaluated with respect to a large collection of relevant parameters, such as rate of synthesis of PG, rate of decrease of breakdown of PG, accumulation of GAG in NP cells, increase of water content in NP, restoration of disc height, and decrease of ODI score in a clinical study.

Several drawbacks associated with the use of bMSCs and ASCs have been highlighted in a previous section of this review. In addition to these, there are other concerns, in particular the possibility of ASCs being involved in cancer metastasis and invasion.[145,187] Thus, the focus of the ninth area of research should be the determination of the viability of using stems obtained from alternative sources, such as umbilical cord blood, for disc regeneration/repair.

The finding that all the cells present in the NP of the mature *ShhCre* mouse are derived from the embryonic notochord cells (NCs)[18] hints at the possibility of using human NCs for regeneration/repair of degenerated discs. There are many technical hurdles to obtaining human NCs. Thus, in the 10th area of research, an alternative approach should be pursued that comprises identification of the proteins that

TABLE III. Commonly Used Material Models and Property Values for Lumbar Spine Tissues and Other Materials: Elastic FEA

Tissue/Material	Material Model	Values	References
Cortical bone	Transversely isotropic, linear elastic[a,b]	$E_{11} = 9600$ MPa; $E_{22} = 9600$ MPa $E_{33} = 17{,}800$ MPa; $G_{12} = 3097$ MPa $G_{13} = 3510$ MPa; $G_{23} = 3510$ MPa $\nu_{12} = 0.55$; $\nu_{13} = 0.30$; $\nu_{23} = 0.30$	86
Cancellous bone	Transversely isotropic, linear elastic[a,b]	$E_{11} = 144$ MPa; $E_{22} = 99$ MPa $E_{33} = 344$ MPa; $G_{12} = 53$ MPa $G_{13} = 45$ MPa; $G_{23} = 63$ MPa $\nu_{12} = 0.23$; $\nu_{13} = 0.17$; $\nu_{23} = 0.11$	167
Bony posterior elements	Isotropic, linear elastic[a]	$E = 3500$ MPa; $\nu = 0.25$	168
Annulus fibrosus—ground substance	Hyperelastic, Mooney–Rivlin[c]	$C_1 = 0.56$ MPa, $C_2 = 0.14$ MPa $d = 0.143$	169
Annulus fibrosus—collagen fibers	Isotropic, linear elastic[a]	$E = 450$ MPa; $\nu = 0.30$	170
Nucleus pulposus	Hyperelastic, Mooney–Rivlin[c]	$C_1 = 0.12$ MPa, $C_2 = 0.03$ MPa $d = 0.0667$	171
Bony endplate	Isotropic, linear elastic[a]	$E = 500$ MPa; $\nu = 0.40$	172
Cartilage endplate	Isotropic, linear elastic[a]	$E = 24$ MPa; $\nu = 0.40$	171
Facet joints	Isotropic, linear elastic[a]	$E = 5$ MPa; $\nu = 0.45$	173
Facet cartilage	Hypoelastic (under compression)[a]	$E = 11.0$ MPa (at 0% strain) to 3500 MPa (at 0.7% strain); $\nu = 0.20$ (at 0% strain) to 0.4 (at 0.7% strain)	171
Uncovertebral joints	Isotropic, linear elastic[a]	$E = 5$ MPa; $\nu = 0.45$	173
Osteophytes	Isotropic, linear elastic[a]	$E = 500$ MPa;$\nu = 0.20$	174
Structure between osteophytes	Hyperelastic, Mooney–Rivlin[c]	$C_1 = 0.19$ MPa, $C_2 = 0.045$ MPa	174
Facet synovial fluid	Fluid[d]	$K = 1667$ MPa	11
Facet synovial membrane	Isotropic, linear elastic[a]	$E = 12$ MPa; $\nu = 0.40$	11
Uncovertebral synovial fluid	Fluid[d]	$K = 1667$ MPa	11
Uncovertebral synovial membrane	Isotropic, linear elastic[a]	$E = 12$ MPa; $\nu = 0.40$	11
Ligaments[e]			
ALL	Nonlinear force deflection	$E = 7.8$ MPa (strain, $\varepsilon < 12\%$)	175
PLL	Nonlinear force deflection	$E = 10.0$ MPa ($\varepsilon < 11\%$)	175
TL	Nonlinear force deflection	$E = 10.0$ MPa ($\varepsilon < 18\%$)	175
LF	Nonlinear force deflection	$E = 15.0$ MPa ($\varepsilon < 6.2\%$)	175
ISL	Nonlinear force deflection	$E = 10.0$ MPa ($\varepsilon < 14\%$)	175
SSL	Nonlinear force deflection	$E = 8.0$ MPa ($\varepsilon < 20\%$)	175
CL	Nonlinear force deflection	$E = 7.5$ MPa ($\varepsilon < 25\%$)	175
PVA/PVP hydrogel[f]	Nonlinear elastic	σ (MPa) $= 0.003 - 0.057\varepsilon + 0.624\varepsilon^2$ $\nu = 0.44$	89,176
NVP-based hydrogel[g]	Nonlinear elastic	σ (MPa) $= 0.023 + 0.608\varepsilon + 1.565\varepsilon^2$ $\nu = 0.49$	93,177
DASCOR device[h]	Isotropic, linear elastic[a]	$E = 4.9$ MPa; $\nu = 0.48$	70,178

[a] E, modulus of elasticity; G, shear modulus; ν, Poisson's ratio.

[b] 11, 22, and 33 refer to the radial, tangential, and longitudinal axes of the bone, respectively.

[c] Incompressible, hyperelastic, two-parameter Mooney–Rivlin formulation, with strain energy function (W) given by

$$W = C_1(I_1 - 3) + C_2(I_2 - 3) + (1/d)(J - 1)^2,$$

where C_1 and C_2 are material constants that characterize the strain energy deviatoric deformation of the material; I_1 and I_2 are the first and second invariants of the deviatoric strain tensors, respectively; d is the material incompressibility factor ($=2/K_o$, K_o being the initial bulk modulus of the material); and J is the local volume ratio.

[d] K, mean value of bulk modulus.

[e] Major ligaments in the lumbar spine. ALL, anterior longitudinal ligament; PLL, posterior longitudinal ligament; TL, transverse ligament; LF, ligamentum flavum; ISL, interspinous ligament; SSL, supraspinous ligament; CL, capsular ligament.

[f] PVA, poly(vinyl alcohol); PVP, poly(pyrrolidone). The values of the coefficients in the stress (σ)-versus-ε relationship for this material were obtained by the present worker by fitting a polynomial equation to the experimental σ–ε results given by Joshi et al.[89] for the hydrogel.

[g] Full composition of NVP-based hydrogel (specifically, N94_2) is 92.1 mol % *N*-vinyl-2-pyrrolidinone (NVP), 5.9 mol % 2-(4′-iodobenzoyl)-oxoethyl methacrylate, and 2.0 mol % allyl methacrylate (Ref. 93). The values of the coefficients in the σ-versus-ε relationship for this material were obtained by the present worker by fitting a polynomial equation to the experimental σ–ε results given by Boelen et al.[93] for the hydrogel.

[h] DASCOR® (Disc Dynamics, Eden Prairie, MN).

are produced by NCs that play a key role in the disc regeneration/repair process.[188]

For the 11th research area, three developments in the field of scaffolds should be investigated for their applicability for use in the case of NP TE work. The first is the use of heparin functionalization as a way to improve the performance of the scaffold. Such functionalization shields and, hence, protects growth factors that bind onto the

TABLE IV. Commonly Used Property Values for Some Lumbar Spine Tissues: Poroelastic FEA

Tissue	Drained Elastic Modulus (MPa)	Poisson's Ratio	Initial Void Ratio	Initial Permeability (10^{-15} m^4 N^{-1} s^{-1})	M[a]
Cortical bone	12,000[b]	0.3[b]	–	–	–
Cancellous bone	100[b]	0.2[c]	0.4[c]	100[c]	18[c]
Nucleus pulposus[d]	0.93[e]	0.1[c]	2.33[f]	2.03[e]	12[c]
Annulus fibrosus[d]					
Matrix	4.44[f]	0.1[c]	1.50[g]	0.18[g]	10[c]
Fibers	500[h]	0.3[h]	–	–	–
Endplate	20[b]	0.1[c]	4.0[c]	7.00[c]	10[c]

[a] For a tissue, void ratio, permeability, and M are related thus:

$$k = k_0 \left[\frac{e(1+e_0)}{e_0(1+e)}\right]^2 \exp\left[M\left(\frac{1+e}{1+e_0} - 1\right)\right],$$

where k is the permeability, k_o is the initial permeability, e is the void ratio, e_o is the initial void ratio, and M is a constant whose value is obtained through curve fitting to experimental results (e.g., see Ref. 179).

[b] Ref. 180.

[c] Ref. 179.

[d] Severely degenerated disc (corresponding to Thompson Grade IV).[181]

[e] Ref. 182.

[f] Ref. 183.

[g] Ref. 184.

[h] Ref. 185.

scaffold.[189,190] Such functionalization is commonly achieved by either (1) covalently linking the heparin to the scaffold[189] or (2) grafting heparin-carrying microcapsules onto the surface of the scaffold.[190] The second development is a proliferation of innovative methods of fabrication of scaffolds, examples being autocatalytic electroless coprecipitation,[191] an embossing ice template technique,[192] hydrospinning,[193] a template-casting technique,[194] indirect phase-changing 3D jet printing,[195] 3D plotting,[196] extrusion free-forming,[197] and cryogenic prototyping.[198] The third development is the design and fabrication of functionally graded scaffolds (FGSs). The rationale for this type of scaffold is the fact that functional gradients exist in the structure of natural tissues, which means that each layer of a tissue performs one or more specific functions and the tissue is composed of several layers.[199] Two types of FGSs are recognized, namely, continuous and discrete types.[199] Each type may be fabricated using either a conventional technique, such as sequential electrospinning,[200] centrifugation followed by fiber bonding,[201] and a twin-screw-extrusion/spiral winding process,[202] or an additive manufacturing technique, such as 3D printing[203] and 3D fiber deposition.[204]

Recently, an approach comprising a combination of topology optimization for the design and an additive method for the fabrication of a scaffold was demonstrated in the case of scaffolds for bone TE applications.[205-207] With this approach, the scaffold obtained has two principal advantages, namely, it does not have directions of low stiffness and its mass is distributed throughout its structure. As the 12th area of research, the feasibility of this combined approach should be explored for scaffolds for NP regeneration/repair.

To date, most TE studies have been on small-animal models of disc degeneration, notably rabbit, rat, and mouse.[98,104,134,138,208-213] These models have a number of

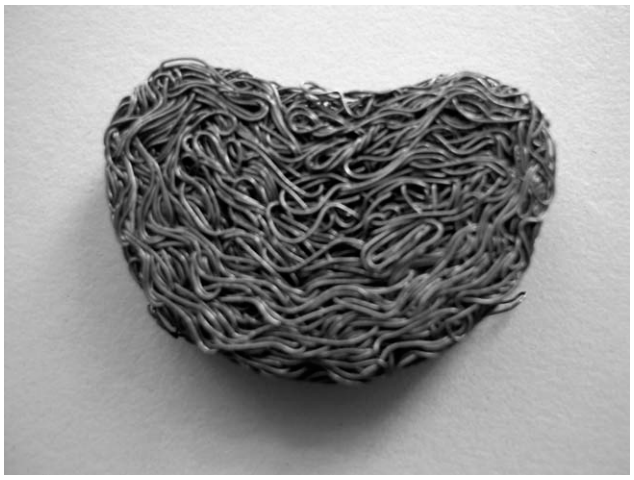

FIGURE 1. Photograph of the Buck nucleus pulposus replacement device (courtesy of Alfred Buck, Buck GmbH & Co KG, Bondorf, Germany).

FIGURE 2. Photograph of the NuBac® Disc Arthroplasty System device (courtesy of Pioneer Surgical Technology, Marquette, MI).

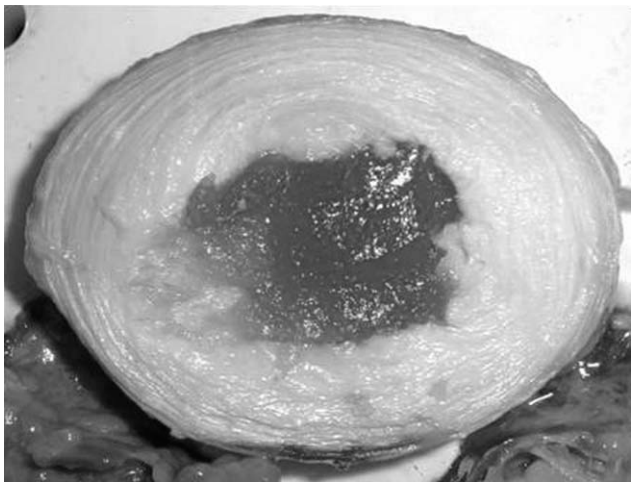

FIGURE 3. Photograph of the NuCore® Injectable Nucleus device in the intradiscal space of a cadaveric test specimen; device dyed black for easy visualization (courtesy of Spine Wave, Shelton, CT).

limitations vis-a-vis the human disc.[209,214] First, many small-animal models retain NC cells, resulting in an increase in disc cell metabolism[215] and, hence, ease of regeneration. In contrast, in human discs, NCs exist only during the development of the embryo and disappear shortly after birth.[42] Second, discs in small-animal models have shorter diffusion distances than in humans, a consequence of which is that there is improved transport of nutrients through and faster removal of waste from the model discs. Thus, there is scope for further work on appropriate large-animal models of disc degeneration, which could then be used to evaluate various aspects of TE methods for disc regeneration/repair, such as determination of optimal concentration of a growth factor that should be used, optimal time for the intervention of a given therapy along the disc degeneration continuum, and the elements to be included in an "effectiveness toolkit" of a given therapy. These issues should be the focus of work in the 13th area of research.

There is growing recognition of the role that oxidative stress [which is a cellular state in which there is an elevated level of reactive oxygen species, such as nitric oxide (NO)[216]] plays in disc degeneration, specifically, (1) herniated discs produce and release higher concentration of NO than healthy discs[217]; (2) increase of exogenous NO promotes cell apoptosis or suppresses synthesis of PG in the disc cell culture[218]; and (3) NO participates in the degeneration induced by mechanical stress or interleukin-1.[219] Thus, the focus of the 14th area of research should be on developing NP regeneration/repair strategies that make use of antioxidative agents or "radical sponges" delivered intradiscally. These agents should, among other things, be long lasting and easily penetrate the cell membrane.

SUMMARY

The following is a summary of the some of the main points made in this review:

- In the case of replacement technologies, (1) the number of available commercial devices is in a state of flux, a consequence of the companies at which these devices were designed going out of business or shifting their focus to other types of biomedical products; (2) limited data on the biomechanical comparison of intact discs (cadaveric lumbar or bovine) and constructs comprising a commercial device implanted in a cadaveric lumbar or bovine disc show that the devices do not, for example, significantly change the ROM or experience expulsion; and (3) there is an abundance of ongoing research on the synthesis and characterization of an assortment of new polymeric materials, especially hydrogels, that may be suitable for fabricating a new generation of commercial devices.
- From the review of the published work on use of TE for the regeneration/repair of degenerated discs, it may be concluded that (1) much is known about many aspects of the three approaches taken in TE (direct injection of an "active" substance, direct gene therapy, and cell therapy), including advantages and drawbacks, and (2) the challenges involved in the use of these approaches are well recognized.
- A large collection of areas for future research is presented, examples being (1) exploration of the feasibility of using the double-network type of hydrogel for fabricating replacement devices; (2) development of an international standard for evaluating the wear performance of a construct (a replacement device implanted in cadaveric disc); (3) performance of FEA of anatomically correct and nonlinear models of the full lumbar spine without and with a replacement device implanted in a disc; (4) determination of the clinical effectiveness of replacement devices through the performance of well-designed, randomized, controlled clinical trials; (5) extension of work on seeding scaffolds with human ASCs; (6) investigation of the feasibility of using a combination of topology optimization and an additive manufacturing method for, respectively, designing and fabricating scaffolds; and (7) expansion of work on identifying appropriate large-animal models for evaluating the performance of replacement devices as well as of TE approaches.

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