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PAIN ASPECTS OF ARTHRITIS (A MOBASHERI, SECTION EDITOR)

Stem Cell Regeneration of Degenerated Intervertebral Discs: Current Status (Update)

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Abstract Low back pain, strongly associated with intervertebral disc (IVD) degeneration, affects a large proportion of the population and has major social and economic costs. Current treatments remain inadequate, targeting the symptoms without addressing the underlying cause. As such, efforts are being directed towards development of therapies aimed at alleviating pain through the restoration of IVD function. The potential of cell-based therapies for the treatment of IVD degeneration are being actively explored, with an emphasis on cell/biomaterial tissue engineering. Adult mesenchymal stem cells, capable of differentiating down the discogenic lineage, have shown promise as a suitable cell source for IVD tissue engineering. However, a number of factors, (discussed in this review), remain to be addressed, including development of a differentiation protocol to produce the correct cell phenotype, identification of suitable biomaterials for cell delivery/implantation, and ensuring cell survival and correct function upon implantation into the degenerate IVD.

Keywords Stem cell regeneration · Degenerated intervertebral discs · Adult mesenchymal stem cells · Low back pain · Cell-based therapy

Introduction

Low back pain (LBP) is a major cause of disability in the developed world [1], with an estimated 84 % of people predicted to experience LBP during their lifetime [2] and a month and point prevalence of 23.2 % and 11.9 %, respectively [3]. As with most musculoskeletal disorders, the prevalence of LBP increases with age [4], suggesting incidences of LBP are likely to increase in the future due to a global aging population, changes in lifestyle and occupational stresses [5, 6]. In addition to the debilitating effect LBP has on individuals, there is also a large social and economic burden on society. It is estimated that LBP costs the United Kingdom economy, through direct healthcare costs, as well as indirect lost productivity and increased disability benefit costs, approximately £12 billion per annum [7].

Although the causes of LBP are multifactorial, increasing evidence implicates intervertebral disc (IVD) degeneration as a major contributor [8–10], with loss of IVD integrity leading to the destabilization of the spinal motion segment, resulting in pain and disability.

Biology of the IVD

The role of the IVD is mechanical, enabling movement of the vertebral bodies through all planes while maintaining the stability of the spine. The IVD is comprised of 3 main regions: the central gelatinous nucleus pulposus (NP), the peripheral collagenous annulus fibrosus (AF), and the superior and inferior cartilaginous end-plates (CEPs). Each region of the IVD has a distinct extracellular matrix (ECM), which is produced by phenotypically distinct cells. The adult NP is comprised of small rounded chondrocyte-like cells embedded within a proteoglycan-rich (PG) matrix, with aggrecan and type II collagen being the predominant ECM constituents [11]. The NP has a high osmotic pressure due to the attraction of cations by the negatively charged glycosaminoglycans (GAGs) attached to the PGs [12]. This ability to imbibe water enables the NP to generate high hydrostatic pressures, which

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are resisted by the surrounding type I collagen-rich lamellar AF, enabling the disc to withstand high axial loads. Unlike NP cells, AF cells are fibroblastic in morphology and orientated in the direction of the highly aligned type I collagen fibers, which alternate between adjacent lamellae. The AF is anchored to the CEPs, which are comprised of chondrocytes embedded in a hyaline cartilage-like matrix [11]. The CEPs connect the IVD to the adjacent vertebral bodies and enable the flow of nutrients and waste products between the vertebral body and IVD (which is normally avascular) and the systemic blood supply [13].

IVD Degeneration

The cause of IVD degeneration is still heavily debated, with many environmental (including mechanical over- and under-load and decreased nutrition) and genetic factors implicated in its pathogenesis [14, 15]. However, the biochemical changes associated with IVD degeneration have been extensively studied, with the hope that this will lead to a better understanding of the etiology of IVD degeneration [16].

With degeneration there is a shift in the homeostatic balance maintained by the resident cells, resulting in decreased tissue anabolism, and increased tissue catabolism [17]. The resultant breakdown of ECM begins in the NP, leading to dehydration of the disc tissue, fissure formation extending through to the AF, and ultimately loss of disc height [18]. There is also a change in cell number, beginning with an increase (possibly as an attempt to regenerate tissue) and then a decrease in cell number, during early and late stages of degeneration, respectively [11].

Although the initiating factors in degeneration are not fully understood, it is known to be a cell driven process. There is an increase in pro-inflammatory cytokines, including interleukin (IL) -1β, -6, tumor necrosis factor (TNF) – α and prostaglandin E2, produced by the IVD cells themselves [17, 19]. This increase in cytokines occurs independently of cytokine antagonists (eg, increased IL -1β expression but no change in IL -1 Receptor antagonist (IL1Ra) expression observed during IVD degeneration), resulting in a shift towards an increased inflammatory response and matrix catabolism [20]. This increase in cytokine profile stimulates the expression of matrix degrading enzymes, namely matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs). Increased expression of MMP -1, -3, -7, -9, -10 [21], -13, and ADAMTS -1, -4, -5, -9, and -15 have been described in degenerate IVDs, predominantly in the NP (see review by Le Maitre et al. [17]). Crucially, this increase in expression of degradative enzymes occurs without a concomitant increase in their endogenous inhibitors, tissue inhibitor of metalloproteinases (TIMPs), resulting in elevated levels of activated enzymes which degrade the ECM components [17].

Alongside the catabolic changes, the excess of cytokines within the disc, particularly IL-1, alters the expression of anabolic genes and matrix proteins [17]. This includes a decrease in aggrecan synthesis in the NP and a shift in collagen expression from type II to type I [17]. This change in matrix composition, in particular the loss of aggrecan, results in a reduction in osmolarity, leading to dehydration of the NP [12]. As the hydrostatic pressure within the NP is lost, abnormal load distribution occurs with greater loading of the AF [22]. This structural and mechanical compromise can potentially lead to micro-traumas and herniation of NP tissue through the damaged AF.

As degeneration progresses, there is also in-growth of blood vessels and nociceptive nerve fibers into the normally avascular disc through the AF and extending into the NP, enabling the infiltration of immune cells and resulting in increased pain, respectively [23, 24]. The precise mechanism which leads to this neovascularization and neoinnervation remains unknown; however, evidence suggests that a combination of the loss of aggrecan (which is known to have inhibitory effects on neurites [25, 26] and endothelial cells [27]) from the NP and decreased expression of semaphorin-3A (a nerve guidance molecule which inhibits neurite ingrowth [28]) in the AF, combined with increased production of growth factors known to promote neurite growth and neovascularization, including nerve growth factor (NGF) [29, 30], brain-derived neurotrophic factor (BDNF) [29, 30] and connective tissue growth factor (CTGF) [31] may be responsible.

Current Treatment Options for IVD Degeneration

Initially, treatment for LBP is often administered through the use of short-term pain relief, with nonsteroidal anti-inflammatory drugs (NSAIDs) shown to have positive results [32]. Other conservative treatments include physiotherapy and exercise therapies, which aim to improve movement of the spine and correct posture. However, when conservative treatments are found to be inadequate, more invasive therapies are considered including epidurals, or injection of corticosteroids and anesthetics directly into local painful regions of the spine, although, the success of these therapies remains questionable [33].

Surgical intervention is considered when less invasive approaches have failed to alleviate the pain, with spinal fusion being the most common procedure. The degenerated or herniated IVD is removed and the vertebral bodies are fused using a metal rod, cement, or through cell-based calcification (bone morphogenic proteins (BMPs) injected into the disc cavity in parallel with autologous iliac crest bone transplant, resulting in ossification and fusion of the vertebral bodies) [34–36]. Although immediate pain relief often follows such

procedures, long term efficacy is disputed with problems in the form of returning pain, reduced spinal mobility and adjacent segment degeneration, often occurring [37]. In addition to vertebral body fusion, the use of artificial prosthetic discs, including Charite [38], ProDisc [39], and Flexicore [40] have been trialed with current data suggesting the benefits of disc prosthetics to be on a par with fusion. Furthermore, many of the disadvantages associated with vertebral body fusions remain with disc replacement, including adjacent segment degeneration (although the incidence appears to be less than with vertebral body fusion (9.2 % and 28.6 % of patients 5 years post total disc replacement and fusion, respectively)) [41]. In addition, prosthetic discs have the potential to become unstable and to suffer wear, increasing the risk of spinal cord damage, and particle-induced inflammation [42]. Thus, given the poor long-term efficacy of current treatments, combined with the increasing prevalence of chronic LBP, there is a growing need for novel biological and cell based therapies to be developed in order to provide an alternative treatment for IVD degeneration.

In light of this, the use of growth factors has been explored for the treatment of IVD degeneration. Studies have shown increased matrix production and decreased catabolic cytokine and enzyme expression, in IVD cells following treatment with recombinant growth factors, including tissue growth factor (TGF) –β [43, 44], insulin growth factor (IGF) -1 [45], epidermal growth factor (EGF) -1 [43], growth differentiation factor (GDF) -5 [46], and bone morphogenic proteins (BMPs) including BMP -2 [47], 7 [48], and 12 [49]. To date, benefits observed following application of growth factors have been demonstrated in animal [48] and human [50] in vitro models, as well as in animal in vivo models [51]. However, there are limitations associated with such therapies, as these treatments rely on the presence of viable and appropriately functioning resident disc cells (which have been shown to decrease in number and alter their phenotype during the progression of the disease [52]) and potentially multiple injections of growth factor (given their short half-life) [53]. Furthermore, the addition of growth factors into a degenerate disc has the potential to increase the metabolic activity of the resident cells, leading to increased consumption of metabolites and production of waste products, potentially exacerbating the problems associated with IVD degeneration [54]. Of interest, recombinant BMP-7 injection is currently being trialed on patients suffering IVD degeneration and while no data is currently available this trial will demonstrate whether such therapies are efficacious in the treatment of degeneration.

Given the current treatment options, there remains a growing need for therapies capable of restoring the cell population, IVD function, and alleviating pain. Increasing efforts have therefore been directed towards investigating the potential of cell-based therapies for the treatment of IVD degeneration.

Regenerative Medicine Strategies

Cell Choice

Proposed cell-based strategies include the implantation of autologous cells into the degenerate NP, with or without a suitable biomaterial scaffold. In order for a biological therapy to be realized, a suitable cell source is required. Autologous NP cells have been shown to halt degenerative changes in an animal model of IVD degeneration [55], and more importantly, a randomized clinical trial has demonstrated improvements in terms of pain relief and disc hydration upon injection into degenerate human IVDs [56]. However, although this cell type appears promising, harvesting of NP cells yields a limited number of cells and requires invasive procedures, which have, themselves, been shown to initiate degenerative changes [57]. Furthermore, NP cells sourced from degenerate IVDs may be inadequate for regeneration purposes, due to increased expression of degradative enzymes [17], reduced expression of matrix proteins [58] and increased cellular senescence [59]. Recently, the use of allogeneic juvenile chondrocytes has been explored as an alternative cell source. Patients receiving the expanded chondrocytes (sourced from articular surfaces of cadavers) showed an improvement at 1 year post treatment in the level of pain and disability experienced, as well as an improvement in the MRI score of the disc [60]. However, caution must be taken as the matrix produced by articular chondrocytes may not be the most appropriate for IVD tissue engineering/repair [61]. Furthermore, while the IVD is considered as a potentially immune-privileged tissue (due to its avascular nature), autologous cells remain the ideal choice for implantation. Thus, the recent evidence suggesting the IVD may harbor endogenous precursor/progenitor cells or stem-like cells has revealed an exciting new avenue of investigation.

Evidence for the presence of progenitor cells in the disc comes from the discovery of highly proliferative cells expressing stem cell markers (Notch1, Delta4, Jagged1, C-KIT, Ki67, and STRO-1) [62, 63], and the ability to isolate a precursor population of cells from the degenerate IVD, which were capable of differentiating down the osteogenic, chondrogenic, and adipogenic lineage [64]. More recently Sakai and colleagues [65••] demonstrated a population of Tie2+/GD2+ 'NP progenitor cells' within the NP of mice and humans; however, the proportion of these cells within the disc was markedly reduced with age and degeneration. While these endogenous stem cells offer huge potential, it remains to be seen whether they can be

harnessed for regenerative purposes, or even stimulated to induce regeneration in situ through stimulation by appropriate exogenous factors.

A more immediate autologous progenitor cell source are adult mesenchymal stem cells (MSCs), which can be isolated easily from multiple sources, most notably bone marrow (BM-MSCs) or adipose tissue (AD-MSCs), divide rapidly, and are capable of differentiating into cells of the mesenchymal lineage. Increasing evidence has demonstrated that they are also capable of differentiating into NP-like cells [61, 66, 67••, 68–70]. Furthermore, implantation of BM-MSCs into a rabbit model of IVD degeneration was shown to reverse some of the degenerative changes when compared with no treatment [68]. Importantly, a small pilot study conducted recently, whereby 10 patients suffering from LBP (associated with IVD degeneration) were injected with autologous BM-MSCs, found that improvements reported were similar to those observed following vertebral body fusion [71••]. This human study, along with numerous animal studies, suggests that MSC implantation could be a useful tool for IVD regeneration.

Understanding the Target Cell

In order to successfully develop an MSC-based therapy for the treatment of IVD degeneration, it is necessary to understand the phenotype of the target cell and ensure correct differentiation and synthesis of an appropriately functioning tissue. NP cells have routinely been described as chondrocyte-like, with traditional chondrogenic gene markers (collagen type II alpha 1 (COL2A1), aggrecan (ACAN), and sex determining region Y (SRY)-box 9 (SOX9)) being used to assess MSC differentiation towards an NP cell phenotype [61, 72]. However, while the NP and articular cartilage (AC) share a similar matrix composition, the ratio of PGs and collagens differs between the 2 tissues, with a PG:collagen ratio of 27:1 and 2:1 in NP and AC tissue, respectively [73], suggesting that NP cells may have a distinctly different phenotype to chondrocytes. Furthermore, the differences in ontogeny between the 2 tissues also suggests that the component cells may be distinctly different (IVD development is beyond the scope of this review, but is an active area of research and more information can be found in excellent reviews by Henriksson and Brisby [74], Ludwinski et al. [75] and Smith et al. [76]).

Recent microarray studies have identified differences in the phenotypic gene profile between NP and AC cells and while the functional significance of these molecules in the IVD (eg, FOXF1, PAX1, KRT-8, -18, and -19, CA12) is largely unknown, their use as phenotypic markers is vitally important in order to help define the NP cell phenotype [67••, 77]. Indeed these markers (particularly CA12 and the cytokeratins) have recently been used to demonstrate MSC differentiation toward NP-like cells (discogenic differentiation) and are being adopted within the research community, as a unique marker profile. While BM-MSCs remain the most widely studied cell type for IVD regeneration, we have recently demonstrated that AD-MSCs may be the more appropriate choice as they differentiate to a phenotype (as depicted by this gene profile) more similar to that of NP cells than BM-MSCs [67••]. In vivo animal studies have also suggested that AD-MSCs may be an appropriate choice for IVD regeneration [78•]. Importantly, they may also avoid the concerns surrounding osteogenic differentiation and osteophyte formation, which have been shown following intradiscal injection of BM-MSCs [78•].

Induction of MSC Discogenic Differentiation

A range of methods have been employed to induce discogenic differentiation of MSCs. These include the addition of growth factors, either in isolation or in combination with specific culture conditions, predominantly 3-dimensional cultures designed to mimic the in vivo environment and maintain the rounded cell morphology found in native tissues. MSCs were first shown to differentiate down the chondrogenic lineage with the addition of TGF-β [79]. In addition to TGF-β, other growth factors also shown to cause chondrogenic differentiation of MSCs include IGF-1, FGF-2, and the BMPs, particularly BMP-7 [80–82]. More recently and following elucidation of NP specific markers, GDF-5 has been demonstrated to induce a more NP-like phenotype than TGF-β [66] and further studies may identify alternative growth factors, which induce a more appropriate phenotype than those currently used.

Stimulation of discogenic differentiation of MSCs can also be achieved by co-culture. MSCs can be cultured with direct contact with IVD cells, and this has been shown to lead to the differentiation of both BM-MSCs [69], and more recently AD-MSCs [83], towards an NP-like phenotype. Furthermore, during co-culture BM-MSCs and NP cells communicate in a bi-directional manner [84], which results in an improvement in the degenerate NP cell phenotype as well as MSC differentiation [69]. This suggests that following implantation MSCs may exert paracrine effects on resident degenerate NP cells to help restore normal disc cell function, thus aiding the repair process.

Biomaterials to Aid Cell Transplantation for Regeneration of the IVD

When implanting cells into a degenerate IVD, particularly where native damaged tissue has been surgically removed, it may be necessary to include a biomaterial scaffold to aid in cell delivery and provide implanted cells with a suitable scaffold/microenvironment while they generate new tissue (this area is

reviewed in detail in [85]). Incorporation of cells into a biomaterial may also aid cell survival post-implantation, enable transduction of mechanical loads, which is important for matrix synthesis, and even induce MSC differentiation. Indeed we have previously demonstrated that thermoreversible chitosan-glycerophosphate (C/Gp) hydrogels are capable of inducing MSC differentiation in vitro without the need for exogenous stimuli [61]. A range of other polymer and polysaccharide based hydrogels have been proposed as ideal materials for IVD regeneration, including pentosan polysulphate-incorporated polyethylene glycol (PEG) / hyaluronan [86], ferulic acid-gelatine chitosan / glycerophosphate [87], laminin-functionalized PEG [88] and type II collagen-hyaluronan [89], due to these biomaterials sharing similar properties with that of the NP (hydrated gelatinous substance), being injectable (through a needle or arthroscope), which would cause minimum damage to the AF and being biocompatible, as evidenced through subcutaneous implantation studies [86]. Another recent study has shown the ability of thermoreversible hyaluronan-based hydrogels, which like C/Gp gels are liquids at room temperature and gels at body temperature, to drive differentiation of MSCs when implanted into a nucleated bovine caudal disc. The authors found that pre-differentiation of MSCs within the hydrogel prior to implantation was not necessary and led to inferior results than direct implantation [90]. In addition to gel thermosensitivity, other important requirements include mechanical integrity as well as biodegradability; this would provide mechanical function to the disc upon implantation and enable implanted cells to replace the hydrogel with de novo matrix over time.

IVD Niche in Health and Disease

The IVD represents a particularly challenging micro-environmental niche for implantation of cells or cell seeded biomaterials. It is the largest avascular tissue in the human body, with a blood supply being up to 8 mm away from the center of some discs [13] and thus has oxygen levels as low as 1 % [91]. It also has a relatively low pH (reducing to pH 5.7 in severely degenerate discs [92]) and low levels of nutrients due to the limited exchange of nutrient and waste products caused by the lack of vasculature and the fact that resident NP cells produce energy predominantly via glycolysis. While evidence is conflicting [93, 94], reduction in vascularity of the end-plates due to smoking [95], atherosclerosis or calcification, which have all been associated with degeneration, is thought to exacerbate the problem. The high GAG content of the disc also results in high tissue osmolarity (between 450 and 550 mOsm, greater than for most other tissues [12]), which is important for generating swelling pressures to enable the IVD to resist mechanical loads [96]. The cells of the disc are also exposed to a variety of mechanical stimuli, including compressive and tensile forces, as well as hydrostatic pressures and shearing forces. These mechanical stimuli have been shown to influence disc cell metabolism, with the response of cells dependent on the magnitude, frequency, and type of loading administered [97]. Indeed all of these factors are affected by and may contribute to the degenerative process, but the NP cells, although reduced in number, are uniquely adapted to survive and function within this harsh microenvironment (see review by Ludwinski et al. [75]). Significantly, MSCs may not have such adaptations to enable them to survive or function in this environment and as such, while studies have shown improvements following implantation of MSCs into animal injury models of IVD degeneration [68], it is unclear whether implantation of MSCs into a more physiologically relevant (larger disc size, low nutrients, low pH, high levels of inflammatory cytokines, high osmolarity and high mechanical loads) model of degeneration or into the human degenerate IVD would be successful. Thus, the question remains as to whether pre-differentiation of MSCs is needed prior to implantation. Recent evidence suggests that microenvironmental factors similar to those found within the degenerate IVD (eg, low glucose, high osmolarity, and low pH) have a detrimental effect on MSC biology, in terms of cell viability, proliferation and expression of matrix markers [98, 99]. Such findings suggest that undifferentiated MSCs are not suitable for direct implantation into a degenerate IVD, and that pre-differentiation may be required in order to pre-condition cells for the harsh microenvironment of the degenerate IVD.

Future for IVD Regeneration

The IVD is a difficult tissue to regenerate due to the unique and harsh microenvironment that resident cells must endure. Adult MSCs appear to offer a promising cell type for IVD regeneration, due to the relative ease of acquisition and their ability to undergo discogenic differentiation (as depicted by the expression of recently described NP phenotypic markers). However, an improved understanding of the effect of the degenerate IVD niche on implanted cells remains important. Although animal models of degeneration are useful, models are required which more accurately reflect natural human IVD degeneration and the associate microenvironmental changes. In light of this, efforts are being directed towards development of ex vivo whole organ IVD models for the study of IVD biology and to examine, in detail, the interactions between implanted cells and the disc microenvironment [100, 101]. These ex vivo systems, in which oxygen tension, nutrients, pH, osmolarity, cytokine levels, and mechanical load can, theoretically, all be independently controlled, are capable of maintaining cell viability and tissue integrity over extended

time periods, and provide a useful model for testing the efficacy of stem cell implantation. However, these models routinely involve enzymatic ablation of the NP and thus do not accurately represent the clinical situation, where stem cells would be implanted into a degenerate IVD, thus further refinements are required. Recent studies have shown that inclusion of a hydrogel carrier appears to be beneficial in encouraging encapsulated cells to differentiate and to produce an appropriate matrix. However, improvements in hydrogel design are required to produce biomaterials, which are mechanically robust, easily administered by injection (liquid outside the body, gel inside the body), direct appropriate cellular differentiation and matrix formation, and are biodegradable over relevant timescales to ensure regeneration of a fully functional tissue.

Interestingly, autologous MSC-based therapies, in a pilot study, have been shown to reduce the pain induced by IVD degeneration but further trials are needed to continue to prove the efficacy and safety of stem cell therapies for the treatment of IVD degeneration [71••]. Further work is also required to understand the role of putative resident progenitor cells and establish methods to activate these cells and initiate endogenous tissue regeneration.

Clinical Application of MSC-Based Therapies for Treatment of Back Pain

MSC-based therapies have demonstrated potential for regeneration of IVD tissue in animal studies, with increases in disc height and hydration observed, as well as deposition of ECM [68, 78•]. However, human clinical trials are essential in determining efficacy of such therapies in alleviation of back pain, an aspect which cannot be meaningfully studied in animals. While the causes of back pain are clearly multifactorial, a correlation with disc degeneration has been demonstrated in 40 % of sufferers [8] and small-scale human trials using both autologous disc cells [56] and MSCs have demonstrated improved pain and disability index scores, as well as increased water content (as assessed by MRI) [71••, 102], although no increases in disc height. These studies are encouraging and suggest that MSC-based therapies may be at least as effective as current clinical interventions, although more extensive trials are needed to assess long-term efficacy. They also suggest that MSC injection is generally safe, although the potential for MSC leakage and peripheral osteophyte formation has recently been raised [103], highlighting the importance of implantation within a suitable biomaterial scaffold and suggesting that care is required when injecting these cells in this region. Assuming long-term efficacy and safety can be demonstrated, these therapies have the potential to revolutionize the treatment of both disc degeneration and chronic back pain.

Conclusions

Cell-based therapies appear to provide a potential answer to this painful, debilitating, and costly disease. It is well recognized that with an aging population, degenerative musculoskeletal disorders will become even more of a burden on society. Through closer collaboration between the different scientific disciplines (biology, chemistry, materials science, etc), with clinicians and with continued financial and government backing, advances in this field of research should continue. The strategy required for the development of a successful cell-based therapy for IVD regeneration is becoming clearer, with cell type, biomaterial, culture conditions, and assessment of outcomes remaining of paramount importance. Recent advances have led to a better understanding of the NP cell phenotype and this has helped in developing appropriate protocols for the discogenic differentiation of MSCs. Understanding the interactions between the degenerate IVD niche and implanted cells will help define the best strategy (in terms of source of MSCs and whether pre-differentiation is required prior to implantation) in order to develop a suitable stem cell therapy for treatment of IVD degeneration.

Compliance with Ethics Guidelines

Conflict of Interest Hamish T. J. Gilbert declares that he has no conflict of interest. Judith A. Hoyland declares that she has no conflict of interest. Stephen M. Richardson declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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