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REVIEW ARTICLE

What is the preclinical evidence on platelet rich plasma and intervertebral disc degeneration?

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Abstract
Purpose Intervertebral disc degeneration is a common disease that usually starts from the third decade of life and it represents a significant cause of socio-economic problems. The accepted surgical treatment for disc degeneration is disc removal and vertebral fusion or, in selected cases, intervertebral disc arthroplasty. Several studies have demonstrated the ability of platelet rich plasma (PRP) to stimulate cell proliferation and extracellular matrix regeneration. However, literature results are still limited and more studies are required to clarify the role of PRP in the prevention or in the treatment of degenerative disc disease. The aim of this review is to summarize and critically analyze the current preclinical evidence about the use of PRP in intervertebral disc degeneration.
Methods Literature search was performed through various combinations of the following keywords: Intervertebral Disc Degeneration, Platelet Rich Plasma, PRP, Intervertebral disc regeneration. Papers included in our review cover the period between 2006 and 2014. The PRISMA 2009 checklist was followed.
Results At the end of the review process, 12 articles were included in our final manuscript, including 6 "in vitro" and 6 "in vivo" studies. All the included studies lead to positive preclinical results. No standardization of methodological analysis was observed.
Conclusion It is not possible to draw definitive evidence about the use of PRP in IVD regeneration. We advise a proper standardization of the methodological analysis in order to compare the available data and achieve definitive results. This should be the cornerstone for future clinical applications.

Keywords Platelet rich plasma · Intervertebral disc regeneration · Intervertebral disc degeneration · PRP

Introduction

Intervertebral disc degeneration (IDD) is considered to be one of the most important causes of low back pain [1]. Differently from the bone, that is a highly vascularized tissue with the ability to repair and regenerate, the intervertebral disc (IVD) is avascular, and when it degenerates there is no intrinsic capacity of remodeling and repair [2–4]. The inner part of the IVD is called nucleus pulposus (NP), which is surrounded by a fibro-cartilaginous ring called anulus fibrosus (AF). The NP is composed of 85 % of water and proteoglycans, and the AF is composed of an extracellular matrix (ECM) mixed with type 1 collagen fibers in the external layer, and of type 2 collagen fibers and chondrocytes in the inner layer [2]. The mechanical function of the disc is strictly dependent on the structures' composition. The NP is able to bear compressive loads thanks to an intrinsic positive hydrostatic pressure, while the AF can resist to tensile stresses [5].

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Disc degeneration usually starts from the degradation of proteoglycans in the NP associated with an alteration of the extracellular matrix [6, 7]. NP dehydration, together with the breakdown of collagen fibers in the AF, results in structural disc deformation, loss of disc height, and segmental instability. Many chemical reactions are involved in the IVD degeneration cascade, such as an increased production of inflammatory cytokines and catabolic enzymes [8–11].

Current treatments for IDD range from conservative therapies, such as bed rest, anti-inflammatory medications, analgesia and physical therapy, to invasive strategies, such as epidural injections, ablation techniques or surgical therapies (spinal fusion and disc replacement technologies) [12, 13]. However, these target the clinical symptoms instead of the degenerative cascade itself. Conversely, the application of technologies aimed to IVD regeneration would have a preventive intention, thus being a curative approach [14].

In regenerative medicine, several biological approaches including the injection of biological substances as growth factors, bioengineering approaches, and cell or gene therapies have been tested in either a preclinical or clinical context [15–18].

A cornerstone of regenerative medicine approach is growth factors. The IVD homeostasis is a complex pathway in which growth factors play a fundamental role. Some of these proteins are supposed to be involved in IVD degenerative cascade [19]. Growth factors already known to have an effect in IVD are TGF-β1 and TGF- β3 [20, 21]. Obviously, several other growth factors, such as Insulin-Like Growth Factor (ILGF), Osteogenic protein (OP-1), Growth and Differentiation factor-5 (GDF-5), have been investigated. Chujo et al. underlined the reparative capacity of GDF-5 on the IVD showing its effects of enhancing ECM production in vitro [22]. Tolonen et al. [23] recently postulated that growth factors, including TGF-β1 and TGF-β2, basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF), activate a molecular pathway in IVD tissue, where they play a role in cellular remodeling from the normal resting stage via disc degeneration to disc herniation. However, single growth factor injection may have limitations, as it seems that no single growth factor is potent enough to reverse degenerative trends. That's why Platelet Rich Plasma (PRP) has gained such popularity. PRP is a fraction of plasma containing a high level of platelets which contain many growth factors: Platelet-derived Grow Factor (PDGF) and Vascular Epithelial Grow Factor (VEGF), Transforming Grow Factors (TGF-B1 and TGF-B2), and Insulin-Like Grow Factor (IGF) [24–26]. Platelet-derived growth factors have been tested in many fields of medicine [26], and in orthopaedics in the management of sport-associated injuries, such as tendinopathies, muscular lesions, and cartilage damages [27, 28]. In many of these, the results of the clinical application of PRP are already available. A multi-center study was carried out by Kon et al. in 2011 the authors compared the clinical efficacy of PRP with low molecular weight Hyaluronate (LWHA) and high molecular weight Hyaluronate (HWHA), and showed a better performance for the PRP group at 6 months of follow-up in both [29]. Sanchez et al. treated 40 patients affected by hip osteoarthritis with 3 weekly ultrasound-guided injections of PRP, and concluded that the treatment was related to a significant reduction in pain level at the first evaluation after 6 weeks, which was confirmed even at the final 6 months' follow-up. However, 11 out of 40 patients did not have any positive effect after injective treatment [30]. Bernuzzi et al. treated 53 patients with grade 2 muscle lesions with 3 weekly injections of PRP. The majority of patients (85 %) reported improvement in pain and function during the first week. All patients resumed sporting activities after 30 days. One patient had a reinjury in the treated femoral biceps muscle, 5 months after, but at a different anatomical site [31].

In the last few years, some authors have suggested the implementation of a regenerative strategy to address IDD, based on the local application of PRP [24, 32], and a clinical application has been proposed. The rationale behind this therapeutic approach is to retard or even reverse the degenerative trend of IDD.

This review aims to explore and summarize the current preclinical evidence that supported the implementation of PRP in intervertebral disc regeneration clinical trials, in order to enhance the transition of this regenerative method from bench to bedside.

Materials and methods

We performed a research of the available English literature on Pubmed, Medline, Google Scholar, Medscape and Embase databases using various combinations of the following keywords: Intervertebral Disc Degeneration, Platelet Rich Plasma, PRP, Intervertebral disc regeneration. Papers included in our review cover the period between 2006 and 2014. The PRISMA 2009 checklist was considered to edit our review. Two reviewers (M.B. and L.C.) independently screened the titles and abstracts from all identified articles to assess their appropriateness to the research focus. References from the identified articles were checked in order not to miss any relevant articles. A total of 44 articles were identified, 23 duplicates were removed. The exclusion criteria were papers not evaluating the regenerative potential of PRP in IDD. Reviews on this topic were excluded. The PRISMA flow 2009 diagram illustrates the

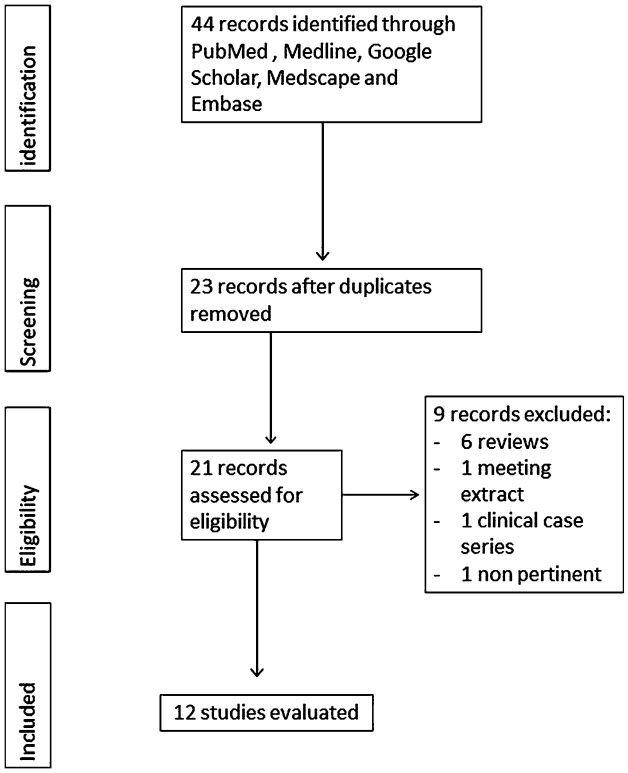

Fig. 1 Flow diagram depicting the number of studies that have been identified, included and excluded as well as the reason for exclusion

number of studies that have been identified, included and excluded as well as the reason for exclusion (Fig. 1). We also included one paper presenting an abstract in English with a Chinese full text. Twelve preclinical studies were evaluated for the current review, and divided into two groups, namely in vivo and in vitro studies. Further, in vivo studies were evaluated for the following variables: study year, size of sample, animal model, evaluation of regeneration, system used to obtain PRP, and results. Papers of in vitro studies were screened for the following variables: study year, system used to obtain PRP/PL, type of cells, cell culture media, time of analysis, analyzed variables, activator, cell scaffold, PRP carriers, and results. We divided carriers from scaffold in order to clearly identify the role of these adjuvant components. The first one aims to simply deliver PRP in the correct site, a scaffold has the objective to support and assist the biological role of PRP during the whole period of PRP activity.

Results

At the end of the manuscript selection, 12 articles concerning the use of PRP in IDD were eventually included in the review. Six of these were "in vitro" studies (Table 1), whereas 6 were "in vivo" studies (Table 2).

In every "in vitro" trial (Table 1) the system used to obtain PRP, the source of cells used, and the cell culture media were different. The main cell type tested was Human NP (hNP) cells. Akeda et al. [33] used porcine NP and AF cells, Mietsch et al. [34] used Mesenchymal Stem Cells (MSC) along with hNP cells and Liu et al. [35] used an immortalized type of hNP cells. Pirvu et al. [36] tested bovine AF cells. No consensus regarding the time of analysis was found among selected studies. The time of analysis ranged from 2 to 9 days. The most employed culture media was Dulbecco's modified Eagle's medium (DMEM) added with different percentage of Fetal Bovine Serum or Fetal Calf Serum (FCS). Akeda et al. [33] used 4 different types of media (Serum-free, 10 % FBS, 10 % Platelet Poor Plasma, 10 % PRP) while Pirvu [36] tested 4 kind of media with different PRP or Platelet Lysate (PL) concentration. Liu et al. [35] used DMEM/F12 exposed to lipopolysaccharide (LPS, 200 ng/mL), LPS added with PRP or none of them (as control). The most frequently analyzed variables were: cell proliferation and extracellular matrix regeneration in terms of different proteoglycan (PG) levels/PG-mRNA expression. Moreover, the PRP activator solution varied significantly, while in three of the included manuscripts no activator was used. A cell scaffold was employed only in two articles [33, 35]. No PRP carriers were utilized. Only one paper analyzed PL regenerative potential [36]. Two trials also reported the role of PRP in mediating the cytokine-induced inflammatory reaction [36, 37]. Chen, Mietsch, Liu and Pirvu [34–37], along with the "in vitro" assessment, carried out a histological analysis respectively at 4 weeks in the first three studies and at 7 days in the last one.

Chen et al. [37] evaluated the effect of PRP in 2D culture human NP cells in terms of proliferation and induction of chondrogenic-specific genes. These authors aimed to determine the proteoglycan accumulation in hNP cells treated with PRP, and its anti-apoptotic effects. They stated that the proliferation of hNP was increased by the use of PRP (about 7–11 times higher than the controls), together with proteoglycans accumulation. Some years later, Akeda et al. [33] performed an in vitro assessment of the effects of PRP on the extracellular matrix of porcine IVD cells (NP and AF). Porcine IVDs cells were separately isolated from both NP and AF tissues by sequential enzymatic digestion. The NP and AF cells were bluntly separated and cultured in a 24-well plate. The synthesis of proteoglycans and collagen, the accumulation of proteoglycans, and the cell proliferation were biochemically assessed. The results showed that PRP had a mild stimulatory effect on cell proliferation of IVD cells and upregulated the synthesis of proteoglycans and collagen. Kim et al. [38] investigated the role of PRP in suppressing the inflammatory response on monolayer with collagen matrix culture of hNPs. After applying

Table 1 Main features of the in vitro included studies

References	Year	System used to obtain PRP/PL	Type of cells	Cell culture media	Time of analysis	Analyzed variables	Activator	Cell scaffold	PRP carriers	Results
Akeda et al. [33].	2006	SYMPHONY 2 Platelet Concentration System (DePuy Spine, Raynham, MA, USA)	Porcine NP and AF cells	Serum-free, 10 % FBS, 10 % PPP, 10 % PRP	3 days	Cell proliferation, proteoglycans content	10 % thrombin solution (vol/vol, 1,000 U/ml in 100 mmol/l CaCl2)	Alginate beads	None	Increased accumulation of glycosaminoglycan; upregulated mRNA expression of Sox9, type II collagen, and aggrecan
Chen et al. [37]	2006	MSC blood cell separation system (Haemonetics Corp., Braintree, MA, USA)	Human NP cells	Various PRP/TGFb 1 concentration in 1 % FBS containing DMEM/F12	7 and 9 days, 4 weeks	Cell proliferation, SOX 9, collagen II, aggrecan mRNA levels, GAG levels, Histological analysis	Bovine thrombin (100 IU bovine thrombin/ 150 ml PRP)	None	None	Induction of human nucleus pulposus proliferation and chondrogenic differentiation
Kim et al. [38]	2013	GPS III System (Biomet, Inc., Warsaw, IN)	Human NP cells	F-12/D-MEM containing 1 % fetal bovine serum (FBS), 1 % penicillin–streptomycin (PS), and 25 mg/ml L-ascorbic acid	2 days	Collagen II, aggrecan, MMP-3, COX-2 mRNA levels	None	None	None	PRP can suppress cytokine-induced pro-inflammatory degrading enzymes enhancing gene expression concerning matrix synthesis, thereby stabilizing NP cell differentiation
Mietsch et al. [34]	2013	Centrifugation and double filtration	HumanNP cells, MSC	D-MEM with 5 % FCS, 1 % L-glutamine, 1 % non-essential amino acids, 1 % penicillin–streptomycin, 0,5 % fungizone	7 days, 4 weeks	mRNA expression of aggrecan, collagen type 2, collagen type 1 and Sox9. Immunohistological evaluation, mechanical analysis	100 ml 2.5 N acetic acid/ 10 M urea	None	None	The mixture of growth factors in PRP promoted proliferation rather than chondrogenic differentiation
Liu et al. [35]	2014	Not decleared	Immortalized Human NP cells (ihNP)	DMEM/F12 and then exposed to LPS (200 ng/mL), LPS + PRP or none of them (as control)	7 days, 4 weeks	Expressions of SOX9, type II collagen and aggrecan, IL-1b, TNF-a and MMP-3. Histologic and immunohistochemical evaluation	None	2D and 3D collagen scaffolds	None	PRP stimulated the expression of chondrogenic markers and inhibited the expression of inflammatory mediators and matrix degrading enzymes in ihNP cells in both 2D monolayer and 3D scaffolds
Pirvu et al. [36]	2014	Centrifugation, suspension and sonication techniques (INTERCEPT Blood System)	Bovine AF cells	25 % PRP-75 % DMEM; 50 % PRP- 50 % DMEM; 25 % PL- 75 % DMEM 50 % PL- 50 % DMEM; 10 % FBS- 50 % DMEM; DMEM (controls)	2,4 and 7 days	Cell proliferation, GAG, collagen I and II, aggrecan, versican, decorin mRNA levels, Histologic assessment	None	None	None	Both PRP and PL have proliferative effects on AF cells and are able to increase ECM production in vitro

DMEM Dulbecco's modified Eagle's medium, *ECM* extracellular cell matrix, *PL* platelet lysate, *FBS* fetal bovine serum, *FCS* fetal calf serum, *MMP-3* matrix metalloproteinase-3, *COX-2* cyclooxygenase-2

Table 2 Main features of the in vivo included studies

Raferences	Year	System used to obtain PRP/PL	Animal model	Size of sample	Time of analysis after PRP injection	Evaluation of regeneration	Activator	PRP carriers	Results
Nagae et al. [39]	2007	centrifugation	Japanese white rabbits	36	2, 4, and 8 weeks	Histological evaluation, PG m-RNA expression, Ultrastructural observation of NP cells	Not declared	Biodegradable Gelatin hydrogel microspheres	Decrease in IVD degeneration rate, increased PG m-RNA expression
Sawamura et al. [40]	2009	centrifugation	Japanese white rabbits	128	2, 4, and 8 weeks	MRI, PG core protein, collagen II, cells proliferation, apoptotic cells	Not declared	Biodegradable gelatin hydrogel microspheres	Increased IVD height, increased expression of PG core protein and collagen II, no proliferative cells and decreased apoptotic cells
Chen et al. [44]	2009	centrifugation	Miniature porcine	14	4, 8 weeks	Cells proliferation, X-ray analysis, chondrogenic and osteogenic matrix proteins m-RNA expression	Not declared	None	PRP promote nucleus pulposus (NP) regeneration, disc height index improvement and osteogenic MSC differentiation
Gullung et al. [43]	2011	Not declared	Sprague–Dawley rats	18	2,4 and 6 weeks	MRI, Histological evaluation	Not declared	None	Preseverd IVD fluid content, decreased IVD degeneration
Obata et al. [41]	2012	centrifugation	New Zealand white rabbits	12	8 weeks	MRI, cell proliferation	Autologous serum and 2 % $CaCl_2$	None	Increased cell proliferation, no statistical differences on MRI findings
Hu et al. [42]	2012	centrifugation	New Zealand white rabbits	45	1 and 2 weeks	MRI, NP cells proliferation, collagen II m-RNA expression	10 % thrombin solution(vol/vol, 1,000 U/ml in 90 mmol/l CaCl2)	None	Reduction of IVD degeneration, extracellular matrix production

MRI magnetic resonance imaging, *PG* proteoglycan

Interleukin-1 (IL-1) and Tumor Necrosis Factor-α (TNF-α), they added PRP, and concluded that PRP administration markedly reduced the pro-inflammatory cytokines that induced degrading enzymes. Simultaneously, PRP enhanced the matrix synthesis by recovering the down-regulated gene expression of collagen type II and aggrecan. Mietsch et al. [34] tested the proliferative potential and the chondrogenic differentiation of hNP cells and mesenchymal stem cells when exposed to Transforming Growth Factor-β1 (TGF-β1) or PRP, in conjunction with hydrostatic pressure. Fresh human bone marrow aspirates were obtained. MSC were isolated and expanded in monolayer in DMEM. hNP were isolated from human disc samples obtained from patients undergoing disc surgeries. Isolation and cell culture were performed. After that, both cell types were seeded into pellet culture or alginate beads. They found a greater chondrogenic response in cells treated with TGF- β1 compared to the PRP-treated group. On the contrary, the expression of proliferation genes was boosted in those cells mixed with PRP. As an adjunctive matter, hydrostatic pressure had a positive effect in a non-standardisable fashion. Some Authors aimed to establish a chondrogenic recovery cell model in order to use it as a screening platform for regenerative drugs. Liu et coll [35] immortalized a hNP cell line and induced an inflammatory response with LPS to evaluate the cell behavior when added with PRP. hNP cells were collected from a 39-year-old male IVD donor. After several suspensions and filtrations NP cells were then seeded into 10 cm tissue culture dishes and incubated at 37 °C in 5 % CO2 before subsequent experiments. In contrast with the work of Mietsch et al. [34], this manuscript underlined the significant effects on promoting chondrogenic differentiation along with the anti-inflammatory potential of PRP. These results, regarding the anti-inflammatory effect of PRP, confirmed the conclusion obtained by Kim et al. [38]. Recently, Pirvu et al. [36] explored the regenerative potential of PRP and PL on bovine AF cells. Bovine AF cells were cultured in 24-well plates with culture media containing different concentrations of PRP or PL. FBS and DMEM were used as control media. They evaluated several proteoglycans mRNA expression and cell proliferation, and performed a histological assessment with an organ culture model. The authors concluded that both PRP and PL induced some proliferative effects on the AF cells, and induced an increase in ECM accumulation.

All the "in vivo" studies were controlled trials, with only one being randomized.

As for the manuscripts about "in vivo" studies (Table 2), the rabbit was the tested animal in 4 out of six studies [39–42]. One experimental study was conducted on rats [43], and one on miniature pigs [44]. 5 of the 6 included studies used centrifugation to obtain PRP [39–42, 44]. In his work, Gullung does not declare how to obtain PRP [43]. The total population of animal samples was 253, with only one study performed using more than one hundred animals [40]. The highest follow- up period was 8 weeks, reached in 4 studies (range 1–8 weeks) [40–43]. The histological evaluation was the most frequently used method to evaluate regeneration rate. When performed, the most utilized type of staining was Hematoxylin and Eosin. Obata et al. [41] stained their sections also with Safranin-O in order to clearly identify chondrocyte-like cells. Chen [44] used Alcian blue staining for detecting synthesized proteoglycan arranged in IVD tissue and von Koss staining to identify mature bone matrix. Immunohistochemistry was performed in 4 studies [40–42, 44]. Sawamura et al. [40] evaluated the proliferative activity of the IVD cells using immunohistochemistry for proliferating cell nuclear antigen (PCNA). Chen et al. [44] quantified the matrix production via type II collagen monoclonal antibody immunochemistry. In the study of Nagae et al. [39], immunochemistry for proteoglycan was performed with a first incubation of the sections with a mouse antihuman cartilage proteoglycan monoclonal antibody. After several washes with Phosphate Buffered Saline (PBS), the sections were incubated with an antimouse immunoglobulin. Lastly, the sections were reacted with a solution containing 3,3′diaminobenzidine-tetrahydrochloride and counter-stained with hematoxylin. An immunofluorescence examination was performed to confirm the staining for proteoglycan. Hu et al. [42], in order to quantify the ECM production, performed a collagen type II immunohistochemical staining. RT-PCR was utilized in 2 trials in order to evaluate gene expression [41, 44]. Sawamura [40], in his work, detected the apoptotic cells with deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) staining. MRI was performed in 4 of the 6 included studies [40–43]. Ultrastructural or X-ray analysis was performed in 2 different studies [39, 44]. When used, the PRP activator varied between studies. Obata [41] employed an autologous serum added with 2 % calcium chloride. Hu et al. [42] used a 10 % thrombin solution added with calcium chloride. Hydrogel microspheres were the only carrier used to transport PRP into the discs, and were employed in 2 trials [39, 40]. To impregnate these microspheres with PRP, in both studies the authors dropped 60 mg PRP onto 3 mg of the sterilized carrier and the mixture was incubated for 1 h at 37 °C. A mixture of MSC and PRP was tested in one study [44].

In 2007, Nagae et al. [39] compared results obtained in groups treated with PRP-Gelatine-Hydrogel-Microspheres, PRP alone, or microspheres impregnated with PBS. The authors noticed a decrease in IVD degeneration rate, together with an increase in proteoglycan expression in the group treated with PRP-Gelatine-Hydrogel-Microspheres.

The histological analysis demonstrated that the group treated with PRP-Gelatine-Hydrogel-Microspheres had significantly less severe lesions in the NP and in the AF compared to the other groups. Two years later, the same authors improved the previous study by associating an MRI evaluation: they confirmed the increase in ECM and cell proliferation after PRP-Gelatine-Hydrogel-Microspheres injection. As a parallel matter, they observed an increase in IVD height on the MRI examination. In both these studies, the PRP was delivered using Gelatine-Hydrogel-Microsphere carriers [39, 40]. Chen et al. [44] realized an "ex vivo" IVD degeneration model to test the IVD regenerative ability of three different therapeutic regimens including: mesenchymal stem cell derived from eGFP-transgenic porcine (MSC-GFP), PRP, and MSC-GFP/PRP combined treatment. These findings were also validated "in vivo". In order to obtain the "ex vivo" model miniature porcine was killed and the complete IVD tissue of lumbar spine from T13 to L5 was harvested. Chymopapain was used to achieve disc degeneration model. They concluded that both in ex vivo and in vivo model, MSC-GFP or PRP, if used alone, lead to chondrogenic-related mRNA expression and matrix synthesis of the injected cells. Unexpectedly, the combination of both resulted in an osteogenic differentiation. These results were also confirmed in the "in vivo" model. In their randomized controlled trial, Gullung et al. [43] explored the effect of a delayed PRP injection in a rat model. They obtained 3 sample groups of 6 animals each, and in 2 of these they induced an intervertebral disc lesion. PRP injection was performed at the time of the lesion (group 1) and 2 weeks later (group 2). The decrease in terms of IVD degeneration was proved in both groups, but animals in group 1 had a faster recovery. Hu et al. [42] performed a preclinical study on 45 New Zealand white rabbits, evaluating the effect of autologous PRP injection in early intervertebral disc degeneration treatment at 1 and 2 weeks after the induction of degeneration. They showed a reduction in IVD degeneration and a promotion of ECM production for the group treated with PRP. Despite these results, some evidence is in contrast with previous results. In 2012, Obata et al. [41] failed to demonstrate any statistical differences on MRI findings, despite the increased cell proliferation demonstrated in the study groups managed by PRP.

Discussion

The mechanical properties of a healthy IVD strictly depend on its histological features. It covers the essential function of load absorption, while maintaining multi-axial flexibility of the spine. The degeneration of the IVD has a well-established histological pattern: increased disc-cell proliferation as well as cell apoptosis or necrosis frequently occurs [45]. The distribution and production of matrix molecules are also altered. Glycosaminoglycans are reduced in disc degeneration by inducing NP dehydration [46, 47]. These molecular changes are responsible for several clinical patterns ranging from disc herniation to symptomatic disc degeneration. At present, the gold standard in IDD treatment is fusion surgery with several techniques [48–50], but these surgeries do not preserve the function of the IVD. Conversely, the conservative treatment is based on physical therapy and cannot reverse the degenerative cascade [51, 52]. Therefore, current research is directed toward an attempt of therapeutic inhibition of IDD at the earlier stages.

However, similarly to the joint cartilage and, to the meniscus, the IVD is traditionally considered an organ with no ability to regenerate. In this context, several regenerative approaches have been attempted to address this issue, including growth factor delivery, gene therapy, tissue engineering, and cell-based therapy [53–56]. One of the theorized methods to promote IVD repair is intradiscal PRP delivery. PRP is a fraction of plasma containing a high level of platelets; however, PRP has a wide range of platelet concentration and platelet granules contain many growth factors which are released from the platelets after their activation by calcium or thrombin, and include PDGF and VEGF, TGF-B1 and TGF-B2, and (IGF) [24, 25]. This variability is due to the fact that in most patients the platelet concentration in the whole blood ranges from 150,000 to 350,000/μL. When concentrated into PRP, the concentration rises up to 1,000,000 platelets/μL even though higher levels are not related to more healing effects [57–59]. Moreover, PRP can also contain a variable amount of other cells, such as red blood cell, white blood cells, as well as a variable amount of plasma: all factors that might influence the PRP-tissue interaction in a dose-depending fashion [60].

In our review, we selected all the available pre-clinical studies regarding the use of PRP applied to IVD regeneration models.

We included 6 "in vitro" trials evaluating regenerative potential of PRP preparations on IVD cells [33–38]. All these in vitro studies showed similar results. Undoubtedly, this evidence represented a valid baseline for the in vivo applications. However, when critically analyzing these results, several differences in methodology can be observed, since these studies were heterogeneous in terms of cell samples, PRP preparation, and methods of evaluation, thereby rendering it very difficult to draw definitive conclusions. Considering the "in vivo" studies, we collected and evaluated 6 articles [39–44]. Similarly to the "in vitro" studies, also the "in vivo" studies lack standardization and homogeneity. All the studies do not

evaluate long-term results, seen that the longest follow-up was 8 weeks after PRP injection. Two studies were performed with carriers for PRP delivery, and this represents another factor that affects analysis of available evidence [39, 40].

Although many issues remain unsolved, clinical Phase I studies are starting worldwide in order to test IVD regenerative strategies. "Ex vivo" expanded autologous bone marrow derived mesenchymal stem cells have been tested [61], and recombinant growth factors and PRP are under investigation, yielding to promising expectations [24]. At the time of writing of this review, only one published congress abstract reports on the preclinical study by Akeda et al. [62], who performed a clinical trial to determine the efficacy and feasibility of PRP released in patients with discogenic low back pain. According to the report, a small sample of patients (three men, three women) were enrolled and completed the 6 month follow-up according to clinical and radiological (radiographic and MRI) parameters, thereby reporting a significant clinical improvement. These results suggest that in selected patients PRP injection is a safe and effective solution with sustained mid-term results.

PRP is known to have intrinsic advantages. Since it is an autologous cocktail of growth factors, it avoids the transmission of diseases and immunological reaction. Moreover, it is simple to prepare, cost-effective, readily available, and easy to use. If the implementation into clinical practice leads to positive results, these features will promote its widespread use in clinical practice [63, 64]. However, at present PRP therapy for IVD degeneration should be used only in controlled clinical trials, mainly in the early stages of degeneration: in fact, in the late stages of IDD only few cells are left, and the calcified IVD limits the biologic potential of growth factors associated to PRP injections.

Our systematic review has several limitations. Due to the methodological heterogeneity of the included studies, we cannot draw definitive results. Moreover, no firm consensus on evaluation of regeneration, use of activators, PRP carriers and the system used to obtain PRP is identifiable. In order to confirm the promising efficacy of PRP in IDD treatment, we advocate for the standardization of preclinical studies, to better move this encouraging regenerative therapy from bench to bedside [65].

In conclusion, with the current review we aimed to summarize and critically analyze the current evidence about the use of PRP in IDD. All the included studies underlined the positive histological results, demonstrating that PRP induces ECM regeneration and cell proliferation. When performed, the MRI analysis of in vivo studies confirmed the histological results in all cases but one. At present, only one clinical trial has been performed with positive clinical and radiological mid-term results. Current studies mainly concentrate on the regenerative potential of PRP, but the possibility of adverse effects for clinical applications needs to be further investigated. We advise a proper standardization of the methodological analysis in order to compare the available data and achieve definitive results. This is the basis for future clinical directions.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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