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Review

Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review

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SUMMARY

Cartilage defects (CDs) and the most common joint disease, osteoarthritis (OA), are characterized by degeneration of the articular cartilage that ultimately leads to joint destruction. Current treatment strategies are inadequate: none results in restoration of fully functional hyaline cartilage, for uncertain long-term prognosis. Tissue engineering of cartilage with auto-cartilage cells or appropriate mesenchymal stem cell (MSC)-derived cartilage cells is currently being investigated to search for new therapies. Platelet-rich plasma (PRP), an autologous source of factors obtained by centrifugation, possesses various functions. For culture of MSCs and cartilage cells, it might be substituted for fetal bovine serum (FBS) with high efficiency and safety. It enhances the regeneration of cartilage cells when added to cartilage tissue engineering constructs for repairing CDs and as regenerative injection therapy for OA. But challenges also remain. Some of the growth factors (GFs) present in PRP have negative effects on the OA joint. It is therefore unlikely that a mix of GFs some of which have negative effects in the OA joint, as present in PRP, will be of benefit in OA. Future directions of PRP application may concentrate on seeking an appropriate and innocuous agent like anti-VEGF antibody that can modulate and control the effect of PRP.

Introduction

Articular cartilage damage is usually caused by sports injuries or accidental trauma and aging. It regularly progresses to more serious joint disorders such as osteoarthritis (OA), necrosis of subchondral bone tissue or arthritis. An estimated 15% of the world's population have joint diseases; more than 39 million people in the European Union and more than 20 million Americans have OA. By 2020, these numbers will probably be doubled[1].

After traumatic or pathological injury, hyaline articular cartilage, the load-bearing tissue of joint, has limited or no intrinsic capacity for repair capacity, and even minor lesions or injuries may lead to progressive damage and joint degeneration.

OA is a chronic degenerative joint disease characterized by progressive destruction of articular cartilage, thinning and eventual wearing of articular cartilage, thus resulting in painful, limited joint movement. The degeneration of articular cartilage, mainly due to changes in the activity of chondrocytes in favor of catabolic activity, which also involves other joint tissues, as alterations of the meniscus, sclerosis and edema in the underlying subchondral bone as well as intermittent inflammation of synovium.

Current treatments for articular cartilage damage, such as surgical intervention (microfracture, osteochondral auto- or allografts), to repair articular cartilage are less than satisfactory and rarely restore full function. To obtain sufficient chondrocytes for therapy, the required *in vitro* expansion usually induces cartilage cell dedifferentiation. Tissue engineering-based cartilage repair has been pursued to provide more functional biological tissue. The chondrocytes are taken from non-weight-bearing parts of intact joint areas and expanded in cell culture, then transplanted into the defective areas of the affected joints. Clinical trials of autologous chondrocyte implantation (ACI) have shown promise[2].

Multipotent adult mesenchymal stem cells (MSCs) can differentiate into cells of the chondrogenic lineage and are isolated from a wide variety of tissue sources; they are easy to isolate without significant donor-site morbidity and are easier to expand *in vitro* than chondrocytes[3]. These cells are also used for cartilage tissue engineering and chronic degenerative disorders and to prevent cartilage degradation in OA because of their trophic/regenerative potential. This therapeutic method needs a large quantity of cells to obtain enough cartilage cells or MSC-derived cartilage cells. Most

Abbreviations: EGF, epidermal growth factor.

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expansion protocols still use fetal bovine serum/fetal calf serum (FBS/FCS) as a growth factor (GF) supplement, which is a potential source of undesired xenogeneic pathogens and raises concerns when used in clinical-grade preparations. A substitute for FBS/FCS is needed.

The first descriptions of the development and use of platelet-rich plasma (PRP) were in the early 1990s, when science focused on developing new "biological glues". PRP has been classically described as "a volume of plasma that has a platelet count above baseline". This definition would suggest a pure mixture of plasma (the acellular, liquid portion of blood that contains proteins for clotting and other bioactive molecules that play a significant role in wound repair) and platelets (and their associated GFs and cytokines). However, the generic term "PRP" has recently expanded to include a variety of final products. To more precisely delineate these products based on their leukocyte and fibrin content, they have been called pure PRP, leukocyte-rich PRP, pure platelet-rich fibrin, and leukocyte- and platelet-rich fibrin[4]. The terms "platelet-rich concentrate" and "platelet concentrate" are also used for PRP. Another platelet product is human platelet lysate (HPL).

Here, we discuss the basic science and applications of PRP and analogue products in cartilage defects (CDs) and OA.

Creating PRP

PRP is prepared by withdrawing peripheral blood and by centrifugation to obtain a highly concentrated sample of platelets. The platelets undergo degranulation to release GFs with healing properties. The plasma contains cytokines, thrombin, and other GFs, with inherent biological and adhesive properties.

The first report of PRP preparation was in the 1970s[5]. A few years later, attention was drawn to gravity forces and times of centrifugation to separate whole blood, which is crucial for platelet counts and volume. Then the focus was on platelet content of the PRP with an objective standard. With the platelet activation method proposed in the last decade, study of the various GFs released from activated PRP became popular, and preparation was diversified. Although peripheral venous blood factors influence the contents of the final PRP product, platelet activation was confirmed to be a key point in platelet counts and GFs: how to obtain more platelet counts with a small volume of PRP, how to improve the concentration of GFs released by PRP and which methods can bring about sustained release of GFs[6].

The most basic method to prepare PRP is centrifugation, divided into a one-step and two-step-centrifugation protocol (Fig. 1). The effect of separation by these two methods is still controversial. According to discarded and retained components, PRP is divided into different categories. The increase in commercial applications led to the development of PRP kits. Whether PRP is prepared by manual centrifugation or by use of kits, platelet concentration is significantly higher than in whole blood. However, the concentration of platelets obtained by each method still differs. In addition, platelet content is affected by the donor's gender (higher with female than male plasma) and personal physical aspects but not age. In contrast, the GF content is not influenced by age or gender. Even the content might differ in the same individual with use of different systems or different manufacturers.

The GFs will release after exogenous or endogenous activation. Different methods of activating PRP probably affect the concentration of GFs. PRPs are commonly activated by calcium chloride, thrombin, chitosan and batroxobin. Calcium chloride and thrombin activation are the two most common methods; 5% calcium chloride treatment for 19 min produces the most effective PRP, which have properties for soft-tissue adhesion[7]. Chitosan can be used instead of thrombin because it enhances aggregation, adhesion and expression of alpha-granule membrane glycoprotein. Furthermore, scaffolds by freeze-drying PRP with chitosan gel can produce sustained release of GFs[6]. This potential contributes to the durability of GFs in clinical and experimental studies. As well as platelet content, concentration of GFs differs between each person.

Bioactivity of PRP

The platelets in PRP range from 2 to 3 μm; proteomic studies have shown that platelets contain more than 800 proteins with numerous post-translational modifications[8], such as phosphorylation, for more than 1,500 protein-based bioactive factors. The physiologic actions of some of the proteins have been studied, including GFs, peptide hormones, and chemoattractants for macrophages, neutrophils, stem cells and several hundred other proteins, such as fibrinogen and fibrin. Fibrin acts as a provisional scaffold for stem or primary cell migration and differentiation and functions as a biological glue. Platelets also store proteins with antibacterial and fungicidal effects, coagulation factors, and membrane glycoproteins that influence inflammation by increasing the synthesis of interleukins (ILs) and chemokines. Dense granules in platelets also store and release adenoside diphosphate (ADP), adenoside triphosphate, calcium ions, histamine, serotonin, and dopamine, which are active in tissue homeostasis.

Platelets actively participate in healing processes by delivering a broad spectrum of GFs and other active molecules (e.g., chemokines, arachidonic acid metabolites, extracellular matrix (ECM) proteins, nucleotides, ascorbic acid) to the injured site by exocytosis following adhesion or stimulation by thrombin and other strong stimuli-like calcium. GFs secreted by platelets include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-I), transforming growth factor β-I (TGFβ-I), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF)[9]. This wide variety of GFs contribute to multifaceted roles of PRP,

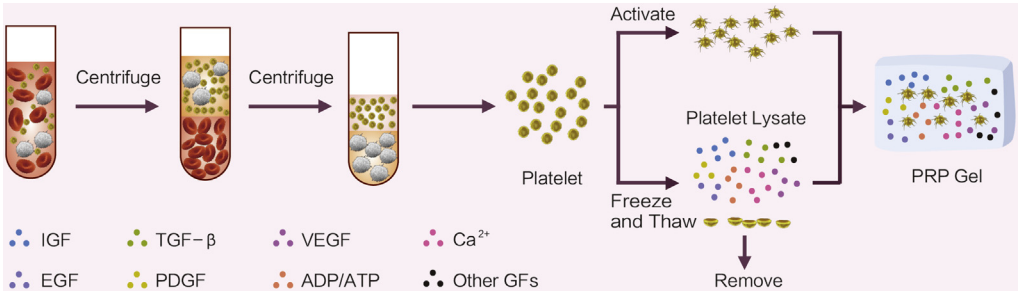

Fig. 1. Two-step-centrifugation protocol. The first centrifugation separate out the red blood cells and the second centrifugation to concentrate the platelets. Growth factors were released from platelet products.

including enhancement of anabolic, bone remodeling, proliferation, vessel remodeling, angiogenesis, inflammation, coagulation and cell differentiation[10]. These substances act in synergy on local cells inducing specific responses: promotion of proliferation, cell migration, and synthesis of ECM proteins including collagen, even changing the cell phenotype and arrangement[11].

Both native and exogenous molecules such as calcium, thrombin, ADP, collagen, and magnesium can activate platelets. Once platelets are activated, an initial burst of GF release is followed by further sustained release, a 3- to 5-fold increase as compared with baseline. Platelet activation increases levels of anti-inflammatory cytokines because of the presence of hepatocyte GF. These GFs have a particular function in bone remodeling and wound healing as well as stimulation of cartilage matrix synthesis and affect catabolic cytokines such as IL-1, IL-4, IGF-1, osteogenetic protein (OP)-1, FGF, TGFs and PDGF[12].

TGF-β is widely considered a promoter for chondrocyte anabolism *in vitro* (enhancing matrix production, cell proliferation, osteochondrogenic differentiation), and intra-articular injections help increase bone formation *in vivo*. It decreases type I collagen (col-I) gene expression, which simultaneously upregulates type II collagen (col-II) and aggrecan gene expression. TGF-β cooperates with bFGF to induce the migration and supplementation of bone-marrow stromal cells (BMSCs) toward the site of injury. It also stimulated cell homing, proliferation and chondrogenic differentiation[13]; sustained release of TGF-β is needed in the entire process of differentiation into cartilage. TGF-β facilitates cell proliferation by changing cell morphologic features. Therefore, both the quantitative and qualitative components of PRP are effective in mimicking the natural processes of soft-tissue wounding. In initiating and facilitating the formation of cartilage, PRP may be a potential candidate for inducing chondrogenesis and sustaining chondrocyte phenotype *in vitro* and *in vivo*.

Some GFs released from PRP may influence chondrocyte phenotype or differentiation. As stated above, the platelet count and leukocyte count in PRP may differ between different preparations from the same individual. TGF-β is a chondrocyte preserver by first regulating sulfation of glycosaminoglycans (GAGs), but along with elevating TGF-β1 activity has been suggested to be associated with elevated bone mass and OA[14]. Because of hypertrophia of articular chondrocytes in the proliferation process triggered by TGF-β1, levels of cartilage marker proteins aggrecan and col-II gradually disappeared[15].

From the bioactivity of PRP, the extensive application of PRP in different fields, including bone, ligaments, rotator cuff repair, and skin damage, is also used for the reduction of postoperative complications, subjective pain and improving tissue healing[16]. In the numerous GFs of PRP release, part of them have a conducive to maintain the phenotype of chondrocytes, others are not, how to use the needed side is the direction of our future study.

Application of PRP for cartilage cell culture (Table Ia)

Articular cartilage, unlike most tissues, has special nutritional requirements and is extremely difficult to repair spontaneously after injury because of lack of blood supply. With the boom in tissue engineering technology, treatment with cultured autologous cartilage cells and engineered tissue for repairing the articular CDs has been effective. Obtaining sufficient chondrocyte numbers for therapy requires *in vitro* expansion. The standard methods for cartilage cell expansion involve culture medium with FBS or serum-free medium. FBS may contain exon-antigen and cause immune-related problems, and serum-free medium is expensive, so a substitution for FBS and serum-free medium is needed. Studies of cultivating chondrocytes in PRP or human platelet supernatant showed the promotion of cell proliferation in monolayer culture and with use of a 3-D environment such as alginate microsphere (Fig. 2); it also increased the expression of GAG and col-II (Table I). The use of PRP even slightly enhanced mitogenic stimulation as compared with FBS. However, some reports indicated that PRP activated only chondrocyte proliferation but not differentiation. The mRNA levels of col-II, aggrecan and bone morphogenetic protein 2 were reduced, but col-I expression was considerably induced[17,18]. If PRP can maintain the morphology and characterization was not clear, dedifferentiation of chondrocytes was still happened. These different results may due to different preparation of PRP and the different GFs in the PRP from different individuals. The standard methods for preparation of PRP need further study.

PRP for MSC differentiation to cartilage cells *in vitro* (Table Ib)

MSCs are multipotent stem cells that can be obtained by numerous approaches. They have multi-differentiation potential, especially high chondrogenic differentiation potential and rapid growth rate, which can be used effectively in damage repair and tissue engineering. Transplanted MSCs seeded with col-I hydrogels were first used to repair CDs in human OA knees. *In vitro*, MSCs can be induced in different cell type directions by use of different induction media and possess potent immunomodulatory and anti-inflammatory effects[19]. PRP added to the culture medium retains the immune-regulatory effect of MSCs. It can decrease alloantigen-induced cytotoxic activity, favors differentiation of $CD4^+$ T-cell subsets expressing a Treg phenotype and increases early secretion of IL-10 as well as induces a striking augmentation of IL-6 production. PRP in medium also showed resistance to spontaneous transformation into tumor cells as demonstrated by molecular karyotyping and maintenance of normal morphology/phenotype after prolonged *in vitro* culture[20].

PRP stimulates adhesion migration and proliferation of stem cells and also enhances chondrogenic differentiation. It significantly stimulates chondrocyte GAG synthesis and tends to increase col-II level. PRP was more reproducibly efficient than FBS in supporting MSC outgrowth. This may be related to the GFs released from PRP.

However, some reports expressed inconclusive results. A tendon bone healing experiment after ACL reconstruction suggests that 60% PRP has good biocompatibility and osteoinductive capacity[21]. Also, Stromal stem cells cultured in 10% PRP show the high proliferation rate and cells return to their regular proliferation rate and osteochondral potential after PRP is withdraw[22].

PRP for OA *in animal model* (Table IIa)

OA is widely considered an age-related degenerative disorder often resulting in chondral lesions, which presents as cartilage wearing away so that the underlying bones are exposed and rub together painfully. Cartilage lacks innate abilities for a sufficient healing response. Thus, damaged tissue is not replaced with functional tissue. Histology shows the inflammatory reaction in the affected joints. OA progression is slow and may occur over 15–30 years. Viscosupplementations, such as intra-articular hyaluronic acid (HA), oral glucosamine, or chondroitin-sulfate, are commonly used as supportive treatment. However, the usefulness remains controversial given the paucity of supporting evidence. Trying to prevent cartilage ECM degenerating is effective in defending OA progress. For economic and methodological considerations, the use of bioactive agents such as recombined GFs in an appropriate carrier to help the defect heal itself has been of interest[23]. Recently, experimental study has identified the effects of PRP on OA in

Table I
PRP application *in vitro*

Cell type	Classification of PRP[58]	PRP application to culture	Outcome	Reference
a. PRP for chondrocyte culture				
Human chondrocytes	P2-x-NA	Cells seeded on gelatin microcarriers sealed with PRP	Improve cell proliferation and ECM synthetize and maintain cell phenotype	59
Porcine chondrocytes	P3-x-Aα	10% PRP release in serum-free medium	Improve cell proliferation and matrix biosynthesis, PG and collagen synthesis	60
Human chondrocytes	P4-B	In mono- and 3-D cultures	Improve cell proliferation and ECM synthetize and maintain cell phenotype	61
New Zealand white rabbit chondrocytes	P4-NA	20% PRP in DMEM	Improve cell proliferation and increase aggrecan, BMP-2, BMP7, col-II expression in the long-term	62
Japanese white rabbit chondrocytes	P4-x-NA	3% PRP with alginate beads	Increase chondrocyte GAG synthesis and maintain cell phenotype.	28
Human articular chondrocytes	NA-x-Bβ	In mono- and 3-D cultures	Improve chondrocyte proliferation but reduce type II collagen, aggrecan and BMP-2	17
Bovine articular chondrocytes	NA-x-Bβ	In mono- and 3-D cultures	Improve chondrocyte proliferation but reduce type II collagen, increase type I collagen	18
b. PRP for MSC culture				
Human mesenchymal stem cell (HMSC)	P2-Aα	10% PRP in DMEM	Improve cell proliferation and induce chondrogenesis	63
BM-HMSCs	NA–NA	10% HPL	Improve cell proliferation	64
BM-HMSCs	P3-NA	5% HPL	Improve cell proliferation and decrease alloantigen-induced cytotoxic activity	20
BM-HMSCs	P3–B	10% HPL	Improve cell proliferation, preserve phenotype and differentiation capacity	65,66
BM-HMSCs	P2-x-Bβ	10% PRP	Improve cell proliferation and migration	67
Human subchondral progenitor cells	P4-NA-B	5% PRP	Improve cell proliferation and induce chondrogenesis	68
Human adipose tissue-derived MSCs	P4-x-NA	10% PRP	Improve cell proliferation, preserves differentiation capacity and immunophenotype	69
Mouse muscle-derived stem cell (MDSC)	P3-x-NA	10% PRP	Improve cell proliferation and upregulate type II collagen	70
Nude rats MDSC	P2-NA	Pellet culture added PRP combined with a VEGF antagonist and BMP-4	Increase type II collagen	24
Sheep MDSC	NA-x-NA	High-density micromass culture in PRP	Improve cell proliferation	71
Human BMSC	P3-NA	Pellet culture with 5% HPL	Increased cell proliferation formed evident and clear cartilage	72

P1, P2, P3, P4 represent platelet concentrations (platelets/μL): P1, ≤baseline levels; P2, >baseline levels to 750,000; P3, 750,000–1,250,000; and P4, >1,250,000. Endogenous activation has no designation. If an exogenous external activator is used, it is documented with an x. Total leukocyte content in buffy coat is identified as A, >baseline level, or B, ≤baseline levels; neutrophil count is identified as α, >baseline level, or β, ≤baseline levels. NA, not applicable.

animal models such as rats[24,25] and rabbits[26–29]. PRP has become an optimal candidate agent for OA because of its milieu of GFs, it is easily obtained and prepared. The effects of PRP include few postoperative complications, alleviating pain, antimicrobial effects and anti-inflammatory effects[30]. Its initial inhibition of macrophage proliferation may explain this phenomenon.

In an OA model induced by formalin, collagenase, or anterior cruciate ligament transfection, treatment with PRP/gelatin hydrogel injected in knee joints increased mRNA expression of proteoglycan core protein in the articular cartilage and decreased chondrocyte apoptosis[24] and suppressed progression of OA[28]. The effects were related to severity of OA[27]. PRP combined with stem cells injected into knees increased col-II content and decreased chondrocyte apoptosis[24] The most common PRP application is injection, which avoids the adverse effects of surgery.

In addition, with osteoarthritic chondrocytes cultured in the presence of IL-1 to mimic the osteoarthritic environment, PRP can diminished multiple inflammatory IL-1 mediated effects. It has analgesic, antibacterial, anti-inflammatory activity; balances joint angiogenesis, coagulation and hemostasis; increases GAG level; and stimulates chondrocyte synthesis cartilage matrix, for stem or primary cell migration, differentiation, as well as wound healing. PRP may become an attractive candidate to remedy and protect against cartilage degeneration in OA.

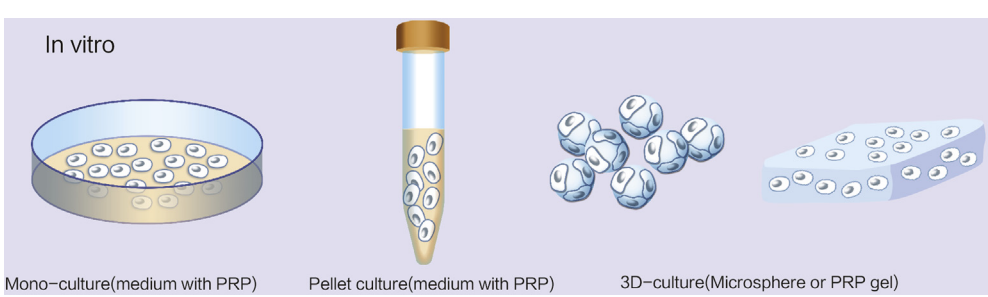

Fig. 2. Chondrocytes expansion *in vitro*.

Table II
PRP application in animal models

Animal model (symptom)	Classification of PRP[58]	PRP application to joint	Study outcome: effect because of PRP	Reference
a. OA				
Male New Zealand white rabbits (OA)	P4-NA	PRP injection	Higher gross morphologic and histologic scores, stimulate cell proliferation and matrix metabolism	27
Nude rats (OA)	P2-NA	PRP + MDSC injected into knee	Increase type II collagen content and decrease chondrocyte apoptosis	24
Wistar rats (OA)	P3-x-NA	PRP gel injection	Moderate degeneration of cartilage layer	25
Japanese white rabbits (OA)	P4-x-NA	PRP + gelatin hydrogel microsphere injection	Lower gross morphological OA scores, suppressed progression of OA	28
b. CD				
Sheep (full-thickness CD)	P2-x-NA	Microfracture + PRP gel	Healthy surrounding cartilage showed intense cell proliferation with formation of cell clusters	31
	P2-NA	Microfracture + Liquid PRP injection	A great amount of chondrocyte-like cells well organized in columns	
Rabbits (full-thickness CD)	P4-x-NA	Microfracture + PRP + polylacticglycolic acid (PLGA) implantation	Induced regeneration of cartilage and subchondral bone into and around the defect	26
New Zealand white rabbits (full-thickness CD)	P4-Bβ	PRP + bilayer collagen scaffold	Forms cartilage tissues, produces more GAGs	29
New Zealand white rabbits (osteochondral defect)	P4-x-Bβ	MDSC + PRP	Better gross appearance and histological and immuno-histochemical characteristics, higher cartilage-specific gene and protein expression	32
		ADSC + PRP	Functional chondrocytes secrete cartilaginous matrix	
New Zealand rabbits (CD)	NA-x-NA	Injection autologous cell-PRP composite	Good macroscopic result, forms mature and homogeneous cartilage with more collagen PG and GAG	73

PRP for CDs *in animal model* (Table IIb)

CDs caused by trauma, acute injury in sports or any other movements forms shaped and regular gaps in cartilage. Such defects can be divided into three depth categories: partial thickness defects, with the defect entirely contained in the cartilage layer; full-thickness defects, with the defect extending down to but not into the subchondral bone; and osteochondral defects that extend into the subchondral bone. PRP for these three types also can be divided into three modalities: (1) acellular repair technology, including PRP injection and infiltration or PRP with polylactic/glycolic acid copolymer polylactic acid-coglycolic acid (PLGA) scaffolds[26]; (2) PRP with cells, including chondrocytes or MSCs; and (3) cell-based tissue engineering, including cells seeded with PRP gel or on PLGA scaffolds, or microfracture and matrix-associated autologous chondrocyte transplantation (MACT) with PRP. PRP enhances the morphological stability of the constructs, maintains chondrocyte phenotypes and induces chondrogenesis,

PRP added to a cartilage ECM scaffold combined with microfractures to treat chondral defects improved mechanical and biochemical quality of repaired tissue[31]. Comparison of the chondrogenic differentiation ability of BMSCs and adipose-derived stem cells (ADSCs) seeded in a PRP scaffold to repair rabbit CDs revealed that a PRP-derived 3-D scaffold has the capacity for endogenous GF release and induces both BMSC and ADSC proliferation and expression of cartilage marker genes and proteins. Cartilage differentiation was better with BMSCs than ADSCs *in vitro*, but both repaired CDs in rabbit[32,33]. Although PRP promote bone reconstruction and mature *in vitro*[34] and sometimes can be found highly amorphous cartilaginous repair tissue and poorly spatially organized underlying bone tissue[35], even a dissatisfied results on cartilage repair in sheep models[31], PRP still give us highly promising. The restriction of repair has been found in PRP alone medium due to angiogenic factors released from PRP. So they use sFlt1 (an angiogenic antagonist) to block the angiogenesis and got the ideal result[24]. In conclusion, the environment of healing site and GFs stimuli are both important to tissue repair.

PRP for clinical application in OA (Table IIIa)

The healing process of OA comprises three phases: (1) inflammation, (2) cell proliferation, and (3) remodeling. Regenerative therapy involves injecting a small volume of solution into multiple sites of painful ligaments and tendons and adjacent joint spaces to reduce pain and promote tissue repair and growth [Fig. 3(b)]. PRP was designed for this purpose. It theoretically augments tissue healing through the natural healing cascade. GFs are released from the granules of platelets and induce chemotaxis, cell migration, angiogenesis, proliferation, differentiation, and matrix production. PRP also enhances HA secretion and increases release of angiogenic GFs[33,36].

Kon *et al.* first reported on intra-articular PRP injections at 21-day intervals to 115 osteoarthritic knees, for a total of three sets of injections. International Knee Documentation Committee (IKDC) scores demonstrated statistically significant improvement at 6- and 12-month follow-up as compared with at baseline[37]. The authors studied PRP vs HA injections in 150 patients, with PRP treatment giving better results than HA in reducing pain and symptoms and recovering articular function[38]. As well, 2-year follow-up of 90 of 181 patients[39] revealed the long-term PRP effect in treating knee degeneration. The same results were found in recent studies[40,41]. In 30 cases, PRP was successful at week 5[42]. PRP is safe and effective for treatment of knee OA[43,44]. More promising results are shown for PRP use in younger than older patients, and milder than more severe cases had better outcomes and longer-lasting results. HA as a scaffold combined with PRP showed a durable property and repair of hyaline cartilage[45].

PRP injection was first proposed by Sanchez *et al.* to improve symptoms and accelerate healing in nontraumatic avulsion fractures of the articular cartilage in soccer players[46]. As a promising way to treat degenerative cartilage lesions (DCL) without surgery, research in this field is popular. Preliminary results of 100 patients indicated that PRP injection is safe and has potential to reduce pain and improve knee function[37]. A 2-year follow-up clinical trial of intra-articular injection of PRP in the knee also found the same results and showed improved quality of life, especially in young males[39]. Similar results were confirmed in recent studies[47,48].

Table III
PRP application in the clinic

Symptom	Cases	Classification of PRP[58]	Treatment/procedure	Time	Outcome	Reference
a. OA						
OA	261	P2-x-Bβ	3 injections of PRP (5.0 mL, 2-week intervals)	3 and 6 months	A significant improvement in pain, stiffness, function, and the Lequesne Index	74
DCL/OA	181	P4-x-NA	3 PRP lesion site injections (5.0 mL, 21 day intervals)	1 and 2 years	IKDC objective score decreased to 59%; IKDC subjective score decreased to 51	39
OA	134	NA–NA	6 injections of PRP (2.0 mL, twice a week)	7, 13 and 26 weeks	WOMAC subscale scores largely reduced, decreased VAS score, improved German Orthokine Osteoarthritis Trial (GOAT), KOOS and knee society clinical rating scale (KSCRS).	75
DCL/OA	72	P4-x-A	3 PRP lesion site injections (5.0 mL, 21 day intervals)	2,6 and 12 months	Level: II. Improved IKDC subjective score and EQ-VAS	47
Kellgren–Lawrence grade I-III OA	65	P3-Aα	PRP injection (3.0 mL)	1, 3, 6, 9, and 12 months	Improved IKDC score and VAS score	76
OA	60	P3–B	Intra-articular injections (3.0 mL)	3 and 6 months	Decreased NRS score and WOMAC scores decreased slower	40
Degenerative joint	54	P3-A	3 PRP injections (5.0 mL, weekly injections)	2, 6, and 12 months	IKDC subjective score increased to 64.9 ± 16.8 at 12 months; Tegner score improved to 3.8 ± 1.3 at 12 months	43
DCL/OA	50	P4-x-NA	3 autologous PRP intra-articular injections (5.0 mL, 14 day intervals)	2 and 6 months	Level: II. Longer efficacy, reducing pain and symptoms and recovering articular function	38
OA	47	P1-x-B	PGA-hyaluronan scaffold + PRP gel + fibrin-like glue	3, 6, 9, 12 months	Level: IV. Improved median KOOS pain subcategory and symptom subcategory	45
OA	40	NA-x-B	PRP injection (6.0 mL, 1–2 weeks intervals)	6–7 weeks and at 6 months	WOMAC scores decreased slower, decreased VAS score	77
OA	33	P4-x-NA	PRP + MSCs injection (3.0 mL)	3 months, 1 year and 24.3 months	Level: IV. WOMAC scores decreased slower, improved Lysholm scores, changed VAS 2.0 ± 1.1. MRI score improved to 21.7 points.	78
Knee OA	30	P2–B	PRP injection (6–8 cc, weekly injections)	5 weeks	WOMAC scores decreased slower	42
Arthritis and degenerative joint disease	27	P3-x-NA	3 PRP intra-articular infiltration (5.0 mL, weekly injections)	6 months	Decrease NRS score and improve WOMAC scores	79
Knee OA	25	P2-A	2 autologous PRP intra-articular infiltration (4.0 mL, 1 month intervals)	6 and 12 months	Improved the KOOS score	80
Early OA	22	P2-NA-Bβ	Intra-articular injections (6.0 mL)	1 week, and 1, 3, 6, and 12 months	Improved WOMAC score and decrease VAS scores	48
Knee OA	14	P4-x-Aα	3 PRP injections (6.0 mL, at 4 weeks intervals)	2, 5, 11, 18 and 52 weeks	VAS showed many improvements, reduced pain.	44
b. CD						
Osteochondral patellar lesions	5	NA-NA-Aα	Microfracture + PRP gel beneath the collagen I/III membrane	2 years	Level: IV. MOCART score remains stable in 2 years	81
Osteochondral lesions	23 26	NA-x-Bβ	Autologous cell concentrate + PRP gel 2 mL of bone marrow concentrate+1.0 mL PRP gel (PRP-fibrin)	24, 36, 48 months	Improved AOFAS score, hyaline cartilage in 4 years	82
Full-thickness CD	5	P1-x-B	PRP gel + PGA-hyaluronan scaffold	3, 6, 9, 12 months	Level: IV. Improved median KOOS pain subcategory and symptom subcategory	45
Osteochondral lesions	25	P2-x-Bβ	2 mL bone marrow concentrate and with 1 mL PR-fibrin glue	12, 36, 130 months	Improved AOFAS score, highlighted hyaline cartilage in biopsy specimens	51
Osteochondral lesions	5	NA-x-Bβ	2 mL bone-marrow concentrate and with 1 mL PR-fibrin glue	6, 12, 18, and 24 months	Improved AOFAS score, PG replace collagen, increased type I and type II collagen	83
Osteochondral lesions	20	NA-x-Bβ	2 mL bone-marrow concentrate and with 1 mL PR-fibrin glue	2 years	Improved AOFAS score, good T2-mapping sequence result and MOCART score	84
Full-thickness CD	5	P3-NA	Bone-marrow mesenchymal stem cells (BM-MSCs) + PR-fibrin glue	6, 12 months	Level: IV. Improved Lysholm and RHSSK scores	49

T2-mapping gives information about the interaction between water molecules and the collagen network within cartilage and represents a fascinating non-invasive diagnostic technique for the qualitative evaluation of cartilaginous tissue. MOCART score is using different variables to describe the constitution of the cartilage repair tissue and the surrounding structures. WOMAC Score: Knee Injury and Osteopaedic Outcome Score. IKDC Score: International Knee Documentation Committee Score. VAS Score: Visual Analogue Scale Score. KOOS Score: Knee injury and Osteoarthritis Outcome Score. AOFAS: American Orthopaedic Foot and Ankle Society. RHSSK Score: Revised Hospital for Special Surgery Knee Score.

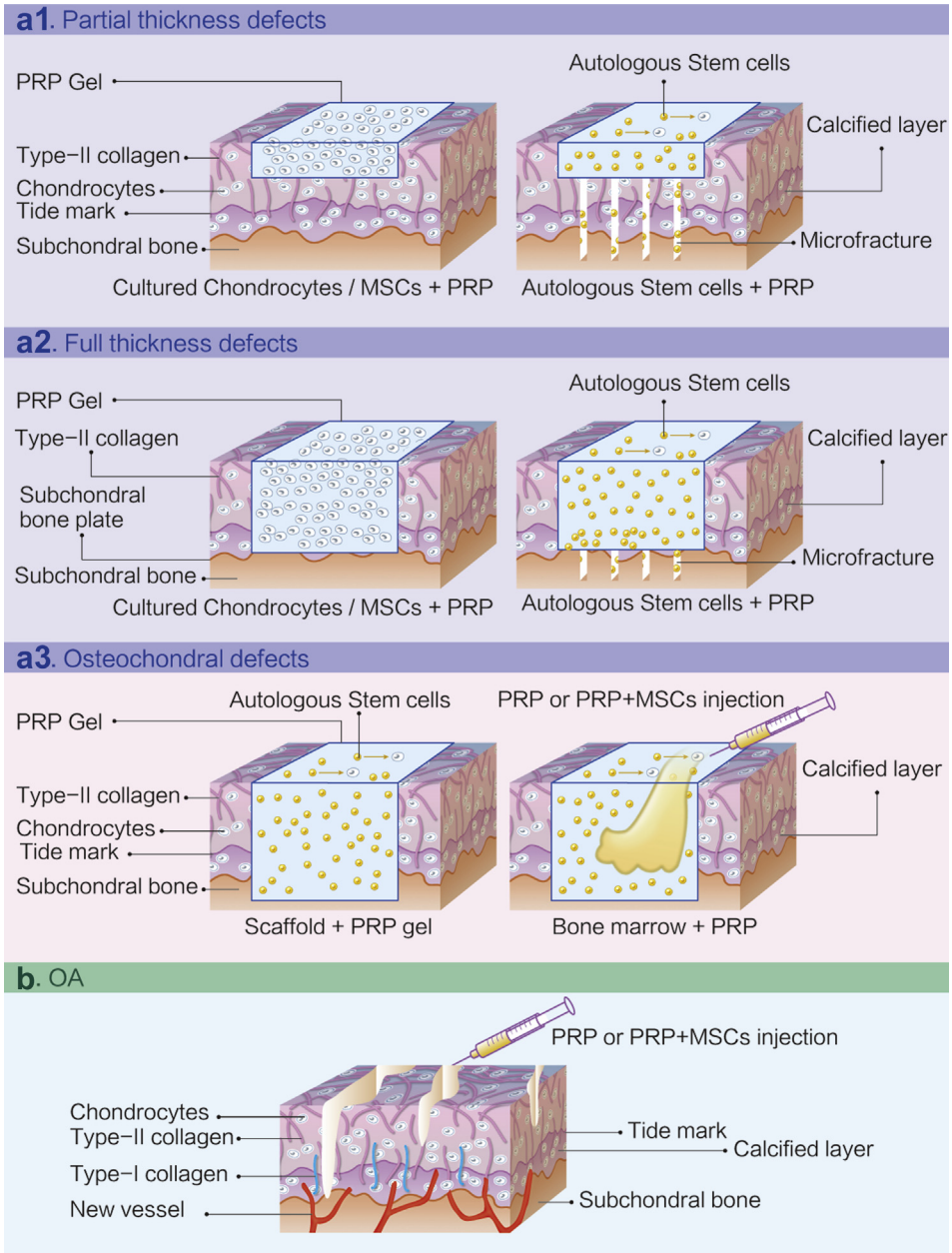

Fig. 3. PRP clinic application. PRP has been wildly used in CDs (a1, a2, a3) and OA (b) now. Autologous stem cells from bone marrow through microfracture with PRP gel (PR-fibrin glue) was frequently be used in partial thickness defects (a1 right figure) and full-thickness defects (a2 right figure). Cultured autologous chondrocyte or MSCs using PRP gel is also utilized for repairing in partial thickness defects (a1 left figure) and full-thickness defects (a2 left figure). As for the therapies of osteochondral defects, PRP gel with scaffold (a3 left figure) and bone marrow concentrate injection with PR-fibrin glue (a3 right figure) were used in most cases. Comparing the therapies of CD, OA has much less choices for its complexity. Reports showed that injection (or infiltration) therapies are the most common choice (b).

PRP has gained popularity in sports medicine and orthopedics to accelerate physiologic healing and a return to function. PRP is used for hemostasis and for total joint arthroplasty for OA. It can be used intraoperatively in conjunction with a fibrin sealant or a gel and even alone during total knee arthoplasty. PRP is used for knee and hip joints. Its use is associated with reduced inflammation, pain relief, improved function, and possible cartilage regeneration. The persistence of the beneficial effects remains to be evaluated. Strong evidence from well-designed clinical trials to support PRP therapy for OA of the knee is needed. Different PRP products might be more or less appropriate to treat different types of tissues, and pathologies also remain too many disputes.

PRP for CDs in the clinic (Table IIIb)

PRP has been found being a good carrier and facilitator for cartilage tissue engineering by reducing inflammation and improving cartilage repair. Especially, it helps stem cells differentiate to cartilage and bone in osteochondral defects [Fig. 3(a)]. MSCs seeded with PRP improving the repair of articular CDs was first proposed by Haleem *et al.*[49].

In recent years, acellular repair technology has used PRP with scaffolds such as HA, hydrogel or PRP gel, the solid state of PRP, to imitate the native environment. A polyglycolic acid (PGA)-hyaluronan scaffold with PRP added in drilling can lead to the formation of hyaline-like cartilage after 1 year[45].

Table IV
Dual character of PRP use for cartilage repair and OA in clinical

The strong facts for the use of PRP in clinical	The poor support for the use of PRP in clinical
Improve WOMAC, AOFAS and VAS scores.	Increased type I collagen which not exist in hyaline cartilage.
Symptomatic relief in early OA and improve clinical outcome[85].	Produce fibrous cartilage.
Inflammation reduction.	Stimulates angiogenesis[86]

PRP with a collagen scaffold in tissue engineering can improve bone-marrow–initiated cartilage repair and stimulate the repair of large articular cartilage in animal models[29]. Also, hydrogel combined with chondrocytes and PRP may be an ideal environment for proliferation and maturation of articular chondrocytes[50]. MACT with PRP can improve cartilage repair in animal models. The same group started the clinical application in 81 patients with osteochondral lesions; the American Orthopaedic Foot and Ankle Society score was improved and expression of col-II and proteoglycan increased after 4 years[51].

Safety of PRP treatment

PRP has antimicrobial effects *in vitro*[30]; it inhibits macrophage proliferation and reduces the inflammatory response[52]. Autologous PRP was found safe for treating knee, foot and ankle lesions in 634 patients[53] and for articular cartilage degeneration[54]. No side effects have been reported. In 261 patients, no adverse effects were found following injection of PRP-fibrin glue into the knee joint at 6 months[55].

Many studies have suggested safety, pain relief, and functional improvements with PRP injections for OA; adverse effects are rare risks, and benefits have been thoroughly reviewed. Risks and benefits are similar to any other intra-articular or peri-articular injection; adverse effects include infection, bleeding, bruising, peripheral nerve injury, allergy to local anesthetics, and temporary exacerbation of stiffness and soreness that may last from 2 to 7 days[56].

Conclusions

Platelet products such as PRP, HPL and platelet supernatants, which can be activated by endogenous or exogenous activators, have high efficiency and multiple functions. Because of the autogenous source, PRP is easy and convenient to extract, and processing is relatively simple and short; it features high-speed recovery potential, easy handling and offers multiple GFs at relatively inexpensive cost. Above all, its use is safe.

Basic science, preclinical, and clinical studies collectively indicate that PRP is promising for treating cartilage injuries and joint pain. PRP in a culture environment has an anabolic effect on chondrocytes and bone-marrow derived stem cells with resulting increases in cell proliferation and matrix production as well as an anti-inflammatory effect via downregulation of known catabolic signaling pathways. It may be a feasible, secure, and economic way to induce MSC differentiation into chondrocytes integrally and expand cartilage cells *in vitro*. It is a more economic and effective culture medium substitute for FBS. When added on scaffolds of cartilage tissue-engineered constructs, it can enhance the regeneration of cartilage cells and repair CDs. The application of PRP for OA in clinical trials has shown promising short-term results (1–2 years), although most of these studies were not randomized controlled trials.

However, challenges remain (Table IV). First, platelet quality influences effectiveness, including platelet content, leukocytes and GF concentration, because preparations of PRP have no selection criterion. Platelet count in PRP may vary from two- to several-fold depending on the donor's physical condition, age or gender, which leads to unstable and non-repeatable PRP treatment. Some of the GFs present in PRP such as TGF-beta and bFGF have negative effects on the OA joint which differ from effects in more normal joints. Because of multifunctional GF effects, chondrogenesis of MSCs or chondrocytes expanded *in vitro* may not retain the chondrocyte phenotype, such as expression of Col-I instead of type II, simultaneous hypertrophy and bone marker expression. So directed stimulation of GFs might be considered in MSCs and chondrocyte culture to maintain the chondrocyte phenotype. When several GFs exist simultaneously, it might inhibit chondrogenesis effect, such as VEGF and bone morphogenetic protein 2. Impairing osteogenesis by PRP via mutual cooperation with other biological molecules may provide new ways that are prompt, stable, and controllable for maintaining cartilage morphologic features or to promote chondrogenic differentiation.

Second, with the wide range of methodologies used in each study and the numerous ways to prepare PRP, we cannot provide firm recommendations regarding the type of PRP to use and for what indications. The methods of platelet application include liquid injection, PRP gel and bonding with bio-scaffolds. Obviously, with liquid injection, the required mechanical environment of cartilage formation is difficult to achieve; moreover, implanted scaffolds may incur unexpected risk and lack integration. The roles for the respective treatment regimens still need to be defined, because many of the questions concerning PRP mechanisms of action remain unanswered.

Future directions of PRP application in OA therapy may concentrate on seeking an appropriate and innocuous agent like anti-VEGF antibody that can modulate and control the effect of PRP by biological integration, when blocking VEGF, defects were repaired mostly with hyaline cartilage[57].

Author contributions

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Competing interests

The authors declare no competing interests.

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