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Perspectives for using platelet-rich plasma (PRP) in the treatment of knee osteoarthritis - can it be improved through modifications of the protocol? (Analytical review).

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Abstract:	Autologous platelet-rich plasma (PRP) injections are widely used in regenerative medicine, including the knee osteoarthritis (OA) therapy. This study reviews methods to enhance PRP therapy for knee OA, aiming to boost articular cartilage recovery. Practices such as combining with hyaluronic acid, pre-injection PRP activation, and multiple administrations are clinically common, while other methods like adjusting growth factors concentration are still in development. Various modifications of this technology allow to use molecular mechanisms involved in the restoration of hyaline cartilage and improve the effectiveness of PRP for the treatment of OA.
Suggested Reviewers:	
Opposed Reviewers:	
Response to Reviewers:	

Cover letter

This review paper is devoted to an important topic, in our opinion, about the use of PRP for the restoration of joints in OA. Autologous platelet-rich plasma (PRP) effectively treats osteoarthritis (OA) through various protocols. This study reviews methods to enhance PRP therapy for knee OA, aiming to boost articular cartilage recovery. Practices such as combining with hyaluronic acid, pre-injection PRP activation, and multiple administrations are clinically common, while other methods like adjusting growth factors concentration are still in development. Various modifications of this technology allow to use molecular mechanisms involved in the restoration of hyaline cartilage and improve the effectiveness of PRP for the treatment of OA. However, there is a limited number of studies specifically addressing PRP technology modifications for OA treatment. We believe that our work will help researchers and practitioners to look at this topic differently and perhaps reconsider (or modify) their treatment protocols.

Reviewer #1:

Dear reviewer. Thank you very much for your time. We are grateful to you for your work. We have taken your comments into account. We have added some to the text, and we are responding to you personally with some.

Since this is a review paper, I would like to know how the authors collect the papers.

Please show the key words used when identified the 5044 papers at beginning.

ANSWER

osteoarthritis, OA, platelet-rich plasma, PRP, knee, hip, vascular endothelial growth factor (VEGF) , epidermal growth factor (EGF), hyaluronic acid , fibroblast growth factor, transforming growth factor beta , platelet growth factor ,gel, chondroitin sulfate , Platelets, leukocyte, CaCl2, activation, thrombin ,volume and THEIR COMBINATION

In Line 74, please describe two stages.

ANSWER

We have fixed it.

In Line 86, only 4 reviewers can do the screening of 2000 papers. Please let me know how long did it take to finish this project.

ANSWER

The task of finding information for this article was non-trivial and difficult. The idea of the work appeared when the authors realized that there is a method for increasing the efficiency of the PRP protocol by removing the VEGF protein from the cocktail. Then we realized that there are other fundamental methods for modifying the procedure, for example, by different activation of PRP. After that, the authors decided to review all available articles on this topic (by keywords), analyze them and identify the fundamental methods of modifying the protocol. The result of this stage (step 1) was the identified fundamental modification options, however, detailed information on each of them was not systematized. Then, the second stage of the search began, when an in-depth search was carried out for each modification option and new information was found that was difficult to identify (or impossible) at the first stage. The work on writing the article was carried out for a year and a half. We were very helped by the fact that the team of authors had multiple publications on related topics, as well as extensive clinical practice in this area.

In each result section, please show the number of papers in each modification method.

ANSWER

We think that it is not necessary to indicate the number of articles for each section directly in the article, for several reasons:

the journal rules limit us to the number of sources in the article (no more than 70),

we are also limited to the amount of text in the article itself.

In this regard, we were forced to take a limited number of publications for each section, choosing only the most important and most recent publications.

However, the number of articles we found for each fundamental method was different. Currently, there are few publications related to changing the protein composition of PRP, while there are many more publications related to the activation of the cocktail and the administration of the number of applications of this procedure.

We believe that this is due to the scientific novelty of this promising area.

==========

Reviewer #2:

Dear reviewer. Thank you very much for your time. We are grateful to you for your work. We have taken your comments into account. We have added some to the text, and we are responding to you personally with some.

First of all, you have to clarify the type of the study (e.g. review?)

ANSWER

We have fixed the title.

.....and to follow the relative format (e.g. you mention "prisma" guidelines in a figure legend but you didn't refer to in the manuscript)

ANSWER

We have fixed the figure1.

Secondly, there is no clear categorization of the "modifications" options and no justification for the choice of the specific one. Your methods have to clearly stated and be explained.

ANSWER

By modification we mean a change in the standard PRP protocol for the recovery of hyaline cartilage in OA by:

a) removing growth factors

b) addition of biologically active substances or other compounds

c) changing the cellular composition of PRP

d) due to the method of activation

e) due to the multiplicity of the mode of administration of PRP

We introduced this classification based on a two-stage search and analysis of modern scientific literature on this topic

The language lacks to express the meaning of your research (e.g. not appropriate words: "cocktail of PRP", "recovery of OA")

ANSWER

You are absolutely right. It was a mistake. We have fixed it.

There are several mismatches and/or wrong references numbers (e.g. line 211 the ref is 50 not 51).

ANSWER

You are absolutely right. We have fixed it.

Methods

Lines 90-98: Could explain what did you do if there was any overlap of the 5 "modification" option in a study?

ANSWER

Articles on the modification options did not overlap. We found enough publications on each of the modification options. We took only the most recent and most important articles, as we were limited in the amount of literature.

However, the number of articles we found for each fundamental method was different. Currently, there are few publications related to changing the protein composition of PRP, while there are many more publications related to the activation of the cocktail and the administration of the number of applications of this procedure.

We believe that this is due to the scientific novelty of this promising area.

Lines 105-107: What "manuscript" do you mean? The present one? If yes the sentence is not clear

ANSWER

You are absolutely right. We have fixed it. We have removed this sentence.

Results

Lines 109-121: It is a little bit confusing this part. How many studies did you finally include in the review? What are these "5" experimental animal studies? Are they part of the total? Why did you not refer to the "bias" of the whole list of the included tudies?

ANSWER

The work itself presents 70 sources of literature. We are limited by the rules of the journal, so we were forced to present only these 70 works. Some of these 70 works (these 5 works) concern experiments on animals and for them we made a bias risk assessment. We believe that it is not necessary to conduct a bias assessment for non-animal articles in the review.

Discussion

This section should start with the most important finding of the study. You do not state it clearly at all, but you present your aim and it is assumed that the reader will conclude to it.

ANSWER

You are absolutely right. We have fixed it in the text.

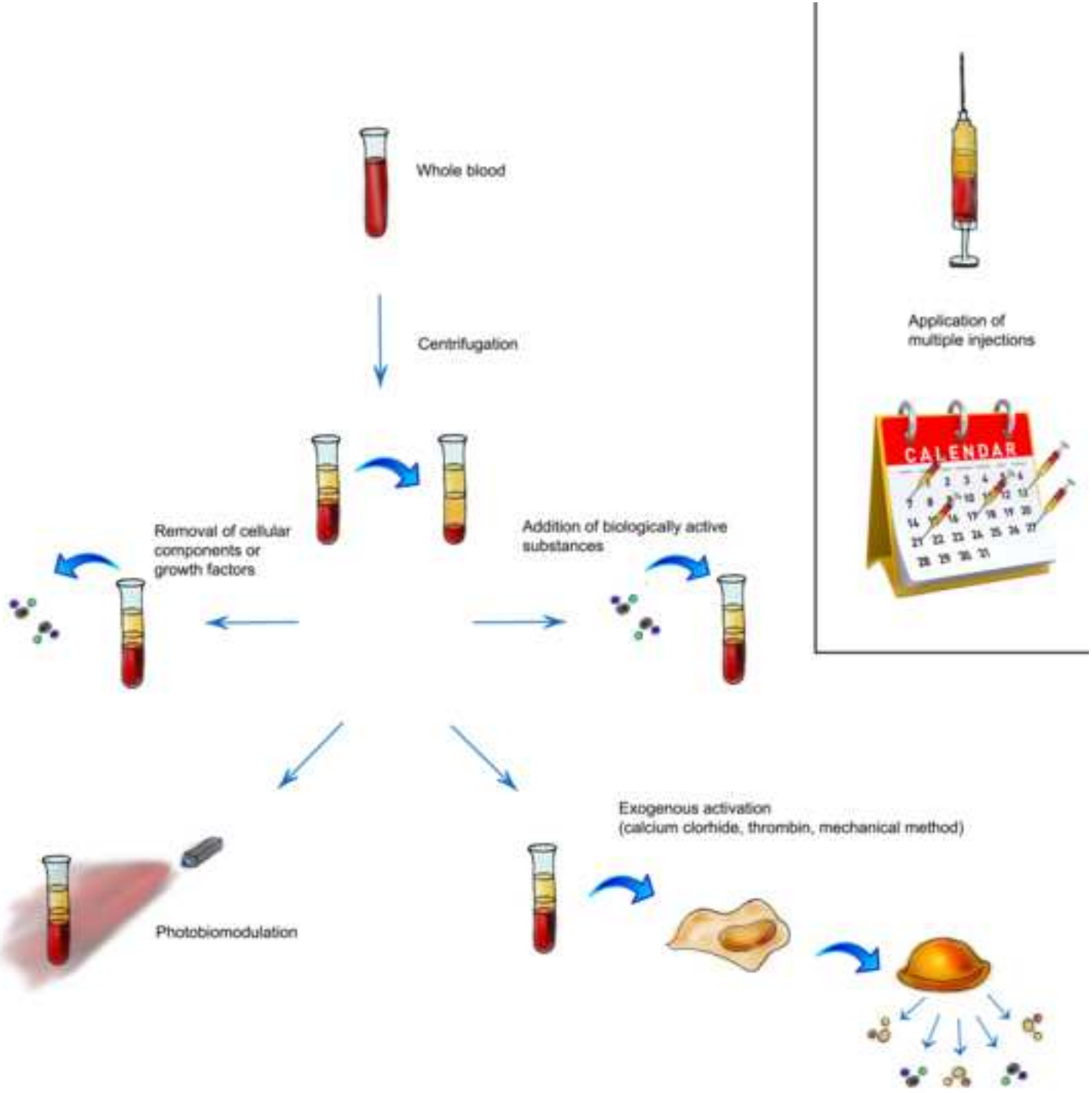
Conclusion

Your conclusion does not answer to your hypothesis/purpose. You have to clearly stated what are the modifications, their use (clinical practice and/or experimentally)

ANSWER

You are absolutely right. We have fixed it in the text.

Graphical Abstract



Perspectives for using platelet-rich plasma (PRP) in the treatment of knee osteoarthritis - can it be improved through modifications of the protocol? (Analytical review).

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No

Statements and Declarations

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Perspectives for using platelet-rich plasma (PRP) in the treatment of knee osteoarthritis - can it be improved through modifications of the protocol? (Analytical review)

ABSTRACT

Autologous platelet-rich plasma (PRP) injections are widely used in regenerative medicine, including the knee osteoarthritis (OA) therapy. This study reviews methods to enhance PRP therapy for knee OA, aiming to boost articular cartilage recovery. Practices such as combining with hyaluronic acid, pre-injection PRP activation, and multiple administrations are clinically common, while other methods like adjusting growth factors concentration are still in development. Various modifications of this technology allow to use molecular mechanisms involved in the restoration of hyaline cartilage and improve the effectiveness of PRP for the treatment of OA.

Keywords: hyaline cartilage; knee; platelet-rich plasma; PRP; modification;

INTRODUCTION

Hyaline cartilage is a connective tissue consisting of extracellular matrix proteins with a small content (no more than 5%) of highly specialized cells –named chondrocytes– that contain a large amount of water, making up more than two-thirds of its weight [1,2]. Among other functions, the hyaline cartilage covers and protects the surfaces of joints. The knee joint is one of the most stressed joints in the body. Traumatic knee injury is prevalent in young adults and contributes significantly to the premature development of knee osteoarthritis (OA)[3]. The risk of OA increases with the age of the patient and the time after the injury[1,4,5,6].

Currently, several surgical methods are used for local surface restoration of the hyaline cartilage in the knee joint, including microfracture, implantation of biodegradable scaffolds, and autologous chondroplasty[7]. Alternatively, various implantation technologies (based on autologous or allogeneic cells) are being actively developed, aiming at production of a hyaline-like cartilage tissue in the joint defect area. However, these methods have not yet been widely introduced into clinical practice due to legal barriers [8].

Intra-articular injection of autologous platelet-rich plasma (PRP) is currently considered an affordable, safe, and effective treatment for many diseases of the musculoskeletal system, however, it is currently one of the most widely discussed topics in regenerative medicine [6,9,10,11,12,13].

PRP therapy is actively used in clinical practice for knee OA treatment. It’s proposed that growth factors secreted from platelets can stimulate chondrocytes viability, proliferation, and migration capabilities [11,14]. For example, substances, containing platelet derivatives, have shown chemoattraction and induction of progenitor

chondrocytes, which contributes to the hyaline cartilage restoration [15]. At the same time, there are some contradictory data on its efficacy [6,13].

Concurrently, high concentrations or just the presence of certain growth factors in plasma can create the opposite effect on cartilage regeneration [16]. Some protocols of PRP preparation involve the removal of growth factors that negatively affect chondrogenesis, e.g., vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF). In other protocols, the PRP is modified by adding biologically active substances, such as hyaluronic acid (HA). To date, there are some pilot clinical studies aimed at using the frozen component of PRP for the restoration of hyaline cartilage, which have shown their efficacy [17].

Different manufacturers of commercial kits have different protocols for preparing PRP, and thus, the resulting products vary greatly, both in the number of platelets and in the presence and concentration of various growth factors, which undoubtedly affects the final efficacy of the PRP technology.

Although many studies describe the use of this technology, there is only a limited number of studies explaining the molecular mechanisms of its influence on chondrogenesis and recovery of hyaline cartilage in OA. Currently, there are only a few studies that show the detrimental effect of individual components of the PRP on chondrogenesis in OA [16,18]. The PRP technology and its use in OA is not standardized, the application protocols differ and have fundamental alterations, which may ultimately affect the final efficacy of the PRP application. At the moment, there is of comprehensive analysis of feasible approaches to modify PRP technology for OA.

The idea of this review is to analyze different experimental and clinical studies of PRP technology for the recovery of hyaline cartilage in OA. The article is aimed at presenting and systematizing different methodological aspects of the modification of

PRP treatment to increase its effectiveness. The paper focuses on the relationship between the molecular mechanisms of PRP technology to increase the effectiveness of the therapy. The work is intended to aid researchers in comprehending in greater depth the primary plausible mechanisms of PRP modification.

MATERIALS AND METHODS

Literature search and selection criteria

The literature review was conducted in the eLIBRARY, PubMed (MEDLINE), Ovid, ScienceDirect, and Google Scholar databases extracting literature available by the end of 2023. The article reviews original works devoted to various modifications of PRP technology in OA of the knee joint in two stages. In the first step, we analyzed articles and looked for fundamental methods of modifying PRP, and in the second step, in the areas found, we deepened the search and detailed the information on each method.The review also includes manuscripts that describe analyses of the molecular mechanisms of each of the basic method's modifications. Studies were included if they simultaneously met the following criteria:

1) effect of using PRP in the recovery of hyaline cartilage after OA

2) at least one way to modify the PRP protocol aimed to increase its efficiency

3) data on the molecular mechanisms underlying the increase in efficiency of the modified PRP therapy in OA

Study selection process

The search results underwent a thorough review process to identify and eliminate duplicates, in accordance with predefined inclusion criteria. This evaluation aimed to select articles that would ultimately be included in the final information extraction. The

review involved four independent reviewers, working in pairs, who meticulously assessed the titles, abstracts, and full texts of manuscripts during two distinct screening phases.

Data collection

The included articles were added to a spreadsheet. The selected studies were classified depending on the principle of the protocol modification, effect, and molecular mechanism of action. The different classification types that were recognized during the data collection were 1) modification of PRP protocol by removing growth factors, 2) modification of PRP protocol by the addition of biologically active substances, 3) modification of PRP protocol by changing the cellular composition, 4) modification of PRP protocol by the method of activation, and 5) modification of the PRP protocol by changing of the administration procedure.

Quality assessment

For experimental articles involving animal manipulations, we used the SYRCLE's Risk of Bias tool to assess the quality of papers and various bias indicators across multiple dimensions.

RESULTS

Search strategy and study selection

The initial search method produced 5044 = (3950 I Stage + 1094 II Stage) records. Figure 1 illustrates the study selection process in a flowchart. Additionally, the main modification methods were identified and categorized.

Quality assessment

Five studies reported an animal model intervention. After applying the SyRCLE's Risk of Bias tool, we detected at least one domain at high risk of bias - for allocation concealment and blinding, indicating a low methodological quality for these records. On the other hand, a low risk of bias was determined for baseline characteristics, incomplete outcome data, and other biases. The complete quality assessment is shown in **Table 1**.

1. Modification of PRP preparation protocol by removing growth factors

One of the approaches claimed to improve the PRP outcome is the elimination of growth factors that negatively affect chondrogenesis. Usually, PRP may contain transforming growth factor beta (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet growth factor (PDGF), VEGF, and EGF [19,20]. As the analysis has revealed, of these six growth factors, only three have a positive effect on chondrogenesis: TGF-β3, PDGF, and IGF. Two more factors, VEGF and EGF, have a negative effect. VEGF and EGF stimulate the new blood vessels growth, attract immune cells and may contribute to the development of chronic osteoarthritis and degradation of hyaline cartilage. Some authors suggest removing these growth factors from the PRP. In addition, it is known that the FGF in high doses can stimulate cell differentiation in both the chondrogenic and osteogenic pathways. The molecular effect of these growth factors on the development of chondrogenesis is described in the in **Table 2**.

VEGF can be removed with clinically approved bevacizumab antibodies [21], which is a soluble form of the sFlt receptor – 1 VEGF is capable of effectively binding [16,22,23]. There are also some approaches to eliminate VEGF via microspheres that adhere to the protein, which was confirmed with animal experiments [23].

2. Modification of PRP with addition of biologically active substances or other compounds

Instead of increasing the concentration of the 'positive' growth factors in the PRP administered, there is another approach to retain the existing growth factors in the area of the damaged hyaline cartilage after injection. There is an option to use the PRP in combination with a biodegradable gel based on chondroitin sulfate [24]. During *in vitro* research, the authors managed to achieve a stable release of growth factors over two weeks compared with the control group, in which the decrease in concentration occurred sharply over three days. *In vivo* experiments on rabbits confirmed the benefits of PRP in the gel form in impaired hyaline cartilage of the knee joint [25], however, no clinical studies have been found on this topic.

PRP therapy performance can be also improved with an addition of substances that positively affect chondrogenesis, for example, HA [11,26]. HA is one of the most important elements of the extracellular matrix of hyaline cartilage and belongs to non-sulfated glycosaminoglycans [27,28]. When HA acid interacts with aggrecan monomers, it forms large aggregates in hyaline cartilage that bind water. This phenomenon dramatically increases the elasticity and shock-absorbing function of the tissue [28]. It is known that HA injected into the joint influences the restoration of the damaged hyaline layer [29]. Pilot studies *in vivo* on animals have shown a better efficacy of HA + PRP compared to HA alone, which, in our opinion, is due to the synergistic effect of two different positive factors on chondrogenesis [11,30]. The same conclusion was formulated by Aw et al. [31] who described greater efficacy of combinational therapy than single PRP technology. The synergistic effect might be achieved because the growth factors remain in the area of damage for a longer time, which prolongs their positive effect. Other authors claim that the effect is achieved by

changing the profile of inflammatory cytokines, through the corresponding mediators, such as CD44 or TGF-βRII, which inhibit the inflammatory response and degeneration of chondrocytes [26].

Chondrogenesis-positive growth factors can be added to the PRP. Results of experimental studies in this area have already been published [32,33]. There is a possibility of using PRP in combination with external physical stimulation to improve the therapy performance. There is an ongoing meta-analysis studying the efficacy of PRP therapy in combination with physical methods for stimulating hyaline cartilage regeneration [34].

3. Modification by changing the cellular composition of PRP

The multiple growth factors, which are the main effecting compounds and have been described in detail above, are secreted by alpha granules of activated platelets and have a synergistic effect on chondrogenesis via modeling inflammation, activating various intracellular signaling pathways that increase the production of hyaline cartilage matrix components and block the action of catabolic enzymes [11,35,36].

Platelets are fragments of the megakaryocyte cytoplasm with a lifespan of up to 10 days. Platelets are formed in bone marrow [1,37]. In a healthy person, their amount normally ranges from 2×10^5/μl to 4×10^5/μl [11,38]. In case of damage to blood vessels, activated platelets release granules with growth factors, that promote tissue regeneration [39].

Despite many existing protocols for PRP preparation, there is still possibility for improvement of the the platelet concentration. Speed, time, and temperature of centrifugation can change the number of platelets in the final product [11,40]. At the same time, additional filters (with a pore size of 1 μm) used at the late stages of the

preparation can retain a larger portion of platelets - up to 92% from the starting number [41].

According to the recommendation, the number of platelets in PRP must be within the 1.0−1.5 million platelets/μl range to stimulate cell proliferation and tissue healing [40,42]. However, there is still a discussion about the optimal number of platelets (and their concentration) required for a therapeutic procedure. One study [34] claims that as much as 10^9 platelets per application are crucial for reaching a stable medium-term therapeutic effect in elderly and (or) overweight patients, which supports an increase of platelet fraction in case of these factors 1[40].

At the same time, the effects of leukocyte concentration in PRP remain uncertain [41,43]. Some studies have shown that the number of leukocytes in a PRP does not affect the efficacy of the hyaline cartilage restoration in the knee joint [44]. According to other authors, leukocytes can damage hyaline cartilage, and the PRP with a reduced number of leukocytes presents better results [41,45,46]. The clinical efficacy of PRP with a low and a high content of leukocytes depends on the specific indications, which is confirmed by the works [1,35,47,48]. For example, leukocytes activate matrix metalloproteinases (MMP13) that destroy hyaline cartilage and cause joint pain and general inflammation, which leads to the death of synovial cells [49,50]. Therefore, a low leukocyte counts PRP (LP-PRP) appears to be more effective for OA patients. To decrease the number of leukocytes, T. Tischer suggests increasing the centrifugation speed at each stage of the PRP component separation [50].

4. Modification of PRP due to the method of activation

To promote the release of growth factors from the platelet granules, the PRP have to be activated. The term "activation" here refers to two key processes: (1) platelet

degranulation to promote the growth factors' release from alpha granules, and (2) fibrinogen cleavage to initiate the matrix formation and to form a gel capturing platelets and spatially restricting the secretion of molecules [51]. It is noteworthy that platelet activation also leads to a rapid translation of remaining mRNA in platelets [52]. There are several ways to activate platelets in PRP: (1) to add $CaCl_2$ 10% (final concentration 22.8 mM), (2) to add autologous thrombin 10% (final concentration 1 U/ml), (3) to add $CaCl_2$ 10% + thrombin mixture, (4) to add type I collagen 10% (final concentration 4 µg/ml) [63], (5) to add calcium gluconate 10% (0.15 ml per ml of PRP) [53], (6) to damage platelets mechanically with a 0.45 µm filter [54], and (7) to activate integrin receptors via gelatin exposure [55]. Among other activation protocols, calcium gluconate in comparison to the rest activation agents, gave the best therapeutic effect. However, the number of patients in the study was small, so this result should be carefully evaluated [53].

Thrombin is a serine protease that plays a critical role in platelet aggregation and activation and blood clotting, thus forming a clot during the PRP application [55]. Early protocols used bovine thrombin to activate PRP, often leading to allergic reactions [56]. Therefore, in modern versions, it has been switched to autologous thrombin from the patient's whole blood [57]. While activating PRP, the thrombin promotes the endostatin release, which is the C-terminal 20 kDa fragment of type 18 collagen. The effect of endostatin on chondrogenesis is being actively studied [58] and beyond the scope of this article. Nevertheless, endostatin plays a role as an anti-angiogenic factor and additionally positively affects chondrogenesis by facilitating type 2 collagen synthesis and increasing the SOX9 expression [59]. Chondrogenic cell proliferation and angiogenic proliferation are two mutually exclusive processes [60]. Therefore, it would

be necessary to provide additional chondrogenic or anti-angiogenic stimuli to the cells in the area of damage.

Calcium chloride activation results in less dense clots compared to thrombin activation and also has the advantage of reducing the burning/tingling sensations experienced by some patients during PRP injections [11,61]. The combination of $CaCl_2$ and thrombin, in turn, creates a dense fibrin-platelet matrix. Platelets inside a dense fibrin clot, in our opinion, undergo degranulation and release of growth factors.

On the other hand, to simplify the activation procedure, many clinicians prefer not to activate PRP exogenously and inject it directly into the joint cavity to let it be activated upon contact with type I collagen receptors. Such an *in vivo* method of activation, according to some authors, leads to a slower and more stable release of growth factors compared to the thrombin method [59,62,63]. In particular, *in vivo* collagen activation results in a longer release of TGF-β1 and an 80% increase in cumulative release over 7 days compared to thrombin activation [62]. Among other advantages of the method is the absence of a preliminary formed fibrin clot and so the possibility of introducing the mixture through a needle of a smaller diameter. Other researchers, on the contrary, insist that the exogenous preliminary activation leads to blood clot formation and, as a result, to a longer release of growth factors [36,64,65].

5. Modification of the protocol due to the multiplicity of the mode of administration of PRP.

To date, there is no consensus on the frequency and volume of injected plasma. However, a few European experts recommend administering 4-8 ml of PRP 1-3 times [66]. In recent studies, a positive effect was reported after 2, 3, or 4 injections [56,67].

DISCUSSION

Over the past decades, the frequency of PRP treatment for various conditions has increased significantly. Among the advantages of the technique are the economic efficiency, the simplicity of the product preparation and administration, the safety due to the use of autologous material, and the ease of technology modification and adaptation to different conditions. The findings of this study highlight the significant role that the composition and modification of PRP protocols play in enhancing chondrogenesis for cartilage repair. Beyond the activation or non-activation of platelets or the inclusion or removal of leukocytes, which are factors that could somehow affect the effectiveness of PRP formulations, the elimination of growth factors such as VEGF and EGF, which negatively influence cartilage regeneration, offers a promising approach to optimize PRP therapy. While growth factors like TGF-β3, PDGF, and IGF have been shown to promote chondrogenesis, the removal of detrimental factors that could contribute to chronic inflammation or OA progression could significantly improve treatment outcomes.

We intended to compile and analyze the principal methods for PRP technology modification and evaluate their efficacy in restoring hyaline cartilage. The logical evolution of this PRP cocktail modification will aim at the alteration of the individual growth factor's concentration to evaluate its optimal effect on chondrogenesis. Since all of these growth factors have an optimal “therapeutic window” for hyaline cartilage regeneration, such a cocktail modification is technologically difficult to research and produce.

Another fundamental direction is related to the method of PRP activation. The effects of clot formation in the knee are poorly documented and not fully analyzed. The rate of the growth factors elution from the PRP cocktail after the exogenous activation and without it has not yet been studied. However, the differences in the therapeutic efficacy of

activated and non-activated PRP, which the researchers write about, may be determined by the different nature of the elution of growth factors. A recent meta-analysis showed that the use of a pre-activated PRP cocktail leads to better therapeutic outcomes (functional state of the joint, pain level) compared to no pre-activation of PRP [36]. The exogenous activation of PRP, would be a logical step to stimulate the growth factors release and to promote chondrogenesis, thus increasing the potential therapeutic effect. However, there are not enough studies that directly compare the efficacy of activated and non-activated PRP and do not unambiguously answer the question of whether pre-activation is needed.

The amount and time interval in which the procedure is performed also plays an important role. However, there are controversial data on the efficacy of PRP technology from the perspective of the number of procedures [68,69,70]. Pan Wang and colleagues [69] are currently analyzing this problem. However, the results of the study have not yet been presented. The delay between the injury and the start of the PRP treatment is also important [11].

Some modification methods related to changing the cellular composition and/or the method of PRP activation are easy to use and are used in clinical practice, as evidenced by clinical studies and comparative analyses. Other methods related to changing the protein composition of the PRP cocktail are only at the stage of experimental work, some have obvious limitations in economic and technological terms.

Another feature of possible modification of PRP, is the age of patients and the volume of damage in OA. Most likely, the possibility of modification and the effectiveness of the entire therapy will be possible only in this limited population of patients.

The complexity of determining the optimal administration regimen is caused by a large number of variables that can affect the result: the patient's age, the duration, and stage of

the disease, the severity of cartilage damage, the intensity of the pain syndrome, the protocol of the PRP preparation, length of the follow-up period. Certain parameters are difficult to take into account due to the complexity of their evaluation and the lack of the study of control groups.

CONCLUSION

The exploration of PRP as a therapeutic modality for knee OA underscores its potential as a regenerative tool, particularly in the restoration of hyaline cartilage. This study highlights the versatility of PRP modifications, including the removal of inhibitory growth factors, the addition of synergistic substances like hyaluronic acid, and advancements in application protocols. Such modifications hold promise for enhancing PRP's therapeutic efficacy by targeting molecular mechanisms of cartilage regeneration and addressing clinical variability. However, significant challenges remain in standardizing PRP preparation and administration protocols. Variability in platelet concentration, growth factor composition, and activation methods complicate the assessment of efficacy and reproducibility. Moreover, the current body of research lacks robust, quantitative analyses and longitudinal studies to determine optimal therapeutic windows and patient-specific factors. Future research should focus on refining PRP cocktails through controlled studies on growth factor optimization, exploring novel delivery systems, and integrating preclinical findings into large-scale clinical trials. Addressing these gaps will not only enhance the reliability of PRP therapy but also position it as a cornerstone treatment for OA and other degenerative joint diseases.

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CONFLICT OF INTEREST

The authors declare that they have no relevant financial or non-financial interests to report.

DATA AVAILABILITY STATEMENT

Data are available on request.

AUTHORS CONTRIBUTIONS

MSB: Conceptualization, Study design, Methodology, Data collection, Reviewing, Writing an original draft.

SAB: Conceptualization, editing the manuscript.

JVS: Study design, Data curation, Writing, and Reviewing.

EIL : Study design, Data curation, Writing, and Reviewing.

MIS: Methodology, Data collection

AAR: Methodology, Data collection

MSM: Conceptualization, Study design, Methodology, Supervision, Writing, Reviewing, and Editing.

ETHICAL COMPLIANCE

This article does not contain a description of studies performed by the authors involving humans or using animals as objects.

Figure 1

The study selection process in a flowchart

Figure 2.

Principal methods for PRP technology modification and evaluated its efficacy in restoring hyaline cartilage

LIST OF ABBREVIATIONS

PRP - platelet rich plasma

OA – osteoarthritis

VEGF - vascular endothelial growth factor

EGF - epidermal growth factor

TGF- β - transforming growth factor beta

PDGF - platelet growth factor

IGF - insulin-like growth factor

FGF - fibroblast growth factor

HA - hyaluronic acid

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Figure1

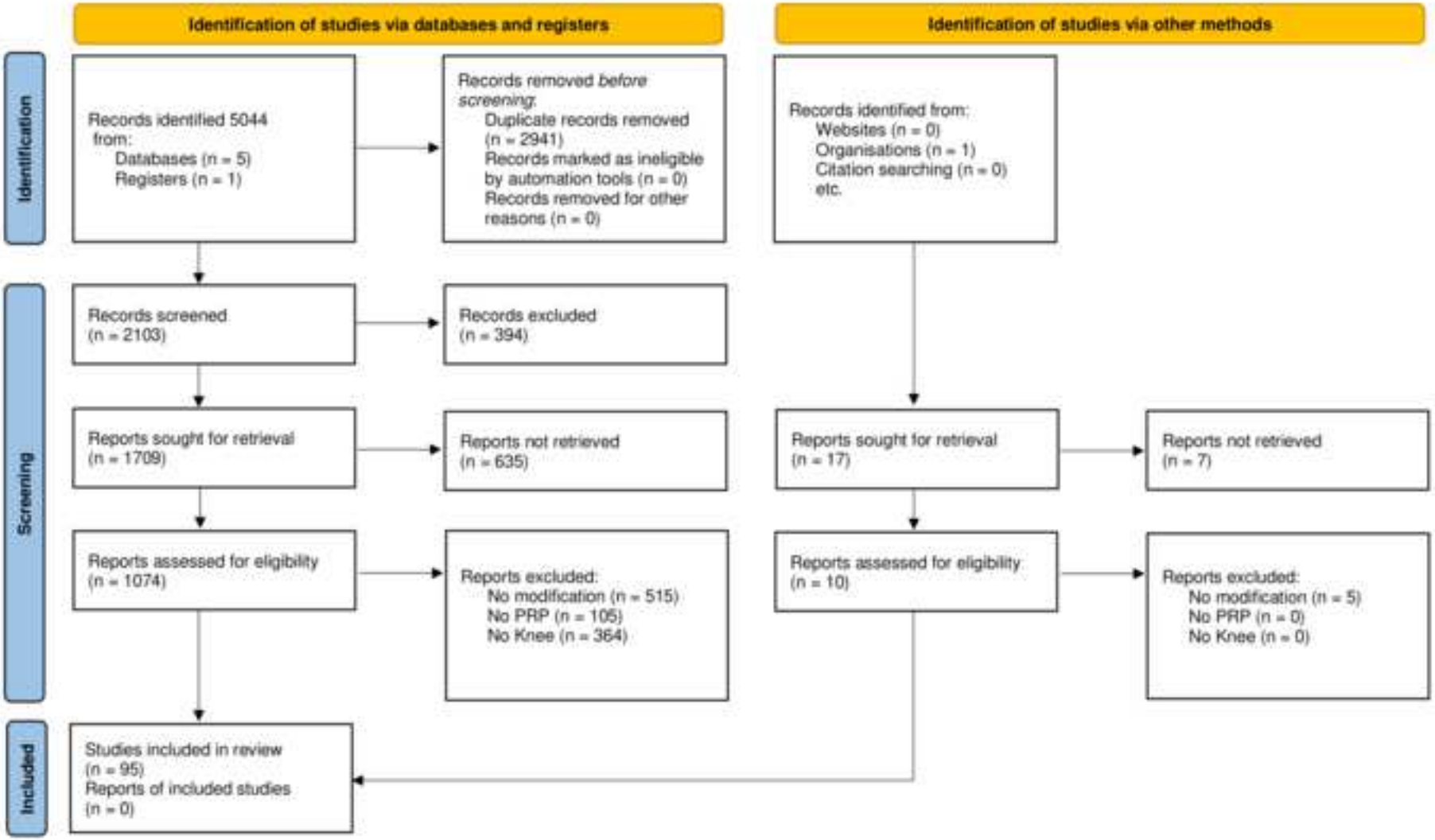

Figure2

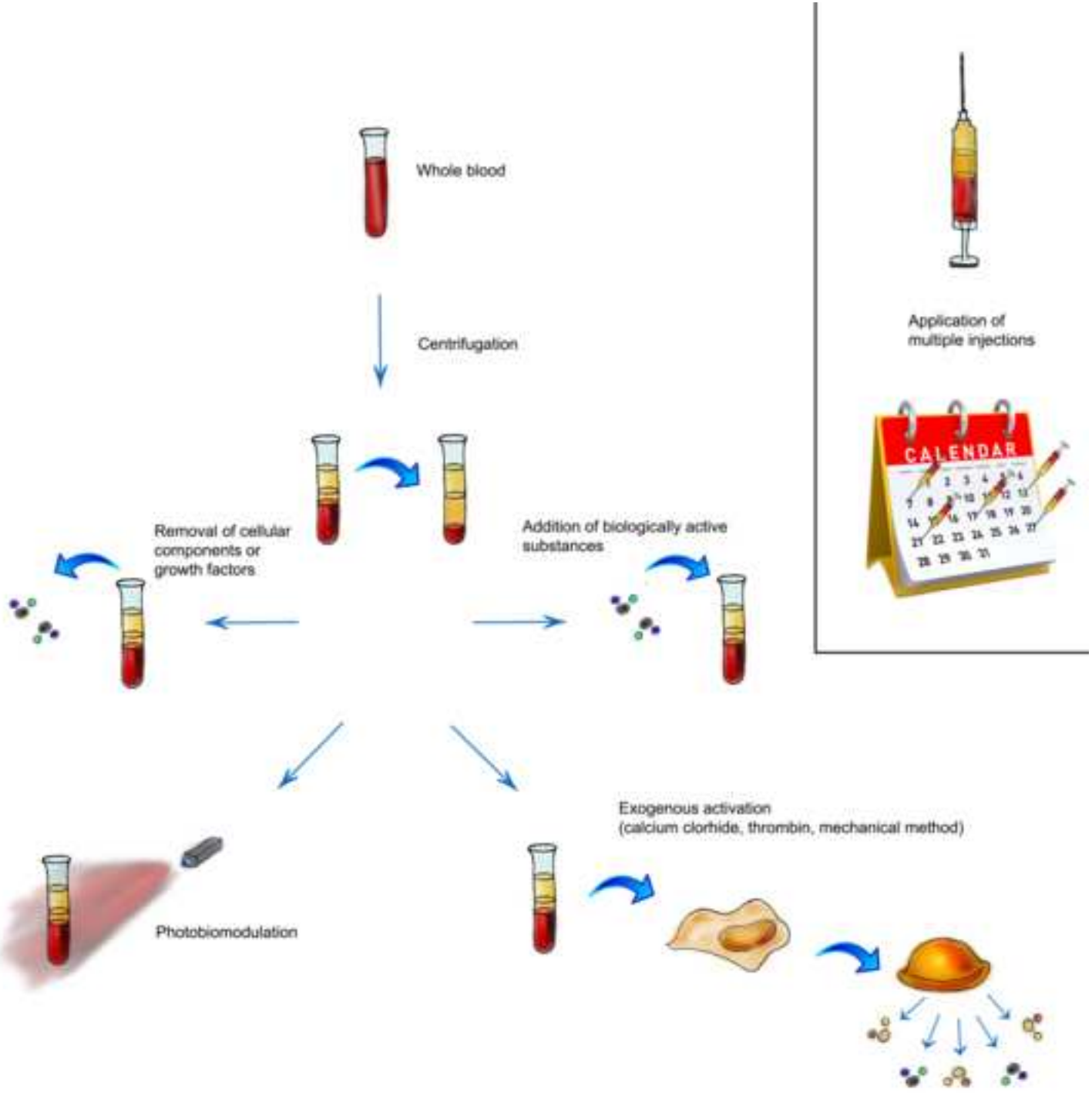

Table 1. Quality analysis using the Systematic Review Center for Laboratory animal Experimentation (SyRCLE) tool.

Author, year	**Model of study**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Bozhokin, 2021	Rat model of induced hyaline cartilage defect	U	L	H	U	H	U	H	L	H	L
Saito, 2021	Japanese white rabbit model of induced knee OA	H	L	H	H	H	U	H	L	U	L
Simi, 2021	New Zealand white rabbit model of osteochondral defect	H	L	H	U	H	U	H	L	U	L
Suyasa, 2020	Wistar rat model of induced knee OA	U	L	H	H	H	H	H	L	U	L
Zhu, 2021	Sprague-Dawley Rat model of induced knee OA	H	L	H	U	H	H	L	L	U	L

OA, osteoarthritis; **H**, high risk of bias; **L**, low risk of bias; **U**, unclear risk of bias.

1. Selection bias, Sequence generation.
2. Selection bias, Baseline characteristics.
3. Selection bias, Allocation concealment.

4. Performance bias, Random housing.
5. Performance bias, Blinding.
6. Detection bias, Random outcome assessment.
7. Detection bias, Blinding.
8. Attrition bias, Incomplete outcome data.
9. Reporting bias, Selective outcome reporting.
10. Other sources of bias.

Table 2. Basic growth factors for chondrogenesis in PRP.

Growth factor	Function	Influence on chondrogenesis	Reference
TGF-β	Increased protein synthesis of the extracellular matrix of hyaline cartilage. Stimulation of chondrogenesis.	positive	[31,32,33]
VEGF	Vascular growth, angiogenesis, endochondral bone formation, promote arthritis.	negative	[34]
EGF	Promotes the degradation of hyaline cartilage, and reduces the expression of extracellular matrix genes.	negative	[24,35,36]
IGF	Increases protein synthesis of the extracellular matrix of hyaline cartilage	positive	[31,33]
FGF	Promotes the proliferation of mesenchymal cells, chondrocytes and osteoblasts, stimulates the growth and differentiation of chondrocytes and osteoblasts.	dose-dependent (context-dependent, alternative splicing)	[33,37]
PDGF	Promotes proliferation, survival and migration of cells, especially cells of mesenchymal origin. Contained in α-granules in platelets, synthesized in megakaryocytes. Increases the synthesis of extracellular matrix proteins	positive	[31,33,38,39,40,41,42]

Transforming growth factor beta (TGF-β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF).

Table 3. Principal methods of modification for PRP technology.

	Type of PRP modification	Effect on cartilage restoration	Experimental	Clinical	Comparative clinical trials
1	Changes in biochemical composition:				
	- a removal of negative growth factors (VEGF, EGF)	Positive	yes	no	no
	- an addition of biologically active substances	Positive	yes	yes	no
2	Changse in cellular composition:				
	- an increase of the platelet concentration	Positive	yes	yes	yes
	- a decrease of the leukocyte concentration	Depends on many factors	yes	yes	yes
3	Physical impact: e.g., laser irradiation	Negative	yes	no	no
4	Exogenous pre-activation	Positive	yes	yes	yes
5	Multiple rounds of administration	Positive	yes	yes	yes

Vascular endothelial growth factor (VEGF), epidermal growth factor (EGF).