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A Systematic Review of Autologous Platelet-Rich Plasma and Fat Graft Preparation Methods

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Background: The addition of platelet-rich plasma (PRP) to adipose tissue may improve fat graft survival, although graft retention rates vary markedly between studies. To what extent this outcome heterogeneity reflects differing methodological factors remains unknown. This systematic review aims to synthesize and critically review methodological approaches to autologous PRP and fat cotransplantation in both human and animal studies.

Methods: In accordance with PRISMA guidelines, Ovid MEDLINE, Scopus, and Cochrane Library databases were searched from inception to April 2017. Data were extracted from all in vivo studies involving autologous PRP and fat cotransplantation. A secondary aim was to assess reporting of technical detail; authors were not contacted to provide missing data.

Results: From 335 articles, 23 studies were included in the qualitative synthesis. Some 21 were performed in humans and 2 in rabbits. Six studies were randomized control trials; the remainder reported on observational data. Methods of PRP extraction and activation varied markedly between studies. Fat graft preparation was comparatively more consistent. Methods of PRP and fat mixing differed significantly, especially with regards to relative volume/volume ratios.

Conclusions: Our study represents the first systematic review of methodological factors in autologous PRP and fat cotransplantation. It demonstrates that technical factors in graft preparation and administration vary significantly between in vivo studies. Such methodological heterogeneity may explain observed differences in experimental and clinical outcomes. Reporting of key procedural information is inconsistent and often inadequate. These issues make meaningful evaluation of the PRP-enhanced fat grafting literature difficult and may limit its translation into clinical practice. *(Plast Reconstr Surg Glob Open 2017;5:e1596; doi: 10.1097/GOX.0000000000001596; Published online 6 December 2017.)*

INTRODUCTION

Autologous fat grafting is used extensively in reconstructive and aesthetic plastic surgery for the correction of soft-tissue volume defects. The popularity of structural free fat transfer comes from the fact that it is nonimmunogenic, versatile, and easily harvested with low donor-site morbidity.[1]

More recently, there has been growing interest in the regenerative potential of free fat transfer.[2,3] Multipotent precursor cells transplanted within the fat stromal compartment—termed adipocyte-derived stem cells (ASCs)—are able to differentiate into various cell lineages[4] and secreting soluble mediators with angiogenic, immunosuppressive, and antiinflammatory properties.[5] Several preliminary studies have demonstrated that autologous free fat transfer may significantly enhance healing of diabetic foot ulcers,[6] pressure sores,[7] and radiotherapy scars.[8]

However, long-term fat graft retention is highly variable with resorption rates in the order of 25% to 80%.[9–11] Fat necrosis and subsequent graft resorption is therefore a significant problem. Various technical factors related to fat preparation and administration have been implicated in graft failure.[12–15] Similarly, adequate neovasculascular-

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ization appears to be an equally important prognostic factor in graft survival. Adipocytes poorly tolerate ischemic conditions,[16] and early angiogenesis is essential for successful transplantation.[17–19]

Platelet-rich plasma (PRP) is an autologous source of concentrated platelets, growth factors, and cytokines used widely in regenerative medicine.[20–24] Its therapeutic efficacy derives from the supraphysiological release of several growth factors and bioactive proteins from platelet α granules.[17,25] These mediators—including platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor β, and insulin-like growth factor—have 2 primary effects: they promote the recruitment and activation of cells responsible for tissue repair, and they encourage new blood vessel formation.[22]

Independently, both PRP and fat transplantation seem to have a role in regenerative medicine; however, the addition of PRP to fat transplants may synergistically enhance graft survival by a number of mechanisms. First, the secretion of proangiogenic factors by PRP may improve early graft vascularization and minimize ischemic injury[26,27]; second, the release of antiinflammatory cytokines is thought to have a role in preventing graft degeneration[27]; and third, PRP-secreted factors have been shown to enhance the differentiation of preadipocytes into their mature form.[28,29]

The majority of human and animal studies suggest that PRP and fat cotransplantation increase graft survival rates. However, the rate of graft resorption varies markedly between studies and, in particular, between research groups. Furthermore, several human[30–32] and animal[33] studies report that the addition of PRP to fat transplants confers no survival advantage when compared with free fat transfer alone. To what extent these variable outcomes reflect differing methodological factors remains unknown.

For PRP alone, different study methodologies produce context-dependent results.[34–36] PRP preparation techniques, composition, mechanism of activation, and outcome measurements vary significantly.[23] This inconsistency is further confounded by poor reporting of key technical factors.[24,37,38] Together, these issues limit the ability to draw overall conclusions about the efficacy of PRP in each setting and hinder its translation into clinical practice. Similarly, methodological heterogeneity in the harvesting and processing of fat transplants affects both experimental[39–42] and clinical[15] outcomes.

It is therefore reasonable to expect that methodological factors in PRP-enriched fat grafting will be important determinants of transplant survival rates. As in individual PRP and fat transfer studies, there is—as yet—no standardized transplantation protocol for their combined use.[24,43,44] Preliminary evidence from both in vitro[28,45] and animal studies[46,47] supports the hypothesis that technical factors significantly influence PRP–fat cotransplantation outcomes.

This systematic review aims to synthesize and critically review the various methodological approaches to autologous PRP and fat cotransplantation in both human and animal studies. In particular, it seeks to assess to what extent technical factors in PRP and fat processing vary between comparable studies.

METHODS

The objective of this systematic review is to critically evaluate the literature on PRP-enriched fat grafting in accordance with PRISMA guidelines.[48] It aims to provide a descriptive synthesis and critical appraisal of technical factors in autologous PRP and fat cotransplantation.

Search Methods

Bibliographic databases (Ovid MEDLINE, Scopus, and The Cochrane Library) were searched for relevant articles from inception to April 2017. Both "free-text terms" and "MeSH term" searches were run sequentially to capture all papers in which PRP was coadministered with fat. Variations on the following keywords were combined using the matrix of Boolean operators detailed in Supplemental Digital Content 1: "platelet-rich plasma," "PRP," "platelet concentrate(s)," "fat graft(s)," "fat transfer," "fat injection(s)," "mixed," "method(s)," "extraction," "preparation," "activated," "human," "animal," "autologous." English language studies from any country of origin were eligible for inclusion. (See appendix, Supplemental Digital Content 1, which displays the electronic database search matrix, http://links.lww.com/PRSGO/A622.)

After merging database search results and discarding duplicate entries, titles and abstracts were screened to eliminate studies unrelated to the current research objective. The remaining articles were read in full to assess their eligibility for qualitative synthesis. Additional articles were identified by routinely screening the reference lists of all included studies. All authors agreed upon the final list of included studies; any disagreement between authors was resolved by discussion and consensus.

Study Selection Criteria

A full set of inclusion and exclusion criteria were prespecified and agreed upon by all authors in advance. This study protocol was not eligible for registration on the PROSPERO database[49] as it constitutes a review of methodological factors only.

All in vivo studies in which autologous PRP was mixed or coadministered with autologous fat grafts were eligible for inclusion. Both preclinical and clinical trials performed for any surgical or medical indication were included. All methods of fat grafting (such as lipofilling, contouring, and solid tissue transplant) were deemed eligible. No differentiation was made between pure PRP and leucocyte-rich PRP.[50] All experimental study designs (irrespective of number of fat grafts or length of follow-up) were included.

In vitro studies only (without a concurrent in vivo human or animal model) were excluded. Studies in which PRP or fat grafting had been performed separately (ie, without mixing or coadministration) were excluded. Animal studies using a pooled isogenous source of PRP were ineligible for inclusion. Studies reporting solely on platelet-rich fibrin (even if added to fat grafts) were excluded. Similarly, those studies involving only secondarily extracted fat products (ie, the stromal vascular fraction or

isolated ASCs) were discarded. Case reports, letters, editorials, book chapters, and reviews were also excluded.

During the shortlisting process, articles were screened for reporting of technical and methodological factors related to PRP and/or fat processing and coadministration. Articles with either a comprehensive or partial description of their methods were included; studies with no methods were excluded. One of the secondary aims of this study was to assess whether authors were reporting sufficient technical detail in their published manuscripts; therefore, no additional contact was made with authors to provide missing data.

Data Collection

All data were extracted by a single author (JL) and recorded on a predesigned bespoke electronic form before being independently verified by a second author (OJS).

Data collected were divided into the following subgroups:

1. baseline study data (title, author(s), year, journal, country, in vivo model)
2. experimental details (study design, number of fat graft procedures, length of follow-up)
3. PRP extraction (whole blood extraction methodology, PRP processing steps, PRP activation details, mean platelet counts, PRP storage)
4. fat extraction (method of fat harvest, donor-recipient site combination, fat processing methods, fat storage)
5. PRP–fat mixing (method and timing of PRP–fat mixing, use of prosurvival adjuncts).

No assumptions were made about the methods used if they were not explicitly reported. If authors referenced supplemental materials, appendices, or other articles in their methods, these were reviewed for relevant data extraction. Methodological factors not reported either directly or indirectly were listed as "unspecified."

In studies with multiple different experimental protocols, data were extracted from those related to the current research objective only.

Quality Assessment and Statistical Analysis

All studies fulfilling the inclusion criteria were eligible for inclusion, and low-quality studies (eg, those performing poorly according to GRADE criteria[51]) were not discarded.

The purpose of this study was to provide a descriptive summary and critical review of variation in experimental technical factors—no attempt to account for risk of bias either in or between studies has been made.

Formal evaluation of experimental and clinical outcomes was beyond the scope of the current research objective; therefore, we have made no attempt to perform pooled statistical comparisons or meta-analyses. Owing to significant study design heterogeneity, such quantitative comparisons would be fundamentally misleading. Where appropriate, we have indicated which studies demonstrate improved graft retention rates with autologous PRP and fat cotransplantation and which do not.

RESULTS

Study Selection

The electronic database search strategy returned 335 results. After the elimination of 58 duplicates and the inclusion of 7 additional records, 286 titles and abstracts were screened against the predefined research objectives. At this stage, 203 articles were excluded; 83 papers were read in full to assess their eligibility for inclusion.

During this process, 60 manuscripts were eliminated for the following reasons: either PRP ($n = 21$) or fat ($n = 10$) was used in isolation; transplants were obtained from nonautologous sources ($n = 8$); reviews, letters, and abstracts ($n = 15$); no reporting of methods ($n = 2$); single case reports ($n = 4$); in vitro experimental studies only ($n = 3$), or the use of cultured ASCs only ($n = 1$).

In total, data were extracted from a final shortlist of 23 articles included in the qualitative synthesis. A comprehensive study attrition diagram is provided in Fig. 1.

Study Characteristics

Of the 23 shortlisted studies, 21 were performed in humans and 2 in rabbits. The majority of human studies were presented as observational case series, although many included a comparator control arm of some description; only one[52] was explicitly described as a case-control study. Four human studies used a randomized control trial design, as did both animal experiments.

All included studies were published since 2009. Studies performed in 4 different continents were included; 11 included articles were published by 2 interrelated Italian research groups.[29,53–62]

Data were extracted from only those experimental protocols where PRP was combined with fat for cotransplantation. The number of grafts fulfilling the inclusion criteria and length of follow-up are provided in Table 1.

PRP Extraction

Methods of PRP extraction varied significantly, particularly between research groups (Table 2). The majority of human studies employed a commercially available PRP extraction system—in these 17 studies, 5 different manufacturer devices were used, each with their own protocol and PRP product characteristics.[35,36,63] Only 4 human trials (and both animal studies) did not use a commercial device to produce PRP.

Most studies used a citrate-based anticoagulant to obtain whole blood; 9 studies did not report on their choice of anticoagulant. Where citrate was used, reporting on its strength and volume/volume (v/v) ratio to blood was inconsistent and often omitted (data not shown).

The volume of whole blood collected varied between studies and depended largely on the anticipated volume of PRP required. However, 16 studies did not specify what volume of PRP was produced for a given volume of blood; in the 9 studies where these data were reported, the ratio of whole blood to PRP varied from 2:1[31] to 13:1.[52] Overall, only 4 studies[52,64–66] provided comprehensive whole blood collection details.

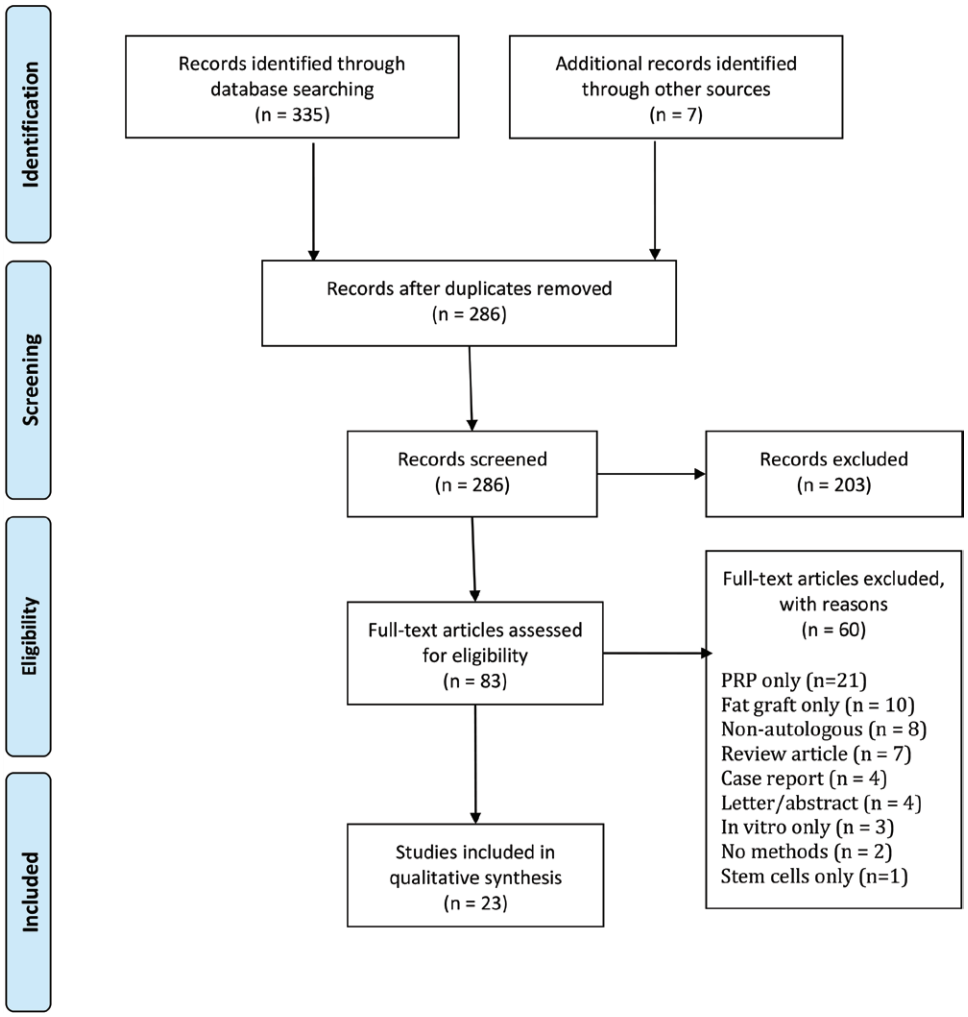

Fig. 1. Study attrition chart.

Reporting of PRP centrifugation protocols was generally better, with only 5 studies providing inadequate methodological detail.[52,54,64,67,68] Most studies used a single centrifugation spin, although the relative centrifugal forces used and the length of time doing so varied between different study designs. Interestingly, this was not necessarily a consequence of using different commercial PRP extraction systems—for example, 4 studies using a commercial RegenKit device employed different centrifugation parameters.[31,54,56,69] Only 2 studies quantified mean PRP platelet concentrations[32,69] before fat grafting; none of the other studies provided either absolute PRP concentrations or change from baseline data.

PRP Activation

Most studies included a PRP activation step (Table 3)—2 human studies did not use activated PRP[52,70] and 2 studies did not report on whether or not activated PRP was used.[57,69]

Studies using commercial devices typically followed manufacturer instructions for activation using calcium chloride, autologous thrombin, or both. However, of the 17 studies using commercial PRP systems, only 2 provided methodological detail within their published manuscript.[54,66] Of the 5 studies using a bespoke extraction method in which activated PRP was used, 2 did not describe the steps involved.[67,71]

In general, authors added the activation reagent to PRP immediately before admixing with fat. Where PRP was injected into the area of fat grafting in vivo, activation was performed either immediately before percutaneous injection[66] or not at all.[52]

The majority of studies extracted PRP at the point of use and did not store PRP in either its active or inactive form for any significant length of time. Where PRP was stored for a short period during fat harvest, it appears that this occurred at room temperature—only one author described maintaining PRP in an ice water bath before implantation.[52]

One study with an optimized experimental PRP extraction protocol activated PRP with calcium chloride and thrombin before cryogenic storage at −80°C.[52] To complete the freeze-thaw cycle, the authors then recentrifuged the product at 18°C to achieve a platelet concentration of $1.4–1.9 \times 10^6$ platelets/μl.

Table 1. Characteristics of Included Studies

Primary Author*	Year	Title	Journal	Country	Model	Study Design	Comparator(s)	No. PRP–Fat Grafts†	Follow-Up (months)
Human studies									
Cervelli	2009	Application of platelet-rich plasma in plastic surgery: clinical and in vitro evaluation	*Tissue Engineering: Part C*	Italy	Human	Observational	PRP only	35	18
Cervelli	2009	Regenerative surgery: use of fat grafting combined with platelet-rich plasma for chronic lower-extremity ulcers	*Aesthetic Plastic Surgery*	Italy	Human	Observational	HA + collagen	20	12
Cervelli	2009	Autologous platelet-rich plasma mixed with purified fat graft in aesthetic plastic surgery	*Aesthetic Plastic Surgery*	Italy	Human	Observational	—	15	12
Cervelli	2010	Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid	*Advances in Skin & Wound Care*	Italy	Human	Observational	—	30	12
Cervelli	2011	Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in posttraumatic lower extremity ulcers	*Stem Cell Research*	Italy	Human	Observational	1. PRP only; 2. HA only; 3. SVF enhanced fat grafting	10	4
Cervelli	2012	Treatment of traumatic scars using fat grafts mixed with platelet-rich plasma, and resurfacing of skin with the 1540 nm nonablative laser	*Clinical and Experimental Dermatology*	Italy	Human	RCT	1. PRP only; 2. PRP–fat with nonablative laser resurfacing	40	6
Cervelli	2012	Platelet-rich plasma greatly potentiates insulin-induced adipogenic differentiation of human adipose-derived stem cells through a serine/threonine kinase Akt-dependent mechanism and promotes clinical fat graft maintenance	*Stem Cells Translational Medicine*	Italy	Human	RCT	Various PRP–fat v/v % ± insulin injections	39	12
Cervelli	2013	P.R.L. platelet rich lipotransfert: our experience and current state of art in the combined use of fat and PRP	*BioMed Research International*	Italy	Human	Observational	—	223	12
Fontdevila	2014	Double-blind clinical trial to compare autologous fat grafts versus autologous fat grafts with PDGF: no effect of PDGF	*Plastic and Reconstructive Surgery*	Spain	Human	RCT	Fat grafting only	20	12
Gennai	2016	Skin rejuvenation and volume enhancement with the micro superficial enhanced fluid fat injection (M-SEFFI) for skin aging of the periocular and perioral regions	*Aesthetic Surgery Journal*	Italy	Human	Observational	—	65	4
Gentile	2012	A comparative translational study: the combined use of enhanced stromal vascular fraction and platelet-rich plasma improves fat grafting maintenance in breast reconstruction	*Stem Cells Translational Medicine*	Italy	Human	Observational	1. Fat grafting only; 2. SVF enhanced fat grafting	13	18
Gentile	2013	Breast reconstruction with autologous fat graft mixed with platelet-rich plasma.	*Surgical Innovation*	Italy	Human	Observational	Fat grafting only	50	12
Gentile	2014	Adipose-derived stromal vascular fraction cells and platelet-rich plasma	*Journal of Craniofacial Surgery*	Italy	Human	Observational	1. Fat grafting only; 2. SVF enhanced fat grafting	10	60

(*Continued*)

Table 1. (*Continued*)

Primary Author*	Year	Title	Journal	Country	Model	Study Design	Comparator(s)	No. PRP–Fat Grafts†	Follow-Up (months)
Keyhan	2013	Use of platelet-rich fibrin and platelet-rich plasma in combination with fat graft: which is more effective during facial lipostructure?	*Journal of Oral and Maxillofacial Surgery*	Iran	Human	RCT	PRF-fat grafting	25	12
Nita	2013	Fat graft, laser CO_2 and platelet-rich-plasma synergy in scars treatment	*Journal of Medicine and Life*	Romania	Human	Observational	—	64	6
Rigotti	2016	Expanded stem cells, stromal-vascular fraction, and platelet-rich plasma enriched fat: comparing results of different facial rejuvenation approaches in a clinical trial	*Aesthetic Surgery Journal*	Italy	Human	Observational	1. SVF enhanced fat grafting; 2. ASC injection	13	3
Salgarello	2011	Breast fat grafting with platelet-rich plasma: a comparative clinical study and current state of the art	*Plastic and Reconstructive Surgery*	Italy	Human	Observational	Fat grafting only	17	9
Sasaki	2015	The safety and efficacy of cell-assisted fat grafting to traditional fat grafting in the anterior mid-face: an indirect assessment by 3D imaging	*Aesthetic Plastic Surgery*	USA	Human	Observational	1. Fat grafting only; 2. SVF enhanced fat grafting; 3. SVF + PRP–fat grafting	106	12
Tolba	2015	Initial experience of face augmentation using fat graft-platelet rich plasma mix	*Surgical Science*	Egypt	Human	Observational	—	40	6
Willemsen	2013	Results and long-term patient satisfaction after gluteal augmentation with platelet-rich plasma-enriched autologous fat	*European Journal of Plastic Surgery*	The Netherlands	Human	Observational	—	24	44
Willemsen	2014	The effects of platelet-rich plasma on recovery time and aesthetic outcome in facial rejuvenation: preliminary retrospective observations	*Aesthetic Plastic Surgery*	The Netherlands	Human	Observational	1. Fat grafting only; 2. MACS-lift + fat grafting; 3. MACS-lift + PRP–fat grafting	40	3
Animal studies									
Pires Fraga	2010	Increased survival of free fat grafts with platelet-rich plasma in rabbits	*Journal of Plastic, Reconstructive and Aesthetic Surgery*	Brazil	Rabbit	RCT	Fat grafting only	30	6
Rodríguez-Flores	2011	Influence of platelet-rich plasma on the histologic characteristics of the autologous fat graft to the upper lip of rabbits	*Aesthetic Plastic Surgery*	Spain	Rabbit	RCT	Fat grafting only	8	3

*Studies listed in alphabetical and chronological order.
†Defined as a single procedure.
HA, hyaluronic acid; MACS, minimal access cranial suspension; PDGF, platelet-derived growth factor; PRF, platelet-rich fibrin; RCT, randomized control trial; SVF, stromal vascular fraction; v/v %, volume/volume percent.

Table 2. PRP Extraction Methods

Primary Author	PRP Extraction Method	Whole Blood Anticoagulant	Whole Blood Volume (ml)	Whole Blood:PRP Produced Ratio	PRP Centrifugation Protocol	Mean Platelet Quantity (platelets/μl)
Human studies						
Cervelli 2009 (1)	Commercial—Cascade system	Sodium citrate	18	Unspecified	1100 g for 10 min	Unspecified
Cervelli 2009 (2)	Commercial—Cascade system	Unspecified	18	Unspecified	1100 g for 10 min	Unspecified
Cervelli 2009 (3)	Commercial—Cascade system	Unspecified	18	Unspecified	1100 g for 10 min	Unspecified
Cervelli 2010	Commercial—RegenKit system	Unspecified	16	Unspecified	1500 g for unspecified time	Unspecified
Cervelli 2011	Commercial—Cascade system	Unspecified	18	Unspecified	1100 g for 10 min	Unspecified
Cervelli 2012 (1)	Commercial—RegenKit system	Sodium citrate	18	Unspecified	1100 g for 10 min	Unspecified
Cervelli 2012 (2)	Commercial—Cascade system	Sodium citrate	18	Unspecified	1100 g for 10 min	Unspecified
Cervelli 2013	Commercial—Cascade system	Unspecified	18	Unspecified	1100 g for 10 min	Unspecified
Fontdevila 2014	Experimental	Sodium citrate	Unspecified	Unspecified	1800 rpm for 8 min	Unspecified
Gennai 2016	Experimental	Citrate (type unspecified)	Unspecified	Unspecified	2000 rpm for 4 min	Unspecified
Gentile 2012	Commercial—Cascade system	Sodium citrate	18	Unspecified	1100 g for 10 min	Unspecified
Gentile 2013	Commercial—Cascade system	Sodium citrate	18	Unspecified	1100 g for 10 min	Unspecified
Gentile 2014	Commercial—Cascade system	Sodium citrate	18	Unspecified	3300 rpm for 10 min	Unspecified
Keyhan 2013	Experimental	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified
Nita 2013	Commercial—Glofin system	Unspecified	8.5	4:1	Two-step centrifugation: details unspecified	Unspecified
Rigotti 2016	Experimental	Sodium citrate	Unspecified	Unspecified	Two-step centrifugation: (1) 300 g for 5 min; (2) 700 g for 17 min	$1.4–1.9 \times 10^6$
Salgarello 2011	Commercial—RegenKit system	Unspecified	16–40	2:1	3500 rpm for 5 min	Unspecified
Sasaki 2015	Commercial—SmartPreP2 system	Adenosine-citrate-dextrose	54	13:1	Unspecified	$4.5–8.8 \times 10^5$
Tolba 2015	Commercial—RegenKit system	Sodium citrate	Unspecified	Unspecified	3000 rpm for 5 min	Unspecified
Willemsen 2013	Commercial—Gravitational Platelet Separation system	Citrate dextrose solution A	108	9:1	3500 rpm for 15 min	Unspecified
Willemsen 2014	Commercial—Gravitational Platelet Separation system	Citrate dextrose solution A	27	9:1	3500 rpm for 15 min	Unspecified
Animal studies						
Pires Fraga 2010	Experimental	Unspecified	10	10:1	Two-step centrifugation: (1) 1450 rpm for 10 min; (2) 2100 rpm for 10 min	Unspecified
Rodríguez-Flores 2011	Experimental	Sodium citrate	10	4:1	Unspecified	Unspecified

g, relative centrifugal force.

Table 3. PRP Activation Methods

Primary Author	PRP Activation	PRP Activation Reagent	PRP Activation Details	Timing of PRP Activation	PRP Storage
Human studies					
Cervelli 2009 (1)	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Cervelli 2009 (2)	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Cervelli 2009 (3)	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Cervelli 2010	Yes	Thrombin	1:9 ratio of thrombin:PRP	Immediately before addition to fat	No
Cervelli 2011	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified
Cervelli 2012 (1)	Yes	Thrombin	Unspecified	Immediately before addition to fat	No
Cervelli 2012 (2)	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Cervelli 2013	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Fontdevila 2014	Yes	$CaCl_2$	1:10 ratio of $CaCl_2$:PRP	Immediately before addition to fat	No
Gennai 2016	No	N/A	N/A	N/A	No
Gentile 2012	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Gentile 2013	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Gentile 2014	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Keyhan 2013	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Nita 2013	Yes	$CaCl_2$	Unspecified	Unspecified	No
Rigotti 2016	Yes	$CaCl_2$ and thrombin	20 mm $CaCl_2$ and 25 IU/ml thrombin added; then incubated at 37°C for 1 h and 4°C for 16 h	Before cryogenic storage	Stored at −80°C; recovered by centrifugation at 3000 g for 20 min at 18°C
Salgarello 2011	Yes	$CaCl_2$	Unspecified	Immediately before addition to fat	No
Sasaki 2015	No	N/A	N/A	N/A	Ice water bath for 2-4 h preimplantation
Tolba 2015	Unspecified	Unspecified	Unspecified	Unspecified	Unspecified
Willemsen 2013	Yes	Thrombin	Unspecified	Unspecified	Unspecified
Willemsen 2014	Yes	$CaCl_2$	1:6 ratio of $CaCl_2$:PRP	Immediately before percutaneous injection	No
Animal studies					
Pires Fraga 2010	Yes	$CaCl_2$ and thrombin	Unspecified	Unspecified	Unspecified
Rodríguez-Flores 2011	Yes	$CaCl_2$	1:20 ratio of $CaCl_2$:PRP	Immediately before addition to fat	No

$CaCl_2$, calcium chloride; g, relative centrifugal force.

Fat Preparation

Fat preparation methods were more uniform between studies (Table 4). All human studies used a version of the Coleman liposuction technique,[72] excluding one[70] in which fat was obtained using microsuperficial enhanced fluid fat injection. Abdominal fat harvest (either with or without additional donor sites) was common to all human studies excluding one[66] where only the thigh was used. In the rabbit animal study using liposuction harvest, adipose tissue was obtained from the groin fat pad.[64] With the exception of the other animal study—in which adipose tissue was harvested from the scapular region and grafted to the ear using an open surgical approach[71]—all studies transplanted fat and PRP via percutaneous injection.

Several authors commented on a decantation step before fat centrifugation.[32,52,64,68] Centrifugation was typically performed for 3 minutes at 3000 rpm. Only 2 studies did not include a fat centrifugation step—in both cases, the authors used either saline[69] or Ringer's lactate[70] to clean the tissue before transplantation. Only one study[52] described routine fat storage—in this case using an ice bath for between 2 and 4 hours to cool fat before implantation, although no rationale was provided for this decision.

Recipient sites varied according to the indication. Most study designs involved a single recipient site for fat grafting (eg, for gluteal augmentation[65] or facial lipostructure[32,52,67]); other study designs were based around an indication for fat grafting, irrespective of target site (eg, for scar improvement[56,68]); 2 studies compiled data from unrelated patient series.[59,60]

PRP–Fat Grafting

Most studies admixed PRP and fat in sterile syringes immediately before cotransplantation. Three studies[52,66,68] transplanted PRP and fat separately, injecting PRP into the fat compartment after lipofilling. One animal study[71] soaked each 0.8 g adipose tissue fragment in 1 ml of activated PRP (for an unspecified length of time) before transplantation.

Relative PRP and fat volumes varied significantly between studies (Table 5). Several studies used a 1:1 ratio of PRP to fat,[32,64,71] whereas others used as little as 0.1 ml of PRP per ml of fat.[31,65–67] Many study designs used a range of PRP volumes for a given quantity of fat (eg, between 0.2 and 0.5 ml of PRP per ml of fat[29,53,56,57,60–62]). Only 3 of these studies provided a rationale for this: 2 were attempting to identify the optimal in vivo v/v concentration[29,60] and 1 selected PRP volume according to the size of the recipient site.[61]

Three studies (2 in humans[52,69] and 1 in rabbits[64]) routinely used prophylactic antibiotics. Several studies used additional regenerative adjuncts for either some or all of their grafts, including nonablative laser therapy,[56,68] hyaluronic acid–medicated biologic dressings,[54] and delayed percutaneous graft site insulin injections.[29]

DISCUSSION

Our article represents the first systematic review of methodological factors in autologous PRP and fat cotransplantation. It demonstrates that technical factors in graft preparation and administration vary significantly between in vivo studies. It also highlights that reporting of key procedural information is inconsistent and, in many cases, inadequate.

Evidence from both the fat[12,14,73] and PRP[35,36,63] literature suggests that methodological factors are critical determinants of experimental and clinical outcomes. It is therefore reasonable to assume that these procedural elements will be equally important in PRP and fat cotransplantation. Most studies included in this systematic review found that the addition of PRP to fat improves graft retention rates[29,52–62,64–71]; however, 3 articles[30–32] report no such survival advantage. It is possible that this variability is at least partially explained by differing study methodologies.

The lack of consistent reporting of essential technical factors further compounds the interpretation of such outcome heterogeneity. Without a minimum description of the qualitative and quantitative characteristics of the PRP–fat transplant used, it is impossible to draw meaningful cross-comparisons between studies.[23] The absence of sufficient experimental methodological detail or standardized preparation protocol may limit the translation of PRP-enhanced fat grafting into clinical practice.

For PRP alone, various authors have proposed descriptive classification taxonomies based upon either preparation methods[23] or final product constituents.[50] The absence of an accepted methodology for the preparation and activation of PRP reflects ongoing disagreement in the preclinical literature.[44] Several experimental studies have attempted to optimize this process,[25,74] but consensus has yet to be reached. The growing use of commercial devices—each with their own unique PRP product characteristics[34,35,63,75]—further compounds the issue.

Comparatively, the preparation of adipose tissue for structural fat grafting is less controversial, and most included studies employed a version of the Coleman liposuction technique.[72] However, greater variability is seen in the v/v mixing ratio of PRP to fat, with up to 10-fold differences in substrate concentrations used. Again, this reflects a lack of consensus in the preclinical literature. Two studies found that a PRP volume fraction of 5% provided the optimal environment for ASC proliferation in vitro[28,45] (with paradoxical inhibition of ASC proliferation at higher concentrations[28]). However, these findings have not been replicated elsewhere: 1 study found that 20% PRP v/v promoted maximal graft viability,[47] whereas a different research group identified a dose-dependent effect for concentrations up to 50%.[29] This inconsistency likely reflects intertrial heterogeneity of the PRP product used.[47] It is also worth considering that, in most clinical scenarios, the most appropriate v/v concentration will be derived from the minimally effective ratio of PRP to fat. In the case of large volume fat transplantation, higher ratios (for example, like those approaching 1:1 in the 2 included animal studies[64,71]) would otherwise require the use of an unfeasibly large volume of whole blood.

This systematic review provides a comprehensive descriptive analysis of the current methodological approaches to in vivo autologous PRP and fat cotransplantation. It

Table 4. Fat Preparation Methods

Primary Author	Fat Harvest Method	Fat Harvest Location	Fat Processing Method	Fat Centrifugation Protocol	Recipient Site	Fat Storage
Human studies						
Cervelli 2009 (1)	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Face, leg	No
Cervelli 2009 (2)	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Leg	No
Cervelli 2009 (3)	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Face	No
Cervelli 2010	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Leg	No
Cervelli 2011	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Leg	No
Cervelli 2012 (1)	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Face, abdomen, arm, leg	No
Cervelli 2012 (2)	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Face, abdomen, arm, leg, breast	No
Cervelli 2013	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Face, leg	No
Fontdevila 2014	Coleman technique liposuction	Unspecified	Unprocessed	3000 rpm for 3 min	Face	No
Gennai 2016	M-SEFFI technique liposuction	Abdomen, hip, thigh, knee	Cleaned with Ringer's lactate; decanted; cleaned with Ringer's lactate	Uncentrifuged	Face	No
Gentile 2012	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Breast	No
Gentile 2013	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Breast	No
Gentile 2014	Coleman technique liposuction	Abdomen	Unprocessed	3000 rpm for 3 min	Face	No
Keyhan 2013	Coleman technique liposuction	Abdomen, knee	Unprocessed	3000 rpm for 3 min	Face	No
Nita 2013	Coleman technique liposuction	Abdomen, flank	Decanted	200 rpm for 5 min	Face, abdomen, arm, leg, breast	No
Rigotti 2016	Coleman technique liposuction	Abdomen	Decanted	Uncentrifuged	Face	No
Salgarello 2011	Coleman technique liposuction	Abdomen, hip, thigh, knee, flank	Unprocessed	3000 rpm for 3 min	Breast	No
Sasaki 2015	Coleman technique liposuction	Abdomen, hip	Decanted	3000 rpm for 3 min	Face	Submerged in ice water bath for 2-4 h preimplantation
Tolba 2015	Coleman technique liposuction	Abdomen, thigh	Cleaned with saline then dried and loaded into syringes	N/A	Face	No
Willemsen 2013	Coleman technique liposuction	Abdomen, hip, thigh, knee, flank, subgluteal	Unprocessed	3000 rpm for 3 min	Buttock	No
Willemsen 2014	Coleman technique liposuction	Thigh	Unprocessed	3000 rpm for 2.5 min	Face	No
Animal studies						
Pires Fraga 2010	Open excision	Scapular region	Unprocessed	Uncentrifuged	Ear	No
Rodríguez-Flores 2011	Coleman technique liposuction	Groin fat pad	Decanted	Uncentrifuged	Lip	No

g, relative centrifugal force; M-SEFFI, microsuperficial enhanced fluid fat injection.

Table 5. PRP and Fat Cotransplantation Methods

Primary Author	Method of PRP–Fat Mixing	PRP–Fat Mixing Timing	PRP:Fat Ratio	Prophylactic Antibiotics	Other Adjuncts
Human studies					
Cervelli 2009 (1)	Admixed in syringe	Immediately before fat transplantation	0.5:1	No	No
Cervelli 2009 (2)	Admixed in syringe	Immediately before fat transplantation	Variable (average 0.5:1)	No	No
Cervelli 2009 (3)	Admixed in syringe	Immediately before fat transplantation	0.3–0.5:1	No	No
Cervelli 2010	Admixed in syringe	Immediately before fat transplantation	0.5:1	No	All wounds covered with hyaluronic acid biologic dressing
Cervelli 2011	Admixed in syringe	Unspecified	0.3–0.5:1	No	No
Cervelli 2012 (1)	Admixed in syringe	Immediately before fat transplantation	0.2–0.3:1	No	1/3 (n = 20) patients received nonablative laser therapy
Cervelli 2012 (2)	Admixed in syringe	Unspecified	0.2–0.5:1	No	≈1/4 (n = 10) received insulin injections on d7 and d15
Cervelli 2013	Admixed in syringe	Immediately before fat transplantation	0.2–0.5:1	No	No
Fontdevila 2014	Admixed in syringe	Immediately before fat transplantation	0.25:1	No	No
Gennai 2016	Admixed in syringe	Immediately before fat transplantation	0.2:1	No	No
Gentile 2012	Admixed in syringe	Immediately before fat transplantation	0.4–0.5:1	No	No
Gentile 2013	Admixed in syringe	Immediately before fat transplantation	0.5:1	No	No
Gentile 2014	Admixed in syringe	Immediately before fat transplantation	0.5:1	No	No
Keyhan 2013	Admixed in syringe	Immediately before fat transplantation	0.1–0.2:1	No	No
Nita 2013	Grafted separately	PRP injected after fat transplantation	Unspecified	No	All patients received nonablative laser therapy
Rigotti 2016	Admixed in syringe	Immediately before fat transplantation	1:1	No	No
Salgarello 2011	Admixed in syringe	Immediately before fat transplantation	0.1:1	No	No
Sasaki 2015	Grafted separately	PRP injected after fat transplantation	0.2:1	Oral (unspecified) for 10 d and topical ointment	No
Tolba 2015	Unspecified	Unspecified	0.25:1	Single prophylactic intravenous dose (unspecified)	No
Willemsen 2013	Admixed in syringe	Immediately before fat transplantation	0.1:1	No	No
Willemsen 2014	Grafted separately	PRP injected after fat transplantation	0.1:1	No	No
Animal studies					
Pires Fraga 2010	Fat graft bathed in activated PRP (time unspecified)	Unspecified	1:0.8	No	No
Rodríguez-Flores 2011	Admixed in syringe	Immediately before fat transplantation	1:1	Oxytetracycline for 3 d	No

is based upon a robust, multiperson search strategy with additional article retrieval from reference lists, but it remains possible that potentially eligible studies were not identified. During the protocol stage, we designed a set of inclusion criteria to address our current research objective; studies using nonautologous and isogenous sources of PRP were excluded, as were trials using second-generation PRP products (such as platelet-rich fibrin). Similarly, experimental approaches using secondarily extracted fat components only (including the stromal vascular fraction and isolated ASCs) were deemed beyond the scope of this review. Finally, we have focused on critically reviewing methodological factors in PRP and fat cotransplantation but have not evaluated other areas that could influence experimental outcome (including outcome measure selection or indication for intervention).

CONCLUSIONS

Methodological factors in autologous PRP-enhanced fat cotransplantation vary significantly between in vivo studies. This is confounded by inconsistent and inadequate reporting of essential procedural information and combined PRP–fat transplant characteristics. The absence of sufficient methodological detail makes meaningful evaluation of the PRP-enhanced fat grafting literature difficult.

PRP and fat cotransplantation is an innovative and potentially transformative therapeutic strategy in regenerative medicine. However, a failure to address the fundamental issues identified in this review may limit its translation into clinical practice.

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