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Venous Blood Derivatives as FBS-Substitutes for Mesenchymal Stem Cells: A Systematic Scoping Review

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Although the biological properties of mesenchymal stem cells (MSC) are well-characterized *in vitro*, MSC clinical application is still far away to be achieved, mainly due to the need of xenogeneic substances for cell expansion, such as fetal bovine serum (FBS). FBS presents risks regarding pathogens transmissions and internalization of animal's proteins, which can unleash antigenic responses in patients after MSC implantation. A wide range of venous blood derivatives (VBD) has been reported as FBS substitutes showing promising results. Thus, the aim of this study was to conduct a systematic scoping review to analyze whether VBD are effective FBS substitutes for MSC *ex vivo* expansion. The search was performed in SciVerse Scopus™, PubMed, Web of Science™, BIREME, Cochrane library up to January 2016. The keywords were selected using MeSH and entry terms. Two independent reviewers scrutinized the records obtained considering specific inclusion criteria. The included studies were evaluated in accordance with a modified Arksey and O' Malley's framework. From 184 found studies, 90 were included. Bone marrow mesenchymal stem cells (BMMSC) were presented in most of these studies. Overall, VBD allowed for either, maintenance of MCS's fibroblast-like morphology, high proliferation, high colony-formation ability and maintenance of multipotency. Besides. MSC expanded in VBD supplements presented higher mitogen activity than FBS. VBD seems to be excellent xeno-free serum for *ex vivo* expansion of mesenchymal stem cells. However, an accentuated heterogeneity was observed between the carried out protocols for VBD isolation did not allowing for direct comparisons between the included studies.

Key Words: human serum, mesenchymal stem cells, platelet, venous blood derivatives, xeno-free.

Introduction

Mesenchymal stromal/stem cells (MSC) have been exhaustively investigated *in vitro* and due to high proliferative, self-renewal, immunomodulatory properties and multipotency, MSC present a high therapeutic potential to be applied in Stem Cell-Based Therapies (SC-BT) (1). Several strategies and approaches to use regenerative therapies in dentistry have been investigated (1-3), since that materials employed by the clinicians are, basically, synthetic (4-6) and can present limited ability to induce regeneration (1,3,7). Thus, the use of MSC could improve the regenerative potential of bone, periodontal and dental pulp regenerative approaches. However, a recent scoping review evaluating the capacity of dental pulp tissue regeneration by strategies to revascularization of root canal has shown limited ability to promote regeneration (3) and this could be improved with the application of MSC. Although MSC's biological properties have been well-characterized *in vitro*, MSC clinical application is still far away to be achieved (8).

To be clinically applied, MSC must be previously isolated and expanded *ex vivo* in order to obtain a needed amount of cells, which will be replanted in patients (8,9). *Ex vivo* MSC expansion relies on solutions composed by a basal medium, basically amino acids, vitamins and inorganic salts, which must be supplemented by Fetal Bovine Serum (FBS) (10,11). FBS is the most applied supplement for MSC culture comprising a complex mixture of growth factors (GF), proteins, carbohydrates and cytokines indispensable for cell development and survival *in vitro* (12-14). However, FBS presents risks regarding pathogens transmissions and internalization of animal's proteins, which can unleash antigenic responses in the patient after MSC implantation (9,15). Animal-derived (or xenogeneic) proteins can be detected in human MSC expanded in FBS, even after consecutive cell washings (9). Additionally, FBS induces changes in MSC surface markers and due to such characteristics. FBS must not be applied in humans (16).

To reduce the barriers arising from the use of xenogeneic materials and to allow the clinical acceptance of SC-BT, venous blood derivatives (VBD) has been widely considered to be applied as FBS substitutes (17-20). Venous blood is a source that can be easily obtained in a large volume from blood banks or from the own patient, decreasing the barriers for the use of SC-BT (2,8,9). In contrast, umbilical cord blood is more difficult to obtain donors and provide few volume of blood available (12,21-23). Therefore, VBD presetting an important source easily accessed to clinicians providing a potential xeno-free supplementation for MSC expansion (24,25). In addition, a wide range of different serum/plasma blood derivatives has been reported as FBS substitutes showing promising results. Thus, the aim of this study was to conduct a scoping review to analyze whether VBD are effective FBS substitutes for MSC *ex vivo* expansion.

Material and Methods

This study was designed following the modified five-stage framework proposed by Arksey and O'Malley (26) denominated scoping review. Recently, several studies applied scoping review to state the current knowledge in a particular area when a systematic review cannot be conducted (3,27). The scoping review design is indicated when the methodologies of studies present a considerable heterogeneity performing a qualitative analysis while a systematic review provides a quantitative analysis. A scoping review presents an exploratory research to respond a broader question through a systematized research, aiming to define concepts and mapping the methodologies used to define gaps in the literature to indicate the need for new studies. A scoping review was carried out to perform a knowledge synthesis regarding VBD as FBS substitutes for *ex vivo* MSC expansion. A complementary search was performed to identify the studies using MSC in humans expanded in VBD.

Conceptual Definition

According to MeSH database (http://www.ncbi.nlm.nih.gov/mesh), serum is defined as "The clear portion of blood that is left after blood coagulation to remove blood cells and clotting proteins by centrifugation". In the meantime, plasma is defined as "the residual portion of blood that is left after removal of blood cells by centrifugation without prior blood coagulation". Unlike plasma, serum naturally contains platelet-derived molecules, such as α-granules-derived growth factors, which become available exclusively after platelet-activation. For Human Serum (HS) the whole blood is collected in an anticoagulant-free plastic bag and stored overnight (4 °C), or at the end of shelf-life, to allow for blood coagulation. Right after, the formed clot must be centrifuged (3000 rpm for 5 min) in order to obtain a supernatant, corresponding to HS (21). Platelet-Rich Plasma (PRP) is stated as "A preparation consisting of platelets concentrated in a limited volume of plasma". While the natural-formed blood clot contains 95% of red blood cells, 5% platelets, less than 1% white blood cells and fibrin strands, PRP holds 4% red blood cells, 95% platelets and 1% white blood cells (28). PRP should be chemically activated by the addition of human/bovine thrombin or Calcium Chloride - $CaCl_2$, affording activated PRP -aPRP (24,25,29). To obtain PRP, whole anticoagulated blood, must be submitted to a double-centrifugation; the first one (soft spin) results in a three-layer suspension where the red blood cells are found at the bottommost layer. Both, topmost, named platelet-poor plasma (PPP), and intermediate (PRP) layers should be transferred to another tube without anticoagulant. Thus, the second spinning (hard spin) is performed, to allows platelets settle the bottom of tube. Then, superficial layer is discarded and the remaining material (PRP) is shaken. To release its platelet content, PRP must be activated (aPRP) by the addition of human/bovine thrombin or Calcium Chloride ($CaCl_2$) (24,25). Human Platelet Lysate (HPL) results from a lysis of high platelet concentrate (traditionally the PRP), being the platelet mechanical lysis induced by susceptive freeze-thaw cycles at -80 or -20 °C (typically 2 or 3 cycles). Thus, the platelet debris must be separated from the clear portion containing platelet released by centrifugation.

Information Sources, Literature Search and Inclusion Criteria

A structured search was performed in SciVerse Scopus™, PubMed/Medline, ISI Web of Science™, and BIREME up to January 2016. The relevant MeSH terms and entry terms (Table 1) were selected based on the PICO-structured

Table 1. Structured search strategy carried out in MEDLINE/PubMed database. The search followed structural of each database

	Search Syntaxes
#1	"mesenchymal stromal cells"[MeSH Terms] OR ("mesenchymal"[All Fields] AND "stromal"[All Fields] AND "cells"[All Fields]) OR "mesenchymal stromal cells"[All Fields] OR ("mesenchymal"[All Fields] AND "stem"[All Fields] AND "cells"[All Fields]) OR "mesenchymal stem cells"[All Fields]
#2	"Culture Media, Serum-Free"[All Fields] OR "Culture Media, Serum Free"[All Fields] OR ("culture media, serum-free"[MeSH Terms] OR ("culture"[All Fields] AND "media"[All Fields] AND "serum-free"[All Fields]) OR "serum-free culture media"[All Fields] OR ("media"[All Fields] AND "serum"[All Fields] AND "free"[All Fields] AND "culture"[All Fields])) OR "Serum-Free Culture Media"[All Fields] OR "Serum-Free Media"[All Fields] OR "Media, Serum-Free"[All Fields] OR "Serum Free Media"[All Fields] OR "Protein-Free Media"[All Fields] OR ("culture media, serum-free"[MeSH Terms] OR ("culture"[All Fields] AND "media"[All Fields] AND "serum-free"[All Fields]) OR "serum-free culture media"[All Fields] OR ("media"[All Fields] AND "protein"[All Fields] AND "free"[All Fields])) OR "Protein Free Media"[All Fields] OR "Low-Serum Media"[All Fields] OR "Low Serum Media"[All Fields] OR ("culture media, serum-free"[MeSH Terms] OR ("culture"[All Fields] AND "media"[All Fields] AND "serum-free"[All Fields]) OR "serum-free culture media"[All Fields] OR ("media"[All Fields] AND "low"[All Fields] AND "serum"[All Fields]))
#3	"Platelet lysate"[All Fields] OR "thrombin activated platelet"[All Fields] OR (allogenic[All Fields] AND pooled[All Fields] AND ("humans"[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]) AND ("serum"[MeSH Terms] OR "serum"[All Fields])) OR "pooled human serum"[All Fields] OR "autologous serum"[All Fields] OR "human serum"[All Fields] OR "thrombin activated platelet"[All Fields] OR "pooled human platelet lysate"[All Fields] OR "platelet rich plasma"[All Fields]

question "Could human venous blood derivatives be applied as FBS substitutive for MSC *ex vivo* expansion?", where:

- P: Human MSC
- I: human serum; Platelet Rich Plasm; Activated Platelet-Rich Plasm ; Human Platelet lysate; Plasma; Platelet Poor Plasma; Human Plasm
- C: Fetal Bovine Serum
- O: MSC biological properties: Cell viability, cell proliferation, multipotency, population doubling time, senescence, telomere shortening

The retrieved records were uploaded into the EndNote™ software, aiming to delete duplicates and to build up a virtual library (VL). Two independent reviewers (LAC and MCMC) read the titles and abstracts of all reports, under predefined inclusion criteria (Table 2). To confirm if the selected studies met the inclusion criteria, the same reviewers independently judged each full text. If any disagreement was found, the reviewers attempted to reach a consensus through discussions. Persistent disagreements have been decided by an intervention from the third reviewer (FFD). Thus, manual evaluation of references from each evaluated study was performed. Twenty percent of the studies were randomly raffled, and the data have been again checked.

Search to identify clinical application of cell therapy using blood derivate serums: The literature was investigated using the keywords: "clinical study", "mesenchymal stem cell", "serum-free medium", "Platelet lysate", "human serum" and "autologous serum" for identify studies employing cell therapy in humans with *ex vivo* expansion in medium supplemented with VBD, to provide an overview of clinical application.

Results

The initial search yielded 272 records corresponding to 184 studies (Table 3). After preliminary titles and abstracts evaluation, 102 studies were select for full-text assessment (Fig. 1). Ninety papers were designated for data extraction.

Table 2. Criteria for selection of the studies

	Inclusion Criteria	Exclusion criteria
Studies	Original papers *in vitro* and *in vivo*	Reviews
Language	English, Spanish, Italian, French, and Portuguese	Other languages
Cell type	Human MSC	not human MSC
VBD from	Human serum and plasma; platelet lysate; thrombin-activated platelet release in plasma; platelet rich plasma	Umbilical cord blood; synthetic
Aim	VBD as FBS substitute	

Excluded studies (10,30-40) and reasons are described in Table 4. Most of the included studies described protocols relying on the platelets lyse after freeze-thaw cycles to obtain VBDs. Bone marrow mesenchymal stem cells (BMSC) were presented in most of this studies (Table 5); adipose stem cells - ASC (24,41-46), dental pulp stem cells - DPSC (47,48), umbilical cord stem cells - UCST (22,25,49-52) and orbital fat-derived stem cells (53) were also tested. VBD concentrations added to culture medium ranged from 0.5 to 30% (54-56). Overall, VBD allowed for either, maintenance of MCS's fibroblast-like morphology (17,43,57), high proliferation (58-60), high colony-formation ability (52,61,62) and maintenance of multipotency (63-67). A linear dose-dependent response regarding medium concentration was not observed for evaluated VBD (54,56,68).

Human platelet lysate (HPL): HPL showed a higher osteogenic potential than MSC in FBS (55,69,70). Besides, MSC expanded in HPL seems presented immunomodulatory ability (52,55,71,72) despite a decrease of ability of inhibition NK and T-cells has also been observed (73). Platelets lyse was triggered by either, freeze-thaw cycles (1 to 5), ultrasound (63) or chemical treatment (43). HPL presented a high growth factors and cytokines content (74-77). HPL seemed to be better than FBS for *ex vivo* MSC expansion (52,72,78,79) since HPL-expanded MSC did not present telomerase shortening (68).

Human Serum (HS): Overall, HS allowed for the isolation and expansion of BMSC, ASC, DPSC maintaining proper cell biological properties both *in vitro* and *in vivo* (48,80-85). Both, 10% HS in DMEM/F-12 or 5%-10% HS in αMEM provided, to ASC and BMMSC, proliferation rates and multipotency as higher as 10% FBS (24,48,51,86-88).

Platelet-rich plasma: Platelets form PRP has been activate with trombin or calcium chloride aiming the increase of bioactive molecules release (24,29,89), however similar properties were observed in PRP (10% freeze thawed human PRP (49,91) / with platelet concentration (92,93)/ platelet and leukocytes concentration (90)). PRP (5% or 10%)-supplemented media heat-inactivated (platelet concentration 79.6 x 10^4/μL) provided similar results for adipogenic and osteogenic differentiation when compared to 10% FBS (92). Besides, PRP supplemented αMEM also provides osteogenenic, chondrogenic and adipogenic differentiation promoting an increase of cell culture

Table 3. Records recovered in each database

PubMed®	Scopus®	ISI web of science®	Cochrane library®	BVS Bireme®
64	121	68	0	19

proliferation (22,49). In this way, 10% aPRP (platelets concentered) has been reported as providing similar (25), or higher proliferation rates than FBS (89). In addition, only in one study reported the presence of leukocyte into PRP (0.3×10^4 mL) (90). Strategies to decreased leukocyte in the PRP even as assessment of activation by flow cytometry did not were reported on included studies. Other VBD: MSC were also expanded by applying platelet-poor plasma (PPP) (94), fresh frozen platelet plasma (FFPP) (56,95) and HS obtained from PPP (42). Either MSC expanded in PPP, FFPP and in FBS had the same phenotype for antigens CD73, CD90, CD105, CD14, CD19, CD34, CD45, HLA-DR. Such MSC presented osteogenic, adipogenic and chondrogenic differentiation and were able to survive in a fibrin clot. BMSC expanded in culture medium containing high (20 and 30%) and low (1%) FFPP concentrations, produced insufficient calcified matrix (56).

Human studies: Seven studies employed MSC expanded in VBD for tissue regeneration (96-102). HPL and HS were used for MSC expansion for clinical application. These studies did not observe neither signal of malign transformation nor some complication associated with HPL or HS.

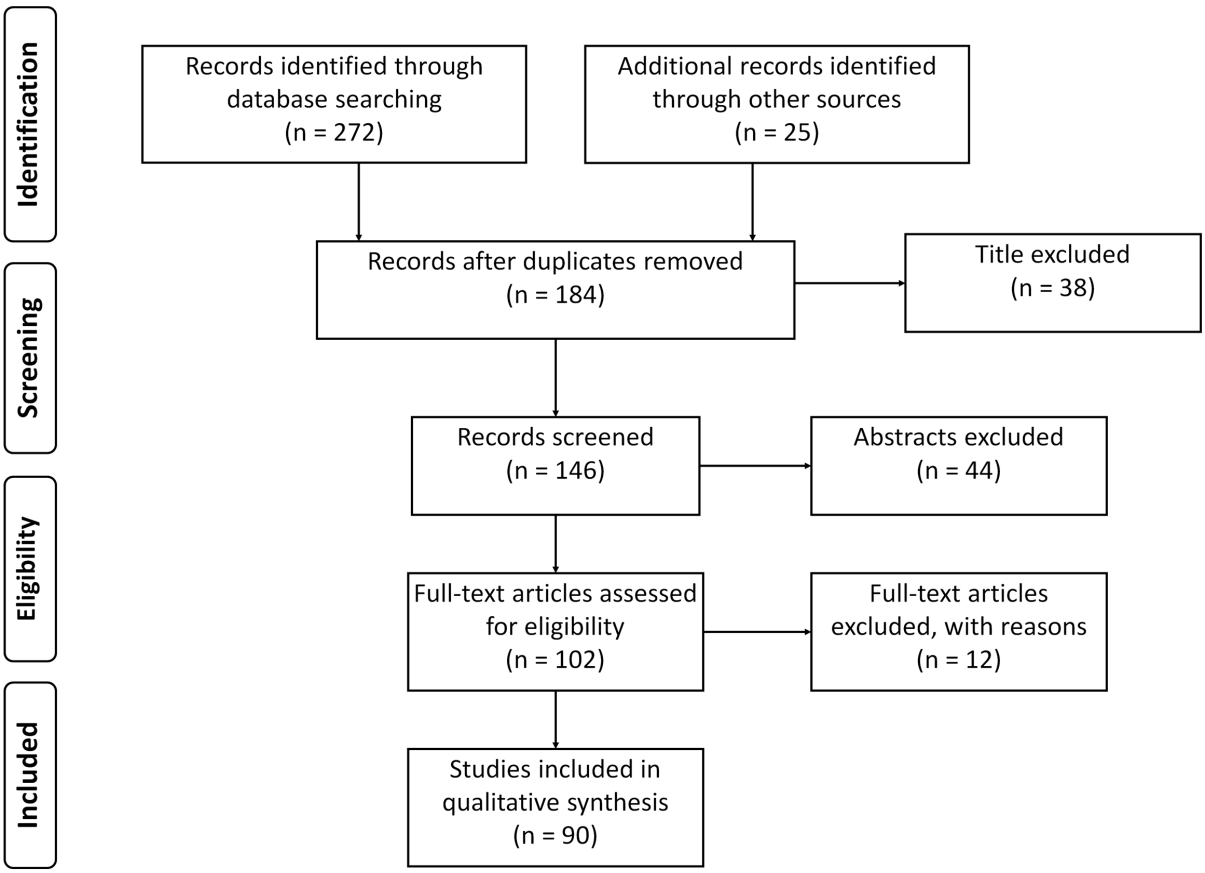

Figure 1. Flow Diagram

Table 4. Excluded studies and reasons for exclusion

Studies	Reason
Cheng et al. (30); Jung et al. (10); Dolley-Sonneville et al. (32); Mark et al. (33); Reza et al. (34); Sato et al. (35); Tan et al. (36); Trubiani et al. (37)	Not defined venous blood derivative serum
Schallmoser et al. (38); Jung et al. (39); Jung et al. (40)	Review

Table 5. Included studies and respective VBD supplements and concentrations, concentration of FBS and stem cells utilized

Author	Xeno-free evaluated and concentration	Contol and concentration	Stem Cells
Stute et al. (21)	1%, 3%, and 10% HS	10% FBS	BMSC
Doucet et al. (62)	5% HPL	10% FBS	BMSC
Shahdadfar et al. (80)	20% HS	FBS 20%	BMSC
Gregory et al. (81)	20% HS and 10% HS + grow factors	FBS 20%	BMSC
Muller et al. (95)	2.5% and 5% HPL	10% FBS	BMSC
Vogel et al. (111)	3% PRP	FBS 2%	BMSC
Le Blanc et al. (82)	10% HS	10% FBS	BMSC
Capelli et al. (60)	5% HPL	FxBS 10%	BMSC
Kocaoemer et al. (89)	10% HS and 10% aPRP	10% FBS	ASC
Lange et al. (78)	5% HPL	10% FBS	BMSC
Lataillade et al. (102) *	8% HPL	-	BMSC
Reinisch et al. (72)	10% HPL	10% FBS	UCMSC and BMSC
Schallmoser et al. (75)	10% HPL	10% FBS	BMSC
Schallmoser et al. (114)	5% and 10% HPL	10% FBS	BMSC
Shayesteh et al. (97) *	20% HS	-	BMSC
Zaky et al. (70)	5% HPL and 5% HPL + 10% FBS	10% FBS and FGF2	BMSC
Behnia et al. (99)	20% HS	-	BMSC
Bieback et al. (29)	10% aPRP and 10% HPL and 10% HS	10% FBS	BMSC
Blande et al. (64)	2.5% or 10% HPL	10% FBS	ASC
Lindroos et al. (86)	10% HS	10% FBS or StemPro®	ASC
Kishimoto et al. (84)	8% 4% 2%, 1%, 0.5% HS with FGF_2 5 ng/mL (F/P MP-Coated plates)	not coated HS	BMSC
Pytlik et al. (88)	10% HS	10% FBS	BMSC
Schallmoser et al. (20)	10% HPL	-	MSC
Bieback et al. (24)	10% HS and 10% aPRP	10% FBS	ASC
Centeno et al. (101) *	10-20% HPL	-	BMSC
Chevallier et al. (59)	5% HPL	10% FBS	BMSC
Felka et al. (56)	FFP with platelet concentrate 0.5, 1%, 5%, 10%, 20% and 30% and human plasma-enriched with platelets media 5% and 10%	10% FBS	BMSC
Hartmann et al. (51)	2% HS	FBS	UCMSC
Horn et al. (69)	10% HPL	10% FBS	MSC
Ichiyanagi et al. (94)	Platelet poor plasma 5%, 10% and 20%	10% FBS	BMSC
Lindroos et al. (87)	10%, 15% and 20% HS	10% FBS	ASC
Lucchini et al. (100) *	5% HPL	-	MSC
Salvade et al. (77)	5% HPL	10% FBS	BMSC
Abdelrazik et al. (73)	10% HPL	10% FBS	MSC
Aldahmash et al. (85)	2.5%, 5.0% and 10.0% HS	10% FBS	BMSC
Capelli et al. (108)	5% HPL	-	UCSC

Cholewa et al. (41)	1%, 2.5%, 5%, 10%, and 20% HPL	FBS 1%, 2.5%, 5%, 10%, and 20%	ASC
Crespo-Diaz et al. (109)	5% HPL	10% FBS	BMSC and ASC
Flemming et al. (71)	10% HPL	10% FBS	BMSC
Goedecke et al. (92)	5% and 10% PRP	10% FBS	BMSC
Govindasamy et al. (47)	10% HPL	10% FBS	DPSC
Hatlapatka et al. (50)	HS 2%, 5%, 10% and HS 2% with 0.5 ng/mL FGF	FBS	UCMSC
Jenhani et al. (65)	5%, 10% HPL or 5% HPL and 10% FBS or 10%HPL and 10%FBS	10% FBS	BMSC and UCMSC
Shih et al. (43)	10% HPL	10% FBS	ASC
Venugopal et al. (106)	5% HS and 2 ng/mL bFGF	10% FBS	WJSC
Xia et al. (76)	7.5% HPL	10% FBS	BMSC
Jenhani et al. (65)	5%,10% HPL 10% FBS 5% HPL	10% FBS 1 ng/mL fibroblast growth factor 2 (FGF2)	BMSC
Behnia et al. (96) *	20% HS	-	BMSC
Fekete et al. (12)	2.5%, 5%, 10%, 15% and 20% HPL	FBS 20%	BMSC
Fekete et al. (13)	10% HPL	-	BMSC
Gottipamula et al. (54)	10% HPL (5 to 20% preliminary screening)	10% FBS	BMSC
Kishimoto et al. (93)	4%, 2%, 1% and 0.5% PRP or 4%, 2%, 1% and 0.5% HS	-	BMSC and ASC
Kruger et al. (90)	10% HS and 5% PRP	-	CSSC
Lohmann et al. (61)	10% HPL	10% FBS	BMSC
Murphy et al. (22)	1% or 10% PRP	10% FBS	UCMSC
Pisciotta et al. (48)	10% HS	10% FBS	DPSC
Poloni et al. (113)	5% HPL and HS 10%	FBS 20%	CVMS and BMSC
Stehlik et al. (11)	10% HS	-	BMSC
Bernardi et al. (63)	10% HPL	10% FBS	BMSC
Griffiths et al. (68)	2%, 5% and 10% HPL	FBS 16%	BMSC
Hemeda et al. (117)	10 % HPL	-	BMSC and ASC
Trojahn Kolle et al. (107)	10% HPL	10% FBS	ASC
Kyllonen et al. (45)	10% HS	10% FBS	ASC
Mojica-Henshaw et al. (118)	10% HPL	10% FBS	MSC
Pawitan et al. (83)	5% and 10% PRP 5% and 10% HS	-	ASC
Rojewski et al. (120)	10% HPL	10% FBS	BMSC
Sandor et al. (98) *	15% HS	-	ASC
Schallmoser et al. (58)	10% HPL	10% FBS	MSC
Shanskii et al. (74)	10% HPL FBS/HPL ratios: 10/0, 7.5/2.5, 5.0/5.0, 2.5/7.5, and 0/10)	FBS	MSC
Castiglia et al. (23)	10% HPL	10% FBS	BMSC
Fekete et al. (14)	5%, 10% and 20% HPL 5%, 10% and 20% HS	FBS 5%, 10% and 20%	BMSC
Gottipamula et al. (104)	2.5 % HS	10% FBS	BMSC
Iudicone et al. (66)	10% HPL	10% FBS	BMSC

Koellensperger et al. (42)	HS 2%, 10%; PPP 2%, 10%; PL 2%, 10%	10% FBS	ASC
Martins et al. (53)	10% HS	10% FBS	OFSC
Muraglia et al. (19)	5% HPL	10% FBS	BMSC
Pham et al. (25)	2%, 5%, 7% and 10% aPRP	10% FBS	UCMSC
Yamauchi et al. (79)	5% HPL	10% FBS	BMSC
Antoninus et al. (17)	HPL 0.031 mg/mL, 0.063 mg/mL, 0.125 mg/mL, and 0.250 mg/mL	20% FBS	WJSC
Budiyanti et al. (49)	PRP 10%	MesenCult®	UCSC
Castren et al. (18)	AB-plasma 2.5% + HPL 0.5%	10% FBS	BMSC
Jonsdottir-Buch et al. (15)	10% HPL	-	BMSC
Laitinen et al. (55)	5% and 10% HPL	10% FBS	BMSC
Laner-Plamberger et al. (67)	10% HPL	10% HPL	BMSC and UCMSC
Li et al. (57)	5% HPL	-	BMSC and ASC
Luzzani et al. (52)	10% HPL	10% FBS	UCMSC and iPS
Oikonomopoulos et al. (46)	10% HPL	10% FBS	BMSC and ASC
Paula et al. (44)	HS 10%	10% FBS	ASC
Pawitan et al. (91)	PRP 10%	10% FBS	BMSC
Riordan et al. (105)	5%, 7.5% and 10% HPL	10% FBS	WJSC

* MSC expanded in VBD for tissue regeneration in humans. MSC: mesenchymal stem cells; UCSC: umbilical cord derived mesenchymal stem cells; ASC: adipocyte stem cells; BMSC: bone marrow stem cells; WJSC: Wharton's Jelly-derived stem cells; OFSC: orbital fat-derived stem cells; CVMS: chorionic villi-derived stem cells; DPSC: dental pulp stem cells; CSSC: human cortico-spongious stem cells; FFP: fresh frozen plasma.

Discussion

The recent literature has been investigated FBS substitutes for MSC *ex vivo* expansion aiming to eliminated the risks inherent to xenogeneic agents for clinical translation of regenerative therapies (103,104). In this systematic scoping review, the studies evaluating VBD as FBS substitutes were summarized. Overall, different protocols were developed and tested to obtain a FBS substitute able to maintain MSC *in vitro*, preserving stemness and multipotency (103,105). VBD were promissors as FBS substitutes, since senescence (68,106) or Karyotype/chromosomal alteration were not detected in MSC expanded in VBD (13,23,107-109). In addition, clinical studies strengthened such evidence by reporting none malign transformation *in vivo* (96,99,101,102).

Despite blood of umbilical cord possess a higher amount of growth factors (platelet-derived growth factors -PDGF, fibroblast growth factor 2 -FGF-2 and vascular endothelial growth factor - VEGF) when compared to venous blood, the application of blood from umbilical cord is restricted due the difficult to obtain large volumes (12,21-23). Besides, the growth factors concentration found in different VBD (12,22,110) was reported as higher than in FBS (22,41,43,111). Autologous and homologous blood have been presented a high amount of platelets, which contains the growth factors responsible VBD therapeutic potential (112). During platelet activation biomolecules such as insulin-like growth factor (IGF), PDGF, transforming grow factor β (TGF-β), and other molecules such as thrombospondin and fibronectin are released (112). HPL presented high concentrations of endothelial growth factor (EGF), PDGF, TGF-β, fibroblast growth factors β (FGF-β) and VEGF when compared to HS (110). Furthermore, the low proteic content observed in HPL may be beneficial by decreasing the risk of immunological reactions for allogenic blood-derived (110). The platelets lyse seems to release all platelet-derived growth factors available, which could not happen during blood coagulation (103). Nonetheless, Poloni (113) showed HS inducing higher cell proliferation than HPL, contrary to the expected. Even so, both were better than FBS.

MSC expanded in VBD supplements have presented major mitogen activity than FBS (8) despite several protocols and concentrations being tested, decreasing larger comparisons. 10% HS-expanded DPSC presented lower initial proliferation rate and population doubling time (PDT) until the fourth day, when compared to 10% FBS-expanded MSC. After the fourth day, an increase in proliferation of cells under HS 10% was reported. HS 10% expanded DPSC were capable of regenerating more mineral tissue than those 10% FBS-expanded *in vivo*

(48). The studies reported 5% (68) and 10% (12) as being the optimal HPL concentration range for MSC expansion (12,54,64,114). Moreover, different VBD concentration (0.5-30%) have been tested presenting different results, which have not been necessarily dose-dependent (54-56). Overall, the selected studies indicated VBD concentrations ranging from 5% to 10% by presenting good results to be applied as FBS substitutive (8,21,25,56,94).

DMEM is currently applied for MSC isolation and expansion (1,5,115,116); DMEM calcium content can stimulate a polymerization of fibrin present in HPL, thereby forming a gel into the solution (8). To avoid this, fibrinogen or heparin has been applied (69). Despite almost studies use 1 or 2 IU/mL heparin, Hemeda, Giebel (8) demonstrated that 0.61 IU/mL or 0.024 mg/mL for low-molecular-weight heparin was sufficient to avoid gel formation. However, high heparin concentrations seem to reduce adipogenic and osteogenic differentiation, as well as cell proliferation (117). In the protocol to obtain the HPL, the Induction of fibrin clotting formation with calcium chloride (instead of thrombin) and posterior centrifugation provides a serum with the same profile of growing factor and cytokines than conventional HPL activated with thrombin, resulting in a serum without free of xenogeneic substances such as porcine thrombin (118). HPL supplemented without anticoagulants tend to form a translucent and viscous gel in 1 h providing a natural scaffold for MSC culture and expansion (61). This matrix, composed by a fibrin network, was biocompatible and biodegradable. Additionally, MSC cultured in this fibrin matrix presented higher proliferation since growth is available in three-dimensional environment, increasing the culture area (8,61).

Interesting findings have been observed regarding to trypsin kinetics of MSC supplemented with VBD. MSC expanded in HS or HPL have been trypsinized with 0.05% Trypsin/EDTA, instead the conventional 0.25% applied for MSC culture in FBS. VBD supplementation provides a decrease in production of adhesion proteins (54,94). An ASC genome gene expression analysis depicted 102 genes were commonly expressed in differentiated ASC being 90 genes, including those responsible for MSC adhesion, high expressed in 10% FBS-supplemented ASC (24), which may be connected to high sensitivity presented to trypsin by MSC expanded in VBD. Changes in surface markers expression has been contradictorily reported in VBD-expanded MSC (25,49,50,92). HS and aPRP provided maintenance of MSC immunophenotype (25).

Currently it is clear that the number of passages reduces MSC differentiation potential and the capacity of proliferation conducting to function alteration (119). MSC senescence has been the focus of some selected studies (41,44,68,106). β-galactosidase, a biomarker for cell senescence, was expressed strongly in FBS cultures when compared with HPL up to 16 passages (68). On the other hand, Venugopal, Balasubramanian (106) showed similar senescence rates for MSC expanded in HS or FBS. Besides, the age of VBD-donor could influence MSC senescence however, controversial results have been described (61,110). MSC expanded in HS from older donors (>45 years old) presented higher β-galactosidase expression than MSC from younger donors (<35 years old) (54). However, donors' age did not influence the GF concentration, hormones content of MSC expanded in HPL (61). Individual variations are expected in VBD, principally in plasm components. Blood contains several components as lipids (HDL, cholesterol), proteins, metabolites of uric acid, creatinine, and albumin; even as several ions: calcium, potassium, resulting from individual diet. Thus, plasm portion may have changes in biochemical composition (74). Growth factor's release profile of HPL and HS were contrasting. HPL seems to contain higher amounts of PDGF, VEGF, EGF, FGF-β, TGF-β and less insulin like growth factor 1 (IGF-1) than HS (110).

A high variation between carried out protocols as well as in the methodologies was detected in the selected studies. Thus, evaluation of quality of the methodologies thought available tools is not possible. However, some points can be highlight about the quality of methodologies. The majority of studies present a high control of surface markers before and after the use of VBD showing the differentiation in almost three cell lineages. Besides, selected studies present control groups (positive and negative) to compare statistically the results. However, variables such as, steps applied to obtain VBD, centrifugation times and culture medium applied were superficially described in the selected studies, which did not allow for direct comparisons between the included studies. In addition, few studies performed a direct comparison between different VBD in the same study. In such a context, it is strongly recommended to perform well-designed randomized controlled trials comparing different VBD obtaining protocols. Besides, protocols standardization should be considered to perform such comparisons.

Although the literature shows a wide variation between methodological studies, VBD presented excellent results as substitute to FBS, seeming to be a supplementation option for MSC in SC-BT. The substitution of animal compounds is highly recommended for good manufacturing practices (GMP) (13,120) guidelines, eliminating the need for animal additives for regenerative therapies in humans (96-102). VBD-supplemented MSC were applied for sinus lift augmentation (97), regeneration of alveolar clefts (96), even as regeneration of large anterior mandibular defect (98) and radiation burn treatment (102). Centeno, Schultz (101) used MSC supplemented in HPL (339 patients) to treat different

orthopedic conditions. Neoplasic transformations were not reported after 3 years of follow-up. Besides, Lucchini, Introna (100) realized an administration of intravenous MSC (expanded in 5% HPL) in eleven patients. However, such clinical results corroborate with *in vitro* and *in vivo* data, showing safe application of expanded MSC in humans. It is important to highlight that no malignant transformation was reported in such studies.

Venous blood derivatives seem to be excellent xeno-free serum for *ex vivo* expansion of mesenchymal stem cells. The replacement of Fetal Bovine Serum by venous blood derivatives can be an important step towards the translation of stem cell-based therapies to the clinic.

Resumo

Embora as propriedades biológicas das células-tronco mesenquimais (MSC) sejam bem caracterizadas *in vitro*, a aplicação clínica das MSC ainda está longe de ser alcançada, principalmente devido à necessidade de substâncias xenogênicas para expansão celular, como o soro fetal bovino (FBS). O FBS apresenta riscos quanto às transmissões de patógenos e à internalização de proteínas animais, o que pode desencadear respostas antigênicas em pacientes após a implantação das MSC. Uma vasta gama de derivados do sangue venoso (VBD) têm sido relatada como substitutos do FBS mostrando resultados promissores. Assim, o objetivo deste estudo foi conduzir uma revisão de escopo sistemática para analisar se VBD poderiam ser substitutos do FBS eficazes para expansão das MSC em condições *ex vivo*. A pesquisa foi realizada no SciVerse Scopus, PubMed, Web of Science, BIREME e biblioteca Cochrane até janeiro de 2016. As palavras-chave foram selecionadas usando MeSH e entre termos. Dois revisores independentes examinaram os registros obtidos considerando critérios de inclusão específicos. Os estudos incluídos foram avaliados de acordo com uma estrutura modificada de Arksey e O 'Malley. Dos 184 estudos encontrados, 90 foram incluídos. As células-tronco da medula óssea (BMMSC) foram utilizadas na maior parte destes estudos. Em geral, o VBD permitiu tanto a manutenção da morfologia semelhante a fibroblastos das MCS, alta proliferação, alta capacidade de formação de colônias e manutenção de multipotêncialidade. Além disso, as MSC expandidas em suplementos derivados do sangue venoso apresentaram uma maior atividade mitogênica do que as expandidas em FBS. Os VBD parecem ser excelentes soro livres de agentes xenogênicos para expansão *ex vivo* de MSC. Entretanto, observou-se uma heterogeneidade acentuada entre os protocolos realizados para o isolamento VBD, não permitindo assim comparações diretas entre os estudos incluídos.

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