REVIEW ARTICLE

Donor to recipient ratios in the surgical treatment of vitiligo and piebaldism: a systematic review

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Abstract

Stabilized vitiligo resistant to conventional therapy (e.g. segmental vitiligo) and piebaldism lesions can be treated with autologous cellular grafting techniques, such as non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). These methods are preferred when treating larger surface areas due to the small amount of donor skin needed. However, the donor to recipient expansion ratios and outcomes reported in studies with cellular grafting vary widely, and to date, no overview or guideline exists on the optimal ratio. The aim of our study was to obtain an overview of the various expansion ratios used in cellular grafting and to identify whether expansion ratios affect repigmentation and colour match. We performed a systematic literature search in MEDLINE and EMBASE to review clinical studies that reported the expansion ratio and repigmentation after cellular grafting. We included 31 eligible clinical studies with 1591 patients in total. Our study provides an overview of various expansion ratios used in cellular grafting for vitiligo and piebaldism, which varied from 1:1 up to 1:100. We found expansion ratios between 1:1 and 1:10 for studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. Pooled analyses of studies with the same expansion ratio and repigmentation thresholds showed that when using the lowest (1:3) expansion ratio, the proportion of lesions achieving >50% or >75% repigmentation after NCST was significantly better than when using the highest (1:10) expansion ratio (χ^2 $P = 0.000$ and χ^2 $P = 0.006$, respectively). Less than half of our included studies stated the colour match between different expansion ratios, and results were variable. In conclusion, the results of our study indicate that higher expansion ratios lead to lower repigmentation percentages after NCST treatment. This should be taken into consideration while determining which expansion ratio to use for treating a patient.

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Conflicts of Interest

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Introduction

Vitiligo and piebaldism are skin disorders in which large depigmented lesions can be present. These skin diseases can severely alter the physical appearance leading to social stigmatization and an impaired quality of life.[1,2] Several methods of autologous skin transplantation are available as treatment for repigmenting stable vitiligo and piebaldism lesions.[3,4] These surgical methods can roughly be divided into tissue grafting and cellular grafting.

The three major techniques of tissue grafting include punch grafting, epidermal blister grafting and split-thickness grafting.[5] Punch grafting is a simple and widely used technique in which a donor skin area of 1 cm^2 can approximately repigment a recipient depigmented skin area of 5 cm^2 (donor to recipient expansion ratio is 1:5).[6] This ratio, however, can vary due to the differences in pigment spread. Moreover, adverse effects such as a cobblestone appearance of the recipient site and scarring of the donor site are not uncommon.[7] The epidermal blister grafting and the split-thickness grafting methods are found to have the highest repigmentation success rates[3,7]; however, the donor to recipient expansion ratios (DR expansion ratios) are approximately 1:1 for both techniques.[8–10] Due to these low expansion ratios, the tissue grafting techniques are poorly suited for treating large depigmented areas, requiring large donor areas to treat large recipient lesions.[11] In addition, repeated surgical operations are needed for treating large surface areas.

Cellular grafting techniques include non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). These methods are preferred when treating larger surface areas due to the small amount of donor skin needed.[11] However, DR expansion ratios and outcomes reported in studies with cellular grafting vary widely, and to date, no overview or guideline exists on which ratio to use. Furthermore, little evidence is available on the correlation of expansion ratios with the repigmentation success rate.

We performed a systematic review to provide an overview of the various expansion ratios used during NCST and CMT. Furthermore, we aimed to identify whether expansion ratios affect the repigmentation success rates and colour matching to the non-lesional surrounding skin.

Methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guideline and is registered in the PROSPERO database (registration number: CRD42020176011).

Search strategy

We performed a systematic literature search in collaboration with a clinical librarian (M.M.) of the Amsterdam University Medical Center in EMBASE and MEDLINE from inception until 20th December 2019. Our search strategy contained main keywords and synonyms of vitiligo, piebaldism and cellular transplantation techniques (see Appendix S1 for entire literature search strategy). Original articles were obtained by excluding editorials, reviews and commentaries in the search. The same approach was used for excluding animal studies. Subsequently, the reference lists of identified relevant publications were screened manually for other relevant articles.

Study selection and eligibility criteria

All articles retrieved by our search were screened independently by 2 reviewers (V.N. and L.B.) using the web-tool *Rayyan*[12], after removing duplicate findings. We performed a first selection by means of screening title and abstract, followed by full text screening based on the predefined eligibility criteria. Any discrepancies between the 2 reviewers were resolved through discussion with a third reviewer (A.W.). The inclusion criteria consisted of: (I) ≥10 vitiligo and/or piebaldism patients, (II) intervention study with cellular transplantation technique(s), (III) known donor and recipient sizes with quantitative repigmentation as outcome measure, (IV) prospective studies including (non)randomized clinical trials and case series and (V) studies written in English. Cellular transplantation techniques consisted of NCST and CMT. We did not exclude studies in which additional therapy (e.g. phototherapy) was given. The selection procedure for eligible studies and exclusion criteria are shown in the PRISMA flow diagram (Fig. 1).

Data extraction and analyses

The 2 reviewers (V.N. and L.B.) extracted the following information independently from each eligible study: author, publication year, study design, number of patients and/or lesions, patient characteristics, (sub)type of depigmentation, disease stability, preparation of recipient site and grafting type of donor site. In addition, for the study results, we extracted information on transplantation technique, adjuvant treatment, DR expansion ratio, number of patients reaching repigmentation based on >90%, 75%, >70%, >65% and >50%, colour match and follow-up duration. Descriptive statistics and pooled analyses of studies with the same expansion ratio and repigmentation thresholds were carried out to meet our study aim. Chi-squared (χ^2) tests were performed to analyse and compare categorical variables. Spearman's rank correlation (r_s) test was used to define the association between the DR expansion ratios and the repigmentation. Statistical analyses were executed using Statistical Package for Social Sciences (SPSS version 26.0 for Windows) and the statistical level of significance was set at $P < 0.05$.

Quality assessment

We performed a risk-of-bias analysis to assess the quality of all included articles. We used the Cochrane Collaboration risk-of-bias tool for randomized trials (RoB 2)[13] to assess the bias (high, some concerns, low) of each included randomized controlled trial (RCT) regarding the (1) randomization process, (2) deviations from the intended interventions, (3) missing data, (4) measurement of the outcome and (5) selection of reported result. For the quality assessment of the included case series, we used the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Case Series.[14] For the included (non-randomized) clinical trials, we used the JBI Critical Appraisal Checklist for Quasi-Experimental Studies.[15] Both checklists comprised of approximately 10 questions on quality appraisal in which the overall appraisal was classified by 'include' for studies with a low or moderate bias and 'exclude' for studies with a high risk-of-bias (Appendix S3).

Results

Study selection and characteristics

A total of 925 unique articles were initially identified by the database searches. Based on our eligibility criteria of title and abstract, 833 articles were excluded leaving 92 studies for full text screening of which 31 studies met our inclusion criteria. Our study selection process and reasons for exclusion are presented in the PRISMA flow diagram (Fig. 1). A total of 1591 patients and 3081 treated leucodermal lesions were investigated. Of the 31 articles, 11 were randomized controlled trials, 6 were (non-randomized) clinical trials and 14 were case series studies. All included studies were prospective in which patients with vitiligo and/or piebaldism were treated with NCST (30

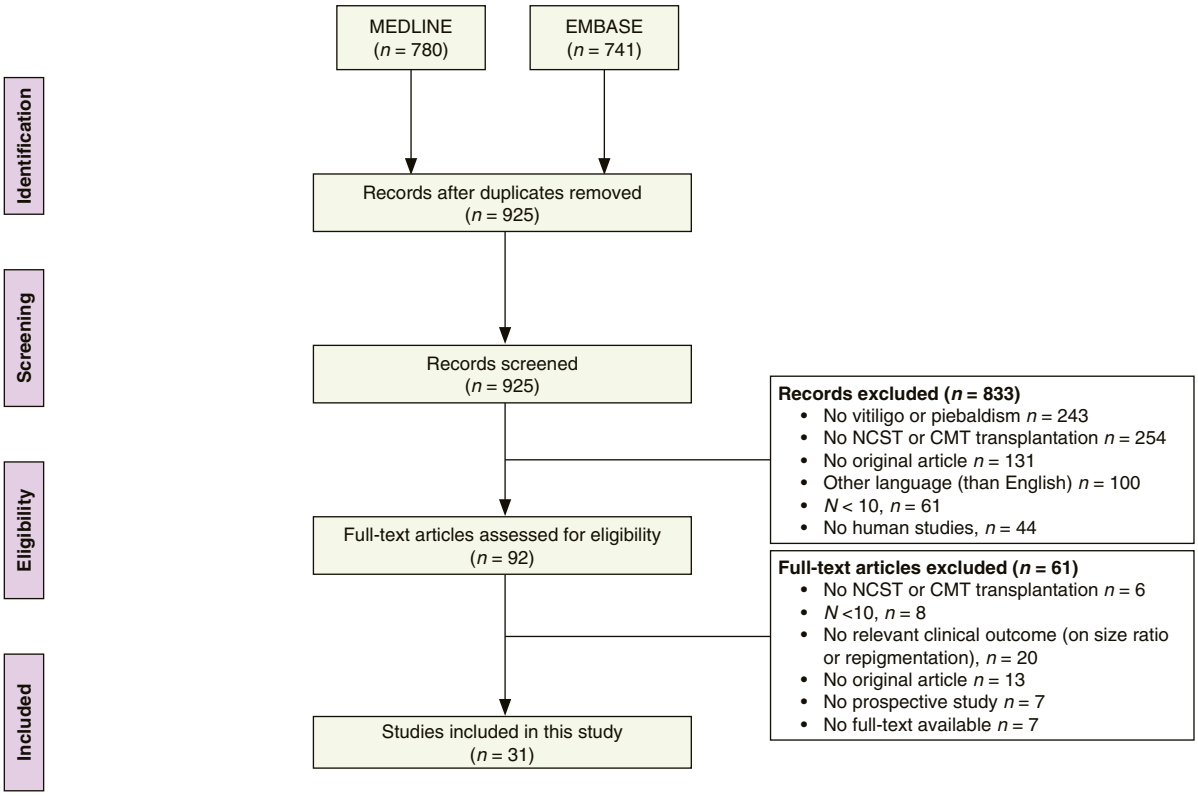

Figure 1 PRISMA flow diagram of selection procedure and included studies. The selection procedure for eligible studies and exclusion criteria are shown in this PRISMA flow diagram.

studies) and/or with CMT (4 studies). Twenty-eight of the 31 studies used the split-thickness skin grafting method to harvest skin from the donor site. Of the eligible articles, 29 studies treated patients with vitiligo and 2 studies treated both patients with vitiligo and piebaldism. In 26 studies, different subtypes of vitiligo (i.e. non-segmental, segmental and focal vitiligo) were included (Table 1). Detailed patient characteristics can be found in Appendix S2, supporting information. Nineteen studies mentioned one specific expansion ratio that was used (marked as 'precise DR expansion ratio' Table 1), and the other 12 studies reported a (wide) range of expansion ratios (marked as 'range of DR expansion ratios' in Table 1). Expansion ratios varied from 1:1 up to 1:100 in the included studies. The study characteristics, DR expansion ratios, results of repigmentation, colour match and treatments are summarized in Table 1.

Studies comparing DR expansion ratios in NCST

Two studies directly compared different expansion ratios and their repigmentation after NCST.[16,17] In the study of Tegta *et al.*,[16] repigmentation after NCST was compared between a 1:3 expansion ratio group (n = 10 patients) and a 1:5 expansion ratio group (n = 10 patients). Three months after transplantation, 5 patients in the 1:3 group had a >75% repigmentation response; whereas, no patients in the 1:5 group reached this response ($P < 0.05$).

Tawfik *et al.*[17] assessed repigmentation after NSCT in 42 patients with non-segmental vitiligo (NSV) randomly allocated to two groups: one group (n = 21 patients) received a 1:3 expansion ratio in which a total of 50 lesions were treated. These lesions were subdivided into two groups: 25 lesions received additional NB-UVB therapy after surgery and the other 25 lesions did not. The other group (n = 21 patients, 52 lesions) was treated with a 1:10 expansion ratio, of which half of the lesions received additional NB-UVB therapy. More patients showed >75% repigmentation in the 1:3 expansion ratio group (88% with NB-UVB and 80% without additional therapy) compared with the 1:10 expansion ratio group (12% with NB-UVB and 8% without post treatment). This repigmentation was significantly better in the 1:3 expansion ratio group compared with the 1:10 group (t-test P = 0.000); however, no significant differences were found between the subgroups. Both studies

Table 1 Study characteristics and results

Author, year	Study design	*n* patients (lesions)	Type of depigmentation	Stability disease (months)	Preparation recipient site	Treatment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n* (% of total)	Follow-up (months)
Precise DR expansion ratio											
Garg, 2019[41]	CS	10 (20)	8 NSV 2 SV	≥6	Er:YAG	NCST		1:3	>75%: 14 lesions (70%)	Unknown	6
Kachhawa, 2017[42]	CS	152 (437)	151 NSV 1 SV	≥12	Dermabrasion	NCST	PUVA	1:4	>75%: 179 lesions (41%)	Unknown	6
El-Zawahry, 2017[19]	CT	A. 10 (61) B. 21 (89)	NSV	≥12	A. CO_2 B. Cryo	NCST	UVB	1:5	A. >75%: 2 patients (20%) B. >75%: 9 patients (43%)	A. 6 patients (60%) B. unknown	18
Razmi, 2018[20],†	RCT	30 (42)	21 NSV 6 SV 3 FV‡	≥12	Dermabrasion	NCST		1:5	>75%: 24 lesions (57%)	25 lesions (60%)	4
Tegta, 2006[16]	RCT	A. 10 B. 10	11 NSV 4 SV 5 FV	≥12	Blister or Dermabrasion	NCST		A. 1:3 B. 1:5	A. >75%: 5 patients (50%) B. >75%: 0 patients (0%)	A. 3 patients (30%) B. 1 patient (10%)	3
Tawfik, 2019[17]	RCT	42 1a. (25) 1b. (25) 2a. (26) 2b. (26)	NSV	≥12	Dermabrasion	NCST	1a. None 1b. NB-UVB 2a. None 2b. NB-UVB	1:3 1:10 	1a. >75%: 20 lesions (80%) 1b. >75%: 22 lesions (88%) 2a. >75%: 2 lesions (8%) 2b. >75%: 3 lesions (12%)	1a. 25 lesions (100%) 1b. 25 lesions (100%) 2a. 17 lesions (65%) 2b. 17 lesions (65%)	6 6
Budania, 2012[21],†	RCT	21 (28)	8 NSV 10 SV 3 FV	≥12	Dermabrasion	NCST	sunlight exposure	1:10	>75%: 25 lesions (89%)	23 lesions (82%)	4
El-Zawahry, 2011[34]	CS	22	19 NSV 1 SV 2 FV‡	≥12	Cryo	NCST	PUVA	1:10	>50%: 12 patients (55%)	Unknown	6-17
Huggins, 2012[22]	CS	23 (29)	19 NSV 2 SV 8 FV‡	≥6	Dermabrasion	NCST		1:10	>65%: 14 lesions (48%)	18 lesions (62%)	3-6
Mulekar, 2006[43]	CS	25 A. 25 B. 25	17 NSV 8 SV‡	≥6	Dermabrasion	NCST	A. None B. Oral beta-methasone	1:10	A. >65%: 8 patients (32%) B. >65%: 22 patients (88%)	Unknown	12
Mulekar, 2005[23]	CS	142	NSV	≥6	Dermabrasion	NCST		1:10	>65%: 95 patients (67%)	125 patients (88%)	12-72
Mulekar, 2003[44]	CS	175	114 NSV 43 SV 18 FV‡	≥12	Dermabrasion	NCST		1:10	>65%: 129 patients (74%)	Unknown	12
Mutalik, 2017[45]	RCT	A. 25 B. 25	14 NSV 10 SV 26 FV	≥24	Dermabrasion	NCST	A. None B. Oral Cyclosporin	1:10	A. >75%: 7 patients (28%) B. >75%: 25 patients (100%)	Unknown	6
Pandya, 2005[33]	CS	A. 4 B. 23	25 NSV 2 SV	≥24	Dermabrasion	A.CMT B.NCST		A. unclear B. 1:10	A. >65%: 2 patients (50%) B. >65%: 16 patients (70%)	Unknown	6
Ramos, 2017[24]	CS	20 (24)	8 NSV 12 SV‡	≥12	Dermabrasion	NCST	sunlight exposure	1:10	>50%: 15 patients (75%)	17 lesions (85%)	3-6

Table 1 *Continued*

Author, year	Study design	*n* patients (lesions)	Type of depigmentation	Stability disease (months)	Preparation recipient site	Treatment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n* (% of total)	Follow-up (months)
Thakur, 2019[25]	RCT	A. 10 B. 10	12 NSV 8 SV	A. 3–6 B. ≥12	Dermabrasion	NCST		1:10	A. >75%: 3 patients (30%) B. >75%: 6 patients (60%)	A. 8 patients (80%) B. 8 patients (80%)	6
Vazquez-Martinez, 2011[46]	CT	11	NSV SV FV§	≥12	Dermabrasion	NCST		1:10	Unclear	Unknown	12
Bao, 2015[31]	CT	83 A. (83) B. (83)	43 NSV 40 SV	≥12	CO_2	A.CMT B.NCST		A. 1:20 B. 1:5	A. >50%: 68 lesions (82%) B. >50%: 67 lesions (81%)	Nearly uniform in both methods	12
Verma, 2014[32]	RCT	A. 6 (50) B. 19 (50)	20 NSV 2 SV 3 FV	≥12	Dermabrasion	A.CMT B.NCST	A. Puvasol B. Puvasol	A. 1:100 B. 1:10	A. >70%: 26 lesions (52%) B. >70%: 31 lesions (62%)	Unknown	6
Range of DR expansion ratios											
van Geel, 2004[26]	RCT	A. 19 (22) B. 9 (11)	NSV	A. ≥12 B. <12	CO_2	NCST	UVB or PUVA	1:1 - 1:4.5	A. >75%: 9 lesions (41%) B. >75%: 0 lesions (0%)	13 lesions (72%)	12
Lommerts, 2017[27]	RCT	10 A. (10) B. (10)	3 SV 7 P	≥12	A.CO_2-209 μm B.CO_2-144 μm	NCST	UVA	1:4-1:5	A. >75%: 5 lesions (50%) B. >75%: 4 lesions (40%)	A. 9 lesions (90%) B. 9 lesions (90%)	6
Khodadadi, 2010[28]	CS	10	6 NSV 4 FV	≥12	Intraepidermal injection	NCST		1:3-1:7	>75%: 4 patients (40%)	4 patients (40%)	6
Gill, 2019[47]	CS	50	40 NSV 4 SV 6 FV	≥12	Dermabrasion	NCST		1:3-1:10	>70%: 31 patients (62%)	Unclear	6
Gupta, 2019[36]	RCT	A. 15 (22) B. 17 (25)	18 NSV 6 SV 8 FV‡	≥12	A. Er:YAG B. Dermabrasion	NCST		1:3-1:10	A. >50%: 9 patients (60%) B. >50%: 10 patients (59%)	Unknown	6
Mulekar, 2004[35]	CS	64	49 SV 15 FV‡	≥12	Dermabrasion	NCST		1:3-1:10	>65%: 55 patients (86%)	Unknown	60
Orouji, 2018[38]	CT	300 (1060)	231 NSV 10 SV 59 FV‡	≥12	Intralesional injection	NCST		1:3-1:10	>75%: 109 patients (36%)	Unknown	6
Sahni, 2011[29]	CS	13 (19)	6 NSV 6 SV 1 FV‡	≥12	Dermabrasion	NCST		1:3-1:10	>75%: 19 lesions (100%)	16 lesions (84%)	4
Olsson, 1998[37]	CS	23 (27)	17 NSV 3 SV 3 P‡	≈89 mean	Dermabrasion	NCST		1:4-1:10	>75%: 23 lesions (85%)	Unknown	6–12
Parambath, 2019[48]	RCT	20 A. (20) B. (20)	13 NSV 7 SV‡	≥12	Dermabrasion	NCST	A. None B. PRP	1:4-1:10	A. >75%: 11 lesions (55%) B. >75%: 16 lesions (80%)	Unknown	6

Table 1 *Continued*

Author, year	Study design	*n* patients (lesions)	Type of depigmentation	Stability disease (months)	Preparation recipient site	Treatment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n* (% of total)	Follow-up (months)
Ebadi, 2015[49]	CT	10 A. (9) B. (10)	NSV	≥12	Dermabrasion	NCST	A. None B. Excimer laser	1:5-1:10	A. >65%: 1 lesion (11%) B. >65%: 4 lesions (40%)	Unknown	3–4
Hong, 2011[30]	CT	A. 35 B. 67	12 NSV 90 FV	≥6	CO_2	CMT		A.<1:10 B.>1:10	A. >50%: 31 patients (89%) B. >50%: 57 patients (85%)	Unknown	6

Abbreviations: CMT, autologous cultured melanocyte transplantation; CO2, CO2-laser; Cryo, cryoblebbing; CS, case series; CT, clinical trial;DR ratio, donor-recipient size ratio; Er:YAG, erbium-doped yttrium aluminium garnet laser; FV, focal vitiligo; *n*, number of patients or lesions; NB-UVB, narrowband ultraviolet-B phototherapy; NCST, autologous non-cultured cell suspension transplantation; NSV, non-segmental vitiligo; P, piebaldism; PRP, platelet-rich plasma; PUVA, psoralen and ultraviolet A; Puvasol, psoralen combined with sunlight exposure; RCT, randomized controlled trial; SV, segmental vitiligo; UVB, ultraviolet-B phototherapy.

†Only 1 (relevant) treatment arm is shown of this study.

‡Analysis of repigmentation was provided per vitiligo subtype in study.

§Number of patients of each subtype unknown.

demonstrate great differences in repigmentation response between the lower and higher expansion ratio groups.

DR expansion ratio related to repigmentation and colour match in NCST

Given the heterogeneity of the studies (predominantly regarding outcomes, disease stability, follow-up and adjuvant treatment), it was not feasible to perform a meta-analysis. Instead, we performed pooled analyses on roughly comparable studies that stated a precise DR expansion ratio and used the same repigmentation outcome thresholds (i.e. 50%, 75% or 90% repigmentation). Studies that provided a form of additional treatment (such as NB-UVB, PUVASOL, excimer laser) were excluded from the pooled analysis since these could influence the outcomes.[18] Tables 2a, b and c show the number of lesions reaching >50%, >75% and >90% repigmentation, respectively, after NCST per DR expansion ratio used. The highest repigmentation rates are seen in the 1:3 expansion ratio group; whereas, the lowest repigmentation percentages are found in the 1:10 expansion ratio group. These differences between expansion ratios were significant in the >50% (χ^2 $P = 0.000$) and > 75% (χ^2 $P = 0.006$) repigmentation groups. Furthermore, a significant correlation was found in these groups between the expansion ratios and the repigmentation percentages ($r_s = -0.228$ $P = 0.000$, $r_s = -0.197$ $P = 0.006$, respectively). For the >90% repigmentation group (Table 2c), no significant association was found between expansion ratio and repigmentation ($r_s = -0.109$ $P = 0.08$).

We identified 13 studies that specified the colour match of the recipient site to the non-lesional normally pigmented skin after treatment. In two studies directly comparing expansion ratios, a significantly better colour match was found in the lower expansion ratio group.[16,17] Other studies illustrated variable results of the colour matching in relation to the expansion ratios (Table 1).[19,20,29,21–28]

CMT

In four studies, repigmentation after CMT was assessed in which the expansion ratios varied from 1:20 until 1:100. Hong *et al.*[30] divided 102 patients into two groups: 35 patients received CMT with a expansion ratio < 1:10 (mean 1:8) and 67 patients were treated with a expansion ratio > 1:10 (mean 1:27). The mean repigmentation was 77% in the low expansion ratio group and 78% for the high expansion ratio group (no significant difference between both groups, *t*-test $P = 0.958$). Three studies compared CMT with NCST using different expansion ratios. In the study of Bao *et al.*,[31] repigmentation in patients ($n = 83$) receiving CMT with an expansion ratio of 1:20 (68 patients >50% repigmentation) did not significantly differ from patients ($n = 83$) receiving NCST with an expansion ratio of 1:5 (67 patients with >50% repigmentation), $P = 0.986$. Verma *et al.*[32] compared repigmentation in 6 patients (50 lesions) after CMT

Table 2 (a) Percentage of NCST treated lesions with >50% repigmentation in three DR expansion ratio groups. (b) Percentage of NCST treated lesions with >75% repigmentation in three DR expansion ratio groups. (c) Percentage of NCST treated lesions with >90% repigmentation in three DR expansion ratio groups

(a)

DR ratio	Author, year	Lesions > 50%	Total lesions	Total percentage lesions with >50% repigmentation
1:3	Garg, 2019	18	20	
	Tegta, 2006	7	10	
	Tawfik, 2019	24	25	
	Total	**49**	**55**	49/55 = **89%** *
1:5	Razmi, 2018	33	42	
	Tegta, 2006	1	10	
	Bao, 2015	67	83	
	Total	**101**	**135**	101/135 = **75%** *
1:10	Tawfik, 2019	6	26	
	Budania, 2012	26	28	
	Mutalik, 2017	11	25	
	Ramos, 2017	15	20	
	Thakur, 2019	8	10	
	Total	**66**	**109**	66/109 = **61%** *

(b)

DR ratio	Author, year	Lesions > 75%	Total lesions	Total percentage lesions with >75% repigmentation
1:3	Garg, 2019	14	20	
	Tegta, 2006	5	10	
	Tawfik, 2019	20	25	
	Total	**39**	**55**	39/55 = **71%** *
1:5	Razmi, 2018	24	42	
	Tegta, 2006	0	10	
	Total	**24**	**52**	24/52 = **46%** *
1:10	Tawfik, 2019	2	26	
	Budania, 2012	25	28	
	Mutalik, 2017	7	25	
	Thakur, 2019	6	10	
	Total	**40**	**89**	40/89 = **45%** *

(c)

DR ratio	Author, year	Lesions > 90%	Total lesions	Total percentage lesions with >90% repigmentation
1:3	Tawfik, 2019	15	25	15/25 = **60%** *
1:5	Razmi, 2018	13	42	
	Bao, 2015	44	83	
	Total	**57**	**125**	57/125 = **47%** *
1:10	Tawfik, 2019	1	26	
	Budania, 2012	20	28	
	Pandya, 2005	12	23	
	Ramos, 2017	5	20	
	Thakur, 2019	4	10	
	Total	**40**	**107**	42/107 = **39%** *

(a) *Chi-squared test $P = 0.000$.
(b) *Chi-squared test $P = 0.006$.
(c) *Chi-squared test $P = 0.158$.

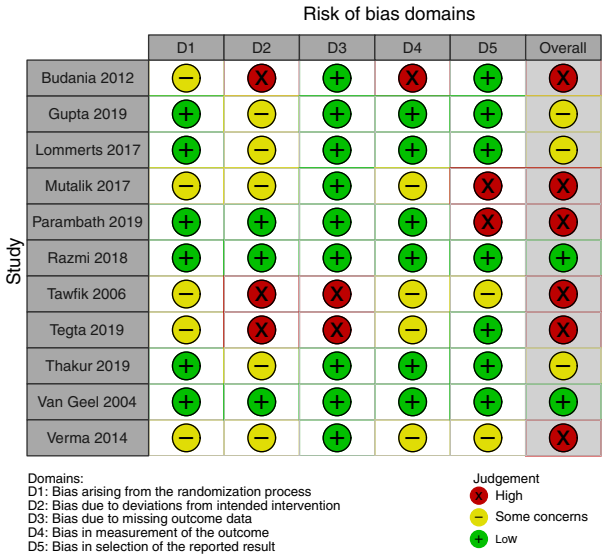

Figure 2 Risk-of-bias assessment of randomized controlled trials. The risk-of-bias assessment of all 12 included RCTs are shown in this 'traffic light' plot accompanied by the explanation of the 5 domains of bias and risk judgement.

with a 1:100 expansion ratio to 19 patients (50 lesions) after NCST with a 1:10 expansion ratio. Although > 70% repigmentation was more frequently seen after NCST (62%) than after CMT (52%), the difference between these two groups was not significant (χ^2 $P = 0.058$). In the case series of Pandya *et al.*,[33] CMT was compared to NCST showing a higher >65% repigmentation response after NCST than CMT (70% vs. 50%). The expansion ratio in the CMT group, however, was not clearly stated in this study.

Quality assessment

The risk-of-bias assessment of the RCTs is shown in Fig. 2. Six (6/11) studies showed an overall high risk due to biases in missing data, selection of reported results and deviations from intended interventions. Other studies had some concerns (3/11) or a low (2/11) risk-of-bias. The critical appraisal of 14 case series studies and 6 (non-randomized) clinical trials is summarized in Appendix S2, supporting information. Four (4/20) studies showed an overall poor quality mainly due to the selective patient population and lack of reporting relevant information.

Discussion

This study provides a systematic overview of donor to recipient expansion ratios, and its relation to repigmentation and colour match in cellular grafting of vitiligo and piebaldism. We reviewed 31 studies evaluating 1591 patients after NCST and/or CMT. We identified expansion ratios from 1:1 to 1:10 in studies investigating NCST and from 1:20 to 1:100 in studies evaluating

CMT. Furthermore, the results of our study indicate that lower expansion ratios lead to higher repigmentation percentages after NCST treatment.

In our pooled analyses of various studies performed in different countries and time periods, we found that studies with a 1:10 expansion ratio had a significant lower >50% and >75% repigmentation than the 1:3 or 1:5 expansion ratio groups after NCST. This relation was not significant for the >90% repigmentation (Table 2c), although a similar trend is seen as the >50% and 75% repigmentation groups. We found 2 RCTs comparing a lower expansion ratio to a higher expansion ratio, demonstrating substantially better outcomes for the lower expansion ratios.[16,17] These studies are in line with the findings of our pooled analyses. On the contrary, no significant differences in repigmentation were found between different expansion ratios after CMT treatment.[30] A possible explanation for this could be that in principle this technique yields a larger number of melanocytes for transplantation depending on the culture time. Remarkably, no significant differences were seen in studies comparing (different expansion ratios between) CMT and NSCT.[31–33] The cultured technique, however, does have a few disadvantages since it is a time-consuming, expensive and complicated procedure, requiring advanced equipment, a sterile lab setup and trained personnel.

Less than half of our included articles state the colour match. Two studies demonstrate a better colour match, when using a lower expansion ratio[16,17]; however, the results of the other studies are variable.

Most studies included different vitiligo subtypes (n = 26); however, in only 12 studies an outcome analysis per subtype was provided (Table 1). Subsequently in seven[22,24,34–37] out of these 12 studies, segmental vitiligo (SV) and/or focal vitiligo (FV) demonstrated a higher repigmentation response after NCST treatment compared with NSV. Solely one study[38] showed the exact opposite. This suggests that a separate analysis per subtype is of importance, since various subtypes respond to treatment in a different manner due to differences in pathophysiology.[39] In NSV, even though stable for >12 months, the persevering auto-immunity against melanocytes can have a negative impact on treatment outcome.

Furthermore, we found differences in preparation of the recipient site (i.e. dermabrasion, C02-laser, Er:YAG-laser, suction blister, cryoblebbing), what could have influenced the outcomes. However, Al-Hadidi et al. have reviewed these methods, stating that there is no evidence-based preference in terms of better outcomes.[40]

One of the limitations in our study was that only two databases were finally used for our search.

In addition, we found a high heterogeneity between our included studies in terms of repigmentation measures, disease stability, vitiligo subtypes, follow-up duration, adjuvant therapy and quality. Moreover, many studies were lacking a control group. As a consequence, we were not able to perform a meta-analysis of the outcomes with regard to the expansion ratios. Instead, we pooled study results with the same expansion ratios and repigmentation threshold outcomes (excluding studies with post-surgical adjuvant therapies). These pooled studies were predominantly comparable in disease stability (>12 months) and follow-up duration (average 6 months). Another limitation is that little under half of all included studies stated a range of expansion ratios, making it somewhat difficult to draw conclusions from these studies.

Given these points, this review once again underlines the importance of establishing a consensus on (core) outcomes. Nevertheless, we attempted to integrate the outcomes of all relevant prospective studies as far as possible, and to our knowledge, this is the first systematic review to summarize different expansion ratios and their related outcomes reported in cellular grafting.

In conclusion, our study provides an overview of various donor to recipient expansion ratios used in cellular grafting for vitiligo and piebaldism. We found expansion ratios between 1:1 and 1:10 for studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. Remarkably, no differences in outcomes were found in studies comparing NSCT with CMT. The results of our study indicate that higher expansion ratios lead to lower repigmentation percentages after NCST treatment. For clinical practice this should be taken into consideration before deciding which DR expansion ratio to use.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Literature search.

Appendix S2. Detailed patient and treatment characteristics table.

Appendix S3. Critical Appraisal of case series and (non-randomized) clinical trials.