Tissue specific stem/progenitor cells for cartilage tissue engineering: A systematic review of the literature

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ABSTRACT

Cartilage derived stem/progenitor cells (CSPCs) have been isolated from a variety of cartilage sources and are suggested to have high chondrogenic potential. However, their role in cartilage engineering has not been well described, in particular, compared to other more widely used cell types such as differentiated chondrocytes and nontissue-specific mesenchymal stem cells (MSCs). The authors performed a systematic review of literature according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [Web of Science (Web of Science Core Collection, BIOSIS Citation Index, KCI-Korean Journal Database, MEDLINE, SciELO Citation Index), PubMed, and Embase] from January 1947 to November 2017, to evaluate CSPC isolation, their characterization, and cartilage regeneration. Two investigators independently reviewed all studies and extracted the data against standardized inclusion/exclusion criteria. A total of 1189 studies were identified, 65 of which met the inclusion criteria, consisting of 69 reports on CSPC isolation from articular (n = 35), intervertebral disk (11), auricular (n = 10), meniscal (n = 5), nasoseptal (n = 5), tracheal (n = 2), and costal (n = 1) cartilages. Despite the heterogeneity in isolation methods, 75% of studies found CSPCs to have trilineage differentiation potential, with consistent but nonspecific cell surface marker expression profiles, being positive for the recognized MSC markers CD90, CD105, CD44, CD166, CD73, and CD29 and negative for hematopoietic markers CD34 and CD45. Four cartilage regenerative outcomes were assessed: chondrogenic gene and protein expression (quantitative polymerase chain reaction, histology, immunohistochemistry, and biochemistry), imaging and structural characterization (gross appearance, scanning electron microscopy, and transmission electron microscopy) and biomechanical testing. CSPCs have been used for cartilage repair in animal models with excellent outcomes that are comparable to chondrocytes and superior to MSCs from unrelated tissue sources. The current review concludes that CSPCs represent a promising cell source for cartilage tissue engineering, but there is currently no consensus on specific cell surface markers or isolation protocols.

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INTRODUCTION

Mesenchymal stem cells (MSCs) derived from adult tissues are a promising cell type for tissue regeneration due to their self-renewal capacity and ability to form multiple tissue types (Bianco, 2001). Despite lacking intrinsic reparative ability due to its avascular, aneural, and immune-privileged nature (Detterline *et al.*, 2005; Cancedda, 2003; and Vinatier, 2009), articular cartilage has been shown to contain a population of cells with progenitor-like qualities that are thought to be involved in tissue homeostasis (Dowthwaite, 2004; Fickert, 2004; Alsalameh, 2004; and Martin, 2003). In recent years, increasing evidence suggests that they share many properties with MSCs, as defined by the International Society of Cellular Therapy (ISCT) (Dominici, 2006) such as adherence to plastic, self-renewal capacity, expression of stem-cell-related surface markers, and multilineage potential, thus functioning as bona fide cartilage derived stem/progenitor cells (CSPCs) (Williams, 2010; Quintin, 2010; Karlsson, 2009; Hattori, 2007; Hayes, 2008; Seol, 2012; Koelling, 2009; McCarthy, 2012; and Jiang, 2015), similar to populations of stem cells found in many other adult tissues (Brack, 2012 and Crisan, 2008).

Similarities in the characteristics of CSPCs and MSCs may be related to the cartilage being a derivative of embryonic mesenchymal cells, arising from various sources including the neuroectoderm (forming the craniofacial skeleton), paraxial mesoderm (forming the axial skeleton), and lateral plate mesoderm (forming long bones) (Olsen, 2000). The mesenchymal cells differentiate to form chondroblasts, and subsequently chondrocytes, with concomitant secretion of extracellular matrix (ECM), characteristic of various stages of development (Fig. 1). Findings suggest that at least two subpopulations of chondrogenic cells coexist in the developing cartilage—multipotent cartilage stem cells and oligopotent chondrogenic cartilage progenitor cells (Wu, 2013)—but definitive differences between these subpopulations in human adult cartilage have remained elusive. Evidence of reduced adipogenicity despite successful osteogenic and chondrogenic induction (Alsalameh *et al.*, 2004 and Grogan *et al.*, 2009), low or no expression of RUNX2, the master transcription factor for chondrocyte terminal differentiation, and enhanced expression of chondrocyte-specific markers in chondroprogenitors (Seol *et al.*, 2012) prevents the synonymity of oligopotent cartilage progenitors and multipotent cartilage stem cells. While other reports suggest that cartilage progenitors

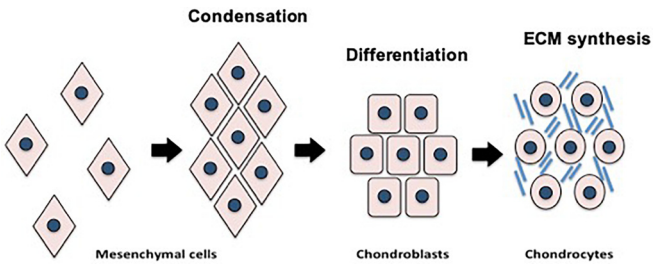

FIG. 1. Stages of cartilage development. Mesenchymal stem cell condensation leading to chondrogenic differentiation and extracellular matrix (ECM) synthesis.

share more features with MSCs than chondrocytes, such as *in vitro* self-renewal and trilineage differentiation, leading to a lack of consensus in nomenclature (Levato *et al.*, 2017 and Seol *et al.*, 2012).

Chondrocytes secrete the cartilage extracellular matrix when cultured in a 3D environment, but their dedifferentiation following expansion has limited their widespread use in cartilage tissue engineering (Hamada *et al.*, 2013 and Darling and Athanasiou, 2005). CSPCs have been implicated in migration and tissue reparative activities in response to native articular cartilage injury (Koelling, 2009 and Seol, 2012) and are identified as a promising renewable cell source for cartilage tissue engineering (Derks, 2013; Dowthwaite, 2004; Henriksson, 2009; and Kobayashi, 2011) due to their niche-specific lineage preference for chondrogenesis (Pizzute, 2015 and Jansen, 2010). Unlike hematopoietic stem cells, for example, which have a well-defined population of cell-surface antigens to identify cellular immunophenotype (Spangrude, 1998), CSPCs lack definitive, stable biomarkers, which has made the developmental origin and correct purification strategy of these cells difficult to elucidate (Jiang, 2015; Quintin, 2010; Lee, 2009; and Diaz-Romero, 2005). To date, systematic reviews describing stem/progenitor cells in cartilage have been lacking and their chondrogenic capacity is debatable.

The purpose of this systematic review is to examine all the published literature looking at progenitor and stem cells from different cartilage types and summarize the available information about their isolation and characterization to conclusively determine whether cartilage contains stem cells according to ISCT criteria (Dominici, 2006) and provide clarity in nomenclature for future research in this field. This review will also assess the cartilage regenerative capacity of these cells to determine their potential utility for cartilage tissue engineering.

METHODS

Search strategy

A systematic search for relevant articles was performed in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, 2015) to evaluate the isolation, characterization, and utilization of CSPCs for cartilage tissue engineering. Preclinical studies of CSPC isolation and characterization, in particular, with respect to plastic adherence, cell surface markers, and multipotency, were identified through a systematic search across electronic databases, Web of Science (Web of Science Core Collection, BIOSIS Citation index, KCI-Korean Journal Database, MEDLINE, and SciELO Citation Index), PubMed, and Embase, from January 1947 to November 2017. Due to the varied nomenclature of CSPCs, broad search terms were used which included: "mesenchymal progenitor cells" AND cartilage OR "articular cartilage"; "cartilage derived stem cells"; "perichondrial progenitor cells"; "cartilage progenitor cells"; "chondroprogenitor cells"; "chondrogenic progenitors"; "chondroprogenitors"; "cartilage stem cells"; and "chondrogenic progenitor cells."

Eligibility criteria

The inclusion criteria were studies that (1) involved the isolation of cartilage stem/progenitor cells; (2) identified stem cells in adult animal or human cartilage; (3) assessed the regenerative capacity of cartilage stem/progenitor cartilage *in vitro* or *in vivo*; and (4) were of English language articles only.

Exclusion criteria

Studies were excluded if they (1) involved stem cells isolated from noncartilaginous tissues; (2) identified stem cells in nonadults or embryonic mesenchyme; and (3) were not available for viewing. Review articles and commentaries were also excluded.

Study selection

Two reviewers (Jessop and Manivannan) independently screened abstracts to identify studies meeting our inclusion criteria, with differences being resolved by the senior author (Whitaker). Titles were initially screened to exclude duplicates and further screened using the abstracts against inclusion and exclusion criteria. Finally, a full text review of the remainder was performed to assess their eligibility. The bibliographies of relevant articles were studied to identify further relevant publications (Fig. 2).

Data extraction and main outcomes

Data were extracted from selected studies using a standardized format (Microsoft Office Excel 2016). The initial tabulated data collection included: cartilage source of CSPCs, species, isolation technique, adherence to plastic, cell surface markers, and multipotency. Because the surface markers for MSCs from other species have not been universally characterized, the ISCT criteria were also applied to both human and nonhuman cartilage derived stem/progenitor cells (Dominici, 2006). CSPC use for cartilage tissue engineering, including scaffolds, signaling factors, and chondrogenicity (evidence for cartilage formation at the gene, protein, structural, or biomechanical level) was recorded. Also noted were study authors, study design (*in vitro*/*in vivo* model), and the year of publication. Studies directly comparing the chondrogenicity outcomes of CSPCs with other commonly used cell sources such as chondrocytes and nontissue-specific MSCs were also evaluated.

RESULTS

Study characteristics

Our search yielded 2071 results, and after the exclusion of duplicates and preliminary screening 278 studies were identified for full text review of which 65 fulfilled the eligibility criteria (Fig. 2). A total of 69 reports describing CSPC isolation in 65 preclinical studies were published between 2004 and 2017. These reports involved the isolation of adult animal or human cartilage stem/progenitor cells, and were all either *in vitro* (n = 50), *in vivo* animal models (n = 13), or both (n = 6), with no first-in-human studies. Of the *in vivo* studies, the majority of them used mice (n = 8), followed by rabbits (n = 5) and rats (n = 3) with only individual studies using large animal models like dogs, goats, monkeys, and horses.

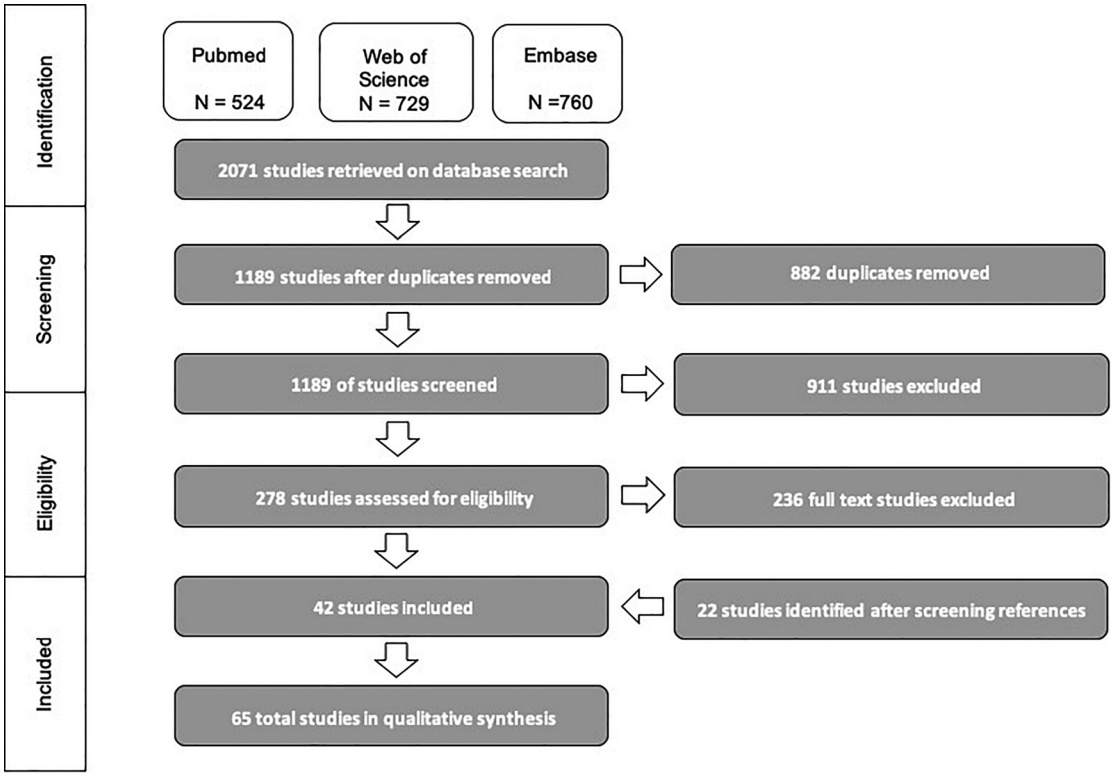

FIG. 2. PRISMA flow literature search summary diagram. The search identified a total of 2071 English articles. All the articles were screened and shortlisted according to the inclusion and exclusion criteria. After initial screening, a total of 1189 abstracts were scrutinized, of which 278 abstracts were then further analyzed by retrieving the full text of the articles. A total of 65 preclinical studies met the inclusion criteria.

CSPC isolation

CSPCs were isolated from both articular (n = 40) and nonarticular cartilage (n = 29) sources (Tables I–III). Articular sources included articular hyaline (n = 35) as well as meniscal (n = 5) cartilage (Tables I and II). Nonarticular cartilage sources included intervertebral disk (n = 11), auricular (n = 10), nasoseptal (n = 5), tracheal (n = 2), and costal (n = 1) locations (Table III).

Various CSPC isolation and cell culture methods have been adopted. Dowthwaite were the first to use differential adhesion to fibronectin (Dowthwaite, 2004), and since then other studies have adopted this technique mainly for the isolation of articular CSPCs (n = 15). Other isolation methods include fluorescence activated cell sorting based on CSPC surface markers, magnetic cell separation as well as identifying migratory and clonogenic subpopulations. Around half (14/29) of the studies isolating nonarticular CSPCs do not have specific isolation techniques and rely on simple tissue digest protocols of the perichondrial/superficial cartilage layer where CSPCs are believed to reside.

CSPC characterization

Of the 69 reports on isolating CSPCs, 53 assessed their cell surface marker expression and 48 assessed their potential to differentiate into additional lineages, namely osteogenic and adipogenic. CD90, CD105, CD44, CD166, CD73, and CD29 were the most common positive cell surface markers for CSPCs across different cartilage locations (Table IV), and the most commonly studied negative markers were CD34 and CD45 (Table V). CSPCs were reported to be positive for HLA-ABC, but lack expression of HLA-DR (Tables IV and V). The only conflicting results were for CD133 cell surface marker expression, wherein four studies found it to be positive in articular (Yu, 2014), nasoseptal (Shafiee, 2011), and IVD CSPCs (Risbud, 2007 and Liu, 2011) and five studies found it to be negative in articular (Joos, 2013 and Su, 2015), auricular (Kobayashi, 2011), and nasoseptal (Shafiee, 2014 and Elsaesser, 2015) CSPCs.

Of the 48 studies that assessed differentiation, only 8 reported failure to differentiate into either adipogenic or osteogenic lineages, with 75% confirming trilineage potential for CSPCs. Only 35% (24/69) reports on CSPCs met the ISCT definition of MSCs due to partial mesenchymal-lineage differentiation potential, most commonly chondrogenic and osteogenic, but failure of adipogenic potential or the lack of immunophenotyping data, particularly assessing for negative CD markers, which left the identity of CSPCs in doubt (Table VI). For instance, Seol (2017) isolated fibroblast-like clonogenic cells from bovine meniscal cartilages that exhibited multilineage (chondrogenic, osteogenic, and adipogenic) potential and plastic adherence, but lack of immunophenotype data means their MSC status was not confirmed. Most commonly, there was a simultaneous lack of both immunophenotype and multilineage data implying that these studies did not attempt to fully characterize CSPCs according to ISCT criteria following isolation rather than actively disproving that they are not adult derived stem cells.

CSPCs for cartilage regeneration

Four cartilage regenerative outcomes were assessed by this review, namely chondrogenic gene and protein expression [including quantitative polymerase chain reaction (qPCR), histology, immunohistochemistry, and biochemistry], imaging and structural characterization (gross appearance, SEM, and TEM) and biomechanical testing. The most common chondrogenesis protocols consisted of pellet culture with TGFb1, TGFb3, or FGF2. Most studies showed that after chondrogenic induction, CSPCs not only had increased expression of chondrogenic genes but also secreted cartilage extracellular matrix *in vitro* and *in vivo*, specific to their cartilage subtype origin, i.e., hyaline (McCarthy, 2012; Yoon, 2013; Williams, 2010; and Anderson, 2017) or elastic (Kobayashi, 2011; Takebe, 2012; and Kagimoto, 2016).

One study reported elastic auricular cartilage CSPCs being able to regenerate hyaline-like articular cartilage in an animal model (Mizuno, 2014), suggesting plasticity between cartilage subtypes. While three studies found evidence to support fibrocartilage rather than functional hyaline articular cartilage formation (Dowthwaite, 2004 and Koelling, 2009) or elastic auricular cartilage formation (Derks, 2013). Only four studies assessed tissue engineered cartilages' biomechanical properties (Levato, 2017; Anderson, 2017; Shen, 2013; and Mizuno, 2014), despite this being a prerequisite for determining their clinical utility (Gleghorn, 2007 and Roy, 2004). Articular and meniscal cartilage studies assessed compressive stiffness using either unconfined uniaxial compression testing (Levato, 2017 and Anderson, 2017) or indentation testing (Shen, 2013). Auricular cartilage elasticity and stiffness were determined by nano(indentation) using atomic force microscopy, with the resulting engineered cartilage from auricular CSPCs more closely matching the mechanical properties of hyaline rather than elastic cartilage (Mizuno, 2014).

Chondrogenesis of CSPCs vs other cell types

CSPC chondrogenicity was directly compared with other cell types, most commonly chondrocytes (n = 21) and bone marrow (n = 21), adipose (n = 4), and synovium (n = 2) derived MSCs. MSCs were found to have increased expression of osteogenic genes resulting in hypertrophic cartilage formation (McCarthy, 2012; Su, 2015; and Shen 2014) compared to CSPCs which exhibited a greater tendency for chondrogenic differentiation (Ding and Huang, 2015; Shafiee, 2011; Baptista, 2013; Togo, 2006; Derks, 2013; Xue, 2015; Liu, 2011; Shi, 2015; and Derks, 2013), with only one study suggesting comparable results (Alsalameh, 2004). Chondrocytes, on the other hand, were either comparable (Elsaesser, 2016; Togo, 2006; Kobayashi, 2011; and Williams, 2010) or superior to CSPCs (Seol, 2017; Marcus, 2014; and Zhou, 2014) in their chondrogenic differentiation potential, with only one study suggesting inferior results (Levato, 2017).

In vivo application of CSPCs for cartilage repair

Of the 19 *in vivo* animal models assessing the use of CSPCs for cartilage regeneration toward tissue engineering applications, most used CSPCs isolated from articular hyaline (n = 6), auricular (n = 6), and meniscal (n = 3) sources, reflecting clinical need for cartilage repair in those locations (Table VII). Clinical disease models for potential future utility of CSPCs included osteoarthritis, degenerative disk disease, as well as facial, tracheal, and thorax reconstruction following trauma, tumor, degeneration, and congenital conditions resulting in cartilage defects (Table VII). 90% of studies demonstrated the capability of CSPCs for generating the cartilage matrix *in vivo*, both in xeno- and auto-transplantation animal models, confirmed using histology, immunohistochemistry, SEM, TEM, and magnetic resonance imaging

TABLE I. Cartilage derived stem/progenitor cells (CSPCs) from articular hyaline cartilage. MACS, magnetic cell separation; O, osteogenic differentiation; A, adipogenic different tion; N, No; Y, Yes; TGFb1/2/3, transforming growth factor b1/2/3; OA, osteoarthritis; rtPCR, reverse transcriptase polymerase chain reaction; Col1/2/10, collagen type 1/2/10; A glycans; COMP, cartilage oligomeric matrix protein; SafO, Safranin O; AB, Alcian blue; TB, toluidine blue; Sox9, SRY-Box 9; MSC, mesenchymal stem cell; BMSC, bone marro TEM, transmission electron microscopy; SEM, scanning electron microscopy; SDF1, stromal cell-derived factor 1; FACS, fluorescence activated cell sorting; IHC, immunohistoch genetic protein 2/6/7; ICRS, International Cartilage Repair Society; ECM, extracellular matrix; DMMB, 1,9-dimethyl-methylene blue assay; HSA-HA, human serum albumin/hy SCID, severe combined immunodeficiency; FGF2, fibroblast growth factor 2; IL1b, interleukin 1 beta.

Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT criteria	*In vitro/ in vivo*	Chondrogenic induction	Control groups	Evidence cartilag regenerati
Alsalameh (2004)	Human	MACS (CD105+ CD166+)	Y	CD105+, CD166+	O, A. C	Y	*In vitro*	Micromass, TGFbl/3	BMSCs OA and non-OA CSPCs	GAG (Al Col1, Col2. (IHC)
Dowthwaite *et al.* (2004)	Bovine	Fibronectin adhesion	Y	CD29+, CD49e+, Notch-1	C,O	N	*In vitro*, *in vivo* (ovine)	Injection into the proximal limb of chick embryo	…	Col1 (IH
Fickert *et al.* (2004)	Human	FACS (CD9+ CD90+CD1 66+)	Y	CD9+, CD44+, CD54+, CD90+, CD166+	O, A, C	N	*In vitro*	Pellet, TGFb3	BMSCs, chondrocytes	Col1, Col COMP (IH GAG (Al
Martin *et al.* (2005)	Bovine	Fibronectin adhesion	Y	…	C	N	*In vitro*	Pellet, TGFb1	+/− TGFbl	GAG (Saf Col2 (IH
Thornemo *et al.* (2005)	Human	Agarose suspension	N	…	C	N	*In vitro*	3D agarose	Different types of cell clusters	GAG (Saf Col2, Ag (IHCQ
Hattori *et al.* (2007)	Bovine	FACS (Hoechst 33342 side population)	Y	ABCG2+	C	N	*In vitro*	Micromass, BMP7 and TGFb1	Untreated CSPCs	GAG (TB) ((IHC)
Ustunel *et al.* (2008)	Human	Fibronectin adhesion	Y	Notch-1+, Notch-2+, Delta+, Jagged-1+	C	N	*In vitro*	Pellet	Chondrocytes	TEM. SE
Grogan *et al.* (2009)	Human	FACS (Hoechst 33342 side population)	Y	CD44+, CD90+, CD105+, CD166+, Stro-1+, Notch-1+, CD106+, ABCG2+	O, C, not A	N	*In vitro*	Pellet, TGFb1	Non side population cells	Col1, Col Agg, Sox (rtPCR) G (SafO)
Khan *et al.* (2009)	Bovine	Fibronectin adhesion	Y	Notch-1+	C	N	*In vitro*	Pellet, TGFb1	Chondrocytes	GAG (Saf Age (IH
Koelling *et al.* (2009)	Human	Migratory subpopulation	Y	CD29+, CD44+, CD73+, CD90+, CD105+, Stro-1+, CD45−, CD17−	O, A, C	Y	*In vitro*	3D alginate, TGFb3 and BMP6	Chondrocytes and osteoblasts	Sox9, Co (ISH)
Williams *et al.* (2010)	Human and Caprine	Fibronectin adhesion	Y	CD105+, CD166+, CD44+, CD29+, CD49e+, Notch-1+, CD90+, Stro-1+, Jagged-1+, Delta-1+	A, C, limited O	N	*In vitro/ in vivo* (caprine)	Pellet, TGFb3 & caprine articular defect	Chondrocytes	GAG (TI SafO) Col Col2 (IH

TABLE I. (*Continued.*)

Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT criteria	*In vitro*/ *in vivo*	Chondrogenic induction	Control groups	Evidence cartilag regenerati
Chang *et al.* (2011)	Human	MACS (CD105+CD166+)	Y	CD105+, CD166+	O, C	N	*In vitro*	TGFb1	CSPCs from fetus, adults, and elderly	Agg, Col (rtPCR)
Pretzel *et al.* (2011)	Human	MACS (CD166+)	Y	CD105+, CD166+	O, A, C	N	*In vitro*	Pellet, TGFb3	CD166+ enriched and depleted	GAG (Al
McCarthy *et al.* (2012)	Equine	Fibronectin adhesion	Y	CD90+, CD166+, Stro-I+, Notch-1+	O, A, C	N	*In vitro*	Pellet, TGFbl	BMSC	Agg. Col Runx2, Matrillin 1(IHC)
Seol *et al.* (2012)	Bovine and human	Migratory subpopulation	Y	ABCG2+, Notch-1+, CD44+	O, C, limited A	N	*In vitro*	Pellet, TGFbl	Chondrocytes and BMSCs	GAG (Saf Microarr
Benz *et al.* (2013)	Human	MACS (W5C5+)	Y	CD49e+, CD73+, CD90+, CD105+, CD140b+, CD166+, CD34−, CD45−, CD271−	C but limited O and A	N	*In vitro*	3D HSA-HA hydrogel, TGFb3	W5C5 enriched and depleted, IVD CSPCS	Sox9. Co (rtPCR) G (DMMB
Bernstein *et al.* (2013)	Human	Enzymatic digest and dedifferentiation	Y	CD9+, CD44+, CD54+, CD73+, CD105+, CD166+, CD 45−	O, C	N	*In vitro*	Pellet, TGFb3	CSPCs from different OA grades	GAG (DM
Joos *et al.* (2013)	Human	Migratory subpopulation	Y	CD29+, CD44+, CD73+, CD90+, CD105+, CD166+, CD54+, MSCA-1+, Stro-l+, CD88+, CD34+, CD133−, CD45	O, A, C	Y	*In vitro*	Micromass	BMSC	Sox9,Col2, Agg, COM (rtPCR) C COMP (IH
Singh *et al.* (2013)	Rabbit	Differential plastic adherence	Y	CD106+, CD44+	C	N	*In vivo* (rabbit)	Pellet, subchondral drilling, then CSPCs in 3D collagen to fill articular defect in rabbits	Subchondral drilling alone	Col2.Ag (rtPCR) G (TB, ICR Arthrosco radiology, SEM
Marcus *et al.* (2014)	Bovine	Fibronectin adhesion	Y	…	C	N	*In vivo* (mouse)	Injection into SCID	Chondrocytes	Col2, Sox (rtPCR) S (IHC) GA (AB. SafO
Nelson *et al.* (2014)	Human	Fibronectin adhesion	Y	Stro-l+	O, A, C	N	*In vitro*	Pellet, TGFb2	…	Sox9, Col2. (rtPCR) G (SafO, T Col2, Ag (IHC)

TABLE I. (*Continued.*)

Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT criteria	*In vitro*/ *in vivo*	Chondrogenic induction	Control groups	Evidence cartilag regenerati
Ozbey *et al.* (2014)	Human	Fibronectin adhesion	Y	CD90+, CD105+, CD166+, Stro-1+	O, A, C	N	*In vitro*	Pellet, TGFbl	…	GAG (IH
Zhou *et al.* (2014)	Bovine	Migratory subpopulation	Y	…	C	N	*In vitro*	Pellet, TGFbl	Chondrocytes and synovial fibroblasts	GAG (DM Microarr
Yu *et al.* (2014)	Bovine	FACS (single cell sorting) and clonogenicity	Y	ABCG2+, CD133+, CD105+, CD90+, CD71+, CD29+, Notch 1+	O, C, limited A	N	*In vitro*	Pellet, TGFbl	CSPCs from different zones	Sox9, Co (rtPCR) G (SatO)
Frisbie *et al.* (2015)	Equine	Fibronectin adhesion	Y	…	C	N	*In vivo* (horse)	3D fibrin, TGFbl and FGF2 injected into horse articular defect	Autologous vs allogenic CSPCs	GAG (Saf Coll, Col (IHC) arth copy, an radiograp
Jiang and Tuan (2015)	Human	NGF cell migration assay	Y	CD271+, CD90+, CD73+, CD105+, CD166+, CD44+, CD29+, CD34−, CD45−	O, A, C	Y	*In vitro*	Pellet, TGFb3	Chondrocytes	Coll, Col2. Sox9 (rtPC GAG (Saf
Neumann *et al.* (2015)	Human	Fibronectin adhesion	Y	Notch-1+	C	N	*In vitro*	3D fibrin and polyurethane, BMP2, and mechanical stimulation	No mechanical stimulation/BMP2 overexpression	Coll, Col2. (rtPCR) N GAG
Su *et al.* (2015)	Human	FACS (CD146+)	Y	CD146+, CD44+, CD73+, CD90+, CD105+, HLA-ABC+, CD34−, CD45−, CD 133−	O, A, C	Y	*In vitro*	Pellet	Chondrocytes and adipose MSCs	Sox9, Agg, (rtPCR) G (TB) Col2.Aeg.S (IHC)
Tong *et al.* (2015)	Rat	Fibronectin adhesion	Y	CD44+, CD90+, CD31−, CD34−, CD45−	O, A, C	Y	*In vitro* and *in vivo* (rat)	Pellet, TGFb1, and IL1b	Chondrocytes and BMSCs	Agg, 5ox9, (rtPCR) G (AB)
Xue *et al.* (2015)	Porcine	Fibronectin adhesion	Y	CD29+, CD44+, CD90+, CD34−, CD35−	O, A, C	Y	*In vitro*	2D monolayer and pellet, TGFbl, and FGF2	BMSCs, auricular/ IVD CSPCs	Agg, Col COMP (rtP GAG (AI Col2 (IH
Li *et al.* (2016)	Rabbit	Fibronectin adhesion	Y	CD90+, CD105+, CD166+, CD34−, CD45−	O, A, C	Y	*In vitro*	3D alginate beads with mechanical stimulation	Chondrocytes and fat pad derived stem cells	GAG (SafC DMMB) C (IHC)
Zhao (2016)	Rat	Clonogenicity	Y	CD29+, CD49e+, CD90+, CD73+, CD146+. CD45−	O, A. C	Y	*In vitro*	3D alginate, TGFb3, and BMP 6	Chondrocytes	GAG (AI Col1. Co (IHC)

TABLE I. (*Continued.*)

Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT criteria	*In vitro*/*in vivo*	Chondrogenic induction	Control groups	Evidence for cartilage regeneration	Outcome
Levato *et al.* (2017)	Equine	Fibronectin adhesion	Y	CD29+, CD44+, CD49d+, CD106+, CD166+CD73+, CD90+, CD105+, CD34−, CD45	O, A, C	Y	*In vitro*	3D gelatin methacryloyl, FGF2, and TGFbl	BMSC and	GAG (DMMB) Col1, Col2, Col10 (IHC) PRG4 (rtPCR) Biomechanical	CSPCs had lower Col10 hypertrophy marker, higher PRG4 lubrication marker, and greater cartilage ECM compared to chondrocytes (MSC > CSPC > chondrocyte)
Anderson (2017)	Human	Fibronectin adhesion	Y	…	C	N	*In vitro*	Condensation on fibronectin membrane, TGFbl, and FGF2	Chondrocytes 5 vs 20% oxygen	GAG (DMMB, TB) Col1, Col2 (IHC) biomechanical	CSPCs are superior in reproducing native anisotropy and greater compressive modulus but less lubricin
Batsehkus (2017)	Human	Migratory subpopulation	Y	…	C	N	*In vitro*	2D and 3D alginate and heavy amino acids	CSPCs in 2D vs 3D	Mass spectrometry	3D culture of CSPCs downregulates catabolic proteins and enhances chondrogenesis

(MRI). One study reported that CSPCs were unable to produce cartilage matrix *in vivo* unlike chondrocytes, despite their expression of chondrogenic genes (Marcus, 2014). Of the studies involving other cell types, CSPCs were found to generate cartilage matrix superior to nontissue-specific MSCs (Ding and Huang, 2015; Wang, 2014; and Togo, 2006), but comparable to chondrocytes (Williams, 2010 and Togo, 2006), thereby confirming *in vitro* findings.

DISCUSSION

To the best of our knowledge, this systematic review is the first to comprehensively summarize the available evidence on cartilage derived stem/progenitor (CSPC) isolation and cartilage regenerative capacity to determine suitability for tissue engineering. Based on the reported studies, CSPCs exist in both adult animal and human cartilages, with the articular hyaline cartilage being the earliest and most well-studied source to date. Increasing evidence suggests the existence of CSPCs in other types of cartilages, including the intervertebral disk, auricular, nasoseptal, tracheal, and costal, in the order of decreasing frequency and across a variety of species. CSPCs have been used for cartilage repair *in vivo* with excellent outcomes that are comparable to chondrocytes and superior to MSCs from unrelated tissue sources. The current review concludes that CSPCs represent a promising cell source for cartilage tissue engineering, but there is currently no consensus on specific cell surface markers or isolation protocols.

CSPCs were initially described to reside in the superficial zone of articular cartilage (Dowthwaite, 2004 and Williams, 2010), and this view has been supported by successful isolation of CSPCs from auricular perichondrium (Kobayashi, 2011 and Takebe, 2012), superficial zone of nasoseptal cartilage (do Amaral, 2012 and Baptista, 2013), as well as tracheal (Yoon, 2013) and costal (Srour, 2015) perichondrium. It is believed that the surface location of CSPCs allows them to respond to soluble factors released into the synovium or blood during injury in order to coordinate *in vivo* cartilage repair. However, other studies refute this theory and have also found CSPCs in deeper zones of cartilage (Ozbey, 2014 and Yu, 2014).

Our results indicate that despite the heterogeneity in CSPC isolation methods, cell surface marker expression profiles are consistent across different studies and cartilage types. CSPCs are positive for recognized MSC markers CD90, CD105, CD44, CD166, CD73, and CD29 and negative for hematopoietic markers CD34 and CD45, with the only conflicting data existing for CD133. This is unlike adult MSCs from other tissue sources which tend to have heterogeneous cell surface marker expression profiles with conflicting data between studies (Mafi, 2011). This suggests that CSPCs represent a relatively homogenous population of cells which could be attributed to the lack of contaminating cell populations with the cartilage being recognized as avascular, aneural, and immune privileged.

Despite the relative overlap in cell surface markers between articular, meniscal, auricular, nasoseptal, tracheal, and perichondral CSPCs, those isolated from the intervertebral disk do express some unique neuronal stem and progenitor cell associated surface markers. These include p75NTR (neurotrophin receptor) (Risbud, 2007), nestin (Feng, 2010), neuron-specific enolase (Feng, 2010), and GD2 (neural ganglioside) (Sakai, 2012). This is likely attributed to the intervertebral disk being a multicomponent structure and

TABLE II. Cartilage derived stem/progenitor cells (CSPCs) from the meniscal cartilage. MHC-II, major histocompatibility complex class II; O, osteogenic differentiation; A, adip genic differentiation; N, No; Y, Yes; TGFb1/3, transforming growth factor b1/3; OA, osteoarthritis; rtPCR, reverse transcriptase polymerase chain reaction; Col1/2/10, collagen ty cans; SafO, Safranin O; Sox9, SRY-Box 9; MSC, mesenchymal stem cell; BMSC, bone marrow derived mesenchymal stem cell; TEM, transmission electron microscopy; SD and FACS, fluorescence activated cell sorting.

Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT criteria	*In vivo/ in vitro*	Chondrogenic induction	Control Group	Evidence cartilage regeneratio
Shen *et al.* (2013)	Rabbit	Clonogenicity	Y	MHC-II−	O, A, C	N	*In vivo* (rabbit)	Pellet, TGFbl, injection postmeniscectomy/OA rabbits	PBS	Col 2 (rtPC GAG (Saf biomechani radiologic
Muhammad *et al.* (2014)	Human	Migratory subpopulation	Y	Strol+, CD29+, CD90+ CD105+. CD106+, CD45−, CD34−	O, A, C	Y	*In vitro*	3D Alginate. TGFb3	Chondrocytes	Sox9, Col (Western
Shen *et al.* (2014)	Human	Clonogenicity	Y	CD44+, CD90+, CD105+, CD166+, CD34−, CD45−	O, A, C	Y	*In vitro* and *in vivo* (rat)	Injected into rats with meniscal defect	Synovial MSCs and BMSCs	GAG (Saf Col1, Col Col10 (IH TEM
Ding and Huang (2015)	Rabbit	Clonogenicity	Y	SSEA-4+, CD44+, CD90+, Stro-1, CD34−	O, A, C	Y	*In vitro* and *in vivo* (nude rat)	Pellet, TGFb3, and implanted in rats on 3D matrigel	BMSCs	GAG (Saf Col2 (Weste
Seol (2017)	Bovine	FACS (Hoechst 33342 side population)	Y	ABCG2+	O, A, C	N	*In vitro*	Pellet, TGFbl	Chondrocytes	GAG (Saf Microarra

TABLE III. Cartilage derived stem/progenitor cells (CSPCs) from nonarticular cartilage sources. MSC, mesenchymal stem cell; BMSC, bone marrow derived mesenchymal st tion; A, adipogenic differentiation; C, chondrogenic differentiation; N, No; Y, Yes; TGFb1/3, transforming growth factor b1/3; FGF2, fibroblast growth factor 2; IGF1, insulin growth netic protein 2; EGF, epidermal growth factor; PLLA/PCL, Poly L-lactide/Polycaprolactone; rtPCR, reverse transcriptase polymerase chain reaction; Col1/2/10, collagen ty glycosaminoglycans; COMP, cartilage oligomeric matrix protein; SafO, Safranin O; AB, Alcian blue; TB, toluidine blue; H&E-hematoxylin and eosin stain; Sox9, SRY-Box 9; TI copy; SEM, scanning electron microscopy; IHC, immunohistochemistry; DMMB, 1,9-dimethyl-melthylene blue assay; EVG, elastic van Gieson; FACS, fluorescence activated separation; ECM, extracellular cartilage matrix; SCID, severe combined immunodeficiency; HUVEC, human umbilical vein endothelial cell; IVD, intervertebral disk; CEP, cartilag AF, annulus fibrosus; HSA-HA, human serum albumin/hyaluronan; and pCT, X-ray microtomography.

Cartilage type	Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT	*In vitro/ in vivo*	Chondrogenic induction	Control groups	E re
Nasoseptal	Shafiee *et al.* (2011)	Human	Clonogenicity	Y	CD90+, CD105+, CD106+, CD166+, HLA-ABC+, CD133+. CD34−, CD45−, HLA-DR−	O, C, N	Y	*In vitro*	Pellet	BMSCs and adipose derived MSCs	G C
	do Amaral *et al* (2012)	Human	Superficial zone digest	Y	CD44+, CD73+, CD105+, CD146−	O, C, not A	N	*In vitro*	Pellet	…	SO G
	Baptista *et al.* (2013)	Human	Superficial zone	Y	…	C	N	*In vitro*	Pellet, TGFb3	Adipose derived MSCs	G C
	Shafiee *et al.* (2014)	Human	Clonogenicity	Y	CD73+, CD90+, CD105+, CD106+, CD166+, HLA-ABC+, CD34−, CD45−, CD133−, HLA-DR−	O, A, C	Y	*In vitro*	3D PLLA/PCL, TGFbl	Aligned vs randomly oriented nanofibers	(rt (I
	Elsaesser *et al.* (2016)	Human	Migratory subpopulation	Y	CD29+, CD44+, CD105+, CD106+, CD90+, CD34−, CD133−, CD45−, CD31−	O, C	N	*In vitro*	3D decellularized ECM	BMSCs and chondrocytes	GA A C
Auricular	Togo *et al.* (2006)	Rabbit	Clonogenicity	Y	…	O, A,C	N	*In vivo* (mouse)	Pellet, then seeded on 3D collagen and injected dorsally in nude mice	Chondrocytes and BMSCs	C (rt (T (E C
	Kobayashi *et al.* (2011) (i)	Human	Perichondrium digest and FACS (CD44+CD90+)	Y	CD44+, CD90+, CD49e+, CD73+, CD 105+, CD34−, CD24−, CD117−, CD133−. CD138−, CD140a−, CD146−, CD271−	C	N	*In vivo*	2D layers, FGF2 and IGF1. injected SCID mice	Chondrocytes	GA Sa C EC
	Kobayashi *et al*2011 (ii)	Murine	Perichondrium digest	Y	CD44+, CD49e+	C	N	*In vitro*	2D	Dermal fibroblast	C

TABLE III. (*Continued.*)

Cartilage type	Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT	*In vitro/ in vivo*	Chondrogenic induction	Control groups	E re
	Takebe *et al.* (2012)	Human	Perichondrium digest	Y	…	C	N	*In vitro*	2D layers, FGF2 Sc IGF1, 3D collagen/ hydroxyapatite/ chondroitin sulfate scaffold	Noncell seeded scaffold only	GA t
	Derks *et al.* (2013)	Porcine	Perichondrium digest	Y	…	O, A, C	N	*In vitro*	Pellet. FGF2 Sc TGFbl	Tracheal CSPCS and BMSCs	C C (rt (
	Sterodimas and de Faria (2013)	Rabbit	Perichondrium and decell hNP chon- drium digest	Y	…	C	N	*In vivo* (rabbit0	Pellet, TGFb3 and BMP2, Seeded on 3D alginate/silk, implanted sack of rabbit	CSPCs from chondrium and perichondrium	C M me
	Mizuno *et al.* (2014)	Canine	Perichondrium digest	Y	CD44+, CD90+	C	N	*In vivo* dog)	2D monolayer. IGF1 and FGF2, cell clus- ters injected into canine articular defect	With and with- out fibrin glue	GA AB bio
	Takebe *et al.* (2014)	Human	Perichondrium digest	Y	…	C	N	*In vivo* (mouse)	Co-cultured with HUVECs, transplanted into the cranial window of SCID mice	Conventional pellet culture	C Ela Col
	Xue *et al.* (2015)	Porcine	Fibronectin adhesion	Y	CD29+, CD44+, CD90+, CD34−, CD 35−	O, A, C	Y	*In vitro*	Pellet, TGFbl and FGF2	BMSC, IVD/ articular CSPCs	C CO C C
	Kagimoto (2016)	Monkey and human	Perichondrium digest	Y	…	O, A, C	N	*In vivo* (mouse and monkey)	FGF2 8c Insulin, trans- planted subcu- taneous and cranio-facial	Human and monkey CSPCs	C Ela C (
IVD	Risbud *et al.* (2007)	Human and rat	Clonogenicity	Y	CD90+, CD73+, p75NTR, CD105+, CD166+, CD63+, CD493+, CD133+, CD34−, CD45−	O, A. C	Y	*In vitro*	Pellet, TGFb3	3D alginate culture	Sox (rt
	Blanco *et al.* (2010)	Human	Clonogenicity	Y	CD90+, CD73+, CD105+, CD166+, CD106+, C034−, CD45−, CD14−, CD19−, HLA-DR−	O, C not A	N	*In vitro*	Pellet	BMSCs	C

TABLE III. (*Continued.*)

Cartilage type	Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT	*In vitro/ in vivo*	Chondrogenic induction	Control groups	E re
	Feng *et al.* (2010)	Human	Migratory subpopulation	Y	CD29+, CD49e+, CD51+, CD73+, CD90+, CD105+, CD166+, CD184+, Stro-l+, Nesting Neuron specific enolase+	O, A, C	N	*In vitro*	Pellet	…	Sox (rt
	Liu *et al.* (2011)	Human	Agarose suspension	N	CD1Q5+, CD73+, CD90+ CD44+, CD166+. Stro-l+, CD133+, CD14−, CD34−, CD19−, CD45−	O, A, C	Y	*In vitro*	3D agarose, pellet, TGFb3	BMSCs	C So GA
	Sakai *et al.* (2012)	Human and mouse	FACS (Tie2+)	Y	Tie2+, GD2+, CD44+, CD49f+, CD56+, CD73+, CD90+, CD105+, 166+	O, A, C	Y	*In vitro* and mouse)	3D methyl cellulose or decell hNP, transplanted into SCID mice	Chondrocytes	G TB
	Xiong *et al.* (2012)	Human	Agarose	N	CD73+, CD90+, CD105+, CD14−, CD19−, CD34−, CD45−, HLA-DR−	O, A, C	Y	*In vitro*	Pellet, TGFb3	+/− macrophage inhibitory factor	C
	Benz *et al.* (2013)	Human	MACS (W5C5+)	Y	CD49e+, CD73+, CD90+, CD105+, CD140b+, CD166+, CD34−, CD45, CD271−	C but limited O and A	N	*In vitro*	3D HSA-HA. TGFb3	W5C5 enriched and depleted, articular CSPCS	S (rt
	Brisby *et al.* (2013)	Human	IVD digest	Y	CD105+, CD90+, Stro-l+, Notch- 1+, Jagged-1	C	N	*In vitro*	Pellet, TGFbl, disk cell/BMSC conditioned media	+/− conditioned	C
	Wang *et al.* (2014)	Human	Agarose suspension	Y	CD90+, CD105+, CD73+, CD34−, CD45−	O, A, C	Y	*In vivo* (rabbit)	3D alginate injected into rabbit IVD degeneration model	BMSCs, CEP/ NP/AF CSPCs	C ra
	Shi *et al.* (2015)	Rat	IVD digest	Y	CD29+, CD44+, CD90+, CD34, CD45−, CD19−, CDllb−	O, A. C	Y	*In vitro*	2D high density	BMSCs	C Sox
	Xue *et al.* (2015)	Porcine	Fibronectin adhesion	Y	CD29+, CD44+, CD90+, CD34−, CD35−	O, A, C	Y	*In vitro*	2D and pellet. TGFbl and FGF2	BMSCs, auricular/articular CSPCs	C CO C C

TABLE III. *(Continued.)*

Cartilage type	Study	Species	Isolation technique	Plastic adherence	Cell surface markers	Multipotency	ISCT	*In vitro*/*in vivo*	Chondrogenic induction	Control groups	Evidence of cartilage regeneration	Outcome
Tracheal	Derks *et al.* (2013)	Porcine	Tracheal digest	Y	…	O, A, C	N	*In vitro*	Pellet, FGF2 and TGFb1	Auricular CSPCs and BMSCs	Col 2. Agg. COMP, Coll (rtPCR) GAG (AB) Col2 (IHC)	CSPCs demonstrated higher chondrogenesis than BMSCs, but lower than auricular CSPCs
	Yoon *et al*2013	Murine	Perichondrium	Y	CD29+, CD90+, CD105+, CD45−	O, A, C	Y	*In vivo* (mouse)	Pellet, FGF2 Sc EGF, injected into midventral tracheal defect (0.30.5 mm)	Untreated defect	BMP2 (IHC) Structural (H&E)	Hyaline cartilage regenerated by CSPCs
Perichondral	Srour *et al.* (2015)	Murine	Perichondrium and chondrium	N	…	C	N	*In vivo* (mouse)	Perichondrium vs no perichondrium in costal cartilage defect	Native costal	GAG (AB) μCT	Cartilage regenerated only if perichondrium is intact (Histo and μCT)

that CSPCs were isolated from both the notochord derived nucleus pulposus and the sclerotome-derived annulus fibrosis and cartilage-end plate (Risbud, 2011).

Interestingly, many of the MSC markers studied e.g., CD73 and CD49e, have also found to be positive in mature chondrocytes (Alsalameh, 2004; de la Fuente, 2004; Dowthwaite, 2004; Diaz-Romero, 2008; and Williams, 2010) indicating lack of specificity. Gogan *et al.* have also reported that the distribution of stem cell markers (Notch-1, Stro-1, and VCAM-1) was not consistent with stem cell distribution, concluding that these surface markers may not be useful for identifying CSPCs (Grogan, 2009). If CSPCs prove to be useful for future cartilage tissue engineering strategies, more efficient and specific isolation techniques will be required to isolate and purify these cells for clinical use.

The progenitor vs stem cell status of CSPCs has been the topic of much debate in the literature. Like chondrocytes, CSPCs have a highly chondrogenic profile, but trilineage potential and MSC marker expression suggest a higher lineage than cartilage committed progenitors (Kobayashi, 2011). Although the limited self-renewal of CSPCs, with senescence after 50PD (Khan, 2009) or 60PD (Koelling, 2009), has been previously used to define them as progenitors rather than true stem cells, there is increasing evidence that many adult stem cell sources also have a limited proliferative history (Foudi, 2009 and Wilson, 2008). Of the 69 reports on CSPC isolation, 24 met the ISCT criteria for an MSC (Dominici, 2006) with no relationship with the type of isolation technique used, which included migration assay, fibronectin adhesion, and flow or magnetic cell sorting. Of those studies that did not meet ISCT criteria, the majority were due to a simultaneous lack of trilineage and immunophenotype assessment rather than actively failing to meet stem cell criteria. This may be because the first progenitor studies predate Dominici criteria in 2006, thereby contributing to confusion in nomenclature. Despite the evidence that these cells may represent a putative cartilage stem/progenitor cell maintaining cartilage homeostasis *in vivo*, a few studies have identified and characterized single cell-derived clonal subpopulations (Barbero, 2003; Williams, 2010; and Yu, 2014) which means that phenotypic "stemness" may be the result of a heterogeneous pool of cells (Janebodin, 2011).

Evidence largely supports subtype-specific extracellular cartilage matrix secretion by CSPCs depending on their cartilage origin using both *in vitro* and *in vivo* chondrogenesis models (McCarthy, 2012; Yoon, 2013; Williams, 2010; Anderson, 2017; Kobayashi, 2011; Takebe, 2012; and Kagimoto, 2016), although there are also studies suggesting that fibrocartilage rather than functional cartilage is formed (Derks, 2013; Dowthwaite, 2004; and Koelling, 2009). One study found that CSPCs of auricular elastic cartilage origin are able to regenerate the hyaline cartilage suggesting their potential for reprogramming these cells (Mizuno, 2014). Nasoseptal CSPCs were shown to differentiate into neuronal lineages which may be attributed to their neuroectodermal origin (Shafiee, 2011). A better understanding of the hierarchy of mesenchymal cell lineage may allow more focused rather than generic characterization of CSPCs and allow them to have other applications beyond cartilage tissue engineering.

We do not yet know whether CSPCs maintain themselves within their niche in native cartilage to promote repair through extracellular

TABLE IV. Positive cell surface markers for cartilage derived stem/progenitor cells (CSPCs). Number of studies reporting positive surface markers for different CSPC cartilage sources.

Cell surface marker	Articular	Meniscal	Auricular	Nasoseptal	Tracheal	IVD	Total
CDS	2						2
CD29	8	1	1	1	1	3	15
CD44	12	2	4	2		4	24
CD49a						1	1
CD49d	1						1
CD49e	4		2			2	8
CD49f						1	1
CD51						1	1
CD54	3						3
CD56						1	1
CD63						1	1
CD71	1						1
CD73	9		1	2		9	19
CD88	1						1
CD90	19	3	3	3	1	11	37
CD105	15	2	1	4	1	9	32
CD106	4	1		3		1	9
CD133	1			1		2	4
CD140b	1					1	2
CD146	2						2
CD166	13	1		2		6	24
CD184						1	1
CD271	1						1
Stro-1	7	2				3	12
Notch-1	9					1	10
Notch-2	1						1
Delta-1	2						2
Jagged-1	2					1	3
ABCG2	5	1					9
HLA-ABC	1			2			3
MSCA-1	1						1
p75NTR						1	1
SSEA-4		1					1
Nestin						1	1
Neuron-specific enolase						1	1
Tie2						1	1
GD2						1	1

signaling factors or differentiate to maintain the cartilage tissue itself. However, the ability of CSPCs to regenerate cartilage *in vitro* even after prolonged expansion (Martin, 2005; Bernstein, 2013; Benz, 2013; and Ozbey, 2014) suggests that tissue homeostasis involves an element of the latter, because any mature chondrocytes within the CSPC enriched population would have been expected to dedifferentiate.

CSPCs unlike other stem cell sources [e.g., adipose derived stem cells (ADSC), BMSC] are capable of spontaneous chondrogenic differentiation *in vitro* (Keoelling, 2009 and Hattori, 2007), even in the absence of growth factors, which makes them a more practical cell source for cartilage tissue engineering (Baptista, 2013 and do Amaral, 2012). Both *in vitro* and *in vivo* studies indicate that BMSCs produce cartilage that is hypertrophic rather than functional due to their lineage preference for osteogenesis (McCarthy, 2012; Shen, 2014; and Ding and Huang, 2015), whereas ADSCs fail to reproduce mature extracellular matrix (Baptista, 2013 and Su, 2015). Although chondrocytes are shown to have comparable (Elsaesser, 2016; Togo, 2006; Kobayashi, 2011; and Williams, 2010) or even superior (Seol, 2017;

TABLE V. Negative cell surface markers for cartilage derived stem/progenitor cells (CSPCs). Number of studies reporting negative surface markers for different CSPC cartilage sources.

Cell surface marker	Articular	Meniscal	Auricular	Nasoseptal	Tracheal	IVD	Totals
CD11b						1	1
CD14						3	3
CD17	1						1
CD19						4	A
CD24			1				1
CD31	1						1
CD34	8	3	2	2		8	23
CD35	1		1			1	3
CD45	10	2		2	1	7	22
CD117			1				1
CD133	2		1	2			5
CD138			1				1
CD140a			1				1
CD146			1	I			2
CD271	1		1			1	3
HLA-DR				2		2	4
MHC-11		1					1

Marcus, 2014; and Zhou, 2016) cartilage regenerative capabilities, unlike CSPCs (Martin, 2005; Bernstein, 2013; Benz, 2013; and Ozbey, 2016), they are not able to maintain their phenotype when expanded in culture (McCarthy, 2012 and Williams, 2010). The presence of meniscal and articular CSPCs has even been demonstrated in diseased states and shown to regenerate cartilage *in vitro* (Alsalameh, 2004; Bernstein, 2013; and Muhammad, 2014), but their susceptibility to various inflammatory mediators i.e., NF-κ B and ILb1 in late stages of osteoarthritis may hamper their utility *in vivo* (Tong, 2015 and Muhammad, 2014).

Limitations and future work

This systematic review has several limitations. Heterogeneity of studies resulted from a lack of standardized CSPC isolation protocols, chondrogenic culture methods (i.e., pellet, micromass or 3D scaffold), experimental animals, and cartilage defects. None of the studies had performed sample size calculations. Future studies should not only characterize cartilage derived cells according to ISCT criteria to determine the progenitor vs stem cell status, but also investigate targeted cell surface markers based on the developmental origin of cartilage type rather than previously established MSC markers alone, to improve our understanding of the overall CSPC immunophenotype. Side-by-side studies directly comparing the chondrogenicity of CSPCs with differentiated chondrocytes and other tissue derived MSCs are required in order to definitively determine the utility of CSPCs for cartilage tissue engineering.

CONCLUSIONS

CSPCs have been shown to have excellent cartilage regenerative ability using both *in vitro* and *in vivo* studies, with outcomes that are

TABLE VI. Summary of included studies on cartilage derived stem/progenitor cells (CSPCs). Stem cell classification based on cartilage cells meeting all three minimal International Society for Cellular Therapy criteria (Dominici,2006). 1. MSC must be plastic-adherent when maintained under standard culture conditions. 2. MSC must express recognized stem cell surface markers (CD105, CD73, and CD90) and lack expression of unrelated markers (e.g., CD45, CD34, CD14, CD11b, CD79alpha or CD19, and HLA-DR). 3. MSC must differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*. Those that did not meet all three criteria were classified as progenitors.

Study type	Classification of cartilage cells	Number of studies							
		Articular	Meniscal	Nasoseptal	Auricular	IVD	Tracheal	Costochondral	Total
In vitro	Stem cells	9	1	2	1	5	…	…	18
	Progenitor cells	20	1	3	3	4	1	…	32
In vitro and *in vivo*	Stem cells	1	2	…	…	1	…	…	4
	Progenitor cells	2	…	…	…	…	…	…	2
In vivo	Stem cells	…	…	…	…	1	1	…	2
	Progenitor cells	3	1	…	6	…	…	1	11

TABLE VII. *In vivo* applications of cartilage derived stem/progenitor cells (CSPCs) for cartilage repair. TGFb3, transforming growth factor b3; TGFb2, transforming growth factor 2; EGF, epidermal growth factor; IHC, immunohistochemistry; Histo, histology; ICRS, International Cartilage Repair Society; rtPCR, reverse transcriptase polymerase chain microscopy; SCID, severe combined immunodeficiency; NF-kB, nuclear factor kappa B; TGFb1, transforming growth factor b1; OA, osteoarthritis; HIF-2a, hypoxia inducible fac 1; BMSC, bone marrow derived mesenchymal stem cell; ECM, extracellular cartilage matrix; BMP2, bone morphogenetic protein 2; HUVEC, human umbilical vein endothelial cartilage endplate; NP, nucleus pulposus; AF, annulus fibrosus; MRI, magnetic resonance imaging; and μCT, X-ray microtomography.

Clinical disease model	Study	CSPC source	Species (no. per treatment)	Study type (follow-up period)	Intervention	Control group	
Osteoarthritis	Williams *et al.* (2010)	Human articular cartilage	Goat (6)	Animal model articular defect (20 weeks)	Pellet and TGFb3 first, then loaded on 3D collagen with TGFb2 El FGF2 (6 mm defect)	Chondrocytes	Cartilag samples
	Dowthwaite *et al.* (2004)	Bovine articular cartilage	Chick embryo (ns)	Animal model cartilage regeneration (10 days)	Injection into the proximal limb of developing chick embryo		Tissue resul
	Singh *et al.* (2013)	Rabbit articular cartilage	Rabbit (24)	Animal model articular defect (IS days)	Pellet first, subchondral drilling, then CSPCs in 3D collagen (3 mm defect)	Subchondral drilling alone	CSPC g firmed tissu
	Marcus *et al.* (2014)	Bovine articular cartilage	SCID mouse (ns)	Animal model (2 weeks)	Injection on the back of mouse	Chondrocytes	CSPCs matri
	Frisbie *et al.* (2015)	Equine articular cartilage	Horse (12)	Animal model articular defect (6 and 12 months)	3D fibrin, TGFbl and FGF2 injected into horse articular defect (16 mm)	Fibrin only	Autol only radiog
	Tong *et al.* (2015)	Rat articular carti1age	Rat (7–10)	Animal model OA (0, 2, 8, 14, 30, 60 or 90 days)	NF-xB inhibitor (BAYL1-7082) injection twice a week	Dimethyl sulfoxide	Inhibit vated
	Shen *et al.* (2013)	Rabbit meniscal cartilage	Rabbit (3, 3, and 12, respectively)	Animal models of meniscal injury (4, 8, and 12)	Pellet, TGFbl, injection into rabbits postmeniscectomy	Phosphate-buffered saline	C5PC for 8 weeks b
	Shen *et al.* (2014)	Human meniscal cartilage	Rat (3 and 3, respectively)	Animal model meniscal defect (4 El 12 weeks)	Injected into meniscal defect rats	Phosphate-buffered saline	CSPC regen compar Histo
	Mizuno *et al.* (2014)	Canine auricular cartilage	Dog (4)	Animal model articular defect (60 days)	2D monolayer, IGF1 and FGF2, cell clusters injected into canine articular defect (4 mm)	With and without fibrin glue	Forma line a auric
	Ding and Huang (2015)	Rabbit meniscal cartilage	Nude rat (4)	Animal model (3 weeks)	Implantation on the back of mouse, CSPCs in matrigel	BMSC	Matrig gen 2 a
Facial Reconstruction	Togo *et al.* (2006)	Rabbit auricular perichondrium	Nude mouse (6)	Animal model (4 weeks)	Pellet then seeded on 3D collagen and injected dorsally in nude mice	BMSCs and chondrocytes	CSI BMS
	Kobayashi *et al.* (2011) (i)	Human auricular perichondrium	SCID mouse (ns)	Animal model (6 and 10 months)	2D layered, FGF2 and IGF1, injected SCID mice	Chondrocytes	CSPCs drium
	Sterodimas and de Faria (2013)	Rabbit auricular cartilage	Rabbit (6)	Animal model (8 weeks)	Pellet, TGFb3 and BMP2, seeded on 3D alginate/silk, implanted dorsally in immunocompetent rabbit	CSPCs from chondrium and perichondrium	Forma

TABLE VII. (*Continued.*)

Clinical disease model	Study	CSPC source	Species (no. per treatment)	Study type (follow-up period)	Intervention	Control group	Outcome
	Takebe *et al.* (2014)	Human auricular perichondrium	SCID mouse (3, 3, and 3, respectively)	Animal model cranial co-cultured with HUVECs, defect (IS, 30 and 60 days) transplanted in the cranial window of SCID mice	Conventional pellet culture	Formation of superior cartilage with condensed progenitors than pellet culture (Histo, IHC)
	Kagimoto (2016)	Monkey and human auricular cartilage	SCID mouse (18) and monkey (3)	Animal models (3 months)	FGF2 and insulin, transplanted subcutaneously in the craniofacial region	Human and monkey CSPCs	CSPCs formed cartilage in both the xeno- and autotransplantation models (Histo, IHC)
Degenerative disk disease	Sakai *et al.* (2012)	Human IVD (NP)	SCID mouse (ns)	Animal model (8 weeks)	Decell h NP transplanted into SCID mice	Decellularized hNP with no cells	Cartilage formation increased in hNP seeded with cells (Histo, IHC)
	Wang *et al.* (2014)	Human IVD (CEP, NP and AF)	Rabbit (21)	Animal model disk 3D alginate injected into rabbit IVD degeneration (6 months) model		MSCs	Cartilage regeneration (CEP > M5C > NP > AF) on histology and MRI
Tracheal reconstruction	Yoon *et al.* (2013)	Murine tracheal cartilage	Mouse (5 untreated and 15 treated)	Animal model tracheal defect, (2, 4 and 20 weeks)	Pellet, FGF2 and EGF, injected into midventral tracheal defect (0.30.5 mm)	Untreated defect	Hyaline cartilage regenerated by 4 and 20 weeks (IHC)
Thorax reconstruction	Srour *et al.* (2015)	Murine rib perichondrium	Mouse (A 33, B12, and C7)	Animal model (3 and 9 months)	A—Perichondrium intact. B—Cartilage and perichondrium removed	C—Native costal cartilage	Cartilage regenerated only if perichondrium is intact (Histo and μCT)

comparable to chondrocytes and superior to MSCs from unrelated tissue sources. The current review concludes that CSPCs represent a promising cell source for cartilage tissue engineering, but further work is required to establish a consensus on nomenclature, specific cell surface markers, and isolation protocols.

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