

# Cartilage Endplate-Derived Stem Cells for Regeneration of Intervertebral Disc Degeneration: An Analytic Study

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**Purpose:** Intervertebral disc degeneration (IDD) is considered the predominant cause of low back pain (LBP) and accounts for global disability and a substantial socioeconomic burden. Given the unsatisfactory outcomes of current treatment strategies, cartilage endplate-derived stem cells (CESCs) are increasingly used in intervertebral disc regeneration. However, comprehensive analyses on CESCs remain rare. Herein, we examined the biological functions and applications of CESCs in IDD.

**Methods:** PubMed, Embase, and Cochrane Library databases were searched to identify studies focused on CESCs. Relevant information from included studies was extracted. Descriptive statistics were performed. Correlation analysis was conducted to determine the relationship among Web of Science (WoS) citations, Dimensions, and Altmetric Attention Score (AAS).

**Results:** Twenty-six studies were included in this study. Most studies (n=20) isolated CESCs from humans, followed by rats (n=5) and rabbits (n=1). Twenty studies were performed in vitro, and the remaining six were implemented both in vivo and in vitro. The findings of this study provide insight into the biological properties of CESCs and clarify their potential application for intervertebral disc regeneration. There was a very high correlation between WoS and Dimensions citation count ( $p < 0.001$ ,  $r = 0.988$ ).

**Conclusion:** This study, for the first time, elaborates biological features of CESCs and analyzes their potential applications in regenerating intervertebral discs. CESCs may be promising candidates for protecting the intervertebral disc from degeneration and contributing to intervertebral disc regeneration.

**Keywords:** intervertebral disc degeneration, low back pain, cartilage endplate-derived stem cells, stem cells, intervertebral disc

## Introduction

Low back pain (LBP), a prevalent musculoskeletal disorder, is encountered frequently in clinical settings.<sup>1-3</sup> It is estimated that approximately 80% of the population will experience LBP at some point in their lifetime.<sup>3-5</sup> LBP is a leading cause of sick leave and work-related disability, thereby imposing a significant economic impact on society.<sup>1,2,4,6</sup> In the United States alone, the annual economic burden of LBP surpasses \$100 billion.<sup>7,8</sup>

Although the pathogenesis of LBP is multifaceted and not fully understood, intervertebral disc degeneration (IDD) is commonly acknowledged as a primary etiological factor.<sup>3,5,6,8-12</sup> The intervertebral disc, a fibrocartilaginous structure situated between adjacent vertebral bodies,<sup>9,10,13-16</sup> is essential for spinal stability and mobility.<sup>11,13,16</sup> This structure comprises three distinct tissues: the annulus fibrosus (AF), nucleus pulposus (NP), and cartilage endplate (CE).<sup>8,11,14,16</sup> The NP, embryologically distinct from the AF and CE, originates from the notochord, whereas the latter two derive from sclerotomes.<sup>5,8-11</sup> This complex architecture facilitates the absorption and distribution of physical forces.<sup>5,11,13-16</sup> The CE, a thin hyaline cartilage layer, both separates the vertebral body from the intervertebral disc and protects it from NP invasion. Given the avascular nature of the intervertebral disc, nutrient and metabolite transport are primarily dependent on CE permeation.<sup>17-19</sup> Degeneration-induced calcification or sclerosis of the CE can impede this critical nutritional and metabolic exchange,<sup>20,21</sup> thus playing a pivotal role in the initiation and progression of IDD.<sup>22-25</sup>

Advancements in stem cell research have led to the discovery of cells with stem-like properties in various tissues and organs.<sup>26–31</sup> These stem cells are integral to the repair or regeneration of damaged structures.<sup>5,8–10,15,16,32</sup> In response to tissue damage or degeneration, endogenous repair mechanisms involve the recruitment of stem cells, which then proliferate and differentiate to restore structural and functional integrity.<sup>5,9</sup> Stem cells in the intervertebral disc were initially isolated from degenerated human NP and AF cells, displaying mesenchymal stem cell (MSCs) characteristics and potential for osteogenic, adipogenic, and chondrogenic differentiation.<sup>5,8–10</sup> Recently, cartilage endplate-derived stem cells (CESCs) have been isolated.<sup>33,34</sup> Based on the distinct anatomical regions within the intervertebral disc, intervertebral disc-derived stem cells are typically categorized into three subsets, including NP-derived stem cells (NPSCs), AF-derived stem cells (AFSCs), and CESCs.<sup>5,8–10</sup> CESCs are considered multipotent stem cells that likely play a significant role in maintaining the integrity and functionality of the cartilage endplate.<sup>8–10,33,34</sup> Furthermore, CESCs have been shown to effectively mitigate IDD by promoting NP regeneration and regulating intervertebral disc homeostasis.<sup>5,9</sup> Failure of endogenous repair by CESCs has been identified as a primary contributor to IDD.<sup>8,23,32–34</sup>

Current IDD interventions include both conservative and operative strategies.<sup>14,16,35,36</sup> Despite significant progress in research and clinical studies, treatment efficacy remains suboptimal.<sup>35–38</sup> This limitation is partly due to these strategies focusing more on alleviating pain than addressing the underlying cause of IDD.<sup>4,13–16,32,35–37,39</sup> Stem cell transplantation has emerged as a novel therapeutic approach,<sup>13–15,37</sup> but the challenging microenvironment of the intervertebral disc, characterized by high osmolarity, mechanical load, poor nutrition, low oxygen tension, and acidic pH, restricts its effectiveness.<sup>40–43</sup> CESCs, as endogenous stem cells, are increasingly utilized in disc regeneration due to their ability to overcome transplantation barriers.<sup>5,8–10,44–46</sup> Despite promising outcomes with CESCs in IDD regeneration, comprehensive studies on CESCs are still lacking. This study aimed to analyze the biological functions and applications of CESCs in IDD and provide insights into CESCs research, proposing future strategies for intervertebral disc regeneration.

## Materials and Methods

### Search Strategy

Given that human and animal subjects were not involved in the present study, approval from the Institutional Review Board was not required. We conducted a comprehensive search to identify papers focused on CESCs published from inception up to April 20, 2023. Two investigators independently retrieved data from the PubMed, Embase, and Cochrane Library databases. The keywords used for the search strategy included “cartilage endplate”, “stem cell”, “progenitor cell”, and “stromal cell”. Titles irrelevant to the topic were first removed. The abstracts of any papers potentially relevant to CESCs were further examined. The full text of each paper was reviewed if the abstract failed to provide sufficient information. Disagreements were settled through discussions, with a third investigator consulted to reach a consensus when necessary. References of included papers and related reviews were screened to identify potential publications.

### Inclusion and Exclusion Criteria

The eligibility criteria for study inclusion/exclusion were as follows: (1) papers were published in English; (2) papers must involve basic research or clinical studies related to CESCs; (3) case reports, editorials, letters, and reviews were excluded.

### Data Extraction and Management

Two investigators reviewed the selected studies separately and performed data extraction using a standardized form. Disagreements were resolved through discussion between investigators. A third investigator was consulted to reach a consensus when necessary. The following information was collected from included studies: authors, year of publication, journal, cell source, study type, CESCs isolation method, the method employed for surface marker detection, positive and negative markers, and key findings. Web of Science (WoS) citations of the included studies were identified in the WoS database. Dimensions citations and Altmetric Attention Score (AAS) were identified in the database of Dimensions ([www.dimensions.ai](http://www.dimensions.ai)).

## Quality Assessment

Two investigators independently evaluated the methodological quality of eligible studies. Disagreements between investigators were resolved by discussion with a third investigator. The quality of included papers was assessed using a quality scoring system,<sup>47</sup> which included eight questions and has been widely used in similar publications with “yes” and “no” answers assigned 1 and 0 points, respectively.<sup>48–51</sup> The maximum achievable score is 8 points. The quality scores for included studies were independently determined. The quality of the studies was graded based on their scores. A score of 7–8 was interpreted as excellent, 5–6 as good, 3–4 as poor, and 0–2 as bad.<sup>48–51</sup>

## Statistical Analysis

In this study, descriptive statistical methods, including total count and proportion, were employed to analyze the data. To examine the association between Web of Science (WoS) citations, Altmetric Attention Score (AAS), and Dimensions citations, Pearson’s correlation analysis was utilized. The Pearson’s correlation coefficient ( $r$ ) was categorized as follows: a coefficient less than 0.3 indicated a poor correlation, 0.3–0.5 a low correlation, 0.5–0.7 a moderate correlation, 0.7–0.9 a high correlation, and greater than 0.9 a very high correlation.<sup>52,53</sup> All statistical analyses were conducted using SPSS software (version 19.0; SPSS, Chicago, IL). A  $p$ -value of less than 0.05 was considered statistically significant.

## Results

### Study Selection

Following the primary retrieval strategy, we identified 284 papers potentially related to CESCc. Twenty papers were removed because they were duplicates. After title and abstract screening, 41 papers progressed to full-text review; subsequently, 15 papers were excluded owing to failure to meet inclusion criteria. The remaining 26 papers were included.<sup>33,34,54–77</sup> Figure 1 presents a flowchart of the screening process.

### General Information on the Papers

The included studies were published between 2011 and 2022 (Table 1). The highest value of WoS citations, Dimensions citations and AAS were 145, 101 and 6, respectively. Twelve works (46%) had no AAS (Table 1). Nineteen journals contributed to 26 papers. Stem cells (3), Molecular Medicine Reports (3), European Spine Journal (2), PLoS ONE (2), and Acta Biochimica et Biophysica Sinica (2) published two or more papers. Most studies ( $n=20$ ) isolated CESCc from humans, followed by rats ( $n=5$ ) and rabbits ( $n=1$ ) (Figure 2). Twenty papers were in vitro studies, while the remaining six involved both in vivo and in vitro experiments (Figure 2).

### Quality of Included Studies

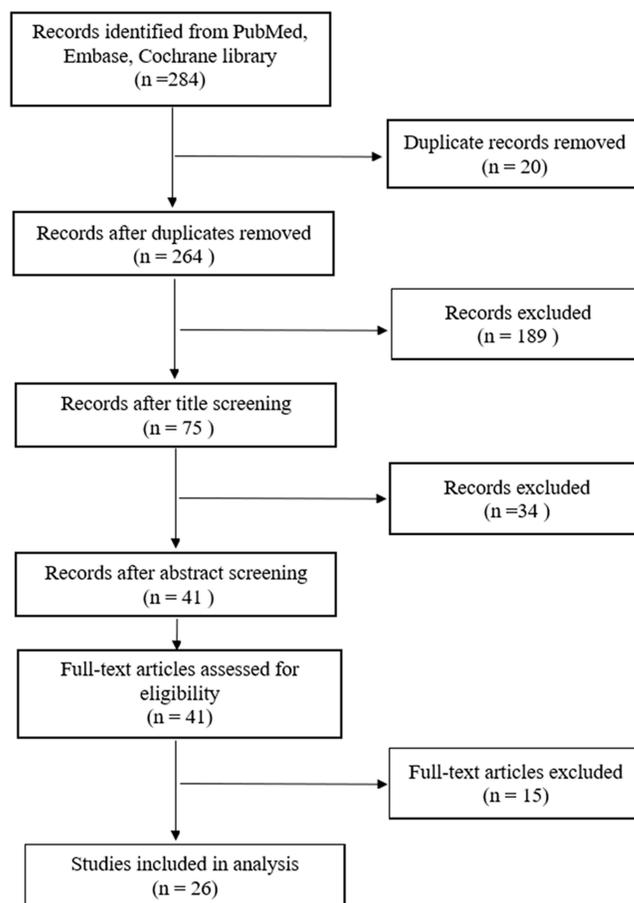
Questions regarding the quality scoring systems are listed in Table 2. The methodological scores of included studies ranged from 5 to 8. Four studies achieved maximum scores. The overall quality of included studies ranged between good and excellent. In total, 17 studies received excellent, and the remaining nine were deemed good. Detailed methodological scores of included studies are presented in Table 3.

### CESCc Isolation

All studies provided a detailed description of the method employed for CESCc isolation (Figure 3). Among included studies, 18 isolated CESCc using the agarose suspension culture system, 5 employed cell culture without any special treatment, 2 used the differential adhesion method, and 1 used low-density plating.

### Method Employed for Surface Marker Detection

Twenty studies performed surface marker detection. Of these, 18 studies used flow cytometry, 1 used immunohistochemical staining, and 1 used both flow cytometry and immunofluorescence assays. Figure 3 presents detailed information regarding the detection of surface markers in CESCc.



**Figure 1** The flow chart of screening process.

## Surface Markers of CESC

Twenty papers reported surface markers for isolated C ESCs; surface markers were not reported in the other six papers. CD90 was the most common positive marker detected in 19 studies (95%), followed by CD105 (15, 75%), and CD73 (13, 65%). The majority of the studies (n=13; 65%) detected CD73, CD90, and CD105. C45 (17, 75%) was the most common negative marker, followed by CD34 (14, 70%) and CD19 (10, 50%). CD45 and CD34 (n=13; 65%) were the most common negative markers reported in the included studies. [Table 1](#) lists C ESCs surface markers detected in the included studies.

## Key Findings of C ESCs

[Table 1](#) summarizes key C ESCs findings of included studies. Two studies described the discovery of C ESCs.<sup>33,34</sup> Five studies compared C ESCs with NPSCs, AFSCs, and bone marrow-derived stem cells (BMSCs) considering their biological properties.<sup>33,55,58,62,65</sup> In addition, five studies reported genome-wide analyses of gene expression profiling and alternative splicing events in C ESCs under normoxic and hypoxic conditions.<sup>56,59,60,64,66</sup> Eight studies discussed the fate of C ESCs in IDD.<sup>54,57,61,63,68–70,76</sup> Eight studies elaborated on the biological potential of C ESCs in intervertebral disc regeneration.<sup>55,67,71–75,77</sup>

## Correlation Analysis

The citation counts of included studies in WoS were very high correlated with their citations in Dimensions ( $r = 0.988$ ,  $p < 0.001$ ). [Figure 4](#) described a clear linear correlation between WoS citation and Dimensions citations. In addition, a poor correlation was demonstrated between the number of citations in WoS and AAS ( $r = 0.280$ ,  $p = 0.220$ ).

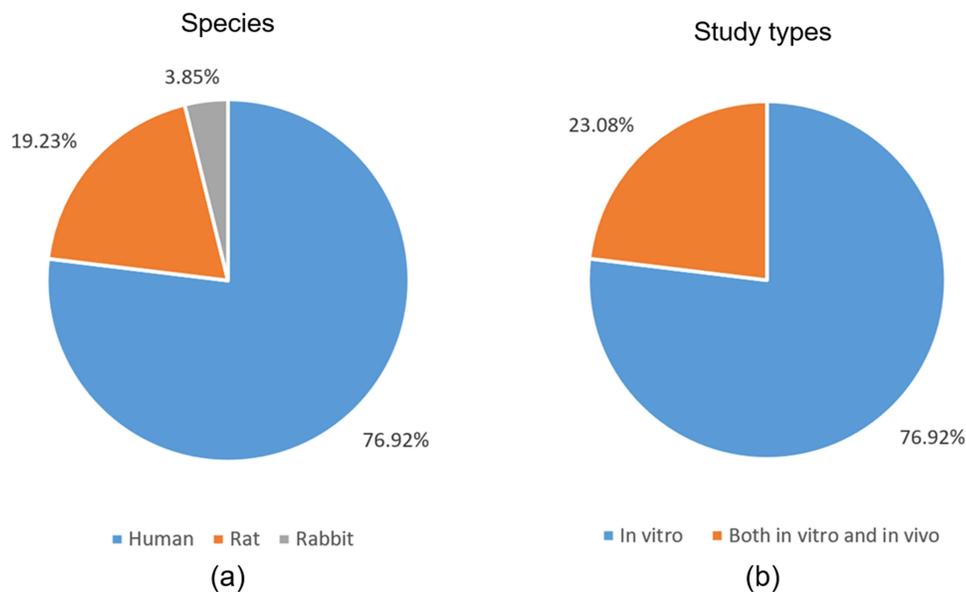
**Table 1** Characteristics and Key Findings of the Included Studies

Author	Year	Species	Study Type	Isolation Method of C ESCs	Detection Method of Surface Marker	Positive Markers	Negative Markers	Key Findings
Liu et al <sup>33</sup>	2011	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105, CD44, CD166, CD133, Stro-1	CD34, CD45, CD19, CD14, HLA-DR	C ESCs could be induced into osteoblasts, adipocytes, chondrocytes, and are superior to BM-MSCs in terms of osteogenesis and chondrogenesis.
Huang et al <sup>34</sup>	2012	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105, Stro-1	CD34, CD45, CD19, CD14, HLA-DR	The presence of progenitor cells in degenerated cartilage endplate.
Xiong et al <sup>54</sup>	2012	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD19, CD14, HLA-DR	Macrophage migration inhibitory factor inhibits the migration of C ESCs by reacting with CD74.
Wang et al <sup>55</sup>	2014	Human	Both in vitro and in vivo	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45	C ESCs have the most powerful ability for nucleus pulposus regeneration compared with AFSCs, NPSCs, and BM-MSCs.
Shang et al <sup>56</sup>	2015	Human	In vitro	Agarose suspension culture system	NA	NA	NA	Changed gene expression patterns and alternative splicing events during chondrogenic differentiation of C ESCs were detected on a genome-wide scale.
Feng et al <sup>57</sup>	2015	Human	In vitro	Agarose suspension culture system	Immunohistochemical staining	CD105, Stro-1	NA	N-Ac-PGP induces the migration of C ESCs from the cartilage endplate into the nucleus pulposus via CXCR1/2 and a pro-inflammatory phenotype in C ESCs, and promotes the inflammatory response in degenerative discs.
Wang et al <sup>58</sup>	2016	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD19, HLA-DR	C ESCs, NPSCs, BM-MSCs, and AFSCs shared similar morphologies, proliferation abilities, and surface immunophenotypes. C ESCs had the strongest osteogenic and chondrogenic potentials.
Yao et al <sup>59</sup>	2016	Human	In vitro	Agarose suspension culture system	NA	NA	NA	Gene expression and alternative splicing events in the hypoxia-regulated chondrogenic differentiation in C ESCs were analyzed in a genome-wide scales.
Shang et al <sup>60</sup>	2016	Human	In vitro	Agarose suspension culture system	NA	NA	NA	Alternative splicing events during the osteogenic differentiation of C ESCs were investigated on a genome-wide scale.
Yao et al <sup>61</sup>	2016	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD14, CD19, HLA-DR	MIF as the downstream target of HIF1A regulates chondro-osteogenic differentiation of C ESCs under hypoxia by interacting with SOX9 and RUNX2.
Liu et al <sup>62</sup>	2017	Human	In vitro	Low density plating	Flow cytometry	CD73, CD90, CD105	CD34, CD45, HLA-DR	C ESCs, AFSCs, and NPSCs have similar characteristics in proliferation, passage, and colony formation capacity. C ESCs had the most powerful properties of migration and invasion when compared with AFSCs and NPSCs.
He et al <sup>63</sup>	2017	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105, CD44	CD34, CD45, CD11b, CD19, HLA-DR	Nutrition deficiency may promote CESC apoptosis partially through upregulating BNIP3, which might lead to activation of the BNIP3-related pathway and apoptosis of C ESCs in a caspase-independent manner.
Yao et al <sup>64</sup>	2017	Human	In vitro	Agarose suspension culture system	NA	NA	NA	Gene expression profiles and alternative splicing events during the hypoxia-regulated osteogenic differentiation of C ESCs were revealed in a on a genome-wide scale.
Liang et al <sup>65</sup>	2017	Human	In vitro	Differential adhesion method	NA	NA	NA	C ESCs, NPSCs, BM-MSCs, and AFSCs shared similar morphologies. C ESCs had the strongest chondrogenic potentials and the weakest cell proliferation capacity.

(Continued)

Table I (Continued).

Author	Year	Species	Study Type	Isolation Method of C ESCs	Detection Method of Surface Marker	Positive Markers	Negative Markers	Key Findings
Yao et al <sup>66</sup>	2017	Human	In vitro	Agarose suspension culture system	NA	NA	NA	Gene expression profiles and alternative splicing events in C ESCs under hypoxia were investigated on a genome-wide scale.
He et al <sup>67</sup>	2018	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD11b, CD19, HLA-DR	C ESCs might promote nucleus pulposus cells proliferation in a paracrine pathway, which was partially mediated by SDF-1/CXCR4 axis via ERK1/2 signaling pathway.
Yuan et al <sup>68</sup>	2018	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD14, CD19, HLA-DR	C ESCs apoptosis induced by cyclic tensile stretch probably mediated by the upregulation of the expression of BNIP3.
Sun et al <sup>69</sup>	2019	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD11b, CD19, HLA-DR	Glucose regulates tissue-specific chondro-osteogenic differentiation of C ESCs via O-GlcNAcylation of Sox9 and Runx2.
Zuo et al <sup>70</sup>	2019	Rat	Both in vitro and in vivo	Differential adhesion method	Flow cytometry	CD29, CD44, CD90	CD34, CD45	Rapamycin induced autophagy inhibits inflammation-mediated cartilage endplate degeneration by enhancing Nrf2/Keap1 signaling of C ESCs.
Luo et al <sup>71</sup>	2021	Rat	Both in vitro and in vivo	Cell culture without any special treatment	Flow cytometry	CD44, CD90	CD45	Autocrine exosomes promote the migration of C ESCs into the intervertebral disc and the transformation of C ESCs into nucleus pulposus cells to inhibit IDD by activating HIF-1a/Wnt signaling pathway.
Guan et al <sup>72</sup>	2021	Human	In vitro	Cell culture without any special treatment	Flow cytometry and immunofluorescence assay	CD73, CD90, CD105	NA	CHST3 overexpression promoted C ESCs to repair IDD by interaction with CSPG4.
Luo et al <sup>73</sup>	2021	Rat	Both in vitro and in vivo	Cell culture without any special treatment	Flow cytometry	CD44, CD90	CD45	Normal C ESCs-derived exosomes inhibited the apoptosis of nucleus pulposus cells and weakened disc degeneration more effectively than degenerated C ESCs-derived exosomes by promoting autophagy via activation of the PI3K/AKT signaling pathway.
Chen et al <sup>74</sup>	2022	Rat	Both in vitro and in vivo	Cell culture without any special treatment	Flow cytometry	CD44, CD90	CD45	MiR-125-5p secreted by C ESCs-derived exosomes may promote the autophagy of nucleus pulposus cells, inhibit apoptosis and extracellular matrix degradation, and alleviate IDD by targeting SUV39H1.
Luo et al <sup>75</sup>	2022	Rat	Both in vitro and in vivo	Cell culture without any special treatment	Flow cytometry	CD29, CD90	CD45	Functional exosomes were sustainably released by injectable cartilage matrix hydrogel loaded with C ESCs that express Sphk2, penetrated the annulus fibrosus, and transported Sphk2 into the nucleus pulposus cells to activate the PI3K/AKT signaling pathway as well as the intracellular autophagy, eventually alleviating IDD.
Zhang et al <sup>76</sup>	2022	Rabbit	In vitro	Agarose suspension culture system	Flow cytometry	CD90, CD105	CD34	Inhibition of JNK and ERK in the MAPK signaling family alleviated tension load-induced C ESCs degeneration by inhibiting the phosphorylation of Raptor and mTOR in the mTOR pathway.
Chen et al <sup>77</sup>	2022	Human	In vitro	Agarose suspension culture system	Flow cytometry	CD73, CD90, CD105	CD34, CD45, CD14, CD19, HLA-DR	MiR-637 inhibits osteogenic differentiation of C ESCs by targeting WNT5A.



**Figure 2** Experimental species (a) and included study types (b).

## Discussion

The expanding comprehension of stem cell biology has underscored the potential of stem cells as formidable therapeutic agents for IDD.<sup>5,9,15,16,37</sup> Among these, CESC are regarded as a promising cellular resource for IDD. However, the biological characteristics of CESC have not been extensively investigated, and their potential applications in disc regeneration warrant further exploration. To our knowledge, this study is the first to concentrate on the biological attributes and potential uses of CESC.

CESC were first identified by Liu et al in 2011.<sup>33</sup> They discovered a cell population within the degenerated human CE of intervertebral discs that exhibited stem cell properties, including the capacity for adipogenic, osteogenic, and chondrogenic differentiation.<sup>33</sup> While CE cells are differentiated cells within the CE, CESC are stem cells characterized by their self-renewal ability and differentiation potential.<sup>9,10</sup> CESC have the potential to replenish the depleting differentiated cell population in the CE.<sup>9,10,14</sup> However, it is crucial to note that not all cells isolated from the cartilage endplate are stem cells. CESC meet the criteria for the definition of MSCs, as defined by the International Society for Cellular Therapy (ISCT), and should be sorted into MSCs.<sup>78</sup> According to ISCT, MSCs must adhere to plastic in standard culture conditions, express specific surface markers (CD105, CD73, and CD90), lack the expression of others (CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-DR), and differentiate into osteoblasts, adipocytes, and chondroblasts in vitro. As endogenous stem cells, CESC play an integral role in maintaining a dynamic equilibrium between tissue repair and damage, modulating their proliferation and differentiation. Furthermore, Huang et al revealed the existence of

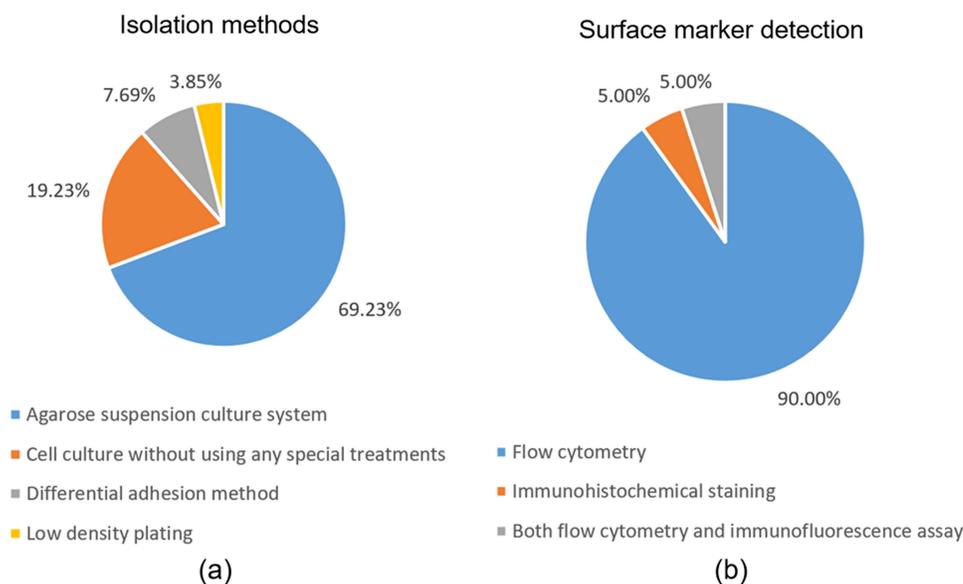
**Table 2** The Criteria of Quality Assessment

Section and Topic	No.	Quality Criteria	Yes	No
Title/keywords/introduction Method	1	Were the study hypothesis/aim/objective being clearly described		
	2	Were the experimental design for the study being well described		
	3	Were the method and materials being well described		
	4	Were the time points of data collection being clearly defined		
	5	Were the main outcome measurements being clearly defined		
Discussion	6	Were the experimental group being well compared with the control group		
	7	Were the results being well described		
	8	Were the limitation of the article being discussed		

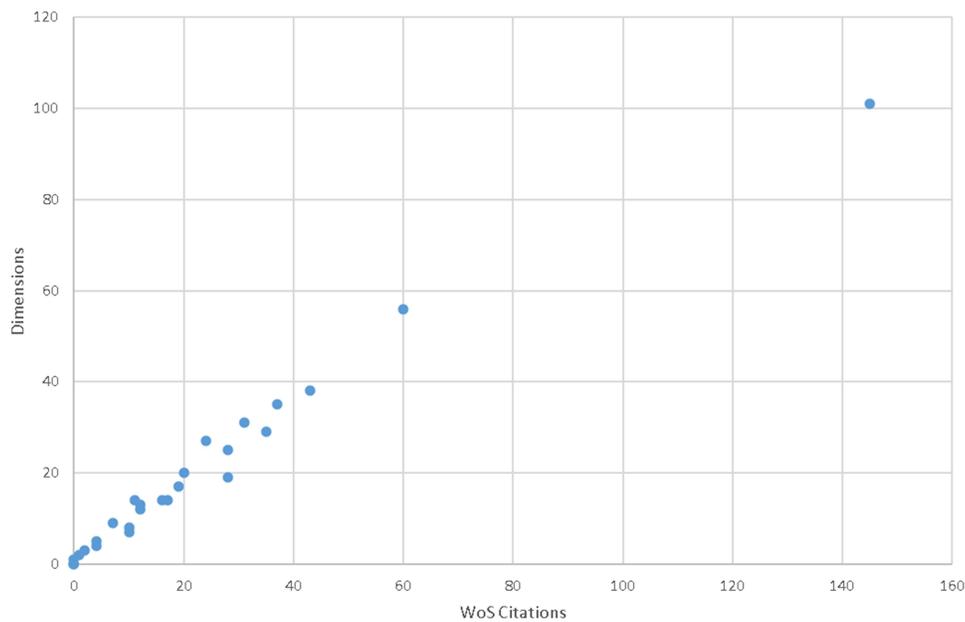
**Table 3** Methodological Scores of the Included Studies

Study	1	2	3	4	5	6	7	8	Quality Score
Liu et al <sup>33</sup>	Yes	8							
Huang et al <sup>34</sup>	Yes	Yes	Yes	No	Yes	No	Yes	Yes	6
Xiong et al <sup>54</sup>	Yes	No	7						
Wang et al <sup>55</sup>	Yes	No	7						
Shang et al <sup>56</sup>	Yes	Yes	Yes	Yes	Yes	No	Yes	No	6
Feng et al <sup>57</sup>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	7
Wang et al <sup>58</sup>	Yes	No	7						
Yao et al <sup>59</sup>	Yes	Yes	Yes	No	Yes	No	Yes	No	5
Shang et al <sup>60</sup>	Yes	Yes	Yes	No	Yes	No	Yes	No	5
Yao et al <sup>61</sup>	Yes	No	7						
Liu et al <sup>62</sup>	Yes	No	7						
He et al <sup>63</sup>	Yes	8							
Yao et al <sup>64</sup>	Yes	Yes	Yes	No	Yes	No	Yes	No	5
Liang et al <sup>65</sup>	Yes	No	7						
Yao et al <sup>66</sup>	Yes	Yes	Yes	No	Yes	Yes	Yes	No	6
He et al <sup>67</sup>	Yes	Yes	Yes	No	Yes	Yes	Yes	No	6
Yuan et al <sup>68</sup>	Yes	No	7						
Sun et al <sup>69</sup>	Yes	Yes	Yes	No	Yes	Yes	Yes	No	6
Zuo et al <sup>70</sup>	Yes	8							
Luo et al <sup>71</sup>	Yes	No	7						
Guan et al <sup>72</sup>	Yes	Yes	Yes	No	Yes	Yes	Yes	No	6
Luo et al <sup>73</sup>	Yes	No	7						
Chen et al <sup>74</sup>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	7
Luo et al <sup>75</sup>	Yes	No	7						
Zhang et al <sup>76</sup>	Yes	8							
Chen et al <sup>77</sup>	Yes	No	7						

progenitor cells in degenerated human CE.<sup>34</sup> These cells expressed stem cell-associated genes such as OCT-4, NANOG, and SOX-2. These findings provide substantial evidence of CESC in the CE. Subsequent research has identified CESC in other species, including rats<sup>70,71,73-75</sup> and rabbits,<sup>76</sup> further broadening the scope of knowledge in this field.



**Figure 3** Isolation method (a) and surface marker detection of CESC (b).



**Figure 4** Scatter plots showing association of WoS and Dimensions citations.

The discovery of resident stem cells has underscored the importance of their successful isolation. For the isolation of cartilage endplate-derived stem cells (CESCs), several methodologies have been developed. Initially, Liu et al introduced the agarose suspension culture system for this purpose.<sup>33</sup> This method has been widely adopted in subsequent studies due to its efficacy in selectively culturing chondrocytes.<sup>79</sup> Additionally, some researchers have utilized the differential adhesion method for CESCs isolation, leveraging the inherent properties of stem cells such as plastic adherence and rapid proliferation.<sup>65,70</sup> This technique takes advantage of the unique characteristics of stem cells to segregate CESCs from other cell types in the intervertebral disc. Another distinctive feature of stem cells is colony formation, which can be exploited for CESCs isolation through low-density plating,<sup>62</sup> based on the premise that stem cells can thrive at lower seeding densities compared to other cells.<sup>10,80</sup> Moreover, several studies have reported the direct isolation of CESCs using standard cell culture techniques without specific interventions.<sup>71–75</sup> Despite these varied techniques, a consensus on the optimal method for CESCs isolation remains elusive, particularly in the absence of comparative studies. Therefore, further investigation is required to develop strategies for the simple and economical isolation of pure CESCs.<sup>10</sup> However, once cells are confirmed as CESCs, the method of isolation may be considered of secondary importance.

Regarding surface markers, CESCs share similarities with MSCs, such as the expression of CD73, CD90, and CD105, and the absence of CD11b, CD14, CD19, CD34, CD45, or HLA-DR, as per the criteria established by the ISCT.<sup>33,34,78</sup> However, the presence of these markers does not unequivocally identify a cell as a CESC,<sup>9,32</sup> as intervertebral disc cells, including AF and NP cells, also exhibit MSC-like surface markers.<sup>81,82</sup> To date, no standardized consensus exists regarding specific surface markers for CESCs,<sup>5,9,10,32</sup> complicating their differentiation from other cells with similar marker profiles.<sup>9,32</sup> Consequently, there is a pressing need to identify unique surface markers for CESCs. Single-cell sequencing is emerging as a potent technique for this purpose, offering the ability to precisely identify distinct CESCs populations at the individual cell level.<sup>9,52</sup>

Advancements in research concerning cartilage endplate-derived stem cells (CESCs) have led to a thorough understanding of their biological properties. Comparative analyses have shown that CESCs possess superior chondrogenic and osteogenic potentials compared to NPSCs, AFSCs, and BMSCs.<sup>55,58</sup> Liu et al<sup>62</sup> conducted a comparative study of CESCs, NPSCs, and AFSCs, revealing that CESCs exhibit enhanced migratory and invasive capabilities. These findings suggest the potential of CESCs as effective cellular sources for stem cell therapy and as seed cells for tissue engineering in the context of IDD.<sup>5,9,10</sup> However, conflicting results have been observed in other studies. Liang et al<sup>65</sup> reported that AFSCs exhibited the most pronounced stem cell-like characteristics, particularly in terms of cell proliferation and

trilineage differentiation capabilities. Such discrepancies in biological properties across different studies could be ascribed to variations in species, isolation techniques, passaging, and culture methods.<sup>5,9,10,55,58,62,65</sup> Moreover, it is noteworthy that most studies on C ESCs have been conducted *in vitro*, raising questions about the translatability of these findings to *in vivo* contexts.<sup>5,9,10</sup> Therefore, there is a pressing need for additional *in vivo* research to elucidate the biological characteristics of C ESCs more accurately. Such studies would be instrumental in validating the potential applications of C ESCs in the treatment of IDD.

Recent advancements in genome-wide analyses have facilitated in-depth investigation into C ESCs. Given the CE's role as a primary channel for nutrient supply, its degeneration, primarily characterized by ossification rather than chondrification, is implicated in the initiation and progression of IDD.<sup>20,21,23,75,83,84</sup> C ESCs, with their inherent chondro-osteogenic differentiation capabilities, may play a crucial role in maintaining the balance between chondrification and ossification in the CE.<sup>33,66</sup> Alternative splicing, a ubiquitous and essential mechanism in eukaryotic cells, leads to gene isoform diversity and protein complexity. Understanding the mechanisms of alternative splicing during stem cell differentiation is vital. High-throughput screening has become the primary method for analyzing transcription profiles and alternative splicing events. Shang et al conducted genome-wide analyses of gene expression profiling and alternative splicing during chondrogenic and osteogenic differentiation of C ESCs, identifying various genes with distinct expression and splicing patterns and investigating related molecular functions and pathways.<sup>56,60</sup> Moreover, hypoxia is known to regulate stem cell proliferation and multi-differentiation potential, suggesting its significant role in modulating C ESCs' biological activities.<sup>85,86</sup> Yao et al investigated the regulatory effects of hypoxia on C ESCs, including differential gene expression and alternative splicing during hypoxia-regulated chondrogenic and osteogenic differentiation.<sup>59,64,66</sup> Overall, these investigations indicate a strong association between alternative splicing events and the regulatory mechanisms of C ESCs. Genome-wide analyses, therefore, could provide a valuable reference framework for future studies aimed at delineating the biological functions of C ESCs. These analyses are instrumental in enhancing our understanding of the underlying mechanisms of IDD and in aiding the development of novel therapeutic approaches for IDD regeneration. This insight into the molecular intricacies of C ESCs could pave the way for innovative strategies in regenerative medicine, potentially transforming the current paradigm of IDD treatment.

C ESCs, as resident endogenous stem cells, are pivotal in the context of IDD.<sup>5,41</sup> However, the mechanisms underlying the compromise of C ESCs during IDD remain elusive. One hypothesis posits that C ESCs may undergo exhaustion following repeated cycles of proliferation and differentiation in an attempt to regenerate the IDD-affected disc. Alternatively, the adverse microenvironmental conditions prevalent in intervertebral discs during IDD could detrimentally impact C ESC function.<sup>5,32</sup> This microenvironment is characterized by hypoxia, acidic pH, high mechanical stress, low glucose concentration, hypertonicity, and suboptimal nutrition supply.<sup>10,12,14,18,87,88</sup> Such environmental stressors could disrupt the physiological milieu of C ESCs, thereby adversely affecting their activity and challenging their survival.<sup>9,10,14,40,44</sup> This understanding highlights the need for further research into the resilience and adaptability of C ESCs within the challenging microenvironment of the degenerated intervertebral disc.

Recent research has extensively explored the fate of C ESCs in the context of IDD.<sup>54,57,61,63,68–70,76</sup> Yao et al discovered that normoxia promotes osteogenesis but inhibits chondrogenesis in C ESCs.<sup>61</sup> Additionally, they identified the macrophage migration inhibitory factor (MIF) as a key nuclear transcriptional regulator influencing the chondrogenic and osteogenic differentiation of C ESCs under hypoxic conditions. The correlation between IDD and inflammatory responses has also been a focal point of investigation.<sup>57,63,68,69,89,90</sup> Xiong et al reported that MIF, a proinflammatory cytokine, was expressed in degenerated discs and hinders the migration of C ESCs to inflammatory sites, potentially exacerbating IDD.<sup>54</sup> Further studies have underscored the impact of inflammatory cytokines on C ESCs. Zuo et al demonstrated that tumor necrosis factor (TNF)- $\alpha$  reduces the proliferation, senescence, and osteogenic differentiation of C ESCs.<sup>70</sup> They also found that rapamycin-induced autophagy mitigates inflammation-mediated cartilage endplate degeneration by enhancing nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) signaling in C ESCs. Feng et al revealed that N-acetylated proline-glycine-proline (N-Ac-PGP), produced by NP cells within the complex microenvironment of the degenerated disc, prompts C ESCs to migrate from the CE into the NP and differentiate into a pro-inflammatory phenotype, accelerating IDD progression.<sup>57</sup> Yuan et al elucidated that cyclic tensile stress induces C ESC apoptosis through the Bcl-2/adenovirus E1B 19-kDa-interacting protein 3 (BNIP3)/Bcl-2

pathway.<sup>68</sup> Moreover, Zhang et al observed that tension load decreases cell viability, enhances apoptosis, and reduces the expression of ACAN, COL-2A, and SOX9 in C ESCs, indicative of C ESC degeneration. They also showed that inhibiting c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) within the MAPK signaling family can alleviate tension load-induced C ESC degeneration by suppressing the phosphorylation of Raptor and mammalian target of rapamycin (mTOR) in the mTOR pathway.<sup>76</sup> He et al demonstrated that nutritional deficiency leads to caspase-dependent apoptosis of C ESCs via the BNIP3-related pathway.<sup>63</sup> Furthermore, Sun et al found that high glucose levels favor osteogenic over chondrogenic differentiation of C ESCs, leading to CE calcification in IDD,<sup>69</sup> possibly due to the intrusion of blood vessels into CE during IDD and the subsequent disruption of the low-glucose microenvironment of C ESCs.<sup>91,92</sup> Overall, the studies underscore that the microenvironment of degenerated discs significantly influences the viability of C ESCs, affecting their quantity, quality, and overall fate. These findings highlight the need for a deeper understanding of potential interventions that can enhance the beneficial effects on C ESCs and mitigate the negative impacts, thereby aiding in the development of effective regeneration strategies for IDD.

Current therapeutic approaches for IDD predominantly focus on symptom management and palliation.<sup>12,14,35,88</sup> Notably, these treatments do not address the fundamental etiology of IDD.<sup>9,10,26,45</sup> Understanding the mechanisms underlying the degeneration of C ESCs could be instrumental in both preventing IDD and developing targeted therapeutic strategies. Recent research efforts have been directed towards elucidating the impact of C ESCs on IDD pathogenesis. Chen et al discovered that miR-637 is downregulated in degenerated C ESCs, and they posited that overexpression of miR-637 might inhibit the osteogenic differentiation of C ESCs by targeting the WNT5A gene.<sup>77</sup> Similarly, Guan et al identified that overexpression of carbohydrate sulfotransferase 3 (CHST3) enhances the proliferation and migration capabilities of C ESCs.<sup>72</sup> Insights gleaned from these studies offer a promising avenue for identifying potential molecular targets, which could inform the development of novel, more effective treatments for IDD. These advances in understanding the molecular dynamics of C ESCs contribute significantly to the evolving landscape of IDD research, potentially leading to breakthroughs in regenerative medicine and therapeutics.

In the rapidly evolving domain of biological therapy, there is an escalating interest in developing novel interventions for IDD that aim to limit, arrest, or potentially reverse the condition, while restoring the biological functions of intervertebral discs.<sup>13,39,45,93–96</sup> The field of regenerative medicine offers promising biological repair strategies for degenerated discs.<sup>13,39,45,93–95</sup> Pioneering this approach, Sakai et al in 2003 investigated the feasibility of MSCs transplantation in IDD using a rabbit model.<sup>97,98</sup> This was followed by Haufe and Mork's 2006 groundbreaking human trial involving intradiscal injections of hematopoietic stem cells.<sup>99,100</sup> In the realm of C ESCs, the application of chemokines and exosomes for recruiting endogenous stem cells or stimulating endogenous repair processes has gained significant traction.<sup>9,10,32,41,44</sup> He et al demonstrated that C ESCs enhance the proliferation of NP cells through paracrine interactions, particularly via the stromal cell-derived factor 1 (SDF-1)/chemokine receptor type 4 (CXCR4) axis.<sup>67</sup> This suggests the potential of the SDF-1/CXCR4 axis to augment the recruitment and interaction of endogenous stem cells with NP cells, thereby contributing to the regeneration of degenerated discs. Moreover, exosomes, as potent mediators of intercellular communication derived from stem cells, have exhibited remarkable regenerative capabilities for tissues and organs.<sup>96</sup> Luo et al conducted studies to assess the therapeutic effects of C ESCs-derived exosomes on IDD.<sup>73</sup> They found that exosomes from normal C ESCs were more effective in inhibiting NP cell apoptosis and mitigating IDD than those from degenerated C ESCs, primarily by enhancing autophagy through the PI3K/AKT signaling pathway. Further exploration in a rat model of IDD revealed that C ESCs-derived exosomes could activate HIF-1 $\alpha$ /Wnt signaling, promoting C ESCs migration into the intervertebral disc and their transformation into NP cells, thus alleviating disc disease.<sup>71</sup> Additionally, Chen et al explored the molecular mechanisms through which C ESCs-derived exosomes impact NP cell function.<sup>74</sup> They discovered that miR-125-5p, secreted by C ESCs-derived exosomes, facilitates NP cell autophagy, inhibits apoptosis and extracellular matrix degradation, and attenuates IDD by targeting SUV39H1. These findings underscore the significant potential of C ESCs-derived exosomes in IDD therapy and highlight the need for further research to optimize their application in clinical settings.

Tissue engineering in the context of IDD provides innovative approaches for functional repair. Wang et al established stem cell-alginate constructs for NP tissue engineering, evaluating the regenerative potential of four different stem cell types, including C ESCs, NPSCs, AFSCs, and BMSCs.<sup>55</sup> Their findings revealed that alginate constructs seeded with

CESCs demonstrated superior regenerative capabilities for IDD, while AFSCs exhibited the least regenerative capacity. NPSCs and BM-MSCs displayed intermediate potential.<sup>55</sup> This evidence positions CESC as a promising candidate for seeding in intervertebral disc tissue engineering, offering an effective biological approach to IDD treatment. Furthermore, Luo et al investigated an innovative noninvasive strategy involving the injection of an injectable hydrogel combined with lentivirus-engineered CESC adjacent to the CE.<sup>75</sup> This study confirmed the sustained release of functional exosomes from the hydrogel, loaded with CESC expressing sphingosine kinase 2 (Sphk2). These exosomes penetrated the AF, delivering Sphk2 to NP cells and activating the PI3K/AKT signaling pathway and intracellular autophagy, thereby contributing to the alleviation of IDD.<sup>75</sup> Despite these promising advancements, the application of CESC in regenerative strategies for IDD is still in its infancy. Several challenges remain, such as limited understanding of the biological characteristics of CESC, the complex pathophysiology of IDD, and the need for scaffolds with optimal biocompatibility, injectability, and sustained-release capabilities.<sup>5,8–10,14,88</sup> Therefore, further research is imperative to explore endogenous repair mechanisms for IDD. Focusing on endogenous repair could potentially overcome several limitations associated with exogenous therapeutic approaches, paving the way for more effective and less invasive treatments for IDD.<sup>8,9,14,32,88,94</sup>

This study possesses certain limitations. Primarily, the sample size was relatively limited, both in terms of the number of studies reviewed and the animal samples considered within these studies. Despite this constraint, the study offers valuable insights into the research surrounding CESC and their role in the regeneration of IDD since their initial discovery. Furthermore, although a comprehensive search strategy was employed, encompassing a review of references in the included papers and related review articles to identify pertinent literature on CESC, it is important to acknowledge that no search strategy is flawless. Consequently, the possibility of selection bias in the study cannot be completely eliminated.

## Conclusion

This study represents the inaugural comprehensive analysis of the biological characteristics of CESC and evaluates their prospective utility in the regeneration of intervertebral discs. CESC appear to be promising candidates for mitigating degeneration and fostering regeneration within the intervertebral disc. However, current research is limited by the absence of specific surface markers and purification methodologies for CESC. Furthermore, there is no established consensus on the most effective approach for employing CESC in the treatment of IDD. Despite these limitations, CESC have emerged as a compelling cellular resource for IDD regeneration. Future investigations should focus on further elucidating the underlying biological mechanisms of CESC and refining their therapeutic potential.

## Data Sharing Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Ethics Approval and Informed Consent

No approval of Institutional Reviewed Board was needed due to not involving human and animals in this study.

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## Disclosure

The authors report no conflicts of interest in this work.

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