Circulating osteogenic precursor (COP) cells constitute a recently discovered population of circulating progenitor cells with the capacity for osteogenesis, or differentiation into mesenchymal tissues. There is a small, but growing body of literature exploring these cells, but with a great deal of disagreement and contradiction within it. This review explores the origins and biological characterization of these cells, including the identification strategies used to isolate these cells from the peripheral blood. It also examines the available knowledge on the in vitro and in vivo behaviour of these cells, in the areas of plastic adherence, differentiation capacity, proliferation, and cellular homing. We also review the implications for future use of COP cells in clinical practice, particularly in the area of regenerative medicine and the treatment and assessment of musculoskeletal disease.
have been identified by several groups, though a variety of terms have been used to reference them, including circulating osteoprogenitors, circulating mesenchymal stem cells (cMSCs), monocyte-derived mesenchymal precursors, and circulating skeletal stem cells, amongst others. However, there is little consensus between the studies regarding their characterization, with individual research groups using a range of different, and at times conflicting, cellular markers and criteria to define these cells. This contradiction and disagreement has diminished the applicability of the current research base and limited progress in the field. Herein, we analyze the literature describing COP cells to provide a clearer understanding of their characterization, as well as to compare them with the better-known bone marrow MSCs. We then identify areas for further research to allow a more comprehensive analysis of the biological identity and behavior of these cells.

2. Search strategy and selection criteria

The studies used in this review were identified through search of the MEDLINE and PubMed databases, using all or part of the search terms ‘Circulating Osteogenic Precursor’, ‘Circulating Mesenchymal Stem cell’ ‘Circulating Osteoprogenitor’, and ‘Circulating Stem Cell’, connected by the Boolean operator ‘OR’, identifying 1048 search items. Examination of paper titles and removal of duplicates yielded 72 included studies. Resulting abstracts were studied for suitability yielding a total of 37 included studies. Finally, reference lists of included the included studies were searched for further possible inclusions, resulting in the inclusion of an additional two papers, bringing the total to 39 (Fig. 1).

3. Origins of the osteoblast – A historical perspective

Ossification and maintenance of the skeleton has long been known to involve a dichotomous relationship between bone forming osteoblasts and resorbing osteoclasts. Osteoblasts were identified on the external surface of bone, but it was unclear where they come from and how they are regenerated, as they are unable to undergo mitotic division. Osteoclasts were identified in the circulation in the 1870’s [1], and thought to be osteoblasts that had fused with a neighboring chondrocyte. This connection sparked the idea of a circulating osteoblastic progenitor cell, as a means of answering the above question, however, despite repeated efforts these were never found. Little progress was made in finding an osteoblastic precursor until nearly a century later, when Urist [2] stimulated histiocytes into osteogenesis via autoinduction, and Tavassoli and Crosby [3] demonstrated that heterotopically transplanted bone marrow formed both blood and bone elements, identifying a source of progenitor cells of the mesenchymal and hematopoietic lineages. This work resulted in the pioneering research by Friedenstein et al., who isolated specific cells within the milieu of the bone marrow responsible for stromal tissues, though the current term “mesenchymal stem cell” was not coined until the early 1990’s [4]. However, despite these discoveries in the 1960’s and 70’s,
some details on the origin of osteoblasts have puzzled scientists. While MSCs have been shown to differentiate into adult osteoblasts, it is unknown how they access sites of bone formation non-contiguous to bone marrow, rekindling the notion of a circulating osteoblastic precursor.

Circulating cells with some capacity for mesenchymal differentiation were identified many years earlier [5], however they were never shown to produce bone tissue. It was not until 1997 that studies identified circulating cells with osteoblastic characteristics in stem cell enriched blood taken from breast cancer patients [6]. These cells were soon demonstrated in healthy individuals at the turn of the 21st century, but could not be prompted to form bone in vitro or in vivo, though they did express several markers of osteogenesis and had hallmarks of the previously identified bone marrow MSCs [7]. Demonstration for bone formation capacity came soon in the pioneering work of Paolo Bianco and Pamela Robey demonstrating in vivo ossification shown after transplantation of the cells into immunocompromised mice, and coining the term circulating skeletal stem cell [8]. As the cells were similar in behavior, appearance and marker expression to the relatively well understood bone marrow MSCs [7], it has been refuted by one study [13]. Due to their similarities, MSCs are typically classified as being (i) plastic adherent, (ii) capable of multilineage differentiation and logarithmic proliferation, (iii) expression of cell surface markers, CD105, CD73, CD90, and (iv) not expressing the hematopoietic marker expression, particularly regarding the presence or absence of hematopoietic markers. Table 1 summarizes the range of marker combinations used to characterize COP cells.

4. Characterization of COP cells

COP cells are known to exist within the peripheral blood mononuclear cell (PBMC) fraction of the blood, estimated to represent approximately 0.42% of this population [11], and it appears that they circulate at a steady level throughout the lifespan in healthy individuals, increasing in times of accelerated bone growth [10,12], however their existence could not be prompted to form bone marrow MSCs, it is unknown how they access sites of bone formation non-contiguous to bone marrow, and initial stimulations of bone formation in the other [19,20]. Once osteogenesis was initiated in the paired mouse, GFP+ cells were found at the site of bone formation, indicating a circulating osteogenic cell, suggesting that COP cells may be an intermediary between HSCs and osteoblasts. Pignolo and Kassem [23] proposed a model of both the hematopoietic and mesenchymal lineages beginning with an unknown common ancestor progenitor cell. It was proposed that this common ancestor gives rise to both HSCs and MSCs, before they both form their accepted terminal cells. The more recently discovered very small embryonic-like (VSEL) stem cells have been shown to have the capacity to regenerate tissue from all germ layers [24], and may be a candidate for this unknown common progenitor. It was also suggested that COP cells could fall directly under a number of ancestors including HSCs, mesangioblasts and MSCs, as a heterogenous population of terminal cell types, providing a possible explanation for the contradictory findings in the literature [23].

5. Origins

Little definitive evidence exists regarding the specific cellular origin of COP cells. However, it is widely believed that the bone marrow is the likely origin. Several studies speculate that COP cells are bone marrow MSCs that have been stimulated to circulate by peripheral tissue demands [6,7,15–18]. This is largely due to their similarities in behavior and initial findings on cell surface marker expression. This has been supported by parabiotic mouse models involving transplantation of green fluorescence protein positive (GFP+) bone marrow into one paired animal and stimulation of bone formation in the other [19,20]. Once osteogenesis was initiated in the paired mouse, GFP+ cells were found at the site of bone formation, indicating a circulating osteogenic cell, though one study of similar methodology did not identify the circulating osteoprogenitors [13]. Despite this evidence that the bone marrow is the tissue of origin, the precise cellular lineage of COP cells remains unclear. It has been suggested that hematopoietic stem cells (HSCs) are possible progenitors for osteoblasts [21,22]. This, combined with newer information on hematopoietic marker expression by COP cells, suggests that COP cells may be an intermediary between HSCs and osteoblasts. Pignolo and Kassem [23] proposed a model of both the hematopoietic and mesenchymal lineages beginning with an unknown common ancestor progenitor cell. The CXCR4/SDF-1 axis is a potent chemotactic pathway, and this study proposed the CXCR4 receptor as a further means of identifying COP cells with markers similar to HSCs. Peripheral tissues factors have been shown to cause mobilization of COP cells with markers similar to MSCs. The CXCR4/SDF-1 pathway has also demonstrated this capacity, stimulating the release of a population of CD29+/CD45-/CD11b- stromal-like cells in the peripheral blood of rabbits after its intravenous administration [17]. Chronic hypoxia has also shown to cause COP cell mobilization with CD44+/CD54+/CD73+/CD90+/CD45- cells mobilized into the
peripheral circulation in a rat model [18]. These studies suggest these cells are involved in processes of tissue injury, inflammation and healing, being released from the bone marrow and circulating to sites of repair, however much more research is required to validate this.

Taken together, this evidence appears to describe cells closely related to bone marrow MSCs which have been stimulated to mobilize into the peripheral circulation, providing a logical explanation for the genesis of these cells. However, a number of studies have noted circulating osteogenic cells which do not conform to the typical MSC identification criteria, expressing a range of hematopoietic markers raising questions regarding the identity, behaviors and functions of COP cells.

Although the majority of research on COP cells has utilized a hematological or vascular marker of some kind either by inclusion or exclusion, a handful of studies have used only the expression of proteins involved in bone formation as the criterion for the identification and isolation of COP cells. As such, OCN+ and AP+ cell behaviors have been studied in experiments related to differentiation and proliferation, including studies related to osteoblasts [36]. Others have simply used OCN expression by PBMCs alone to classify COP cells and their relationship to vascular calcification [28,29]. Whilst this approach has the benefit of simplicity, the lack of characterization of additional marker expression makes the relationships of the cells studied unclear.

### 6.2. COP cells with hematopoietic and vascular markers

The first cells to be identified as COP cells with hematopoietic markers were coined monocyte-derived mesenchymal precursors. They were shown to be CD45+/CD34+/CD14- and CD11b+ cells capable of multilineage mesenchymal differentiation and expansive proliferation [9]. In another study, a similar CD45+/CD34+ (low) population was identified as COP cells, due to their expression of the gene coding for osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL), both important markers of bone formation. The cells identified in this study were heterogenous in their expression of CD14 with a small proportion of the cells expressing the marker, and the rest not [30]. More recently, the co-expression of CD45, OCN and/or Col1 have been used to identify and characterize COP cells. Expression of CD45+/OCN+ alongside the exclusion of CD3+ (T lymphocyte) and CD19+ (B lymphocyte) cells, has been used to study COP cells in a series of experiments on their behavior in frail older adults [11,31,32]. Addition of the marker Col1 to the above criteria has also been used to isolate COP cells in studies showing the role of COP cells in heterotopic ossification [33] and vascular calcification [34]. Furthermore, the role of CD45+/Col1+ COP cells have been studied in the hereditary condition Fibro dysplasia Ossificans Progressiva (FOP) [35]. This growing body of evidence clearly shows the presence of COP cells with hematopoietic markers, though the range of expression patterns studied makes it difficult to identify whether there is one, or many populations present.

Moreover, the biological behavior and functional significance of these cells is still unknown. Indeed, a recent study using a novel triple transgenic mouse model has shown that while CD45+ COP cells were recruited to sites of bone formation, they did not contribute to ossification via differentiation into osteoblasts [36]. They used mice with three gene modifications – CD45-Cre, Z/RED and Col2.3GFP, which allowed them to identify cells which had expressed CD45 at any time, even if it was not currently present. While they showed the presence of these CD45+ cells, and that their capacity to home to a site of bone morphogenetic protein-2 (BMP2) mediated heterogenous ossification (HO), they did not differentiate into osteoblasts. The parallels between the expression of the hematopoietic lineage COP cells and the myeloid lineage osteoclast precursors coupled with this finding raises interesting questions about their biological role. Their expression of markers of bone formation implies an osteogenic role, even if it is not to differentiate osteoblasts. It could be possible that they instead regulate the balance between osteoclastic resorption and osteoblastic activity, directing the formation of bone. Thus, while hematopoietic cells with osteogenic potential seem to exist in the circulation and be implicated in the regulation of bone formation, they do not contribute to the actual formation of bone.

Therefore, the following the finding that the CD34+ fraction of the bone marrow more reliably produced osteoblasts [37], expression of this endothelial stem cell marker in conjunction with osteogenic markers has been employed to isolate COP cells. One study found that there are two distinct populations amongst OCN+ cells: (i) CD34+ population, and (ii)

| Study | Hematopoietic Markers | Other Markers | Study | Hematopoietic Markers | Other Markers |
|-------|-----------------------|--------------|-------|-----------------------|--------------|
| Kuznetsov et al., (2001) [8] | CD45- CD34- CD14- | CD44- OCN+ Col1+ Stro1- | Eggen et al. (2011 & 2018) [33,34] | CD45+ | OCN+, Col1+
| Zwaifler et al., (2000) [7] | CD45-, CD34-, CD14- | CD105+ OCN+ AP+ Col1+ | Otsuru et al., (2017) [36] | CD45+ | Nil
| Fernandez et al., (1997) [6] | CD45-, CD34-, CD14- | Col1+ AP+ | Suda et al., (2009) [35] | CD45+ | Col1+
| Undale et al., (2010) [25] | CD45-, CD34-, CD14- | AP+ | Fadini et al., (2011) [47] | CD45+ | OCN+ AP+
| Otsuru et al., (2008) [15] | CD45- | CD44+ OCN+ AP+ Stro1+ CXCR4+ | Eghbali-Fatourechi et al., (2007) [12] | CD34+ | OCN+ AP+
| Alm et al., (2010) [16] | CD45-, CD34-, CD14- | Col1+ AP+ CD3133+, CD105+, CD90+ | Pirro et al. (2010, 2011 & 2012) [38–40] | CD45+ | OCN+ AP+
| Dalle Carbonare et al., (2009) [30] | CD45+, CD34+, CD14+ | Nil | Rattazzi et al., (2016) [41] | CD34+ | OCN+ CD46+
| Kovalenko et al., (2003) [9] | CD45+, CD34+, CD14+ | AP+ Col1+ CD31+ | Manivalan et al., (2012) [44] | CD34+ | OCN+ AP+
| Matsumoto et al., (2006) [78] | CD45+, CD34+, CD14+ | AP+ Col1+ CD3133+, CD105+ | Rubin et al. (2011 & 2012) [43,45] | CD34- | OCN+ CD46+
| Ritz et al., (2014) [61] | CD45+, CD34+ | CD31+ CD3133+ | Eghbali-Fatourechi & al., (2005) [10] | Nil | OCN+ AP+
| Al Saeid et al., (2017) [32] | CD45+ | OCN+ | Boban et al., (2010) [13] | Nil | GFP+
| Gunawardene et al. (2015 & 2017) [11,31] | CD45+ | OCN+ | Komagai et al., (2008) [20] | Nil | OCN+ AP+
| Otsuru et al., (2007) [19] | CD45- | OCN+ | Kuznetsov et al., (2007) [46] | Nil | OCN+ AP+ BSP+
| Eghbali-Fatourechi et al., (2007) [12] | CD45+ | OCN+ | Boban et al., (2010) [13] | Nil | GFP+
| Rubin et al. (2011 & 2012) [43,45] | CD34+ | OCN+ | Manivalan et al., (2012) [44] | CD34+ | OCN+ AP+
| Boban et al., (2010) [13] | Nil | OCN+ | Rattazzi et al., (2016) [41] | CD45+ | OCN+ AP+
| Kunimori et al., (2010 & 2011) [38–40] | Nil | OCN+ | Rubin et al. (2011 & 2012) [43,45] | CD34+ | OCN+ AP+
| Rattazzi et al., (2016) [41] | Nil | OCN+ | Manivalan et al., (2012) [44] | CD34+ | OCN+ AP+

OCN: Osteocalcin, AP: Alkaline Phosphatase, Col1: Type 1 collagen, CXCR4: C-X-C chemokine receptor type 4, OPN: Osteopontin, BSP: Bone sialoprotein, ON: Osteonectin.
CD34+ population. The CD34+ cells are smaller and less granular, whilst the CD34− cells are large and dense. They proposed that they are not different populations, but that the change in expression occurs as the cells mature and differentiate towards their terminal tissue, losing expression of CD34 as they lose their ‘stem’ capacity [12]. They also found that the proportions of CD34+−/− cells changed as a function of ages, with less CD34− cells present in older populations. Co-expression of CD34 and OCN or AP has also been used to identify these cells in osteoporotic women, exploring the association between COP cell numbers and rates of bone loss [38], arterial stiffness [39], thyroid hormones [40] and the effect of the hypercholesterolemia medication atorvastatin [41]. Cells with a CD34+/−/OCN+ phenotype in rates with artificially induced tibial fractures. They described these cells as peaking in number at three weeks post fracture, proposing them to have a role in the transition between the cartilaginous and mineralized callus [42]. Several studies also used the endothelial and mesenchymal stem cell marker CD146 to isolate COP cells to study their behavior in diabetic patients, seeking to provide a rationale for the decrease in bone mass in type 2 diabetes mellitus [43–45]. The addition of markers classically associated with vascular endothelium further complicates the landscape of COP cells. The interaction between the bone, blood, and vascular lineages remains unclear, however there seems to be some association between the three in COP cell biology.

While there is a wide variance in the characterizations of COP cells, some unifying themes can be identified. The literature appears to describe at least two distinct populations of COP cells, one with a very similar pattern of marker expression to bone marrow MSCs, and at least one other displaying surface receptors associated with the hematopoietic/vascular lineages. These populations require further characterization before they are fully understood. Additionally, the inconsistencies within the literature should be explored, and each identified population further studied for their functionality to determine their biological behavior and roles within normal physiology, as well as in pathological conditions.

7. Plastic adherence

Plastic adherence is one of the central characteristics used to identify bone marrow MSCs. By commonly agreed on definition, MSCs must adhere to plastic in culture – a characteristic often shown to be shared by COP cells [6–9,16,25,30,35,40,46–48]. However, the discovery of a non-adherent cell population in the marrow expressing bone specific markers [49,50], which has significantly greater capacity for regenerating bone [21] has prompted the examination of the non-adherent PBMC fraction as well. Three other studies used this same methodology when analyzing COP cell behavior [15,25,43]. These differences in adherence raise further questions regarding the nature of these cells and their relationship to bone marrow MSCs, as more inconsistencies between their biological behavior arise. It is currently unclear whether the differences between adherent behavior correlates to different patterns of observed marker expression, as well its functional significance.

8. Morphology

COP cell morphology has been documented in a number of studies. They are typically described as small round cells initially, but become elongated, “spindle shaped” or fibroblast-like cells over 3–7 days in general growth media [7,9,16,30]. Some studies have also reported a second population of flat or polygonal COP cells [8,48], though in one study it was noted that these cells displayed a nearly identical panel of markers to their fibroblastic counterparts [8]. These spindle and flat morphologies have been demonstrated in bone marrow MSCs, being shown to represent different stages of differentiation, with the flat cells being further differentiated than their spindle shaped counterparts [51]. The flat cell morphology has not been identified in hematopoietic COP cells, though their morphology has only been described in a single study [9]. Upon osteogenic differentiation, both hematopoietic and MSC-like COP cells take on the characteristic rounded, cuboidal or “cobble stone” appearance of osteoblasts [7,9], consistent with the morphological behavior of bone marrow MSCs. It is also yet unknown how the morphologies of these cells change in relation to external factors, such as physiological stress, aging or time in passage. It has been demonstrated that with increased number of passages, bone marrow MSCs become broader and flatter, with more numerous podia [52]. Additionally, they appear to show decreased affinity for the spindle morphology with increasing lifespan, with a correlating decrease in differentiation and proliferation capacity [53]. It is yet unknown whether these patterns of aging are seen also in COP cells, or how they may affect the cells functionally.

9. Differentiation potential

Differentiation into different mesenchymal tissues by COP cells has been established in a number of studies. As might be expected, numerous studies have shown osteoblastic differentiation potential of COP cells, both in vitro and in vivo. Osteoblastic differentiation in vitro has been demonstrated via a number of means including: staining of calcified nodules after culture [10,15,16,25,40,43,47,48], upregulation of genes related to bone formation [30,43], and increased secretion of factors associated with osteogenesis [7,9,19]. Several studies have also shown that these cells are osteogenic when injected or implanted on pellets into mice, forming trabecular bone and some hematopoietic elements [8,15,35,46]. Evidence demonstrating multilineage capacity is less frequent, but some studies have been able to demonstrate COP cells forming tissues other than bone, showing capable of in vitro adipogenesis and chondrogenesis in humans [16–18,48] and guinea pigs [46]. Interestingly, these authors were all experimenting on MSC-like cells, with evidence demonstrating the same stem capacity in hematopoietic COP cells scarcer. Only one study has shown multilineage differentiation in COP cells with hematopoietic markers, demonstrating the in vitro formation of fat, muscle, and cartilage, in addition to bone [9]. These authors provide support to the “stemness” of COP cells, however further investigation is necessary to understand the full extent of their capacity for differentiation, particularly if and how it varies between the different subcategories of identified COP cells by marker expression. It is yet unknown how the differentiation of COP cells compares with their bone marrow or adipose tissue counterparts either in vitro or in vivo in terms of the rate, extent and functionality. It has also yet to be demonstrated how their differentiation is affected by external variables such as patient age, culture conditions and number of passages. Bone marrow MSCs are known to undergo changes in differentiation behavior throughout the lifespan, seemingly more inclined to undergo adipogenesis than bone or muscle formation with increasing age [54,55]. Additionally, bone marrow MSCs have been shown to have the capacity to form tissues outside the traditional mesenchymal lineages, such as neural cells [56], a capacity yet to be investigated in COP cells. This information is essential in the evaluation of COP cells as a potential target for diagnostic or therapeutic intervention.

10. Proliferative capacity

High capacity for proliferation is one of the hallmarks of a stem cell, as it allows for generation of a large number of cells from a relative few, enabling the maintenance of constant cellular turnover throughout the life span. Proliferation is commonly assessed by calculation of population doublings over time or with immunofluorescent measures such as the bromodeoxyuridine (BrdU) assay, giving an indication of how rapidly, and expansively, a cell type can multiply [57,58]. Despite the important role proliferation plays in the definition of a stem cell, only few studies have assessed this quality in COP cells. COP cells have been shown to have a similar capacity for proliferation as bone marrow...
MSCs [16], with a population doubling time of 2.5 days, with 5 × 10^5 cells becoming 6.7 × 10^7 [7] in 17 days [7]. Another study was able to passage COP cells more than 6 times, and generate millions of additional cells over that time, though no specific measurements were reported [46]. The rate of proliferation over time has also been assessed in COP cells. Using the BrdU assay one study showed that at first passage approximately 50% of the COP cells were dividing, but declined to 5% after five passages [9]. Of these four studies, three were assessing COP cells which were similar in expression to MSCs [7, 16, 46], with only one study examining COP cells with hematopoietic markers [9]. The functional effect of proliferation has yet to be explored in COP cells. For example, it is known that as the number of passages increase, the ability of bone marrow MSCs to differentiate into their terminal cell types is diminished as the cells become senescent [59]. It has also been demonstrated that the secretome of MSCs changes depending on the number of passages, with later passage cells having increased inflammatory activity, cancer cell genesis and migration [59]. It is also important to note that proliferation in vitro is tightly regulated, with stem cells multiplying in response to peripheral demand, and entering a quiescent state when the need has been met. This contrasts with a dedicated progenitor cell, which is able to multiply a certain number of times in order to regenerate local cells [60]. While it appears that the MSC-like COP cells exhibit behaviour more akin to stem cells, it is yet unclear how their hematopoietic counterparts behave. These behaviors must be explored in COP cells in order for them to safely and effectively become a viable option for medical use.

11. Cell homing

A key piece of information required for utilization of ex vivo cellular therapies is the mechanism of cell homing, as it allows for locally targeted therapies and reduces the risk of unintended effects in distant tissues. Both hematopoietic and MSC-like COP cells have been shown to be mobilized by a range of peripheral tissue states and factors including fracture [16, 20, 27, 61], thyroid hormones [43], substance P [17], and hypoxia [18]. The mechanisms by which they home to sites of tissue formation or repair still require much investigation, although it is clear that the CXCR4/SDF-1 plays a role in the process. A number of studies have suggested this by identifying the CXCR4 surface membrane receptor on these cells [15, 35], by selectively inhibiting this receptor [15, 47] or demonstrating expression of the gene coding for the SDF-1 protein [7]. Additionally, COP cells have been shown to be mobilized by several growth factors used medically to stimulate HSC migration in cancer patients [6]. That the CXCR4/SDF1 axis is also involved in the trafficking of the HSC and leukocyte populations [62] is yet another intriguing parallel between cells of the hematopoietic and mesenchymal lineagesworthy of investigation. Despite this evidence supporting the CXCR4/SDF1 axis as a homing mechanism for these cells, there are numerous other avenues requiring investigation. It is not yet known how COP cells respond to other common chemotactic factors related to tissue injury, growth or development. MSCs are known to migrate in response to a vast range of factors including platelet derived growth factor AB (PDGF-AB), insulin-like growth factor 1 (IGF-1), and macrophage derived chemokine (MDC) [63]. Additionally, the changes in cell homing with increasing number passages must be evaluated, as bone marrow MSCs are known to undergo a decrease in homing ability with increasing expansions [64]. Full evaluation of how these cell targets tissue viscosity is vital to progress them to medical therapeutics, as well as to provide insights into their physiological behaviors.

12. COP cells and bone marrow MSCs: outstanding questions

The similarity between COP cells and bone marrow MSCs is clear, and as such they provide the most likely comparison population on which to base future research. They appear to have similar morphologies and capacity for differentiation into mesenchymal tissues, however their patterns of surface marker expression seem to vary. There are, however, many properties of MSCs that are unexplored in COP cells. Perhaps most noteworthy of these are the effects and relationships with the human immune system. Bone marrow MSCs are known to have a strong immunomodulatory effects in humans, with suppressive effects on a range of immune cells, including T, B and natural killer cells and enhancement of macrophage activity via delay of apoptosis [65]. Additionally, it has been shown that MSCs possess the capability of immune cell evasion, allowing them to be administered to non-host organisms without risk of immune response and rejection [66]. This is mediated by a number of mechanisms, including lack of the major histocompatibility complex class II (MHCII) and related co-stimulatory molecules [67], local immunosuppressive mechanisms inhibiting immune cell activation via prostaglandin and interleukin suppression [68], and direct modulation of lymphocyte and dendritic cell function [69, 70].

These immune effects are vital in the application of MSC therapeutics, however no studies have evaluated these functions in COP cells. It is also known that MSCs have a strong paracrine function in humans, enabling them to have physiological or therapeutic effects in a diverse range of tissues and diseases [71]. For example, paracrine effects of MSCs have been credited for a large percentage of the cardioprotective benefit they confer in post-myocardial infarction patients [72]. It is also demonstrated that the secretory and functional activity of MSCs changes when the cells are placed under stress. For example, culture of MSCs in an oxidative environment results in increased secretion of antioxidant factors, providing evidence of their ability to respond to local tissue demands [73].

The secretome of COP cells is currently completely unexplored, but research in this area may identify mechanisms of therapeutic action thus far unidentified, as well as potentially broadening the scope of conditions in which they have use. It is currently unknown whether COP cells secrete any factors, or whether this behavior changes according to local conditions. The evaluation and use of MSC therapies has also been limited by the so called “pulmonary first pass effect” in which significant numbers of intravenously injected MSCs become trapped in the lungs before reaching the target tissue [74]. Local therapeutic application of COP cells has been investigated in an animal model with positive results on fracture healing [48], however the fates of the injected cells has not been assessed, so whether they are subject to this effect is unknown.

While the literature surrounding COP cells is still sparse, some preliminary conclusions can be drawn regarding their characterization. Firstly, it is apparent that there are at least two major populations present – a cell population that is very similar in most areas to a bone marrow MSC, and another discrete population with similar behavior, but expressing hematopoietic markers (Fig. 1). The MSC-like COP cells appear to be rarer [7, 46] and they may only circulate in response to peripheral factors associated with tissue damage and repair, such as substance P [17], hypoxia [18], or SDF-1 [15]. These cells express a very similar panel of surface markers to bone marrow MSCs, do not express hematopoietic markers such as CD45, 34, or 14, and are similar, if not identical, to MSCs in behavior and appearance. The second population has been more extensively researched but is also possibly well understood due to the divergent methods of identification and analysis. It is currently unclear as to whether this group is made up of one cell type or multiple, as researchers have not used consistent strategies for identification and isolation. It appears that the population expresses some combination of CD45, CD34, and CD14, as well as the markers of bone formation OCN, Col1, and AP. This pattern of expression is unique compared to other known circulating adherent cell types such as endothelial stem cells (CD34+/CD45−) [75], and macrophages (CD34−) [76] though is similar to that of fibrocytes. While the behavior of fibrocytes and COP cells is distinct, more research is required to identify the relationships and roles of these two cell types, particularly given the ability of fibrocytes to differentiate into osteoblasts in vitro [77].
Preliminary evidence on the COP cells of hematopoietic lineage suggests they behave similarly to MSCs, however, confirmation and exploration of their proliferative and differentiation capacity is required, alongside careful characterization of their marker expression. Hematopoietic COP cells have been associated with several states of physiological or pathological bone formation, such as puberty and fracture [10], osteoporosis [38–40], and frailty [31,32], indicating that these cells may be a diagnostic or therapeutic target for use in managing these conditions. It has frequently been identified that the isolation and extraction of COP cells is a difficult task due to their poorly understood expression of markers, and their low frequencies [23]. While MSC-like COP cells are consistently shown to be rare, and likely circulate only in response to peripheral injury or demand [8,16], hematopoietic COP cells have been shown to circulate in greater numbers [12] and at a steady state throughout the lifespan [11] perhaps providing a greater reservoir to draw upon for culture. There is also minimal evidence demonstrating that they can be sufficiently expanded and manipulated once cultured, providing further hurdles to their use in therapeutic settings, particularly in the case of hematopoietic COP cells. While much remains unknown, it is clear that cells of the blood, vascular and bone lineages are more physiologically connected than once thought, and further study must be done to explore the interplay between these tissues.

13. Conclusions

COP cells have profound and exciting implications for future use, particularly in the area of regenerative medicine and the treatment and assessment of musculoskeletal disease. An easily accessible reservoir of stem cells in adult patients would overcome many hurdles in the therapeutic or diagnostic application of these cells. Removal of blood carries significant advantages over bone marrow biopsy in risk and patient comfort, making stem cell therapies a more feasible treatment strategy. However, before they can be applied in medical settings, significantly further understanding of their biological nature, morphological, and functional properties is required. Future research must therefore, further examine the different cell populations identified in the literature, fully characterizing both, as well as determining their respective physiological roles, behaviors, and interactions. Once this is achieved, it will provide a foundation upon which future research on the role of COP cells in pathology can be based, providing consistency amongst researchers as to the precise identity of the populations being studied.

Author contributions

Jack Feehan performed the literature search, prepared the manuscript and figures. Gustavo Duque, Vasso Apostolopoulos and Kulmira Nurgali edited the review and supervised the preparation of the manuscript. Ahmed Al Saedi edited the review and assisted with the literature search.

Declaration of interests

Mr. Jack Feehan has nothing to disclose.
Dr. Kulmira Nurgali has nothing to disclose.
Dr. Vasso Apostolopoulos has nothing to disclose.
Dr. Ahmed Al Saedi has nothing to disclose.
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References

[1] Geddes A. The origin of the osteoblast and of the osteoclast. J Anat Physiol 1913;47 (Pt 2):159.
[2] Urist MR. Bone: formation by autoduction. Science 1965;150(3689):893–9.
[3] Tavassoli M, Crosby WH. Transplantation of marrow to extra-medullary sites. Science 1968;161(3836):54–6.
[4] Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9(5):641–50.
[5] Maximow AA. Cultures of blood leucocytes. From lymphocyte and monocyte to connective tissue. Gustav Fischer 1928:5 189-178.
[6] Fernandez M, Simon V, Herrera G, Cao C, Favero H, Minguell J. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. Bone Marrow Transplantation 1997;20(4):265.
[7] Zvaifler NJ, Marinova-Mutafchieva L, Adams G, et al. Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res Ther 2000;2(6):477.
[8] Kuznetsov SA, Mankani MH, Grontos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. J Cell Biol 2001;153(5):1133–40.
[9] Kowana M, Okazaki Y, Kodama H, et al. Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. J Leukoc Biol 2003;74(3):833–45.
[10] Eghbali-Fatourechi GZ, Modder UI, Charatcharoenwitthaya N, et al. Characterization of circulating osteoblast-lineage cells in humans. N Engl J Med 2005;352(19):1959–66.
[11] Gunawardene P, Pfeiffer SM, Singh L, et al. Age-related, percentage of circulating osteoprogenitor (COP) cells: the COP Study. Exp Gerontol 2017;96:68–72.
[12] Eghbali-Fatourechi GZ, Modder U, Charatcharoenwitthaya N, et al. Characterization of circulating osteoblast lineage cells in humans. Bone 2007;40(5):1570–7.
[13] Roban I, Barisic-Dujmovic T, Clark SH. Parabiosis model does not show presence of circulating osteoprogenitor cells. Genesis 2010;48(3):171–82.
[14] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(5):315–7.
[15] Otsuru S, Tamai K, Yamazaki T, Yoshikawa H, Kaneda Y. Circulating bone marrow-derived osteoblast-derivated osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4-stromal cell-derived factor-1 pathway. Stem Cells 2008;26(1):223–34.
[16] Alm JI, Koivu HM, Heino TJ, Hentunen TA, Laitinen S, Aro HT. Circulating plastic adherent mesenchymal stem cells in aged hip fracture patients. J Orthop Res 2010 12(6):1634–42.
[17] Hong HS, Lee J, Lee E, et al. A new role of substance P as an injury-inducible messenger for mobilization of CD34(+)-stromal-like cells. Nat Med 2009;15(4):425–35.
[18] Rochefort GY, Delorme B, Lopez A, et al. Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. Stem Cells 2006;24(10):2202–8.
[19] Otsuru S, Tamai K, Yamazaki T, Yoshikawa H, Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. Biochem Biophys Res Commun 2007;354(2):453–8.
[20] Kumauchi K, Vanaj C, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice. J Orthop Res 2008;26(2):165–75.
[21] Dominici M, Pichard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic stem cells: the COP Study. Exp Gerontol 2017;96:68–72.
[22] Otani S, Tamai K, Yoshikawa H, Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. Biochem Biophys Res Commun 2007;354(2):453–8.
[23] Kumauchi K, Vanaj C, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice. J Orthop Res 2008;26(2):165–75.
[24] Dominici M, Pichard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic stem cells: the COP Study. Exp Gerontol 2017;96:68–72.
[25] Otani S, Tamai K, Yoshikawa H, Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. Biochem Biophys Res Commun 2007;354(2):453–8.
[26] Kumauchi K, Vanaj C, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice. J Orthop Res 2008;26(2):165–75.
[27] Dominici M, Pichard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic stem cells: the COP Study. Exp Gerontol 2017;96:68–72.
[28] Otani S, Tamai K, Yoshikawa H, Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. Biochem Biophys Res Commun 2007;354(2):453–8.
[29] Kumauchi K, Vanaj C, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice. J Orthop Res 2008;26(2):165–75.
[30] Dominici M, Pichard C, Garlits JE, Hofmann TJ, Persons DA, Horwitz EM. Hematopoietic stem cells: the COP Study. Exp Gerontol 2017;96:68–72.
[31] Otani S, Tamai K, Yoshikawa H, Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. Biochem Biophys Res Commun 2007;354(2):453–8.
[32] Kumauchi K, Vanaj C, Drazba JA, Butler RS, Muschler GF. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice. J Orthop Res 2008;26(2):165–75.
[33] Egan KP, Duque G, Keenan MA, Pignolo RJ. Circulating osteogenic precursor cells in human postmenopausal osteoporosis. Arthritis Rheum 2009;60(11):3365–6.
[63] Eggenhofer E, Luk F, Dahlke MH, Hoogduijn MJ. The life and fate of mesenchymal stem cells. Front Immunol 2014;5:148.
[64] Sohni A, Verfaillie CM. Mesenchymal stem cells migration homing and tracking. Stem Cells Int 2013;2013:130763.
[65] Ghannam S, Bouffi C, Djojad F, Jorgensen C, Noel D. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res Ther 2010;1(1):2.
[66] Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm 2005;2(1):8.
[67] Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003;31(10):890–6.
[68] Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol 2014;32(3):252.
[69] Zhang W, Ge W, Li C, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev 2004;13(3):263–71.
[70] Krampera M, Glennie S, Dyson J, et al. Bone marrow mesenchymal stem cells inhibit the response of naïve and memory antigen-specific T cells to their cognate peptide. Blood 2003;101(9):3722–9.
[71] Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008;103(11):1204–19.
[72] Gnecchi M, He H, Noisieux N, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 2006;20(6):661–9.
[73] Lanza C, Morando S, Voci A, et al. Neuroprotective mesenchymal stem cells are endowed with a potent antioxidant effect in vivo. J Neurochem 2009;110(5):1674–84.
[74] Fischer UM, Harting MT, Jimenez F, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev 2009;18(5):683–92.
[75] Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. Circ Res 2004;95(4):343–53.
[76] Woltman AM, de Fijter JW, Kamerling SW, et al. Rapamycin induces apoptosis in monocyte-and CD34-derived dendritic cells but not in monocytes and macrophages. Blood 2001;98(1):174–80.
[77] Choi YH, Burdick MD, Strieter RM. Human circulating fibrocytes have the capacity to differentiate osteoblasts and chondrocytes. Int J Biochem Cell Biol 2010;42(5):662–71.
[78] Matsumoto T, Kawamoto A, Kuroda R, et al. Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing. Am J Pathol 2006;169(4):1440–57.
