# Biologics in orthopaedics
## concepts and controversies



### BACKGROUND

Biological approaches to treat orthopaedic injuries seek to improve clinical outcomes by promoting tissue regeneration and healing. These approaches can be used in isolation or as an augment to surgical interventions. They include, but are not limited to, preparations of growth factors, autologous blood products, and cells. Despite considerable research effort, the majority of biological approaches have not yet achieved a sufficient evidence base to warrant widespread clinical application, and inappropriate use is a growing problem. This article aims to outline concepts underlying some of the key biological approaches, namely growth factors, autologous blood products (including platelet-rich plasma or PRP), and mesenchymal stem/stromal cells (MSCs), highlighting current challenges that limit clinical translation and application.

#### *The race to the clinic has been at the expense of scientific understanding*

There is a good rationale for using biological strategies to accelerate tissue healing. Nature uses cells and growth factors as the building blocks for tissue repair and, as such, approaches that seek to emulate this process seem, on the face of it, intuitive. Unfortunately, the race to clinical use has often been at the expense of scientific understanding or supportive data. Promising preliminary results from laboratory studies,[1-3] modest regulatory barriers of certain autologous preparations, and public appetite for new treatments have all fuelled a rush to find new applications in which biological treatments may incur clinical benefit.[1,2,4] Patient popularity has been further driven by public celebrity endorsements from athletes including Tiger Woods[5] in golf and Peyton Manning[6] in American football. Many biological strategies are now being used to treat a wide range of clinical conditions in mainstream orthopaedic practice, despite a lack of robust clinical evidence supporting efficacy.[7] This is not only a problem in so-called 'pirate clinics' where some physicians are seizing an opportunity to make a fast buck, but also in larger teaching institutions. To put the problem into perspective, there are now over 400 complete or ongoing clinical trials evaluating the use of PRP and over 800 evaluating the use of MSCs in a range of clinical applications (see clinicaltrials.gov). Many of these trials have been designed and started with little knowledge of what preparations contain, and without comprehensive scientific understanding of the mechanisms by which it may produce benefit.[7] There is an evident danger here that potentially beneficial treatments will be dismissed as ineffective simply because scientifically questionable and suboptimized preparations are being rushed directly into a clinical setting. We will only truly know if biological therapies can be of therapeutic benefit if the scientific/clinical community accepts that shortcuts cannot be taken, and adopts a responsible approach to the use of biological therapies including the generation of both an evidence base to support their use and an understanding of the principles of use.

#### *First consider the injury microenvironment*

Biological approaches should always begin with an understanding of the underlying injury microenvironment. From the injury perspective, specific deficiencies in cells, cytokines, or the mechanical environment that contributes to pathology should be identified, and, from a regeneration perspective, opportunities to enhance particular components of the healing response can then be exploited. Once the biological targets for a specific scenario are identified, the ideal biological formulation should then be matched to the clinical setting. The effect of biological strategies relies on a complex interplay between the injury microenvironment and the biological preparations being delivered. Important injury factors contributing to variability include tissue type, and the mechanism and chronicity of injury. The therapeutic 'needs' of each injury will therefore be different. For example, ligaments are relatively acellular and avascular, while skeletal muscle is highly

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**Table I.** Biological effects of a selected number of growth factors with activity relevant to musculoskeletal tissue engineering

| Growth factor | Selected biological effects |
|---|---|
| BMP2 | MSC proliferation; promotes differentiation of MSCs to osteoblasts and chondrocytes |
| BMP4 | Promotes differentiation of MSCs to osteoblasts and chondrocytes |
| BMP6 | Promotes differentiation of MSCs to osteoblasts and chondrocytes |
| BMP7 | Promotes differentiation of MSCs to osteoblasts and chondrocytes |
| CTGF | Pro-angiogenic; promotes wound healing by increasing fibroblast proliferation |
| EGF | MSC proliferation; regulates bone and cartilage formation |
| FGF1 | Fibroblast proliferation; pro-angiogenic |
| FGF1 | Pro-angiogenic; regulates osteogenic, myogenic, and chondrogenic differentiation |
| HGF | Pro-angiogenic; antifibrotic; reduces local immune response |
| IGF1 | Increases MSC proliferation and differentiation to osteoblasts and chondrocytes |
| IL-1 | Pro-inflammatory |
| PDGF | Promotes MSC proliferation and differentiation to osteoblasts; pro-inflammatory |
| NELL1 | Induces osteoblastic differentiation of MSCs |
| SDF1α | Pro-inflammatory; increases homing of MSCs |
| TGFβ-1 | Fibroblast activation and proliferation; pro-angiogenic; MSC proliferation; chondrogenic and osteogenic differentiation |
| TGFβ-2 | MSC proliferation; chondrogenic and osteogenic differentiation |
| TGFβ-3 | MSC proliferation; chondrogenic and osteogenic differentiation |
| VEGF | Stimulates angiogenesis; attracts macrophages and granulocytes |
| Wnt3a | MSC proliferation and survival; regulates osteogenic and chondrogenic differentiation of MSCs |
| Wnt5a | Regulates osteogenic and chondrogenic differentiation of MSCs |

BMP, bone morphogenetic protein; MSCs, mesenchymal stem/stromal cells; CTGF, connective tissue growth factor; EGF, endothelial growth factor; FGF, basic fibroblasts growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL-1, interleukin 1; PDGF, platelet-derived growth factor; NELL1, NEL-like protein 1; SDF1α, stromal cell derived factor alpha; TGFβ, transforming growth factor beta; VEGF, vascular endothelial growth factor



cellular and generously supplied with blood vessels. Ligaments are largely static constraints, while skeletal muscle is designed to contract. The archetypal cell within ligament (fibroblast) is the cell least welcome in the long term following skeletal muscle injury, and the presence of fibroblast-produced tissue (fibrosis) is a principal predictor of re-injury. This highlights that injuries affecting different tissue types, and of different chronicity, may not be best served by the same formulations, and treatments must therefore be individualized.

## GROWTH FACTORS AND CYTOKINES

Growth factors are part of the normal response to injury, acting to promote tissue regeneration and healing. Most growth factors are pleiotropic, causing multiple biological effects in a variety of cell types. Although the term 'cytokine' is sometimes used interchangeably with 'growth factor', cytokines are a unique family of growth factors secreted primarily from leucocytes that stimulate both the humeral and immune responses, as well as activation of phagocytes. Growth factors bind with specific receptor molecules on the surface of target cells and the presence of these receptors define a cell's capacity to respond to the signals. A large number of growth factors have effects relevant to musculoskeletal regeneration and therefore represent potential therapeutic targets (Table 1), not just cytokines. It is imperative to note that not all factors will promote healthy regeneration in all tissues at all times – moreover, some may do more harm than good. For example, cues such as transforming growth factor beta (TGFβ)-1 that encourage the persistence of matrix-producing fibroblasts are undesirable in skeletal muscle,[8] yet this cytokine has been shown to be beneficial for healing of ligament.[9] TGFβ-1 also has angiogenic actions that may be of particular value in tissues where healing is delayed secondary to reduced vascularity.[10]

Growth factors can be delivered either individually or in synergistic combinations. They can be used to pre-condition cells prior to delivery or combined with cells at the time of delivery.[11] The number of commercially available purified cytokine products is low: platelet derived growth factor (PDGF) is the sole protein in three United States Food and Drug administration (FDA)-cleared products for patient use, while bone-morphogenetic protein (BMP)-2 is the sole protein in another FDA-approved product. BMP-2 has demonstrated beneficial effect on fracture healing in randomized controlled trials.[12,13] Other agents such as fibroblast

**Table II.** Fractions of PRP and their contents

| PRP fraction | Content or releasate |
|---|---|
| **Plasma** | |
| Proteins | Albumin, fibrinogen, globulins, complement, clotting factors |
| Electrolytes | Sodium, chloride, potassium, calcium |
| Hormones | IGF1, ACTH, HGH estrogens, progesterone, androgens |
| **Platelets** | |
| Alpha granules | Growth factors (over 300 including TGFβ1, IGF1, bFGF, PDGF, PDAF, PF4, EGF, VEGF, CTGF, HGF, SDF1α) |
| | Clotting factors: Factor V, vWF, fibrinogen |
| Dense granules | ADP, calcium, serotonin |
| Lysosomes | Lysosomal enzymes |
| **Leucocytes** | |
| Neutrophils | Cytokines (e.g. IL-4, IL-8, TNFα), proteases, bactericidal molecules, lysozyme |
| Eosinophils | Growth factors (including VEGF, PDGF, TGFα, TGFβ, interleukins), plasminogen |
| Basophils | Histamine, proteases, heparin, leukotrienes |
| Monocytes | Growth factors (including TGFβ, IL-1, FGF, PDGF) |
| **Red blood cells** | Hemoglobin, nitric oxide, ATP, S-nitrosothiols, and free radicals |

IGF1, insulin-like growth factor 1; ACTH, adrenocorticotropic hormone; HGH, human growth hormone; TGFβ, transforming growth factor beta; bFGF, basic fibroblastic growth factor; PDGF, platelet derived growth factor; PDAF, platelet-derived angiogenesis factor; PF4, platelet factor 4; EGF, endothelial growth factor; VEGF, vascular endothelial growth factor; CTGF, connective tissue growth factor; HGF, hepatocyte growth factor; SDF1α, stromal cell derived factor; vWF, von Willebrand factor; ADP, adenosine diphosphate; IL, interleukin; TNFα, tumour necrosis factor alpha; TGFα, transforming growth factor alpha; ATP, adenosine triphosphate

**Table III.** Differences in platelet count, white cell count and neutrophil count of selected commercial platelet-rich plasma (PRP) systems

| PRP preparation | Platelets (fold change) | Leucocytes (fold change) | Neutrophils (fold change) |
|---|---|---|---|
| GPS (Zimmer Biomet)* | ×8.21 | ×4.87 | ×2.04 |
| Magellan autologous platelet system (Medtronic perfusion systems, now Arteriocyte)† | ×9.29 | ×5.4 | ×1.99 |
| Arthrex ACP Double Syringe (Arthrex)† | ×2.40 | ×0.54 | ×0.06 |
| Genesis (EmCyte)† | ×5.53 | ×4.30 | ×2.86 |
| Smart prep 2 system (Harvest autologous hemobiologics)† | ×7.25 | ×4.3 | ×1.57 |
| Regen (Stryker)† | ×1.70 | ×0.95 | N/A |

*Data provided by Zimmer Biomet
†Data provided by Arthrex
N/A, not available



growth factor (FGF) have shown promise in clinical studies,[14] while others, including TGF-β, have been evaluated in small animals.[15,16]

## AUTOLOGOUS BLOOD PRODUCTS/ PLATELET-RICH PLASMA

### *Not all PRP preparations are the same*

The term PRP can be, and is, used to describe any autologous blood preparation with a platelet concentration higher than baseline. This is normally achieved through differential centrifugation, with collection of the PRP from above the white blood cell layer. A second spin is frequently performed and has the effect, in those preparations in which it is used, to further concentrate the platelets. Platelets are recognized to release a host of growth factors and cytokines that are able to induce pro-regenerative attributes in laboratory studies, including promoting proliferation and recruitment of progenitor cells, modulation of inflammatory responses, and stimulation of new blood vessel formation.[17] In addition to platelets, these preparations can contain leucocytes, red blood cells, and over 300 different growth factors and cytokines in variable numbers (Table II).[18,19] The heterogeneous nature of PRP preparations is widely underappreciated. There are over 20 commercially available systems, with considerable differences in the PRP composition even among the most widely used brands (Table III).[17] In addition to cell composition, variability is introduced by different methods of 'activating' platelets prior to delivery to encourage release of their contained growth factors. PRP composition also varies considerably between individuals and even the same individuals can have variable amounts of growth factors in the PRP produced on a day-to-day basis, and this can even be affected by recent exercise or meals.[20] Since the introduction of PRP, a number of other autologous blood products have emerged, including platelet poor plasma (PPP) and other autologous protein solutions. Regardless of the method of preparation, all autologous blood products should be considered highly variable and intrinsically different for each individual.

### *Matching conditions with the 'ideal' formulation*

Autologous blood products should be selected based on the formulation most suited to the pathology being treated. PRP preparations can be potentially and theoretically manipulated in this way by enriching or depleting certain

growth factors or cell types. In osteoarthritis, a meta-analysis of six randomized controlled trials (evidence level 1) and three prospective comparative studies (evidence level 2) with a total of 1055 patients indicates that leucocyte-poor PRP, but not leucocyte-rich PRP, can result in symptomatic improvement over treatment with hyaluronic acid or placebo control.[21] This may relate to the significant increase in anti-inflammatory mediators in leucocyte-poor PRP (interleukin (IL)-1ra, IL-4, IL-10), and the significant increase in inflammatory mediators seen in leucocyte-rich PRP (IL-1β, IL-6, interferon (IFN)-γ, tumour necrosis factor (TNF)-α).[22] Conversely, multiple randomized control trials and meta-analyses have found that leucocyte-rich PRP is an efficacious treatment for lateral epicondylitis (tennis elbow).[23-25] At present, there is little evidence to suggest an optimal formulation for acute ligament or muscle injury. Interestingly, laboratory studies suggest that autologous blood preparations depleted of platelets (platelet-poor plasma) may have therapeutic benefit in the setting of muscle injury.[26]

### *Current studies fail to characterize PRP and under-report methods*

A major challenge in interpreting the currently published studies is the inadequate reporting of experimental detail or compositions of PRP formulations used.[27] This precludes proper interpretation of results, prevents comparison between studies, and does not permit others to replicate experimental or clinical conditions to verify results. A recent systematic review concluded that only 16% of published clinical studies provided any quantitative metrics of the composition of PRP delivered.[27] To address inadequacies in reporting, Murray et al[28] facilitated an international expert consensus to establish minimum reporting standards for studies evaluating PRP. These are now approved by the EQUATOR network of journals (including *The BMJ* and *The Lancet*) and a growing number of orthopaedic journals.[29]

## CELL THERAPIES

### *Autologous chondrocyte implantation*

Autologous chondrocyte implantation (ACI) is currently the only cell therapy recommended by the National Institute for Clinical Excellence (NICE) to treat musculoskeletal defects.[30] ACI involves the harvesting of chondrocytes from a healthy non-weight-bearing portion of the knee followed by implantation of culture-expanded autologous chondrocytes under a periosteal flap (first-generation ACI) or a bioabsorbable collagen membrane (second-generation ACI), or onto a porcine membrane (MACI).[31] MACI has now received approval from the FDA and is widely available in Europe. MACI was recently recommended by NICE as an option for treating large (> 2 $cm^2$) symptomatic articular cartilage defects of the knee. Confirmation of cell viability, identity, and potency are critical quality assessments of seeded cells, and it is, of course, important that a characterized strain of chondrocytes, selected for their improved ability to generate hyaline cartilage, is used. The recently published five-year outcomes of the SUMMIT (Superiority of MACI Implant Versus Microfracture Treatment) randomized controlled clinical trial demonstrated that symptomatic cartilage knee defects 3 $cm^2$ or larger can be treated safely with MACI resulting in clinically and statistically improved outcomes at five years compared with microfracture alone.[32]

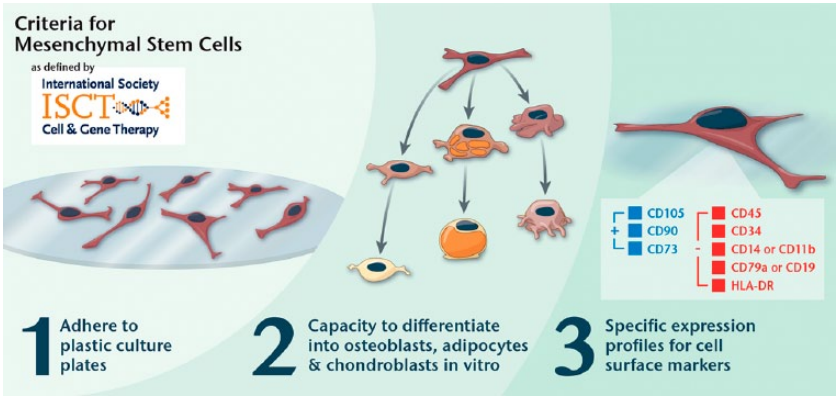


**Fig. 1** International Society for Cell Therapy (ISCT) defining criteria for mesenchymal stem/stromal cells (MSCs).

### *Mesenchymal stem/stromal cells (MSCs)*

Mesenchymal stem (or stromal) cells (MSCs) are multipotent cells with the capacity to multiply ('self-renew') and differentiate into osteocytes, chondrocytes, and other mesodermal cell types. They also release pro-regenerative growth factors and cytokines with immune modulating effects. Friedenstein et al[33] first described cells from bone marrow that could replicate and become bone in the 1960s. Observations that these cells could also form fat, cartilage, and muscle led to the term 'mesenchymal stem cells' being introduced in 1991.[34] The International Society for Cellular Therapy (ISCT) have since proposed minimum defining criteria that included a propensity to adhere to laboratory culture plastic and the capacity to differentiate into bone, cartilage, and fat and to express certain cell surface markers (Fig. 1).[35]

It has since been reported that MSCs reside around blood vessels in almost every tissue, including subcutaneous fat, and adipose tissue isolated from the knee fat pad.[36] While all these populations fulfil ISCT criteria, they have distinctive features reflecting their tissue of origin. For example, MSCs from bone marrow exposed to TGF-β form chondrocytes and make cartilage extracellular matrix, while fast-derived MSCs require both TGF-β and bone BMP-6 to make cartilage.[37] A timeline of the discovery of MSCs and their application in clinical studies is outlined in Figure 2. Interestingly, MSCs were being used in clinical trials long before the native identity of MSCs within blood vessel walls was known.

### *MSC preparations*

Irrespective of their tissue of origin, MSC preparations can be broadly categorized on how separated they are from other cell types (homogeneity) and whether they have been exposed to laboratory culture. Although bone marrow represents the most widely used source (Fig. 3), these concepts can be equally applied to MSCs isolated from different tissues including adipose tissue which offers an attractive alternative because it is plentiful and largely dispensable.[38] The yield of MSCs harvested from adipose tissues is reported to be higher than from bone marrow.[39-40] The infrapatellar fat pad is also recognized as a source for adipose-derived stem cells. When considering commercial systems, clinicians should consider where in this framework any product fits as aspects of preparation are known to reflect regenerative characteristics.

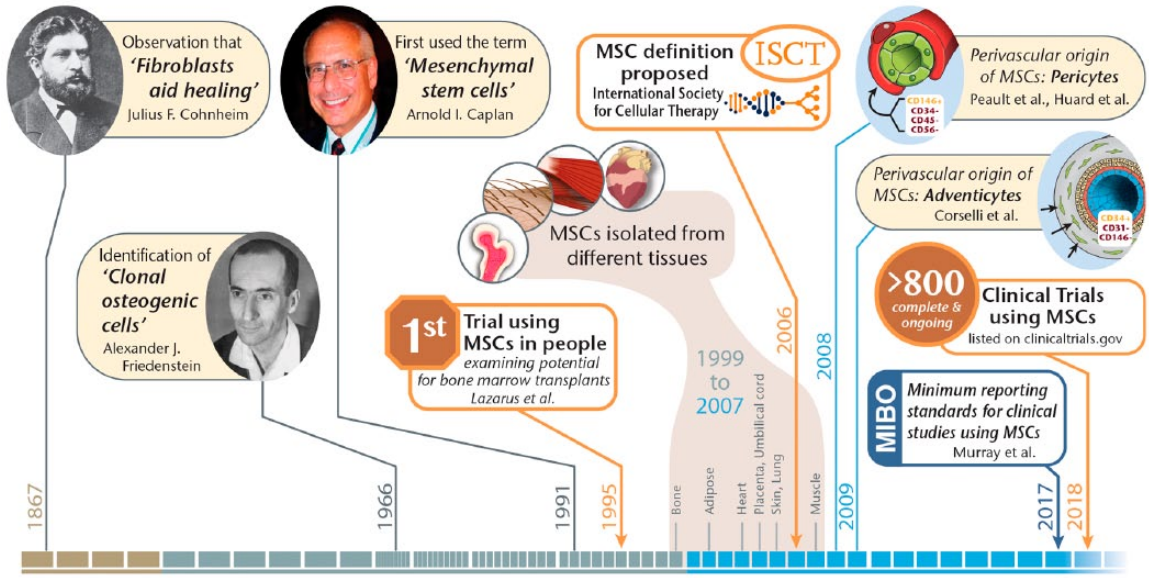


**Fig. 2** A timeline of mesenchymal stem/stromal cells. Note that clinical trials using these cells have been running long before it was known where these cells resided within native tissues.

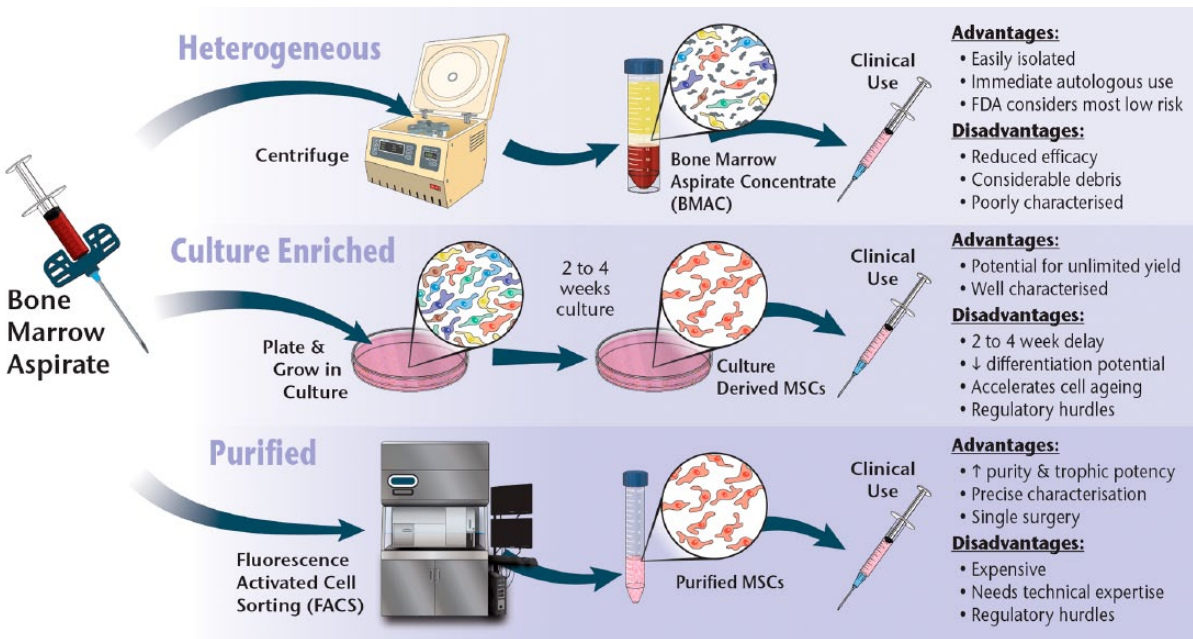


**Fig. 3** Mesenchymal stem/stromal cell (MSC) preparations can be broadly categorized based on whether the preparations are heterogenous (contained MSCs remain mixed with other cell types), the preparations have been enriched for MSCs through laboratory culture, or the MSCs have been purified through cell sorting techniques. Similar categories of cells can be prepared from multiple different tissue types including bone marrow (shown here), adipose tissue and periosteum.

1) Minimally manipulated 'heterogenous' preparations (e.g. bone marrow aspirate concentrate, or BMAC). Although MSCs make up a small minority of cells within bone marrow (less than 1/10000 cells) or fat, preparations that have undergone 'minimal manipulation' processing techniques such as centrifugation or mechanical disruption only (e.g. concentrated bone marrow aspirate) have been used directly with the aim of harnessing the potential of contained progenitors.[41] These strategies are widely available and now represent the majority of clinical studies evaluating MSCs in the clinical literature.[42] They are attractive to clinicians and patients because unlike culture preparations, harvest, and delivery can occur under the same procedure. Minimally manipulated preparations can also be utilized from fat.[43] However, available studies demonstrate that these heterogeneous populations, including inflammatory cells, hematopoietic cells, endothelial cells, and

nonviable cells, may result in poor and inconsistent tissue formation compared with enriched MSC preparations, as the contaminating cells may have inhibitory effects on the MSCs.[44-47]

2) Culture-derived MSCs. A period of laboratory culture can be used to both enrich for MSCs, and increase their number, as MSCs tend to outgrow other cells in these conditions.[48] A number of clinical studies have demonstrated that MSC implantation can be used effectively to repair and regenerate bone[49] and cartilage.[50] Promising results have been demonstrated in the treatment of chondral[51,52] and osteochondral defects,[53,54] osteonecrosis of the femoral head[55] and to aid spinal fusion[56] to name a few. However, controversy remains as to whether long-term culture is associated with genetic instability and a reduction in therapeutic potency.[57]
3) MSCs purified through affinity-based separation. Using fluorescent activated cell sorting (FACS) or magnetic activated cell sorting (MACS), MSCs can now be rapidly separated from other cells in suspension using antibodies that target known cell surface markers of MSCs. This process does not require extended periods of laboratory culture and harvest and delivery of pure populations of MSCs can theoretically be performed under the same anaesthetic. Using this strategy, 200 ml of lipoaspirate can theoretically yield 31 million MSCs, sufficient for healing a mid-diaphyseal femoral defect measuring 2 cm in diameter.[38] In addition, for the need for robust clinical trials, this strategy is currently limited by a lack of formal accreditation from the relevant regulatory bodies and high costs.

### *Ambiguous nomenclature and misrepresentation of biologics*

There is growing concern about the misrepresentation of uncharacterized, minimally manipulated cell preparations as 'stem cells' leading to widespread clinical use of unproven biological therapies. Misinformation is frequently delivered by direct-to-consumer marketing. From 2014 to 2016, approximately 90 to 100 new stem cell business websites appeared per year, many of which offered services without any orthopaedic surgeon involvement. There is concern that the current environment may erode the trust in biological therapies and the investment and grant funding required to bring legitimate cell therapies to patients.[58] There is a clear need to define terminology to clearly distinguish uncharacterized cell products from rigorously characterized, culture expanded, and purified 'stem cell' and progenitor cell populations.

## SUMMARY

The pro-regenerative characteristics of growth factors and regenerative cell types hold great promise for musculoskeletal tissue engineering. Early favourable results from *in vitro* work are now being supported by level 1 studies in a limited number of clinical settings. Irrespective of the setting, clinical use should be based on a comprehensive understanding of injury, environment, and the biological therapy being delivered.

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