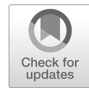

12 Advancing Quantitative Stem Cell Dosing for Veterinary Stem Cell Medicine

Samuel R. Boutin and James L. Sherley

Abstract

Because of the important role of preclinical animal studies in the development of innovative medicines for human patients, many stem cell therapies have been evaluated in animals. However, the last decade has seen the beginning of a shift from stem cell treatments in animals only for the benefit of human patients to including new therapeutic development of tissue stem cells primarily for animal care. Not surprisingly, given their historical dependency, the new field of veterinary stem cell medicine faces many of the same challenges as human stem cell medicine. In this chapter, a shared major deficiency, the lack of stem cell-specific dosing, is considered from the perspective that implementing dosing would accelerate progress in veterinary stem cell medicine and human stem cell medicine as well, as a follow-on. Since the vast majority of present-day veterinary stem cell treatments utilize preparations of mesenchymal stem cells (MSCs), the well-recognized uncertainties about this treatment source are discussed. The challenges of quantifying the stem cell-specific dose of MSC preparations exemplify the general problem of determining the stem cell dose of all stem cell treatments. Particular consideration is given to previous veterinary MSC treatment studies that include measures that might relate to stem cell dosage. Kinetic stem cell counting, a first potential solution to the tissue stem cell dosing problem, is described, and the potential benefits of its future use are discussed. Adoption of kinetic stem cell counting into the general practice of veterinary stem cell medicine is presented as the key that can unlock the full potential of stem cells in veterinary medical practice and perhaps human stem cell medical practice as well.

S. R. Boutin · J. L. Sherley (✉)
Asymmetrex, LLC, Boston, MA, USA
e-mail: jsherley@asymmetrex.com

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R. K. Choudhary, S. Choudhary (eds.), *Stem Cells in Veterinary Science*,
https://doi.org/10.1007/978-981-16-3464-2_12

Keywords

Tissue stem cells · Stem cell medicine · Stem cell dose · Asymmetric self-renewal · Kinetic stem cell count · Hematopoietic stem cell · Mesenchymal stem cell

12.1 Introduction

The existence and role of stem cells in mature vertebrate tissues have been known for more than a half-century. From the very beginning of their discovery, the potential of tissue stem cells for medical applications has been recognized and pursued. In fact, the discovery of the first tissue stem cells and the demonstration of their function in mature tissue cell renewal, which occurred in experimental animal models, were essentially in the format of future human stem cell replacement therapies (Till and McCulloch 1961). Those earliest origins led to the present only successful stem cell therapy in routine clinical practice, hematopoietic stem cell transplantation (HSCT). After many decades of research and technological development, additional effective stem cell therapies have been slow to emerge despite significant past and continuing investments of research and development resources.

Like its origins, much of the progress made in stem cell medicine can be attributed to research and development in animal models for stem cell therapy. Earlier investigations with animals were targeted primarily to the development of new approaches and applications for human stem cell medicine and not veterinary medicine per se. However, in the recent decade, stem cell therapeutics, first modeled in preclinical animal studies to gain approval for subsequent human clinical trials, have begun to be evaluated as primary treatments for animal patients in veterinary stem cell medicine (Gugjoo et al. 2018).

Because of the essential role of stem cells in the cellular tissue homeostasis of vertebrates, the same challenges that slow progress in the achievement of effective human stem cell therapies beyond HSCT are also faced in the development of effective veterinary stem cell medicine. In this chapter, an important but often unappreciated or understated challenge that greatly limits progress in both human and veterinary stem cell medicine is presented and discussed with the perspective that overcoming it would accelerate progress in veterinary stem cell medicine. Remarkably, the challenge at issue is a long-standing deficiency in stem cell medicine of a fundamental principle of medicine. For both animal patients and human patients, stem cell therapies are administered without knowing the stem cell dosage of the treatments (Sherley 2018a; Dutton et al. 2020).

In this chapter, the authors discuss the need for and benefits of advancing veterinary stem cell medicine to being dosage-based like traditional veterinary pharmaceutical medicine. The validation of the need for dosage-based stem cell medicine accrues readily from consideration of the fundamental medical concept that knowing the dosage of medicines is essential for the highest quality medical treatment. In modern medicine, the importance of the utilization of medication

dosage for effective development of new medicines and for the safe and efficacious administration of approved medicines is a self-evidentiary principle. Yet, presently, in both human medicine and veterinary medicine, stem cell clinical trials and even approved stem cell therapies like HSCT are performed without knowing the dosage of the stem cells in the treatments. In many human stem cell therapy clinical trials using expanded cell treatments, stem cells are presumed to be present, but they could be altogether absent (Paré and Sherley 2006; Taghizadeh and Sherley 2009; Sherley 2014).

Until very recently, the dosage limitation of tissue stem cell medicine was, for the most part, unavoidable. No technologies were available for accurately determining the dose of tissue stem cells that are characteristically a small fraction of the cells in treatment preparations (Sherley 2018a; Dutton et al. 2020). In this chapter, the authors describe a recently developed method for specific and accurate counting of therapeutic tissue stem cells. Called "kinetic stem cell counting," this new method for counting therapeutic tissue stem cells has the potential to open a first path to the implementation of quantitative dosing in veterinary stem cell medicine. The predicted benefits from such innovation in veterinary stem cell medical practice are discussed herein, with respect to their potential to compel similar modernization of human stem cell medical practice.

12.2 Therapeutic Tissue Stem Cells in Animal Tissues

Tissue-specific stem cells (TSCs) have a well-defined role in animal tissues for continuously renewing expiring mature differentiated tissue cells (Sherley 2005, 2006, 2013). Such tissue maintenance stem cells also have functions in repairing and restoring injured or diseased tissues. These abilities of tissue stem cells account for the effectiveness of HSCT, which reconstitutes the diseased or destroyed production of mature hematopoietic tissue cells.

Another important class of tissue stem cell does not appear to be tissue-specific in the usual sense. These mesenchymal stem cells (MSCs) are found in the interstitial and perivascular spaces of many different tissues. Unlike TSCs, which, although unipotent or multipotent, produce mature cells whose phenotypes are limited to the differentiated lineages of their specific tissue or residence, MSCs exhibit multitissue multipotency. In cell culture, their isolated preparations can produce differentiated cells with adipogenic, osteogenic, or chondrogenic phenotypic properties (Pittenger et al. 2019).

Both TSCs and MSCs have been shown to undergo asymmetric self-renewal division (Fig. 12.1; Dutton et al. 2020). Asymmetric self-renewal is gnomonic for vertebrate tissue stem cells (Sherley 2005, 2013). The unique and defining property of TSCs and MSCs is their ability to divide continuously with the production of one sister cell that retains all the stem cell properties of the parental stem cell and another sister that is committed to producing maturing, differentiating, and expiring, lineage-committed cells.

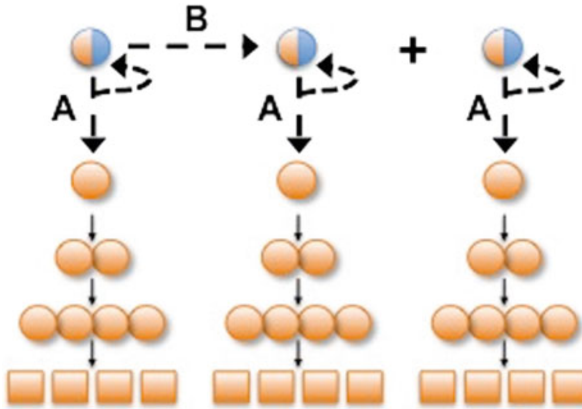

Fig. 12.1 Tissue stem cell asymmetric self-renewal division. (**A**) To maintain cellular tissue homeostasis, tissue stem cells (bivalent circles) undergo asymmetric self-renewal divisions. In the net, these divisions maintain the tissue fraction and stemness properties of stem cells while simultaneously producing committed progenitor cells (uniform circles). Committed progenitor cells continue division to produce mature differentiated functional cells (uniform squares). Mature cells have a finite lifetime in tissues before their loss to tissue wear or cell death. (**B**) In a regulated fashion (e.g., in response to tissue loss by injury), stem cells may undergo symmetric self-renewal divisions to produce new asymmetrically self-renewing tissue units

TSCs and MSCs can divide symmetrically as well, in which case two stem cells are produced. However, asymmetric self-renewal is the default state for tissue stem cells, and they are inherently resistant to frequent symmetric division (Sherley 2013). These properties are consistent with their established role in tissue cell homeostasis. They maintain a relatively undifferentiated state while continuously generating cells committed to tissue-specific differentiation. As mature cells lose function and undergo apoptosis, the asymmetric self-renewal of tissue stem cells maintains a balancing supply of new differentiating cells. With tissue injury, stem cells have the potential to symmetrically self-renew and establish new tissue units for tissue repair (Fig. 12.1B).

Although most TSCs appear to have limited mobility, HSCs and MSCs are naturally mobile stem cells. A significant body of literature describes the property of these cells to migrate preferentially to sites of tissue damage (Kavanagh and Kalia 2011; Nitzsche et al. 2017; Szydlak 2019; Liesveld et al. 2020). Both have been implicated for secreting cytokines and growth factors that induce processes that promote the repair of injured tissues (Gnecchi et al. 2016; Liesveld et al. 2020). For this reason, both HSCs and MSCs, and in particular MSCs, have become major treatment foci for human stem cell clinical trials (Li et al. 2014) based on their potential paracrine functions (Gnecchi et al. 2016; Liesveld et al. 2020). They are also favorable for clinical investigation because of the availability of effective methods for their isolation in higher yield than immobile TSC types. Essentially all veterinary stem cell clinical trials focus on evaluating the potential therapeutic paracrine effects of MSCs.

12.3 Therapeutic M[S]Cs in Veterinary Medicine

Veterinary medicine faces the same challenges as human medicine when it comes to identifying and quantifying tissue stem cells of therapeutic interest. The recent controversy around the nature of human MSCs extends to veterinary medicine as well (Gomez-Salazar et al. 2020). Stem cells are often defined by an ensemble of features that include both physical features and functional properties. For example, MSCs are defined as in Table 12.1.

Though accurately descriptive, these properties are quite inadequate for quantifying the effective stem cell dose of an MSC treatment. Unstated in the criteria as presented is the crucial shortcoming that none of the listed biomarkers identify MS(tem)Cs *specifically*. They also identify and co-quantify committed progenitor cells that are produced by the division of stem cells. Committed progenitor cells, though possessing many tissue precursor cell properties, do not have the unique long-term tissue cell renewal properties of tissue stem cells. Whether they share observed paracrine tissue reparative functions is unknown. For that matter, it could be that the committed progenitor cells are the major sources of tissue repair factors. This distinction could be an important consideration for sound evaluations of the effectiveness of MSC treatments. It may also better inform current ideas on how to explain the otherwise paradoxical long-term effects of MSCs because the cells appear to have characteristically short lifetimes in tissues after transplantation (Gnecchi et al. 2016).

Recent increased focus on the usually understated uncertainty about the specific identities of the cellular constituents of MSC preparations has inspired calls to change the name of these tissue cell preparations to something more representative of their uncertain cellularity. Suggestions like MS(tromal)Cs and medicinal signaling cells have been suggested (Caplan 2017; Gomez-Salazar et al. 2020). Although these choices avoid giving the erroneous impression of homogenous stem cell populations, they somewhat obscure the contribution of what many believe to be the key therapeutic factors, the stem cells in the treatment preparations. Hereafter, in this chapter, these cell populations will be referred to as *M[S]Cs* as a reminder of this important uncertainty about their cellular constituents and their therapeutic potential.

Table 12.1 Properties used to define MSCs[a]

• "Be plastic adherent
• Express the cell surface antigens CD105, CD90, and CD73
• Not express the cell surface antigens CD45, CD19, CD14, CD11b, CD34, CD79a, and HLA-DR
• Have the capacity to differentiate into osteoblasts, chondrocytes, and adipocytes"

[a]Source: From Gomez-Salazar et al. (2020)

12.4 Previous Attention to Indicators of Stem Cell Dose in Veterinary M[S]C Treatments

Many animals have been treated with human stem cells as models for developing human stem cell therapies (Pittenger et al. 2019; Gomez-Salazar et al. 2020). This is particularly true for the clinical development of human M[S]Cs. Herein, the purpose is to address stem cell therapies with species homologous animal stem cells administered in veterinary medicine to improve the healing and health of animals, as human pets, sport animals, and agricultural animals. In particular, studies are highlighted that considered how available measures of tissue stem cell dosage relate to treatment outcomes.

The vast majority of stem cell therapies in development for veterinary medicine use M[S]Cs derived primarily from allogeneic sources. Examples include species-specific M[S]C therapy for inflammatory conditions in pet cats (Quimby and Borjesson 2018; Arzi et al. 2020), pet dogs (Gallant.com 2019–2020), and agricultural animals like milk goats (Costa et al. 2019); diverse disorders (Barberini et al. 2018; Saldinger et al. 2020) and injuries in horses (Delco et al. 2020); and a diverse range of treatments for companion animals, including wound healing, tissue restoration, and inflammatory disorders (Kang and Park 2020). Though mostly species-specific M[S]Cs have been used for companion animal therapies, human-derived M[S]C treatments have also been evaluated (Kang and Park 2020). Human-derived M[S]Cs have been investigated for the treatment of disorders in horses as well (Barberini et al. 2018).

As for human stem cell medicine, veterinary medicine stem cell clinical trials must address the need for evaluating the stability of stem cell treatments during storage and transport. Similarly, veterinary studies are also challenged by the lack of effective and convenient tests to monitor stem cell-specific stability, viability, and function (Arzi et al. 2020).

There has been interest in defining metrics for veterinary stem cell treatments that can be used to identify more effective choices for cell therapies. Human M[S]C studies in animals and humans have established that the definable efficacy of M[S]C preparations has a high degree of variability that has been attributed to many obvious clinical factors and features like tissue source, isolation procedure, age, gender, expansion culture conditions, storage procedures, etc. (Gomez-Salazar et al. 2020). The importance of standardizing and optimizing the cell dose of stem cell treatments has also been discussed (Kang and Park 2020). However, despite the understanding that M[S]C preparations have heterogeneous cellularity (Costa et al. 2019; Pittenger et al. 2019), variation in stem cell-specific fraction has gone unevaluated as a possible cause of the variability or as an important factor for optimization and standardization.

In veterinary investigations, Zhan et al. (2019) evaluated five different sources of canine M[S]Cs derived from adipose tissue, bone marrow, umbilical cord, amnion membrane, and placenta. Although all sources had similar expression of the surface biomarker CD44, they differed in their cell proliferation rates, transcriptome, and rate of multipotent differentiation. In earlier studies, Russell et al. (2016) reported a

similar observation to that of Zhan et al. (2019) that MSCs derived from canine adipose tissue had a significantly higher proliferative rate than those derived from canine bone marrow. In dogs, differences in the proliferative rates of M[S]Cs derived from young and old animals show associated differences in immunomodulatory activity and osteogenic gene expression (Taguchi et al. 2019). Studies of equine M[S]Cs showed that those derived from equine umbilical cord blood produced mechanically superior cartilage tissue in vitro compared to those derived from equine bone marrow. However, a possible relationship to differences in the stem cell fraction was not considered (White et al. 2018).

There is also evidence that properties of veterinary M[S]Cs that suggest differences in stem cell fraction are correlated with differences in the multilineage differentiation capacity. Compared to canine adipose tissue M[S]Cs derived in serum-supplemented medium, those derived in the serum-free medium had a shorter lag phase for growth, a higher colony-forming efficiency, and an accelerated population doubling time. These properties are expected for cultures with a higher stem cell fraction (Devireddy et al. 2019). The serum-free M[S]Cs also maintained multipotency to higher culture passages (Liu et al. 2018; Devireddy et al. 2019). Recent studies in which human tissue stem cells were counted specifically have now established that reductions in the proliferative rate of primary tissue cell cultures are indicative of decreases in stem cell fraction (Dutton et al. 2020). Therefore, previous differences in veterinary M[S]C functional capabilities that were associated with differences in proliferative culture rate may, in fact, reflect differences in stem cell dose.

12.5 The Tissue Stem Cell Counting Problem

Although the tissue stem cell counting problem has existed since the beginning of stem cell biology history, it is either not recognized or poorly understood by many who work in stem cell science and stem cell medicine, including veterinary stem cell medicine (Sherley 2018b; Dutton et al. 2020). Until very recently, there has been no method available to count vertebrate tissue stem cells *specifically*, meaning without also counting committed progenitor cells, which invariably occur in tissue stem cell compartments and their isolated preparations at a much higher fraction than the stem cells.

Two very common misconceptions account for the state of general confusion on capabilities for determining specific tissue stem cell number and, corresponding, tissue stem cell-specific dose. The first is the misbelief that isolated tissue stem cell populations are composed of high fractions of nearly homogeneous stem cells. Such a constituency never exists. As tissue stem cells exist as rare fractions in intact tissues, they continue to exist as a rare cell fraction in isolated tissue cell preparations used for research and stem cell therapies. Applications that seek to expand stem tissue stem cells in culture further aggravate this problem. Because tissue stem cells continue to divide with asymmetric self-renewal division in culture, their fraction has been shown to decrease dramatically with cell culture as more committed

progenitor cells are produced and proliferate (Paré and Sherley 2006; Sherley 2014; Dutton et al. 2020).

The second pervasive misconception is the belief that stem cells can be counted specifically by flow cytometry. This error in understanding is partly due to flow cytometry's widely known sensitivity for identifying and quantifying rarified cell subpopulations. The missing information that results in the error is a failure to appreciate that no stem cell-specific biomarkers have been identified to date. Flow cytometry requires cell type-specific biomarkers for specific cell detection and quantification. All current tissue stem cell biomarkers (e.g., CD34, CD133, CD90) are misnamed. The targeted molecules are expressed on tissue stem cells, but they are also expressed on more abundant committed progenitor cells. The presence of high numbers of committed progenitor cells in essentially all tissue stem cell preparations precludes any chance of flow cytometry to be used to determine specific stem cell number or stem cell-specific dose.

The extent of entrenchment of these misconceptions is evident from a review of data obtained from a recent online survey conducted by the authors (https://asymmetrex.com/stem-cell-counting-study/). The survey was conducted over approximately a 1-year period and collected answers to a series of questions designed to probe respondents' level of knowledge about the current state of tissue stem cell counting technologies. To date, 116 respondents completed the ongoing survey and self-identified in the following occupational categories: undergraduate student (19%), graduate student (12%), physician (9.5%), CEO (8.6%), postdoctoral associate (6.9%), corporate research technical staff (6.9%), lab head (6.0%), project manager (4.3%), investor (3.5%), academic research technical staff (2.6%), CSO (2.6%), and other (18%). "Other" included respondents who self-reported in a variety of academic, administrative, business, and industrial professions.

Of these respondents, about 40% (45) answered that "*Homogenous tissue stem cells are the cellular constituents of isolated or expanded tissue stem cell preparations that are currently marketed and used for FDA-authorized stem cell clinical trials, private stem cell clinic treatments, or research*;" and about 56% (66) answered that "*Flow cytometry can be used to count tissue stem cells specifically*." Although respondents identifying as physicians were only 9.5% (11) of the total, their responses are suggestive of a high degree of misinformation about the stem cell dosing problem in stem cell medicine. Seven (64%) of the 11 responding physicians stated that stem cell treatments were *homogeneous* stem cell preparations, and 8 (73%) stated that stem cells could be counted *specifically* with flow cytometry.

If these early estimates are faithful representations of the current state of academic, medical, and industry knowledge about the cellular make-up of tissue stem cell treatments and the quantification of their dosage, then the ideas developed in this chapter are both needed and timely for inspiring a crucial awareness required for greater progress in veterinary stem cell medicine.

12.6 A Solution for the Tissue Stem Cell Counting Problem

Though confusion does exist regarding the state of technology for counting therapeutic tissue stem cells and determining their dosage, in surprising contrast, the importance of these metrics in stem cell science and stem cell medicine is generally well appreciated. In the same online survey described earlier, 82% (95 of 116 respondents) selected "*Without knowing the stem cell-specific treatment dose, it is not possible to soundly interpret the outcomes of tissue stem cell clinical trials*" as the reason "*Why the tissue stem cell-specific dose is important for stem cell clinical trials*." In 2020, The FDA's Standards Coordinating Body (SCB 2020) listed methods for determining the cell-specific dose of stem cell treatments as needed standards for regenerative medicine.

Until 2020, there were no technologies available for specific and accurate determination of the stem cell dose of therapeutic stem cell treatments. All the available in vitro methods described score both stem cells and committed progenitor cells. Colony-forming unit (CFU) assays cannot distinguish cell colonies produced by stem cells from those produced by early committed progenitor cells (Rich 2015). As noted earlier, the lack of stem cell-specific biomarkers precludes quantification of stem cell fraction by flow cytometry. Methods deploying assays for cellular enzymes and metabolites also score the activities of both stem cells and committed progenitor cells (Patterson et al. 2015).

In vivo cell transplantation assays allow detection of stem cells without confounding with detection of committed progenitor cells. These assays detect cells that can confer long-term reconstitution of human tissues in immunodeficient animals. Tissue stem cells have this ability, but committed progenitors do not. These assays have been limited primarily to applications for human HSCs using immunodeficient mice as cell transplant recipients; but there are also examples of their use to study human HSCs in larger animals like sheep (Almeida-Porada et al. 2004). In the case of human HSC assays in immunodeficient mice (typically NOD/SCID strains), performing a limiting dilution series of the evaluated cells before transplantation allows the application of Poisson statistical modeling to estimate the number of HSCs in the starting sample. The assays are commonly called limiting dilution SCID mouse repopulating cell (LDSRC) assays (Purton and Scadden 2007).

Though affording the requisite stem cell specificity for tissue stem cell counting, the LDSRC assay has significant quantitative limitations and shortcomings. First, it is expensive and takes a long time to perform. A single "count" for one treatment sample may require as many as 50 mice to achieve sufficient statistical power for the Poisson estimates, and these animals must be maintained for 16 weeks after transplantation to confirm long-term tissue reconstitution. Second, there is a significant quantitative shortcoming of the method that is often overlooked. The readout is a function of both HSC number and engraftment efficiency. The quantitative modeling assumes engraftment efficiency is 100% because there is no way to measure or estimate it independently. To the extent that engraftment efficiency is less than ideal, the LDSRC assay will underestimate the actual HSC number and dose; and because

it varies from mouse to mouse and time to time, it undermines the quantitative precision of HSC determinations.

Two other methodologies for specific quantification of tissue stem cells have been described more recently. The first is a morphological approach based on detecting a special characteristic of tissue stem cells that was first envisioned by John Cairns in the 1970s (Cairns 1975). When they undergo asymmetric self-renewal division, both animal and human tissue stem cells have been shown to nonrandomly co-segregate the same oldest complement of chromosomal DNA strands (Panchalingam et al. 2020). Since asymmetric self-renewal is a tissue stem cell-exclusive feature, the corresponding "immortal DNA strands" have been proposed as specific biomarkers that might be targeted for specific quantification of tissue stem cells (Huh et al. 2015). This approach has been used to detect tissue stem cells in preparations of tissue cells from agricultural animals like cows (Choudhary and Capuco 2012; Capuco and Choudhary 2020). To date, immortal DNA strand detection has not been validated as a means for determining the dose of stem cells in either human or veterinary stem cell treatments. Though promising in concept, the detection of cells with immortal DNA strands is technically challenging. Only recently have suitable technical methods been achieved that might enable this method's clinical validation and future use (Huh et al. 2015).

A second quantitative methodology for specific and accurate determination of tissue stem cell fraction, number, and dosage was reported last year (Dutton et al. 2020). The new approach, "kinetic stem cell counting," provides specific quantification of the tissue stem cells in complex tissue cell preparations. Kinetic stem cell counting is an in vitro cell culture method that uses computational simulation to discover the number of tissue stem cells responsible for the total cell proliferation of serially passaged tissue cell cultures. The method is based on a stem cell-driven cell production model that incorporates principles of in vivo tissue cell homeostasis. After an initial foundational computational simulation analysis based on 3–4 weeks of serial cell culture, kinetic stem cell counting yields simple mathematical algorithms for specific stem cell counting. Thereafter, the kinetic stem cell counting algorithms require only culture population doubling time data, produced from a few days of cell culture, to compute the stem cell-specific fraction, number, and dose of a tissue cell sample. Though not yet clinically validated, the kinetic stem cell counting method has the potential to become a routine clinical tool for the determination of stem cell dose for both veterinary stem cell medicine and human stem cell medicine (Dutton et al. 2020).

12.7 Benefits of Quantitative Stem Cell Dosing for Stem Cell Veterinary Medicine

Though still early in its development, the new kinetic stem cell counting technology promises to make the long-needed implementation of stem cell dosing in stem cell medicine not only feasible but also practical. Many benefits can be envisioned for

Table 12.2 Future benefits of stem cell dosing in stem cell veterinary medicine

Benefit of stem cell dosing	Current problem addressed
1. Increased treatment fidelity	Unknown stem cell dosing reliability[a]
2. Increased statistical power	Unknown dosage variance
	Efficacy evaluation
3. Direct potency metric	Potency
4. Improved biomanufacturing	Uncertain production
5. Improved quality control and assurance	Stem cell preservation, stability, transport

[a]In particular for expanded stem cell treatments

both veterinarians and their patients when stem cell dosing is a routine practice for veterinary stem cell medicine. Table 12.2 highlights several predicted benefits.

The most significant benefit is the increased treatment fidelity. Knowing the dosage of the therapeutic agent in administered medicines is a fundamental tenet of clinical medicine (Sherley 2018a). The stem cell treatment dosage is an important factor for delivering reproducible stem cell treatments among different patients and for the same patients over time. Poor treatment fidelity is a particular worry for M[S]C therapies that use expanded stem cell populations. It is generally recognized that culture expansion of all types of tissue stem cells proceeds with a reduction in stem cell activity by all available measures (Shakouri-Motlagh et al. 2017). Though various explanations are considered to account for this loss—including stem cell differentiation, loss of tissue factors, stem cell senescence, and stem cell dilution—the resulting challenge is the same. The reduced dose of stem cells in expanded cell populations is unknown. It is certainly variable from expansion lot to expansion lot, and in some cases, unknowingly, it might be too low to be therapeutically effective.

Stem cell dosage data could have a major impact on the progress and success of stem cell medicine clinical trials. Of course, improved treatment fidelity would be advantageous for clinical trial success. However, in addition, the availability of precise quantification of stem cell dosage would improve the design of clinical trials and the interpretation of their outcomes. Since compared patients rarely get treated with ideally replicate treatment samples, currently, stem cell dose is an unknown denominator in all stem cell clinical trial outcome data analyses. Beyond improving overall treatment fidelity, knowledge of how stem cell dose varies among patients, between treatment arms, among trial sites, and among trials would provide greater statistical power for soundly detecting treatment effects.

Throughout the practice of stem cell medicine, potency is a controversial topic (Rich 2015). The potency is a prediction of the effects of treatment *before* its administration to patients. It is ideal for potency to be a quantitative measurement that is able to predict the degree of clinical response with respect to the amount of the treatment. Stem/progenitor biomarkers, like CD34 and CD90 for instance, are qualitative indicators of the potency for HSC and M[S]C treatments, respectively. However, neither provides a quantitative prediction of the effectiveness of stem cell treatment (Ivanovic 2010). Other commonly applied characterizations of stem cell

populations, like CFU and LDSRC assays, fall short as potency measures as well (Purton and Scadden 2007; Rich 2015). Stem cell-specific fraction, number, or dose determined by kinetic stem cell counting may prove to be the first effective potency measures for stem cell medicine.

General implementation of effective kinetic stem cell counting by ancillary industries that support veterinary stem cell medicine will also benefit veterinarians and their patients. Companies engaged in biomanufacturing of expanded stem cell treatments can improve their bioprocess engineering by monitoring, for the first time, the stem cell-specific fraction of starting tissue sources, processing stages, and final expanded production lots. Companies that store and ship either primary tissue stem cell preparations or biomanufactured cells can better ensure the viability and potency of stem cell treatments after storage and shipment. Currently, quality control and quality assurance of such services are based on assessments of total cells, for which the crucial stem cells are only a small fraction. Kinetic stem cell counting could be used to investigate the widely applied but nonvalidated belief that such total cell measurements are quantitatively informative about the status of the stem cells in the preparations. If kinetic stem cell counting invalidates this belief, its stem cell dose determinations can become the new basis for quality control and quality assurance evaluations.

12.8 Conclusions and Future Perspective

Current veterinary stem cell medicine operates with the same deficiency presently accepted by human stem cell medicine. Stem cell medicine treatments, whether approved for a current medical practice or in clinical trials, proceed without knowledge of the dosage of the administered stem cells. In this long-standing state of a principal deficiency, unknowingly, treatments may occur without any stem cells being delivered at all. This lack of qualitative, though more often quantitative, treatment fidelity undermines the success of veterinary stem cell medicine. It renders approved therapies uncertain and unreliable, and it confounds the design and outcome interpretation of stem cell clinical trials and research studies. The way out of the chronic widespread resignation to dose-less stem cell medicine is through the implementation of new technologies that provide an effective means for specific and accurate counting of therapeutic tissue stem cells. Recently described kinetic stem cell counting may be such a technology (Dutton et al. 2020).

So far, human stem cell medicine has been slow to move to evaluating kinetic stem cell counting as a solution for introducing specific dosing for stem cell treatment production, storage, shipping, and administration to patients. As outlined in this chapter focused on veterinary stem cell medicine, important benefits are predicted to accrue to veterinarians, and more importantly to their patients, wherever stem cell-specific dosing information is introduced in current stem cell medical practice or its ancillary supporting industries. If this advantage proves true for veterinary stem cell medicine, it will also prove true for human stem cell medicine. Because of its historical role as the gateway through which medical innovations must

travel to reach human patients, veterinary medicine has the positioning and opportunity to lead to way to advancing quantitative stem cell dosing for stem cell medicine.

Acknowledgments Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number R43HL154900. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Ratan Kumar Choudhary
Shanti Choudhary *Editors*

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Ratan Kumar Choudhary •
Shanti Choudhary
Editors

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Editors
Ratan Kumar Choudhary
Department of Bioinformatics
College of Animal Biotechnology
Ludhiana, Punjab, India

Shanti Choudhary
Department of Microbial and Environmental Biotechnology
College of Animal Biotechnology
Ludhiana, Punjab, India

ISBN 978-981-16-3463-5 ISBN 978-981-16-3464-2 (eBook)
https://doi.org/10.1007/978-981-16-3464-2

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