Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy
ISCT®

Variation in primary and culture-expanded cells derived from connective tissue progenitors in human bone marrow space, bone trabecular surface and adipose tissue

MAHA A. QADAN[1,2,3], NICOLAS S. PIUZZI[1,4,5], CYNTHIA BOEHM[1], WESLEY BOVA[1], MALCOLM MOOS JR.[6], RONALD J. MIDURA[1], VINCENT C. HASCALL[1], CHRISTOPHER MALCUIT[2] & GEORGE F. MUSCHLER[1,4]

[1]Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA, [2]School of Biomedical Sciences, Kent State University, Kent, Ohio, USA, [3]Department of Biotechnology and Genetic Engineering, Philadelphia University, Amman, Jordan, [4]Department of Orthopaedic Surgery, Cleveland Clinic Foundation, Cleveland, Ohio, USA, [5]Instituto Universitario del Hospital Italiano de Buenos Aires, Buenos Aires, Argentina, and [6]FDA/Center for Biologics Evaluation and Research, Division of Cellular and Gene Therapies, Office of Cellular, Tissue, and Gene Therapies, Silver Spring, Maryland, USA

Abstract

Background aims. Connective tissue progenitors (CTPs) embody the heterogeneous stem and progenitor cell populations present in native tissue. CTPs are essential to the formation and remodeling of connective tissue and represent key targets for tissue-engineering and cell-based therapies. To better understand and characterize CTPs, we aimed to compare the (i) concentration and prevalence, (ii) early *in vitro* biological behavior and (iii) expression of surface-markers and transcription factors among cells derived from marrow space (MS), trabecular surface (TS), and adipose tissues (AT). *Methods.* Cancellous-bone and subcutaneous-adipose tissues were collected from 8 patients. Cells were isolated and cultured. Colony formation was assayed using Colonyze software based on ASTM standards. Cell concentration ([Cell]), CTP concentration ([CTP]) and CTP prevalence (P_{CTP}) were determined. Attributes of culture-expanded cells were compared based on (i) effective proliferation rate and (ii) expression of surface-markers CD73, CD90, CD105, SSEA-4, SSEA-3, SSEA-1/CD15, Cripto-1, E-Cadherin/CD324, Ep-CAM/CD326, CD146, hyaluronan and transcription factors Oct3/4, Sox-2 and Nanog using flow cytometry. *Results.* Mean [Cell], [CTP] and P_{CTP} were significantly different between MS and TS samples ($P = 0.03$, $P = 0.008$ and $P = 0.0003$), respectively. AT-derived cells generated the highest mean total cell yield at day 6 of culture—4-fold greater than TS and more than 40-fold greater than MS per million cells plated. TS colonies grew with higher mean density than MS colonies (290 ± 11 versus 150 ± 11 cell per mm^2; $P = 0.0002$). Expression of classical-mesenchymal stromal cell (MSC) markers was consistently recorded (>95%) from all tissue sources, whereas all the other markers were highly variable. *Conclusions.* The prevalence and biological potential of CTPs are different between patients and tissue sources and lack variation in classical MSC markers. Other markers are more likely to discriminate differences between cell populations in biological performance. Understanding the underlying reasons for variation in the concentration, prevalence, marker expression and biological potential of CTPs between patients and source tissues and determining the means of managing this variation will contribute to the rational development of cell-based clinical diagnostics and targeted cell-based therapies.

Key Words: *adipose tissue, bone, colony flow cytometry, forming unit assay, connective tissue progenitor, mesenchymal stromal cell, stem cell, surface markers, trabecular bone, transcription factor*

Introduction

Stem and progenitor cells in native tissues are essential for the formation and remodeling of new tissues. They constitute a target cell population for a broad range of tissue engineering applications [1]. They also represent a therapeutically useful starting material for the generation of culture-expanded progeny for

Correspondence: **George F. Muschler**, MD, Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA. E-mail: muschlg@ccf.org

(Received 30 August 2017; accepted 29 November 2017)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2017.11.013

cellular therapies, including cartilage, bone, and soft tissue regeneration [2].

The term "connective tissue progenitors" (CTP) has been used to define the heterogeneous populations of stem and progenitor cells present in native connective tissues that are able to proliferate and differentiate into one or more connective tissue phenotype [3,4]. Colony-founding CTPs are found in virtually every connective tissue in adults [3–7].

Stem and progenitor cells can be assayed from any tissue to characterize their concentration, prevalence and range of biological phenotypes using colony-forming unit (CFU) assays, based on the assumption that each colony is formed by the progeny of one stem cell or progenitor [8]. Tissue-resident stem cells and progenitors are by nature heterogeneous in biological state and potential [9]. Differences between the colonies formed under standardized conditions reflect the heterogeneity in biological potential among the colony-founding cells [10–14].

CFU assays are traditionally performed by a skilled operator by performing a manual colony count. However, evidence suggests that manual counting provides poor repeatability and reproducibility [15]. Moreover, subjective manual methods are unable to capture biologically important metrics of colony formation and differentiation systematically. This information, which is not being collected by manual-colony counting, if obtained, could be used for better biological characterization of these colonies and cells, which potentially could identify clinically relevant differences between colony-founding cells and their clonal progeny [15,16].

The ASTM International (previously American Society for Testing and Materials), as a globally recognized leader in the development and delivery of consensus standards, has provided a Standard Test Method for Automated Colony-Forming Unit Assays that address the limitations of manual methods (F2944–12 Standard) [15]. The main benefit of the use of automated methods is that it reduces the variation in measurement that results from subjective differences between observers. The improved repeatability and reproducibility of automated CFU assays enhances the utility of these assays for a variety of applications, including (i) comparison and selection of optimal anatomic sites and methods for stem and progenitor cell harvest; (ii) assessment of the effect of *in vitro* processing on CFU concentration, prevalence, and biological performance; (iii) exploring the relationship between CTP concentration, prevalence, and biological performance to local tissue health or the progression of disease; (iv) predicting cell quality or potency and the likelihood of clinical efficacy if used for treatment; and (v) enabling systematic rational discrimination and selection among CFU subtypes to enhance control over cell-source quality and outcomes.

Several studies have described significant differences between tissue sources with respect to biological potential for the harvest of stem and progenitor cells [17–19]. Variation has been reported between patients related to gender, age [20,21], surgical site and harvesting techniques [6,22–24]. Even among cultured-expanded populations, there have been reports of variations between tissues and among separate cells isolated from the same tissue [10–13]. Heterogeneity has even been reported within an apparent clone [14].

Bone marrow [7,17,23], trabecular bone [17,20,25,26] and adipose tissue [17,27,28] are the most common sources of CTPs for both research and clinical applications. However, these sources have been reported to vary significantly in cell concentration, prevalence and biological attributes. Nancarrow-Lei *et al.* [17] provided a systematic review of cell source options. The data reported in the field are not sufficiently homogeneous in methods of analysis or reporting to allow a meta-analysis to systematically quantify the magnitude and extent of variation between sources. Therefore, this article may only provide qualitative comparisons and consensus statements. Bone marrow aspirates are considered to be the reference standard against which all other tissue sources are compared. Adipose tissue is accepted to provide the highest prevalence of CFUs among tissue resident cells, but adipose-derived cells tend to lag in differentiation potential toward bone and cartilage phenotypes compared with bone marrow–derived cells. Even within a given donor or tissue, heterogeneity within and between donors is large [29,30]. Rational clinical development demands further investigation and direct comparison of these cell sources with respect to their concentration, prevalence and the biological performance.

When culture expanded *in vitro*, cells from each of these sources can be used to generate populations of culture-expanded cells that can be categorized as "mesenchymal stromal cells" (MSCs). It has been shown that culture-expanded MSCs may be indistinguishable from fibroblasts, based on conventional markers [31,32]. However, the International Society for Cellular Therapy (ISCT) has defined standardized terminology and minimal criteria for classification of culture-expanded cells as MSCs based on the presence of surface markers CD73, CD90 and CD105 and the absence of hematopoietic markers CD34, CD45, CD14, CD19 and HLA-DR [33]. The ISCT MSC Committee has also proposed the need to add functional analysis, including the immunological modulatory effects, to enable standardization of clinical cell-based therapies [34]. There is evidence that *in vitro* expansion induces or selects for the expression of MSC