Location, allocation, relocation: isolating adult tissue stem cells in three dimensions

Won Jae Huh[1,*], Xiaoou O Pan[1,*], Indira U Mysorekar[2] and Jason C Mills[1]

The literature on isolation of adult tissue stem cells is vast and disparate. To better organize the field, we redefine 'isolation', re-expressing it as the sum of three component vectors: location, allocation and relocation. Location is the isolation of stem cells *in situ* by anatomical features. Allocation is physical isolation by cell sorting. Relocation is isolation of the functional properties of a stem cell to regenerate its normal progeny when relocated to a new environment. Techniques for the allocation and relocation of stem cells from certain tissues (e.g. hematopoietic) are currently better defined than their location, whereas those of other tissues (e.g. mammary glands) have had recent advances along all three vectors. Yet another group (e.g. gastric glands), have stem cells with well characterized location, emerging techniques for allocation but still rudimentary techniques for relocation.

Addresses
[1] Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
[2] Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA

Corresponding author: Mills, Jason C (jmills@pathology.wustl.edu)
*These authors contributed equally to this work.

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Introduction

Here, we review techniques for isolating adult tissue stem cells, a topic on which there is a vast, varied, controversial literature. Controversy and misunderstanding often result from imprecise language, so our review proposes a more precise definition of 'stem cell isolation'. We posit that, regardless of tissue, there are three dimensions or component vectors to isolation that we have mnemonically dubbed: 'location, allocation and relocation' (Figure 1). 'Location' measures how well scientists can isolate the stem cell by anatomical features (e.g. using microscopy) *in situ* within the *in vivo* three-dimensional structure of the tissue. 'Allocation' quantifies the sophistication of tools (e.g. antibodies to stem-cell-specific cell-surface proteins) available for dissociating a tissue stem cell from its *in vivo* microenvironment and physically sorting it from other cells. 'Relocation' denotes techniques available for proving that a given cell or cell population can be transplanted to a new niche and regenerate both itself and all the appropriate progeny cells of its tissue of origin (i.e. has functional stem cell properties).

Each tissue with a proven, constitutively active adult stem cell is currently at a different state of development with regard to the biotechnology available for its isolation; in other words, the tools available for location, allocation, relocation (LAR) vary widely from tissue to tissue. Rather than attempt to review this literature comprehensively, we will simply describe three LAR patterns and give two tissues that exemplify each (Table 1).

Location <(allocation and relocation)

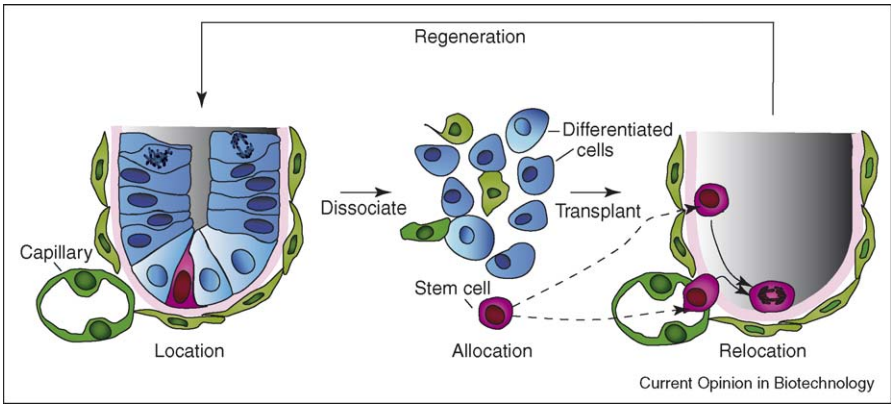
Hematopoietic and mesenchymal tissues are examples for which much is known about stem cell allocation and relocation, but less is understood about location.

Location

Although the concept of the hematopoietic stem cell (HSC) 'niche' was proposed by Schofield in 1978 [1], the microanatomy of the HSC within its niche is still relatively uncharacterized; however, there has been recent progress [2,3,4•] (reviewed in [5]). For example, a recent study has shown that murine HSCs localize to the inner lining of multiple bones, suggesting that the endosteal surface of epiphyseal bone is the HSC niche [3]. Along the endosteal surface, HSCs attach to spindle-shaped N-cadherin$^+$, CD45$^-$ osteoblastic (SNO) cells via a complex including two adherens junction-associated molecules: N-cadherin and β-catenin [2]. Proliferating mouse HSCs seem to line the sinusoidal endothelium in the spleen and bone marrow, but the quiescent reservoir of HSCs might be the endosteal niche [4•]. The calcium-sensing receptor (CaR) could help retain HSCs along the endosteal surface, because HSC alignment along the endosteal surface was perturbed in CaR$^{-/-}$ mice [6•].

Mesenchymal stem cells (MSCs) can differentiate into multiple structural tissues, such as adipose, bone, cartilage and various loose connective tissues throughout the body. MSCs have been isolated from a variety of tissues throughout the body, complicating localization of their niche. Human MSCs have been found residing in bone

Figure 1



The three component vectors to isolation of adult stem cells. A hypothetical tissue is depicted to illustrate the concepts discussed in the text. Stem cells can be isolated by anatomical features *in situ* (location). They can also be isolated by dissociation from the tissue using sorting (allocation). Finally, they can be isolated by transplantation to a new environment where, if they are stem cells, they will divide and differentiate to regenerate a functionally and anatomically equivalent tissue (relocation).

marrow and dental pulp microvasculature [7] and also associated with trabecular bone in both humans and mice [8,9••]. When associated with trabecular bone, they are quiescent but proliferate upon leaving it [8], implicating trabecular bone as an MSC reservoir. The niche for MSCs and HSCs might be linked: MSCs are also associated with the endothelium and endosteal surfaces, and both histological and gene expression studies indicate that most immature MSC-like stromal cells contact and support HSCs in both humans and mice [9••,10].

Allocation

Molecular methods for sorting HSCs have been extensively investigated and well-characterized (e.g. [11]), and HSC sorting is sufficiently established to become an aspect of common therapeutic protocols. However, techniques are continually being refined; for example, it was recently reported that SLAM (signaling lymphocytic activation molecule) genes could be used to differentiate murine cells at multiple stages of hematopoietic potential [4•]. HSCs were $CD150^{+}CD244^{-}CD48^{-}$, multipotent hematopoietic progenitors were $CD244^{+}CD150^{-}CD48^{-}$, and the most restrictive progenitors were $CD48^{+}CD244^{+}CD150^{-}$. Another method isolated mouse HSCs using regulatory elements 5′ to the *Gata2* gene to direct green fluorescent protein (GFP) expression specifically to a $CD34^{+}$ HSC-like population in bone marrow [12]. Cells sorted as GFP^{+} and Sca-1^{+} (an HSC marker) were enriched for HSC capacity and, following transplantation to new hosts, homed to endosteum in contact with osteoblasts [12]. Human HSCs are enriched in aldehyde dehydrogenase (ALDH) expression, and $ALDH^{high}CD133^{+}Lin^{-}$ cells demonstrated increased stem-cell capacity (versus $CD133^{+}Lin^{-}$ cells) following relocation into mouse NOD/SCID hosts [13].

MSC isolation techniques based on physiochemical properties (e.g. adherence in tissue culture) have been well

Table 1

Tissue stem cells and the current state of techniques for their isolation based on location, allocation and relocation.

Pattern	Stem cell/tissue	Location	Allocation	Relocation
Location < (allocation and relocation)	Hematopoietic cells	Recent advances	Well characterized	Well characterized
	Mesenchymal cells	Elusive	Relatively well characterized	Relatively well characterized
Location∼allocation∼relocation	Mammary epithelial cells	Well studied but not unequivocally established	Recent advances	Relatively well established
	Epidermis and appendages	Well studied, with ongoing controversy	Recent advances	Well established *in vitro* methods
Location > (allocation and relocation)	Stomach epithelium	Well established location and ultrastructure	Emerging	Elusive
	Urinary tract transitional epithelium	Relatively well established	Emerging	Emerging