One size does not fit all: developing a cell-specific niche for in vitro study of cell behavior

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Abstract

For more than 100 years, cells and tissues have been studied in vitro using glass and plastic surfaces. Over the last 10–20 years, a great body of research has shown that cells are acutely sensitive to their local environment (extracellular matrix, ECM) which contains both chemical and physical cues that influence cell behavior. These observations suggest that modern cell culture systems, using tissue culture polystyrene (TCP) surfaces, may fail to reproduce authentic cell behavior in vitro, resulting in "artificial outcomes." In the current study, we use bone marrow (BM)- and adipose (AD)-derived stromal cells to prepare BM-ECM and AD-ECM, which are decellularized after synthesis by the cells, to mimic the cellular niche for each of these tissues. Each ECM was characterized for its ability to affect BM- and AD-mesenchymal stem cell (MSC) proliferation, as well as proliferation of three cancer cell lines (HeLa, MCF-7, and MDA-MB-231), modulate cell spreading, and direct differentiation relative to standard TCP surfaces. We found that both ECMs promoted the proliferation of MSCs, but that this effect was enhanced when the tissue-origin of the cells matched that of the ECM (i.e. BM-ECM promoted the proliferation of BM-MSCs over AD-MSCs, and vice versa). Moreover, BM- and AD-ECM were shown to preferentially direct MSC differentiation towards either osteogenic or adipogenic lineage, respectively, suggesting that the effects of the ECM were tissue-specific. Further, each ECM influenced cell morphology (i.e. circularity), irrespective of the origin of the MSCs, lending more support to the idea that effects were tissue specific. Interestingly, unlike MSCs, these ECMs did not promote the proliferation of the cancer cells. In an effort to further understand how these three culture substrates influence cell behavior, we evaluated the chemical (protein composition) and physical properties (architecture and mechanical) of the two ECMs. While many structural proteins (e.g. collagen and fibronectin) were found at equivalent levels in both BM- and AD-ECM, the architecture (i.e. fiber orientation; surface roughness) and physical properties (storage modulus, surface energy) of each were unique. These results, demonstrating differences in cell behavior when cultured on the three different substrates (BM- and AD-ECM and TCP) with differences in chemical and physical properties, provide evidence that the two ECMs may recapitulate specific elements of the native stem cell niche for bone marrow and adipose tissues. More broadly, it could be argued that ECMs, elaborated by cells ex vivo, serve as an ideal starting point for developing tissue-specific culture environments. In contrast to TCP, which relies on the "one size fits all" paradigm, native tissue-specific ECM may be a more rational model to approach engineering 3D tissue-specific culture systems to replicate the in vivo niche. We suggest that this approach will provide more meaningful information for basic research studies of cell behavior as well as cell-based therapeutics.

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Introduction

The origins of cell and tissue culture date to the early 1900's, with studies reported by Carrel, Burrows, and others describing the culture of mammalian tissue outside the human body [1]. Initially, tissue explants were placed on glass coverslips in a drop of hypotonic plasma that was allowed to clot before being inverted over a concave microscope slide. Considerable progress has been made over the last 100 years, including the isolation and growth of single cells [2], the seminal studies of Harry Eagle leading to the successful development of defined media [3], and the development of glow discharge treatment of tissue culture polystyrene (TCP) to facilitate cell attachment [4], that have facilitated many discoveries in modern medicine and the award of several Nobel prizes. Today, despite the achievement of many significant milestones in methods of cell and tissue culture, our studies of cell behavior continue to be constrained by an inadequate replication of the in vivo environment in culture.

An ideal tissue culture system should not only support the growth of cells in vitro but, more importantly, cause them to faithfully retain and express their in vivo phenotypic characteristics. However, because of their simplicity, TCP plates have been widely used for this purpose. Although much has been learned using such "two-dimensional" (2D) culture surfaces, it has become increasingly clear that these classical culture systems are inadequate for replicating in vivo cell behavior and studying cell and molecular biology, understanding the mechanisms of diseases, and improving diagnosis and treatment of diseases [5–7].

Within the body, cells reside in tissues that contain distinct physiological microenvironments or niches. Cells receive not only biochemical and physical cues from this local environment, but also interact with and remodel it, by secreting extracellular matrix (ECM) proteins and growth factors that play a role in cell signaling pathways and producing proteolytic enzymes capable of modifying the ECM. This "give and take" relationship between cells and their niche has the effect of directing changes in cell behavior that include cell quiescence, migration, proliferation (symmetric or asymmetric division), differentiation, survival, and senescence [8]. Over the last decade, it has become increasingly clear that this relationship cannot be studied in classical 2D culture systems.

The local microenvironment (niche) of the cell is mainly comprised of ECM components that form a three-dimensional (3D) network which anchors cells and provide a reservoir of growth factors/cytokines that direct cell fate and function [9–11]. For this reason, we have focused on developing an authentic niche *ex vivo*, instead of optimizing growth media, for controlling the fate of mesenchymal stem cells (MSCs). With this approach, we were the first to establish a culture system using cell-free native ECM made by bone marrow stromal cells. Although this bone marrow stromal cell-derived ECM (BM-ECM) contained very few growth factors, it dramatically promoted MSC replication, retention of multipotency, and significantly increased responsiveness to differentiation stimuli when compared to MSCs cultured on TCP [12,13]. We have demonstrated that this native BM-ECM is composed of, at least in part, collagens, fibronectin, small leucine-rich proteoglycans (SLRPs), and basement membrane components [12,13]. These matrix proteins are known to play a key role in regulating cell adhesion, migration, proliferation, differentiation, and survival [14–16]. Importantly, many of these cell functions are controlled by growth factors and cytokines (TGFβ, TNFα and PDGF) that are stored in the ECM and presented to cells as they interact with and remodel the matrix. The types of control mechanisms employed include secretion of growth factors in latent form, storage in the ECM by use of specific binding proteins to prevent receptor binding, activation of latent or bound forms via proteolytic processing [17–19], and modulation of receptor activity [20,21]. Indeed, our previously published studies have shown that ECM proteins regulate MSC behavior by modulating both endogenous (stem cell-derived) and exogenous (externally added or serum-derived) growth factor stores [10,12,14,22].

To extend these observations, we hypothesized that native ECM, replicated *ex vivo* by this technology, contains a unique collection of key effective components (or cues) that direct MSC differentiation to the cell lineage that originally synthesized the ECM. In the current study, we tested this hypothesis by investigating whether BM-ECM was unique in its ability to preserve MSC properties by comparing it to ECM produced in a similar fashion by adipose tissue-derived stromal cells. We compared the response of bone marrow- or adipose-derived MSCs (BM-MSCs, or AD-MSCs, respectively), as well as cancer cell lines, to culture on the same substrates including BM-ECM, adipose-derived ECM (AD-ECM), and TCP. We also characterized the biomolecular composition, architectural and mechanical properties of BM-ECM versus AD-ECM in an effort to identify physical and chemical cues that may uniquely characterize the ECM present in the bone marrow and adipose-tissue microenvironments. The results provide evidence that cells are extremely sensitive to their culture substrate and raise questions/concerns regarding both potential inherent bias and the reliability of results obtained with cells maintained on the classical 2D culture system (TCP).