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Solid-supported lipid bilayers to drive stem cell fate and tissue architecture using periosteum derived progenitor cells

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ABSTRACT

A challenge to mimicking nature's "bottom up" approach to generate tissue is the coordination of cellular self-assembly and emergent phenotype. Here we create a biosynthetic platform to mimic native cell–cell interactions and to drive emergent tissue behavior by human multipotent cells from the periosteal niche, *i.e.* PDCs, whose regenerative capacity is equal or greater to those from the bone marrow niche. Western blots showed that human PDCs express proteins for both N-cadherin, a hallmark of mesenchymal condensation, as well as for ZO-1, a tight junction membrane protein conferring epithelial barrier membrane properties. Hence, we functionalized a solid supported lipid bilayer (SLB) membrane with recombinant N-cadherin and investigated the short term phenotype of PDCs seeded on unfunctionalized and N-cadherin functionalized SLBs compared to that of PDCs seeded on glass coverslips. After 24 h, SLB functionalization promoted aggregation of PDCs seeded at high density (35,000 cells/cm^2), with no significant concomitant changes in transcription of N-cadherin (*CDH2*) as measured by rtPCR. In contrast, cells seeded on unfunctionalized SLBs remained non-adherent but showed a significant upregulation in transcription of N-cadherin. Furthermore, culture of PDCs at high density on N-cadherin functionalized SLBs was negatively correlated with expression of ZO-1, while culture on unfunctionalized SLBs was positively correlated with the expression of the tight junction membrane protein. High density seeding on N-cadherin functionalized and unfunctionalized SLBs places PDCs in distinct cellular contexts and relates to emergent behavior typical for mesenchymal condensation. These studies demonstrate a biosynthetic *in vitro* cell culture platform to elucidate and guide emergent tissue architectures by PDCs.

1. Introduction

Nature manufactures tissues through self-assembly by pluripotent cells. The formation of molecular patterns (patterning) allows for the subsequent specification of cell lineage (differentiation) and the formation of specialized tissues that form the templates of organs and organisms (morphogenesis) [1–5]. Processes of cellular self-assembly are evident not only during development *in utero* but are also recapitulated during postnatal healing [1,6]. Recent studies designed to harness nature's tissue engineering paradigms emphasize molecular approaches to understand cell–cell junction assembly and its role in emergent structural and functional properties at the tissue length scale [1–4]. Yet, to our knowledge, no technological platform exists to study nascent formation of cell–cell junctions and emergent tissue template architecture by primary embryonic or adult tissue-derived stem cells.

Solid-supported lipid bilayers (SLBs) provide an *in vitro* biological membrane model system to investigate the dynamic molecular processes occurring at the interface of biological membranes [7], which modulate cell structure and function. In the past decade, SLBs have been used to characterize interactions between proteins or viruses and the cell membrane surface as represented by the

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lipid bilayer [8–13]. SLBs have also been utilized as a platform to build biomimetic interfaces with multiple functionalities [14] and as cell membrane models to study cell–cell interactions [15–17]. A limited number of studies have used SLBs to create semi-3D, biomimetic surfaces for culture of neural progenitors [18] and terminally differentiated cells [19,20]. For optimal functionalization, SLBs should be decorated with junctional adhesion or ECM proteins to which native cells are known to attach. Previous studies creating SLBs for use in cell culture have functionalized the surface with E-cadherin [19], a cell–cell adhesion protein, as well as type I collagen [20] and a peptide derived from laminin, both of which are proteins key to the extracellular matrix (ECM) of connective tissues [18].

The goal of our study was to develop the SLB platform to elucidate the role of cell–cell junctions in differentiation and *de novo* tissue generation by primary adult periosteum derived multipotent cells (PDCs). We were interested in the heterogeneous, multipotent cell population of PDCs, because PDCs have been shown to exhibit regenerative capacity equal to or greater than that of multipotent cells derived from the bone marrow [23]. Furthermore, *de novo* tissue generation in bone defects recapitulates processes of tissue formation during development, via both endochondral and intramembranous bone formation [2,5,21,22]. The periosteum is a bilayered tissue (Fig. 1), comprising a cambium layer that provides a niche for PDCs, (zone I) and an outer fibrous sheath (comprising zones II, III) that serves a putative structural function [4].

As the initial step in formation of skeletal tissues, mesenchymal condensation is the leading step in skeletogenesis [2,24,25]. Mesenchymal multipotent progenitor cells such as PDCs are capable of committing to *i.a.* chondrogenic, osteogenic, and adipogenic lineages, and the relative stage of lineage commitment can be assessed through patterns of gene expression over time (Fig. 2) [2]. Bone formation *in utero* and during postnatal healing occurs either indirectly via endochondral ossification, where a cartilage template is first formed and then mineralized to form skeletal elements, or directly via intramembranous ossification. The mode and spatiotemporal patterns of ossification are major determinants of subsequent tissue architecture, which emerges over time from the molecular to the cellular and tissue length scales [1,30–32].

Tissue template architecture is first determined by spatiotemporal patterning of cells, which in turn is highly dependent on the expression of cell–cell junctional proteins such as those of the cadherin protein family [26,33–36]. In addition, cadherins play a key role in regulating cell proliferation, differentiation, migration, and apoptosis [26,37,38]. N-cadherin is a classic type I cadherin responsible for the formation of junctional cell–cell adhesions and joining cells together in the appropriate configuration to form tissues. N-cadherin is one of a limited number of cell–cell adhesion molecules expressed by skeletal cells. N-cadherin plays a critical role in events of mesenchymal condensation during limb bud development and appears to be under tight spatiotemporal control in development of the cartilage template of the skeleton. Both mesenchymal condensation and chondrogenesis are perturbed in N-cadherin conditional knockouts [26,39]. N-cadherin is also "expressed at all stages of bone formation, although at various level [s]," [26,40,41] and its expression "persists in periosteal cells in adult rat tibia" [26,42]. Further, inhibition of the extracellular domain of N-cadherin in human trabecular bone-derived cells leads to loss of cell–cell adhesions and a reduction in size or even absence of cell pellet [43]. Therefore, N-cadherin plays an important role in pre-mesenchymal condensation and expression is necessary to initiate mesenchymal condensation. Based on these and other data, in 2002, Marie proposed that N-cadherin serves a decisive role in differentiation of mesenchymal stem cells toward chondro, osteo and adipogenic fates (Fig. 3), where the magnitude and spatiotemporal expression of N-cadherin are "associated with parallel changes in cell–cell adhesion and differentiation" [26].

Hence, in the current study, we targeted N-cadherin for SLB functionalization, as the expression of N-cadherin is a hallmark of mesenchymal cells. Furthermore, since periosteal tissue exhibits directional transport characteristics consistent with an epithelial barrier membrane [4], we hypothesized that periosteal cell express the cell–cell junctional protein, ZO-1 (*zona occludens I*), which is responsible for the formation of tight junctions between cells and confers barrier properties to epithelial membranes [44]. Since no studies to date have characterized the junctional adhesion proteins by which human PDCs interact or the behavior (phenotype and genotype) of PDCs seeded on a representative cell membrane, our approach was first to determine the cell–cell junctional adhesion proteins expressed by human PDCs. We then sought to create a model membrane system functionalized with recombinant N-cadherin, creating a functionalized surface for cell culture with the ability to mimic cell–cell interactions. As a first step toward the

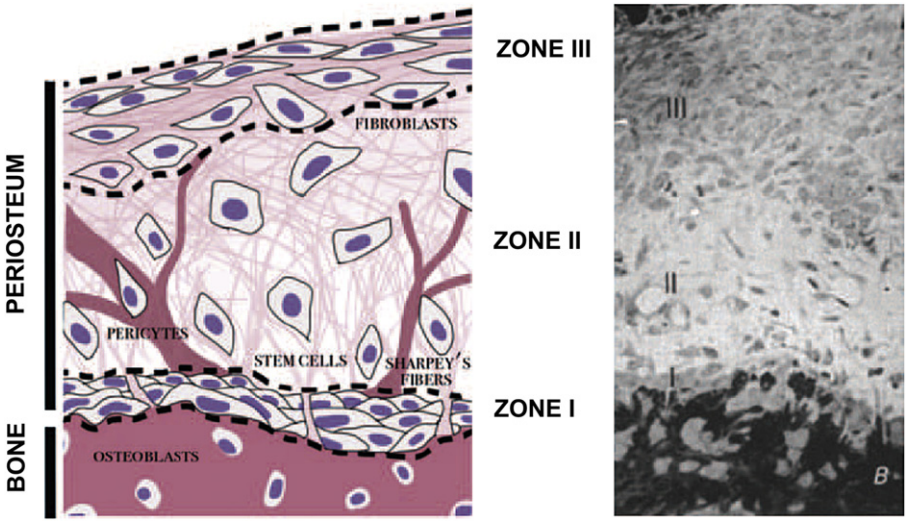

Fig. 1. Structural relationships of the periosteum as observed in a transverse section of tissue. *After* [23,73], *used with permission.*