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Non-invasive tracking of injected bone marrow mononuclear cells to injury and implanted biomaterials

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ABSTRACT

Biomaterial scaffolds enhancing the engraftment of transplanted bone-marrow mononuclear cells (BM-MNC) have enormous potential for tissue regeneration applications. However, development of appropriate materials is challenging given the precise microenvironments required to support BM-MNC engraftment and function. In this study, we have developed a non-invasive, real-time tracking model of injected BM-MNC engraftment to wounds and implanted biomaterial scaffolds. BM-MNCs, encoded with firefly luciferase and enhanced GFP reporter genes, were tail vein injected into subcutaneously wounded mice. Luciferase-dependent cell bioluminescence curves revealed our injected BM-MNCs homed to and engrafted within subcutaneous wound sites over the course of 21 days. Further immunohistochemical characterization showed that these engrafted cells drove functional changes by increasing the number of immune cells present at early time points and remodelling cell phenotypes at later time points. Using this model, we subcutaneously implanted electrospun polycaprolactone (PCL) and PCL/Collagen scaffolds, to determine differences in exogenous BM-MNC response to these materials. Following BM-MNC injection, immunohistochemical analysis revealed a high exogenous BM-MNC density around the periphery of PCL scaffolds consistent with a classical foreign body response. In contrast, transplanted BM-MNCs engrafted throughout PCL/Collagen scaffolds indicating an improved biological response. Importantly, these differences were closely correlated with the real-time bioluminescence curves, with PCL/Collagen scaffolds exhibiting a ~2-fold increase in maximum bioluminescence compared with PCL scaffolds. Collectively, these results demonstrate a new longitudinal cell tracking model that can non-invasively determine transplanted BM-MNC homing and engraftment to biomaterials, providing a valuable tool to inform the design scaffolds that help augment current BM-MNC tissue engineering strategies.

Statement of Significance

Tracking the dynamic behaviour of transplanted bone-marrow mononuclear cells (BM-MNCs) is a long-standing research goal. Conventional methods involving contrast and tracer agents interfere with cellular function while also yielding false signals. The use of bioluminescence addresses these shortcomings while allowing for real-time non-invasive tracking *in vivo*. Given the failures of transplanted BM-MNCs to engraft into injured tissue, biomaterial scaffolds capable of attracting and enhancing BM-MNC engraftment at sites of injury are highly sought in numerous tissue engineering applications. To this end, the results from this study demonstrate a new longitudinal tracking model that can non-invasively determine exogenous BM-MNC homing and engraftment to biomaterials, providing a valuable tool to inform the design of scaffolds with implications for countless tissue engineering applications.

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1. Introduction

One of the most widely used cell therapies for tissue regenerative applications are the transplantation of exogenous bone marrow mononuclear cells (BM-MNC), having been employed as treatments for various medical conditions including myocardial infarction [1,2], limb ischemia [3] and traumatic brain injury [4]. The demonstrated tissue regenerative effects of BM-MNC are thought to arise from three major classes of stem cells that exist within the BM-MNC fraction: hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs) [5]. However, BM-MNC therapy has not yet met expectations for clinical efficacy due to their poor survival and engraftment at sites of injury following transplantation [6]. Indeed, BM-MNC function is closely related to their local microenvironment niche, which provides both mechanical and chemical cues that support growth and control differentiation. Importantly, necrotic tissue at sites of injury are largely void of these cues, accentuating the challenge of effective therapy [7]. Tissue engineering aims to provide artificial microenvironments using biomaterial scaffolds which better regulate BM-MNC behaviour and survival [8].

The physical and mechanical properties of implanted biomaterials largely determine the nature of ensuing BM-MNC responses. More closely mimicking the native bone marrow niche *in vivo* through tailoring of scaffold parameters including material composition, dynamic control of soluble and surface-bound cytokines, and physicochemical cues is a favoured approach [8]. However, appropriate mimicry of the extracellular matrix and BM-MNC niche is complex and elusive [7]. In combination with an optimal blend of mechanical properties and signalling cues, candidate scaffolds would ideally be available off-the-shelf to make them clinically applicable. The most effective current strategies involve pre-seeding of BM-MNC subpopulations onto scaffolds prior to implantation [9]. However, this severely diminishes the feasibility of clinical application, due to lengthy time requirements for cell harvest, culture, and seeding and the high regulatory burden and cost. Additionally, *ex vivo* expanded BM-MNC subpopulations often display altered signalling responses, function and phenotype [10]. Together, these shortcomings have prompted investigation into the cellularization of scaffolds by endogenous recruitment of BM-MNC cells *in vivo*, an approach known as *in situ* tissue engineering [11]. Similarly, approaches combining exogenous BM-MNC injections with implanted scaffolds tuned to recruit and engraft BM-MNCs at the site of injury hold significant promise [12].

The model application for *in situ* tissue engineering strategies would see biomaterial scaffolds implanted prior to BM-MNC injection, serving structural functions in the short-term, then facilitate recruitment of injected BM-MNCs in the midterm, and eventually lead to long-term remodelling and being entirely replaced by new functional tissue. However, as previously mentioned, biomaterial scaffolds possessing appropriate parameters that favourably regulate BM-MNC function must first be developed. To more readily achieve this, *in vivo* responses of transplanted BM-MNC populations towards these biomaterials must first be better understood.

In this study, we developed a model which tracks the temporal and spatial distribution of intravenously injected BM-MNCs, specifically their homing and engraftment. By measuring the luciferase-induced bioluminescence of these cells in real-time, we were initially able to model injected BM-MNC engraftment responses to injury. In addition we show, through immunohistochemical analysis, that engraftment of these cells leads to functional effects by elevating the presence of cell phenotypes known to participate in the native response to injury. Extending the utility of this model, we further examined its ability to distinguish *in vivo* engraftment of injected BM-MNC to two model scaffolds. We demonstrate the bioluminescent curve of each scaffold closely relates to the ensuing BM-MNC engraftment responses towards the scaffold post-implantation. Furthermore, we reveal that differing engraftment responses elicit distinct scaffold remodelling changes. These findings represent an effective tool to determine, in real-time, biomaterial scaffolds more favourable to injectable BM-MNC therapies.

2. Materials and methods

2.1. Reagents

D-luciferin potassium salt was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Lympholyte®-M cell separation media was purchased from Cedarlane Labs (Burlington, ON, CA). Endothelial Basal Media (phenol red free) was purchased from Lonza (Switzerland). Polycaprolactone (PCL) polymer and 1,1,1,3,3,3-hexafluoro-2-proponal (HFP) solvent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Purified, soluble ovine collagen was obtained from CollTech (Osborne Park, WA, Australia) and lyophilised as previously described [13]. Multiple Stain Solution (MSS) and JB-4 resin were purchased from Polysciences Inc. (Warrington, PA, USA).

2.2. FVB-L2G mice

Donor firefly luciferase and enhanced green fluorescent protein (eGFP) co-expressing transgenic mouse strains (FVB-L2G) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) (FVB/NJ-Tg(Hspa1a-luc,-EGFP)2Chco/J, stock no. 012370) [14,15]. Recipient FVB neutral wildtype (WT) mice were purchased from Australian BioResources (Moss Vale, NSW, Australia).

2.3. Density gradient isolation of BM-MNCs

Female FVB-L2G mice, aged 7–8 weeks, were anaesthetised under 2% methoxyfluorane and sacrificed by cervical dislocation. To collect the bone marrow, tibias and femurs were explanted and flushed using a 21-gauged needle with sterile PBS. The bone marrow was then layered on top of Lympholyte®-M cell separation media (6 ml per one pair of tibia and femur) and centrifuged at 1250×*g* for 25 min with deceleration off. The resulting buffy coat was aspirated and washed three times in sterile PBS: 800×*g* for 10 min, 50×*g* for 10 min, and 200×*g* for 10 min. Following the last wash, the pellet of BM-MNC was resuspended in EBM (phenol free) to desired cell concentrations [6].

2.4. Bioluminescence imaging

D-luciferin was reconstituted at a concentration of 40 mg/ml. Binding of the luciferin substrate to the luciferase enzyme results in bioluminescence, quantified using the IVIS Series pre-clinical *in vivo* imaging system apparatus (Perkin Elmer). To induce bioluminescence, luciferin was given at a total volume of 50 μl for cells and 200 μl (intra-peritoneal) for mice. Bioluminescence was measured in units of radiance (photos/s/cm^2/steradian). All bioluminescence measurements were calculated within a pre-defined region of interest (ROI). The mean radiance values within an ROI were recorded. For BM-MNC tracking to wounds, the mean radiance for each of the four wound ROIs were averaged to give a single bioluminescence value for each mouse. Bioluminescent profiles represent average measurements of n = 6 mice per group per time point. For BM-MNC tracking to scaffolds, mean radiance measure-