In vivo imaging and evaluation of different biomatrices for improvement of stem cell survival

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Abstract

Therapeutic effects from injection of stem cells are often hampered by acute donor cell death as well as migration away from damaged areas. This is likely due to the fact that injected cells do not have the physical and biochemical cues for ordered engraftment. Here we evaluate 3 common biomatrices (Matrigel, Collagen I, Puramatrix) that has the potential of providing suitable scaffolds needed to enhance stem cell survival. The longitudinal fate of transplanted stem cells was monitored by reporter imaging techniques. Copyright © 2007 John Wiley & Sons, Ltd.

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In recent years, tissue engineering has emerged as a new and ambitious approach that aims to restore damaged organs or tissues by delivering functional cells, supporting scaffolds and biologically active molecules [1]. One of the challenges yet to be overcome in this field is to find a suitable biomaterial scaffold capable of creating enriched microenvironments to improve cell survival in vivo. In order to address this issue, we compared three different matrices: Matrigel, an extract of basement membrane proteins derived from the Engelbreth–Holm–Swarm (EHS) tumour; collagen I, a fibrillar extracellular matrix protein which is also the most abundant collagen type; and Puramatrix, a self-assembling peptide with sequence AcN–(RADA)4–CNH2 [2]. These matrices were used in conjunction with bone marrow-derived mesenchymal stem cells isolated from L2G85 transgenic mice that constitutively express firefly luciferase (Fluc) and enhanced green fluorescent protein (eGFP) (BM-MSCsFluc+/eGFP+) under the constitutive β-actin promoter (from Dr Christopher Contag, Stanford University) [3,4]. Using longitudinal bioluminescence imaging (BLI), we demonstrate here that the combination of Matrigel with BM-MSCsFluc+/eGFP+ has the best long-term survival.

At present, two critical problems limit the field of cell-based tissue engineering: (a) poor survival of transplanted cells; and (b) lack of a non-invasive modality to monitor cell fate. In this study, we hypothesize that biomaterial scaffolds can furnish the biomechanical and structural characteristics of the seeded cells until they are able to fully incorporate into their new environment, and that BLI can be adapted to image the efficacy of different biomatrices in living subjects. We evaluated three common biomatrices, including Matrigel (BD Biosciences, San Jose, CA, USA), collagen I (BD Biosciences, San Jose, CA, USA) and Puramatrix (BD Biosciences, San Jose, CA, USA). We focused on BM-MSCsFluc+/eGFP+ because these cells have the potential to differentiate into many clinically relevant cell types, such as muscle, liver, brain and epithelial lineages [5,6].

BM-MSCsFluc+/eGFP+ were isolated from adult male L2G85 transgenic FVB mice (n = 6) using a modified protocol [3,7]. Fluorescence microscopy showed typical mesenchymal spindle-like morphology of BM-MSCsFluc+/eGFP+...
Figure 1. Isolation and characterization of bone marrow derived mesenchymal stem cells from L2G85 transgenic mice (MSCsFluc+/eGFP+). (a) Morphology of MSCsFluc+/eGFP+ in brightfield and fluorescence microscopy. (b) MSCsFluc+/eGFP+ show robust correlation of firefly luciferase activities and cell numbers. Bioluminescence imaging was performed on varying numbers of MSCsFluc+/eGFP+ plated on 24-well plates. (c) MSCsFluc+/eGFP+ showed 87 ± 8% GFP-positive, 94 ± 11% CD44, 90 ± 5% CD90, 3 ± 0.4% CD34, and 0.1 ± 0.05% CD45. All samples were performed in triplicate. (d) MSCsFluc+/eGFP+ morphology after culturing in the presence of different biomatrices for 48 h. The same culture condition and same cell number was tested for all groups. Scale bars: 50 µm in (a) and 100 µm in (d).

(Figure 1a). Ex vivo BLI of cultured cells demonstrated a robust correlation between cell numbers and Fluc signal activity ($r^2 = 0.98$), suggesting that BLI could be used to track cell fate in vivo (Figure 1b). Cell characteristics were further analysed by LSR Flow Cytometry (Becton-Dickinson Immunocytometry Systems) and FlowJo analysis software (TreeStar, San Carlos, CA, USA). These cells expressed high levels of MSC-specific markers such as CD44 and CD90, but were negative for CD34 and CD45 markers present on haematopoietic stem cells and white blood cells (Figure 1c). Overall, the patterns of cell surface markers seen in BM-MSCsFluc+/eGFP+ were consistent with those reported in other studies [6,8]. Furthermore, there is no significant change of BM-MSCGFP+/GFP− morphology after culturing in the presence of different biomatrices for 48 h under the same culture conditions (Figure 1d).

To assess stem cell viability in different biomatrices in vivo, the same numbers (5 × 10^5) of MSCsFluc+/eGFP+ were suspended with: (a) PBS as a control; (b) Matrigel alone; (c) Collagen I alone; (d) Puramatrix alone; or (e) with an equal mixture of Matrigel, collagen I, and Puramatrix (20 µl total volume for all groups). Cells and biomatrices were implanted subcutaneously into the backs of adult athymic nude mice ($n = 10$) at five different sites. After transplantation, the mice were imaged repeatedly using the Xenogen In Vivo Imaging System (IVIS; Xenogen, Alameda, CA, USA). The reporter probe was d-luciferin (375 mg/kg body weight). BLI was acquired at 1 min intervals and activities expressed as photons/s/cm²/steradian (p/s/cm²/sr), as described previously [9]. BLI signals were normalized to day 0 and expressed as % activity of day 0. Within 2 h (day 0) after cell transplantation, BLI signals (mean ± SD) in all five groups were similar: 5.57 ± 0.58 × 10^5 p/sec/cm²/sr in PBS control; 6.90 ± 0.43 × 10^5 in Matrigel; 5.35 ± 0.62 × 10^5 in collagen I; 2.76 ± 0.23 × 10^5 in Puramatrix; and 4.44 ± 0.41 × 10^5 in the mixture group ($p = $ not significant; Figure 2a). However, the BLI signals subsequently decreased progressively in all groups. By 1 month, the PBS control and Puramatrix groups had significantly lower activities (1.6–1.8%) compared to the others (5.1–8.2%) ($p < 0.05$) as shown in Figure 2b. Interestingly, after 5 months of follow-up, cell signals in the Matrigel and mixture groups (5–9%) maintained significantly higher signal activities compared to the
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(a)

(b)

(c)

Figure 2. Bioluminescence imaging of transplanted MSCsFluc+/eGFP+ in living animals. (a) To assess longitudinal cell survival, animals were imaged for 5 months after subcutaneous injection of $5 \times 10^5$ MSCsFluc+/eGFP+ mixed with PBS control, Matrigel, Collagen 1, Puramatrix, and mixture. (b) Quantification of BLI signals showed a drastic decrease of Fluc activities from day 2 to month 1. After that, the BLI signals in the Matrigel and mixture groups remained stable compared with the other three groups. BLI signals were all normalized to day 0 in each group. (c) Postmortem immunohistochemistry staining of eGFP by confocal fluorescence microscopy revealed more robust engraftment of MSCsFluc+/eGFP+ within the Matrigel and mixture groups compared to the PBS control and Collagen 1 groups, consistent with the non-invasive BLI data. Scale bars: 50 µm

In summary, we have demonstrated that Matrigel-supported stem cell engraftment is superior to cells alone (PBS control) or cells supported with other matrices (Collagen 1 and Puramatrix). This can be attributed to the special properties of the Matrigel basement membrane matrix, which is known to release various growth factors and to provide structural support for cell seeding and differentiation [10]. These data support the notion that loss of transplanted cells occurring within the heterotopic sites is likely due to the lack of appropriate extracellular matrix components needed for the cells to attach and develop in their new environment. We succeeded in assessing the longitudinal survival course and proliferation of engrafted cells by performing high-throughput in vivo BLI. This technology is superior to the traditional histology-based assessment of cell grafts, which cannot provide longitudinal information within the same animal [11]. To fully realize the clinical potential of tissue engineering, careful in vivo evaluation of the viability of engrafted cells and/or tissues will be needed. Further optimization and validation of both the supporting biomatrices and imaging technology will advance the development of this burgeoning and exciting field.

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References


