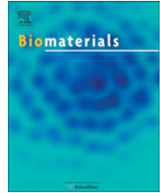
Biomaterials 31 (2010) 3244–3252

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials



Extracellular matrix-mediated osteogenic differentiation of murine embryonic stem cells

Nicholas D. Evans [a,b], Eileen Gentleman [a,b], Xinyong Chen [d], Clive J. Roberts [d], Julia M. Polak [c], Molly M. Stevens [a,b,*]

[a] Department of Materials, Tissue Engineering and Regenerative Medicine Centre, Imperial College London, South Kensington, London SW7 2AZ, UK
[b] Institute of Biomedical Engineering, Tissue Engineering and Regenerative Medicine Centre, Imperial College London, South Kensington, London SW7 2AZ, UK
[c] Department of Chemical Engineering, Tissue Engineering and Regenerative Medicine Centre, Imperial College London, South Kensington, London SW7 2AZ, UK
[d] School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK

A R T I C L E I N F O

Article history:
Received 3 November 2009
Accepted 10 January 2010
Available online 9 February 2010

Keywords:
Embryonic stem cells
Extracellular matrix
Osteogenic
Decellularised matrix
Tissue engineering
Differentiation

A B S T R A C T

Embryonic stem cells (ESCs) are pluripotent and have the ability to differentiate into mineralising cells in vitro. The use of pluripotent cells in engineered bone substitutes will benefit from the development of bioactive scaffolds which encourage cell differentiation and tissue development. Extracellular matrix (ECM) may be a suitable candidate for use in such scaffolds since it plays an active role in cellular differentiation. Here, we test the hypothesis that tissue-specific ECM influences the differentiation of murine ESCs. We induced murine ESCs to differentiate by embryoid body formation, followed by dissociation and culture on ECM prepared by decellularisation of either osteogenic cell (MC3T3-E1) or non-osteogenic cell (A549) cultures, or on defined collagen type I matrix. We assessed osteogenic differentiation by formation of mineralised tissue and osteogenic gene expression, and found it to be significantly greater on MC3T3-E1 matrices than on any other matrix. The osteogenic effect of MC3T3-E1 matrix was reduced by heat treatment and abolished by trypsin, suggesting a bioactive proteinaceous component. These results demonstrate that decellularised bone-specific ECM promotes the osteogenic differentiation of ESCs. Our results are of fundamental interest and may help in tailoring scaffolds for tissue engineering applications which both incorporate tissue-specific ECM signals and stimulate stem-cell differentiation.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Although bone has a remarkable capacity to heal itself, disease or injury often results in a loss of tissue too significant for the body to replace naturally. Bone autografts [1], which provide the best clinical outcome, are associated with severe pain and morbidity at the site of removal [2]. Allogenic transplants are also used, but are often of poor quality, carrying the risk of rejection and the transmission of disease from donor to recipient [3]. Due to these drawbacks, the development of new treatments – ideally those which simulate the low immunogenicity and healing effects of autografts – is a pressing need.

One possible solution involves engineering new tissue using an artificial biomaterial scaffold containing a source of cells, which may develop into a tissue when implanted into the body [4]. Marrow stromal cells (or mesenchymal stem cells; MSCs) are a potential cell source for such strategies, but they have limited proliferative potential (which decreases with age [5]), and it may prove difficult to expand enough cells for regenerating large tissue defects. Pluripotent cells are potential alternative candidates because they can self-renew (and so are potentially unlimited in supply), they may be tissue-matched to the recipient [6], and because diffusible moieties including β-glycerophosphate, ascorbate and dexamethasone [7,8], compactin [9], retinoic acid and bone morphogenic proteins (BMPs) [10,11], can induce stem cells to differentiate into cells and tissue which exhibit molecular and ultrastructural features of mature bone tissue [12]. Biomaterial scaffolds can incorporate extracellular matrix (ECM) components which are likely to play a critical role in bone tissue engineering where cells are intimately associated with a scaffold. To our knowledge, the effect of the insoluble ECM on osteogenic ESC differentiation has not yet been investigated, despite the fundamental insights that such a study may reveal. Biomaterial scaffolds provide an opportunity not only for physical support but also for the controlled presentation of appropriate biological cues.

* Corresponding author at: Department of Materials, Tissue Engineering and Regenerative Medicine Centre, Imperial College London, South Kensington, London SW7 2AZ, UK. Tel.: +44 (0)20 7594 6804; fax: +44 (0)20 7594 6757.
E-mail address: m.stevens@imperial.ac.uk (M.M. Stevens).

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.01.039

Improving bioactivity through the incorporation of tailored tissue-specific ECM signals is therefore of importance in scaffold-based bone regeneration applications.

ECM is established as a potent regulator of cell function and differentiation (for reviews see [13,14]), and there is already evidence that ECM affects differentiation in stem cells. For example, studies have shown that purified and cell-derived ECM proteins are important in the differentiation of ESCs to columnar and squamous epithelia [15], trophectoderm [16], pancreatic beta cells [17], and skin [18]. Although the effect of ECM on the differentiation of ESCs to bone has not yet been investigated, several previous studies have documented the effect of ECM proteins such as collagen type I, vitronectin and laminin-5 on the differentiation of marrow stromal cells (MSCs) [19–23], and demonstrated that such matrix-induced differentiation is partially dependent on signalling via the integrin family of cell-surface receptors [20,24]. In another study, an ECM derived from MSCs grown in osteogenic supplements increased bone formation significantly when MSCs were re-cultured on this matrix [25]. These observations are perhaps unsurprising as demineralised bone matrix (DBM) – the proteinaceous part of bone – readily mineralises to form new bone tissue when it is implanted into muscle tissue [26], indicating that it can play a role in inducing osteogenic differentiation. Indeed, demineralised, decellularised bone ECM is also routinely used to correct bone defects [27], and many commercial products including these ECM components have approval for clinical use in Europe and the USA [28]. Decellularised ECM is also used routinely for a number of other applications, including intestinal, bladder and skin reconstruction (see [4,29] for recent reviews).

ECM proteins therefore have the potential to play a significant role in the design of scaffolds in stem-cell based tissue engineering applications. Here we test the hypothesis that a bone-specific ECM, derived from a osteogenic cell line (MC3T3-E1), enhances the differentiation of ESCs to osteoblasts as compared to either collagen type I alone or non-bone-specific ECMs derived from alveolar epithelium (A549), pre-adipocyte (3T3-L1) and cardiomyocyte (HL-1) cell lines. The resulting findings are of fundamental interest and also have important consequences in the design of bioactive and biomimetic scaffolds to support bone formation and to direct osteoblast differentiation.

2. Methods

2.1. Cell culture

The murine embryonic stem cell (ESC) line E14 TG2α was cultured on surfaces coated with gelatin (0.1% w/v in phosphate buffered saline [PBS]) in DMEM supplemented with 10% (v/v) batch-tested FBS, 2 mM L-glutamine, 50 U/ml penicillin, 50 mg/mL streptomycin (Invitrogen, Paisley, UK) and 100 μM β-mercaptoethanol (Sigma, Poole, UK). Medium was further supplemented with leukaemia inhibitory factor (LIF) at 1000 U/ml (Chemicon, Chandler's Ford, UK). Cells were fed every day and were passaged every 3–4 days at around 50% confluence.

MC3T3-E1 cells (clone 4, ATCC catalogue number CRL-2593; ATCC Teddington, UK) were seeded at 10 000 cells/cm^2 and were grown in αMEM supplemented with 10% (v/v) FBS, 50 U/ml penicillin, and 50 mg/mL streptomycin. Cells were fed every 2–3 days. For differentiation, at confluence, cells were maintained for a further 10-days in the presence of 280 μM ascorbate, 10 mM β-glycerophosphate and 1 μM dexamethasone (Sigma, Poole, UK).

3T3-L1 and A549 cells (ATCC, Teddington, UK) were were seeded at 10 000 cells/cm^2 and grown in DMEM supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 mg/mL streptomycin. Cells were fed every 2–3 days.

HL-1 cells (a kind gift of Prof WC Claycomb, Louisiana State University, USA) were seeded at 10 000 cells/cm^2 and grown in Claycomb Medium (Sigma–Aldrich, Poole, UK) with 10% (v/v) FBS, 2 mM L-glutamine, 0.1 mM norepinephrine and 50 U/ml penicillin, and 50 mg/mL streptomycin. Wells were pre-coated with gelatin (0.1% w/v in PBS).

2.2. Cell removal and preparation of ECM

Cell-specific ECM was prepared using a previously reported protocol [29]. Confluent MC3T3-E1, A549, 3T3-L1 and HL-1 cells were washed twice in PBS at 4 °C, and were then incubated with 12 mM sodium deoxycholate (Sigma, Poole, UK) in 10 mM Tris–HCl, pH 8.0 at 4 °C for 10 min. ECM was also prepared using two other protocols [18,30] where sodium deoxycholate solution in the above protocol was replaced either with Hank's Balanced Saline Solution (HBSS) containing 20 mM EDTA, 10 mM EGTA and 20 mM HEPES, or 15 mM NH_4OH, respectively. Matrices were then washed with 4 changes of PBS, and were stored for up to 2 weeks at 4 °C. Where indicated, matrices were treated either by heating matrices at 70 °C for 15 min by floating matrices in a water bath, or by treating matrices with 0.05% (w/v) trypsin in Hank's buffered salt solution (Invitrogen, Paisley, UK) for 15 min at 37 °C.

Collagen coating was performed by spreading 5 μg collagen (from a 2.2 mg/mL of collagen type I in 0.6% (v/v) acetic acid [First Link Ltd, West Mids, UK]) per cm^2 of tissue culture plastic (TCP). Following collagen coating, surfaces were allowed to dry thoroughly for 24–48 h before storage at 4 °C for up to 2 weeks.

2.3. Osteogenic differentiation of ESCs

ESCs were induced to differentiate by embryoid body (EB) formation. LIF was removed and ESCs were partially dissociated into clumps of 15–20 cells, transferred to bacteriological-grade 90 mm Petri dishes and were then cultured in suspension for 5 days in the presence of αMEM supplemented with 15% (v/v) FBS, 50 U/ml penicillin, and 50 mg/mL streptomycin with feeding after three days. 5 days following EB formation (day 0), EBs were washed in PBS and dissociated into single cells with trypsin-EDTA (Invitrogen, Paisley, UK). Cells were then used to assess attachment (see below) or were seeded onto various matrices at a density of 30 000 cells/cm^2 in αMEM supplemented with 10% (v/v) FBS, 50 U/ml penicillin, and 50 mg/mL streptomycin. Cells were then fed after 24 h with the same medium supplemented with 280 μM ascorbate, 10 mM β-glycerophosphate and 1 μM dexamethasone (differentiation medium) and were then fed every 2–3 days for a period of up to 21 days. In some experiments arginine-glycine-aspartate (RGD) peptide or arginine-glycine-glutamate (RGE) peptide (BA Chem, Weil am Rhine, Germany) dissolved in PBS were added 2 days following seeding to a final concentration of 0.1 or 1 mM. Where indicated, hamster anti-rat CD29 (integrin β_1 chain; BD Biosciences, Germany) dissolved in PBS with 0.1% (w/v) bovine serum albumin (BSA) was added at confluence (six days following seeding) to a final concentration of 1 μg/mL.

2.4. Cell attachment assay

Cells obtained from dissociated EBs (see above) were seeded at various concentrations in wells of a 96-well plate in the presence of αMEM supplemented with 10% (v/v) FBS, 50 U/ml penicillin, and 50 mg/mL streptomycin and with or without 0.1 mM RGD peptide or 1 μg/mL hamster anti-rat CD29 antibody. After 24 h, medium was removed and cells were washed twice in PBS to remove non-adherent cells. Adherent cells were then fixed for 20 min with 70% ethanol and washed twice in PBS. 50 μL of a 5 mg/mL solution of

Download English Version:

https://daneshyari.com/en/article/8129

Download Persian Version:

https://daneshyari.com/article/8129

Daneshyari.com