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Role of injured endothelial cells in the recruitment of human pulp cells

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Human pulp stem cells;
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Summary In restorative dentistry, deep cavity preparation may lead to partial destruction of the odontoblastic layer. However, newly formed odontoblast-like cells can replace the necrotic odontoblasts and secrete a reparative dentine matrix. While growth factors such as transforming growth factor β1 (TGFβ1) and bone morphogenetic proteins (BMP-2 and BMP-4) seem to be involved in the proliferation and differentiation of pulp cells, little is known about the migration of the newly proliferating stem cells to the injury site. Our hypothesis was that endothelial cell injury may be involved in directing these cells towards the injury site.

For this study, human pulp fibroblasts and L929 cells were fluorescence-labeled by transduction with the Enhanced Green Fluorescent Protein (EGFP). Similarly, human umbilical vein endothelial cells (HUVEC) were labeled with the Discosoma Red Fluorescent Protein-2 (DsRed2). Cell migration was then studied in an insert cell culture system. The HUVEC cells were cultured in the lower compartment while the human pulp fibroblasts or L929 were in the upper compartment.

After artificial injury to the HUVEC cells, only human pulp fibroblasts migrated to the lower compartment. At early time periods (4 days), migrating cells were randomly localized on the HUVEC layer. However, after 14 and 20 days, they were perfectly aligned along the injury site. In the absence of injury, no migration was observed.

These results suggest that, the endothelial injury is involved in the recruitment of odontoblast-like cells at the injury site.

Introduction

In pathological conditions, such as mild carious dentine lesions, odontoblastic activity is stimulated to elaborate reactionary dentine. Severe lesions or deep cavity preparation may lead to local odontoblast death. However, newly formed cells attracted

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to the injury site, can differentiate into odontoblast-like cells and secrete a reparative dentine matrix. Several lines of evidence suggest the presence of progenitor or postnatal stem cells in the pulp capable of differentiation into odontoblast-like cells and secretion of reparative dentine in vitro.[1] After transplantation into immunocompromised mice, a dentine-like structure lined with odontoblasts and surrounding a pulp like interstitial tissue was obtained.[2]

Growth factors such as transforming growth factors (TGFβs), bone morphogenetic proteins (BMPs) fibroblast growth factors (FGFs) and insulin growth factors (IGFs) are secreted by functional odontoblasts and pulp fibroblasts, and are sequestered in the dentine matrix which can be considered as a reservoir of growth factors.[3,4] After carious injury, these factors are released as the dentine is demineralised with the acidic environment resulting from plaque bacterial metabolism.[5,6] Among these growth factors, TGFβ1 and BMP-2 and BMP-4 seem to be involved in the proliferation and differentiation of pulp cells, thus providing chemotactic signals to recruit progenitor pulp cells at the injury site and to initiate tissue repair.[7,8]

On the other hand, after surgical pulp amputation, healing can occur with hard tissue formation in germ-free animals independently of a local acidic environment.[9,10] This indicates the high healing potential of the dental pulp. However, under these conditions, very little is known about the migration and recruitment of the newly proliferating progenitor cells to the injury site. Moreover, pulp injury is likely to also involve the blood vessels within this organ. It is well established that injured endothelial cells release signaling molecules to initiate inflammatory reactions and the healing process.[7] Our hypothesis was that endothelial cells injury may also be involved in directing progenitor/stem cells towards the injury site.

Methods

Materials

The pEGFP-N1 and pDsRed2-N1 vectors were from Clonetech (Palo Alto, CA, USA). These plasmids carry the Enhanced Green Fluorescent Protein (EGFP)[11] and the Discosoma Red Fluorescent Protein (DsRed2),[12] respectively.

Culture media and reagents were purchased from BioWhittaker (Gagny, France). Chemicals were obtained from Sigma Chemicals Corporation (St. Louis, MO) unless otherwise stated.

The pL1-EGFP and pL1-DsRed2 constructs were prepared as described in Mathieu et al.[13] Human pulp fibroblasts prepared from immature third molars[1] and the L929 fibroblastic cell line (NCTC, Paisley, UK) were fluorescence-labeled by transduction with the Enhanced Green Fluorescent Protein according to Naldini et al.[14] as modified by Mathieu et al.[13] Similarly, human umbilical vein endothelial cells (HUVEC) (Cambrex Bioscience, MD, USA) were fluorescence-labeled by transduction with the Discosoma Red Fluorescent Protein-2. The insert cell culture system (Corning, NY, USA) containing a polycarbonate membrane (8 μ pore size) was used to study cell migration in response to injury. In the Insert culture system, the DsRed2-labeled HUVEC cells were cultured in EBM-2 medium (Cambrex Bioscience, MD, USA) in the lower compartment while the EGFP-labeled human pulp fibroblasts or L929 were cultured in DMEM medium (4.5 g/L glucose) in the upper compartment.

When the cells reached confluence, both cell types were incubated in the EBM-2 medium and mounted in the insert culture system. Injuries were performed mechanically with sterile scalpels on the HUVEC cells in the lower compartment. The scalpels

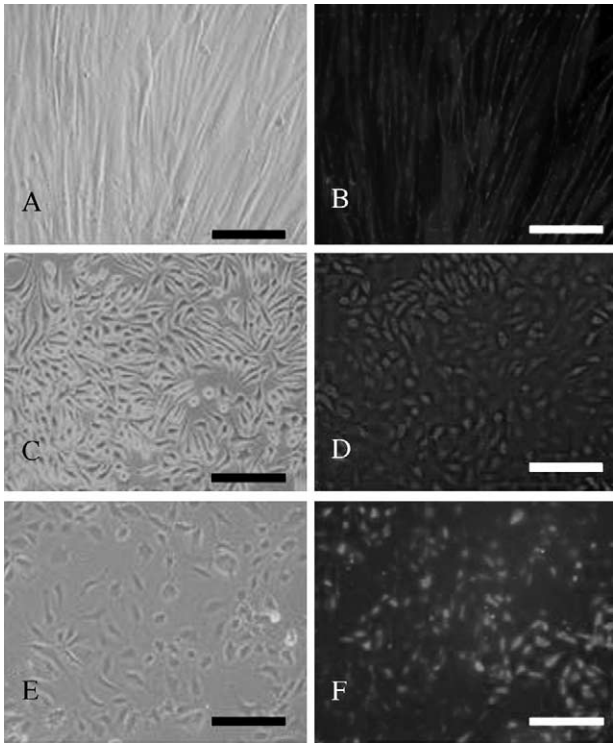

Figure 1 Fluorescence transduction of the cells: pulp fibroblasts and L929 fibroblastic cell were EGFP-labeled. Human endothelial HUVEC cells were DsRed2-labeled. Micrographs represent the phase contrast and fluorescence labeling of pulp fibroblasts (A and B), L929 (C and D) and HUVEC cells (E and F). Scale bars: 100 μm.