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The influence of elastin-like recombinant polymer on the self-renewing potential of a 3D tissue equivalent derived from human lamina propria fibroblasts and oral epithelial cells

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ABSTRACT

Three-dimensional epithelial tissue equivalents tend to lose their self-renewing potential progressively during culture as their epithelial cells lose their proliferative capacity with time. Even though the tissue engineered construct can mimic the native tissue well, it rapidly degrades after implantation due to the insufficient number of proliferating cells in the equivalent. In the present study we demonstrate for the first time that the use of an elastin-like recombinant polymer (ELR) engineered to contain the cell adhesion peptide RGD can result in a 3D tissue equivalent with high self-renewing potential, containing as many proliferative cells as the native tissue itself. The 3D tissue equivalent was reconstructed by the coculture of human lamina propria fibroblasts and oral epithelial cells in the nanofibrous ELR-collagen scaffold. Histological, immunohistological and transmission electron microscopic analyses of this oral mucosa equivalent demonstrated the expression of markers characteristic of epithelial proliferation (Ki67) and differentiation (keratin 13), and also the presence of a pluristratified epithelium and an ultrastructurally well-organized basement membrane expressing laminin 332. The synthesis of new extracellular matrix by the fibroblasts was also demonstrated. The scaffold proposed here presents great potential for tissue engineering applications, and also for studies of epithelial proliferation, and epithelial disorders including carcinogenesis.

1. Introduction

Recombinant polymers (which have also been termed 'Recombinamers' in recent publications [1]), are macromolecules produced using recombinant DNA technology by introducing a desired gene into the genetic content of a host organism such as microorganisms, plants or other eukaryotic organisms. This way, it is possible to bioengineer protein-based polymers of well-defined and complex structure [1]. Elastin-like recombinant polymers (ELRs), which form a subclass of protein-based recombinant polymers, are composed of the pentapeptide repeat Val-Pro-Gly-Xaa-Gly (VPGXG), which mimics from the hydrophobic domain of tropoelastin where X represents any natural or modified amino acid, except proline [2]. The first ELR products were simple peptides, to which the cells did not attach. Soon after, they were enriched with short peptide sequences having specific bioactivity [3] and have been used as coatings [4], films [5] for improved cell attachment, and as hydrogels to promote chondrogenesis [6].

The major challenge for tissue engineering in terms of success after grafting is to secure the survival of the cultured cells [7]. Indeed, oral mucosal epithelial cell sheets successfully reconstructed *in vitro* were reported to degenerate one week after transplantation [8]. It was suggested that host subcutaneous tissues were unable to promote maintenance of stem and progenitor cells and therefore could not produce long-term survival [8]. This indicates the importance of the presence of highly proliferative cells such as stem or progenitor cells in the tissue engineered construct to ensure its self-renewal and therefore its post-transplantation viability. The native oral mucosa itself has all its basal cells in proliferative stage, which are responsible of the high turnover and

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