Biomaterials 33 (2012) 5287–5296

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Characterization of natural, decellularized and reseeded porcine tooth bud matrices

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ARTICLE INFO

Article history:
Received 12 March 2012
Accepted 1 April 2012
Available online 30 April 2012

Keywords:
Decellularization
Second harmonic generation (SHG)
Tooth bud
Extracellular matrix

ABSTRACT

Dental tissue engineering efforts have yet to identify scaffolds that instruct the formation of bioengineered teeth of predetermined size and shape. Here we investigated whether extracellular matrix (ECM) molecules present in natural tooth scaffolds can provide insight on how to achieve this goal. We describe methods to effectively decellularize and demineralize porcine molar tooth buds, while preserving natural ECM protein gradients. Natural tooth ECM composition was assessed using histological and immunohistochemical (IHC) analyses of fibrillar and basement membrane proteins. Our results showed that Collagen I, Fibronectin, Collagen IV, and Laminin gradients were detected in natural tooth tissues, and retained in decellularized samples. Second harmonic generation (SHG) image analysis and 3D reconstructions were used to show that natural tooth tissue exhibited higher collagen fiber density, and less oriented and less organized collagen fibers, as compared to decellularized tooth tissue. We also found that reseeded decellularized tooth scaffolds exhibited distinctive collagen content and organization as compared to decelluarized scaffolds. Our results show that SHG allows for quantitative assessment of ECM features that are not easily characterized using traditional histological analyses. In summary, our results demonstrate the potential for natural decellularized molar tooth ECM to instruct dental cell matrix synthesis, and lay the foundation for future use of biomimetic scaffolds for dental tissue engineering applications.

1. Introduction

Current efforts in whole tooth tissue engineering focus on identifying methods to accurately control bioengineered tooth size and shape, create functional tooth roots, and eliminate ectopic mineralized tissue formation in *in vivo* implanted bioengineered tooth and bone constructs. To date, strategies for tooth tissue engineering have utilized a variety of scaffold materials, growth factors, and cell sources, achieving some level of success [1–4]. We hypothesized that detailed characterizations of extracellular matrix (ECM) composition and organization in natural tooth development could facilitate human tooth tissue engineering efforts. Evidence in support of this includes the fact that amelogenin and its associated natural cleavage products have been shown to direct the proper self-assembly of enamel crystals into microribbons [5], and that biglycan decorated nanofiber scaffolds can induce amelogenin expression and subsequent enamel formation and maturation [6–8]. These and other reports indicate that functional characterizations of tooth expressed ECM molecules, including their respective developmental and spatial organization, may facilitate the design of effective scaffolds for tooth regeneration.

Based on the fact that the ECM provides morphogenetic cues that guide proper cellular interactions during natural and bioengineered organogenesis, recent reports have focused on elucidating roles for natural ECM molecules and gradients in craniofacial tissues and organs [9–11]. In the tooth bud, dental epithelial and dental mesenchymal cell layers develop into enamel and pulp organs, respectively. As the tooth matures, dental mesenchymal cells differentiate into odontoblasts and secrete a matrix that eventually mineralizes to form dentin, and dental epithelial cells differentiate into ameloblasts, which secrete an enamel matrix. To date, the fabrication of biomimetic scaffolds that support robust dentin and enamel formation in a predictable manner remains an elusive goal. Recently, tissue decellularization methods have been used to preserve natural tissue-specific ECM composition and spatial organization, creating acellular scaffolds for a variety of

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doi:10.1016/j.biomaterials.2012.04.010