Viscoelastic Properties of Dental Pulp Tissue and Ramifications on Biomaterial Development for Pulp Regeneration

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Abstract

Introduction: A critical step in biomaterial selection effort is the determination of material as well as the biological properties of the target tissue. Previously, the selection of biomaterials and carriers for dental pulp regeneration has been solely based on empirical experience. **Methods:** In this study, first, the linear viscoelastic material functions and compressive properties of miniature pig dental pulp were characterized using small-amplitude oscillatory shear and uniaxial compression at a constant rate. They were then compared with the properties of hydrogels (ie, agarose, alginate, and collagen) that are widely used in tissue regeneration. **Results:** The comparisons of the linear viscoelastic material functions of the native pulp tissue with those of the 3 hydrogels revealed the gel-like behavior of the pulp tissue over a relatively large range of time scales (ie, over the frequency range of 0.1–100 rps). At the constant gelation agent concentration of 2%, the dynamic properties (ie, storage and loss moduli and the tanδ) of the collagen-based gel approached those of the native tissue. Under uniaxial compression, the peak normal stresses and compressive moduli of the agarose gel were similar to those of the native tissue, whereas alginate and collagen exhibited significantly lower compressive properties. **Conclusions:** The linear viscoelastic and uniaxial compressive properties of the dental pulp tissue reported here should enable the more appropriate selection of biogels for dental pulp regeneration via the better tailoring of gelation agents and their concentrations to better mimic the dynamic and compressive properties of native pulp tissue. *(J Endod 2015;41:1711–1717)*

Key Words

Biomaterial, compression, pulp, regeneration, tooth, viscoelastic

Pulp tissue is the only soft tissue in a tooth and serves primarily to maintain its own physiological functions as well as those of dentin through blood supply and nerves. Dental pulp tissue is a reservoir of multiple cell types including odontoblasts that reside on mineralized dentin surface in addition to abundant fibroblasts that are populated in a matrix of blood vessels and nerve endings. The extracellular matrix of dental pulp is also rich in terms of collagenous (collagens type 1: 56%, type 3: 41%, and type 5: 2%) and noncollagenous (chondroitin 4- and 6-sulfate: 60%, dermatan sulfate: 34%, keratan sulfate: 2%, and glycosaminoglycans as proteoglycans) proteins (1). The cells and the organic components of the dental pulp together determine the structural and functional nature of pulp tissue, with collagen type I likely contributing to its biomechanical properties, such as stiffness and strength (2), and proteoglycans mostly contributing to its viscoelasticity (1).

Despite reported clinical success, endodontically treated teeth become devitalized and brittle as well as susceptible to reinfections because of coronal leakage or microleakage, leading to considerable structural deformations, including removal of part of the enamel, dentin, and pulp during endodontic treatment, possibly resulting with tooth fracture and trauma (3). Because of extraction of the pulp tissue, endodontically treated teeth lose pulpal sensation and are unable to detect microbial challenges. If dental pulp can be regenerated, these complications may be avoided, and many teeth can be saved to function as native teeth.

Biomaterials for tooth regeneration need to be biocompatible and biodegradable; provide a suitable environment for cells that regenerate dental tissues; allow functionality for a variety of cells including ameloblasts, odontoblasts, cementoblasts, fibroblasts, vascular cells, and/or neural endings; be clinically applicable and easily handled by clinicians; and involve multiple structural characteristics because of diverse structures and functions of dental tissues. Although there are some insights for the selection of biomaterials for tooth regeneration based on experimental findings (4, 5), existing literature lacks benchmark data for the material properties of dental pulp tissue. The lack of data may stem from a lack of pertinent interest considering that dental pulp is confined and is shielded from direct exposure to mechanical stresses by several layers of hard mineralized tissues such as dentin, cementum, and enamel. However, many investigations have shown that the migration, proliferation, and differentiation of cells are intimately related to various physical properties of the scaffolding materials (6) including their viscoelasticity (7). Obviously, the viscoelastic material functions and biomechanical behavior of the native pulp tissue need to be known to allow their mimicry as a tool for biomaterial selection and development for regenerative dentistry. Such properties can then be used for the selection and

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tailoring of biogels to act as scaffolds for tissue engineering of dental pulp, and collagen, agarose, and alginate can be considered important candidates (3).

Collagen macromolecules, major constituents of the extracellular matrix, exist ubiquitously in diverse tissues including tooth (8) and have been used as scaffolding material for dental pulp regeneration (9). Chemical cross-linking by the addition of cross-linking agents such as glutaraldehyde or diphenylphosphoryl azide can enhance the mechanical stiffness of collagen scaffolds (10), yet only at the expense of cell survival and biocompatibility (9). One of the major challenges in tissue engineering is vascularization. Research showed that *in vivo* implantation of endodontically treated human teeth in mouse dorsum yielded recellularized and revascularized connective tissue upon delivery of the basic fibroblast growth factor and/or the vascular endothelial growth factor in a collagen carrier (11). Similarly, delivery of dental pulp stem cells and dentin matrix protein-1 with collagen scaffolds in mice led to ectopic formation of dental pulp–like tissue (12).

Another hydrogel that is considered as a scaffolding material for pulp regeneration and that can allow cell infiltration and growth is agarose (13–15). Agarose derives from seaweed and forms thermally reversible gels (9). Agarose was earlier used for the regeneration of prismlike enamel tissue (13). It was also investigated for the effect of mechanical loading on osteogenesis upon seeding with human dental pulp stromal cells (14). Findings suggested that mechanical loading through a bioreactor mimicking the biting force to enhance human dental pulp stromal cell osteogenesis in an agarose scaffold promoted bone formation and/or prevented bone resorption. Agarose hydrogels can also accommodate 3-dimensional neurite extension from primary sensory ganglia *in vitro* (15).

A third hydrogel widely used as a scaffolding material in tissue regeneration is alginate, which is a naturally derived polysaccharide. Alginate is biocompatible and permeable to small molecular-weight proteins (16). The mechanical strength of alginate can be modified by altering the calcium content and cross-linking density (17). Alginate hydrogel has been loaded with exogenous transforming growth factor beta 1 for regeneration of the dentin-pulp complex and found to promote odontoblastlike cell differentiation with subsequent secretion of tubular dentin matrix (18).

Thus, previous investigations using these 3 hydrogels have indicated that there is a significant degree of freedom in altering the properties of gels via compounding them with various bioactives at various concentrations, cross-linking to different degrees, and generation of tissue constructs using various cell lines. However, effective tailoring of biomaterials for tissue regeneration also requires the availability of the properties of the native tissues to allow the matching of the properties of the biomaterials. Here, the linear viscoelastic properties and uniaxial compression behavior of miniature pig dental pulp tissue were characterized and compared with those of collagen, agarose, and alginate gels.

Materials and Methods

Dental Pulp Harvesting

Pulp tissue was obtained from the mandibular canine of a 28-month-old miniature pig (single donor, $n = 3$) following a protocol approved by the Institutional Animal Care and Use Committee of Columbia University. Briefly, after the animal was euthanized, the tooth was extracted and cut into half approximately at equal distances from the coronal and apical regions using a saw (Fig. 1*A*). The fresh pulp

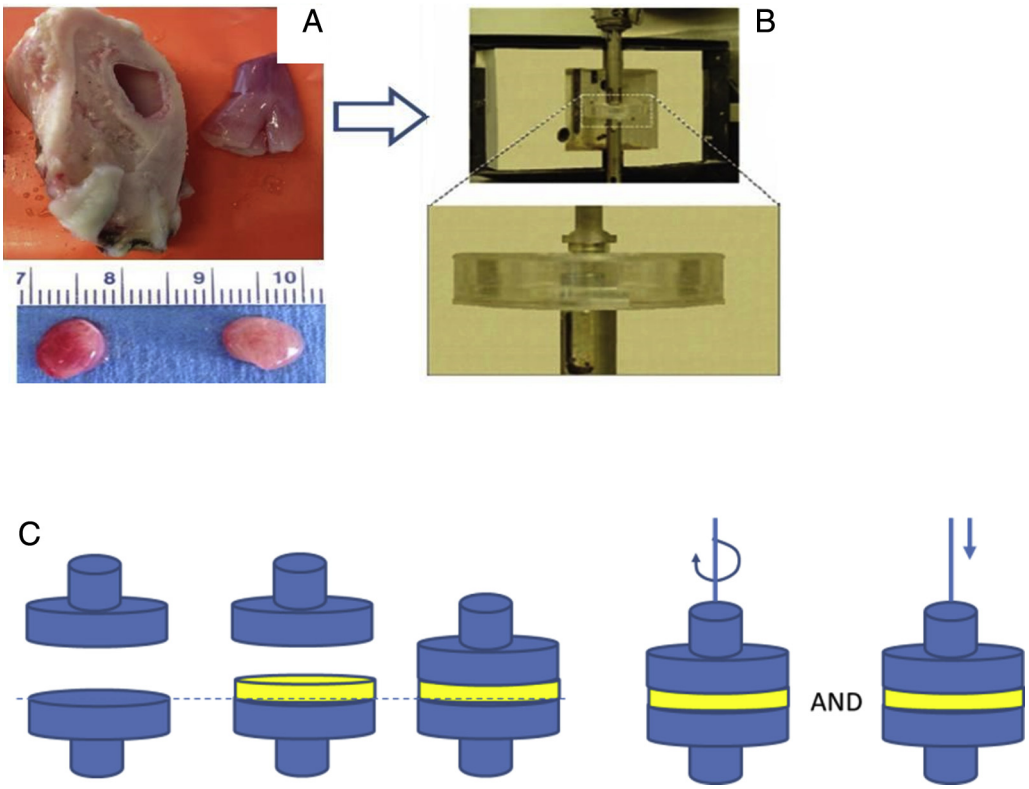

Figure 1. Harvesting and characterization of dental pulp tissue obtained from different locations ($n = 3$) of the canine of a 28-month-old miniature pig. (*A*) Extraction and sampling of dental pulp from the pulp chamber and rheological characterization in PBS using a (*B*) custom-made hydration chamber under (*C*) shear and compression.