DENTAL MATERIALS 30 (2014) e349–e361

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

The type and composition of alginate and hyaluronic-based hydrogels influence the viability of stem cells of the apical papilla

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ARTICLE INFO

Article history:
Received 28 February 2014
Received in revised form 8 July 2014
Accepted 8 August 2014

Keywords:
Tissue engineering
Stem cells
SCAP
Dental pulp
Hydrogel
Alginate
Hyaluronic acid
Regenerative endodontics

ABSTRACT

Objective. The goal of the present work was to evaluate *in vitro* and *in vivo* the influence of various types and compositions of natural hydrogels on the viability and metabolic activity of SCAPs.

Methods. Two alginate, three hyaluronic-based (Corgel™) hydrogel formulations and Matrigel were characterized for their mechanical, surface and microstructure properties using rheology, X-ray photoelectron spectroscopy and scanning electron microscopy, respectively. A characterized SCAP cell line (RP89 cells) was encapsulated in the different experimental hydrogel formulations. Cells were cultured *in vitro*, or implanted in cyclosporine treated mice. *In vitro* cell viability was evaluated using a Live/Dead assay and *in vitro* cellular metabolic activity was evaluated with a MTS assay. *In vivo* cell apoptosis was evaluated by a TUNEL test and RP89 cells were identified by human mitochondria immunostaining.

Results. Hydrogel composition influenced their mechanical and surface properties, and their microstructure. *In vitro* cell viability was above 80% after 2 days but decreased significantly after 7 days (60–40%). Viability at day 7 was the highest in Matrigel (70%) and then in Corgel 1.5 (60%). Metabolic activity increased over time in all the hydrogels, excepted in alginate SLM. SCAPs survived after 1 week *in vivo* with low apoptosis (<1%). The highest number of RP89 cells was found in Corgel 5.5 (140 cells/mm^2).

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http://dx.doi.org/10.1016/j.dental.2014.08.369

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Significance. Collectively, these data demonstrate that SCAP viability was directly modulated by hydrogel composition and suggest that a commercially available hyaluronic acid-based formulation might be a suitable delivery vehicle for SCAP-based dental pulp regeneration strategies.

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1. Introduction

In the past decade, regenerative endodontics have become accepted clinical procedures that aim at regenerating the pulp tissue after necrosis. These procedures have been generally focused on immature teeth [1,2], in order to allow for the immature root to develop both in length and in thickness, hoping to reduce the risk of ulterior fracture. Currently, the clinical endodontic regenerative procedure consists of chemical cleaning of the root-canal space using low concentration sodium hypochlorite, with little or no mechanical instrumentation of the root-canal surface. Then, after placement of an inter-appointment medication (either an antibiotic mixture or as suggested more recently calcium hydroxide [3]), the root canal is rinsed with EDTA, dried, and bleeding is induced by instrumentation beyond the apex [4]. The blood clot formed will then serve as an *in situ* scaffold for the migration – proliferation – differentiation of progenitor stem cells, originating from the apical papilla, which can remain vital even in the presence of periapical radiolucency [4,5]. An alternative strategy would consist in directly delivering progenitor stem cells from the apical papilla (SCAPs) into the root canal by incorporating them in hydrogels that would undergo gelification *in situ*. As mentioned by Murray et al. [6], more research is needed to develop hydrogels for this specific application, and provide the appropriate conditions for survival, proliferation and differentiation of the SCAPs.

One important feature for the hydrogel to be used in regenerative procedure is to provide appropriate conditions to ensure cell viability by mimicking the microenvironment of target tissues. For soft tissue as for dental pulp regeneration, injectable hydrogels are clinically convenient and seem appropriate in terms of cell differentiation through mechanotransduction [7,8]. The properties of hydrogels, including chemical composition, stiffness and network porosity and permeability, have been shown to have a significant impact on the survival and differentiation of encapsulated mesenchymal stem cells [9,10]. Alginates have been used as injectable cell transplantation vehicles due to their biocompatibility, low toxicity, and relatively low cost [11]. Alginate hydrogels are formed by cross-linking the polysaccharide chains by ionic bridges with divalent cations to form a water-insoluble network. Cells may be encapsulated during the cross-linking process to create cell-hydrogel constructs [12]. Natural materials such as hyaluronic acid have also been widely used for tissue regeneration. Hyaluronic acid is a high molecular weight glycosaminoglycan composed of D-glucuronic acid and N-acetyl-D-glucosamine. It interacts with hyaluronic acid-binding proteoglycans as well as collagen [13]. Hyaluronic acid is a natural component of the extracellular matrix (ECM) [14] and cells, including human SCAPs, possess CD44 [15] and other receptors that interact with hyaluronic acid and initiate signaling cascades [9]. Recently, a modified hyaluronic acid-based hydrogel has become commercially available under the name Corgel™. It presents the advantage to combine the potentials of natural and synthetic polymers and also, to be a reliable source of well-characterized hydrogel with controlled properties. This type of hydrogel is indicated for surgical applications and regenerative medicine, but few data are available to date [16].

Currently, there is no formal comparison of the influence of various injectable hydrogels on the survival of SCAPs. The hydrogel mechanical and chemical properties as well as its network structure interacting with the cells are known to affect the cell viability and behavior [17]. We hypothesized that hydrogel properties will influence SCAP viability and metabolic activity. Hence, the goal of the present work was to evaluate *in vitro* and *in vivo* the influence of various types and compositions of alginate and hyaluronic-based hydrogels on the viability and metabolic activity of SCAPs, with special focus on several key characteristics of the hydrogels: mechanical properties, pore size, chemical surface composition. A fully characterized cell line population of SCAPs (RP89 cells) was used in this study [18].

2. Materials and methods

2.1. *Hydrogels formulation*

Alginates of different compositions were purchased from Pronova (FMC BioPolymers, NovaMatrix, Philadelphia, USA). Alginate UP MVG (MVG: medium viscosity, >60% guluronate monomer units) and SLM100 (SLM: sterile, low viscosity, >50% mannuronate monomer units) have a molecular weight > 200 kDa and a G/M ratio >1.5 and <1, respectively. Corgel™ hydrogels (Corgel™ BioHydrogel Lifecore kit, Lifecore Biomedical, Chaska, USA) are hyaluronic acid-based hydrogels which form covalent bonds under the action of peroxidase and hydrogen peroxide [19]. Corgel™ polymers with three different tyramine substitution degrees were used (1.5%, 2.8% and 5.5% for Corgel 1.5, Corgel 2.8 and Corgel 5.5, respectively). 0.5% (w/v) Alginate MVG [20] and 1% (w/v) alginate SLM [21] were prepared by dissolving alginate in 3-(N-morpholino)propanesulfonic acid, 4-morpholinepropanesulfonic acid (MOPS) buffer (Sigma–Aldrich, Saint Louis, USA). Hydrogels were formed by addition of a 50 mM $CaCl_2$ in MOPS (Sigma–Aldrich) solution on the alginate solution. Corgel hydrogels were

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