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Photopolymerization of cell-laden gelatin methacryloyl hydrogels using a dental curing light for regenerative dentistry

Nelson Monteiro[a], *Greeshma Thrivikraman*[a], *Avathamsa Athirasala*[a], *Anthony Tahayeri*[a], *Cristiane M. França*[a,b], *Jack L. Ferracane*[a], *Luiz E. Bertassoni*[a,c,d,*]

[a] *Division of Biomaterials and Biomechanics, Department of Restorative Dentistry, OHSU School of Dentistry, Portland, OR, USA*

[b] *Biophotonics Applied to Health Sciences Post-Graduation Program, Nove de Julho University, São Paulo, SP, Brazil*

[c] *Department of Biomedical Engineering, School of Medicine, Oregon Health and Science University, Portland, OR, USA*

[d] *Center for Regenerative Medicine, Oregon Health and Science University, Portland, OR, USA*

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ABSTRACT

Photopolymerized hydrogels, such as gelatin methacryloyl (GelMA), have desirable biological and mechanical characteristics for a range of tissue engineering applications.

Objective. This study aimed to optimize a new method to photopolymerize GelMA using a dental curing light (DL).

Methods. Lithium acylphosphinate photo-initiator (LAP, 0.05, 0.067, 0.1% w/v) was evaluated for its ability to polymerize GelMA hydrogel precursors (10% w/v) encapsulated with odontoblast-like cells (OD21). Different irradiances (1650, 2300 and 3700 mW/cm^2) and photo-curing times (5–20 s) were tested, and compared against the parameters typically used in UV light photopolymerization (45 mW/cm^2, 0.1% w/v Irgacure 2959 as photoinitiator). Physical and mechanical properties of the photopolymerized GelMA hydrogels were determined. Cell viability was assessed using a live and dead assay kit.

Results. Comparing DL and UV polymerization methods, the DL method photopolymerized GelMA precursor faster and presented larger pore size than the UV polymerization method. The live and dead assay showed more than 80% of cells were viable when hydrogels were photopolymerized with the different DL irradiances. However, the cell viability decreased when the exposure time was increased to 20 s using the 1650 mW/cm^2 intensity, and when the LAP concentration was increased from 0.05 to 0.1%. Both DL and UV photocrosslinked hydrogels supported a high percentage of cell viability and enabled fabrication of micropatterns using a photolithography microfabrication technique.

* *Corresponding author at*: Biomaterials and Biomechanics, School of Dentistry, Oregon Health and Science University—OHSU, 2730 S.W. Moody Ave, Portland, OR 97201, USA.
E-mail address: bertasso@ohsu.edu (L.E. Bertassoni).

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Significance. The proposed method to photopolymerize GelMA cell-laden hydrogels using a dental curing light is effective and represents an important step towards the establishment of chair-side procedures in regenerative dentistry.

1. Introduction

Tissue engineering and regenerative medicine consist of delivering cells and bioactive agents (i.e. growth factors, nucleic acids) to injured sites to promote and restore tissue function [1–3]. Hydrogels, which are highly hydrated natural and synthetic biomaterials that closely replicate the structural and biological characteristics of the native extracellular matrix (ECM), have long been proposed as ideal candidates for cell delivery in regenerative medicine and dentistry [4]. Their characteristics, such as biocompatibility, biodegradability, tunable physical and chemical properties, and ease of fabrication, have made them attractive biomaterials for biomedical applications [5–7].

Various natural and synthetic materials have been chemically modified with photocrosslinkable functional groups, including gelatin, alginate, chitosan, collagen, polyethylene glycol, and many others [5]. These materials can be mixed with a photoinitiator that absorbs an appropriate wavelength of light and decomposes into free radicals to initiate photopolymerization and form hydrogels [5]. Photocrosslinkable hydrogels allow control over mechanical properties, swelling ratios and degradation rates [6,8,9], while being compatible with cell encapsulation, which allows for precise tuning of the 3D microenvironment surrounding cells in tissue engineering constructs. This, in turn, enables precise regulation of cell behavior, which may lead to more predictable outcomes in regenerative strategies [8–10]. Gelatin methacryloyl (GelMA), in particular, has additional desirable properties for tissue engineering. GelMA has been shown to possess matrix metalloproteinase (MMP) and RGD (Arg-Gly-Asp) responsive peptide motifs, which are known to enhance cell-mediated matrix degradation and binding, respectively [7,11,12].

Although several photoinitiators have been proposed for hydrogel cell encapsulation and photocrosslinking, Irgacure 2959 (2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone) has been the most commonly used for cell encapsulation and tissue engineering applications [13–17]. However, in addition to its low water solubility, the requirement for exposure to light at ultra-violet (UV) (365 nm) wavelengths is a significant limitation. UV light has been shown to have potential detrimental consequences for both delivered cells and host tissues, hence, the formation of free radicals upon longer UV exposure may lead to DNA damage and impair cellular function [5,14,18–20]. As a result, photoinitiators that absorb light in the visible region are considered advantageous over conventional UV photoinitiators. It was demonstrated that the visible light photoinitiator lithium acylphosphinate salt (LAP) has high water solubility and permits cell encapsulation at lower photoinitiator concentrations and longer light wavelength (405 nm), enabling efficient polymerization compared to Irgacure 2959 [14]. Also, visible light is expected to cause less damage to cells and to be more efficiently transmitted through tissues, allowing greater depth of cure [13,21]. Moreover, many devices, such as dental lamps, endoscopic probes, microscope imaging lamps and lasers emit light in the short wavelength visible spectrum, but not in the UV spectrum [14]. Especially, dental curing light devices that use light emitting diode (LED) technology have become the dominant visible light source for photopolymerizations due to their high energy [22,23].

Recently, we have demonstrated a novel strategy to engineer pre-vascularized, cell-laden hydrogel pulp-like tissue constructs in full-length root canals *in vitro* by sequential GelMA polymerization using UV-light [10]. Such techniques for oral regeneration can benefit from hydrogel polymerization using dental curing lights operating in the visible range (Fig. 1).

Therefore, to bring this new tissue engineering strategy in regenerative dentistry a step closer to clinical practice, a polymerization process using visible light dental curing devices (DL), which are FDA approved and well established in dental practice, is desirable. Considering the advantage of visible light relative to UV light, we carried out a study comparing the effect of visible DL and UV light polymerization methods on GelMA encapsulating odontoblast-like cells (OD21). First, we evaluated the effect of dental light irradiance, exposure time and photoinitiator concentration on OD21s encapsulated in GelMA. After determining the optimized condition to photopolymerize cell-laden GelMA with a DL instrument, we then compared the physical and mechanical properties of DL and UV light photopolymerized GelMA hydrogels. Lastly, we compared the viability of OD21 cells encapsulated in GelMA hydrogels polymerized with either DL or UV light polymerization.

2. Materials and methods

2.1. Gelatin methacryloyl (GelMA) synthesis

The syntheses of GelMA was performed as described previously [11]. Type A gelatin (10% w/v) from porcine skin (Sigma, St. Louis, MO, USA) was dissolved in Dulbecco's phosphate buffered saline (DPBS, Sigma). While stirring, the solution was heated to 50 °C. Then, 8% (v/v) methacrylic anhydride (Sigma) was added to the solution in a dropwise manner, allowing the reaction to proceed for 2 h at 50 °C, and subsequently stopping it by diluting 5 times with DPBS at 40 °C. The solutions were dialyzed against distilled water using 12–14 kDa dialysis tub-

URL: http://www.bertassonilab.com (L.E. Bertassoni).

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