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Hydrogels for light delivery in *in vivo* optogenetic applications

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ABSTRACT

Biocompatible hydrogels present interesting opportunities for *in vivo* waveguiding for optogenetic or photomedical applications. Here, we investigate the applicability of poly(ethylene glycol) diacrylate hydrogels in combination with scattering particles as optical diffusors. Gel characteristics and bioactivity can be tuned to achieve controlled light distribution and tissue interaction.

Keywords: hydrogel waveguide, light delivery, optogenetics, diffusor

1. INTRODUCTION

Continuing advances in the field of optogenetics are creating an unmet need for waveguides that are suitable for *in vivo* applications. Devices that are currently evaluated to fulfill the requirements include fiber-based systems and microLEDs[1]. For some applications, hydrogels constitute an interesting alternative. Hydrogel waveguides have not yet been thoroughly evaluated for use in optogenetics or photomedicine, although some studies already show their potential[2,3]. Transparent hydrogels possess good flexibility and waveguiding properties, and can be fabricated into optical fibers[4]. Their versatility and ease of production and modification make them ideal candidates for highly specialized applications. Here, we explore possibilities to make use of a hydrogel waveguide from poly(ethylene glycol) dimethacrylate (PEGDMA) as an optical diffusor. In photomedicine as well as optogenetics, it can be necessary to illuminate larger tissue areas than can be reached by fibers or microLEDs alone. Polystyrene particles can be embedded in the hydrogel and scatter the incident light, significantly extending the illuminated area. Characteristics such as elasticity, refractive index and transparency can be easily tuned by changing monomer length and concentration. Careful adjustment of all relevant properties enables production of specialized waveguides for controlled target illumination. Hydrogels from PEGDMA are bioinert, but cell attachment and tissue interaction can be achieved by chemical modification of the polymers and addition of biomolecules[5]. We present a waveguide that realizes efficient light diffusion and tissue integration at the surface by combining optimized waveguiding and scattering with controlled bioactivity. This waveguide can be adjusted to specific requirements in optogenetics or biomedicine.

2. MATERIALS AND METHODS

2.1 Hydrogel production

Poly(ethylene glycol) diacrylate (PEGDA, molecular weight 700; Sigma Aldrich, USA) or dimethacrylate (PEGDMA, molecular weight 8000 or 20000; Polysciences Inc, USA) was mixed with demineralized water at weight concentrations between 10 and 50%. To initialize polymerization, ammonium persulfate (APS, 10% w/v in H_2O; 1:100 final concentration) and TEMED (1:1000 final concentration; both Merck, Germany) were added to the PEGD(M)A/H_2O mixture, which was then poured into silicone molds and placed into a plastic chamber flooded with N_2. Gels were placed in demineralized water after approx. one hour and not used before the next day. Where particles were used, they were mixed with the solution before addition of APS/TEMED. In this study, polystyrene (PS) particles (0.625, 1 or 10 µm diameter; Kisker Biotech, Germany) were used for light diffusion and titanium dioxide (TiO_2) particles (~5 µm; Sigma Aldrich) for reflection.

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2.2 Hydrogel characterization

Refractive indices of hydrogels were measured via optical coherence tomography (central wavelength: 1325 nm; spectral bandwidth: 100 nm; swept source OCT, Thorlabs, USA). The refractive index was calculated as the ratio of optical thickness (distance between upper and lower hydrogel surface as displayed by OCT) and actual thickness.

Attenuation in PEGDMA 8000 and 20000 (10 %) was determined with cut-back measurements. Hydrogels were produced inside a silicone hose with 2 mm diameter. Light from a blue laser diode (450 nm) was coupled into the hydrogels and power output from the gel was measured. Then, the gel was shortened by 10 mm and output was measured again.

Transmission and reflection of different hydrogel compositions was measured in a PerkinElmer spectrophotometer (Lambda 900) with an integrating sphere. Different compositions were measured in transmission and reflection mode. Additionally, samples from PEGDMA 8000, 10 % containing TiO_2 particles at high concentrations were tested.

To evaluate light distribution in different hydrogel composites with or without scattering particles, the sample surface was observed with a CMOS camera. The imaging axis was perpendicular to the direction of the incident light.

2.3 Cell and tissue attachment

For collagen coating, hydrogels were produced as described above with additional 1 wt.-% methacrylic acid (Merck). Gels were washed in water for at least one week and then incubated for 30 minutes with 200 mM EDC and NHS (Merck) in 20 mM HEPES followed by at least four hours of incubation with rat tail collagen type I (5 mg/mL). Samples were washed with PBS and 3T3 fibroblasts were carefully pipetted on top of the hydrogel and cultivated in DMEM/F12 medium + 1% penicillin/streptomycin and 10 % fetal bovine serum. Imaging was performed with a confocal laser scanning microscope after staining the cells with 2 µM calcein-AM green (ThermoFisher, USA), a live cell calcium dye. To mimic tissue integration, a protein matrix from collagen and matrigel was produced on EDC/NHS-treated hydrogels.

3. RESULTS AND DISCUSSION

3.1 Tuning hydrogel properties

Varying monomer length and concentration evokes changes in the gel's elasticity, refractive index and transparency. Hydrogels became softer and more elastic with increasing water content. For successful tissue integration, the gel's elasticity needs to be tuned to match that of the target tissue[5]. Refractive index could be tuned in the range from 1.344 (20000, 10 %) to 1.437 (700, 90 %). This range is large enough to achieve waveguiding in a core-clad structure but will not yield total reflection at larger angles of incidence, allowing for controlled light distribution using gel-in-gel constructs

Figure 1: PEGDA 700 (illuminated from the right) in PEGDMA 20000. The PEGDA 700-gel bends towards the top, coupling light into the PEGDMA 20000-block where the incident angle is too large (white arrow).

(see Fig. 1). Transmission in the visible range as well as the near UV and near IR varied only slightly in samples with long polymer chains (8000 and 20000). Overall, the values were lower for PEGDA 700, which only becomes transparent above concentrations of 20 %. PEGDMA 8000 and 20000 samples showed >90 % transmission at wavelengths of interest for optogenetics (450-480 nm for Channelrhodopsin-2) or PDT sensitizers (red). Cut-back measurements confirmed low losses within the material. The attenuation coefficient for PEGDMA 8000, 10 % and 20000, 10 % was determined at 0.80 ± 0.22 dB/cm and 0.80 ± 0.27 dB/cm, respectively. Since *in vivo* illumination would be expected to take place on a centimeter scale, these characteristics constitute a good basis for an efficient waveguide.

3.2 Waveguiding and light diffusion

The favorable optical properties of a transparent hydrogel sample allow for light propagation on an undisturbed path through the gel (see Fig. 2a). Differences in the gels' refractive indices can be used to guide the direction of incident light in a more complex construct (see Fig. 1). To distribute the light to a larger area, polystyrene particles were dispersed inside the hydrogels (see Fig. 2b). In combination with a reflective TiO_2 layer (> 90 % reflection between 430 and 915 nm), the whole sample surface could be illuminated with a single point source (see Fig. 2c). At the same time, losses at the lower surface could be minimized (see Fig. 2d). For applications where the waveguide is to be placed besides or around the target tissue rather than inside of it, this architecture enables efficient illumination of a larger area while minimizing losses through the bottom surface. Using multiple and/or structured fibers in combination with a hydrogel diffusor ensures

sufficient light intensity over longer distances (Fig. 2e). Further studies will need to determine the distance over which light can be delivered to the desired target area with relevant intensities.

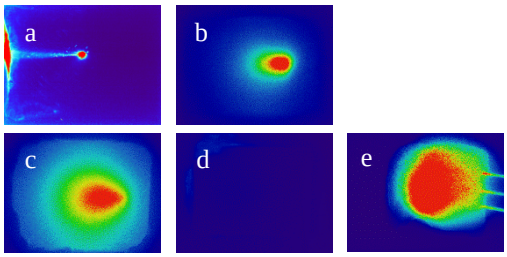

Figure 2: Pseudocolor images of light distribution on the surface of PEGDMA gels. a: no particles, brightness enhanced. b-d: 0.625 µm PS particles, same exposure time, c+d with TiO_2 layer (same sample). c shows enhanced light diffusion by introducing a reflective bottom TiO_2 layer; d shows the gel's bottom surface shielded by TiO_2 layer. e: Three structured fibers inserted into one hydrogel sample containing 1 µm PS particles (1 mg/mL). Sample size 6x8 mm.

3.3 Cell and tissue interaction

Fibroblasts were able to attach to and proliferate in a monolayer on collagen coated but not on untreated hydrogels. After 3-4 days, high confluency and good viability could be observed on coated samples (see Fig. 3). Cells grew only on the coating and did not invade the hydrogel samples. This modification of the otherwise bioinert hydrogel matrix allows for controlled tissue integration while ensuring stability of the waveguide. Instead of one collagen layer, a 3D protein matrix could be immobilized on EDC/NHS-treated PEGDMA gels. Therefore, the hydrogel waveguide is also suitable as a carrier matrix for 3D cell culture. Protein coating needs to be optimized with respect to the targeted cell type[6]. In principle, any type of protein can be coupled to the modified hydrogel surface. Depending on the application, cells can be embedded in the hydrogel as well[2]. It is therefore possible to combine versatile waveguiding, tunable elasticity and tailored bioactivity to produce hydrogel constructs that cover many special needs arising in optogenetics or photomedicine.

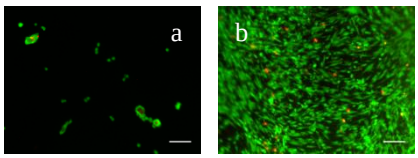

Figure 3: Calcein-stained 3T3 fibroblasts on uncoated (a) and collagen-coated PEG-DMA 8000, 10%. Cells in b display high confluency and good viability. Margins are out of focus due to slight unevenness of the hydrogel. Scale bar 100 µm.

4. ACKNOWLEDGMENTS

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