2024-06-18

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Funder: Irish Research Council, Science Foundation Ireland

Photopolymer Material Durability and Safety in Holographic Diffusers for Visual Applications

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ABSTRACT

This study introduces novel holographic diffuser applications employing acrylamide- or diacetone acrylamide-based photopolymers, patterned within the volume on a micron scale by a single-beam holographic recording process. These diffusers have previously been presented as potential treatments for amblyopia and diplopia. This work has now been extended to spectrometric analysis to determine their properties under broadband light. Diffusive elements with higher diffusion efficiencies exhibited a marginal reduction ($< 5\%$) in diffusion efficiency across most of the visible spectrum. Given the intended application of these holographic diffusers, cytotoxicity assessments were also performed. This is significant as there is a difference in toxicity between the crystalline acrylamide (classified as a category 3 material) and diacetone acrylamide (classified as a category 4 material). The findings indicated substantially lower toxicity in holograms produced with diacetone acrylamide-based photopolymer. The accelerated ageing of both formulations of holographic diffusers indicated that the acrylamide-based holographic diffusers did not reduce efficacy in the 292 days of ageing. The diacetone acrylamide-based holographic diffusers exhibited reduced efficacy by day 182. Despite this, both formulations have been shown to perform for prolonged periods as the treatment modality would require. These results emphasise that holographic diffusers exhibit minimal spectral impact, and longevity on the scale of treatment regimes which are crucial considerations for their prospective use case as treatments for amblyopia and diplopia.

Keywords: Holographic diffusers, hologram, holography, acrylamide photopolymer, diacetone acrylamide photopolymer, photopolymer ageing study, cytotoxicity.

1. INTRODUCTION

Amblyopia is a significant eye health issue for children around the world. It is a visual development impairment in which one eye has a significantly reduced ability to perceive than the other and is commonly referred to as 'lazy eye'. The common clinical definition for amblyopia is a reduction in best-corrected visual acuity (BCVA) of 2 lines (0.2 logMAR) or greater between the eyes in the absence of further ocular pathologies.[1–5] Further, *mild* amblyopia is classified as 0.2 or 0.3 logMAR difference between the eyes, *moderate* amblyopia as 0.3 - 0.8 logMAR difference, and *severe* amblyopia as >0.8 logMAR difference.[1]

The prevalence of amblyopia is estimated to be between 2% and 4%, with the incidence in different regions varying significantly. An Australian study (N = 4721) found a prevalence of unilateral amblyopia of 3.06%.[3] A study in India (N = 4020) found an overall prevalence of 1.1%.[4] A central China study (N = 2260) determined an incidence of 2.5%.[6] An Irish study (N = 1626) found an incidence of 5.1%.[7]

The Pediatric Eye Disease Investigator Group have conducted trials to investigate the efficacy of patching, atropine, and Bangerter foils and determined that they are effective treatments (adjunct to refractive error

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correction) for amblyopia. It was determined that at the 6-month mark in treatment the baseline improvement in visual acuity was 3.16 ± 0.21 (95% CI) lines for patching (N = 215), and 2.84 ± 0.23 (95% CI) lines for atropine use (N = 204).[8] In a separate study, it was determined that the baseline acuity improvement at 24 weeks was 1.9 lines for Bangerter foils (N = 89) and 2.3 lines in the patching group (N = 97).[9]

With relation to the downsides of current treatment methodologies, one significant issue with patching relates to compliance. The Monitored Occlusion Treatment of Amblyopia Study (MOTAS) showed that the average adherence to the prescribed patching regime was 49% (N = 94), with only 14% of participants achieving a mean adherence within 30 minutes of the prescribed dosage.[10] It was also separately determined using dose monitors that for 3-hour treatment levels (N = 20), there was a mean compliance of 57.5%, and for 6-hour treatment levels (N = 20), there was 41.2% adherence.[11] A disadvantage to atropine is that it can induce systemic side effects, which include dry mouth, tachycardia, delirium, photophobia, ocular pain, headache, and lowered seizure threshold, and these were present in 26% of patients.[2,8] For Bangerter foils, it has been shown that the effect on visual acuity has considerable variability across the various grades of foils, with the 0.3 through 0.6 graded foils producing similar results.[12] A further study showed that the 0.3 and 0.4 graded foils induced similar visual function deterioration.[13] A study by Odell *et al.* showed that the degree of visual degradation was not to the expected levels based on the labelling of the foils when considering near and distance optotype acuity, vernier acuity, and contrast sensitivity.[14] These issues highlight that the grading on a Bangerter foil may not accurately predict an individual's visual acuity penalisation as the label describes, which may affect the ability of clinicians to prescribe the treatment with confidence.

Diplopia is when a patient has the perception of a second image in another location and is due to a misalignment of the visual axes and can be a consequence of strabismus. This becomes intractable diplopia when the patient cannot suppress the unwanted image via neuroadaptation,,[15] and it can only be eliminated by occluding one eye.[16] A study in the UK found that there was an incidence of 53 cases of diplopia per year, with causes ranging from surgery (38%), spontaneous/no known triggering event (25%), to severe head trauma (8%).[16] The most effective treatment methods were opaque intraocular lenses (IOLs) (86%) and opaque contact lenses (50%),[16] noting these were small sample sizes, and three recipients of the IOLs had complications from the surgery. A further study on IOLs in the UK found that of 892 consultant ophthalmologists questioned, 72% would consider the implant, with 48 surgeons having implanted one.[17]

Holographic diffusers have been presented as a potential treatment modality for both amblyopia and diplopia.[18] This work will investigate the spectral response of the holographic diffusers, any potential cytotoxicity concerns, and the impact of ageing on the holographic diffusers.

2. METHODS

Both acrylamide- and diacetone acrylamide-based photopolymers were analysed during this work. The composition of each is highlighted in Table 1. The resulting liquid photopolymer was pipetted onto a substrate and allowed to gravity settle and dry for approximately 24 hours in a low-humidity environment. The photopolymer volume remained consistent with 560 μL deposited per LEXAN 8010 substrate for a thickness of 70 ± 5 μm. The substrate was laser cut to a circular shape with a square tab for handling. Once dry, they were covered using a 50 μm Melinex cover layer.

Table 1. Composition of the acrylamide and diacetone acrylamide photopolymer.

Component	Constituent	AA qty	DA qty
Monomer	Acrylamide—Diacetone acrylamide	5.71 g	5.00 g
Cross-Linker	Methylbisacrylamide	1.14 g	1.00 g
Binder	Polyvinyl Alcohol (in 100 ml DI water)	10 g	10 g
Electron Donor	Triethanolamine	11.4 ml	10.0 ml
Dye	Erythrosine B (Conc: 1.1 mg/cm^3)	23 ml	23 ml

The recording methods used have been shown previously[18,19] and utilise a single beam recording of a controllable speckle pattern into a photosensitive material. The power used at the recording plane was 1.00 mW/cm^2

± 5%. The recording times were varied from 20-80 s to obtain a range of diffusion efficiencies to perform the ageing, cytotoxicity, and spectroscopy analysis. The holographic diffusers were recorded to a diffusion efficiency of 92% ± 2% for the ageing analysis.

2.1 Spectroscopic Analysis

Spectroscopic profiles through the holographic diffusers were obtained to determine how different wavelengths propagated through the elements. The optical setup shown in Figure 1 was used.

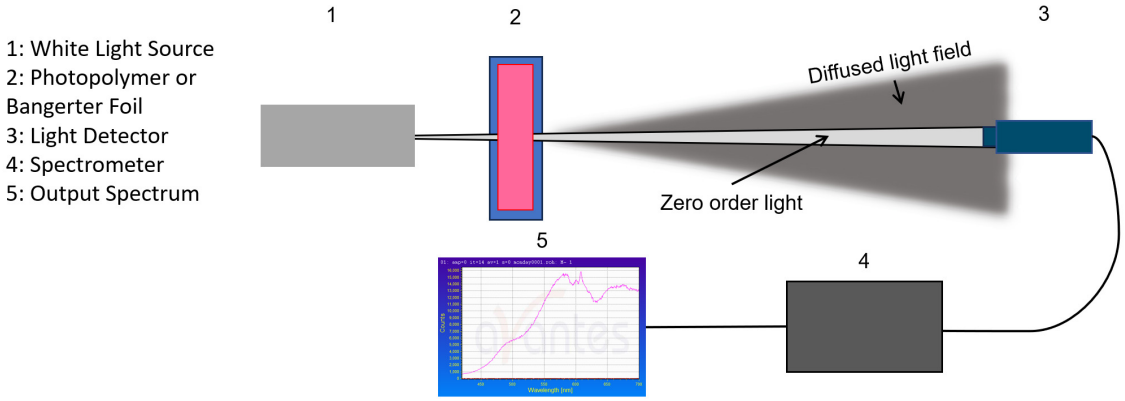

Figure 1. Optical configuration for the spectroscopic analysis of holographic diffusers effect on white light.

An Avaspec-2048-spu spectrometer was arranged so the white light from a fibre source (Avalight-HAL-S) was coupled into the spectrometer. Within the white light beam path, a sample was positioned so that the beam was 6 mm in diameter when incident upon the sample to mimic the human pupil size. The light detector was placed 20 cm away, where the zero-order light overfilled the lens of the light detector. A dark reading was taken with the light source blocked off, and then the spectrum through the control was taken three times. Following this, recorded samples were placed within the beam path, and the spectrum was averaged over 50 data times each to reduce the impact of read noise.

2.2 Cytotoxicity

The chemical materials used to determine the cytotoxicity of the holographic diffusers are highlighted in Table 2. HaCaT, an immortal cell line, were cultured in DMEM-F12 medium supplemented with 10% FBS (fetal bovine serum) at 37 ^{o}C in a humidified incubator at 5% CO_2.

Table 2. Materials used in the cytotoxicity analysis.

Material	Supplier	Identifier
Phosphate buffered saline (PBS)	Sigma Aldrich	P4417
Ethylenediaminetetraacetic acid (EDTA)	Sigma Aldrich	60-00-4
Fetal Bovine Serum (FBS)	Thermo Fisher	10270106
DMEM F12	Thermo Fisher	21331046
Dimethylsulfoxide (DMSO)	Sigma Aldrich	D2650
Trypsin	Thermo Fisher	15090046
Alamar Blue	Thermo Fisher	DAL1025

The holographic diffusers were cut into squares of ≈ 1 cm side length to adequately expose the cells. This was performed with holographic diffusers, which consisted of the substrate, the recorded photopolymer, and a cover layer, as well as the substrate and the recorded photopolymer, then finally only the substrate (36 of each grouping per photopolymer). The holographic diffuser samples were UV bleached to kill any microbes that could affect the experiment, and one piece was placed in each well. After this, a cell solution density of 1x10^5 mL^{-1}

was prepared for seeding. 100 μL of this stock was added to each substrate and allowed 1 hour to attach. After attachment the media was topped up with an additional 100 μL media and the cells were incubated for the desired timepoint.

Following 24 and 48 hours of cell attachment, plates were washed with 100 μL per well of phosphate buffered saline (PBS). The alamar blue (AB) assay was carried out in accordance with the manufacturer's instructions. Briefly, control media or test exposures were removed; the cells were rinsed with PBS, and 100 μL of AB medium (5% [v/v] solution of AB) prepared in fresh media (without FBS or supplements) were added to each well. After 3 hours of incubation, AB fluorescence was measured at the respective excitation and emission wavelength of 531 nm and 595 nm in a SpectraMax M3 Multi–Mode Microplate Reader (Molecular Devices, USA). Wells, having only AB and media, were used as blanks.

2.3 Ageing Studies

The ageing study used a Q-Sun Xe-3 machine, a xenon arc chamber that simulates the radiation exposure caused by full-spectrum sunlight over a condensed time frame. The ageing was performed over 160 hours, equivalent to approximately 300 days of exposure. Each experiment cycle involved 20 hours of exposure within the Q-Sun apparatus, approximating 36.5 days' equivalent per data point. After each cycle, the angular diffusion efficiency and point spread functions were obtained as described in an earlier work.[18]

This analysis was carried out on holographic diffusers composed of acrylamide- (AA) and diacetone acrylamide-based (DA) photopolymers with 11 samples of each and one control sample with no recorded diffuser present. A further unaged control was also analysed alongside the aged samples.

3. RESULTS & DISCUSSION

3.1 Spectroscopy Results

Spectroscopic analysis of the holographic diffusers showed a wavelength dependency on the diffusion efficiency (Figure 2A). There is also an association between the diffusion efficiency of the element and the rate at which the diffusion efficiency reduces with increasing wavelength. This was the expected behaviour as while Kogelnik Coupled Wave Theory (KCWT) does not directly apply to the holographic diffuser, both the Raman-Nath[20,21] and KCWT[22] possess a wavelength dependence.

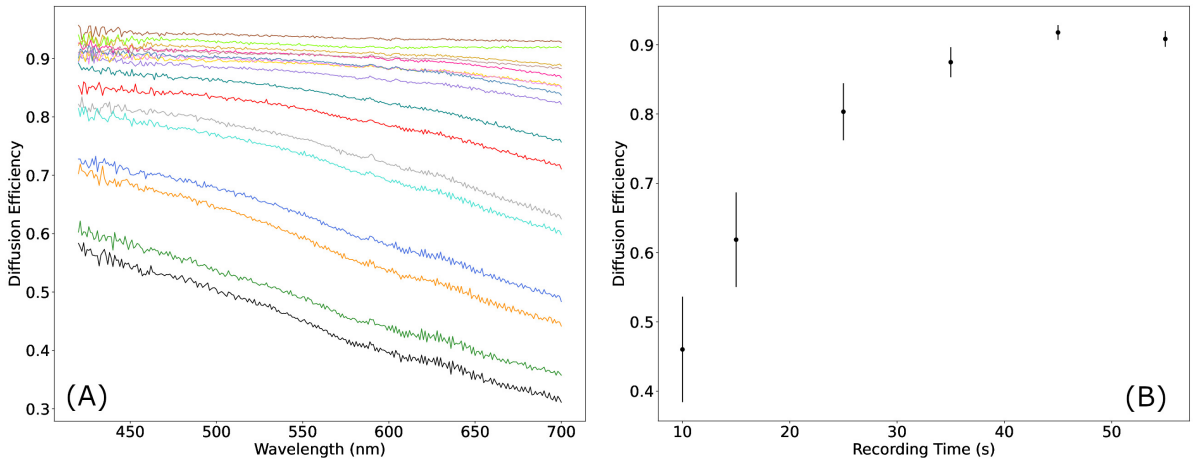

Figure 2. (A) Diffusion efficiency of a range of holographic diffusers as a function of wavelength. (B) Diffusion efficiency of a range of holographic diffusers as a function of recording time. Each data point is a combination of three diffusers recorded under the same conditions (error bars represent ± 1 standard deviation).

Grouping the holographic diffusers by recording condition (such as recording time) confirms previous findings regarding diffusion efficiency and recording time[18] where increased recording time leads to an increase in diffusion efficiency and a minimising of the standard deviation of the dataset.

3.2 Cytotoxicity Results

Whilst the toxicology of constituent parts of the holographic diffusers has been analysed previously,[23] the toxicology of the single whole element is reported for the first time here. The cytotoxicity study of the acrylamide- and diacetone acrylamide-based holographic diffusers involved analysing a covered sample (recorded holographic diffuser), an uncovered sample, and the substrate (LEXAN polycarbonate), as well as a control. Table 3 shows the Alamar blue dye's testing results, where the values are normalised to the average control wells.

Table 3. The mean difference in viability of HaCaT cells compared to the reference control.

Photopolymer	Covered	Uncovered
AA 24 hours	0.33	0.23
AA 48 hours	0.19	0.16
DA 24 hours	0.82	0.67
DA 48 hours	0.37	0.29

As can be seen in Fig 3, the covered diacetone acrylamide-based holographic diffuser had significantly less effect on the assay after 24 hours, with 82% of the cells still viable compared to the control. This contrasts with the covered acrylamide-based holographic diffusers, which returned 33% of the cells viable. After 48 hours, the covered diacetone acrylamide sample returned 37% viability, which agrees with the covered acrylamide within experimental uncertainties of both readings. It is of note that for 24 and 48 hours, the substrate returned an average value of 77%, which indicates that there may be an issue with the LEXAN polycarbonate cell adhesion. The difference between uncovered and covered samples for both formulations is within the experimental error of this dataset; therefore, there may be no difference between covered and uncovered samples in terms of toxicology. The proposed use case for the holographic diffusers as a treatment upon spectacles means that the contact time expected with skin would be significantly below 24 hours. As such, it is suggested that the viability of the diacetone acrylamide holographic diffusers is sufficient.

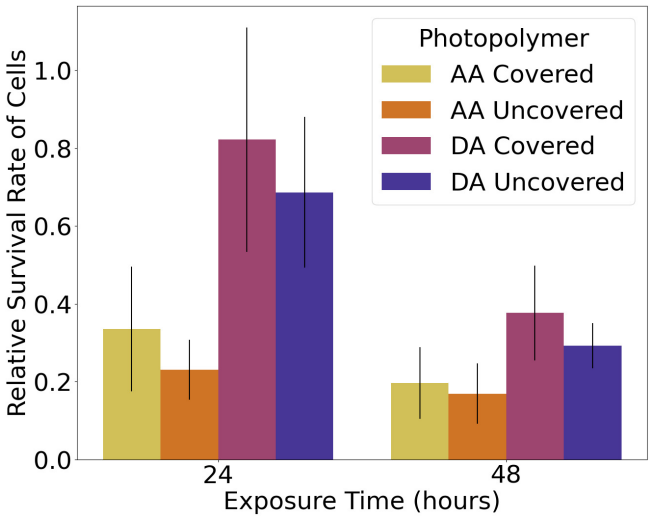

Figure 3. A bar chart of the viability of the cells post-exposure, where survival rates are proportionally normalised as the difference between the sample and the control.

3.3 Ageing Results

Figure 4 shows the diffusion efficiency of each acrylamide-based sample, which in all instances of the AA holographic diffusers maintained a diffusion efficiency of 92.5 ± 2%. There is some variation between readings; however, this is expected to be related to the angular selectivity of the samples rather than a reduction in the inherent diffusing capability of the elements. Comparisons between Figure 4 and 5A shows that there is a significant difference in behaviour between the AA and diacetone acrylamide-based holographic diffusers.

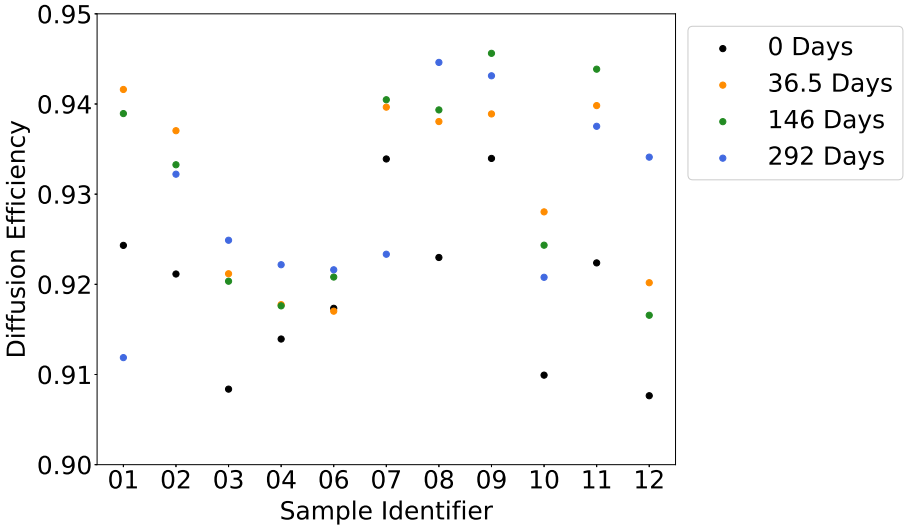

Figure 4. The diffusion efficiency of individual acrylamide-based samples.

From a reasonably uniform diffusion efficiency (≈92%) at 0 hours of simulated ageing, the diffusion efficiency of five samples reduced more than 5% by 36.5 days of ageing. These samples were recorded for the least time to achieve their diffusion efficiency, highlighting that the polymerisation of the photopolymer plays a significant role in the diacetone acrylamide-based holographic diffuser maintaining a high diffusion efficiency. There is a drop-off in diffusion efficiency after day 146 for all DA holographic diffusers as shown in Figure 5B; this was present regardless of recording time as demonstrated in 5A.

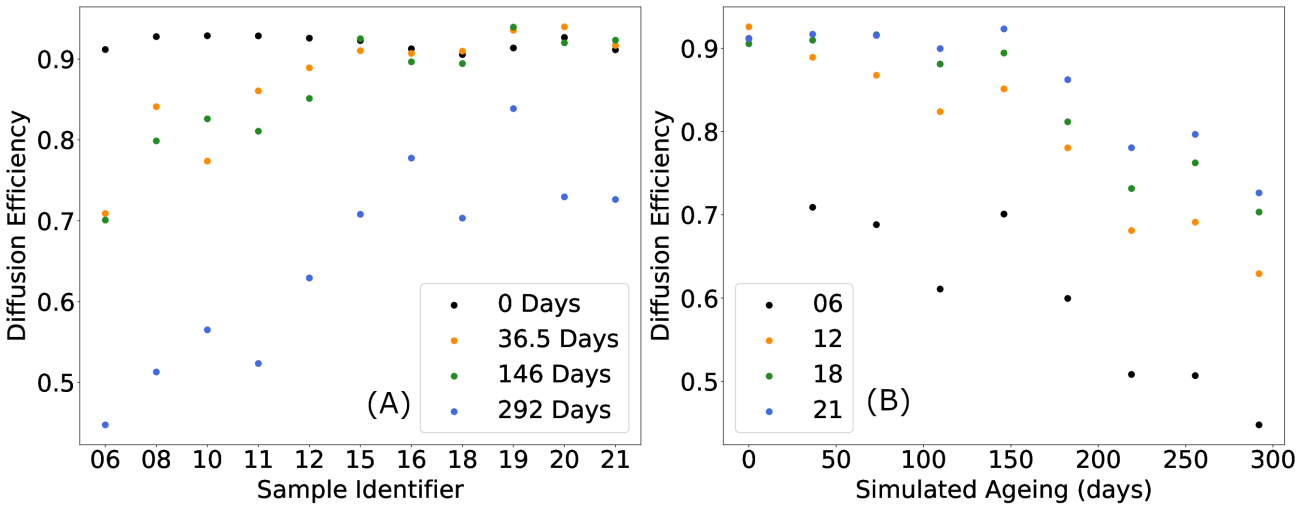

Figure 5. (A) The diffusion efficiency of individual diacetone acrylamide-based samples. (B) The stepwise ageing of individual diacetone acrylamide-based samples (recording time: 25, 35, 50, 60 s respectively).

The AA holographic diffusers' Modulation Transfer Functions (MTFs) are shown in Figure 6. Figure 6A and C indicate the MTF in direct comparison to the control, and Figure 6B and D show the individual MTFs. 6A and B are the unaged samples, and 6C and D are the samples after 292 days of simulated ageing. It is noteworthy that after 292 days of accelerated ageing, the aged and unaged blanks show remarkably similar MTFs. This highlights that the ageing of the photopolymer itself is not likely to impact the performance of the holographic diffusers. Figure 6A and C shows that across the 292 days of accelerated ageing (C), the holographic diffusers maintained their ability to remove the spatial frequency information acting akin to an occluder. Figure 6B and

D shows that while there is some spatial frequency information in the system, the magnitude of it is reduced to the extent that the noise in the system is potentially significant.

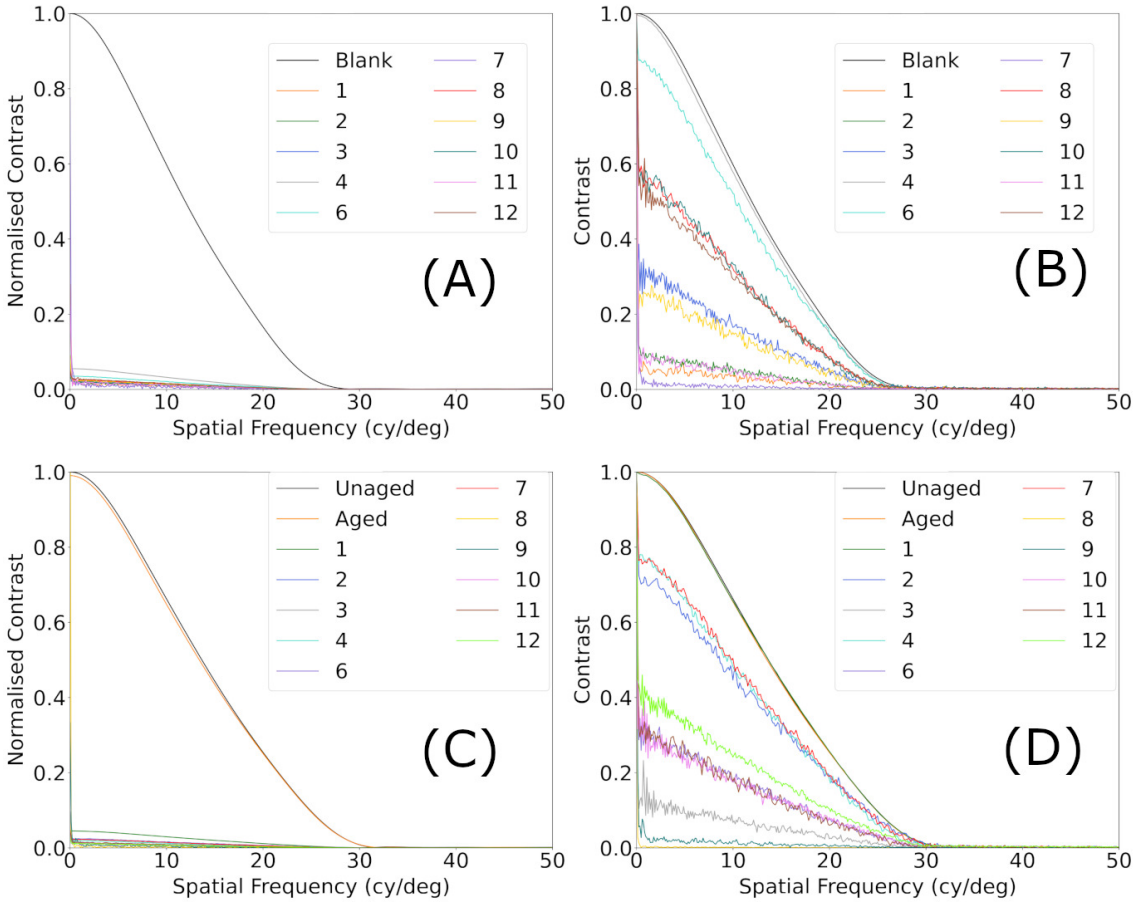

Figure 6. The Modulation Transfer Functions (MTF) of the acrylamide-based holographic diffusers. (A & B) show the MTF before simulating the ageing, (C & D) show the MTF of the samples after simulated ageing of 292 days. (A & C) are the MTFs with reference to the blank/unaged sample, (B & D) are the individual MTFs.

Figure 7 shows the MTFs of the DA holographic diffusers. Figure 7A and C indicate the MTF in direct comparison to the control, and Figure 7B and D show the individual MTFs. 7A and B are the unaged samples, and 7C and D are the samples after 292 days of simulated ageing. Once again, the aged and unaged blank samples of diacetone acrylamide-based holographic diffusers produced similar MTFs, indicating that the ageing of the photopolymer itself is not likely to impact the performance of the holographic diffusers. Figure 7A shows a similar starting point for the holographic diffusers compared to 6A. The holographic diffusers remove the spatial frequency information in most instances (sample 6 being an outlier here). In contrast to the behaviour of the AA holographic diffusers, the DA diffusers displayed a pronounced difference once aged to 292 days. This is shown in Figure 7B and D, which indicates a difference in spatial frequency response between day 0 (B) and day 292 (D); specifically, by day 292, the holographic diffusers were not producing any impingement on the spatial frequencies present in any holographic diffuser, only reducing the contrast of the images as shown in (C).

The above indicates that the diacetone acrylamide-based holographic diffusers have a shorter lifespan than the acrylamide version. It is of note that some of the diacetone acrylamide-based holographic diffusers maintained their diffusion efficiency until between day 146 and 182. Therefore, it would be estimated that the useful lifetime of a diacetone acrylamide-based holographic diffuser is in this range. As it would be standard procedure to review progress in this period, the diacetone acrylamide-based holographic diffusers are expected to still be useful as a treatment modality.

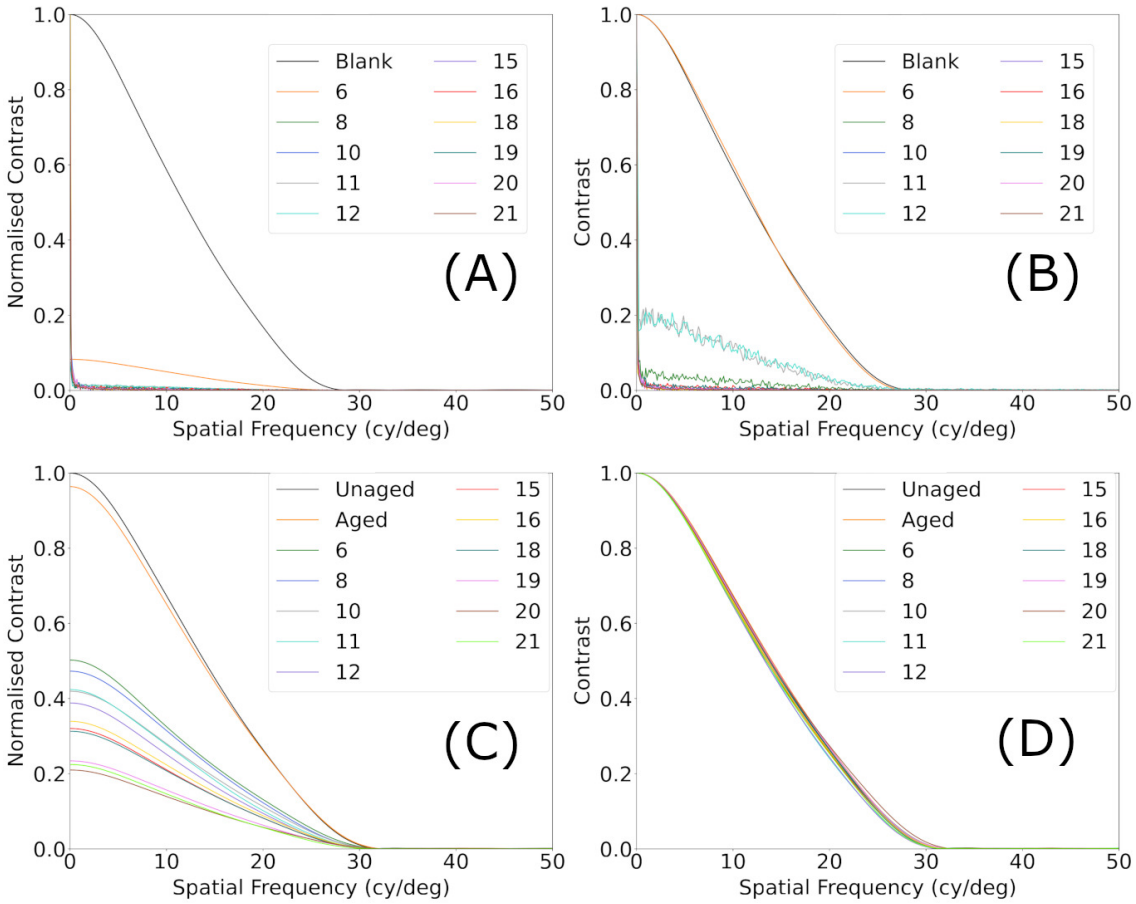

Figure 7. The Modulation Transfer Functions (MTF) of the diacetone acrylamide-based holographic diffusers. (A & B) show the MTF before simulating the ageing, (C & D) show the MTF of the samples after simulated ageing of 292 days. (A & C) are the MTFs with reference to the blank/unaged sample, (B & D) are the individual MTFs.

4. CONCLUSION

Holographic diffusers have a minimal spectral impact at higher diffusion efficiencies, with samples above 90% diffusion efficiency have a <5% deviation, between 420 nm and 700 nm. The lower diffusion efficiency samples exhibited a more pronounced spectral response. However, there is no significant drop out of specific wavelengths or wavelength ranges.

Holographic diffusers composed of the diacetone acrylamide-based photopolymer have a much lower toxicity than those composed of acrylamide-based photopolymer, with a viability of 82% for covered samples after 24 hours constant exposure. While the times examined are significantly more than expected patient interaction, having a lower level of toxicity would be preferable for the treatment (assuming equal effectivity between treatments).

The accelerated ageing of both holographic diffusers showed the ability to maintain their diffusion efficiency over prolonged periods. The acrylamide-based holographic diffusers showed no deterioration due to simulated ageing up to 292 days. However, the diacetone acrylamide versions exhibited diffusion efficiency and spatial frequency reduction after simulated exposure times of 182 days or more. Neither formulation showed cause for concern regarding their longevity as typical treatment regimes would involve examination by the practitioner within these timeframes.

Holographic diffusers in acrylamide- and diacetone acrylamide-based photopolymer materials had minimal spectral influence, relatively low toxicity, and enduring performance. All of these qualities are supportive of their potential application as a personal visual appliance.

5. ACKNOWLEDGEMENTS

The authors wish to acknowledge the FOCAS Research Institute for the use of facilities, equipment, and technical support.

5.1 Funding

Irish Research Council (GOIPG/2021/1547); Science Foundation Ireland (18/SIRG/5666).

5.2 Author Contributions

MH Obtained funding for the project. KM supervised project direction. Experimental plans devised by MH, SM, MS, AC, and KM. Experimental work by MH (all), AC (cytotoxicity), ES (cytotoxicity). All figures produced by MH. Manuscript written by MH. Feedback and editing of manuscript performed by SM, MS, AC, ES, and KM.

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