Poly(ethylene glycol)-based magnetic hydrogel nanocomposites for hyperthermia cancer therapy

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ABSTRACT

Hyperthermia, the heating of cancerous tissues to between 41 and 45 °C, has been shown to improve the efficacy of cancer therapy when used in conjunction with irradiation and/or chemotherapy. Here a novel method for remotely administering heat is presented, which involves the heating of tumor tissue using hydrogel nanocomposites containing magnetic nanoparticles which can be remotely heated upon exposure to an external alternating magnetic field (AMF). Specifically, this research explores the use of hydrogel nanocomposites based on poly(ethylene glycol) methyl ether methacrylate and dimethacrylate with iron oxide as implantable biomaterials for thermal cancer therapy applications. Swelling analysis of the systems indicated a dependence of ethylene glycol (EG) content and cross-linking density on swelling behavior where greater EG amount and lower cross-linking resulted in higher volume swelling ratios. Both the entrapped iron oxide nanoparticles and hydrogel nanocomposites exhibited high cell viability for murine fibroblasts, indicating potential biocompatibility. The hydrogels were heated in an AMF, and the heating response was shown to be dependent on both iron oxide loading in the gels and the strength of the magnetic field. As proof of concept of these systems as a thermal therapeutic the ability to selectively kill M059K glioblastoma cells in vitro with hydrogel nanocomposites exposed to an AMF was demonstrated.

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1. Introduction

The development of cancer therapeutics is an important component of biomedical research today because even though much has been done to overcome and treat the disease, there are still many types of cancer, such as glioblastoma and pancreatic cancer [1], that have extremely poor treatment success rates. Therefore, multiple modality treatment has become the preferred treatment approach. Hyperthermia, the heating of cancer tissues to between 41 and 45 °C, has been proven to have the potential to provide a straightforward and effective way of treating cancer in combination with well-developed therapeutics such as irradiation and chemotherapy [2]. Extensive pre-clinical data have shown that the combination of hyperthermia with irradiation and/or chemotherapy improves the efficacy of the prescribed treatment without producing additional systemic toxicity [3,4]. Aside from having a cytotoxic effect on cancerous tissues, hyperthermia has been shown to increase tumor blood flow, which induces hypoxia, acidosis and energy deprivation at the tumor site, thereby increasing therapy efficacy [5]. Thermoablation, the thermal destruction of cells at temperatures above at least 50 °C, is another potential cancer therapy method involving heat therapy [6]. It induces cell necrosis rather than the apoptotic death usually attributed to hyperthermia, thereby effectively killing cancer cells. Currently there are a number of obstacles facing hyperthermic treatment, including restriction of local heating to the tumor without damaging surrounding tissue [7] and an inability to heat the cancer tissue locally without using invasive and uncomfortable heating probes [8]. This can potentially be overcome through the development of systems which can be delivered to and/or implanted at tumor sites and remotely heated from outside the body. The hydrogel nanocomposite composed here will be fixed in place due to compression by the surrounding tissue, hence providing local heating and overcoming less specific whole-body and regional heating. A novel class of biomaterials based on nanocomposite hydrogels, specifically systems composed of poly(ethylene glycol) (PEG) and iron oxide, have the potential to be used in such hyperthermic applications. Exposure of the gels to a high frequency magnetic field will lead to remote heating due to the magnetic particles in the nanocomposite.

Hydrogels are three-dimensional, hydrophilic, polymeric networks that can absorb up to thousands of times their dry weight in water or biological fluids [9–11]. They consist of polymeric chains with either physical or chemical cross-links preventing their dissolution while allowing swelling upon interaction with aqueous...
solutions. Hydrogels are advantageous for many biomedical applications due to their resemblance to natural living tissue and inherent biocompatibility, which can be partially attributed to their soft, flexible nature and high water content [10]. They have been utilized in a wide variety of biomedical and pharmaceutical applications, such as drug delivery systems, contact lenses and tissue engineering [9,12,13]. In particular, PEG-based hydrogels have been widely investigated and are considered “stealth” systems due to their high water content and the presence of PEG chains which exhibit high biocompatibility. The biocompatibility of PEG stems from its ability to repel protein adsorption, due to the hydrophilic nature of the polymer [14,15]. The PEG polymer component in this research showed an ability to shield iron oxide nanoparticules incorporated into the polymer matrix.

Despite the many advantages of using conventional hydrogels, their application is often limited due to their poor mechanical strength and somewhat limited response and actuation properties [17–20]. Hydrogel nanocomposites involve the incorporation of various nanoparticle materials within a hydrogel matrix which provides easy, straightforward methods to enhance the properties of hydrogels. Thus far a number of nanoparticulates have been utilized in nanocomposite hydrogel systems, including metallic nanoparticles, carbon nanotubes, clays, ceramics, magnetic nanoparticles, hydroxyapatite and semiconducting nanoparticles [21–26]. The incorporation of magnetic nanoparticles such as iron oxide nanoparticles into hydrogels can create tunable nanocomposites that can be remotely controlled by a magnetic field [23,27]. In recent work by Satarkar and co-workers [27,28] remote controlled heating and drug release using hydrogel nanocomposites based on N-isopropylacrylamide (NIPAAm) and Fe₃O₄ nanoparticles have been successfully demonstrated. This controlled release was due to the thermally responsive nature of the NIPAAm and remote controlled heating by applying an alternating magnetic field (AMF) which heated the magnetic nanoparticles present in the hydrogel matrix. Similarly, PEG methyl methacrylates demonstrate a thermal response with tunable lower critical solution temperature (LCST) values depending on the PEG chain length which may also allow them to be used in drug delivery applications [29]. At temperatures below the LCST hydrogel systems are swollen, whereas above this temperature the polymer matrix collapses. For these studies iron oxide nanoparticles (20–30 nm diameter) were incorporated into a PEG methyl ether methacrylate/dimethacrylate hydrogel matrix. Upon exposure to an AMF superparamagnetic iron oxide nanoparticles have the ability to heat, primarily due to Brownian movement (frictional losses) and Neel relaxation losses [30,31]. With regard to the biocompatibility of hydrogel nanocomposites, a limited number of cytocompatibility or hemocompatibility studies have been reported. However, a wide variety of literature is available regarding the biocompatibility of the hydrogels and nanoparticulate components that make up a hydrogel nanocomposite [21]. One of the advantages of hydrogel nanocomposites is that hydrogels can provide increased biocompatibility over exposed, uncoated nanoparticulates because they encapsulate the particulate matter in the composite matrix and provide a barrier between sensitive tissues and the more harmful nanoparticulates. In a recent publication the encapsulation of uncoated iron oxide nanoparticles within a poly(N-isopropylacrylamide) hydrogel [24] was shown to exhibit a more favorable cell viability than the nanoparticles themselves. As more hydrogel nanocomposites are fabricated and characterized for various applications it is important that biocompatibility issues and the safety of these materials are examined.

The objective of this research was to develop biocompatible magnetic hydrogel nanocomposites that exhibit favorable swelling and cytotoxicity properties and have the ability to be remotely heated via an AMF. Swelling analysis showed the thermally responsive nature of the hydrogels, which exhibited deswelling behavior at higher temperatures. In addition, murine fibroblasts exposed to the nanocomposites showed viability similar to that of controls on polystyrene. Also, it was shown that the thermal response of the nanocomposites can be controlled through the AMF field strength so that they can exhibit temperatures for either hyperthermic and thermoablative treatment. As proof of concept of these systems as a thermal therapeutic the ability to selectively kill M059K glioblastoma cells in vitro with hydrogel nanocomposites exposed to an AMF was demonstrated. Ultimately these hydrogel systems have the potential to more effectively treat localized tumors via both drug delivery and thermal therapy via hyperthermia.

2. Materials and methods

2.1. Materials

The macromers poly(ethylene glycol) (N = 200) methyl ether methacrylate (PEG200MMA) and poly(ethylene glycol) (N = 1000) methyl ether methacrylate (PEG1000MMA) and cross-linkers tetra(ethylene glycol) dimethacrylate (TEGDMA) and poly(ethylene glycol) (N = 400) dimethacrylate (PEG400DMA) were obtained from Polysciences (Warrington, PA). The Fe₃O₄ nanoparticles (20–30 nm diameter, 0.2% PVP-coated) were obtained from Nanostructured and Amorphous Materials (Los Alamos, NM). The initiator, ammonium persulfate (APS), accelerator, N,N,N,N-tetramethylethane-1,2-diamine (TEMED) and ethanol (95%) were obtained from Sigma Aldrich (St Louis, MO) at 99% and 98% purity, respectively. All materials were used as received.

2.2. PEGMMA–TEGDMA hydrogel fabrication

Magnetic hydrogel nanocomposites were fabricated via free radical polymerization with various macromer (PEGMMA) and cross-linking (TEGDMA) amounts, as shown in Table 1, resulting in various cross-linking densities. The structures of these compounds are shown in Fig. 1. Ethanol was added to the macromer solution at a 1:1 by weight basis based on the macromer and cross-linker. For hydrogels with iron oxide nanoparticles the particles were added at 5 wt.% based on the macromer and cross-linker and were exposed to an ultrasonic bath for 30 min to facilitate dispersion of the iron oxide nanoparticles throughout the solution. After sonication, 2 wt.% APS and 4 wt.% TEMED were then added

Table 1

<table>
<thead>
<tr>
<th>Nanocomposite system</th>
<th>Abbreviation</th>
<th>Macromer feed</th>
<th>Cross-linker feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>50PEG200MMA–50TEGDMA</td>
<td>AM</td>
<td>50 mol.%</td>
<td>50 mol.%</td>
</tr>
<tr>
<td>80PEG200MMA–20TEGDMA</td>
<td>BM</td>
<td>80 mol.%</td>
<td>20 mol.%</td>
</tr>
<tr>
<td>95PEG400MMA–5PEG400DMA</td>
<td>CM</td>
<td>95 mol.%</td>
<td>5 mol.%</td>
</tr>
<tr>
<td>50PEG200MMA–50TEGDMA</td>
<td>DM</td>
<td>50 mol.%</td>
<td>50 mol.%</td>
</tr>
<tr>
<td>50PEG1000MMA–50TEGDMA</td>
<td>EM</td>
<td>50 mol.%</td>
<td>TEGDMA</td>
</tr>
<tr>
<td>50PEG400DMA–50TEGDMA</td>
<td>FM</td>
<td>50 mol.%</td>
<td>PEG1000MMA</td>
</tr>
<tr>
<td>50PEG1000MMA–50TEGDMA</td>
<td></td>
<td>50 mol.%</td>
<td>PEG1000MMA</td>
</tr>
</tbody>
</table>

For the nanocomposite system column the numbers before the PEG constituents refer to the mol.% added to the feed solution. All gels were made with 5 wt.% iron oxide nanoparticles based on the mass of the macromer and cross-linker.
to the mixture to initiate free radical polymerization. This solution was then sonicated further for 2 min before being loaded into a template consisting of two 15 x 15 cm glass plates with a 1.5 mm thick Teflon spacer. The gels were kept in this template for at least 2 h (usually overnight) to allow completion of polymerization. To remove any potentially unreacted and potentially toxic macromer, cross-linker and initiator residues the hydrogels were washed with deionized water for at least 1 week.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis was performed to determine the conversion of available C=C double bonds present in the PEG constituents of the hydrogel using a Varian Inc. 7000e step-scan spectrometer. Upon addition of initiator to the gel solution a sample of the solution was placed on the diamond ATR crystal and IR spectra were obtained for 90 min at 2 scans min\(^{-1}\) at 8 cm\(^{-1}\) spectral resolution between 700 and 4000 cm\(^{-1}\). The data were analyzed using Varian Resolutions software.

2.3. Thermal gravimetric analysis (TGA) of hydrogel nanocomposites

TGA measurements were made to determine the actual iron oxide nanoparticle loading in the hydrogel nanocomposites using a TA Instruments Q500 thermogravimetric analyzer. A dry hydrogel sample of approximately 10 mg was exposed to heat at a rate of 20 °C min\(^{-1}\) under nitrogen flow conditions. At 120 °C the sample was heated isothermally for 10 min to vaporize residual water and other volatile compounds before being heated to a final temperature of 700 °C. Final TGA data were obtained after normalizing the results with respect to the gel mass at 120 °C.

2.4. Swelling characterization

Equilibrium swelling characteristics of the PEG–Fe\(_2\)O\(_4\) hydrogel nanocomposites and pure PEG hydrogels were measured using a gravimetric method based on a comparison of the density measurements of both swollen and dry gels at equilibrium, as described previously [23]. Upon completion of synthesis and washing hydrogel discs 13.8 mm in diameter were cut from the bulk hydrogel films. The discs were then placed in phosphate-buffered saline solution (PBS) (pH 7.4) for at least 3 days to reach equilibrium. The masses of the discs were measured in air and then in n-heptane (non-solvent) in their swollen state at 22, 37, 43 and 63 °C and in their dry state. Heptane was used for volumetric swelling studies as it interacts little, if at all, with water in the hydrogel system. The densities of the hydrogels in both the dry and swollen states in air and n-heptane then allowed calculation of the volume swelling ratio (Q) at equilibrium:

\[
Q = \frac{V^s}{V^d} = \frac{M_{air}^s - M_{heptane}^s}{M_{air}^d - M_{heptane}^d} = \frac{M_{air}^s - M_{heptane}^s}{M_{heptane}^d - M_{heptane}^d}
\]

where \(V\) is the volume of the sample, \(\rho_{heptane}\) is the density of n-heptane, \(M_{air}\) is the mass of the sample in air and \(M_{heptane}\) is the mass of the sample in n-heptane. The superscripts \(s\) and \(d\) refer to the swollen and dry forms of the samples, respectively.

2.5. Cytotoxicity analysis of iron oxide nanoparticles and hydrogel nanocomposites

The iron oxide nanoparticles and hydrogel nanocomposites were analyzed for tissue cytotoxicity using NIH 3T3 murine fibroblasts (ATCC, Manassas, VA). Fibroblasts were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10 vol.% calf bovine serum, 100 IU ml\(^{-1}\) penicillin, 100 μg ml\(^{-1}\) streptomycin (ATCC) and 1 μg ml\(^{-1}\) antimycotic Fungizone\(^{+}\) (Invitrogen, Carlsbad, CA) at 37 °C and 5% CO\(_2\) in a humidified incubator. The cells were used from passages 5–8.

For iron oxide nanoparticle analysis fibroblasts were seeded at 2500 cells cm\(^{-2}\) in a polystyrene 12-well plate. After 24 h the medium was aspirated and 1 ml of nanoparticles suspended in complete medium (at 100, 500 and 1000 μg ml\(^{-1}\)) was added to each well. The well plates were then returned to the incubator for a 24 or 48 h exposure time period, after which the fibroblasts were assayed for cell viability. A two color fluorescence Live/Dead Viability Assay\(^{+}\) (Molecular Probes, Carlsbad, CA) was used to assess the cell viability of both attached and unattached fibroblasts. Stained samples were observed using a Nikon Eclipse 80i microscope at 50× magnification. The percent cell viability (live cells/total cells) was determined by manually counting the live and dead cells imaged with NIS-Elements software. Both attached and unattached fibroblasts were analyzed in three separate samples with 10 images (5 live and 5 dead) taken from each.

For hydrogel analysis the gels were cut into discs (12.5 mm diameter), placed in complete fibroblast medium and incubated at 37 °C for 48 h. NIH 3T3 murine fibroblasts were seeded at 2500 cells cm\(^{-2}\) for 24 h in 12-well plates. The medium was then aspirated from the wells and discarded. Next, the medium that the hydrogels had been soaked in was placed over the fibroblasts. The well plates were then returned to the incubator for 24 or 48 h. Afterwards both attached and unattached cells were assayed for cell viability using the method described above.

2.6. Remote controlled heating of nanocomposite hydrogels

Prior to heating hydrogel nanocomposites were cut into discs (8.2 mm in diameter) and equilibrated in PBS at room temperature (22 °C). Remote controlled heating of the hydrogel nanocomposites was completed using an alternating electromagnetic field induced by a Taylor Winfield induction power supply (model MMF-3-135/400-2) equipped with a solenoid of 15 mm diameter with five turns. The hydrogel discs in their equilibrium swollen state were covered with Saran wrap, placed on top of the solenoid coil and exposed to the AMF. Two types of thermal analyses were performed. First, all six hydrogel nanocomposite systems were exposed to the same magnetic field power of 297 kHz and 25 kA m\(^{-1}\). Then the hydrogel systems were heated to either the hyperthermic temperature range (41–44 °C) or thermoablative temperature range (61–64 °C) by controlling the magnetic field strength. Thermal images and data were acquired using an infrared camera (Agema Thermovision 470) which recorded the surface temperature of the hydrogels. The surface temperature was recorded continuously for 5 min.
2.7. M059K glioblastoma cell thermoablation via hydrogel heating

PEG-based magnetic hydrogels were used to demonstrate that cancer cells can be killed by heat generated by hydrogel nanocomposites upon exposure to an AMF (via thermoablation). M059K glioblastoma cells (ATCC) were cultured in DMEM/Ham’s F-12 medium supplemented with 0.05 mM non-essential amino acids and 10% fetal bovine serum at 37 °C and 5% CO₂ in a humidified incubator. The cells were then seeded in 35 mm culture dishes at 20,000 cells cm⁻² and incubated for 24 h. 50 mol.% PEG400MMA/50 mol.% TEGDMA hydrogel nanocomposites were cut at 8.2 mm and equilibrated in PBS overnight at room temperature. Prior to heating the gels were placed in Saran wrap to prevent water loss and then placed on the AMF coil. M059K cells were removed from the incubator, medium was aspirated from the culture dish and the dish was placed directly on the hydrogel for heat treatment. The gel and cells were then exposed to the AMF (297 kHz, 18 kA m⁻¹) for 5 min. An IR camera was used to collect still IR images at the end of the heating experiment to show the actual temperature of the cells after exposure. Controls were done with the cells only (with no hydrogel) exposed to the AMF and with cells exposed to no field, both with medium removed for 5 min. Upon heat and AMF exposure the cells were returned to the incubator for 2 h to allow time for a cellular response to the heat treatment and then assayed using the Molecular Probes Live/Dead Assay and fluorescence imaging.

2.8. Statistical analysis

All experiments were performed at least in triplicate. MSTAT 12 for Windows (12.02.00) was used for t-tests (paired t-test with unequal variances) to determine any significance in the observed data. P < 0.05 was considered statistically significant.

3. Results

3.1. Polymerization analysis and TGA characterization of hydrogel nanocomposites

ATR-FTIR was used to determine the conversion of C=C double bonds present in the hydrogels. Both PEGMMA and PEGDMA have C=C bonds which undergo free radical polymerization to form the cross-linked hydrogel systems, which decreases the number of C=C bonds available. Conversion of the double bonds of the PEG constitutes was determined using standard baseline techniques from the peak area of 1713 cm⁻¹ for C=C vibration, using the area of 1711 cm⁻¹ for C=O stretching as a reference. The percent conversion of the double bonds was calculated from:

\[
\% \text{ Conversion} = \left(1 - \frac{R_f}{R_0}\right) \times 100\%
\]

where \(R_f\) is the ratio of the peak area of the C=C to the reference peak area of C=O at the final time (90 min) and \(R_0\) is the ratio of the same peak at the initial time. Conversion for all of the hydrogel nanocomposites after 90 min was at least 93%, as shown in Table 2, indicating that for the majority of the hydrogels the reaction was nearly complete.

TGA was performed to determine the actual iron oxide nanoparticle loading in the hydrogel nanocomposites. For all hydrogel nanocomposites the iron oxide loading was found to be in the range 5.48 ± 0.65 wt.% (results not shown), which is close to the initial loaded amount.

3.2. Characterization of hydrogel nanocomposite swelling behavior

Analysis of the swelling behavior of hydrogels is necessary to determine the ability of the gels to retain fluids for drug delivery applications. The release of hydrophobic drugs such as estradiol, insulin and bovine serum albumin from PEG-based hydrogels has already been successfully controlled [32,33]. The swelling behavior of the nanocomposite gels was analyzed to determine the effects of iron oxide nanoparticle loading, cross-linking ratio, cross-linking and macromer types and temperature. The mesh size of the hydrogel systems was calculated using the Flory–Rehner equation incorporating the Peppas–Merrill modification [34,35]. The mesh size was determined to be of the order of 10−20 Å, which is an order of magnitude smaller than the iron oxide nanoparticles incorporated into the hydrogel matrix. As such, it is hypothesized that the nanoparticles are physically entrapped in the nanocomposite and, therefore, particle loss from the systems is negligible (nor has it been observed).

The following descriptions apply for the hydrogels at 22 °C. For the PEG200MMA/PEG400DMA systems the amount of cross-linking varied from 5 to 50 mol.%, and as the amount of cross-linking increased the swelling ratio decreased, as seen in Fig. 2. As the amount of cross-linking increased the mesh structure of the hydrogel became tighter, which did not allow as much water into the system. For 50 mol.% PEG200MMA cross-linked with 50 mol.% PEG400DMA or TEGDMA the volume swelling ratio was the same irrespective of the cross-linker used. For 50 mol.% PEG1000MMA systems with either cross-linker the volume swelling ratio was higher for the gel with TEGDMA versus the system with PEG400DMA. This is in contrast to what was expected. TEGDMA has a much shorter PEG chain length in comparison with PEG400DMA (~4.5 vs. 9 PEG groups, respectively) and it is expected that a system cross-linked with TEGDMA would exhibit a tighter gel mesh and

### Table 2

<table>
<thead>
<tr>
<th>System</th>
<th>C=C Conversion (%)</th>
<th>Fibroblast cell viability (%)</th>
<th>Final T at 25 kA m⁻¹ (°C)</th>
<th>Fe₃O₄ mass/gel volume (mg cm⁻³)</th>
<th>Hyperthermia AMF strength (kA m⁻¹)</th>
<th>Thermoablative AMF strength (kA m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50PEG1000MMA–50TEGDMA</td>
<td>96.3</td>
<td>95.7 ± 1.4</td>
<td>97.2 ± 3.3</td>
<td>60.7 ± 0.7</td>
<td>1.58</td>
<td>17.3</td>
</tr>
<tr>
<td>95PEG200MMA–5PEG400DMA</td>
<td>94.2</td>
<td>97.3 ± 0.9</td>
<td>97.4 ± 2.5</td>
<td>59.5 ± 1.1</td>
<td>1.92</td>
<td>17.4</td>
</tr>
<tr>
<td>50PEG1000MMA–50PEG400DMA</td>
<td>99.4</td>
<td>97.9 ± 0.5</td>
<td>96.8 ± 0.8</td>
<td>65.7 ± 1.7</td>
<td>2.74</td>
<td>16.5</td>
</tr>
<tr>
<td>8PEG200MMA–20PEG400DMA</td>
<td>97.3</td>
<td>96.0 ± 2.0</td>
<td>97.8 ± 2.2</td>
<td>66.1 ± 0.7</td>
<td>5.32</td>
<td>14.7</td>
</tr>
<tr>
<td>50PEG200MMA–50PEG400DMA</td>
<td>93.3</td>
<td>97.0 ± 2.5</td>
<td>97.8 ± 2.5</td>
<td>73.8 ± 0.8</td>
<td>7.24</td>
<td>14.3</td>
</tr>
<tr>
<td>50PEG200MMA–50TEGDMA</td>
<td>95.0</td>
<td>96.9 ± 1.0</td>
<td>98.0 ± 2.1</td>
<td>79.6 ± 1.3</td>
<td>7.93</td>
<td>12.7</td>
</tr>
<tr>
<td>100 μg ml⁻¹ Fe₃O₄</td>
<td>98.3 ± 0.5</td>
<td>99.1 ± 0.1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>500 μg ml⁻¹ Fe₃O₄</td>
<td>98.1 ± 0.4</td>
<td>98.1 ± 0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 μg ml⁻¹ Fe₃O₄</td>
<td>98.0 ± 0.2</td>
<td>98.0 ± 0.5</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>97.9 ± 0.6</td>
<td>98.3 ± 1.3</td>
<td></td>
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</tr>
</tbody>
</table>
therefore have a smaller swelling ratio. It is hypothesized that in this case primary chain cyclization may have an effect on the structure of the hydrogel. This occurs when a propagating radical reacts intramolecularly with a pendant double bond on the same chain, [36]. This results in a decrease in the cross-linking density and an increase in the space between cross-links. For these systems the solvent (ethanol) concentration was relatively high (1:1 by weight of the macromer and cross-linker), which is known to increase the probability of primary cyclization.

For the PEG200MMA and PEG1000MMA systems cross-linked with PEG400DMA the volume swelling ratio was smaller for the system containing the PEG200MMA macromer. This is because the ethylene glycol (EG) content of PEG200MMA is much less than that of PEG1000MMA (by a factor of five), which makes the system less hydrophilic. For the same macromers cross-linked with TEG-DMA the swelling ratio for PEG200MMA versus PEG1000MMA was also lower, resulting from the decreased EG content. Iron oxide nanoparticle loading had a slight effect on swelling of the PEG-based hydrogel nanocomposites. The gels with iron oxide nanoparticles exhibited slightly higher volume swelling ratios than those without nanoparticles (data not shown). This may be attributed to the particles interfering with the macromers and cross-linkers to cause less effective cross-linking during polymerization.

The PEG-based hydrogels were shown to be thermally responsive, as seen by the change in their swelling properties when exposed to various temperatures. The swelling ratios for all systems decreased as they reached their approximate LCST. For the PEG200MMA hydrogels the volume swelling ratio was approximately 2.2 at 63 °C, which is above the LCST [29] of this compound, explaining the partial collapse of the hydrogel structure. This attribute of the hydrogels may allow them to be used in controlled drug delivery applications when switching the temperature of the system from below their LCST at room temperature to above it in the body.

3.3. Cytotoxicity analysis of Fe3O4 nanoparticles and hydrogels

The effects of iron oxide nanoparticles on the cell viability of fibroblasts after 24 and 48 h are shown in Table 2. For all nanoparticle concentrations the cell viability was statistically insignificant in comparison with the control sample, regardless of the amount of nanoparticle loading in the medium (P > 0.05). This indicates that the iron oxide nanoparticles have the potential to be biocompatible (most likely due to their polymeric coating) and thus should not be recognized by the body as foreign objects should they leach from the hydrogel nanocomposites.

After the hydrogels were soaked in complete fibroblast medium for 48 h the medium was transferred to fibroblasts for 24 and 48 h. The results determined whether or not any harmful substances leached from the hydrogels into the medium. As seen in Table 2, for all hydrogel systems the cell viability was favorable and close to that of the control (P > 0.05). This indicates minimal or no leaching of substances from the hydrogels, which was as expected. Fig. 3 shows the favorable responses of fibroblasts exposed to both the nanoparticles and gel-exposed medium in comparison with a control on polystyrene. The cells had good morphology and looked similar to those of the control. A similar cell morphology was seen for cells exposed to all nanoparticle concentrations and hydrogel systems (results not shown). Overall, these results indicate that magnetic PEG-based hydrogel nanocomposites have the potential to be biocompatible.

3.4. Characterization of the remote controlled heating of nanocomposite hydrogels

Swollen hydrogels equilibrated at 22 °C were exposed to an AMF for 5 min at 297 kHz and 25 kA m$^{-1}$ to induce heating within the system. As seen in Fig. 4, the hydrogel nanocomposites reached
their maximum temperature after 180 s. Hydrogels without magnetic particles were also exposed to the AMF (data not shown) and exhibited minimal heating, confirming that heat generation was due to the Fe$_3$O$_4$ nanoparticles. The amount of heating of the hydrogel systems was dependent on the swelling ratio of the gel and, subsequently, the iron oxide nanoparticle loading per gel volume. The estimated number of Fe$_3$O$_4$ nanoparticles for a given swollen nanocomposite disc was calculated from the following:

$$\text{Fe}_3\text{O}_4 \text{ mass} = \frac{M_{\text{heat}}}{q_w} W_i = \frac{M_{d}^\text{is}}{M_{s}^\text{is}} W_i$$

where $M_s$ refers to the gels in the swollen state, $M_d$ in their dry state and the superscripts heat and is refer to the gels prior to heating and the initial swelling data, respectively. $q$ is the mass swelling ratio calculated from the mass of swollen gels over dry gels and $w_i$ refers to the initial wt.% of iron oxide loaded into the gels. The volume for a swollen nanocomposite disc prior to heating was then determined from:

$$V_{\text{disc}} = \left( \frac{M_{\text{air}}^\text{heat} - M_{\text{heptane}}^\text{heat}}{\rho_{\text{heptane}}} \right)$$

where $M_{\text{air}}$ and $M_{\text{heptane}}$ are the mass of the gels prior to heating in air and in n-heptane, respectively. The iron oxide mass per hydrogel volume was then calculated by taking the ratio of the iron oxide mass and gel volume calculated above. As expected, as the amount of iron oxide per volume in the gels increased the final maximum temperature of the systems increased, as shown in Table 2. The variance in heating can be attributed to the fact that the gels with a higher volume swelling ratio had a looser mesh and fewer particles present when cut at the same size as a gel with a lower $Q$ value that had a tighter mesh and more particles. A demonstration of this phenomenon can be seen in the insert to Fig. 5.

For hyperthermic applications the temperature of cancerous tissue needs to reach 42–45 °C for effective therapy, whereas temperatures above 50 °C cause damage to cancer cells via thermoablation. It was demonstrated that the temperature of the hydrogels can be controlled by changing the AMF strength so that the gels either reached hyperthermic (42–45 °C) or thermoablative (60–63 °C) temperatures. Fig. 5 demonstrates that the final temperature the hydrogel nanocomposites reach can be tailored to either one of these temperature ranges. The insert shows IR images of the gels heated to hyperthermic temperatures (a–c) and thermoablative

![Fig. 4. Thermal response of hydrogel nanocomposites exposed to AMF at 297 kHz and 25 kA m$^{-1}$ for 5 min. The AM to FM gel abbreviations are defined in Table 1. The insert represents the hydrogel mesh structures and iron oxide particles for a gel with a higher volume swelling ratio (left) and a lower swelling ratio (right).](image)

![Fig. 5. Thermal analysis of hydrogel nanocomposites exposed to varied AMF strengths to control gel temperatures in the hyperthermia and thermoablative temperature ranges, where Th and Hy after the gel abbreviations represents thermoablative and hyperthermia heating, respectively. Transparent boxes represent the thermoablative (top) and hyperthermia (bottom) temperature range goals for heating. The insert show IR images of gels for hyperthermia (a–c) and thermoablative (d–f) at 15 s and 1 and 5 min.](image)
temperatures (d–e) for 15 s and 1 and 5 min, respectively. The variance in the temperatures reached by the hydrogels was directly controlled by the strength of the AMF, as seen in Table 2. As expected, for the hydrogel nanocomposites with less iron oxide per hydrogel volume the AMF strength needed to heat the nanocomposites to the appropriate temperature range increased. This was true for both the hyperthermic and thermoablative temperature ranges and the field strength needed to heat gels for thermoablation was higher than that for hyperthermia.

3.5. Thermoablation demonstration with M059K glioblastoma cells exposed to hydrogels heated in an AMF

M059K glioblastoma cells were heated with hydrogel nanocomposites exposed to an AMF to both demonstrate the ability of the gels to kill cells via thermoablation and prove the safety of exposure to the field. Fig. 6 (top) shows the heating set-up, with the hydrogel placed in Saran wrap on the solenoid with the Petri dish containing cells on top of the gel-wrap. Previous studies showed negligible heat loss through the Petri dish, so the cells received direct heat through the polystyrene. An 8.2 mm hydrogel was placed under a 35 mm dish so that only the center area of cells was affected by the heat. This resulted in an acute cellular response, as indicated in Fig. 6 (bottom), where the center cells were killed by the heat and the outer cells were unaffected, with a distinct interface between the live and dead cells. A 50 mol.% PEG200MMA, 50 mol.% TEGDMA hydrogel was used as it showed an ability to produce the greatest heat with the lowest field strength. Fig. 7 confirms the success of these experiments, with the left column of images showing M059K cells heated by the gel, the center cells exposed to the field only and the right controls. The cells heated with the gel showed distinct cell death at the center and an interface, whereas both of the controls showed favorable cell morphology. The IR images show the final temperatures the cells were exposed to [63 °C for thermoablation (j) and 24 °C for AMF control (k)]. Overall, these results show the ability of the gels to kill cancer cells without the magnetic field causing harm.

4. Conclusions

It was demonstrated that PEGMMA/PEGDMA magnetic hydrogel nanocomposites can potentially be used in thermal cancer therapy through remote heating via application of an AMF. Swelling analysis of the hydrogels indicated a dependence of the swelling properties on both the EG content in the gels and...
the cross-linking density of the system. As either the amount of EG increased or cross-linking decreased the volume swelling ratio of the hydrogels increased. The hydrogel systems were shown to be slightly temperature responsive. Increasing the temperature of the hydrogels decreased their volume swelling ratio upon reaching the LCST. The exposure of murine fibroblasts to the hydrogel nanocomposites and iron oxide nanoparticles was carried out and favorable cell viability was seen for both the gels and particles, indicating their safe use as in vivo systems. This is due to both the high EG content and high water content of the hydrogels. Both hyperthermia and thermoablation are therapies that increase the efficacy of both irradiation and chemotherapy. Upon exposure to an AMF the hydrogels showed an ability to heat to both hyperthermic and thermoablative temperatures, which can be controlled by the content of the iron oxide in the hydrogel or by changing the strength of the applied alternating magnetic field. For gels with a higher iron oxide content a lower field strength was needed to get the desired temperature and as the field strength was increased for a particular system the temperature also increased.

A proof of point demonstration was done to show the ability of these hydrogel nanocomposites to kill M059K glioblastoma cells in vitro by exposing the cells to hydrogels at thermoablative temperatures. This concept can be further extended to systems for the delivery of heat at hyperthermica temperatures with the delivery of chemotherapeutics as a dual delivery system for cancer therapy. These systems could potentially be used to target cancer in two primary applications, including implantation after surgical resection of a tumor for prevention of metastasis or recurrence and injection for in situ formation of the hydrogels in difficult to reach locations. For this to occur in vivo, testing of these materials must be completed, including those demonstrating biocompatibility of the nanocomposites (before and after heating), potential iron oxide nanoparticle release during implantation and the overall effect of heating on the surrounding tissues. The limitations of these systems that must be overcome may include, but are not limited to, non-specific formation of the hydrogels during in situ implementation, encapsulation of the hydrogel system, the need to surgically remove the implant after its useful life and the possible need for long-term drug delivery.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 2–7, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi: 10.1016/j.actbio.2009.10.017.

References

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