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Evaluation of Two Chemical Crosslinking Methods of Poly(vinyl alcohol) Hydrogels for Injectable Nucleus Pulposus Replacement

Valerie R. Binetti,[1] Garland W. Fussell,[2] Anthony M. Lowman[3]

[1]Department of Materials Science and Engineering, Drexel University, Philadelphia, Pennsylvania 19104
[2]Depuy Synthes Biomaterials, West Chester, Pennsylvania 19382
[3]Rowan University, Glassboro, New Jersey 08028
Correspondence to: V. R. Binetti (E-mail: valeriebinetti@gmail.com)

ABSTRACT: Low back pain caused by intervertebral disc degeneration is one of the most common spinal disorders among patients seeking medical treatment. The most common surgical treatments are spinal fusion and total disc arthroplasty, both of which are very invasive surgical procedures. Nucleus pulposus replacement is an earlier stage intervention for disc degeneration. One of the material classes being studied for this application is hydrogels: a three-dimensional hydrated network of polymer(s), which mimics the mechanical and physiological properties of the nucleus. Poly(vinyl alcohol) (PVA), poly(vinyl pyrrolidone) (PVP), and poly(ethylene glycol) (PEG) hydrogels have previously been shown to be great candidate materials for injectable nucleus pulposus replacement, but have experienced issues with swelling and mass retention. The addition of chemical crosslinking to the PVA/PVP/PEG hydrogel system will allow tailoring of the swelling, mechanical, injectability, and mass loss properties of the hydrogel network. Two chemical crosslinking methods were evaluated for the PVA/PVP/PEG hydrogel system by characterizing the hydrogels with compression, swelling, and spectroscopy experiments. The results of these experiments led to the selection of the difunctional crosslinking strategy using PEG functionalized with terminal epoxide group (PEG diglycidyl ether) as the preferred crosslinking method. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40843.

KEYWORDS: biomedical applications; crosslinking; irradiation; mechanical properties; swelling

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INTRODUCTION

Nucleus pulposus replacement is a treatment option for patients with early stages of disc degeneration, before any significant annular damage. Replacement of the degenerating nucleus pulposus allows for proper mechanical loading of the spine in which the annulus fibrosus is loaded in tension instead of compression, which happens as a result of disc degeneration. Hydrogel materials have been investigated for this application due to their three-dimensional polymer networks with water content and mechanical properties similar to that of the natural nucleus material.[1–8] An injectable hydrogel for nucleus replacement is an ideal design feature, due to the ability for a minimally invasive procedure. Previously investigated hydrogels for injectable nucleus replacement have been freeze/thawed poly(vinyl alcohol) (PVA)–poly(vinyl pyrrolidone) (PVP) copolymers which were formed into a string and freeze/thawed before injection or the *in situ* forming poly(*N*-isopropylacrylamide) (PNIPAAm)-based materials which could not match the properties of the nucleus tissue.[9] Ideally, an injectable hydrogel network will gel within minutes of injection in to the nuclear cavity for a cohesive implant with similar properties to the native nucleus tissue.

Chemical crosslinking of PVA hydrogels has been previously done with radiation (e.g., gamma radiation and ebeam radiation)[10–17] and difunctional crosslinking agents (e.g., glutaraldehyde and diepoxides).[7,18–23] Chemical crosslinking of PVA typically eliminates the ability to inject the material. However, it can be molded into a string similar to PVA/PVP physical network gels, but this does not create a cohesive implant. Physical PVA hydrogels have been produced using the theta-gel method[24–29] using poly(ethylene glycol) (PEG) as the gellant, which does result in an injectable hydrogel that forms a cohesive implant. The biggest limitations for PVA theta-gels are high swelling ratios and low mass retention in biological simulated environments. This work will combine the ideas of using PEG to create PVA theta-gels and chemically crosslinking these hydrogels by either radiation or difunctional crosslinkers to overcome the swelling and mass retention issues experienced with current PVA theta-gels.

Table I. Sample Compositions for Radiation Crosslinked Formulations

	Sample composition (% w/w)		
Components	9.5% PVA	12.1% PVA	14.8% PVA
PVA (145 kDa)	9.5	12.1	14.8
PVP (58 kDa)	0.1	0.1	0.1
Deionized H_2O	75.0	70.0	65.0
$BaSO_4$ (1–10 μm)	7.0	7.0	7.0
PEG (4.6 kDa)	8.4	10.7	13.1

In this work, two families of chemically crosslinked hydrogels were synthesized: one using radiation and one using a difunctional crosslinker. The radiation crosslinked hydrogels were created using electron beam radiation; PVA content and electron beam radiation dosage were varied to determine the effects of each variable. For the difunctional crosslinked gels, PEG diglycidyl ether (PEG-DGE) was used to crosslink PVA; basic catalyst volume and reaction time were varied to determine the effect of each variable on the hydrogel properties. In the following experiments, the swelling and mechanical properties of these crosslinked hydrogels were studied as a function of PVA content, irradiation dosage, catalyst volume, and reaction time. It is hypothesized that a material candidate from this family of chemically crosslinked PVA/PEG-DGE hydrogels could serve as a synthetic nucleus pulposus replacement.

EXPERIMENTAL

Materials

PVA (99.0–99.8% hydrolyzed, molecular weight 145 kDa) and PEG-DGE (molecular weight 526 Da) were purchased from Aldrich. PVP (molecular weight 58 kDa) was purchased from ISP Technologies. PEG (molecular weight 4.6 kDa) was obtained from Aldrich and PEG (molecular weight 20 kDa) was obtained from Crescent Chemical Company. Barium sulfate ($BaSO_4$) with a 1–10 μm particle size was purchased from J.T. Baker. Sodium hydroxide (reagent grade, ≥98%) and sodium chloride (reagent grade, ≥99%) were obtained from Sigma–Aldrich. Dialysis tubing purchased from Fisher had a nominal molecular weight cut-off of 3500 g/mol.

Hydrogel Synthesis

Radiation Crosslinked. PVA/PVP/PEG hydrogel compositions (Table I) were made by preparing an aqueous PVA/PVP solution (ranging from 9.6 to 14.9% w/w) by mixing PVA, PVP, and deionized water in a sealed glass bottle and heating to 121°C for 30 min in an autoclave. The ratio of PVA to PVP was 99 : 1. After the autoclave cycle, solutions were removed from the autoclave and equilibrated to 75 ± 5°C in a water bath. $BaSO_4$ (7.0% w/w) was then mixed into the PVA/PVP solution and the mixture was autoclaved again at 121°C for 30 min. Previous work has shown that a 4–15% concentration of $BaSO_4$ in the resultant hydrogel composition was sufficient to make the hydrogels radiopaque. The addition of $BaSO_4$ was an optional step.

After the second autoclave cycle, solutions were again removed from the autoclave and equilibrated and maintained at 75 ± 5°C in a water bath during the addition of PEG (MW = 4.6 kDa) by manual stirring. After the addition of PEG, the mixtures were left to equilibrate at RT for 3 ± 0.25 h. During the equilibration time, the solution separated into a polymer-rich gel and a solvent-rich liquid phase, at which point the liquid phase was decanted, leaving just the polymer-rich gel. The gel was then autoclaved for a third time at 121°C for 30 min, after which the gel was again separated from the additional solvent-rich liquid phase that has formed; the gel was then loaded into a 60 mL syringe and injected into 15 mL centrifuge tubes. The resulting tubes of hydrogel were then irradiated at RT with a 10 MeV electron beam to the desired dosages of 15 or 20 kGy at Sterigenics (Salem, NC), or reserved as 0 kGy controls. Following irradiation, the hydrogel was autoclaved again at 121°C for 30 min, after which the gel was loaded into a 60 mL syringe and molded into a 15 mL centrifuge tube or a test specific mold. The chemical reaction for the radiation crosslinking of PVA is shown in Figure 1.[30]

Difunctional Crosslinked. PVA/PVP/PEG-DGE hydrogel compositions (Table II) were made by preparing an aqueous PVA/PVP solution (14.4% w/w) by mixing PVA, PVP, and deionized water in a sealed glass bottle and heating to 121°C for 30 min in an autoclave. $BaSO_4$ was added to hydrogels used for swelling, mechanical testing, and cytotoxicity, but it was not used

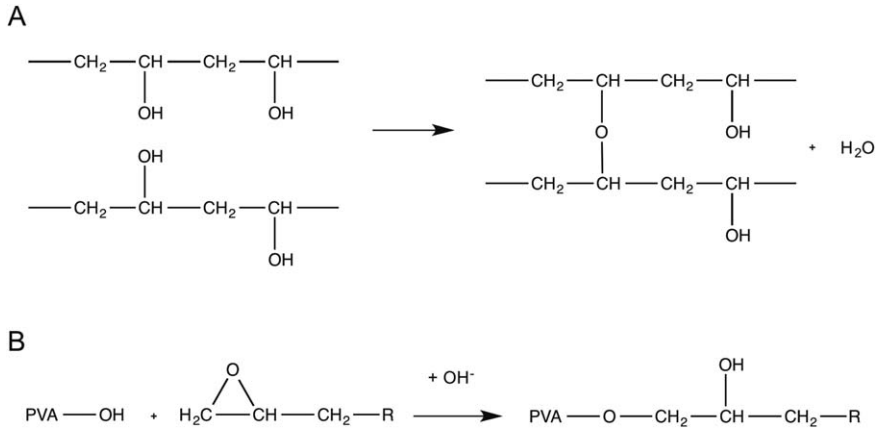

Figure 1. Chemical reactions: (A) radiation crosslinking of PVA, (B) difunctional crosslinking of PVA.

Table II. Sample Compositions for Difunctional Crosslinked Formulation

	Sample composition (% w/w)
Components	29% PEG-DGE
PVA (145 kDa)	14.27
PVP (58 kDa)	0.13
Deionized H_2O	49.60
$BaSO_4$ (1–10 μm)	7.00
PEG-DGE (526 Da)	29.00

for other testing due to issues with overpowering the signal of the polymers in the system. When used, $BaSO_4$ was added before the first autoclave cycle at 7.0 wt %.

After the autoclave cycle, solutions were equilibrated to 75 ± 5°C in a water bath. PEG-DGE (29.0% of the total solution mass) was then stirred into the solution with 100 μL of 10*M* sodium hydroxide (per 75 g batch, if another volume is not specified) to form a gel. NaOH was added to create a basic condition for the ring opening reaction of the PEG-DGE to enable it to crosslink to PVA (Figure 1).[31] The solution was left to react for 24 h (if another time is not specified), the supernatant was then decanted and the gel was autoclaved again at 121°C for 30 min. After this last autoclave cycle, the material was loaded into a 60 mL syringe and molded into a 15-mL centrifuge tube or a test specific mold.

Swelling Mechanics

Osmotic solutions were made by dissolving PEG (20 kDa) in 0.15*M* sodium chloride to achieve osmotic pressures mimicking the swelling pressure of the IVD. Equation (1) was used to calculate the PEG concentration, c_2, required to for an osmotic pressure, Π:

$$\Pi = RT\left(\frac{c_2}{M_2} + Bc_2^2 + Cc_2^3 + \ldots\right), \quad (1)$$

where R is the universal gas constant, T is the absolute temperature, and M_2 is the polymer molecular weight. The second and third virial coefficients, B and C, for 20 kDa PEG are 2.59×10^{-3} and 13.5×10^{-3}, respectively.[32] The osmotic pressure used for this study, 0.2 MPa, is the midpoint of the range measured by Urban and McMullin for cadaver IVDs[33] and has a PEG concentration of 128.2 g/mL.

After the final autoclave cycle of the hydrogel synthesis procedure, the hydrogel was loaded into a 60 mL syringe and injected into poly(vinyl chloride) (PVC) tubing with an inner diameter of 9.5 mm. Cylindrical samples, approximately 0.5 cm^3 in volume, were sliced from the PVC tubing, the tubing was removed and the sample was weighed in air and heptane to determine the initial density of the hydrogel using eq. (2):

$$\rho_{\text{hydrogel}} = \frac{\rho_{\text{heptane}} \times m_{\text{air}}}{(m_{\text{air}} - m_{\text{heptane}})}, \quad (2)$$

where ρ_{hydrogel} is the density of the hydrogel, ρ_{hep} is the density of heptane, m_{air} is the mass of the hydrogel in air, and m_{heptane} is the mass of the hydrogel in heptanes.[3] Using the density and initial mass of each sample, the initial volume was calculated by dividing the mass by the density. The samples were then placed in dialysis tubing to prevent uptake of PEG (20 kDa) by the hydrogels. Hydrogels in tubing were placed in the 0.2 MPa PEG solutions for 1 week at 37°C. The volume of swelling medium was 100× larger than the volume of hydrogel samples to prevent significant changes in the pressure of the osmotic solution due to changes in the hydrogel water content over the length of the study.

Samples were removed from the PEG solution and the dialysis tubing at each time point (0, 1, 4, and 7 days), after which each sample was weighed in air and heptane to determine the osmotic volume change (V/V_o) by comparing the volume of swollen samples to the volume of the initial samples [eq. (3)]. After swelling, hydrogel samples were dried in an oven at 50°C; the mass of the dried hydrogel samples was compared to the initial mass to calculate the initial water content [eq. (4)].

$$\text{Osmotic volume change} = \frac{\text{swollen volume}}{\text{initial volume}} \quad (3)$$

$$\text{Initial water content} = 1 - \frac{\text{dry mass}}{\text{initial mass}} \times 100\%. \quad (4)$$

Mechanical Properties

Unconstrained, uniaxial compressive modulus was measured to determine if the hydrogel would be a suitable nucleus pulposus replacement material. Testing was conducted using an Instron Materials Testing System Series 4442 (Norwood, MA) bench-top mechanical testing system with a 50 N load cell.

After the hydrogel was allowed to react for the specified amount of time it was autoclaved at 121°C for 30 min. After the autoclave cycle, the hydrogel was loaded into a 60 mL syringe and injected into PVC tubing with an inner diameter of 9.5 mm. The hydrogel was then removed from the tubing in sections 20 cm in length, placed in dialysis tubing and swollen in 0.2 MPa osmotic PEG solutions for up to 4 weeks at 37°C. After swelling, cylindrical hydrogel samples are sliced ($n = 5$), to a thickness of 7–8 mm and cut to ensure flat surfaces. The samples were preloaded to 0.1 N and compressed to a total strain of 30% at a strain rate of 100% min^{-1}; a chord from the initial linear portion of the stress versus strain curve (10–20% strain) was used to calculate elastic modulus. At least five independent samples were tested for each set of hydrogels ($n = 5$).

FTIR

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) analysis was conducted to examine the hydrogel crosslinking, scission, and final polymer composition using a Thermo Nicolet 6700 with ATR attachment (Madison, WI). After the hydrogel was allowed to react for the specified amount of time, it was autoclaved at 121°C for 30 min. Following the autoclave cycle, the hydrogel was loaded into a 60 mL syringe and injected onto polyethylene terephthalate copolymer with cyclohexylene dimethylene segments (PETG) sheeting with spacers of 0.1 mm thickness to control thickness of the film. Samples larger than the diameter of the ATR crystal were cut from the hydrogel film and then tested ($n = 3$). The spectra of the as-prepared films were obtained with 4 cm^{-1} resolution

(1.928 cm^{-1} data spacing) and 64 scans. Spectral analysis was performed using the Nicolet OMNIC software package.

Statistical Analysis

All data points are represented as the mean ± one standard deviation for at least three independent samples. Statistical significance was determined by one-way analysis of variance (ANOVA) with post hoc analysis by Bonferroni correction with a 95% confidence interval. *p*-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Swelling Mechanics

Radiation Crosslinked. The osmotic volume changes for the electron beam irradiated hydrogels are shown in Figure 2. The 9.5% PVA hydrogel formulation formed a gel at a dosage of 20 kGy, while a gel was not formed at 0 or 15 kGy. Both the 12.1 and 14.8% PVA hydrogel formulations formed gels at dosages of 0, 15, and 20 kGy. There was no difference in osmotic volume change for the 12.1% PVA formulation at each irradiation dosage ($p > 0.05$), nor for the 14.8% PVA formulation ($p > 0.05$). At 0 kGy dosage, the osmotic volume change increased from 12.1% PVA to 14.8% PVA ($p < 0.001$). There was no significant difference in osmotic volume change at 15 kGy between the 12.1 and 14.8% PVA formulations. At 20 kGy, the osmotic volume change increased from 9.5% PVA to 12.1% PVA ($p < 0.001$), and from 12.1% PVA to 14.8% PVA ($p < 0.01$).

The initial water content values for the electron beam irradiated samples are shown in Figure 3. For the initial water content of the 12.1% PVA formulation, there was no significant difference ($p > 0.05$) between 0 and 15 kGy or 15 and 20 kGy, but there was an increase in water content from 0 to 20 kGy ($p < 0.05$). For the 14.8% PVA formulation, there was no significant difference ($p > 0.05$) in initial water values between the three-irradiation dosages tested: 0, 15, and 20 kGy.

Difunctional Crosslinked. The osmotic volume change of hydrogel formulations with increasing basic catalyst volume from 0 to 200 μL is shown in Figure 4 and the initial water content values are shown in Figure 5. As catalyst volume was increased from 0 to 200 μL there was an increase in osmotic volume change from 0.96 ± 0.01 to 1.05 ± 0.02 ($p < 0.01$).

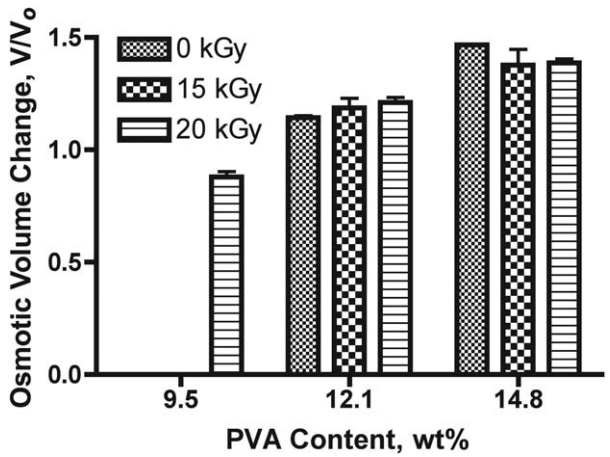

Figure 2. Osmotic volume change of the electron beam crosslinked samples. At 9.5% PVA, a gel is only formed at 20 kGy. At 12.1% PVA and 14.8% PVA there is no significant change ($p > 0.05$) in osmotic volume change with increase in radiation from 0 to 20 kGy ($n = 3$).

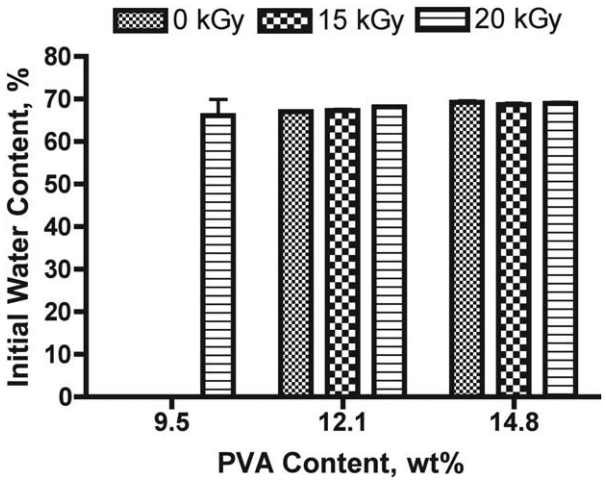

Figure 3. The initial water content for electron beam crosslinked hydrogels. The 12.1% PVA formulation increases from 0 to 20 kGy ($p < 0.05$) but there is no significant difference in the initial water content for the 14.85 PVA formulation ($p > 0.05$) ($n = 3$).

The osmotic volume changes of hydrogel formulations with varying reaction times are shown in Figure 6, while Figure 7 shows the initial water contents. As reaction time was increased from 1 to 72 h, the osmotic volume change decreased from 1.17 ± 0.01 to 1.01 ± 0.02 ($p < 0.01$). There was no significant difference in initial water content values as reaction time varied ($p > 0.05$).

Mechanical Properties

Radiation Crosslinked. The compressive moduli for the radiation crosslinked hydrogels are shown in Figure 8. For the 12.1 and 14.8% PVA there was no change in stiffness between 0, 15, and 20 kGy irradiation dosages ($p > 0.05$). The 9.5% PVA

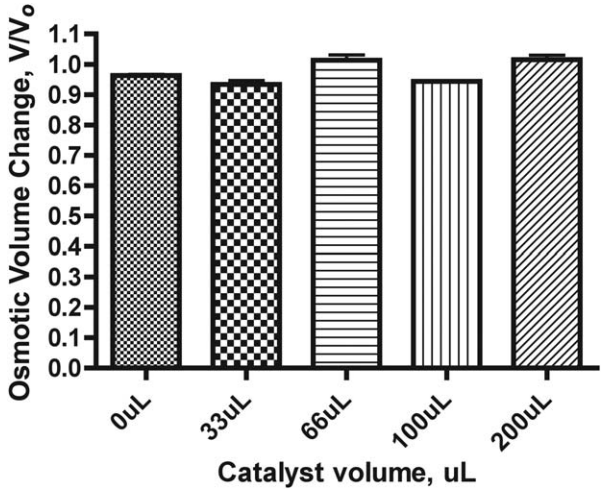

Figure 4. Osmotic volume change of difunctional crosslinked hydrogels varying basic catalyst volume. As the basic catalyst volume is increased from 0 to 200 μL the osmotic volume change increases ($p < 0.01$) ($n = 3$).

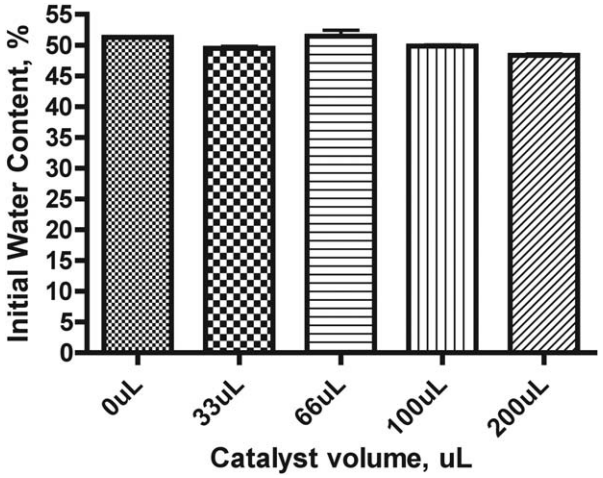

Figure 5. Initial water content of hydrogel formulations varying basic catalyst volume. As the basic catalyst volume is increased from 0 to 200 μL the initial water content of the gel decreases ($p < 0.05$) ($n = 3$).

hydrogel formulation only formed a gel at 20 kGy. There was also no difference between the compressive moduli of the 12.1 and 14.8% PVA formulations at any dosage. The only difference measured in electron beam irradiated gel moduli was between the 9.5 and 14.8% PVA formulations at 20 kGy ($p < 0.01$).

Difunctional Crosslinked. The compressive moduli are shown for the varying catalyst volumes in Figure 9 and for varying reaction times in Figure 10. As catalyst volume increased, from 0 to 200 μL, compressive moduli at 2 weeks increased ($p < 0.001$). For the reaction time formulations moduli increased from 1 to 24 h ($p < 0.001$) and from 24 to 48 h ($p < 0.001$), but did not increase from 48 to 72 h ($p > 0.05$).

FTIR

Radiation Crosslinked. Figure 11 shows the FTIR spectra for 14.8% PVA at 0, 15, and 20 kGy. As irradiation increased, from 0 to 20 kGy, the PEG—CH_2— stretch (2851 cm^{-1}) and the PVA—CH— stretch (2908 cm^{-1}) increased indicating the scission of the polymer chains.[34,35] Zhang and Yu[36] show how CH is increased in irradiated PVA via chain scission.

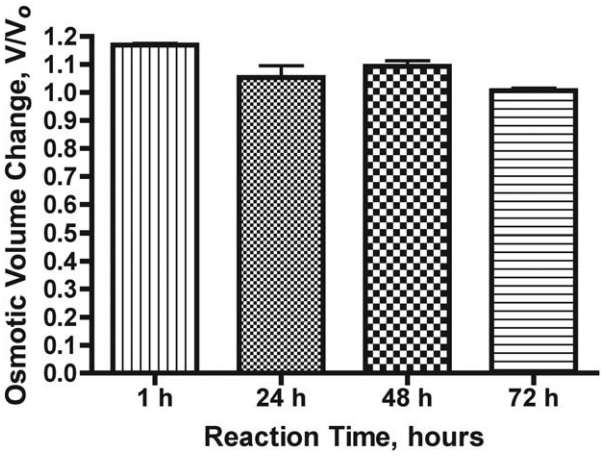

Figure 6. Osmotic volume change of difunctional crosslinked hydrogel formulations varying reaction time. As reaction time is increased from 1 to 72 h, the osmotic volume change decreases ($p < 0.01$) ($n = 3$).

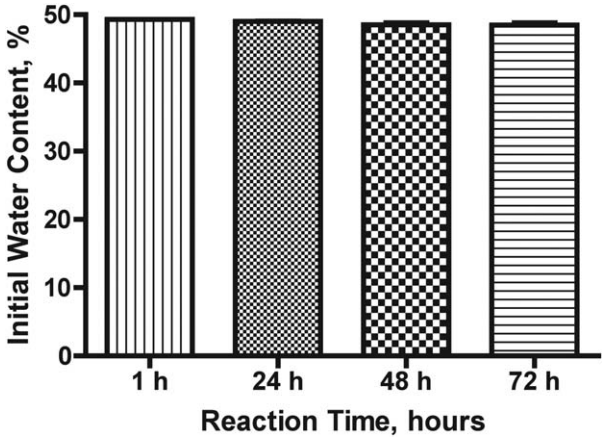

Figure 7. Initial water content of hydrogel formulations varying reaction time. As the reaction time is increased from 1 to 72 h, there is no significant difference in initial water content ($p > 0.05$) ($n = 3$).

Difunctional Crosslinked. Figure 12 shows the FTIR spectra for basic catalyst volume. As catalyst volume increased from 0 to 200 μL there was a decrease in the peaks where the CH stretch of the PVA (2906 cm^{-1}) and the CH_2 stretch of the PEG-DGE (2880 cm^{-1}) overlap. In addition, there is a decrease in the peaks where the C—O stretch of the PVA (1023, 1087, 1142 cm^{-1}) and the C—O—C out of phase stretch of the PEG-DGE (1059, 1093, and 1145 cm^{-1}) overlap as catalyst volume increased from 0 to 200 μL.

DISCUSSION

Hydrogels mimic the behavior of the nucleus pulposus, in particular in their ability to swell and release water throughout the course of the day. Proteoglycan and hydration levels in the nucleus material are at a concentration sufficient to produce an osmotic pressure of between 0.05 and 0.3 MPa.[33] This high

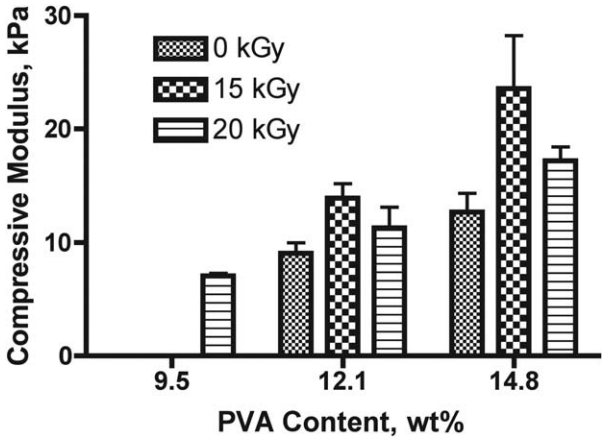

Figure 8. Compressive moduli of electron beam hydrogel formulations. For the 12.1 and 14.8% PVA hydrogel there is no difference in the compressive modulus as the electron beam dosage is increased from 0 to 20 kGy ($p > 0.05$). There is an increase in compressive modulus from the 9.5% PVA 20kGy to the 14.8% PVA 20 kGy hydrogel ($p < 0.01$) ($n = 5$).

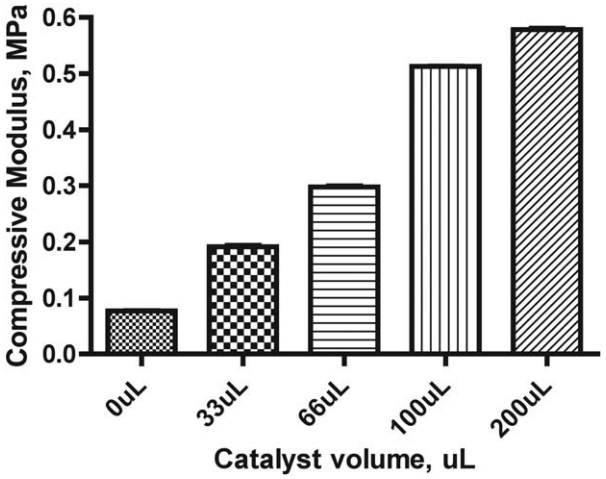

Figure 9. Compressive moduli of difunctional crosslinked hydrogel formulations varying basic catalyst volume. With each increase in catalyst volume there is an increase in compressive modulus ($p < 0.001$) ($n = 5$).

pressure arises from the loading conditions within the disc and the spine. When the material, natural nucleus, or hydrogel is at equilibrium there is no net fluid loss or gain.

The changes in osmotic volume change for the electron beam gels were a combination of increasing polymer concentration between formulations and the effects of scission. The increase in initial water content values in the 12.1% PVA formulations with increasing irradiation was due to increased crosslinking with increased irradiation dosage. The lack of change in the 14.8% PVA formulations was due to the increased crosslinking and scission that was occurring in this formulation as a result of the higher polymer concentration. The osmotic volume change decrease from 1 to 72 h reaction time for the difunctional crosslinked gels was due to increased crosslinking with increased reaction time.

Normal nucleus tissue properties vary with state of degeneration and have been described in some cases as fluid[37] and in others as an isotropic solid.[3,38] Because of the variation in tissue, it is difficult to match the mechanical properties of the tissue with

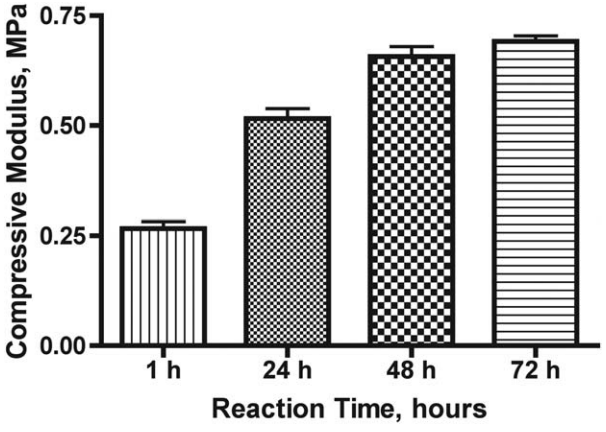

Figure 10. Compressive moduli of difunctional hydrogel formulations varying reaction time. There is an increase in compressive modulus as reaction time is increased from 1 to 24, 48, and 72 h ($p < 0.001$) ($n = 5$).

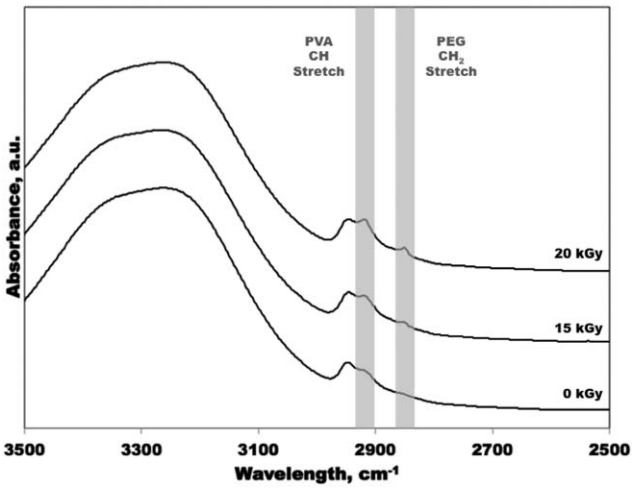

Figure 11. FTIR of electron beam 14.8% PVA hydrogel formulation; with increased irradiation, from 0 to 20 kGy, the PEG—CH_2— stretch (2851 cm^{-1}) and the PVA—CH— stretch (2908 cm^{-1}) increases indicating scission of the polymer chains.

the hydrogel material. However, cadaver testing and finite element modeling have shown that a polymeric hydrogel implant with a compressive modulus of at least 50 kPa at 15% strain can restore tension in annulus fibers.[3,39] The compressive modulus for each electron beam crosslinked formulation was below 50 kPa, therefore they were not considered as potential candidate materials for nucleus replacement. For the difunctional crosslinked gels, the compressive modulus increased significantly with each increase in catalyst volume from 0 to 200 μL, which was due to increased crosslinking with each increase in catalyst volume. As stated before, there was also a decrease in water content as the catalyst volume increased, due to hydrolysis of the PEG-DGE in the alkaline environment. More PEG-DGE and water were removed from the system with each increase in

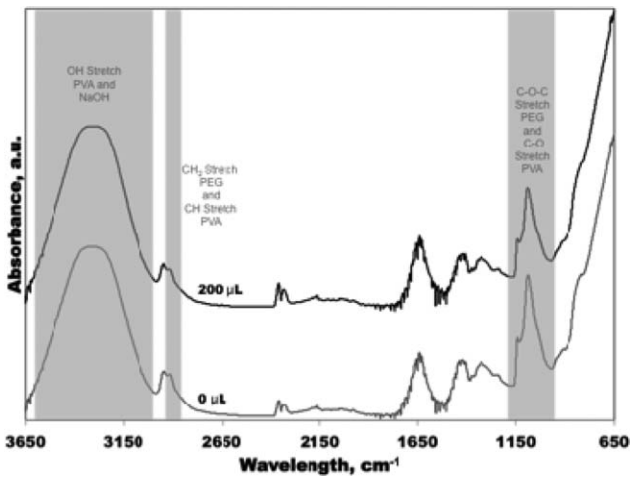

Figure 12. FTIR of difunctional crosslinked chemically crosslinked hydrogels varying basic catalyst volume shows a decrease in the PVA CH stretch (2906 cm^{-1}) and the PEG-DGE CH_2 stretch over lap (2880 cm^{-1}) over lap and a decrease in where the PVA C—O stretch (1023, 1087, 1142 cm^{-1}) and the PEG-DGE C—O—C out of phase stretch (1059, 1093, 1145 cm^{-1}).

Figure 13. Electron beam crosslinked hydrogel formulations with radiation dosages ranging from 0 to 100 kGy (right to left) showing how electron beam dosages above 20 kGy lead to non-injectable hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

catalyst volume, which resulted in a more polymer dense hydrogel network. As the reaction time was increased from 1 to 24, 48, and 72 h the modulus also increased. These additional reaction times allowed for an increase in crosslinking within the hydrogel network. All the difunctional crosslinked hydrogels had compressive moduli above 50 kPa at each of the time points tested.

The FTIR for the electron beam irradiated gels explained why there was not a significant increase in compressive modulus and a significant decrease in the osmotic volume change of the hydrogel formulations with increasing irradiation dosage. Although the gels were crosslinking, which was mostly noticeable due to their change in texture and their injectability, there was also scission of the polymers within the network. Scission introduced shorter chain polymer chains in addition to the longer/branched chains formed by the chemical crosslinking, which produced no change in properties with each dosage increase. The FTIR for the difunctional crosslinked hydrogels showed that in addition to the crosslinking in the system, there was a shift in polymer content with additional catalyst volume. The additional catalyst did not just provide a more alkaline environment for the ring opening reaction between the PEG-DGE epoxide and the PVA hydroxyl, it also allowed for additional hydrolysis of the PEG-DGE epoxide ring. This end group change increased the solubility of the PEG, allowing for it to pull additional water out of the system in the supernatant that was formed with the addition of PEG or PEG-DGE. This conclusion was validated by the decrease in water content from the 0 μL formulation ($51.3 \pm 0.1\%$) to the 200 μL formulation ($48.3 \pm 0.4\%$) ($P < 0.05$).

CONCLUSIONS

Because of the increased scission in the radiation crosslinked hydrogels, the compressive modulus was below 50 kPa, the minimum modulus established to restore healthy tension in the annulus fibers.[3,39] Hydrogel samples were irradiated with dosages ranging from 0 to 100 kGy, but only the formulations from 0 to 20 kGy were flowable after irradiation (Figure 12). The low modulus value removed electron beam irradiation as a potential method for chemically crosslinking the PVA/PEG hydrogels for injectable nucleus replacement.

All the difunctional crosslinked PVA/PVP/PEG-DGE hydrogels had modulus values above the necessary 50 kPa, in addition to having low osmotic volume changes when swollen in 0.2 MPa osmotic pressure solution. The hydrogels were also easily injected through a 10-gauge needle on a hand-depressed syringe. Similar to the electron beam gels with dosages over 20 kGy shown in Figure 13, with catalyst volumes greater than 200 μL the hydrogels were not flowable again after the 24-h reaction period. This was due to chemical crosslinking and the decreased water content in the hydrogel as additional catalyst was added. The results from this study show that a family of injectable chemically crosslinked hydrogels was developed that show promise as potential materials for nucleus pulposus replacement.

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