Original Research Report

IN VITRO CHARACTERIZATION OF POLY(ETHYLENE GLYCOL) DIMETHACRYLATE-NANOFIBRILLATED CELLULOSE AS AN INJECTABLE BIOMATERIAL FOR HERNIATED NUCLEUS PULPOSUS SUBSTITUTE

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ABSTRACT

Herniated nucleus pulposus develops when the intervertebral disc portudes through the annulus fibrosus due to the rupture of the annulus fibrosus or a decrease in proteoglycans. Hydrogel implant material can be injected into the disc space to restore disc thickness caused by disc degeneration with minimal invasiveness. This study aimed to characterize poly(ethylene glycol) dimethacrylate-nanofibrillated cellulose (PEGDMA-NFC) in vitro as a potential biomaterial for herniated nucleus pulposus substitute. This study utilized PEGDMA-NFC to treat first-degree herniated nucleus pulposus using the photopolymerization method. PEGDMA was selected because of its hydrophilic ability to produce hydrogel. The addition of NFC to the PEGDMA precursor was expected to show mechanical properties as a hydrogel bio composite candidate. The characterization of PEGDMA-NFC was conducted using three tests: Fourier-transform infrared spectroscopy (FTIR), viscosity assessment, and an in vitro injection testing model. The normal distribution of the data was analyzed using the Kolmogorov-Smirnov test, while the homogeneity was assessed using Levene's test. Homogenous and normally distributed data were analyzed using a one-way analysis of variance (ANOVA) with a p-value of <0.05. The explored concentrations of PEGDMA-NFC included a ratio of 1:0 for the control samples and ratios of 1:0.5 (K1), 1:0.75 (K2), and 1:1 (K3) for the experimental samples. The FTIR analysis revealed the presence of various functional groups in PEGDMA-NFC, indicating its potential classification as a hydrogel biomaterial. The characterization data showed that the K3 sample yielded the most favourable outcome with a viscosity value of 74.67 dPa·s. From the in vitro injection testing result, the addition of NFC demonstrated that the hydrogel would not rupture when released from the mold. The hydrogel could be injected with an 18 gauge needle. The statistical analysis results showed a significant difference among the samples ($p<0.05$). This study concludes that the PEGDMA-NFC hydrogel biocomposite can be effectively applied in herniated nucleus pulposus cases.

Keywords: Illness; biomaterial; nucleus pulposus substitute; hydrogel; poly(ethylene glycol) dimethacrylate-nanofibrillated cellulose (PEGDMA-NFC)

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Highlights:
1. Novel synthetic biopolymer hydrogels were successfully prepared from pristine poly(ethylene glycol) dimethacrylate (PEGDMA) and nanofibrillated cellulose (NFC) using the photopolymerization method.
2. PEGDMA-NFC biocomposite hydrogel can be developed as an affordable biomaterial for herniated nucleus pulposus substitute, with the potential to meet clinical application standards.

INTRODUCTION

Low back pain is one of the leading causes of work-related illness and disability worldwide. The prevalence of low back pain in Indonesia is estimated to be 18–29%, while globally it is approximately 15-20% (Purnamasari et al. 2010, Liu et al. 2014). According to Chamradová et al. (2012), the United States of America (USA) has spent as much as 80 million USD per year on these cases. High degrees of low back pain can lead patients to experience motor weakness, sensory disturbances, and decreased physiological reflexes. Low back pain often manifests in the L5-21 lumbar intervertebral disc area. Meanwhile, the L5-S1 area is more resistant to high loads than other lumbar segments. The ratio of men to women for low back pain is 2:1 (Schmocker et al. 2015). Several diseases that cause low back pain include spasms in the lumbar muscles, osteoarthritis, herniated nucleus pulposus, and infections of the spine. As many as 30–80% of low back pain cases are caused by herniated nucleus pulposus (Campbell 2013).

Herniated nucleus pulposus is a condition when the nucleus pulposus shifts and presses on the spinal nerves. The protrusion of the nucleus pulposus on the intervertebral disc can occur due to the rupture of the annulus fibrosus or a decrease in proteoglycans (PGs). Herniated nucleus pulposus disease is one of the causes of spinal cord pain (DiStefano et al. 2020). Protrusion of the nucleus pulposus in the annulus fibrosus can cause compression of the spinal cord, resulting in neuropathy. Based on the degree of pain, the herniated nucleus pulposus is divided into four, i.e., protrusion, prolapse, extrusion, and sequestration. Nucleotomy is a method of treating herniated nucleus pulposus, which is the surgical removal of the nucleus pulposus tissue. This procedure does not restore the original disc structure and function (de Lamo-Rovira et al. 2008). The disadvantage of this method is that it is invasive and can change the biomechanical properties of the spine. Numerous patients have reported pain after surgery (Aichmair et al. 2014). On the other hand, hydrogel-based regenerative medicine is a minimally invasive method. This option is able to restore the biomechanical properties of the nucleus pulposus and has the potential to reduce the risk of herniation (Cramer 2014).

Injectable hydrogel can restore the thickness of the disc that has been reduced due to disc degeneration. The advantage of hydrogel is that it contains bioactive molecules, which can increase cell viability, cell differentiation, and tissue regeneration (Chan et al. 2013, Benhamou et al. 2014). Furthermore, hydrogel is easy to form and can fill the entire empty space of the disc. The material has good mechanical properties and biocompatibility (Sivashanmugam et al. 2015). However, Joshi et al. (2006) demonstrated that hydrogel has low mechanical properties and is not able to withstand high pressure. A hydrogel biocomposite can be formed by combining nanofibrillated cellulose (NFC) biopolymer, which has excellent biocompatibility and mechanical properties, with poly(ethylene glycol) dimethacrylate (PEGDMA) synthetic polymer (Baker et al. 2012, Schmocker et al. 2015).

Previous studies have investigated the photopolymerization of PEGDMA for the purposes of tissue engineering and drug delivery. As PEGDMA is hydrophilic, it can be applied to the nucleus pulposus, which contains 90% water (Cortes et al. 2014, Schmocker et al. 2015, Molladavoodi et al. 2020). The proteoglycans (PGs) bond in the nucleus pulposus can be formed by a protein link present in the PEGDMA chain 7. An addition of NFC to PEGDMA has been found to be able to improve the mechanical properties of the hydrogel biocomposite. Previous research has synthesized PEGDMA-NFC with the most favorable outcome at a concentration ratio of 1:0.75 (Schmocker et al. 2015). The findings pertaining to compressive strength, swelling, and the in vitro injection model are applicable to the nucleus pulposus, with the exception of the viscosity test. Nevertheless, additional characterization and optimization at varying concentrations are still necessary. This research aimed to characterize poly(ethylene glycol) dimethacrylate-nanofibrillated cellulose (PEGDMA -NFC) in vitro as a potential biomaterial for herniated nucleus pulposus substitute. The use of this biomaterial is expected to be beneficial for patients with herniated nucleus pulposus.

MATERIALS AND METHODS

This study employed a true experimental research design, involving the fabrication of poly(ethylene glycol) dimethacrylate-nanofibrillated cellulose (PEGDMA-NFC) characterization using the photopolymerization method and in vitro assay. The characterization procedures encompassed Fourier-transform infrared spectroscopy (FTIR), viscosity assessment, and an in vitro injection testing model. The hydrogel photopolymerization process was carried out utilizing visible light (Hola et al. 2023). The materials used in this study were poly(ethylene glycol) dimethacrylate (PEGDMA) with Chemical Abstracts Service (CAS) number 25852-47-5 (Sigma-Aldrich, USA), Irgacure 2959 Photoinitiator with CAS number 106797-53-9 (Advanced BioMatrix, USA), and nanofibrillated cellulose (NFC) with CAS number 9004-34-6 (Performance BioFilaments, Canada). Phosphate buffered saline

(PBS) and distilled water were produced in Indonesia by CV Chemical Indonesia.

The PEGDMA-NFC hydrogel preparation was produced by combining 10% (w/v) of PEGDMA, NFC, and Irgacure 2959 (0.1 wt%) with PBS. The concentrations of PEGDMA-NFC for the control samples were at a ratio of 1:0, whereas the experimental samples had ratios of 1:0.5 (K1), 1:0.75 (K2), and 1:1 (K3). The solution was homogenized using a magnetic stirrer for 30 minutes. Afterwards, it was placed in the TLC Visualizer (CAMAG, Muttenz, Switzerland) and exposed to 366 nm of light waves for 60 minutes (Widiyanti et al. 2020).

The characterization of PEGDMA-NFC was initially conducted using FTIR analysis. The use of infrared spectroscopy as an analytical technique was able to identify functional groups by utilizing electromagnetic radiation (Zeng et al. 2016). The results were presented in a graphical format and analyzed using the correlation table. The samples identified were control, K2, and K3.

The characterization procedures also involved viscosity assessment to determine the viscosity value of PEGDMA-NFC hydrogel. This test was performed to determine the hydrogel's resistance to flow. The tool utilized in this test was the Rion Viscotester. The standard viscosity value for injectables was established at 80 dPa·s (Widiyanti et al. 2020).

An in vitro injection testing model was used in the characterization of PEGDMA-NFC to figure out the process of gel formation in the body. This was achieved by creating a mold model using agarose. The gel was prepared using 3% (w/v) of agarose dissolved in PBS solution (Frith et al. 2013, Schmocker et al. 2015). The PEGDMA-NFC hydrogel was mixed with food coloring and then placed into agarose molds. The molds were incubated at 37°C for two hours (Schmocker et al. 2016).

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA). The normality and homogeneity of the data were assessed using the Kolmogorov-Smirnov test and Levene's test, respectively. One-way analysis of variance (ANOVA) was employed for data that exhibited homogeneity and a normal distribution, with a significance value established at $p<0.05$ (Kim 2017).

RESULTS

A total of 10 wt% of PEGDMA, NFC and irgacure-2959 were included in PBS was synthesized using the TLC Visualizer. The synthesis results indicated that the K1, K2, and K3 samples produced a higher degree of hydrogel gelation compared to the control samples. In the TLC Visualizer, the interaction between visible light and the Irgacure 2959 Photoinitiator resulted in the formation of free radicals, which initiated the process of photopolymerization (Nicol 2021).

Characterization of PEGDMA-NFC using FTIR analysis

The range of wavelength used was 4000–500 cm^{-1}. Figure 1 shows a peak at 2,947.23 cm^{-1}, indicating the stretching of C-H, which is the main chain of PEGDMA. The presence of a C-O ether chain in PEGDMA was suggested by a peak at 1,085.92 cm^{-1}.

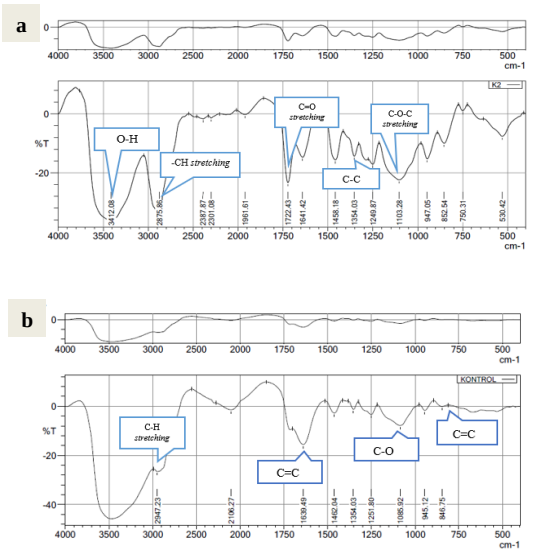

Figure 1. FTIR analysis of the (a) control sample (1:0) and (b) K2 sample (1:0.75).

The absorption of PEGDMA was observed at 846.75 cm^{-1}, and a C=C bond was identified at 1,639.49 cm^{-1} (Burke et al. 2019). The absorption area of NFC was found to span from 3,660 to 2,900 cm^{-1}. The peak at 3,412.08 cm^{-1} exhibited the distinctive characteristics of the polysaccharide hydroxyl group, namely the NFC chain. As shown in Table 1, the absorption area of each functional group was present for all concentration ratios. Figure 2 depicts the photopolymerization process involving PEGDMA, NFC, and Irgacure 2959.

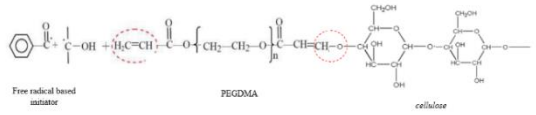

Figure 2. Illustration of PEGDMA-NFC chain reaction.

Table 1. Absorption area of each functional group.

Functional groups	Wavenumber (cm^{-1})		
	PEGDMA	NFC	PEGDMA-NFC
C-H stretching	2,947.23	-	2,875.86
C=C	1,639.49 846.75	- -	1,639.49 846.75
C-O	1,085.92	-	1,085.92
O-H	-	3,412.08	3,423.65
C=O stretching	-	-	1,722.43
C-C	-	-	1,354.03
C-O-C stretching	-	1,103.28	1,103.28

Characterization of PEGDMA-NFC through an in vitro injection testing model

An in vitro injection test was employed as a form of qualitative analysis by assessing the ability of PEGDMA-NFC hydrogel to completely fill a given space and maintain its intactness upon release from the agarose molds. All of the samples were incubated in PBS at 37°C for two hours.

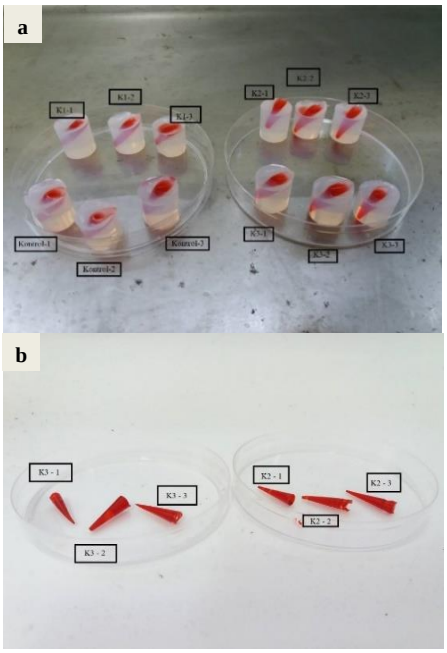

Figure 3. In vitro injection test results: (a) hydrogels in the molds; (b) hydrogels after being released from the molds.

The qualitative testing showed that in the control samples, two samples partially occupied the agarose molds, while the rest completely occupied the agarose space (Figure 3). The K1, K2, and K3 samples remained intact when released from the agarose molds. This result indicated that all concentration ratios of the hydrogel met the required standard and could be injected through an 18 gauge needle at a temperature of 37°C (Liu et al. 2014, Zeng et al. 2016, Schmocker et al. 2016).

Characterization of PEGDMA-NFC by employing viscosity assessment

As demonstrated in Figure 4, the addition of NFC resulted in an increase in the viscosity of the sample. The measured viscosity values were 60.33±1.45 dPa·s for the control sample, 71.67±0.45 dPa·s for the K1 sample, 72.33±0.59 dPa·s for the K2 sample, and 74.67±0.44 dPa·s for the K3 sample. The concentration ratio of the K3 sample, which was 1:1, closely matched the standard viscosity of 80 dPa·s (Widiyanti et al. 2020). The resulting color of the K3 sample was darker and denser compared to the other three samples with different concentration ratios.

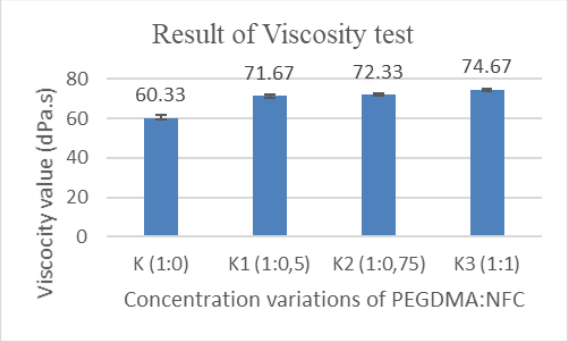

Figure 4. Results of the viscosity test on PEGDMA-NFC at different concentration ratios.

The data obtained from the tests were subjected to statistical analysis. The viscosity assessment data were found to be homogeneous and normally distributed. The results of the one-way ANOVA revealed a significance value of $p<0.05$, showing a significant difference across the samples. Furthermore, it was indicated that the addition of NFC at different concentration ratios had an influence on the test results.

DISCUSSION

Previous research conducted by Culbert et al. (2022) has proposed the requirements for the effective use of biomaterials as nucleus pulposus substitutes. The

biomaterials needed have to meet three specific criteria. Firstly, they should be minimally invasive to prevent damage to surrounding tissue. Secondly, the injected biomaterials should be in the form of a gel capable of filling the rupture. Thirdly, the hydrogels must be biocompatible and have mechanical properties similar to those of a healthy disc, enabling the restoration of disc function.

Depending on the severity of the herniation, the herniated nucleus pulposus may advance to stage four. First-degree herniated nucleus pulposus is characterized by intervertebral disc protrusion. However, the nucleus protrudes without causing any damage to the annulus fibrosus. Second-degree herniated nucleus pulposus is defined as the prolapse of the intervertebral disc, where the nucleus has shifted but remains within the annulus fibrosus. Extrusion of the intervertebral disc occurs when the nucleus protrudes outward and the annulus fibrosus is under the posterior longitudinal ligament. Intervertebral disc sequestration refers to a condition in which the nuclei of the disc have penetrated the posterior longitudinal ligament (Ren et al. 2023).

PEGDMA-NFC hydrogels used for herniated nucleus pulposus patients require good physical properties. The nucleus pulposus serves as a shock absorber and is frequently subjected to strenuous activities, necessitating high compressive strength (Schmocker et al. 2016). PEGDMA is a readily soluble derivative of polyethylene glycol (PEG) that easily swells. However, the physical properties of PEGDMA are subpar, thus requiring the incorporation of NFC to improve its physical properties (Widiyanti et al. 2020).

During the photopolymerization process, a gelation of the hydrogel occurred. This was possible because the PEGDMA compound, which contains an alkene of PEG, reacted with Irgacure 2959. Subsequently, it resulted in the formation of a crosslinked hydrogel (Schmocker et al. 2016). The dispersion properties of NFC enhanced the gelation process. The advantages of using the photopolymerization method to synthesize hydrogels are its control-ability and rapid rate of hydrogel formation (Zeng et al. 2016, Karami et al. 2018). In this study, the -CH stretching vibration observed at 2,875.86 cm^{-1} indicated the presence of a hydrocarbon in polysaccharides. The characteristics of ethyl cellulose were identified by the -C-O-C- stretching observed at a wavenumber of 1,103.28 cm^{-1} (Wang et al. 2018, Widiyanti et al. 2020). The transition from 1,639.49 cm^{-1} to 1,722.43 cm^{-1}, representing a shift from C=O to C-C caused by Irgacure 2959, indicated that the hydrogel reached stability (Widiyanti et al. 2020).

The viscosity value of hydrogels is a crucial factor, as it determines the hydrogel's ability to be ejected from a syringe during the injection procedure. According to prior research conducted by Doench et al. (2018), NFC has the ability to enhance the viscosity value of hydrogels. The viscosity assessment results from this study demonstrated that the addition of NFC led to an increase in the viscosity value. The viscosity of the K3 sample (74.64±0.44 dPa·s) was close to the standard viscosity value. This finding suggests that PEGDMA-NFC hydrogel is applicable as a biomaterial for substituting herniated nucleus pulposus.

The addition of NFC affected the results of multiple tests conducted in this study. As the concentration of NFC increases, the viscosity value also increases. The hydrogel was able to fill the agarose mold with the incorporation of NFC. The physical bond of NFC functions as an entanglement that forms a network between cellulose and PEGDMA (Karami et al. 2018). NFC nanostructures have a large surface area that can increase the bonds between molecules. This aspect suggests that the addition of NFC led to an increase in the density of the hydrogel. Furthermore, NFC exhibits excellent crystallinity due to its orderly molecular structure (Atikah et al. 2019, Aristri et al. 2021). According to this explanation, NFC can serve as a reinforcement for a polymer matrix. As the diameter of the NFC fibers reduces, the mechanical strength of the NFC increases, which enhances the water retention value (Benhamou et al. 2014). In short, NFC is capable of acting as a reinforcing component in hydrogels.

The statistical analysis enhanced the results by providing stronger evidence of the effect of increasing NFC concentration. The analysis revealed that the data were homogeneous and normally distributed. The subsequent analyses using one-way ANOVA and two-way ANOVA for degradation data yielded significantly different data. Finally, the statistical analysis resulted in the rejection of the null hypothesis (H0). The rejection of H0 is possible if the error size (p) is less than the maximum error tolerance (α). The results of each test consistently demonstrated statistical significance ($p<\alpha$) (Widiyanti et al. 2020).

Strength and limitations

The strength of this research is that the hydrogel was specifically formulated to be used as an injectable, which means it is minimally invasive. The hydrogel offers several benefits due to its bioactive molecules, which have the potential to enhance cell viability, cell differentiation, and tissue regeneration. The PEGDMA-NFC hydrogel is capable of completely filling the defect caused by

the herniated nucleus pulposus. The limitation of this research was the absence of scanning electron microscopy (SEM) to examine the hydrogel morphology. Further research utilizing thermogravimetric analysis (TGA) and in vivo testing for animal trials is necessary.

CONCLUSION

The poly(ethylene glycol) dimethacrylate-nanofibrillated cellulose (PEGDMA-NFC) hydrogel has the potential to be a biomaterial replacing herniated nucleus pulposus. This hydrogel can be effectively synthesized using the photopolymerization method in the Thin Layer Chromatography (TLC) Visualizer. The addition of NFC can increase the viscosity of the hydrogel, allowing it to fill the herniated nucleus pulposus.

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Conflict of interest

None.

Ethical consideration

The Research Ethics Committee of Rumah Sakit Universitas Airlangga, Surabaya, Indonesia, issued the ethical clearance for this study under reference No. 012/KEPK/2024 dated 7/2/2024.

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Author contribution

PW and A contributed to the conceptualization and design. PW and YF contributed to the analysis and interpretation of the data. YF contributed to the drafting of the article. PW contributed to the acquisition of funding. All authors have read and provided their final approval of the article.

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