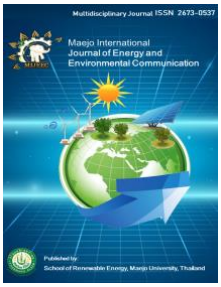
Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Enhanced biocompatibility: A comparative approach on polyethylene glycol and zinc-incorporated hydrogels

Vaidhegi Kugarajah[1,*], Pradeesh[2], Vignesh[2]

[1] School of Life Sciences, B. S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Tamil Nadu 600048, India
[2] Department of Nanobiomaterials, Saveetha Institute of Medical and Technical Sciences, Chennai-602015, India

ARTICLEINFO

Article history:
Received 23 August 2024
Received in revised form
17 September 2024
Accepted 21 September 2024

Keywords:
Hydrogels
Antibacterial activity
Biocompatibility
Zinc
Nanocomposites

ABSTRACT

With recent medical technology developments, hydrogels have gained vast interest due to their soft tissue-like mechanical properties, injectability, high water content, etc. Poly (ethylene glycol) (PEG) hydrogels are highly commendable due to their synthetic structure, tunable architecture, biocompatibility, and reproducibility. In the present research, zinc nanoparticles at various concentrations (0.025, 0.05, 0.075 wt%) were infused in PEG hydrogels to enable better biocompatibility. The prepared nanocomposites are evaluated for their morphological, functional, and structural characteristics compared to naïve PEG hydrogel. Antibacterial activity revealed that PEG + 0.075 wt% Zn exhibited the maximum zone of inhibition of 0.09 ± 0.2mm compared to plain hydrogel (0.02 ± 0.5 mm). Statistical analysis through independent T-test exhibited a statistical significance of the nanocomposite hydrogel with p= 0.001, ($p<0.05$) when tested with a G-power of 80%, 0.5 alpha error, and 95% confidence interval. The present research introduces novel PEG-Zn nanocomposite hydrogels and offers a future scope for bioengineering applications.

1. Introduction

Hydrogels, versatile three-dimensional interconnected networks, have recently been widely explored in tissue engineering, drug delivery, and medical device equipment due to their soft tissue-like characteristics, hydrophilicity, and tuneable mechanical properties (Hussain et al., 2024; Isaac et al., 2024). Additionally, the biocompatibility, drug-encapsulating tendency, solubility, and negligible cytotoxicity make them appealing in many biological studies (Wang et al. 2023). Polyethylene glycol (PEG) hydrogels are among the widely explored biomaterials in vast applications including soft tissue regeneration, drug delivery, and wound healing. With its synthetic nature, these hydrogels are mechanically and architecturally tuneable compared to natural hydrogels derived from polysaccharides and proteins (Isaac et al., 2024). Hydrophilicity in PEG hydrogels can be related to their –NH2, –COOH, –CONH2, –SO3H, –OH polar groups within the chemical structure of (H−(O−CH2−CH2)n−OH) that provide excellent properties to covalent links, encapsulate drugs with required solubility (Rodriguez - Rivera et al., 2024). Further, PEG hydrogels have been proven to be highly stable during internal circulation due to their low toxicity and relatively low immune response (Li et al., 2012; Vaezifar et al., 2013).

Another interesting feature of PEG hydrogels is that the size of water–swelling hydrogels can be precisely tuned into the suitable range, resulting in an enhanced permeability and retention (EPR) effect (Iyer et al. 2006). Moreover, PEG has been regarded as bioinert, often termed as blank slates due to its resistance to protein adsorption, and has been declared a safe additive by the Food and Drug Administration

* Corresponding author.
E-mail address: vaidhegi@crescent.education (V. Kugarajah)

(FDA), allowing its application in many products, including processed foods, toothpaste, tissue sealants, cosmetics, etc. (Pasut & Veronese 2012). Considering the promising features above, PEG hydrogels have been widely investigated for developing various therapeutic strategies for generalized, local drug delivery and several biological applications. Although the application of PEG has been highly promising, in recent times, concerns have been raised regarding anti-PEG antibodies in the general public. Studies have proven the prevalence of anti-PEG antibodies in about 23-71% even in healthy populations (Isaac et al., 2024; Lubich et al., 2016; Yang et al., 2016) which can be attributed to the frequent contact with PEG-based products in everyday life.

Nanomaterials have gained vast interest, offering various technological advancements in broad areas, including medicine, energy conversion, water treatment, and catalysis (Kugarajah et al., 2022). The ability to alter these materials and unique properties even when administered in lower quantities has been a boon to scientists to develop various products to improve human welfare. The intrinsic features of these nanomaterials depend on their size and shape (Kazeminava et al. 2021). Among the various nanomaterials, Zinc oxide (ZnO) has been explored due to its antibacterial efficiency and low toxicity and has been employed in various fields, including drug delivery, catalyst, membranes, etc. (Kugarajah et al., 2021; Siddiqi et al., 2018; Sirelkhatim et al., 2015). Being an essential trace element required for the effective functioning of the human system, Zinc is essential for eukaryotes as it modulates certain physiological functions.

The absence of Zinc in the human body leads to the toxicity of many enzymes, including carboxypeptidase, alcohol dehydrogenase, and carbonic anhydrase, as they become inactive. For instance, bamboo salt contains zinc and has been prescribed as an herbal medicine to regulate caspase-1 activity in treating inflammation. In addition, Zinc oxide nanoparticles have been reported to inhibit the nuclear factor kappa B cells (NF-kB) activation by reducing the mRNA expression of inflammatory cytokines (Kim et al., 2014). Zinc oxide has been reported since the regime of Pharaohs and has been rapidly investigated in many ointments, sunscreen lotions, supplements, etc., after FDA recognition (CFR 182.8991) (Brown, 1978; De Romana et al., 2002; Szabó et al., 2003). Although research on zinc nanoparticles has been attempted, its application can be further investigated in wider areas of medicine.

Considering the above, the present research introduces a novel hydrogel nanocomposite comprised of PEG hydrogel infused with 0.025, 0.005, and 0.075wt% of zinc oxide (ZnO) for its biomedical applications. The proposed nanocomposite hydrogel was analyzed for its functional and morphological characteristics. Antibacterial activity was performed to evaluate the biocompatibility of the prepared hydrogels. In addition, statistical analysis using SPSS was performed to evaluate its statistical significance compared to native PEG hydrogel. The present research proposes a preliminary study to optimize a novel hydrogel combination and provides a future scope for improved biomedical applications.

2. Material and Methods

2.1. Materials

Polyethylene Glycol (PEG) was procured from Sigma Aldrich, Chennai. All chemicals including Zinc nitrate (≥99% pure), Sodium Hydroxide pellets (99.5 % pure), Citric acid, and Isopropanol were procured from Ravi Scientific Company, India. All chemicals were of analytical grade and required no extra processing. Distilled water was used for all experiments unless otherwise specified.

2.2 Preparation of PEG hydrogels

Polyethylene glycol hydrogels were prepared by a modified method adopted from an existing protocol as follows (Maddu, Winarti, and Kurniati 2019); dried grass was submerged in water in a clean beaker and placed in a stirrer at 60℃ for about 2 hours. The beaker was then filtered to remove chlorophyll content. The grass was desiccated, and about 1 g of grass was taken in a beaker, onto which about 20 mL of isopropanol was added and stirred for 10 mins. Progressively, 3 mL of PEG was added and stirred for 10 mins, followed by 10 mL of 0.5 g citric acid. The mixture was stirred for 1 hour and left undisturbed for cooling. The resultant was then filtered in the clean Petri plate and placed in a hot air oven to allow the evaporation of excess solvents to result in PEG hydrogel.

2.3 Preparation of Zinc oxide nanoparticles and nanocomposite hydrogels

Zinc nanoparticles were prepared as follows: about 2.97g of 0.1M zinc nitrate was dissolved in 100 mL of distilled water (solution A). In a separate beaker, about 0.4 g of 0.1 M Sodium Hydroxide (NaOH) was added to 100 mL of distilled water (solution B). 50 mL of solution B was introduced into Solution A and stirred continuously for 10 minutes with a magnetic stirrer. After mixing, the mixture was allowed to settle. The resultant was washed 5-6 times using distilled water, filtered, and then dried in a hot air oven at 60℃ to produce zinc nanoparticles. Composite hydrogels were prepared by dispersing the required wt.% (0.025, 0.5, 0.075) of ZnO nanoparticles in 10 mL of PEG hydrogel using an ultrasonic probe sonicator for 2 minutes. The obtained composite hydrogel was stored for further studies.

The schematic representation for portraying the formation of the PEG – x ZnO (x =0.025, 0.05, 0.075wt%) is illustrated below (Fig 1).

2.4 Swelling Ratio

The prepared hydrogels were subjected to swelling ratio studies. A modified approach of our prior work was experimented (Kugarajah et al. 2021) as follows: About 5 mL of the composite hydrogel was lyophilized, of which about 1 x 1 cm was dried for 12 hours, and the weight was measured as (Sd). Similarly, the sample was soaked in water for 12 hours and was weighed and labeled as (Sw). The water absorption was calculated from the following equation (1);

$$\text{Swelling Ratio (\%)} = ((S_w - S_d)/S_d) \times 100 \quad (1)$$

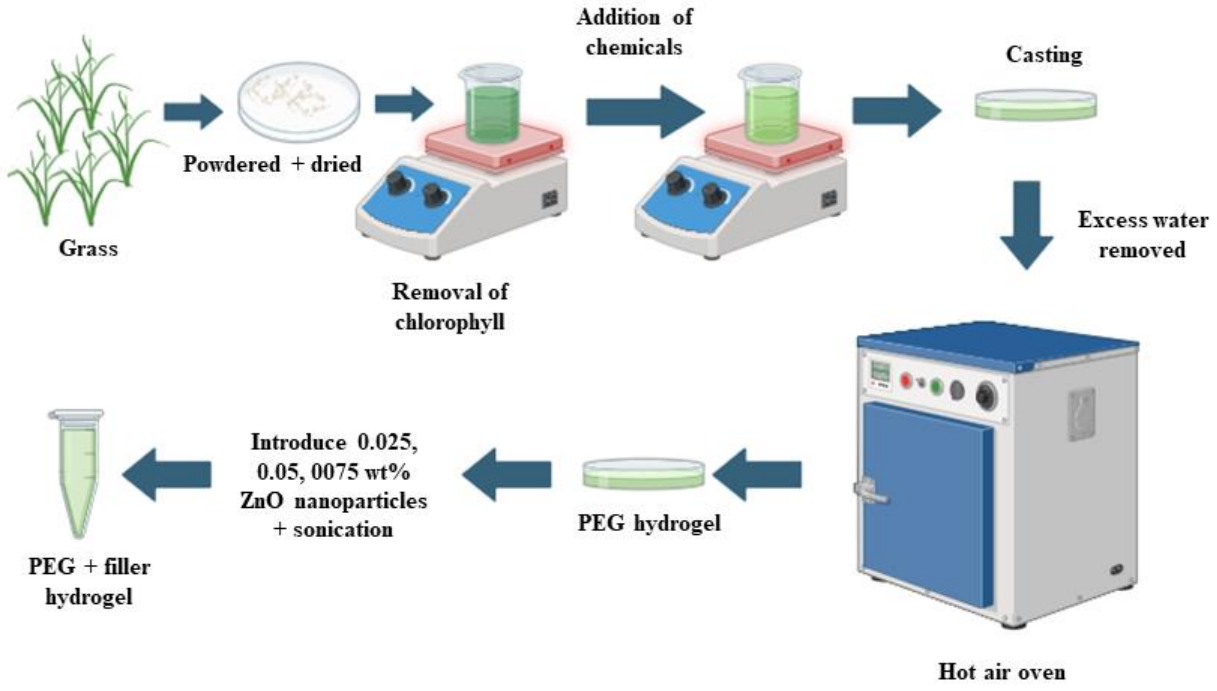


Figure 1. Schematic representation for the preparation of PEG + Zn composite hydrogels

2.5 Instrumental Analysis

The prepared hydrogels were analyzed for their morphological characteristics through Scanning Electron Microscopic (SEM, ZEISS) studies. Their functionality was investigated through Fourier Transform Infrared Spectroscopy (FTIR, Alpha Bruker) in transmittance mode at a scan range of 4000 – 500 cm^{-1}. The wettability of the prepared hydrogels was examined through contact angle measurement (sessile drop method).

2.6 Antibacterial Activity

Petri plates containing 20 mL nutrient agar medium were seeded with 24 hr E. coli culture of bacterial strain and adjusted to 0.5 OD value according to McFarland standard (*E. Coli*-443). Wells were cut, and different concentrations of the sample (0.025,0.05,0.075 wt.%) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

2.7 Data analysis

The biocompatibility of the prepared novel polyethylene glycol-zinc composite was evaluated through IBM-SPSS (Meyers, Gamst, and Guarino 2013)The control group comprises naive polyethylene glycol (PEG) hydrogel, whereas the study group contains polyethylene glycol hydrogel incorporated with zinc nanoparticles (PEG + x ZnO, x = 0.025, 0.05, 0.075 wt. %). There are no dependent variables. An independent sample T-test was done to determine the maximum biocompatibility, and the mean and standard deviation were considered.

3. Result and Discussion

3.1 Studies on Swelling ratio and wettability

One of the attractive features of PEG hydrogels is their improved hydrophilicity. Studies on the swelling ratio and wettability were evaluated on the prepared nanocomposite hydrogels to investigate whether adding ZnO nanoparticles affected its inherent tendency. Figure 2 showed that native PEG hydrogel possessed a swelling ratio of 22 ± 1.1 % and a contact angle of 30.37°, which was more than the existing literature (Zhang et al., 2021; Zhu et al., 2015). The variation may be attributed to preparing PEG hydrogel using plant cellulose. Upon addition of filler into the PEG hydrogel, the swelling ratio was found to increase gradually to 48±2.4, 59±2.9 and 61±3% in contrast to the water contact angle, which exhibited minor variations (28.2°, 27.2°, 26.5°) for 0.025, 0.05 and 0.075 wt.% of PEG + ZnO hydrogel. The obtained results thus exhibited improved hydrophilicity and were under earlier studies (Salvekar et al., 2017).

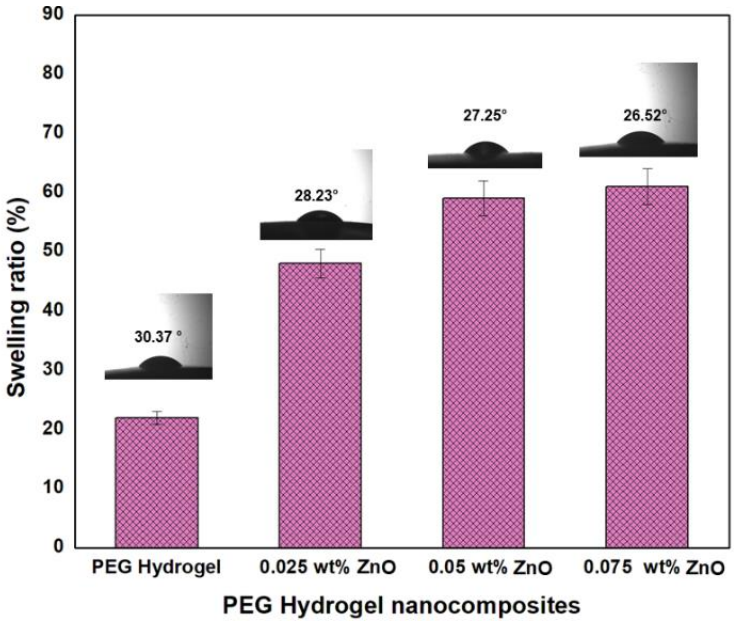


Figure 2. Swelling ratio and wettability studies of the prepared PEG hydrogels

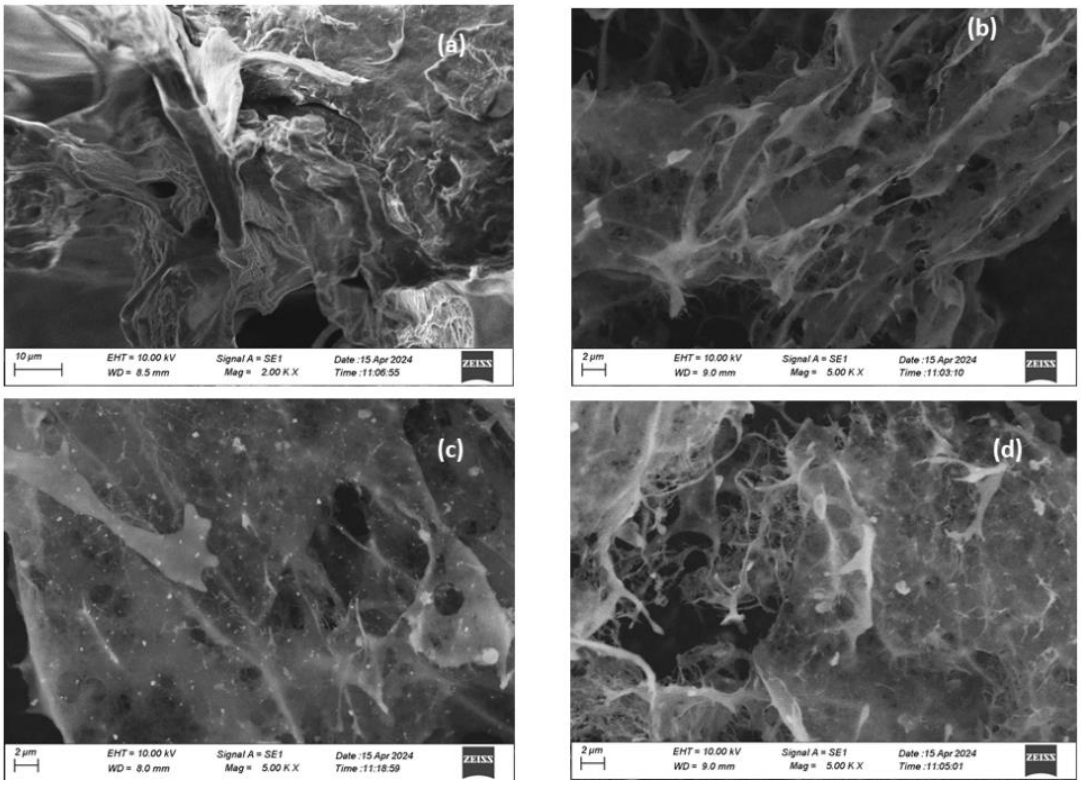


Figure 3. SEM Micrographs of (a) PEG Hydrogel, (b) PEG hydrogel + 0.025 wt.% ZnO, (c) PEG hydrogel + 0.05 wt.% ZnO and (d) PEG hydrogel + 0.075 wt.% ZnO

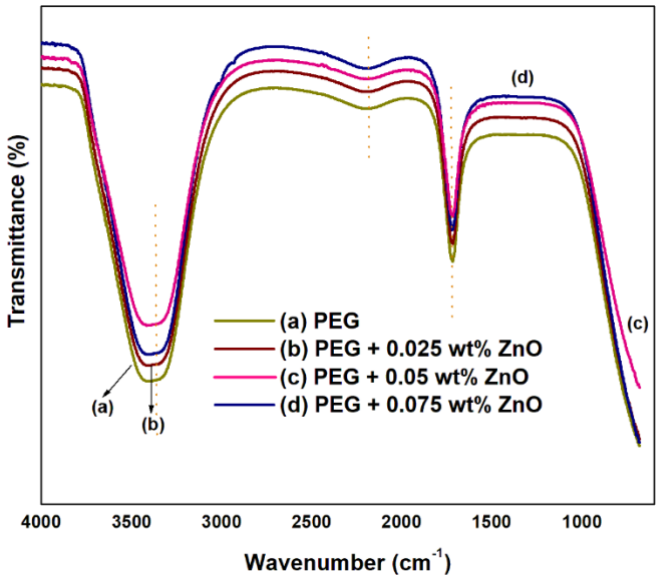


Figure 4. FTIR analysis of (a) PEG Hydrogel, (b) PEG hydrogel + 0.025 wt.% ZnO, (c) PEG hydrogel + 0.05 wt.% ZnO and (d) PEG hydrogel + 0.075 wt.% ZnO

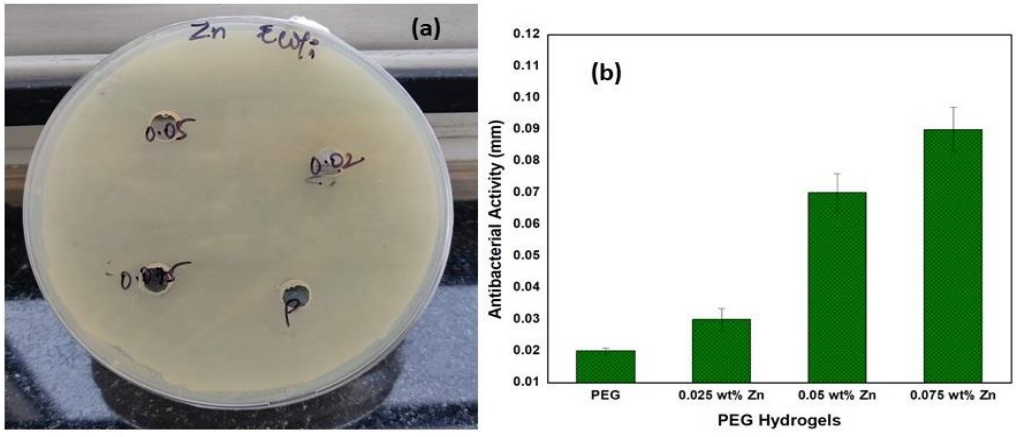


Figure 5. Antibacterial activity representing (a) Zone of inhibition (ZOI) and (b) ZOI plate count of PEG Hydrogels

3.2 Studies on Morphology and Functionality

The morphology of the prepared hydrogels was examined through SEM analysis; the results are depicted in Figure 3. Figure 3 (a) represents the plain PEG hydrogel with random interactions due to the hydrophilicity and chemical structure (Bryant et al. 2004). Upon addition of ZnO nanoparticles, the SEM micrographs exhibited random agglomerations attributing to the incorporation of ZnO, which were found to increase with the increase in filler concentration. Figure 4 (a-d) represents the FTIR analysis of the prepared hydrogel. From Figure 4(a), it was observed that plain PEG hydrogel exhibited a peak at 3500 -3400 cm^{-1}, which can be attributed to OH bond present in PEG hydrogel in accordance with an earlier report (Askari et al., 2019). The C=C aliphatic double band was observed in 1643 cm^{-1} and a minuscule peak at 1845 cm^{-1} attributed to the C=O group present in PEG hydrogels. Upon filler addition, the intensity of the peaks exhibited slight variation owing to the addition of ZnO nanoparticles.

3.3 Antibacterial Studies

The zone of inhibition (ZOI) of PEG hydrogen and nanocomposite hydrogels was evaluated through the well diffusion method, and the results are represented in Figure 5. From the figure, it was observed that PEG hydrogel exhibited a ZOI of 0.02 ± 0.5 mm. Upon incorporation of Zinc oxide nanoparticles, the ZOI was found to increase with the increase in concentration to reach a maximum of 0.09 ± 0.2 mm. The observed results were in accordance to certain earlier reports (Kugarajah et al., 2021; Siddiqi et al., 2018). The increased surface area and the size of the nanoparticles lead to an improved particle surface reactivity (Sirelkhatim et al. 2015; Dogra et al., 2023). Thus, upon addition of zinc nanoparticles, react with the bacteria cell wall leading to cell wall degradation and release of cellular contents. In addition, the bio-safe nature of ZnO further allows an added advantage when opting for biomedical applications.

3.4 Statistical Analysis

The present research introduces PEG hydrogel and ZnO-infused PEG hydrogels for their improved biocompatibility. In comparison to plain PEG hydrogel, the composite PEG hydrogel showed better properties, indicating a statistical significance between the two groups ($p=0.01$, $p<0.05$) (Figure 6). The current study aims to improve biocompatibility using ZnO nanoparticles infused PEG hydrogel. Table 1 depicts the mean and standard deviation of the prepared hydrogels, whereas Table 2 represents the statistical variables. The prepared composites showed statistical significance through independent T-tests, further prompting their suitability for biomedical applications.

Table 1. Comparison of mean standard deviation for PEG hydrogel and PEG hydrogel + ZnO in terms of biocompatibility (N=4)

Hydrogel	N	Mean	Standard deviation	Standard error mean
PEG hydrogel	4	.0200	.00000	.00000
PEG hydrogel with ZnO	4	.0525	.03304	.01652

Table 2. Independent sample T-test comprising the significance for PEG hydrogel and PEG hydrogel with ZnO for improved biocompatibility ($p=0.01$, $p<0.05$) where there exists statistical significance between the two groups

Parameters	Test of Equality of Variances		T-test for equality of means					95% confidence interval of the difference	
	F	Sig	T	df	Significance (2-tailed)	Mean Difference	Std. Error	Lower	Upper
Equal variance assumed	36.3	.001	-1.96	6	.097	-.03250	.01652	-.072	.00792

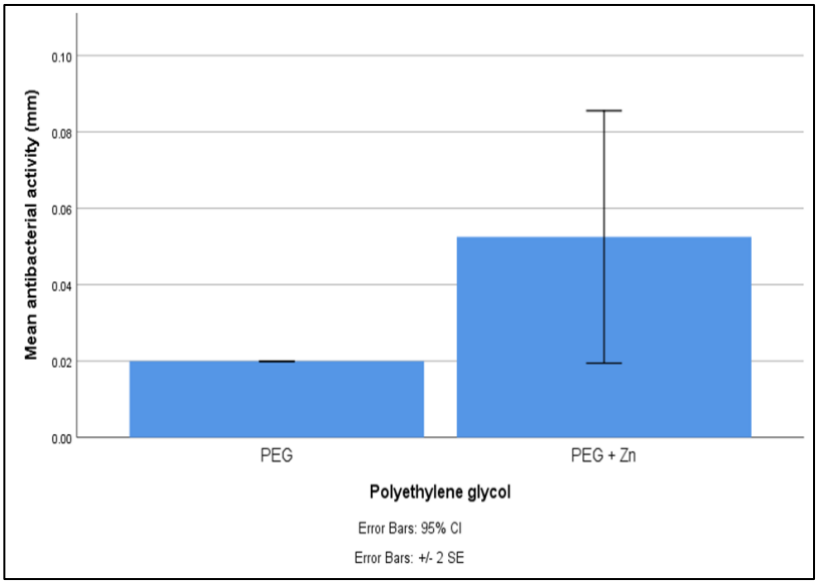


Figure 6. Bar chart representing the mean biocompatibility of PEG hydrogel and PEG hydrogel + ZnO X-axis: Samples with PEG hydrogel and PEG hydrogel +ZnO (wt%), Y-axis: Mean biocompatibility of PEG hydrogel and PEG hydrogel + ZnO/- 2 SE

4. Conclusion

The present research introduces a comparative study between PEG hydrogel and ZnO-infused PEG hydrogels for their improved biocompatibility. The results suggested that PEG hydrogel + 0.075 wt.% of ZnO exhibited the highest maximum antibacterial activity, suggesting its rapid application in biomedical applications. Statistical

analysis further revealed a statistical significance between the two groups. However, the present study merely provides preliminary insights on nanocomposite hydrogel. Enhancing the potential of composite hydrogel for tissue engineering and other biomedical uses will require more research and analysis.

Acknowledgment

The authors thank the Department of Nanobiomaterials, Institute for Biomedical Engineering, Saveetha School of Engineering, for providing the necessary facilities to carry out the research and The 6th International Conference on Renewable Energy, Sustainable Environmental and Agricultural Technologies

References

Askari F, Zandi M, Shokrolahi P, Tabatabaei MH, Hajirasoliha E. (2019). "Reduction in Protein Absorption on Ophthalmic Lenses by PEGDA Bulk Modification of Silicone Acrylate-Based Formulation." Progress in Biomaterials, 169-83.

Brown, Harvey E. (1978). "Zinc Oxide--Properties and Applications." International Lead Zinc Research Organization, Inc., New York. 1978, 218.

Bryant, Stephanie J, Kristi S Anseth, David A Lee, and Dan L Bader. (2004). "Crosslinking Density Influences the Morphology of Chondrocytes Photoencapsulated in PEG Hydrogels during the Application of Compressive Strain." *Journal of Orthopaedic Research* 22(5): 1143–49.

Dogra, S., Sharma, M. D., Tabassum, S., Mishra, P., Bhatt, A. K., & Bhuyar, P. (2023). Green biosynthesis of silver nanoparticles (AgNPs) from Vitex negundo plant extract and its phytochemical screening and antimicrobial assessment next to pathogenic microbes. Journal of Microbiology, Biotechnology & Food Sciences, (4).

Hussain S, Salman M, Youngblood JP, Farooq U, Yasmeen S, Al-Ahmary KM, Ahmed M (2024) Enhanced adsorption of Congo red dye by CS/PEG/ZnO composite hydrogel: Synthesis, characterization, and performance evaluation. Journal of Molecular Liquids. Oct 1;411:125704.

Isaac, Alisa H, Recalde Phillips SY, Ruben E, Estes M, Rajavel V, Baig T, Paleti C, Landsgaard K, Lee RH, Guda T, Criscitiello MF(2024). "Impact of PEG Sensitization on the Efficacy of PEG Hydrogel-Mediated Tissue Engineering." Nature Communications 15(1): 3283.

Iyer, Arun K, Greish Khaled, Jun Fang, and Hiroshi Maeda. (2006). "Exploiting the Enhanced Permeability and Retention Effect for Tumor Targeting." Drug discovery today 11(17–18): 812–18.

Kazeminava F, Arsalani N, Ahmadi R, Kafil HS, Geckeler KE (2021). "A Facile Approach to Incorporate Silver Nanoparticles into Solvent-Free Synthesized PEG-Based Hydrogels for Antibacterial and Catalytical Applications." Polymer Testing 101: 106909.

Kim, Min-Ho, Jun-Ho Seo, Hyung-Min Kim, and Hyun-Ja Jeong. (2014). "Zinc Oxide Nanoparticles, a Novel Candidate for the Treatment of Allergic Inflammatory Diseases." European journal of pharmacology 738: 31–39.

Kugarajah V, Sugumar M, Swaminathan E, Balasubramani N, Dharmalingam S. (2021). "Investigation on Sulphonated Zinc Oxide Nanorod Incorporated Sulphonated Poly (1,4-Phenylene Ether Ether Sulfone) Nanocomposite Membranes for Improved Performance of Microbial Fuel Cell." International Journal of Hydrogen Energy. 2021 Jun 18;46(42):22134-48.

Kugarajah V, Ojha AK, Hadem H, Dasgupta N, Mishra BN, Ranjan S, Dharmalingam S. (2022). Nanoparticles and nanofluids: Characteristics and behavior aspects. InFood, Medical, and Environmental Applications of Nanomaterials pp. 41-71.

Li A, Luehmann HP, Sun G, Samarajeewa S, Zou J, Zhang S, Zhang F, Welch MJ, Liu Y, Wooley KL (2012). Synthesis and in vivo pharmacokinetic evaluation of degradable shell cross-linked polymer nanoparticles with poly (carboxybetaine) versus poly (ethylene glycol) surface-grafted coatings. ACS nano, 6(10):8970-82.

Lubich C, Allacher P, de la Rosa M, Bauer A, Prenninger T, Horling FM, Siekmann J, Oldenburg J, Scheiflinger F, Reipert BM. (2013). The mystery of antibodies against polyethylene glycol (PEG)-what do we know?. Pharmaceutical research. (3) 2239-49.

Maddu, A, C Winarti, and M Kurniati. (2019). "Cellulose-Based Nano Hydrogel from Corncob by Gamma Irradiation." In IOP Conference Series: Earth and Environmental Science, IOP Publishing, 12003.

Meyers, Lawrence S, Glenn C Gamst, and A J Guarino. (2013). Performing Data Analysis Using IBM SPSS. John Wiley & Sons.

Pasut, Gianfranco, and Francesco M Veronese. (2012). "State of the Art in PEGylation: The Great Versatility Achieved after Forty Years of Research." Journal of controlled release 161(2): 461–72.

Rodriguez-Rivera GJ, Green M, Shah V, Leyendecker K, Cosgriff-Hernandez E. (2024). "A User's Guide to Degradation Testing of Polyethylene Glycol-based Hydrogels: From in Vitro to in Vivo Studies." Journal of Biomedical Materials Research Part A 112(8): 1200–1212.

De Romana, D Lopez, Kenneth H Brown, and J-X Guinard. (2002). "Sensory Trial to Assess the Acceptability of Zinc Fortificants Added to Iron-fortified Wheat Products." Journal of Food Science 67(1): 461–65.

Salvekar, Abhijit et al. (2017). "Water-Responsive Shape Recovery Induced Buckling in Biodegradable Photo-Cross-Linked Poly(Ethylene Glycol) (PEG) Hydrogel." Accounts of Chemical Research 50.

Siddiqi, Khwaja Salahuddin, Aziz ur Rahman, null Tajuddin, and Azamal Husen. (2018). "Properties of Zinc Oxide Nanoparticles and Their Activity against Microbes." Nanoscale research letters 13: 1–13.

Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Ann LC, Bakhori SK, Hasan H, Mohamad D (2015). "Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism." Nano-micro letters (7): 219–42.

Szabó, Tamás, József Németh, and Imre Dékány. (2003). "Zinc Oxide Nanoparticles Incorporated in Ultrathin Layer Silicate Films and Their Photocatalytic Properties." Colloids and Surfaces A: Physicochemical and Engineering Aspects 230(1–3): 23–35.

Vaezifar S, Razavi S, Golozar MA, Karbasi S, Morshed M, Kamali M (2013). Effects of some parameters on particle size distribution of chitosan nanoparticles prepared by ionic gelation method. Journal of Cluster Science.24:891-903..

Wang, Zihan, Qinzhou Ye, Sheng Yu, and Behnam Akhavan. (2023). "Poly Ethylene Glycol (PEG)-Based Hydrogels for Drug Delivery in Cancer Therapy: A Comprehensive Review." Advanced healthcare materials 12(18): 2300105.

Yang Q, Jacobs TM, McCallen JD, Moore DT, Huckaby JT, Edelstein JN, Lai SK.(2016). Analysis of pre-existing IgG

and IgM antibodies against polyethylene glycol (PEG) in the general population. Analytical chemistry. 88(23):11804-12.

Zhang Z, Ye Z, Hu F, Wang W, Zhang S, Gao L, Lu H (2022). Double-network polyvinyl alcohol composite hydrogel with self-healing and low friction. Journal of Applied Polymer Science. 139(4):51563.

Zhu, Xiaomin, Tian Zhong, Ran Huang, and Ajun Wan. 2015. "Preparation of Hydrophilic Poly(Lactic Acid) Tissue Engineering Scaffold via (PLA)-(PLA-b-PEG)-(PEG) Solution Casting and Thermal-Induced Surface Structural Transformation." Journal of biomaterials science. Polymer edition 26: 1-12Length3593.