Journal of Dental Sciences 21 (2026) 410–418

Available online at www.sciencedirect.com

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Original Article

Development of bio-compatible isosorbide-based polyurethanes exhibiting eucalyptol solubility for advanced root canal filling applications

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Received 25 September 2025; Final revision received 30 September 2025
Available online 25 October 2025

KEYWORDS
Biocompatibility;
Eucalyptol;
Isosorbide;
Retreatment;
Root canal filling materials;
Thermoplastic polyurethane

Abstract *Background/purpose:* Gutta-percha (GP) has long been the standard root canal filling material, but its poor removability, limited mechanical properties, and potential cytotoxicity restrict clinical performance. To address these shortcomings, this study aimed to develop bio-based D-isosorbide thermoplastic polyurethanes (IPUs) with improved removability in eucalyptol oil, superior mechanical performance, and enhanced cytocompatibility, providing a potential alternative to GP.

Materials and methods: D-isosorbide-based IPUs were synthesized from polycarbonate polyol and D-isosorbide via catalyst-free, two-step polymerization. Four formulations with different hard segment contents (HS10–HS40) were prepared and evaluated for thermal stability (thermogravimetric analysis and differential scanning calorimetry), mechanical properties (tensile testing), solubility in eucalyptol oil, and cytocompatibility (CCK-8 and LDH assays), compared with GP.

Results: All IPUs showed high thermal stability ($T_5d > 270$ °C) and melting transitions between 44 and 48 °C, similar to GP. Among the formulations, IPU-30 demonstrated optimal performance, achieving a tensile strength of 11.8 ± 1.0 MPa and elongation at break of 721 ± 144 %, markedly surpassing GP. Solubility in eucalyptol increased with D-isosorbide

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https://doi.org/10.1016/j.jds.2025.10.002

content, with IPU-40 exhibiting up to 13 % mass loss. In cytocompatibility assays, IPU-30 displayed lower early-phase cytotoxicity and maintained higher cell viability than GP over 6 days. *Conclusion:* D-isosorbide-based IPUs combine favorable thermal and mechanical properties with partial solubility in eucalyptol, enabling easier and safer retreatment. Their excellent cytocompatibility further supports their potential as sustainable and clinically viable alternatives to GP in root canal obturation.

Introduction

The primary goal of root canal treatment (RCT) is to eliminate microorganisms and infected tissue from the root canal system, followed by three-dimensional sealing with filling materials to prevent reinfection and promote periapical healing.[1] Gutta-percha (GP) remains the most widely used root canal filling material, typically combined with sealers to enhance sealing performance.[2,3] The American Association of Endodontists defines obturation as "the method used to fill and seal a cleaned and shaped root canal using a root canal sealer and core filling material." The purpose of obturation is to create a complete barrier between the root canal system and surrounding periodontium, thereby preventing microbial penetration.[4] Clinically, obturation materials must satisfy key requirements: ease of handling, thermal and dimensional stability, biocompatibility, radiopacity, and removability to facilitate retreatment when necessary.[5,6]

Although GP has long been regarded as the gold standard for obturation due to its moldability and removability, its nonpolar, hydrophobic nature limits adhesion to dentin, increasing the risk of microleakage and reducing long-term sealing integrity.[7—9] Polymer-based alternatives such as Resilon/Epiphany and EndoREZ™ have been introduced to improve bonding to dentin.[10,11] However, their crosslinked resin structures make removal during retreatment challenging, particularly in narrow or curved canals. From a clinical perspective, this difficulty can extend chair time, increase dentin removal, and potentially weaken the tooth.

Root canal retreatment—performed when persistent periapical infection or reinfection occurs—requires complete removal of the existing filling material to allow effective reshaping, disinfection, and re-obturation.[12] Current removal strategies combine mechanical instrumentation, ultrasonic or thermal techniques, and solvent application. Among solvents, eucalyptus oil and its major component, eucalyptol, are favored for their low toxicity and biocompatibility compared with chloroform.[13,14] Eucalyptol can soften GP within clinically acceptable times, especially when warmed to physiological temperatures.[15—17] However, its limited ability to dissolve highly crystalline or crosslinked materials often necessitates aggressive instrumentation, increasing the risk of procedural errors such as ledge formation or perforation. This highlights the clinical need for obturation materials that provide reliable sealing yet can be efficiently and safely removed in biocompatible solvents like eucalyptol.

D-isosorbide (1,4:3,6-dianhydrohexitol), a bio-based diol derived from glucose, has unique stereochemical properties—two cis-fused tetrahydrofuran (THF) rings in a rigid, puckered V-shaped conformation—that disrupt molecular packing, reduce crystallinity, and increase polarity.[18—26] These structural features can enhance flexibility, solvent interaction, and biocompatibility when incorporated into polyurethane (PU) matrices.[27—30] Polycarbonate polyols (PCPOs), synthesized from carbon dioxide, offer excellent mechanical strength, thermal stability, and environmental benefits, making them suitable as soft segments in thermoplastic polyurethane (TPU) formulations.[31] Our previous work demonstrated that PCPO-based polyurethane acrylates had strong mechanical properties and biological stability, but their crosslinked networks hindered removability.[32]

In this study, we replaced the conventional linear chain extender 1,4-butanediol with rigid, polar D-isosorbide in PCPO-based TPUs to enhance eucalyptol compatibility while retaining favorable thermal and mechanical properties. We hypothesized that this design would yield an obturation material with high strength and flexibility, partial solubility in eucalyptol for easier retreatment, and excellent cytocompatibility. A series of isosorbide-based thermoplastic polyurethanes (IPUs) with varying hard segment ratios were synthesized and characterized for thermal behavior, mechanical performance, eucalyptol solubility, and cytocompatibility, with conventional GP as a reference. Our goal was to develop a sustainable, clinically adaptable root canal filling material that meets the demands of both primary treatment and retreatment—maximizing sealing performance while minimizing retreatment complexity and risk.

Materials and methods

Materials

Polycarbonate diol (PCPO2000, Mw ≈ 2000 g/mol) was purchased from Sigma—Aldrich (St. Louis, MO, USA). Hexamethylene diisocyanate (HDI, 99 %) was obtained from Merck (Darmstadt, Germany). D-isosorbide (≥98 %) was supplied by Tokyo Chemical Industry (Tokyo, Japan). Eucalyptol oil was purchased from Sigma—Aldrich. All materials were used as received without further purification.

Instruments

Fourier transform infrared spectroscopy (FT-IR, Jasco Inc., Tokyo, Japan) was used to monitor urethane bond

formation. Thermogravimetric analysis (TGA, Q50, TA Instruments, New Castle, DE, USA) was employed to evaluate thermal stability under a nitrogen atmosphere at a heating rate of 10 °C/min. Differential scanning calorimetry (DSC, Q20, TA Instruments) was used to analyze thermal transitions of the synthesized polymers. Mechanical properties were measured using a universal testing machine (Landmark 370.02 Test System, MTS Systems Corp., Eden Prairie, MN, USA) according to ASTM D638. Cell assays were performed using a microplate reader (Synergy HT, BioTek Instruments, Agilent Technologies, New Taipei City, Taiwan).

Synthesis of thermoplastic polyurethanes (IPUs)

PCPO 2000, HDI, and THF were mixed at 60 °C and stirred under nitrogen for 1PCPO 2000, HDI, and THF were mixed at 60 °C under nitrogen and stirred for 1.5 h to form prepolymers. The reaction temperature was then increased to 70 °C, and D-isosorbide was added to achieve target hard segment ratios of 10 %, 20 %, 30 %, and 40 %, corresponding to IPU-10, IPU-20, IPU-30, and IPU-40. The reaction mixture was stirred for an additional 3 h.

The resulting solution was cast into Teflon trays and left at room temperature for 6 h, followed by curing at 40 °C, 50 °C, and 60 °C for 6 h each to remove residual THF. Polymerization was confirmed by FT-IR analysis through the disappearance of the isocyanate (N=C=O) peak and the appearance of the urethane C=O peak.

Thermal and mechanical properties

Thermal stability was evaluated by thermogravimetric analysis (TGA) using approximately 5 mg of each IPU sample, heated from 110 °C to 800 °C under nitrogen at 10 °C/min. Melting temperatures were determined by differential scanning calorimetry (DSC) under nitrogen at the same heating rate.

Tensile tests were performed according to ASTM D638 using dog-bone-shaped specimens at 23 ± 2 °C with a crosshead speed of 100 mm/min. Five specimens per group (IPU-10 to IPU-40) were tested.

Solubility evaluation in eucalyptol oil

IPU discs (6 mm diameter, 2 mm thickness) were immersed in 4 mL of eucalyptol oil at 40 °C for 2 h. Samples were then filtered, rinsed with ethanol, dried under vacuum at 40 °C overnight, and weighed. Solubility (%) was calculated as:

$$\text{Solubility}\,(\%) = (\text{Initial weight} - \text{Residual weight})/\text{Initial weight} \times 100$$

Cell cytotoxicity tests

MG63 osteoblast-like cells (ATCC® CRL-1427™, Manassas, VA, USA) were cultured under standard conditions. Extracts were prepared according to ISO 10993-12 by incubating IPU specimens in culture medium at 37 °C for 24 h, followed by filtration.

CCK-8 assay

Cells (1×10^5 cells/mL) were seeded in 96-well plates for 24 h, then exposed to 100 μL of IPU extract. Cell viability was measured at 1, 3, and 6 days using the CCK-8 kit. Controls were maintained in fresh medium.

Lactate dehydrogenase (LDH) cytotoxicity assay

Cells (1×10^5/well) were seeded in 96-well plates for 24 h and then exposed to IPU extracts for 1, 3, or 6 days. LDH release was quantified, with 1 % Triton X-100 as the positive control and fresh medium as the negative control.

Statistical analysis

All experiments were performed in triplicate unless otherwise stated. Results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using Student's t-test (SPSS 18.0, SPSS Inc., Chicago, IL, USA), with $P < 0.05$ being considered to be statistically significant. Significance levels were indicated as: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns = not significant.

Results

Synthesis of IPUs-based on D-isosorbide

A series of D-isosorbide-based thermoplastic polyurethanes (IPUs) were synthesized via a catalyst-free, two-step, one-pot solvent polymerization (Fig. 1). Prepolymers were first obtained by reacting HDI with PCPO2000 at 60 °C, followed by chain extension with D-isosorbide at 70 °C to yield IPUs with hard segment (HS) contents of 10 %, 20 %, 30 %, and 40 % (IPU-10 to IPU-40). The soft segments consisted of PCPO-HDI, while the hard segments were formed from D-isosorbide and HDI. Unlike conventional TPU synthesis using dimethylformamide (DMF) and tin-based catalysts, this method employed less toxic THF and omitted catalysts to improve biocompatibility. The absence of toxic catalysts may reduce the risk of residual cytotoxic components in the final product, which is advantageous for materials in direct contact with periapical tissues.

Fourier transform infrared spectroscopy (FT-IR) characterization of TPU polymerization

FT-IR spectra confirmed the successful formation of urethane linkages in the synthesized IPUs. In Fig. 2(a), the spectrum at the initial stage showed a distinct N=C=O stretching peak at 2260 cm^{-1}, corresponding to unreacted HDI. As the reaction proceeded, this peak gradually diminished and completely disappeared after approximately 3 h, while a new C=O stretching peak at 1740 cm^{-1} emerged and intensified, indicating the formation of

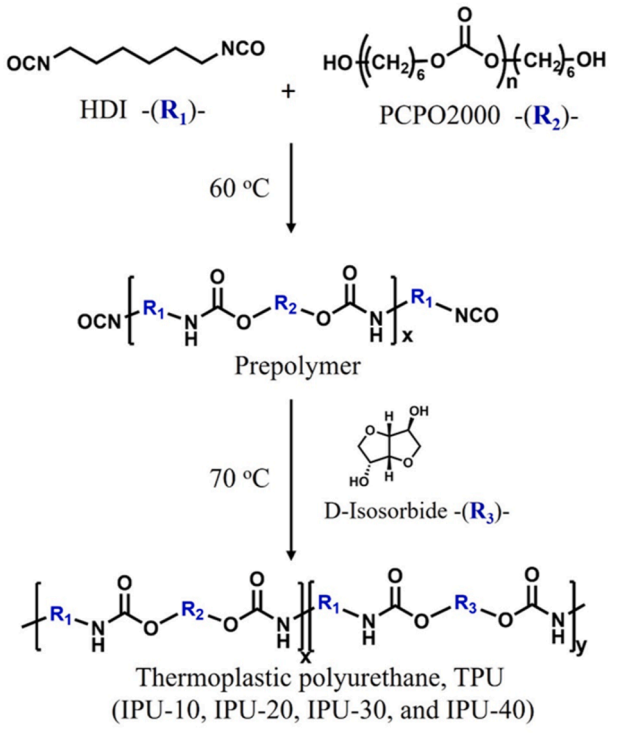

Figure 1 Schematic illustration of the preparation process for isosorbide-based thermoplastic polyurethanes (IPUs). Abbreviations: IPU, isosorbide-based polyurethane.

urethane bonds through the reaction between isocyanate and hydroxyl groups.

In Fig. 2(b), spectra of IPUs with different D-isosorbide contents demonstrated that increasing D-isosorbide proportion slowed the disappearance of the N=C=O peak. This is likely due to steric hindrance from the rigid bicyclic structure of D-isosorbide, which reduces the accessibility of hydroxyl groups for reaction. This observation is consistent with previous reports describing how rigid diols influence polyurethane reaction kinetics.

The complete disappearance of the NCO peak across all formulations indicates full conversion of isocyanate groups, ensuring chemical stability and minimizing the risk of residual reactive species that could compromise the material's long-term performance in clinical applications.

Thermal characterization of IPUs via thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC analyses)

Thermal properties were assessed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). As shown in Fig. 3, the decomposition temperatures at 5 wt % weight loss (T_5d) ranged from 270 °C to 302 °C. Increasing hard segment content slightly decreased T_5d. This reduction may result from disruption of polymer chain packing by the rigid D-isosorbide units. Nevertheless, all IPUs exhibited sufficient thermal stability for clinical use, as root canal filling procedures typically occur below 200 °C.

Fig. 4 revealed melting points (Tm) between 44.7 °C and 48.2 °C, corresponding to soft segment crystalline transitions. As hard segment content increased, Tm decreased, indicating reduced crystallinity. This effect is consistent with previous studies showing that the asymmetric V-shaped conformation of isosorbide units hinders chain packing and limits phase separation.

Importantly, the melting transition range of the synthesized IPUs was comparable to commercial materials such as gutta-percha (GP) and Resilon, which typically exhibit Tm values between 40 °C and 65 °C without sharp phase transitions. These results highlight the suitability of D-isosorbide-based IPUs for clinical applications requiring moderate-temperature softening.

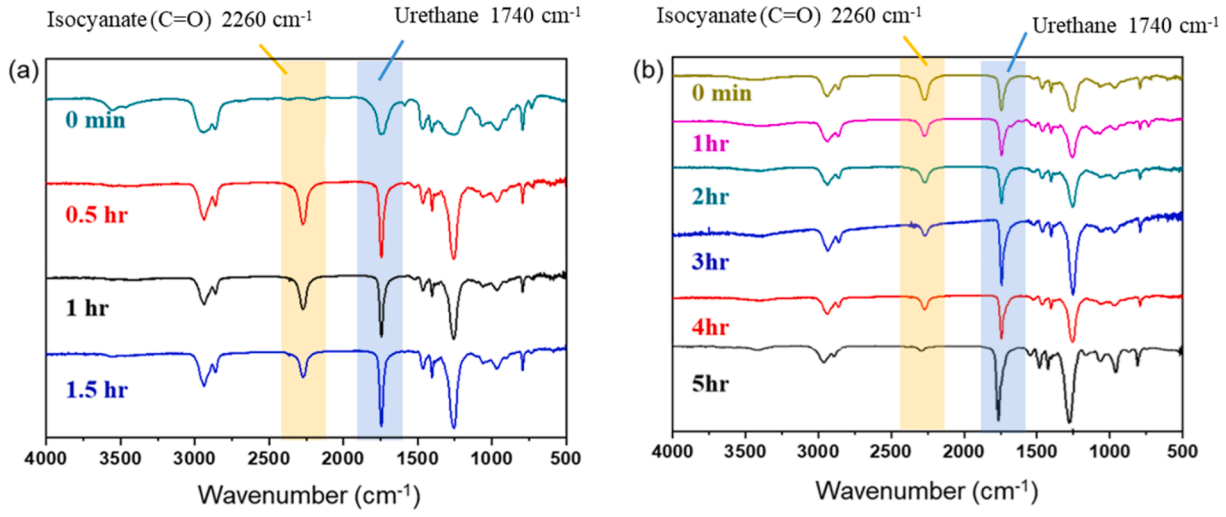

Figure 2 FT-IR spectra of IPUs. (a) Spectra of IPU-30 at different reaction times, showing the decrease of the N=C=O peak (2260 cm^{-1}) and the increase of the urethane C=O peak (1740 cm^{-1}). (b) Spectra of IPUs with varying D-isosorbide content, where higher D-isosorbide slows NCO consumption. Yellow and blue shaded areas indicate isocyanate and urethane peaks, respectively. Abbreviations: FT-IR, Fourier transform infrared spectroscopy; IPU, isosorbide-based polyurethane; NCO, isocyanate.

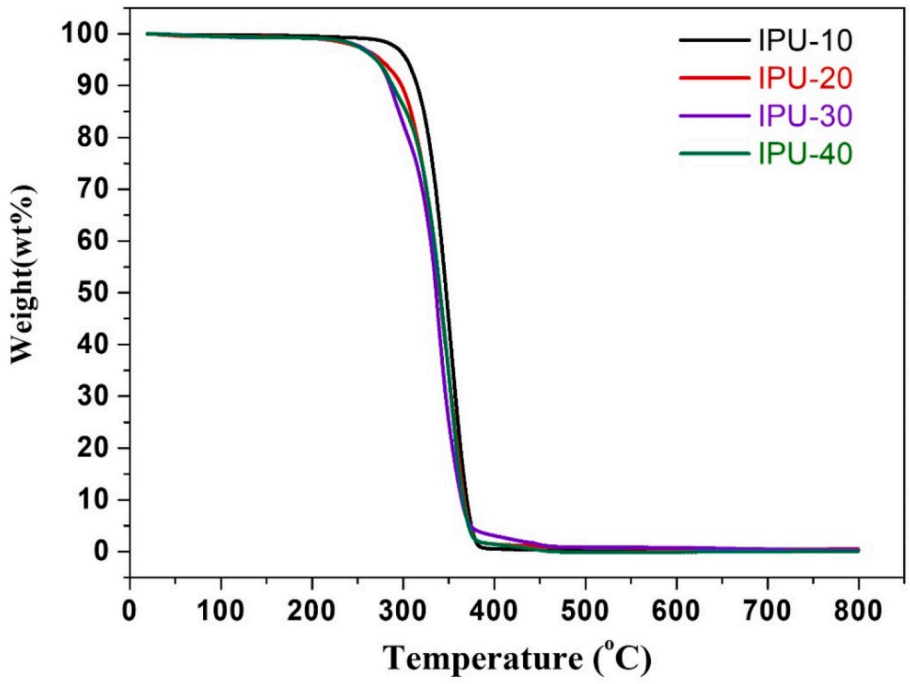

Figure 3 TGA thermograms of IPU-10, IPU-20, IPU-30, and IPU-40, showing thermal decomposition behavior and the corresponding 5 wt% weight loss temperatures (T_5d). Abbreviations: TGA, thermogravimetric analysis; IPU, isosorbide-based polyurethane; T_5d, temperature at 5 % weight loss.

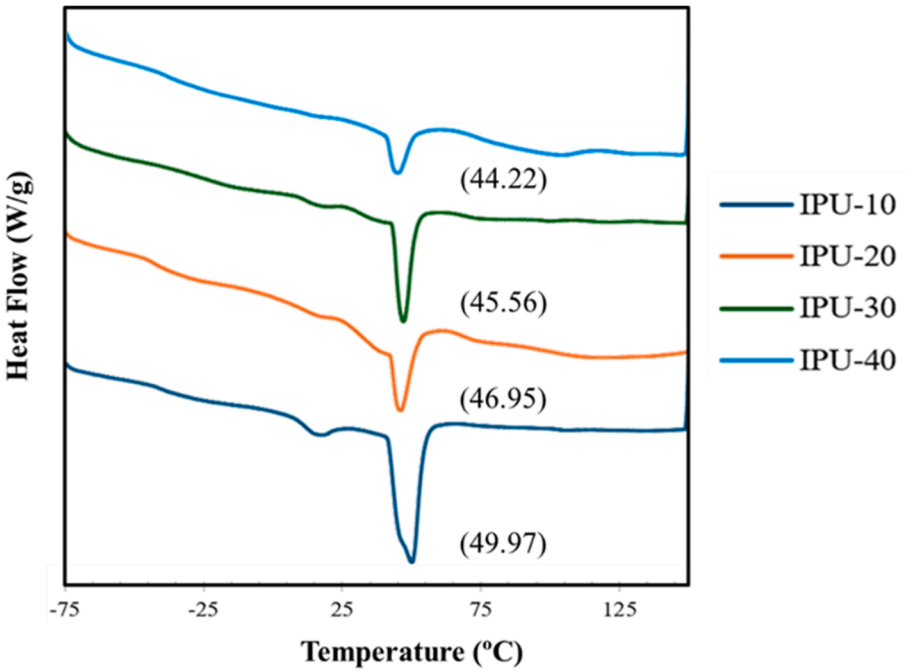

Figure 4 DSC thermograms (second heating scan) of IPU-10, IPU-20, IPU-30, and IPU-40, indicating melting transitions associated with soft segment crystallinity. Abbreviations: DSC, differential scanning calorimetry; IPU, isosorbide-based polyurethane.

Solubility evaluation of D-isosorbide-based TPUs in eucalyptol oil

Solubility in solvents such as eucalyptol oil, xylene, and chloroform is a critical factor in root canal retreatment. These solvents are typically non-polar or weakly polar, which limits the dissolution of polar polymers like IPUs. Chloroform is effective but associated with potential periapical tissue irritation when extruded beyond the apex. In contrast, eucalyptol oil, a naturally derived terpenoid oxide, offers lower cytotoxicity and a safer clinical profile.

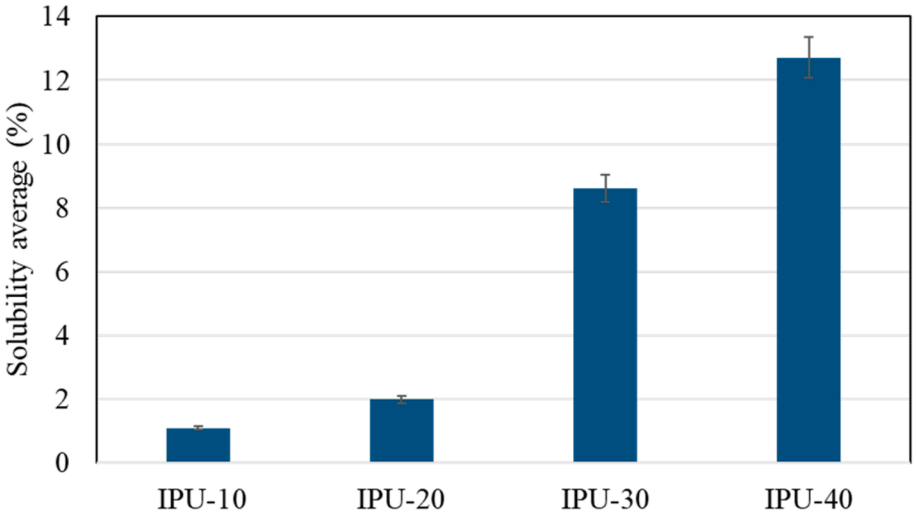

Figure 5 Solubility of IPU-10, IPU-20, IPU-30, and IPU-40 in eucalyptol oil. Residual weight percentage was determined after 2 h immersion at 40 °C. Higher D-isosorbide content enhanced solubility, facilitating removability. Abbreviations: IPU, isosorbide-based polyurethane.

As shown in Fig. 5, increasing D-isosorbide content improved IPU solubility in eucalyptol oil. IPU-30 and IPU-40 retained only 91.4 % and 87.3 % of their initial weight, respectively, whereas reference TPUs containing linear chain extenders remained largely insoluble.

Mechanical properties of D-isosorbide-based IPUs

Mechanical performance is critical for ensuring long-term sealing and durability in root canal applications. Conventional gutta-percha (GP) exhibits poor mechanical strength, making tensile testing by ASTM D638 difficult. In contrast, the synthesized IPUs exhibited markedly different behavior depending on formulation. IPU-10 and IPU-20 were too brittle for testing, likely due to the high PCPO2000—HDI soft segment content. However, IPU-30 and IPU-40 retained sufficient integrity for tensile evaluation (Fig. 6).

IPU-30 achieved a tensile strength of 11.8 ± 1.0 MPa and an elongation at break of 721.4 ± 143.6 %, while IPU-40 exhibited 8.4 ± 0.7 MPa and 20.9 ± 4.2 %. The enhanced flexibility of IPU-30 can be attributed to reduced

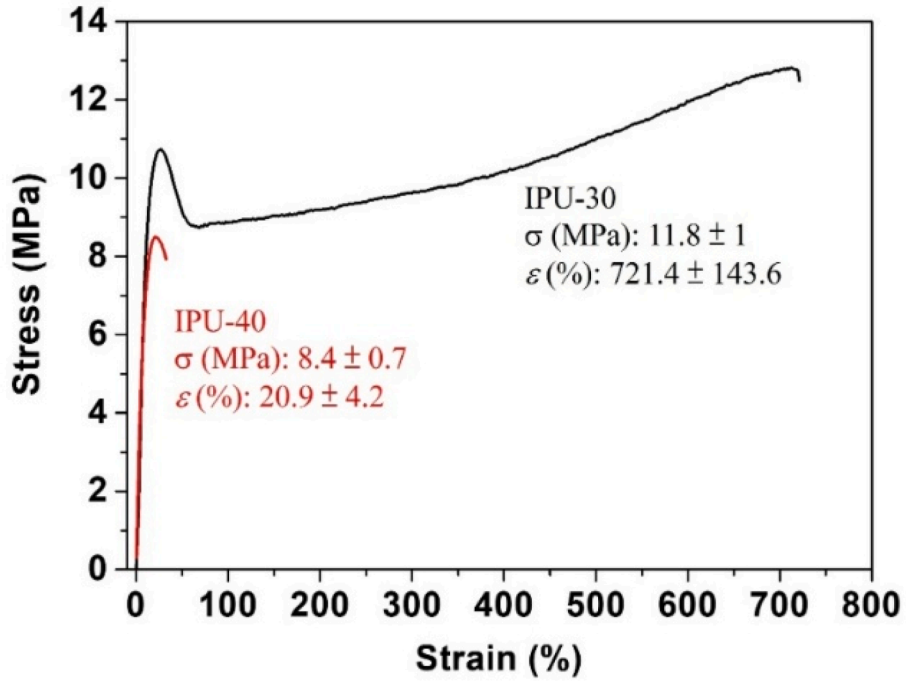

Figure 6 Representative stress—strain curves of IPU-30 and IPU-40 obtained from tensile testing according to ASTM D638 at 23 ± 2 °C and 100 mm/min crosshead speed. IPU-30 exhibited higher tensile strength (11.8 ± 1.0 MPa) and elongation at break (721.4 ± 143.6 %) compared to IPU-40 (8.4 ± 0.7 MPa, 20.9 ± 4.2 %). Abbreviations: IPU, isosorbide-based polyurethane; ASTM, American Society for Testing and Materials; MPa, megapascal.

crystallinity and increased hydrogen bonding, which provide partial physical crosslinking and mechanical adaptability.

Cell toxicity test of D-isosorbide-based IPU materials

Cell vitality and toxicity analysis for root canal sealing materials was performed using the CCK-8 assay, in accordance with ISO 10993 guidelines, as shown in Figs. 7 and 8. For comparison, conventional gutta-percha (GP) was assessed alongside the D-isosorbide-based polyurethane IPU-30. On Day 1, GP exhibited ~80 % cell viability, while IPU-30 showed nearly 100 %. Over time, GP viability gradually declined to ~50 % by Day 6, whereas IPU-30 consistently maintained higher viability, indicating superior long-term cytocompatibility.

Discussion

D-isosorbide-based thermoplastic polyurethanes (IPUs) were successfully synthesized using a catalyst-free process,

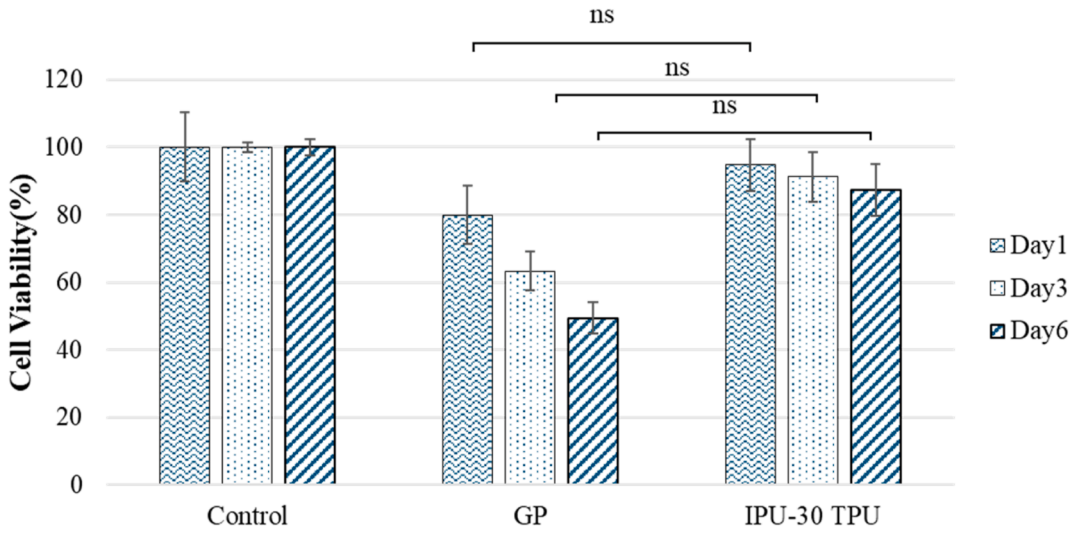

Figure 7 Cytotoxicity analysis of control, gutta-percha (GP), and IPU-30 using the CCK-8 assay after 1, 3, and 6 days of incubation with MG63 cells. Cell viability was expressed as a percentage relative to the untreated control. Data are presented as mean ± SD (n = 3). Statistical significance was evaluated by two-tailed unpaired Student's t-test. Abbreviations: GP, gutta-percha; IPU, isosorbide-based polyurethane; CCK-8, cell counting kit-8; SD, standard deviation.

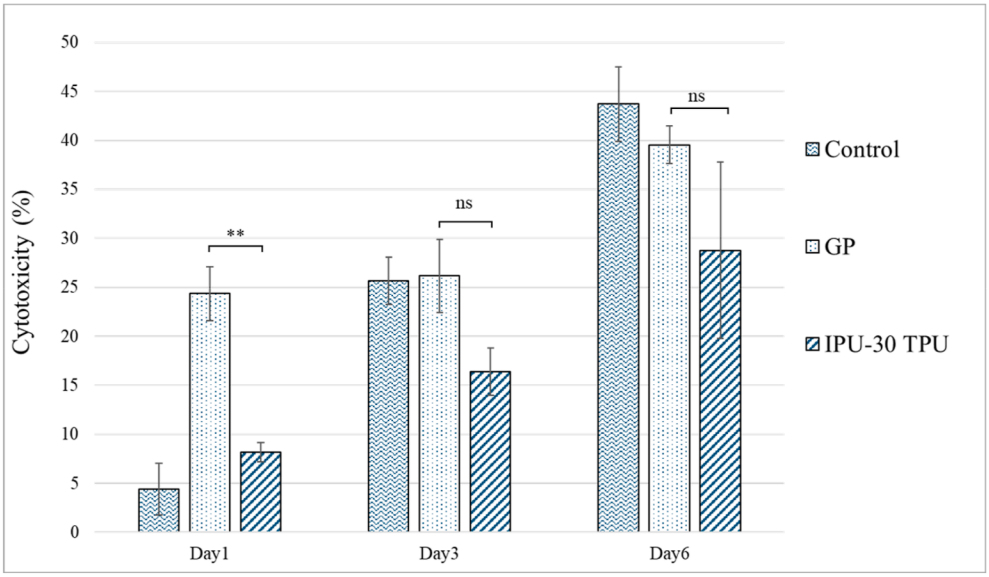

Figure 8 Cytotoxicity (%) of gutta-percha (GP) and IPU-30 samples measured by LDH assay after 1, 3, and 6 days of incubation with MG63 cells. GP showed significantly higher cytotoxicity than IPU-30 on Day 1 ($P = 0.0043$), while no significant differences were observed on Days 3 and 6 ($P > 0.05$). Abbreviations: GP, gutta-percha; IPU, isosorbide-based polyurethane; LDH, lactate dehydrogenase.

demonstrating favorable thermal stability, mechanical performance, solubility, and cytocompatibility. FT-IR analysis confirmed complete consumption of isocyanate groups, ensuring chemical stability and eliminating concerns about residual reactive species. Compared with conventional polyurethane synthesis routes, omission of toxic catalysts may reduce risks of cytotoxicity, which is particularly relevant for materials in direct contact with periapical tissues.

Thermal characterization showed decomposition temperatures above 270 °C and melting transitions between 44 and 48 °C, values comparable to gutta-percha (GP) and other clinically used root canal filling materials. These results suggest that synthesized IPUs can withstand clinical operating conditions while providing thermal behavior suitable for obturation. Reduced crystallinity caused by the rigid, V-shaped structure of isosorbide contributed to moderate softening, facilitating clinical handling.

Solubility studies indicated that incorporation of D-isosorbide enhanced dissolution in eucalyptol oil. IPU-30 and IPU-40 exhibited partial mass loss, while TPUs with linear chain extenders remained largely insoluble. Improved solubility is attributed to disrupted molecular packing and reduced crystallinity, which allow solvent penetration. From a clinical perspective, this characteristic is advantageous, as removability is essential for safe retreatment procedures.

Mechanical testing highlighted differences among formulations. IPU-10 and IPU-20 were brittle and unsuitable for tensile evaluation, whereas IPU-30 achieved an optimal balance of strength (11.8 MPa) and flexibility (721 % elongation). This combination is superior to GP, which typically exhibits low strength and negligible elongation. Enhanced flexibility of IPU-30 is likely due to partial hydrogen bonding and physical crosslinking, which improve adaptability to irregular root canal geometries.

Cytocompatibility evaluation further supported the potential of IPU-30 as a safer alternative to GP. While GP showed reduced cell viability and higher early-phase cytotoxicity, IPU-30 maintained higher viability over six days. Lower toxicity is attributed to the aliphatic polyurethane backbone and absence of additives such as zinc oxide and waxes. Sustained biocompatibility is critical for avoiding periapical inflammation and ensuring long-term clinical success.

Overall, findings suggest that isosorbide-based IPUs, particularly IPU-30, combine durability, removability, and excellent biological performance. This balance of properties highlights their potential as sustainable alternatives to conventional GP for root canal obturation and retreatment procedures.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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