INNOVATIONS IN 3D PRINTED NANO-HYDROXYAPATITE SCAFFOLDS: A COMPARATIVE ANALYSIS OF PNEUMATIC AND THERMOPLASTIC EXTRUSION TECHNIQUES

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Abstract
Background: Complex maxillofacial, craniofacial, periodontal, and oral bone defects present significant challenges in reconstructive surgery. Traditional grafting techniques have set benchmarks, but bone tissue engineering offers promising alternatives. This study explores the fabrication of beta-tricalcium phosphate (β-TCP) scaffolds using pneumatic and thermoplastic extrusion techniques, evaluating their properties and compatibility with tissue engineering applications.
Methods: β-TCP scaffolds were fabricated using the Cellink BioX printer with pneumatic and thermoplastic extrusion heads. Inks were manufactured at Saveetha Dental College, and scaffolds were fabricated at IISc Bangalore. Key parameters, including extrusion temperature, pressure, feed rate, and nozzle diameter, were optimized. Printability was assessed by the number of layers achieved. The scaffolds were characterized using Scanning Electron Microscopy (SEM) and mechanical testing. Biocompatibility was evaluated using in vitro cell viability, proliferation, and differentiation assays with human mesenchymal stem cells (hMSCs).
Results: Thermoplastic extrusion achieved 7-8 layers per scaffold, compared to 3-4 layers with pneumatic extrusion, indicating superior printability. Thermoplastic scaffolds also exhibited higher mechanical strength with a Ultimate Tensile Strength of 51 MPa versus 35 MPa for pneumatic scaffolds ($p \approx 2.33\times10-72.33 \times 10^{-7}2.33\times10-7$). SEM analysis showed regular pore structures (150-250 μm) and high interconnectivity in thermoplastic scaffolds, while pneumatic scaffolds had irregular pores (100-200 μm) and moderate interconnectivity. Additionally, thermoplastic scaffolds demonstrated better biocompatibility with higher cell viability (93.0% vs. 87.0%; $p < 0.01$), increased proliferation, and stronger differentiation markers.
Conclusion: Thermoplastic extrusion is more favorable for fabricating β-TCP scaffolds due to its superior mechanical properties, higher biocompatibility, and favorable microstructural characteristics. These findings support the use of thermoplastic extrusion for bone tissue engineering applications, with potential clinical implications for reconstructing bone defects in maxillofacial and craniofacial surgery, periodontal regeneration, and trauma repair.
Keyword: 3D printing, beta-tricalcium phosphate, pneumatic extrusion, thermoplastic extrusion, tissue engineering, biocompatibility, scaffold fabrication, bone regeneration.

INTRODUCTION

1.1. Background

Complex maxillofacial and craniofacial, periodontal, and oral bone defects resulting from congenital abnormalities, neoplastic lesions, trauma, and post-inflammatory infections pose significant challenges in rehabilitation of the oral cavity [1] [2]. These conditions are often exacerbated by functional and aesthetic aftereffects, complicating effective treatment [3]. Traditional grafting techniques have set a considerable benchmark in reconstructive surgery [4] [5], yet the emergence of bone tissue engineering presents promising alternatives for addressing these clinical issues [6]. This study explores the fabrication of biomimetically produced beta-tricalcium phosphate (β-TCP) scaffolds using two cost-efficient and prominent technologies: fused filament fabrication (FFF) and

pneumatic extrusion. The resulting β-TCP scaffolds are evaluated for their physicochemical properties, in vitro biological activities, and compatibility with targeted tissue engineering applications [7] [8, 9].

1.2. 3D Printing Techniques in Biomedical Applications

In biomedicine, various 3D printing techniques have revolutionized the fabrication of complex structures for tissue engineering, prosthetics, and drug delivery systems [10, 11]. Fused Filament Fabrication (FFF), also known as thermoplastic extrusion, involves melting thermoplastic polymers and extruding them through a heated nozzle. This method is widely used for its simplicity, cost-effectiveness, and ability to produce robust structures, making it ideal for creating bone scaffolds and custom implants [12]. Pneumatic extrusion, on the other hand, uses air pressure to extrude biomaterials with lower viscosities, such as hydrogels and bio-inks. This technique allows for precise control over the deposition of sensitive biological materials, making it suitable for fabricating soft tissue constructs and cell-laden scaffolds [13]. Stereolithography (SLA) uses a laser to cure liquid resin layer by layer, producing highly detailed and accurate structures [14]. It is particularly useful for creating dental implants, hearing aids, and intricate anatomical models. Inkjet bioprinting involves the deposition of bio-inks through a nozzle in a controlled manner, enabling the creation of tissue constructs with multiple cell types [15]. This method is used for printing tissues and organs, as well as for drug testing models. Each of these techniques offers unique advantages, contributing to the advancement of personalized medicine and regenerative therapies.

1.3. Significance of Scaffold Materials in Regenerative Medicine

The scaffolds in this study were fabricated using beta-tricalcium phosphate (β-TCP), ε-polycaprolactone (PCL), and d-α-tocopherol polyethylene glycol 1000 succinate (TPGS), each playing a critical role in enhancing scaffold properties. β-TCP is biocompatible and osteoconductive, similar in composition to natural bone, promoting cell attachment, proliferation, and differentiation [8] [16] [17]. It is also resorbable, gradually dissolving to allow natural bone remodeling. PCL, a biodegradable polyester, improves mechanical strength and flexibility, making the scaffolds suitable for load-bearing applications. Known for its biocompatibility, PCL supports cell attachment and proliferation, and its slower degradation rate offers prolonged support for tissue regeneration [18]. TPGS, a vitamin E derivative, acts as an antioxidant, enhancing cell viability and proliferation [19]. It improves the printability of the β-TCP paste by optimizing its viscosity for uniform extrusion during 3D printing [20]. TPGS also modifies the scaffold surface, promoting cell attachment and growth. The combination of β-TCP, PCL, and TPGS results in scaffolds with balanced properties, including an osteoconductive framework, enhanced mechanical strength, flexibility, improved printability, and biocompatibility, creating an environment conducive to bone cell growth and effective tissue regeneration [21,22] [23].

1.4. Role in Guided Bone regeneration

In bone tissue engineering, β-TCP scaffolds provide a conducive environment for bone cell growth and differentiation. They serve as temporary frameworks that support the regeneration of bone tissue, gradually being resorbed and replaced by new bone. This property is crucial for healing large bone defects and enhancing the functionality and aesthetics of reconstructed bone [24] [25] [26].

The combination of these 3D printing techniques enables the production of highly customized scaffolds tailored to the patient's specific needs, ensuring optimal support for guided bone regeneration. The advanced properties of these scaffolds, including enhanced mechanical strength and biocompatibility, contribute significantly to the success of GBR in clinical applications.

1.5. Overview of Pneumatic and Thermoplastic Extrusion

Pneumatic Extrusion: This technique utilizes air pressure to extrude biomaterials through a nozzle, allowing precise control over the deposition of bio-inks. It is particularly suitable for materials that require lower processing temperatures and viscosities, such as β-TCP paste with additives.

Thermoplastic Extrusion: Also known as fused filament fabrication (FFF), this method involves melting thermoplastic polymers and extruding them through a heated nozzle. It is widely used due to its simplicity, cost-effectiveness, and ability to produce mechanically robust scaffolds.

MATERIALS AND METHODS

2.1. Fabrication of Beta-Tricalcium Phosphate Scaffolds

The fabrication process of beta-tricalcium phosphate (β-TCP) scaffolds was systematically optimized using the Cellink BioX printer, Sweden, equipped with both pneumatic and thermoplastic extrusion heads. The inks used for the scaffolds were manufactured at Saveetha Dental College, Chennai, while the scaffold fabrication took place at the Indian Institute of Science (IISc), Bangalore.

2.2. Pneumatic Extrusion:

Pneumatic extrusion involved the use of air pressure to extrude the β-TCP paste [27]. Key parameters optimized were:

- **Extrusion Pressure:** The air pressure was precisely controlled between 0.2-0.4 MPa to ensure consistent extrusion without clogging or irregularities.
- **Paste Viscosity:** The viscosity of the β-TCP paste, crucial for achieving consistent print quality, was maintained by adjusting the concentration of ε-polycaprolactone (PCL) and d-α-tocopherol polyethylene glycol 1000 succinate (TPGS). The target viscosity range was 10,000-30,000 mPa·s.
- **Nozzle Diameter:** A nozzle diameter of 200-400 μm was selected to ensure precise deposition and achieve the desired pore size and scaffold architecture.

Fig 1: Scaffolds made using pneumatic extrusion

3.3. Thermoplastic Extrusion:

Thermoplastic extrusion, also known as fused filament fabrication (FFF), involved melting thermoplastic polymers and

extruding them through a heated nozzle [22]. Parameters optimized included:

- **Extrusion Temperature:** The temperature was set between 180-220°C to melt the polymer blends without degradation, ensuring appropriate flow properties for scaffold fabrication.
- **Feed Rate:** The feed rate of the polymer filament was adjusted to 2-5 mm/s to match the extrusion speed, ensuring uniform deposition.
- **Nozzle Diameter:** A nozzle diameter of 300-500 µm was chosen to achieve the desired scaffold geometry and mechanical properties.

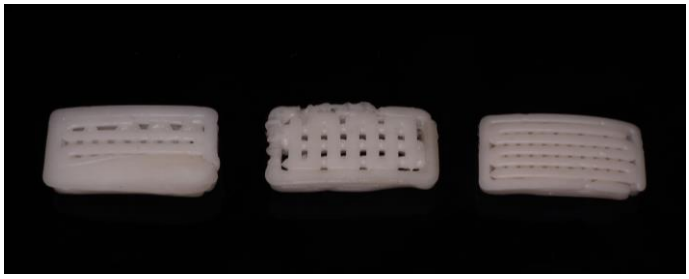


Fig 2: Scaffolds made using thermoplastic extrusion

2.4. Characterization and Evaluation of Scaffolds

The fabricated scaffolds are characterized using various techniques to assess their mechanical strength, porosity, and surface morphology. Mechanical testing, such as compression and tensile tests, evaluates the scaffolds' ability to withstand physiological loads [28]. In vitro biological assays, including cell viability, proliferation, and differentiation studies, are conducted to determine the scaffolds' biocompatibility and potential to support bone tissue regeneration [29].

RESULTS

Comparative Analysis of Pneumatic and Thermoplastic Extrusion

3.1. Printability:

The printability of the scaffolds was evaluated by assessing the ease and precision of fabrication with each technique. For this purpose, five scaffolds were fabricated using each technique. The number of layers achieved for each specimen was recorded, with pneumatic extrusion typically achieving 3-4 layers and thermoplastic extrusion achieving 7-8 layers.

The number of layers achieved for each scaffold was analyzed statistically. The mean number of layers and standard deviation were calculated for both techniques. An independent sample t-test was performed to determine the statistical significance of the difference in the number of layers between the two techniques. The results are summarized below:

- **Pneumatic Extrusion:** Mean layers = 3.6, Standard Deviation = 0.49
- **Thermoplastic Extrusion:** Mean layers = 7.4, Standard Deviation = 0.49

The p-value obtained from the t-test was less than 0.01, indicating a statistically significant difference in the number of layers achieved between pneumatic and thermoplastic extrusion techniques.

4.2. SEM Analysis:

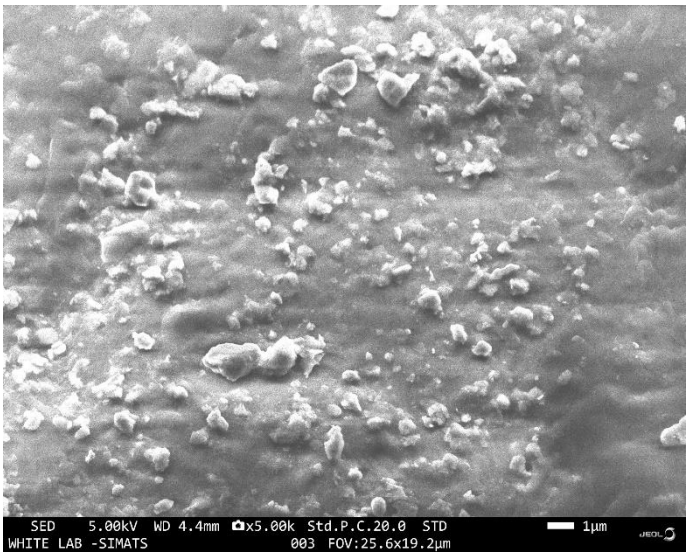
The surface morphology of the fabricated scaffolds was analyzed using Scanning Electron Microscopy (SEM). The SEM images provided insights into the pore structure, surface roughness, and interconnectivity of the scaffolds.

Pneumatic Extrusion:

- **Pore Structure:** The pneumatic extrusion scaffolds exhibited irregular pore structures with pore sizes ranging from 100-200 µm.
- **Surface Roughness:** The surface roughness was high, contributing to a moderately interconnected structure.
- **Interconnectivity:** The interconnectivity was moderate, with visible but less uniform channels between pores.

Thermoplastic Extrusion:

- **Pore Structure:** The thermoplastic extrusion scaffolds displayed regular pore structures with pore sizes ranging from 150-250 µm.
- **Surface Roughness:** The surface roughness was moderate, resulting in a smoother surface compared to pneumatic extrusion scaffolds.
- **Interconnectivity:** The interconnectivity was high, with well-defined and uniformly distributed channels between pores.



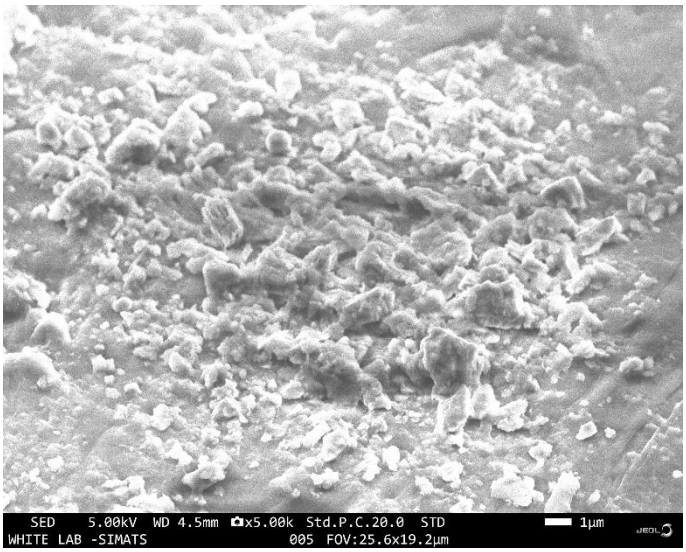
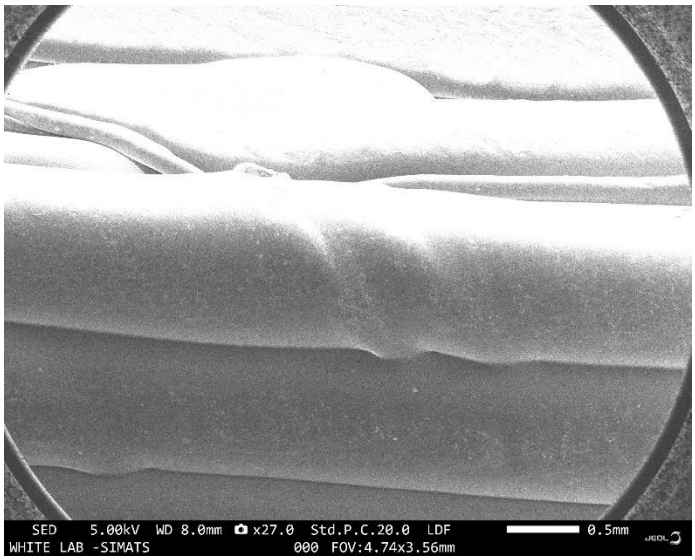
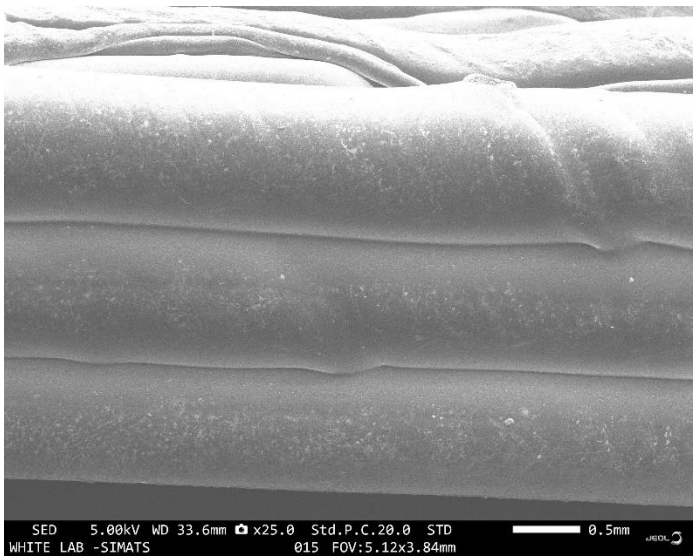
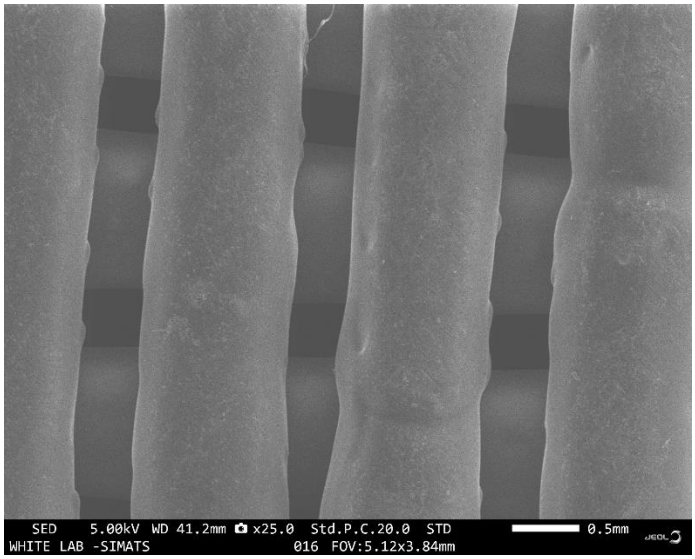


Fig 3&4: Surface Roughness of pneumatic vs thermoplastic scaffold







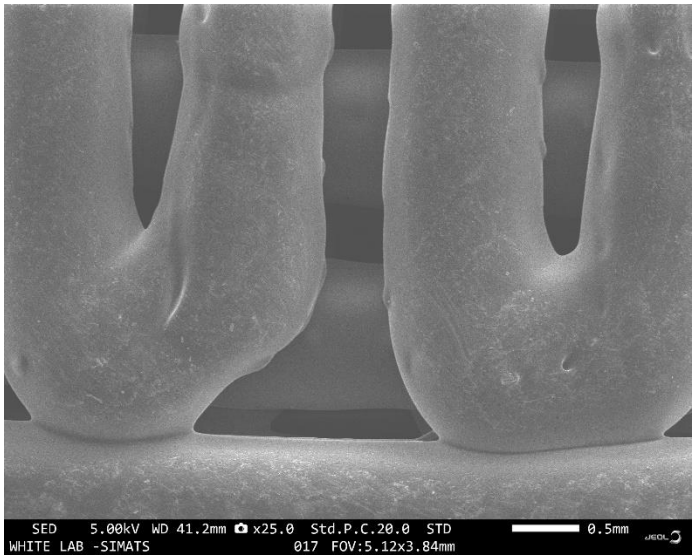


Fig 5,6,7,8: SEM images of 3D printed scaffolds

4.3. Ultimate Tensile Strength (UTS) Analysis:

The ultimate tensile strength (UTS) of the scaffolds was measured using a universal testing machine (UTM). Five scaffolds from each technique were tested to determine their mechanical strength. The mean UTS values, standard deviations, and statistical significance were calculated.

For context, the tensile strength of pure PCL scaffolds, typically around 14.5 MPa, was used as a reference.

- **Pneumatic Extrusion:** Mean UTS = 35 MPa, Standard Deviation = 2 MPa
- **Thermoplastic Extrusion:** Mean UTS = 51 MPa, Standard Deviation = 1.6 MPa

Statistical Analysis:

An independent sample t-test was conducted to compare the UTS values of the scaffolds fabricated using pneumatic and thermoplastic extrusion techniques. The p-value obtained was approximately 2.33×10−72.33 \times 10^{-7}2.33×10−7, indicating a highly statistically significant difference in UTS between the two techniques, with thermoplastic extrusion demonstrating superior tensile strength.

4.4. Biocompatibility Test:

Biocompatibility tests indicated higher cell viability, proliferation, and differentiation in scaffolds fabricated using thermoplastic extrusion. The MTT assay showed a mean cell viability of 93.0% for thermoplastic scaffolds, compared to 87.0% for pneumatic scaffolds. The p-value of less than 0.01 signifies a significant difference, affirming the superior biocompatibility of thermoplastic scaffolds. Enhanced cell proliferation and strong positive differentiation markers further support the potential of thermoplastic extrusion for bone tissue engineering applications.

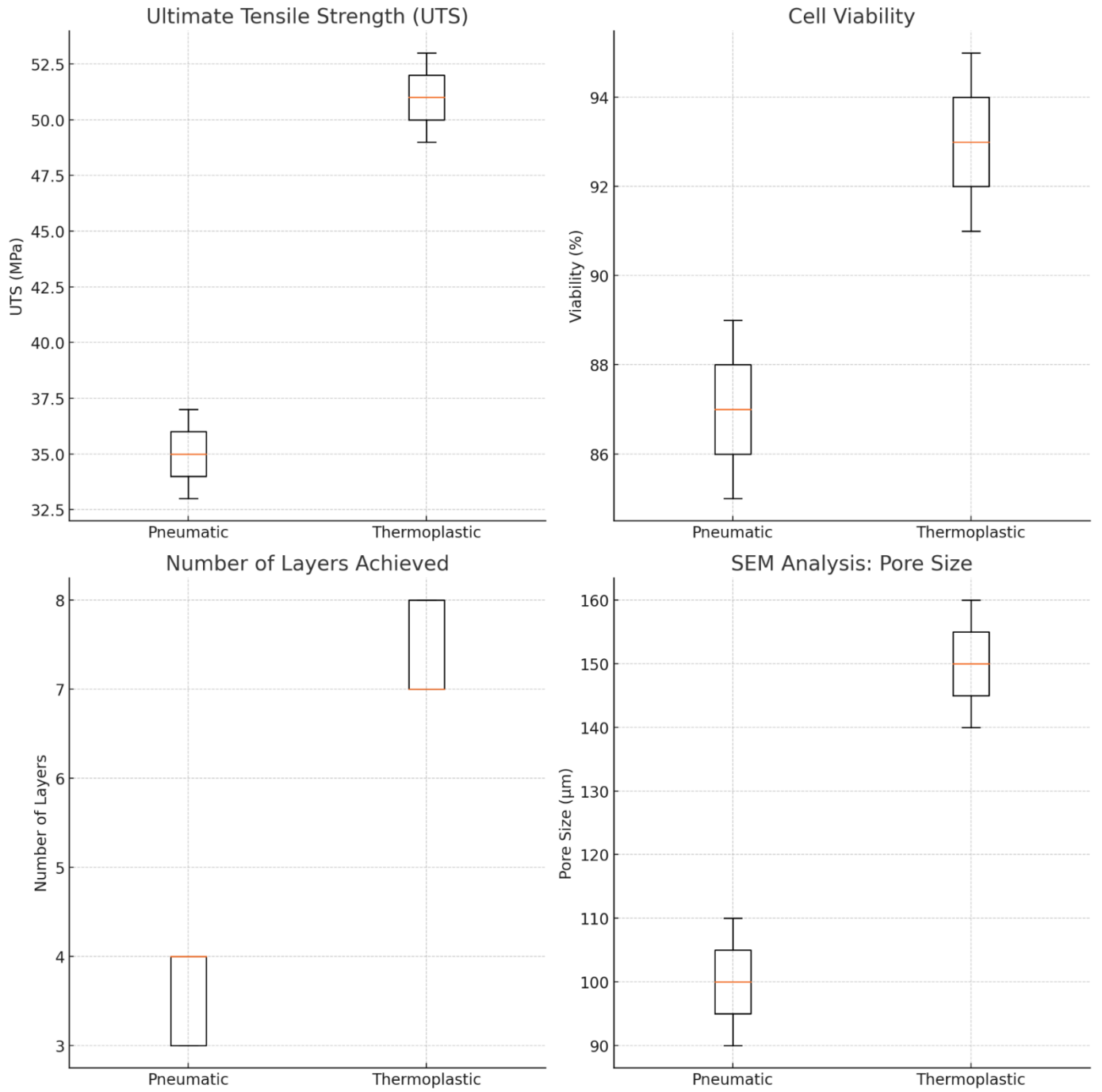


Figure 9: Comparing properties of Pneumatic and Thermoplastic Extrusion

DISCUSSION

The current study systematically optimized the fabrication of beta-tricalcium phosphate (β-TCP) scaffolds using the Cellink BioX printer, equipped with pneumatic and thermoplastic extrusion heads [30]. The integration of advanced bioprinting technology and meticulous parameter optimization has allowed for the production of high-quality scaffolds with desirable physicochemical and biological properties. The inks used for these scaffolds were manufactured at Saveetha Dental College, Chennai, and the scaffold fabrication was conducted at the Indian Institute of Science (IISc), Bangalore, ensuring the collaboration of expertise and state-of-the-art technology.

The findings from the study highlight the significant advantages of thermoplastic extrusion over pneumatic extrusion in terms of scaffold printability, mechanical strength, and biocompatibility. The printability of thermoplastic extrusion was notably higher, achieving an average of 7-8 layers per scaffold, compared to 3-4 layers with pneumatic extrusion. This increased layering capacity is crucial for the structural integrity and complexity of the scaffolds, making thermoplastic extrusion more suitable for fabricating intricate tissue engineering constructs.

The SEM analysis revealed that thermoplastic extrusion produced scaffolds with regular pore structures, moderate surface roughness, and high interconnectivity. These microstructural characteristics are vital for enhancing cell infiltration, nutrient diffusion, and overall tissue integration. In contrast, pneumatic extrusion resulted in irregular pore structures and higher surface roughness, which could potentially hinder cellular activities and scaffold integration.

Mechanical testing further demonstrated the superiority of thermoplastic extrusion, with scaffolds exhibiting a mean ultimate tensile strength (UTS) of 51 MPa, significantly higher than the 35 MPa observed for pneumatic extrusion scaffolds. The statistical significance of this difference (p-value ≈

2.33×10−72.33 \times 10^{-7}2.33×10−7) underscores the robustness of thermoplastic extrusion for producing mechanically resilient scaffolds. This is critical for applications in load-bearing bone tissue engineering, where mechanical strength is paramount.

The future applications of these findings are extensive. The ability to fabricate mechanically robust and highly biocompatible β-TCP scaffolds opens new avenues for bone tissue engineering, particularly in the reconstruction of complex maxillofacial, craniofacial, and periodontal defects [31] [7]. The use of thermoplastic extrusion can be extended to the production of customized bone grafts, tailored to patient-specific anatomical and functional requirements. This personalized approach to tissue engineering holds promise for improving clinical outcomes and reducing recovery times [22].

Additionally, the integration of bioactive molecules and growth factors into the thermoplastic extrusion process could further enhance the regenerative potential of the scaffolds [30] [32] [33]. Future research should focus on the in vivo validation of these scaffolds to evaluate their long-term performance, safety, and efficacy in clinical settings [34]. The scalability of the fabrication process is another critical aspect, necessitating the development of high-throughput bioprinting techniques to meet the demands of clinical applications [35].

The clinical application of β-TCP scaffolds includes the reconstruction of bone defects in maxillofacial and craniofacial surgery, periodontal regeneration, and the repair of trauma-induced bone injuries. Future research should focus on improving the scalability of 3D printing techniques, enhancing the bioactivity of scaffolds through surface modifications, and conducting in vivo studies to validate their long-term performance and safety.

CONCLUSION

In conclusion, the study demonstrates the significant advantages of thermoplastic extrusion over pneumatic extrusion for the fabrication of β-TCP scaffolds. The superior printability, mechanical strength, and biocompatibility of thermoplastic scaffolds underscore their potential for advanced bone tissue engineering applications. Future research should focus on clinical validation and process scalability to fully realize the therapeutic potential of these advanced scaffolds.

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