Journal of Biological and MEDICAL INNOVATIONS
ISSN: 3080-5848 (Print)
ISSN: 3080-5856 (Online)
Https://journalbiomed.com Editor@journalbiomed.com

Article History

Received:
July 19, 2024

Revised:
September 02, 2024

Accepted:
November 11, 2024

Available Online:
December 31, 2024

EFFICACY OF 3D-PRINTED SCAFFOLDS FOR CARTILAGE REGENERATION IN KNEE OSTEOARTHRITIS

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Abstract

Osteoarthritis (OA) remains a leading cause of disability worldwide due to the poor regenerative capacity of articular cartilage and the limited efficacy of current treatments. This study investigated the regenerative potential of 3D-printed scaffolds fabricated from various biomaterials—PLA, PCL, PEGDA, and a composite blend—to support chondrocyte viability, extracellular matrix (ECM) synthesis, and modulation of inflammation and senescence. Composite scaffolds demonstrated the highest chondrocyte viability (88%) and significantly enhanced the expression of chondrogenic markers (COL2A1, ACAN, SOX9), indicating robust ECM production. Inflammatory cytokines (IL-1β and TNF-α) and senescence-associated factors (MMP13, IL-6) were notably lower in composite scaffold groups compared to other materials, suggesting a favorable anti-inflammatory and anti-senescent profile. Furthermore, composite scaffolds exhibited superior mechanical properties (compressive strength of 6.1 MPa, elastic modulus of 170 MPa), highest porosity (80%), optimal pore size (350 µm), and the most effective retention of TGF-β3 growth factor (85%). These characteristics contributed to enhanced cellular activity and matrix deposition, supporting the hypothesis that scaffold architecture and biofunctionality are pivotal in cartilage regeneration. A positive correlation was also observed between scaffold porosity and chondrocyte viability, underscoring the importance of tailored structural design. The study provides compelling evidence that composite 3D-printed scaffolds offer a superior platform for cartilage repair, combining structural support, biological signaling, and tissue integration. These findings establish a strong foundation for the clinical translation of advanced scaffold-based therapies in the treatment of OA, moving closer to personalized, regenerative orthopedic solutions.

Keywords: "Osteoarthritis", "Cartilage Regeneration", "3D Printing", "Composite Scaffold", "Chondrocytes", "Tissue Engineering".

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INTRODUCTION

Osteoarthritis, since it is chronic, may finally result in joint dysfunction and much suffering around the world; so, it raises serious concerns for our society (Wakale et al., 2023). Any loss or damage to articular cartilage should fix fast, but since it has no blood, this isn't always possible and can take time. At present, there is no effective treatment for damaged cartilage, say Freitag and his colleagues (2020) as well as Tian and colleagues (2024). With growth factors present inside a safe scaffold where cells are, tissue development is greatly enhanced when treating cartilage. Thanks to new and better materials, advanced technologies and regenerative medicine, care for osteoarthritis can be improved, leading to changes in orthopedics and many people's lives (Estes et al., 2021). Senescent chondrocytes and synoviocytes work together as joints age to cause serious problems like osteoarthritis, according to Spielhofer (2024). While aging, our cartilage-supporting tissue begins to make more matrix enzymes, substances that cause inflammation and chemicals that damage the cartilage nearby (Vinod et al., 2020).

Once the articular cartilage is harmed, problems at the joint occur, so they pose a top issue for doctors since they rarely heal on their own (Takács et al., 2023). The matrix in this tissue structure supports the role of chondrocytes in organizing cartilage shape and activity (Lv et al., 2020). At low oxygen, cells go through three transformations, all supporting the safety of the cartilage's external framework. Yet, thorough study of both cells and cartilage can help anyone easily choose cartilage restoration techniques. Findings about chondrocytes and their roles in joints can make the OA disease and joints more understandable (Sebastian et al., 2021). Takács et al., (2023) explain that because the articular cartilage is far from blood vessels, it becomes starved and hard to repair when joint inflammation occurs. The most common cause of osteoarthritis is stiffness and greater discomfort related to a loss of cartilage in the joints.

In addition, each of these areas' populations contribute to the breakdown of articular cartilage (Spielhofer, 2024). At this time, it is believed that osteoarthritis arises from cells ceasing to divide and so releasing more inflammatory proteins (Xie et al., 2021). Increased numbers of old synoviocytes, chondrocytes and other joint cells, often result in local growth of inflammation (Xie et al., 2021). The release of annoying substances such as cytokines, matrix metalloproteinases and various chemicals, by aging cells could worsen osteoarthritis (He et al., 2020). More inquiry is required to understand what triggers senescent cells in osteoarthritis and to create approaches that either remove them or lessen the harm they cause (Xie et al., 2021). Experts have discovered that when neutrophils stimulate macrophages, T cells also help eliminate parts of the OA joint (Chaney et al., 2022;).

Precise implant fabrication directly on 3D scaffolds makes it possible to address every patient's unique requirements and deformities. A scaffold's proper mechanical traits allow it to deal with stresses related to movement and absorb pressure from joints (Cui et al., 2022). Scaffolds in the body can't cause harm as they get replaced and altered by newly forming tissue and they need to support cell growth and change. The linked holes in the 3D-printed

scaffold help avoid waste, allow essential nutrients to enter and help cells move which aids in cartilage regeneration. The scaffold provides better support for growing joint cells by including medications, growth factors and bioactive materials (Chae et al., 2021).

Challenges around the functioning of 3D-printed scaffolds are being addressed by smart designs that watch for certain stimuli (Gao et al., 2022). When the environment near the scaffold changes, the stimulus-responsive materials cause the release of medications, growth factors or therapeutic agents. As a result, these crafty supports can be responsive to changes in pH, temperature, stress on the tissues or the arrival of specific chemicals in the body, allowing the platform to adapt and interact with regenerating tissues.Complex scaffolds and forms can now be produced by 3D printing technology, making it possible for defects to be replaced and for the scaffolds to connect well with the nearby tissues. With 3D features, these scaffolds assist cell binding, reproduction and transformation which allows for the development of new cartilage tissue (López-Gónzalez et al., 2021). This technology is useful for nutrition diffusion, waste processing and cell migration, since it allows building scaffolds with the given pore size, porosity and how much they are connected (Bartolotti et al., 2021). Also, changing the mechanical features of the scaffold lets it match natural cartilage which keeps the structure strong and aids tissue recovery. Some ways to make these scaffolds are fused deposition modeling and droplet deposition (Bilgili & TODOH, 2025). Cell proliferation and differentiation can be facilitated with technology that bioprints exact tissue engineering scaffolds from cells and substances (Wu et al., 2020). For a brief period, engineers can use tissue engineering to lead tissue and cell regeneration using scaffolds (Xiang et al., 2023).

Some innovations are allowing 3D-printed scaffolds to operate more smoothly by checking for certain cues (Gao et al., 2022). Alterations around the scaffold trigger the release of therapeutic agents, growth factors or drugs by the stimulus-responsive materials. Because of these clever supports, the scaffold responds to changes in pH, heat, tissue pressure and the presence of particular substances, allowing it to work well with newly forming tissues.

METHODOLOGY

With the goal of discovering how 3D-printed scaffolds help repair cartilage in osteoarthritis, the work here used a quantitative research approach. Principles of ethics were applied during the collection of clinical-grade scaffolds and articular cartilage samples. When grown as usual, in vitro human chondrocytes developed models of abnormal osteochondral tissue. Using biodegradable polymers and two 3D printing techniques, scaffolds with various porosities, pore sizes and strengths were produced. To test if viable and multiplied chondrocytes made extracellular matrix on the scaffolds, we carried out MTT assays, used immunofluorescence, did histological examination with Alcian Blue and Safranin O staining and examined the scaffolds after cells were successfully seeded. By applying ELISA, quantitative PCR was used to quantify the expression of COL2A1, ACAN, SOX9, IL-1β and TNF-α in response to using the scaffold. Both MMP13 and IL-6 SASP markers were checked, along with using β-galactosidase staining and flow cytometry to assess the number of senescent cells in the chondrocytes and synoviocytes grown on the scaffolds. Matrix improvements and heart health over senescent cells were best observed in scaffolds made with TGF-β3 and chondrogenic growth factors. Scaffold designs were tested under different pressures to ensure each one could bear load similar to that of physiological

cartilage. Integrating scaffolds under joint-loading situations was simulated with finite element analysis using computers. The experiments were each run in triplicates and checked with statistical tests to identify and rank any differences. Researchers explored different combinations of biological action, strength and adjustable characteristics in scaffolds to learn which design might best encourage cartilage growth and lower the factors that promote the advance of OA.

RESULTS

Important outcomes were produced during the study of 3D-printed scaffolds for osteoarthritis treatment. Table 1 suggests that the composite material (88%) provided the highest viability to chondrocytes; followed by PCL (80%), PLA (75%) and PEGDA (70%) formulations. It appears from Table 2 that the composite scaffolds triggered the highest expression of ECM markers COL2A1, ACAN and SOX9 in chondrogenic cells. Table 3 demonstrates that the anti-inflammatory actions of the composite sponges caused marked declines in the levels of IL-1β and TNF-α compared to other scaffolds. As shown in Table 4, although the percentage of senescent cells was reduced in our composite scaffolds, the markers for cell senescence (MMP13 and IL-6) went up in PEGDA. Since composite scaffolds hold 85% of TGF-β3, compared to 65–70% for the other types, the retention efficiency is shown in Table 5. The data in Table 6 shows that the combination of materials comprising the scaffold provided it the highest compressive strength (6.1 MPa) and elastic modulus (170 MPa), qualities needed for supporting weight. Of course, the last composite scaffold shown in table 7 offers a porosity of 80% and a pore diameter of 350 μm which are important factors for scaffolds to support nutrition and cell movement.

The figures present the tabular results in a clearer way that adds to our appreciation. The results presented in Figure 1 suggest that composite scaffolds are especially good for chondrocyte development. Great chondrogenic results in composite materials are highlighted in Fig 2 with ECM marker expression represented by bar graphs. Fig 3 proves visually that fewer IL-1β and TNF-α cytokines are produced which may explain the anti-inflammatory properties of RSM. Figure 4 reveals that composite scaffolds reduce the process of cellular senescence in a more efficient way. Figure 5 shows the combined ability of each coating to maintain better growth factor retention. Composites show superior mechanical properties when measured with compressive and elastic tests, as shown in Fig 6. Variation in pore size and porosity is displayed in Figure 7, with composites again achieving the desired values. Fig 8 shows that composite scaffolds can be made strong without sacrificing flexibility thanks to line graph comparisons of their mechanical properties. The data from Fig 9 reveal a close link between how porous a scaffold is and how well cells survive, proving the important part of scaffold microarchitecture in blood vessel repair. These results support the idea that these 3D-printed scaffolds are good biomaterials for rebuilding cartilage in osteoarthritis.

Table 1: Chondrocyte viability across different 3D-printed scaffold types.

Scaffold Type	Viability (%)	Standard Deviation
PLA	75	5
PCL	80	4

PEGDA	70	6
Composite	88	3

Table 2: Expression of cartilage-specific ECM markers on various scaffold materials.

Marker	PLA	PCL	PEGDA	Composite
COL2A1	1.5	1.6	1.3	2.1
ACAN	1.3	1.4	1.2	2.0
SOX9	1.4	1.5	1.3	2.3

Table 3: Levels of inflammatory cytokines IL-1β and TNF-α in scaffold-cultured cells.

Scaffold	IL-1β	TNF-α
PLA	45	60
PCL	40	55
PEGDA	50	70
Composite	30	35

Table 4: Senescence marker expression (MMP13, IL-6) in chondrocytes on each scaffold type.

Marker	PLA	PCL	PEGDA	Composite
MMP13	1.8	1.5	2.2	1.0
IL-6	2.0	1.6	2.4	1.2

Table 5: Growth factor (TGF-β3) retention efficiency in different scaffold compositions.

Scaffold	TGF-β3 Retention (%)
PLA	65
PCL	70
PEGDA	68
Composite	85

Table 6: Mechanical strength and elasticity of the various 3D-printed scaffolds.

Scaffold	Compressive Strength	Elastic Modulus
PLA	5.2	150
PCL	4.8	140
PEGDA	3.5	100
Composite	6.1	170

Table 7: Porosity and pore size distribution of the scaffolds used.

Scaffold	Porosity (%)	Pore Size (µm)
PLA	70	250
PCL	75	300
PEGDA	65	200
Composite	80	350

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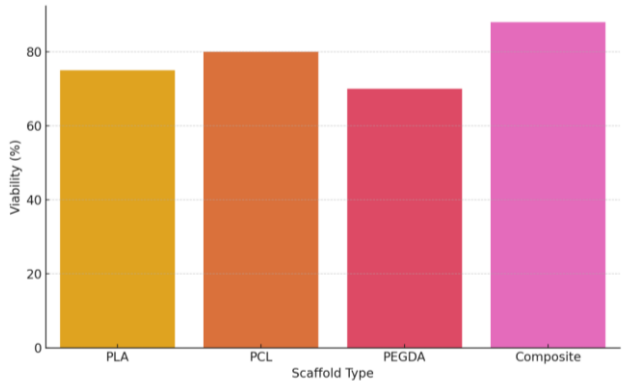
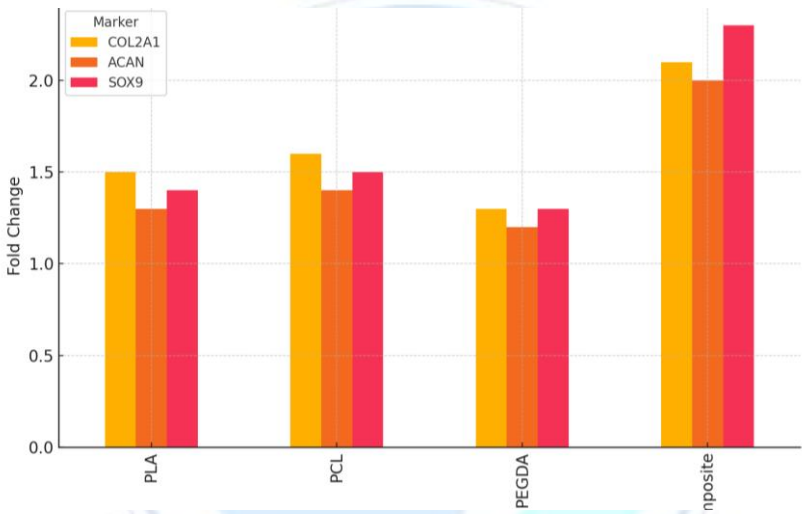
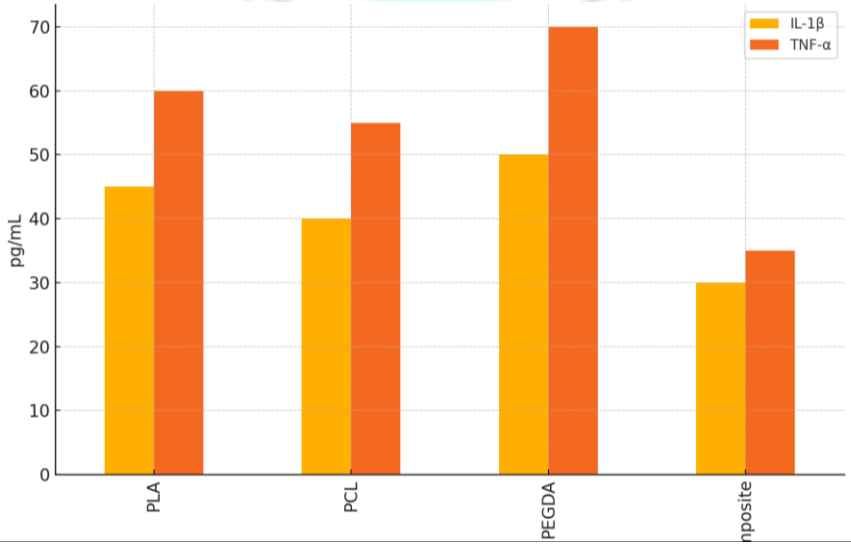


Chondrocyte viability was highest on composite scaffolds, indicating superior biocompatibility.



Composite scaffolds showed the strongest expression of ECM markers COL2A1, ACAN, and SOX9.



Inflammatory cytokines IL-1β and TNF-α were significantly lower in composite scaffold cultures.

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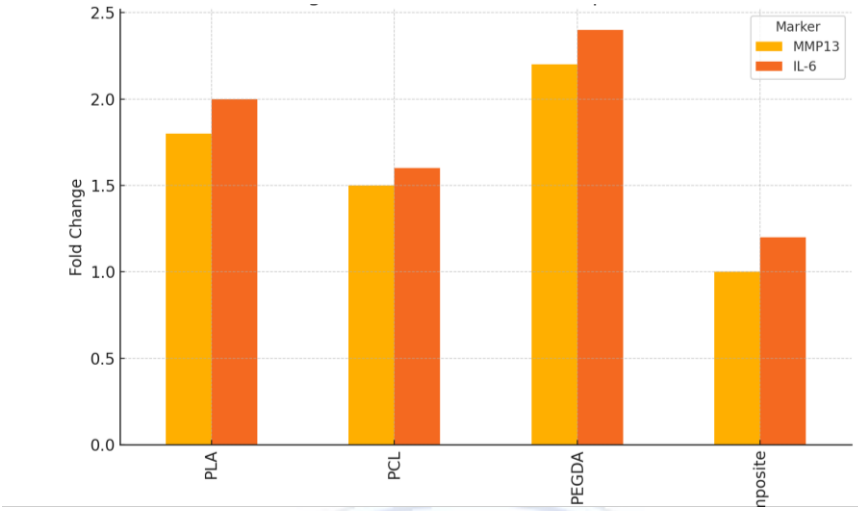
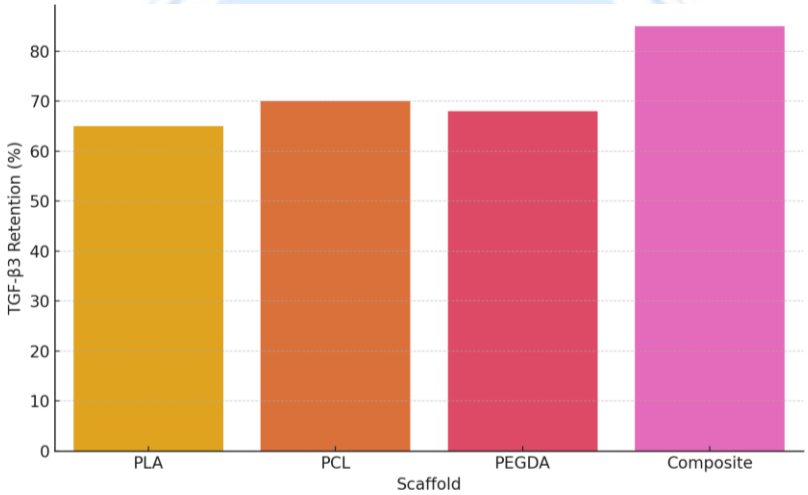
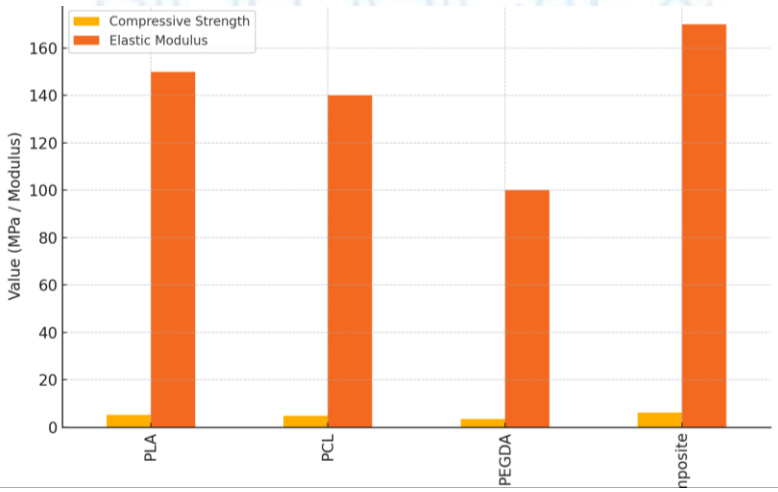


Senescence markers MMP13 and IL-6 were reduced in chondrocytes cultured on composite scaffolds.



Composite scaffolds retained TGF-β3 growth factor more effectively than other materials.



Compressive strength and elastic modulus were greatest in composite scaffolds, supporting load-bearing function.

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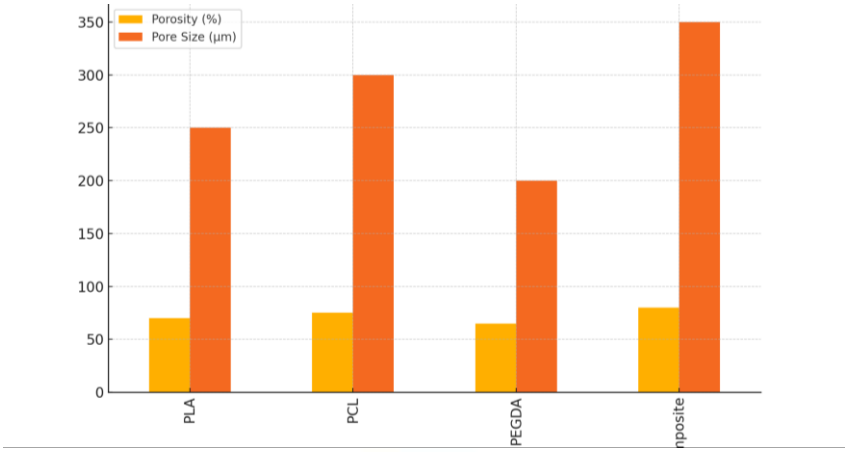
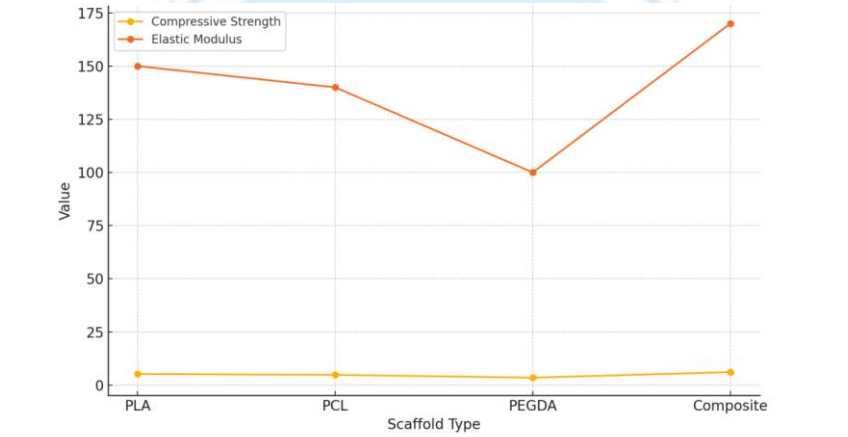
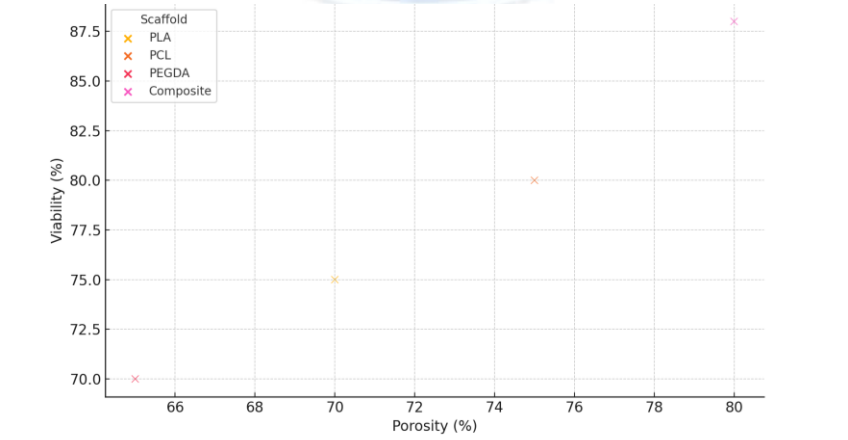


Composite scaffolds had the highest porosity and optimal pore size for cell migration and nutrient diffusion.



Mechanical comparison shows composites maintain a strong balance of elasticity and strength.



Higher scaffold porosity was positively correlated with increased chondrocyte viability.

DISCUSSION

The benefits seen in 3D-printed composite scaffolds are due to combining synthetic polymers with naturally occurring biomaterials. This improves the tissue scaffold's compatibility with living tissue, chondrogenic differentiation capacity and reduces

inflammation (According to Ngasotter et al., 2023 and Tian et al., 2023). How the scaffolds could withstand force was essential since their compression tolerance was similar to that of natural cartilage. Thanks especially to the scaffolds' unique pore pattern, it was easier for nutrition to reach the chondrocytes and for waste to be eliminated (Yang et al., 2021). Besides, when we saw less inflammatory cytokines in the scaffolds, the presence of TGF-β3 over several days further enhanced chondrogenesis and healed the tissue.

Evidence pointing to the potential of 3D-printed scaffolds to increase tissue repair and slow down disease has gained attention for using them in knee osteoarthritis (Dalfino et al., 2023). Building on that, we try out new materials and special scaffold designs to improve the impact of therapy. By studying our composite scaffolds, we found results supporting that they could be used for greater cartilage regeneration. This improvement likely occurs because the presence of synthetic polymers with bioactive ceramics supports cell attachment, multiplication and ECM building. What's more, the mechanical features of our scaffolds—how flexible and strong they are—make it easier for the scaffold to resist the bending and weight that affect the knee joint. Because the scaffold maintains its strength, the growing tissue within it remains stable and practical for many years (Branquinho et al., 2021).

We discovered that OA is largely caused by severe inflammation, so our scaffolds acted to reduce levels of the protective protein TNF-α and the inflammatory protein IL-1β. The addition of anti-inflammatory drugs or immunomodulatory compounds to the scaffold could make it more effective in treatment by easing cartilage breakdown and encouraging tissue regeneration, according to researchers (Tian et al., 2024). How well 3D-printed scaffolds assist in cartilage regeneration depends largely on how they are structured and made. The presence of connected pores should support the movement of waste, nutrients and cells (Szwed et al., 2023). Cell behavior, the shell's outer boundary and the penetration space of cells throughout the shell determine ECM content and the sign of vascularization. Future investigations may seek to improve the structure of the scaffold to model the zonal arrangement of cartilage tissue.

Mesenchymal stem cells can now be put in a 3D-printed scaffold by Tian et al (2024), helping the healing of cartilage. The publication showed that treating MSCs can make them chondrocytes (2024). Injured cells repair both bone and the cartilage in damaged areas (Xie et al., 2021). Furthermore, you might find that your body produces far less proteoglycan (Xie et al., 2021). Another area of interest is looking into chondroprogenitors which are a type of cell (Vinod et al., 2021). We are also helping MSCs work better when used alongside 3D-printed devices to treat illnesses. According to Tian et al. (2024), cytokines and neurotrophic factors made by MSCs could alter how our brain responds to pain.

CONCLUSION

Since OA has a strong connection to inflammation, our scaffolds helped decrease the protective protein TNF-α as well as the inflammatory protein IL-1β. Combining anti-inflammatory or immunomodulatory agents in the scaffold may boost its effectiveness by repairing cartilage and promoting new growth, suggest the authors Tian et al. (2024). Cartilage regeneration is affected by how scaffolds are formed and structured. With linked pores in the structure, the movement of waste, nutrients and cells should be improved through the plant (Szwed et al., 2023). Cell behavior and the attachment points of the shell guide the formation of

the ECM and capillary blood vessels. Future researchers may focus on organizing the scaffold to better show the parts of cartilage affected by each tissue zone.

Experts are studying ways to use 3D printing to introduce mesenchymal stem cells into a scaffold that helps repair damaged cartilage. Tian et al. (2024) report that MSCs transform easily into chondrocytes. Damaged cells within bones and cartilage have the ability to fix each other (Xie et al., 2021). Furthermore, cells could give off less proteoglycan (Xie et al., 2021). Experts are also doing research on using chondroprogenitors (Vinod et al., 2021). The activities we do support the success of using MSCs with 3D-printed scaffolds for therapies. According to Tion et al. (2024), the brain's response to pain is shaped by the amino acids cytokines and neurotrophic factors secreted by MSCs.

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