doi.org/10.36721/PJPS.2025.38.6.REG.14584.1

Effects of PCL/PLA/HA nanocarrier on apoptosis, growth and functional metabolism of osteoblasts

Zhiwen He[1], Shuang Wang[2]* and Xiaoxuan Xia[2]*

[1]Department of Rehabilitation, Wuhan Fourth Hospital, Wuhan, Hubei, China
[2]Department of Rehabilitation Medicine, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Abstract: Bone tissue engineering has emerged as a key approach to address orthopedic disorders. This study aimed to evaluate the effects of a composite nanofiber scaffold composed of polycaprolactone (PCL), polylactic acid (PLA) and hydroxyapatite (HA) on osteoblast viability, apoptosis and functional metabolism. Electrospun scaffolds of PCL/PLA, PCL/HA and PCL/PLA/HA were fabricated and analyzed by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Cell proliferation was assessed via MTT assay, apoptosis through flow cytometry and alkaline phosphatase (ALP) activity via ATP assay. The PCL/PLA/HA scaffold exhibited smaller fiber diameters and promoted more prominent cell extensions compared to other groups. FTIR analysis confirmed component integration, with specific PCL peaks disappearing after composite formation. The PCL/PLA/HA scaffold significantly enhanced osteoblast proliferation and ALP expression ($P<0.05$) while reducing apoptosis rate. The PCL/PLA/HA composite nanoscaffold improves osteoblast function by promoting proliferation and metabolic activity while minimizing apoptosis. These findings support its potential application in bone tissue engineering.

Keywords: Apoptosis; Functional metabolism; Osteoblasts; Growth; PCL/PLA/HA nano

Submitted on 16-05-2025 – Revised on 01-07-2025– Accepted on 31-07-2025

INTRODUCTION

Bone defects, arising from congenital malformations, trauma, infection, or tumor resection, are among the most prevalent conditions in orthopedic practice (Wan, Qin *et al*., 2021). Clinically, autologous and allogeneic bone grafts remain the standard treatment modalities(Wang, Liu *et al*., 2024). While autologous grafting offers superior biocompatibility and osteogenic potential without immune rejection, it is limited by donor site morbidity and insufficient bone volume (Feng, Zhu *et al*., 2021). Allogeneic grafts, though readily available, are associated with immunogenic responses and increased infection risk (Smeets, Matthies *et al*., 2022). These limitations have driven the development of alternative bone scaffold materials that can support bone regeneration while minimizing complications (Yuan, Zhu *et al*., 2024).

Bone regeneration is a tightly regulated process involving three primary cell types: osteoblasts, osteoclasts and osteocytes (Yudoh, Sugishita *et al*., 2023). Bone remodeling relies on the coordinated activity of osteoclast-mediated bone resorption and osteoblast-driven bone formation (Xu, Yu *et al*., 2023). Osteoblast differentiation and survival are critical in maintaining skeletal homeostasis. Disruption of this balance-such as excessive apoptosis of osteoblasts-can impair bone formation and ultimately lead to skeletal deterioration (Ponzetti and Rucci, 2021).

Recent advances in nanotechnology have led to the design of nanocarrier-based scaffolds with enhanced bioactivity and biodegradability (Piszko, Piszko *et al*., 2024). Among these, composite materials combining polycaprolactone (PCL), polylactic acid (PLA) and hydroxyapatite (HA) have shown promising properties for bone tissue engineering (Shu, Zhang *et al*., 2023). Hydroxyapatite, a principal inorganic component of natural bone, offers excellent osteoconductivity and biocompatibility(Ran, Liu *et al*., 2023). However, its brittleness and poor degradability limit its standalone application. Polymers like PCL and PLA are commonly co-electrospun with HA to improve mechanical integrity and degradation profiles (Ianhez, de Goés *et al*., 2024). PCL is a slow-degrading, hydrophobic polymer with excellent biocompatibility, while PLA offers faster degradation and better mechanical strength. However, each material used independently falls short in fully promoting osteoblast proliferation and differentiation. Studies suggest that their combination may synergistically enhance scaffold functionality, but research on PCL/PLA/HA ternary nanocomposites remains scarce (Fang, Zhang *et al*., 2010). Therefore, understanding the biological performance of PCL/PLA/HA scaffolds-particularly their influence on osteoblast apoptosis, proliferation and metabolic activity-is essential.

In this study, we fabricated electrospun PCL/PLA/HA nanocomposite scaffolds and investigated their effects on osteoblast function. By assessing apoptosis rates, cell proliferation and alkaline phosphatase activity, we aimed to evaluate the scaffold's potential as a candidate material

**Corresponding author:* e-mail: duvdd4931@163.com

for bone regeneration and provide a theoretical foundation for its clinical translation in bone defect repair.

MATERIALS AND METHODS

Instruments and equipment

Electrospinning apparatus (Qianyun Biotech, China), scanning electron microscope (SEM; BY-3000, Nanjing Beiyu, China), microplate reader (Boqi Instruments, Hong Kong) and Fourier-transform infrared spectrometer (FTIR; Thermo Scientific Nicolet iS10, USA) were used throughout the study.

Materials and reagents

Polycaprolactone (PCL, Mw = 80,000 Da) was purchased from Shanghai Fantai Chemical Co., Ltd., China. Polylactic acid (PLA, Mw = 70,000 Da) was obtained from NatureWorks LLC, USA. Hydroxyapatite (HA, particle size <100 nm, purity >99%) was supplied by Beijing Ita Biotechnology Co., Ltd., China. MTT reagent and alkaline phosphatase (ALP) assay kits were procured from Shanghai Xinfan Biotech. Osteoblasts were purchased from ALLCELLS Co., Ltd., Shanghai, China.

Preparation of nanofiber scaffolds

Nanofiber scaffolds were fabricated by electrospinning, based on modified protocols from previous studies (Hamaya, Fujisawa *et al*., 2019, Li, Zhang *et al*., 2020, Ou, Wang *et al*., 2021, Sun, Deng *et al*., 2013).

(1) PCL/PLA group

0.5 g of PCL was dissolved in 10 mL trifluoroethanol (TFE). After 48 hours, 0.5 g of PLA was added. The mixture was sonicated for 1 hour and stirred for 12 hours to achieve homogeneity. Electrospinning was performed at 18 kV, with a feeding rate of 0.5 mL/h and a collector distance of 15 cm. The resulting nanofibers were vacuum freeze-dried for 7 days.

(2) PCL/HA group

Prepared similarly by replacing PLA with HA (0.5 g), sonicated in TFE for 1 hour before spinning.

(3) PCL/PLA/HA group

Equal masses (1:1:1, w/w) of PCL, PLA and HA were combined. HA was ultrasonicated for 1 hour in TFE to obtain a suspension. It was mixed with the PCL/PLA solution and stirred for 24 h. The solution was electrospun under the same parameters and freeze-dried for 7 days.

Scaffold characterization and cell experiments

Scanning electron microscopy (SEM)

Scaffold morphology and cell attachment were observed using SEM. Samples were sputter-coated with gold prior to imaging. Osteoblast-seeded scaffolds were fixed in 2.5% glutaraldehyde for 6 hours, dehydrated in graded ethanol, freeze-dried, coated and imaged at 15 kV acceleration voltage (Jiang, Zhan *et al*., 2024).

Fourier-transform infrared spectroscopy (FTIR)

Chemical composition and functional groups were analyzed using FTIR (resolution 4 cm^{-1}, range 400-4000 cm^{-1}). Characteristic peaks of PCL, PLA and HA were identified and compared across groups (Lodoso-Torrecilla, Konka *et al*., 2024).

Cell proliferation (MTT assay)

Osteoblasts (5×10^4 cells/mL) were seeded onto the scaffolds in 48-well plates and incubated at 37°C with 5% CO_2. After 3, 7 and 10 days, 50 µL of MTT (5 mg/mL) was added and incubated for 4 h. Supernatants were discarded and 150 µL of DMSO was added. After 12 h of incubation, absorbance was measured at 570 nm and 630 nm using a microplate reader (Liu, Ma *et al*., 2022).

Osteogenic differentiation (ALP activity assay)

Cells were seeded and cultured under osteogenic induction medium (supplemented with dexamethasone, β-glycerophosphate and ascorbic acid) for 10 days, with medium changes every 2 days. ALP activity was quantified using a commercial kit following cell lysis and ultrasonic disruption. Results were normalized to protein content.

Apoptosis detection by flow cytometry

Cells cultured on different nanofiber scaffolds were trypsinized, centrifuged at 2000 rpm for 5 min, washed three times with PBS and resuspended in binding buffer. The cells were then stained with FITC-conjugated Annexin V and propidium iodide (PI) according to the manufacturer's protocol and analyzed by flow cytometry (BD FACSCalibur, USA). Based on Annexin V and PI staining, cell populations were classified into four quadrants: Q1 (Annexin V^-/PI^+, necrotic cells), Q2 (Annexin V^+/PI^+, late apoptotic cells), Q3 (Annexin V^+/PI^-, early apoptotic cells) and Q4 (Annexin V^-/PI^-, viable/live cells).

Statistical analysis

Statistical analysis was performed using SPSS v23.0 (IBM Corp., USA). All results are expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Scanning electron microscopy (SEM) analysis

As shown in fig. 1, the PCL/PLA nanofiber scaffold exhibited a smooth and uniform surface morphology, whereas the PCL/HA and PCL/PLA/HA scaffolds displayed rougher surfaces. This roughness is likely due to the homogeneous dispersion of HA nanoparticles within or on the surface of the polymer fibers.

In addition, the average fiber diameters of the scaffolds decreased in the order of PCL/PLA > PCL/HA >

PCL/PLA/HA. After 1 day of cell culture, osteoblasts began to adhere, extend and spread on all scaffolds. Notably, after 15 days, the PCL/PLA/HA scaffold showed the most extensive cellular filopodia and protrusions, indicating enhanced cell-scaffold interaction compared to the PCL/HA and PCL/PLA scaffolds (Fig. 2).

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra (Fig. 3) of all three scaffold types exhibited characteristic absorption peaks, with the intensity and position of peaks varying according to the component ratios. The disappearance of PCL-associated peaks at 1238 and 1297 cm^{-1} in the PCL/PLA/HA composite indicates a reduction in crystallinity following the incorporation of PLA and HA, which may enhance scaffold bioactivity and cell affinity.

Osteoblast proliferation assay

As shown in fig. 4, the MTT assay revealed a time-dependent increase in cell proliferation across all scaffold groups. Among them, the PCL/PLA/HA group exhibited a significantly higher proliferation rate compared to PCL/PLA and PCL/HA scaffolds at corresponding time points ($P<0.05$), suggesting superior support for osteoblast growth.

Osteoblast differentiation assay

ATP analysis of ALP expression is presented in fig. 5. The PCL/HA scaffold showed significantly higher ALP activity than the PCL/PLA group ($P<0.05$), indicating improved osteogenic potential. Furthermore, the PCL/PLA/HA scaffold exhibited the highest ALP expression among the three groups, significantly exceeding that of the PCL/HA scaffold ($P<0.05$), thereby confirming its enhanced osteoinductive capability.

Osteoblast apoptosis analysis

Fig 6 show the apoptosis rates at 24 and 48 hours. There were significant differences ($P<0.05$) among the three groups at different time points. And the PCL/PLA/HA scaffold demonstrated the lowest apoptosis rate among the three, which was significantly lower than those of the PCL/PLA and PCL/HA scaffolds ($P<0.05$), suggesting that the ternary composite more effectively supports cell survival and reduces apoptotic events.

DISCUSSION

Bone defect is a common clinical condition. In recent years, lifestyle changes and aging populations have led to an increasing incidence of bone defects. However, traditional treatments, such as autografts or allografts, present limitations including donor site morbidity and immune rejection. Bone tissue engineering, therefore, has become an important alternative approach for regenerating bone tissue (Junka, Quevada *et al*., 2020, Salifu, Obayemi *et al*., 2020). Among the various biomaterials explored, nanomaterials are particularly attractive due to their large surface area and favorable bioactivity. Osteoblasts play a critical role in bone regeneration and their behavior on scaffolds is essential for evaluating the success of bone substitutes. This study investigates the effects of a PCL/PLA/HA composite nanofiber scaffold on osteoblast proliferation, apoptosis and functional metabolism.

SEM observations revealed that the average fiber diameter decreased sequentially across the PCL/PLA, PCL/HA and PCL/PLA/HA scaffolds, which is favorable for cell adhesion due to increased surface area. The PCL/PLA/HA scaffold showed the most pronounced cellular filopodia and extensions after 15 days of culture, suggesting a higher degree of cell spreading and interaction with the scaffold. This aligns with previous findings that nanostructured scaffolds, particularly those incorporating hydroxyapatite, enhance osteoblast adhesion and proliferation (Li, Fu *et al*., 2022).

FTIR analysis showed the disappearance of PCL peaks at 1238 and 1297 cm^{-1} after the addition of PLA and HA, indicating reduced crystallinity. Lower crystallinity has been associated with improved cell attachment and proliferation due to a more amorphous structure (Radwan-Pragłowska, Janus *et al*., 2020). This supports the hypothesis that the PCL/PLA/HA composite exhibits improved osteoconductive potential. Similar observations were reported by (Radwan-Pragłowska, Janus *et al*., 2020), where reduced crystallinity in PLA-based scaffolds led to better osteoblast response.

MTT assay results showed significantly higher osteoblast proliferation on the PCL/PLA/HA scaffold, with absorbance values indicating greater metabolic activity over time. This suggests that the scaffold composition and nanoscale morphology promote cellular activity. The range of MTT values obtained in our study (absorbance: ~0.65-0.89 at 48h) is consistent with previous reports using PLA/HA or collagen/HA scaffolds (Jia, Ma *et al*., 2023).

ALP expression was highest in the PCL/PLA/HA group, demonstrating superior osteoinductivity. Higher ALP activity typically correlates with early-stage osteogenic differentiation, which supports the role of HA and PLA in promoting osteoblast maturation. Our results align with studies showing that scaffolds composed of HA and biocompatible polymers can significantly upregulate osteogenic markers (Zhang, Chen *et al*., 2022).

Flow cytometry results showed minimal apoptosis across all scaffold groups, with the PCL/PLA/HA scaffold yielding the lowest apoptosis rates (~4.5% at 48h), indicating better cytocompatibility. These values are within the expected range for non-toxic biomaterials and are comparable to previously reported systems such as collagen/HA and traditional Chinese medicine-nHA composites (Tihăuan, Pircalabioru *et al*., 2022).

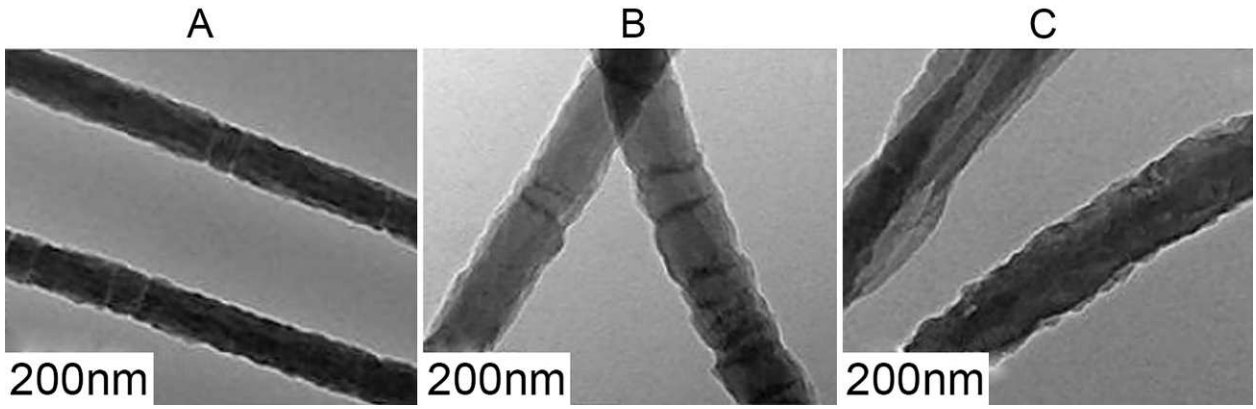

Fig. 1: SEM image of electro spun nanofibers: (a) PCL/PLA, diameter: 280 nm; (b) PCL/HA, diameter: 212 nm; (c) PCL/PLA/HA, diameter: 177 nm.

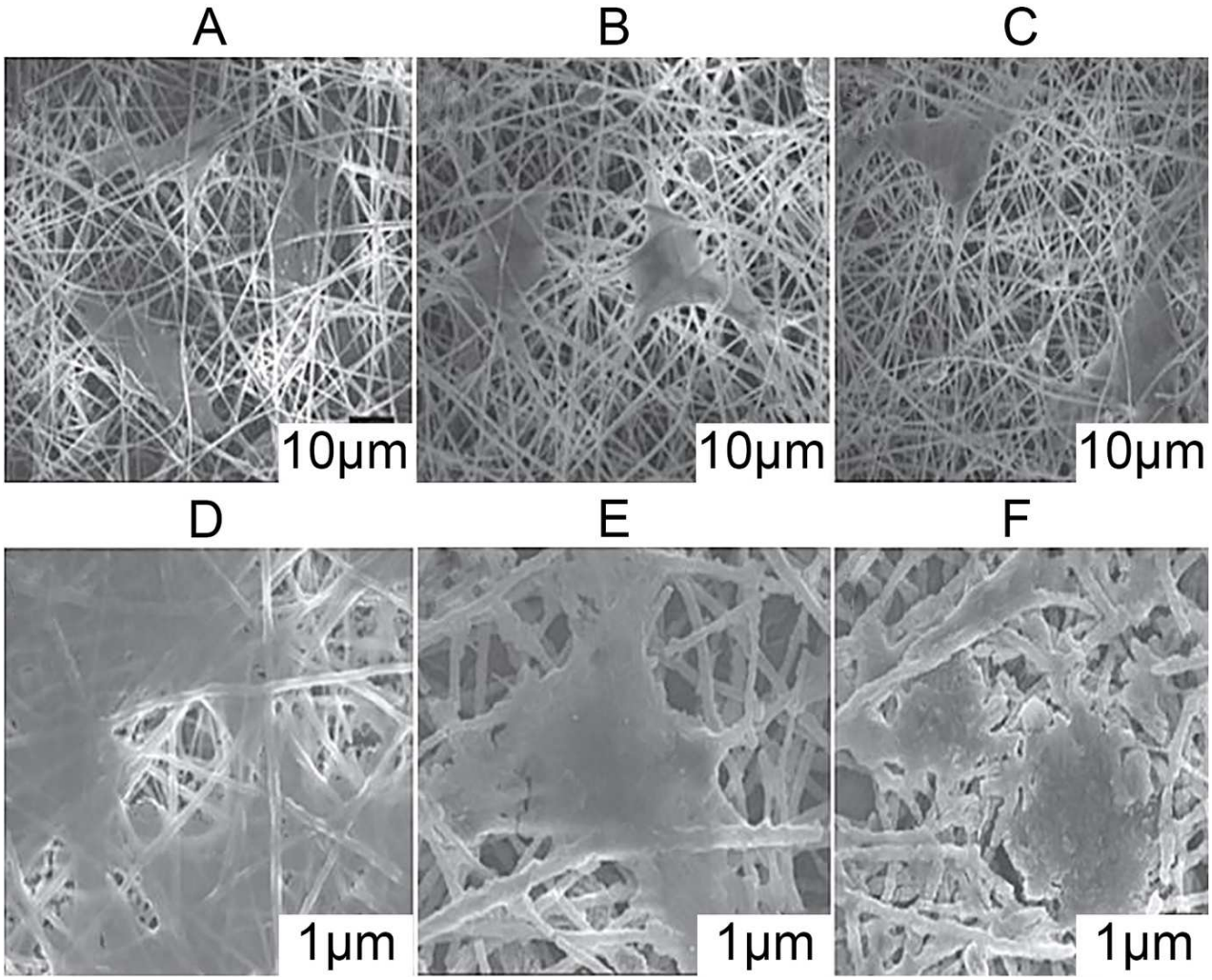

Fig. 2: SEM images of osteoblasts cultured on different scaffolds for 1 day and 15 days (a, d) PCL / PLA (b, e) PCL / HA (c, f) PCL / PLA / HA.

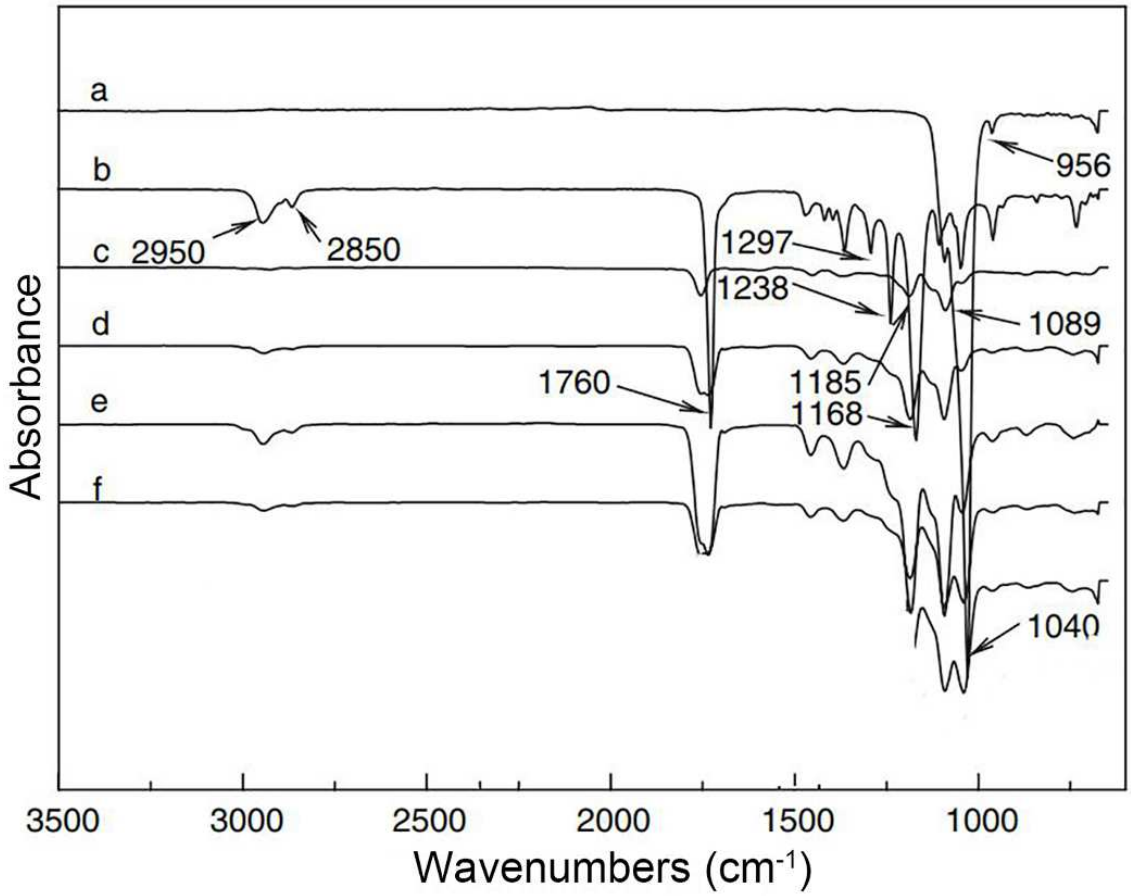

Fig. 3: FTIR: (a) HA (b) PCL (c) PLA (d) PCL / PLA (e) PCL / HA (f) PCL / PLA / HA.

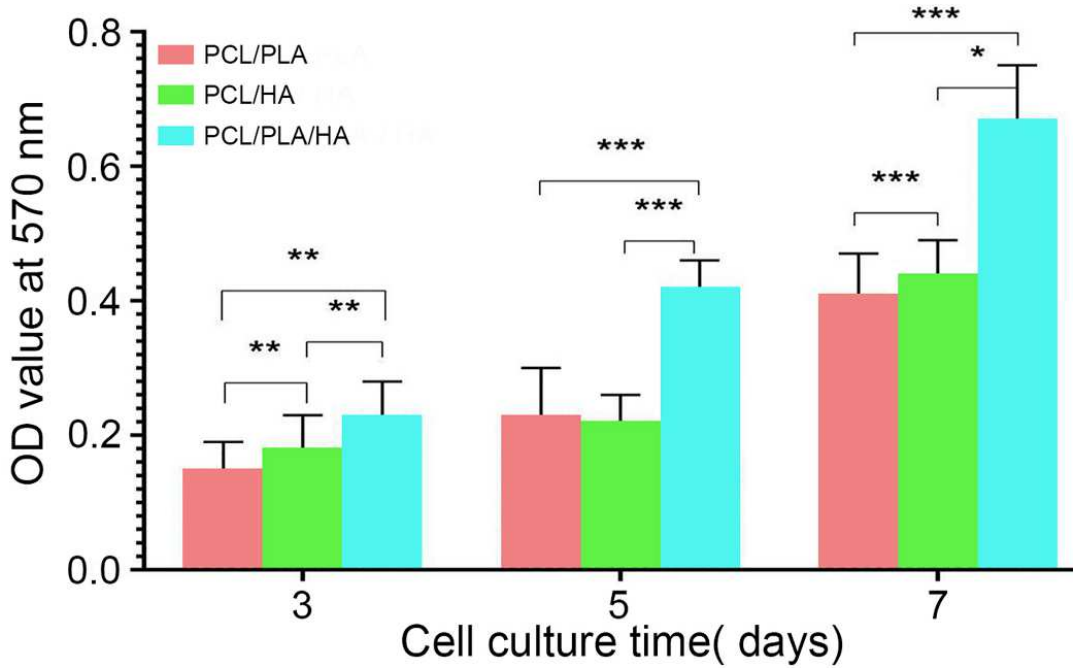

Fig. 4: MTT results of osteoblasts in the three groups for 3, 5, and 7 days, * is P<0.05.

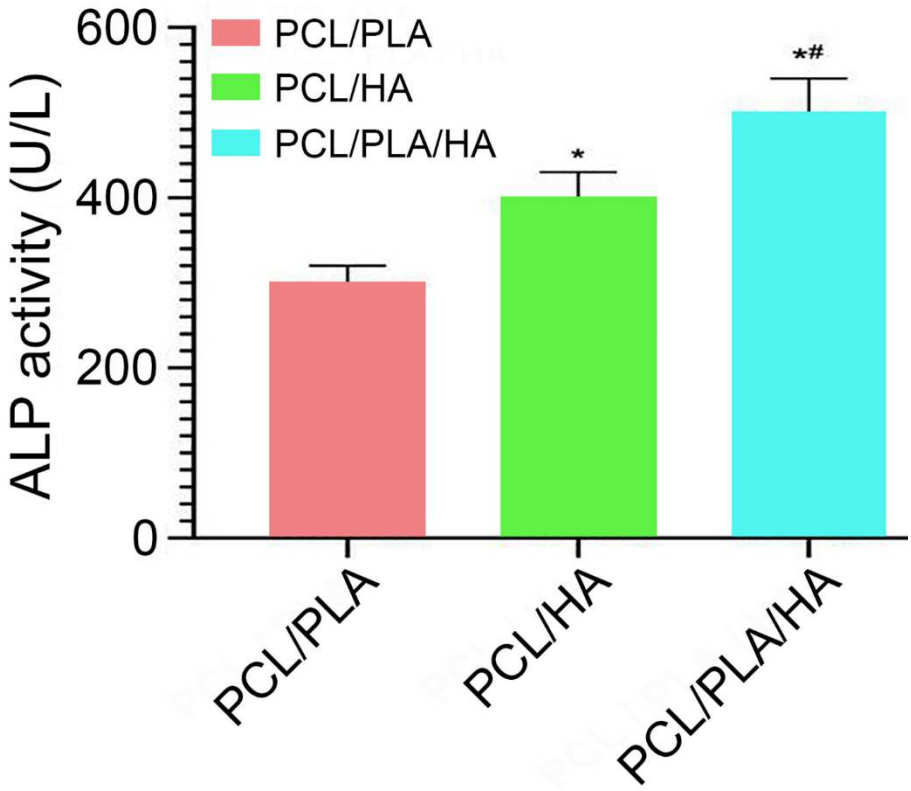

Fig. 5: The effect of three kinds of nanofiber scaffolds on the viability of osteoblast ALP, * is P<0.05, # is P<0.05.

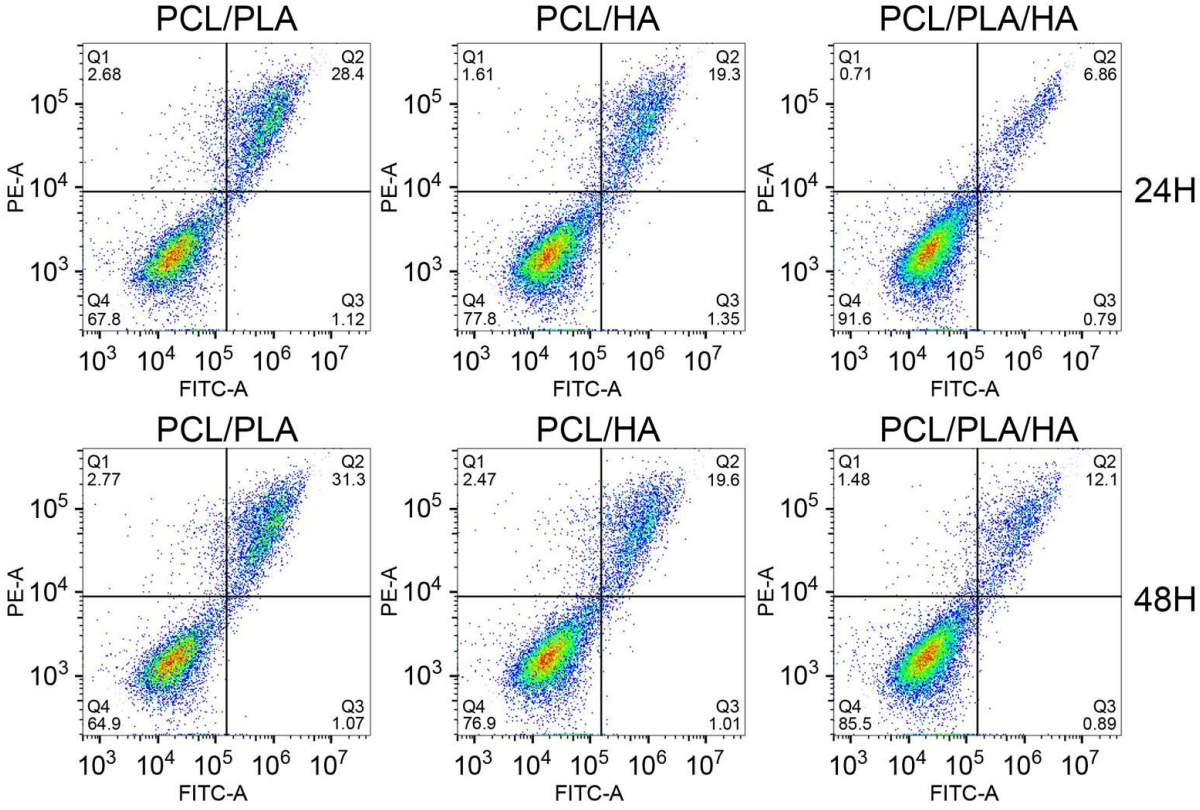

Fig. 6: Flow cytometric diagram of apoptosis of three groups of cells at 24h and 48h.

While this study demonstrates the superior performance of the PCL/PLA/HA scaffold in vitro, we acknowledge some limitations. Quantitative assessments such as mechanical strength, contact angle, pH variation and *in vitro* degradation profiles were not performed and will be considered in future studies. Moreover, the current investigation is limited to short-term *in vitro* testing with a modest sample size and no *in vivo* validation was conducted. These factors will be addressed in subsequent research.

CONCLUSION

In summary, the PCL/PLA/HA composite nanofiber scaffold exhibited excellent biocompatibility, enhanced osteoblast proliferation, reduced apoptosis and promoted early osteogenic differentiation. Compared to binary systems (PCL/PLA or PCL/HA), the ternary composite offered superior performance in supporting osteoblast growth and function. This suggests that the synergistic effects of combining three biomaterials-PCL for structural support, PLA for biocompatibility and HA for osteoconductivity-can provide a more favorable environment for bone tissue regeneration.

While most prior studies have focused on binary composites, our research adds to the relatively limited body of work on ternary nanocomposites. Notably, our findings are consistent with previous reports such as (Fang, Zhang *et al*., 2010) and (Firoozabadi and Ramazani S.A *et al*., 2023), which also demonstrated the benefits of PCL/PLA/HA scaffolds in supporting osteoblast activity. By extending these findings, our study highlights the potential of such composites in bone tissue engineering applications.

However, this study has some limitations. No mechanical, degradation, or pH change analyses were conducted; the sample size and culture duration were also limited. Future studies will address these aspects and include *in vivo* experiments to further validate the scaffold's performance. Nonetheless, our results provide a solid foundation for future exploration of PCL/PLA/HA composites as promising candidates for bone defect repair.

Acknowledgment
We gratefully acknowledge the Union Hospital of Tongji Medical College, Huazhong University of Science and Technology for providing the necessary equipment for this study.

Author's contribution
Zhiwen He and Shuang Wang contributed equally to the experimental design, data collection and analysis, and participated in the drafting of the manuscript. Xiaoxuan Xia was responsible for research supervision, manuscript revision, and final approval. All authors have read and approved the final manuscript.

Funding
There was no funding.

Data availability statement
All data generated or analyzed during this study are included in this article.

Ethical approval
This study was approved by the Ethical Committee of Wuhan Fourth Hospital (Ethical approval NO: KY2021-068-02).

Conflict of interest
There are no conflicts to declare.

REFERENCES

Fang R, Zhang E, Xu L and Wei S (2010). Electrospun PCL/PLA/HA based nanofibers as scaffold for osteoblast-like cells. *J. Nanosci. Nanotechnol.,* **10**(11): 7747-7751.

Feng Y, Zhu S, Mei D, Li J, Zhang J, Yang S and Guan S (2021). Application of 3D printing technology in bone tissue engineering: A review. *Curr. Drug Deliv.,* **18**(7): 847-861.

Firoozabadi F, Ramazani SAA and Asefnejad A (2023). Improving cell proliferation using polylactic acid, polycaprolactone, hydroxyapatite and zinc oxide nanocomposite for cancellous bone substitutes. *Polym.-Plast. Technol. Mater.,* **63**: 1-25.

Hamaya E, Fujisawa T and Tamura M (2019). Osteoadherin serves roles in the regulation of apoptosis and growth in MC3T3-E1 osteoblast cells. *Int. J. Mol. Med.,* **44**(6): 2336-2344.

Ianhez M, de Goés ESFG, Sigrist RMS, Colpas PT, de Faria IA, Parada M and Miot HA (2024). Complications of collagen biostimulators in Brazil: Description of products, treatments and evolution of 55 cases. *J. Cosmet. Dermatol.,* **23**(9): 2829-2835.

Jia Z, Ma H, Liu J, Yan X, Liu T, Cheng YY, Li X, Wu S, Zhang J and Song K (2023). Preparation and Characterization of polylactic acid/nano hydroxyapatite/nano hydroxyapatite/human acellular amniotic membrane (PLA/nHAp/HAAM) hybrid scaffold for bone tissue defect repair. *Materials,* **16**(5): 1937.

Jiang W, Zhan Y, Zhang Y, Sun D, Zhang G, Wang Z, Chen L and Sun J (2024). Synergistic large segmental bone repair by 3D printed bionic scaffolds and engineered ADSC nanovesicles: Towards an optimized regenerative microenvironment. *Biomaterials,* **308**: 122566.

Junka R, Quevada K and Yu X (2020). Acellular polycaprolactone scaffolds laden with fibroblast/endothelial cell-derived extracellular matrix for bone regeneration. *J. Biomed. Mater. Res. A.,* **108**(2): 351-364.

Li L, Zhang J and Akimenko MA (2020). Inhibition of mmp13a during zebrafish fin regeneration disrupts fin growth, osteoblasts differentiation and Laminin organization. *Dev. Dyn.,* **249**(2): 187-198.

Li Y, Fu Y, Zhang H, Wang X, Chen T, Wu Y, Xu X, Yang S, Ji P and Song J (2022). Natural plant tissue with bioinspired nano amyloid and hydroxyapatite as green scaffolds for bone regeneration. *Adv. Healthc. Mater,* **11**(12): e2102807.

Liu J, Ma L, Dong W, Du G and Dang X (2022). Effect of urolithin a on bone repair in mice with bone defects. *Tissue. Eng. Regen. Med.,* **19**(1): 151-159.

Lodoso-Torrecilla I, Konka J, Kreuzer M, Jimenez-Pique E, Espanol M and Ginebra MP (2024). Quality assessment of regenerated bone in intraosseous and intramuscular scaffolds by spectroscopy and nanoindentation. *Biomater Adv.,* **164**: 213982.

Ou L, Wang H, Wu Z, Wang P, Yang L, Li X, Sun K, Zhu X and Zhang R (2021). Effects of cadmium on osteoblast cell line: Exportin 1 accumulation, p-JNK activation, DNA damage and cell apoptosis. *Ecotoxicol. Environ. Saf.,* **208**: 111668.

Piszko PJ, Piszko A, Kiryk S, Kiryk J, Horodniczy T, Struzik N, Wiśniewska K, Matys J and Dobrzyński M (2024). Bone regeneration capabilities of scaffolds containing chitosan and nanometric hydroxyapatite-systematic review based on *in vivo* examinations. *Biomimetics,* **9**(8): 503.

Ponzetti M and Rucci N (2021). Osteoblast differentiation and signaling: Established concepts and emerging Topics. *Int. J. Mol. Sci.,* **22**(13): 6651.

Radwan-Pragłowska J, Janus L, Piątkowski M, Bogdał D and Matysek D (2020). 3D hierarchical, nanostructured chitosan/PLA/HA scaffolds doped with TiO(2)/Au/Pt NPs with tunable properties for guided bone tissue engineering. *Polymers,* **12**(4): 792.

Ran L, Liu L, Gao J, Pan Y, Ramalingam M, Du X, Liu Y, Cheng L and Shi Z (2023). Strontium-doped hydroxyapatite and its role in osteogenesis and angiogenesis. *Int. J. Dev. Biol.,* **67**(4): 137-146.

Salifu AA, Obayemi JD, Uzonwanne VO and Soboyejo WO (2020). Mechanical stimulation improves osteogenesis and the mechanical properties of osteoblast-laden RGD-functionalized polycaprolactone/ hydroxyapatite scaffolds. *J. Biomed. Mater. Res. A.,* **108**(12): 2421-2434.

Shu Z, Zhang C, Yan L, Lei H, Peng C, Liu S, Fan L and Chu Y (2023). Antibacterial and osteoconductive polycaprolactone/polylactic acid/nano-hydroxyapatite/Cu@ZIF-8 GBR membrane with asymmetric porous structure. *Int. J. Biol. Macromol.,* **224**: 1040-1051.

Smeets R, Matthies L, Windisch P, Gosau M, Jung R, Brodala N, Stefanini M, Kleinheinz J, Payer M, Henningsen A, Al-Nawas B and Knipfer C (2022). Horizontal augmentation techniques in the mandible: A systematic review. *Int. J. Implant. Dent.,* **8**(1): 23.

Sun Y, Deng Y, Ye Z, Liang S, Tang Z and Wei S (2013). Peptide decorated nano-hydroxyapatite with enhanced bioactivity and osteogenic differentiation via polydopamine coating. *Colloids Surf B. Biointerfaces,* **111**: 107-116.

Tihauan BM, Pircalabioru GG, Axinie Bucos M, Marinaș IC, Nicoara AC, Maruțescu L, Oprea O, Matei E and Maier SS (2022). Crosslinked collagenic scaffold behavior evaluation by physico-chemical, mechanical and biological assessments in an *In vitro* Microenvironment. *Polymers,* **14**(12): 2430.

Wan QQ, Qin WP, Ma YX, Shen MJ, Li J, Zhang ZB, Chen JH, Tay FR, Niu LN and Jiao K (2021). Crosstalk between Bone and Nerves within Bone. *Adv. Sci.* **8**(7): 2003390.

Wang J, Liu M, Yang C, Pan Y, Ji S, Han N and Sun G (2024). Biomaterials for bone defect repair: Types, mechanisms and effects. *Int. J. Artif. Organs.,* **47**(2): 75-84.

Xu J, Yu L, Liu F, Wan L and Deng Z (2023). The effect of cytokines on osteoblasts and osteoclasts in bone remodeling in osteoporosis: A review. *Front. Immunol.,* **14**: 1222129.

Yuan X, Zhu W, Yang Z, He N, Chen F, Han X and Zhou K (2024). Recent advances in 3D printing of smart scaffolds for bone tissue engineering and regeneration. *Adv. Mater,* **36**(34): e2403641.

Yudoh K, Sugishita Y and Suzuki-Takahashi Y (2023). Bone development and regeneration 2.0. *Int. J. Mol. Sci.,* **24**(10): 8761.

Zhang X, Chen JL, Xing F and Duan X (2022). Three-dimensional printed polylactic acid and hydroxyapatite composite scaffold with urine-derived stem cells as a treatment for bone defects. *J. Mater. Sci. Mater. Med.,* **33**(10): 71.