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Experimental Trial

Polycaprolactone (PCL) as an Adipose-Derived Stem Cells (ADSCS) Stimulator – the Experimental Trial

O. Turkevych[1] and D. Turkevych[1]

[1]*Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*

Corresponding author:
Prof. Oleksandr Turkevych
Danylo Halytsky Lviv National Medical University,
Pekarska St, 69,
Lviv, Ukraine
e-mail: aturkevych@gmail.com

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ABSTRACT

This study investigates polycaprolactone (PCL, 25–50 µm microspheres in carboxymethylcellulose gel) as a stimulator of adipose-derived stem cells (ADSCs) for collagen production and tissue regeneration. Histological, immunohistochemical, and electron microscopy analyses in a rat model (n=25; 20 treated, 5 controls) over 21 days demonstrated significant ADSC activity (95% CD34 positivity), fibroblast proliferation (>50% increase), angiogenesis (55% CD31 positivity), controlled inflammation (low CD45), and organized collagen formation. Results indicate PCL's superiority in regenerative medicine applications, offering minimal adverse reactions compared to traditional stimulators.

INTRODUCTION

Collagen, the most abundant structural protein, supports skin integrity and elasticity but declines with aging and UV exposure, leading to compromised dermal structure. Traditional biostimulators like calcium hydroxyapatite (CaHA), hyaluronic acid (HA), and polylactic acid (PLA) have limitations including inflammatory reactions, transient effects, and granuloma formation (1–3).

CaHA has shown inconsistent collagen stimulation and often leads to foreign body reactions and fibrosis (4). HA provides temporary volumizing and hydration but lacks the ability to stimulate sustained neocollagenesis (5). PLA stimulates fibroblast activity through controlled inflammation, which can result in granuloma formation and delayed adverse reactions (6).

A promising alternative, polycaprolactone (PCL), activates adipose-derived stem cells (ADSCs) through a regenerative rather than inflammatory pathway. ADSCs play a central role in tissue regeneration by promoting extracellular matrix (ECM) remodeling, angiogenesis, and collagen production (7). PCL forms a mesh-like scaffold in subcutaneous white adipose tissue (sWAT), fostering a microenvironment conducive to stem cell activation. This promotes balanced type I and III collagen synthesis, improved dermal architecture, and sustained tissue regeneration without inducing chronic inflammation (8, 9).

The hypothesis of this study is that PCL stimulates collagen production through ADSC activation rather than direct fibroblast stimulation, thereby circumventing the inflammatory complications associated with CaHA, HA, and PLA. By leveraging the regenerative capacity of ADSCs, PCL represents a novel approach to aesthetic and regenerative medicine with superior outcomes and reduced inflammatory responses (10).

METHODS

The experiment used 25 female Wistar rats obtained from the certified Vivarium of Danylo Halytsky Lviv National Medical University. All procedures strictly complied with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes, ARRIVE guidelines, the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No.123), the Law of Ukraine "On Protection of Animals from Cruelty" (2006, No. 3447-IV), and guidelines provided by the Ministry of Education and Science of Ukraine (Order No. 249, 01.03.2012). All procedures were approved by the Ethical Committee of Danylo Halytsky Lviv National Medical University.

Study Design

Rats were divided into two groups:

- 20 rats received a single intradermal injection (0.1 ml at 1.5 mm depth) of ELLANSE S (PCL + carboxymethylcellulose gel) into the dorsal skin under sterile conditions.
- 5 control rats received a 0.1 ml saline injection under identical conditions.

The injection technique was standardized (single injection per rat, dorsal skin, sterile conditions). After 21 days (equivalent to approximately 12 human weeks based on established metabolic scaling factors), standardized biopsies (20 ± 1 mm diameter) were taken from the central dorsal zone.

Rat-to-Human Time Scaling

To translate the findings in rats to potential human applications, a scaling factor was applied based on established physiological differences. Rats have a faster metabolic rate and accelerated tissue turnover compared to humans. Studies suggest that one unit of time in human developmental terms is approximately equivalent to four to five units in rats (11, 12). This scaling accounts for variations in metabolic rate, tissue respiration, and mitochondrial oxidative processes between the two species. For instance:

- The tissue respiration rate in rats is approximately 14.17 ml/kg/min, compared to 3.5 ml/kg/min in humans (a ratio of ~4:1) (13).
- Research correlates these metabolic disparities to biochemical and regenerative processes such as collagen biosynthesis and biodegradation (14).

Using this scaling factor, the 21-day experimental duration in rats corresponds to approximately 12 weeks in human physiology. This conversion ensures that the timeline for observing effects on collagen synthesis, angiogenesis, and extracellular matrix remodeling aligns with the expected progression in human tissue regeneration.

Histological Analysis

Tissue samples were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 μm thickness using Leica RM2125, and stained with hematoxylin and eosin (H&E) following standard protocols.

Immunohistochemical Analysis

To assess cellular activity and specific regenerative markers, immunohistochemical staining was performed using monoclonal antibodies targeting key proteins:

- CD31: A marker for endothelial cells, used to evaluate angiogenesis (Fig. 1a-d).
- CD34: Indicative of hematopoietic stem cells and endothelial progenitor cells, reflecting tissue repair and progenitor cell activity (Fig. 2a-d).
- CD45: A leukocyte common antigen, identifying immune cell infiltration and response (Fig. 3a-d).
- CD117 (c-kit): A stem cell marker, suggesting the presence of active stem cells and their role in tissue remodeling (Fig. 4a-d).

Immunohistochemistry employed a chromogenic detection system, visualizing antigen-antibody reactions under a Leica DMLB optical microscope. Positive and diffuse staining patterns for these markers were

carefully analyzed to determine the degree of angiogenesis, stem cell recruitment, and immune modulation around the injection sites (Fig. 5a-d).

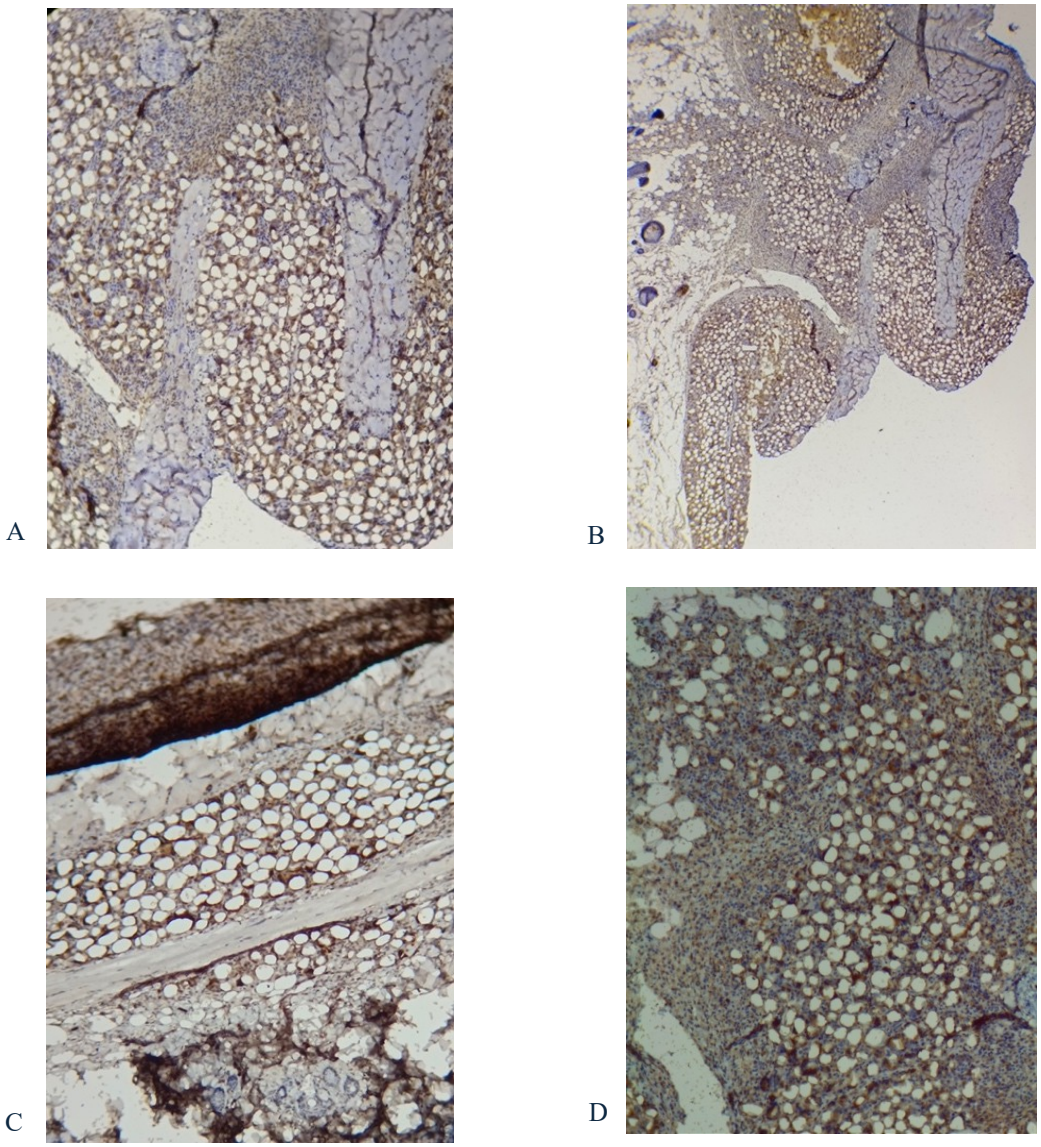

Fig. 1. *Immunohistochemistry with monoclonal antibodies at 21 days. A): IHC staining for CD31 (brown) in rat skin after PCL treatment; B): IHC staining for CD31 (brown) in rat skin after PCL treatment; C): IHC staining for CD31 (brown) in rat skin after PCL treatment, showing a distinct linear staining pattern. This indicates vascular remodeling and the presence of more mature blood vessels; D): Immunohistochemical staining for CD31 (brown) in rat skin post-PCL treatment, revealing a dense network of CD31-positive blood vessels.*

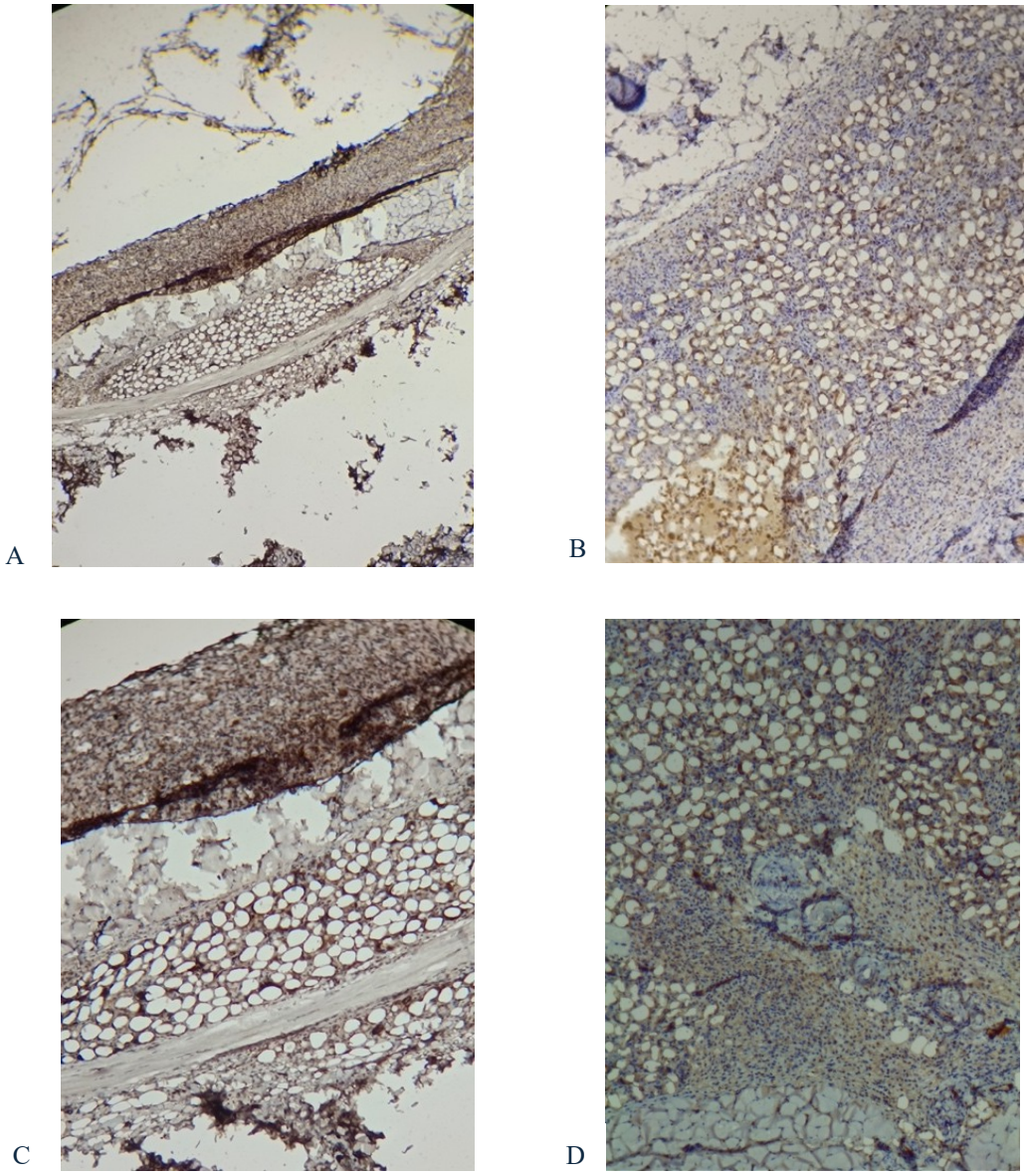

Fig. 2. *A): The presence of CD34-positive cells, likely hematopoietic stem cells and/or endothelial progenitor cells, indicates the potential for tissue regeneration and repair; B): The abundance of CD34-positive cells, including potential hematopoietic stem cells and endothelial progenitor cells, highlights the regenerative capacity of the tissue; C): Hematopoietic stem cells and endothelial progenitor cells (brown) in rat skin after PCL treatment. These CD34-positive cells contribute to the regenerative capacity of the tissue; D): Visualization of CD34-positive cells in rat skin following PCL treatment. These cells are crucial for tissue regeneration and repair.*

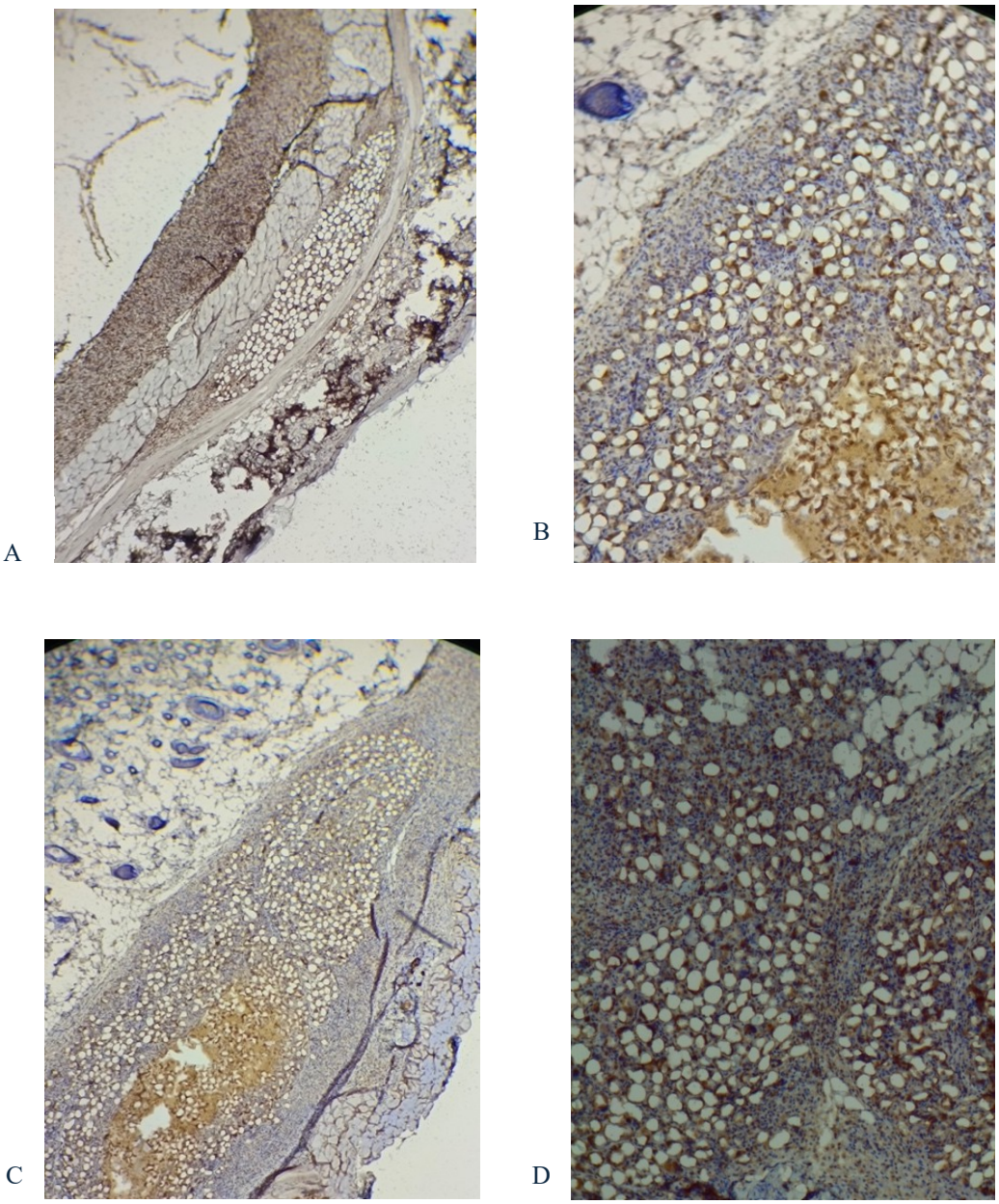

Fig. 3. *A): Immunohistochemical staining for CD45 (brown) in rat skin after PCL treatment. CD45-positive leukocytes are present, indicating an immune response; B): CD45-positive leukocytes are present, indicating an immune response; C): Visualization of leukocytes (brown) in rat skin following PCL treatment. CD45 staining highlights the immune response. D): CD45 staining reveals leukocytes (brown) in rat skin after PCL treatment.*

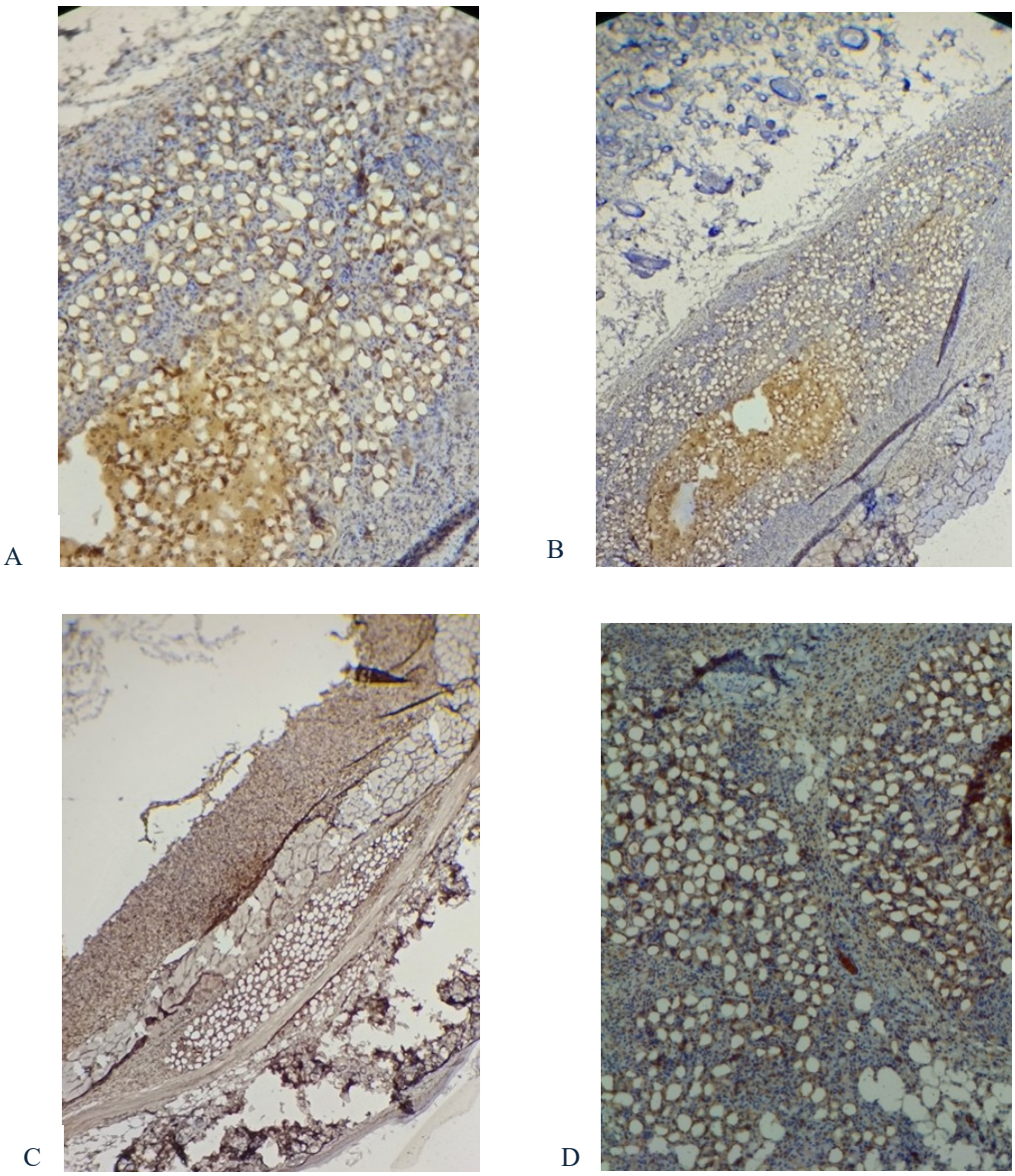

Fig. 4. *A): Immunohistochemical staining for CD117 (brown) in rat skin after PCL treatment. CD117-positive cells are present, suggesting the presence of stem cells that contribute to tissue regeneration; B): CD117-positive cells are present, indicating a potential role for stem cells in the tissue's response to the treatment; C): CD117-positive cells (brown) in rat skin post-PCL treatment, suggesting a potential role for stem cells in the tissue's response; D): Stem cell activity indicated by CD117 staining (brown) in rat skin after PCL treatment.*

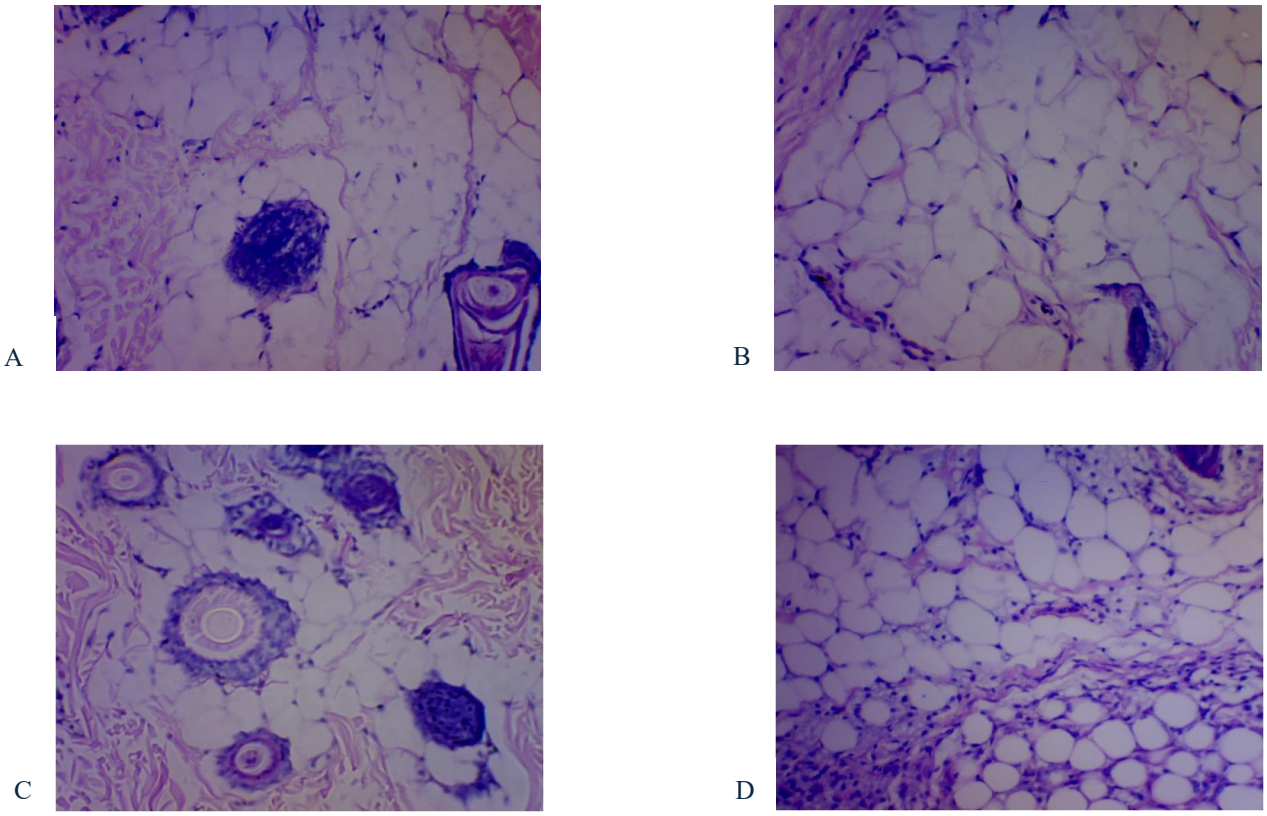

Fig. 5. *Total fibres and adipose tissue at 21 days. A): Histological section of rat skin after PCL treatment (H&E stain). Increased collagen fibers (pink) indicate a regenerative response, contributing to improved skin structure; B): Rat adipose tissue after PCL treatment (H&E stain); C): Possible adipose-derived stem cells (ADSCs) in rat skin after PCL treatment. These cells play a key role in tissue regeneration and repair. D): Adipose tissue from a rat treated with PCL (H&E stain). Presence of some smaller adipocytes and potential vascular structures (stained purple). These may indicate ongoing tissue remodeling and angiogenesis.*

Electron Microscopy

To supplement histological findings, electron microscopy was used to observe ultrastructural details of tissue regeneration. This included identifying extracellular matrix remodeling, collagen fibril organization, and the presence of exosomes released by adipose-derived stem cells (ADSCs). The proximity of ADSCs to vascular structures and fibroblasts was also evaluated to understand the interactions facilitating tissue repair (Fig. 6a-d).

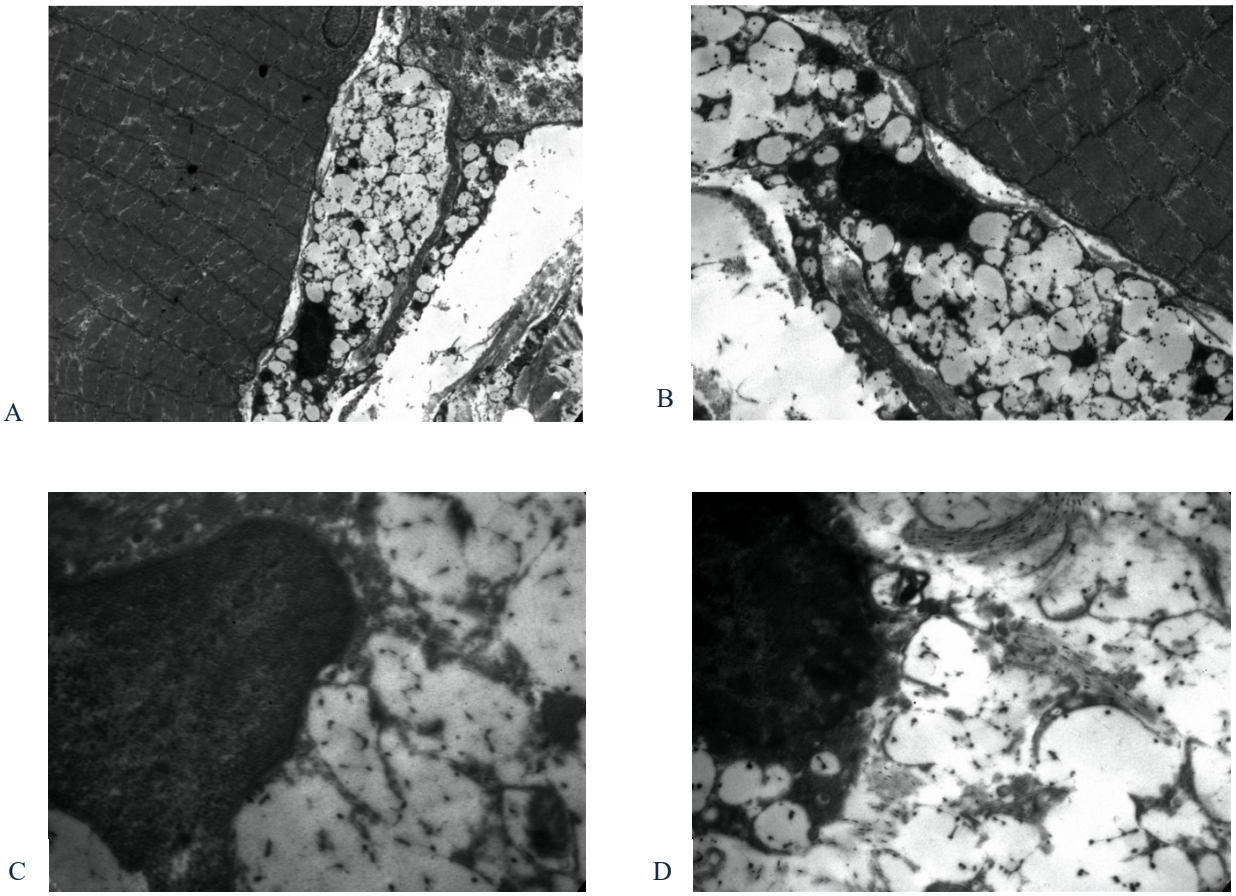

Fig. 6. *Electron microscopy at 21 days. A): Electron micrograph highlighting potential ADSCs and exosomes in rat skin after PCL treatment.(x5500); B): Electron micrograph showcasing the extracellular matrix (ECM) in rat skin after PCL treatment. The abundance of collagen fibers and the presence of exosomes indicate active extracellular matrix (ECM) remodeling and intercellular communication, which contribute to the regenerative effects of PCL. (x7500); C): Electron micrograph showcasing the extracellular matrix (ECM) in rat skin after PCL treatment. The abundance of collagen fibers and the presence of exosomes indicate active extracellular matrix (ECM) remodeling and intercellular communication, which contribute to the regenerative effects of PCL. (x15000); D): Electron micrograph showcasing the extracellular matrix (ECM) in rat skin after PCL treatment. The abundance of collagen fibers and the presence of exosomes indicate active extracellular matrix (ECM) remodeling and intercellular communication, which contribute to the regenerative effects of PCL. (x9000).*

DISCUSSION

The findings of this study underscore the exceptional potential of polycaprolactone (PCL) as a regenerative agent, setting it apart from traditional collagen stimulators such as calcium hydroxyapatite (CaHA) and hyaluronic acid (HA). Unlike these agents, which often rely on inflammatory processes to induce tissue responses, PCL operates through a biostimulatory mechanism, leveraging the natural activity of adipose-derived stem cells (ADSCs) to promote collagen biosynthesis and tissue regeneration. This unique mechanism minimizes adverse reactions and establishes PCL as a superior option for long-term skin rejuvenation.

At the core of PCL's efficacy is its ability to activate ADSCs, as evidenced by increased CD34 and CD117 marker expression in treated samples. While PLA and PCL both serve as effective collagen stimulators, their mechanisms differ significantly. PLA primarily relies on an inflammatory pathway to activate fibroblasts, which can lead to the formation of granulomas and other adverse events. In contrast, PCL activates ADSCs to

foster a regenerative environment, minimizing inflammation and supporting long-term tissue remodeling. This distinction positions PCL as a more balanced and sustainable biostimulator for aesthetic and regenerative applications.

ADSCs are key drivers of tissue repair and regeneration, contributing to extracellular matrix (ECM) remodeling, angiogenesis, and fibroblast stimulation. By creating a supportive microenvironment, PCL enhances ADSC activity, facilitating their secretion of exosomes and other bioactive factors that promote collagen production and dermal structure enhancement. This indirect collagen stimulation mirrors physiological processes, ensuring natural tissue remodeling without the risks associated with direct fibroblast stimulation.

In contrast, traditional collagen stimulators such as CaHA and HA have demonstrated significant limitations:

- CaHA often induces inflammatory foreign body reactions, including lymphocytic infiltration and giant cell formation, which can hinder tissue regeneration and lead to fibrotic encapsulation (22, 23). Moreover, some studies report that CaHA may show little to no evidence of actual collagen production several months after administration (9).
- HA fillers frequently trigger transient inflammatory responses and provide only temporary volumizing effects without contributing to sustained ECM remodeling (5).
- PLA, while effective in stimulating collagen synthesis, relies on an inflammatory mechanism that can result in granuloma formation and fibrotic encapsulation (6).

In this study, minimal CD45 expression in PCL-treated tissues reflected a controlled immune response, highlighting PCL's ability to avoid the excessive inflammation seen with other stimulators. This balance between regeneration and immune modulation underscores PCL's unique advantage in minimizing fibrosis and adverse reactions.

PCL's effects on tissue regeneration extend beyond collagen production. The enhanced angiogenesis observed in this study, evidenced by increased CD31 marker expression and vascularization in treated tissues, is critical for sustaining oxygen and nutrient delivery to regenerating areas. This vascular support not only strengthens the regenerative microenvironment but also ensures the longevity of PCL's effects.

Additionally, the slow biodegradation of PCL microspheres allows for sustained stimulation of ADSC activity and collagen biosynthesis, further differentiating it from rapidly metabolized HA fillers or potentially inflammatory CaHA formulations. Exosomes, secreted by ADSCs in response to PCL, have been shown to facilitate intercellular communication and accelerate tissue remodeling by delivering growth factors, cytokines, and microRNA.

The comprehensive improvements observed in dermal thickness, collagen density, and skin quality following PCL treatment validate its role as a balanced regenerative agent. By promoting ADSC-mediated repair while avoiding inflammatory pathways, PCL achieves significant and lasting skin rejuvenation. These findings not only establish PCL as a superior alternative to traditional stimulators but also pave the way for its broader application in aesthetic medicine and regenerative therapies.

Future research should focus on optimizing the PCL microsphere size and delivery system to enhance the duration and intensity of its biostimulatory effect, exploring combination treatments with other regenerative agents (such as PRP or mesenchymal stem cells), and expanding clinical trials to assess long-term safety, efficacy, and patient satisfaction across diverse patient populations and treatment areas.

CONCLUSION

Polycaprolactone (PCL) represents a significant advancement in regenerative medicine, particularly for skin rejuvenation. Unlike traditional collagen stimulators, which often rely on inflammatory responses to achieve tissue remodeling, PCL operates through a biostimulatory mechanism that activates adipose-derived stem cells (ADSCs). By leveraging the natural reparative capacity of ADSCs, PCL promotes balanced and sustained collagen production, extracellular matrix (ECM) remodeling, and angiogenesis, resulting in improved skin quality and structural integrity.

The efficacy of PCL is evident through increased dermal thickness, enhanced collagen density, and the formation of organized collagen networks in treated tissues. Robust ADSC activity, indicated by CD34 and CD117 positivity, drives these regenerative effects, while angiogenesis marked by CD31 expression ensures adequate vascular support. Ultrastructural observations highlight active ECM remodeling, with abundant exosomes facilitating intercellular communication and coordination among ADSCs, endothelial cells, and fibroblasts.

In contrast to PCL, traditional stimulators such as calcium hydroxyapatite (CaHA) and hyaluronic acid (HA) fillers exhibit significant limitations:

- CaHA often induces inflammatory foreign body reactions, leading to lymphocytic infiltration and fibrotic encapsulation.
- HA fillers, while providing temporary hydration and volume, lack the ability to stimulate sustained collagen production or ECM remodeling.
- Polylactic Acid (PLA) stimulates collagen production but relies on inflammatory fibroblast activation, which can result in delayed adverse effects such as granuloma formation and fibrosis.

PCL circumvents these challenges by fostering a regenerative microenvironment with minimal inflammation, as evidenced by low CD45 expression. This balance between regeneration and immune modulation underscores PCL's unique advantage as a long-term biostimulator.

Controlled degradation further enhances PCL's benefits, enabling prolonged stimulation of ADSCs and sustained collagen biosynthesis. Unlike rapidly metabolized HA fillers or the potential complications associated with CaHA, PCL's slow biodegradation ensures consistent and reliable results over time. Its role as a safe and effective tool for skin rejuvenation sets it apart as a superior option in aesthetic medicine.

PCL's potential extends beyond aesthetic applications to broader areas of regenerative medicine. From wound healing to tissue engineering and reconstructive surgery, its ability to promote balanced and sustained tissue regeneration opens new avenues for innovation and therapeutic advancements.

Polycaprolactone exemplifies a new paradigm in regenerative therapies. By harnessing the natural capabilities of stem cells and minimizing inflammatory pathways, it achieves superior and long-lasting outcomes. PCL's role as a transformative biostimulator offers a safer, more effective, and more sustainable alternative to traditional collagen stimulators. Future exploration of optimized formulations and synergistic combinations with other regenerative agents will further unlock its vast clinical potential.

Conflict of interest statement

DISCLOSURES: Both authors are not Sinclair Pharma consultants or speakers and do not receive any honorarium from Sinclair Pharma. Costs of rats, injectables, and laboratory investigations were covered by Medical Clinic Adassa LTD, Ukraine.

REFERENCES

1. Lu F, Xing M, et al. Polycaprolactone nanofibrous mesh reduces foreign body reaction and induces adipose flap expansion in tissue engineering chamber. Int J Nanomedicine. 2016;11:6471–6483.
2. Craven NM, et al. Breakdown of collagen during aging and its relationship with UV exposure. Br J Dermatol. 1997;137:344–350.
3. Smith JG, et al. Lysosomal activity in dermal aging. J Invest Dermatol. 1962;39:347–356.
4. Holzapfel AM, Mangat DS, Barron DS. Soft-tissue augmentation with calcium hydroxyapatite: histological analysis. Arch Facial Plast Surg. 2008;10(5):335–338.
5. Yutskovskaya Y, et al. A randomized, split-face, histomorphologic study comparing volumetric calcium hydroxylapatite and a hyaluronic acid-based dermal filler. J Drugs Dermatol. 2014;13(9):1047–1052.
6. Yutskovskaya YA, et al. Improved neocollagenesis and skin mechanical properties after injection of diluted calcium hydroxyapatite in the neck and decolletage: a pilot study. J Drugs Dermatol. 2017;16(1):62–67.
7. De Francesco F, Ricci G, D'Andrea F, Nicoletti GF, Ferraro GA. Human adipose stem cells: from bench to bedside. Tissue Eng Part B Rev. 2015;21(6):572–584.
8. Turkevych A, Turkevych D. Influence of calcium hydroxylapatite on the soft tissues – a critical viewpoint. J Appl Cosmetol. 2022;40(1):19–27.
9. Nicolau P, Marijnissen-Hofsté J. Neocollagenesis after injection of a polycaprolactone-based dermal filler in a rabbit. Eur J Aesth Med Dermatol. 2013;3(1):19–26.
10. Kim JS. Changes in dermal thickness in biopsy study of histologic findings after a single injection of polycaprolactone-based filler into the dermis. Aesthetic Surg J. 2019;39(12):484–494.
11. Thanaphum O, et al. The responses of human adipose-derived mesenchymal stem cells on polycaprolactone-based scaffolds: an in vitro study. Tissue Eng Regen Med. 2014;11(3):239–246.
12. Stander BA, Van Vollenstee FA, Kallmeyer K, et al. An in vitro and in vivo study on the properties of hollow polycaprolactone cell-delivery particles. PLoS One. 2018;13(7):e0198248.
13. Lavker RM, et al. Ultrastructural changes in aging skin and the effects of UV. J Invest Dermatol. 1979;73:559–566.
14. Redaelli A, et al. Neocollagenesis in vivo with injectable fillers: clinical evaluation. J Clin Aesthetic Dermatol. 2014;7(2):21–25.
15. Tomasek JJ, et al. Fibroblast-mediated collagen remodeling during tissue repair. J Cell Biol. 2002;160:635–644.
16. Yang S, et al. ADSCs-mediated angiogenesis and dermal regeneration with PCL scaffolds. Adv Healthc Mater. 2019;8(4):180–191.
17. Blum JC, Schenck TL, Birt A, et al. Artificial decellularized extracellular matrix improves the regenerative capacity of adipose tissue-derived stem cells on 3D printed polycaprolactone scaffolds. J Tissue Eng. 2021;12:20417314211001536.
18. Marijnissen J, Nicolau P. Comparing the long-term efficacy of polycaprolactone-based fillers versus other biostimulatory fillers. J Cosmet Dermatol. 2015;14(3):324–332.
19. Heinemann S, et al. Polycaprolactone-based scaffolds for tissue regeneration: properties and applications. Biomater Sci. 2013;1:926–935.
20. Taylor M, et al. Polycaprolactone as a scaffold for dermal regeneration. Regener Biomater. 2019;6(1):35–41.
21. Nicolau P. Long-lasting and natural collagen stimulation with polycaprolactone-based dermal fillers. Clin Cosmet Investig Dermatol. 2017;10:431–439.

22. Marijnissen-Hofsté J, Nicolau P. Polycaprolactone in aesthetic medicine: from dermal filler to scaffold material. J Cosmet Dermatol. 2020;19(1):31–37.
23. D'Este M, et al. From filler to scaffold: polycaprolactone (PCL)-based platforms for tissue engineering. Mater Sci Eng C. 2018;84:40–52.
24. Lo KW, Ulery BD, Ashe KM, Laurencin CT. Studies of poly(lactic-co-glycolic acid) (PLGA) nanoparticle delivery systems for sustained release of therapeutic agents. Adv Drug Deliv Rev. 2020;107:1–2.
25. Yeo DC, et al. Biodegradable PCL-based scaffolds with built-in vascularization channels for enhanced tissue regeneration. Tissue Eng Part A. 2016;22(3–4):114–125.
26. Phan SH. Biology of fibroblasts and myofibroblasts. Proc Am Thorac Soc. 2008;5(3):334–337.
27. Kim YJ, et al. In vivo degradation behavior of PCL scaffolds with different pore structures. J Biomed Mater Res A. 2014;102(11):3854–3862.
28. Gombotz WR, Pettit DK. Biodegradable polymers for protein and peptide drug delivery. Bioconjug Chem. 1995;6(4):332–351.
29. Mosahebi A, et al. Adipose-derived stem cells: biology and potential for therapy. World J Stem Cells. 2012;4(4):46–60.
30. Cervelli V, Gentile P, De Angelis B, et al. Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in post-traumatic lower extremity ulcers. Stem Cell Res. 2011;6(2):103–111.