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Effect of Polycaprolactone Nanofiber Membrane Versus Concentrated Growth Factor on Periodontal Ligament Model Defect.

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ABSTRACT

Purpose: This study was carried out to evaluate the effect of two different regenerative materials on periodontal Ligament Model Defect through histological examination of rabbit's mandible. **Material and Methods:** Thirty-six (36) adult male New Zealand rabbits (weight 3 to 3.5 kg) were used and then divided randomly into 3 groups according to material received (n=12).Group I (GI) control group that did not receive any treatment, Group II (GII) that received polycarolactone nanofiber membrane (PCL), Group III (GIII) that received concentrated growth factor membrane (CGF), surgical procedures were done to create a bony defect of 5x5mm size in the right side on buccal mandibular bone, the rabbits were euthanized at day 7,30 and 45. **Results:** The histological evaluation showed that bone healing in bony defects of group III was faster than that of the other two groups. Group III showed the statistically significantly highest mean % increase in bone density than Group I and II. **Conclusion:** The regenerative therapy using (CGF) membrane showed an improvement in the treatment of periodontal ligament defects than those treated with (PCL) nanofiber membrane.

KEYWORDS

Polycarolactone, concentrated growth factor, periodontal ligament defect.

INTRODUCTION

Periodontitis is a prevalent oral disease which incorporate impairment in the supporting tissue around tooth structure as alveolar bone, periodontal ligament (PDL), and cementum. Pathogenic organisms in plaque, genetic cause, and environmental factors such as

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smoking, all these factors have a role in periodontitis and therefore bone loss. Loss of bone structure around a tooth may result in tooth movement and displacement leading to tooth loss which affect the patient function and aesthetic [1].

Guided tissue regeneration (GTR) depends on renewal of the periodontal apparatus, involving cementum, periodontal ligament, and alveolar bone. GTR includes positioning of a membrane barrier over the affected area to prevent migration of the gingival epithelium. This permits the stem cell to differentiate and regenerate the defect [2].

Recently, nanofiber membrane has been developed as scaffold method of tissue regeneration with the advantage of biocompatibility properties. Polycaprolactone (PCL) nanofiber membrane is employed as a material for tissue engineering that used for regeneration of bone, ligament, cartilage, skin, nerve, and vascular tissues. It also used as a material for guided tissue regeneration as it exhibits biocompatibility estates, not toxic, can be degraded and safe in clinical use [3,4]

New technology was developed as a regenerative treatment called concentrated growth factor (CGF). It is a third generation of platelet concentrate achieved with long continuous centrifugal technology. It contains different kinds of growth factor and fibrins, capable to facilitate the regeneration of soft and hard tissues. Damaged periodontium results in formation of long junctional epithelium (LJE) to reduce pocket depth without PDL fibers regeneration. Ultimate regeneration requires restoring the fiber which will attach to the adjacent cementum and bone. CGF membrane was used to guide regeneration of soft tissue that enhance the proliferation of periodontal ligament stem cell (PDLSC) and creation of PDL and bone [5-7].

This study was aimed to evaluate and compare the effect of PCL nanofiber membrane versus CGF on healing of PDL model defect in buccal bone of mandible of rabbits histologically.

MATERIAL AND METHODS

Animal:

Thirty-six (36) adult male New Zealand rabbits (weight 3 to 3.5 kg) were selected from (Modern Veterinary Office, Al Haram - Giza). The rabbits were following the rules and regulations of the animal experimental studies approved by ethical committee (code: REC-BI-22-01) - faculty of Dental Medicine for Girls, Al-Azhar University including their diet facilities and method of euthanization. The animals were housed in standard cages individually and were fed with standard rabbit chows.

Material

1. Polycaprolactone:

The material was purchased from Sigma-Aldrich company. It was prepared by electrospinning machine. PCL was transmitted into a 5ml syringe with straightforward needle. The tip of the blunt needle was connected to high power supply, electrospun fiber was ejected from the syringe on metallic accumulator [8]. The morphology of the fibers was examined by scanning electron microscopy (SEM) at Desert Research Center, the diameter of resulting fibers was measured randomly. Fig (1)

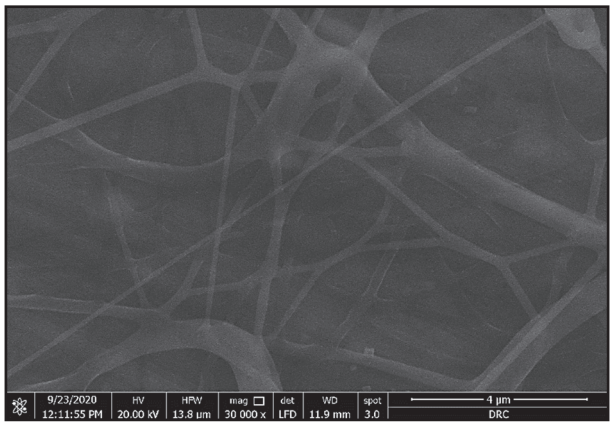

Figure (1) Scanning electron microscope of PCL fibers. (Mag. X 30000)

2. Concentrated growth factor:

The skin at the site of sample collection was prepared by shaving and wiping it with alcohol swab. A local anesthetic cream was applied over ear lobes and 5 ml blood was gathered from the auricular artery. It was taken immediately into a sterile tube without anticoagulants and instantaneously centrifuged in a centrifuge device for nearly 13 minutes. The fibrin buffy coat phase of CGF was separated using scissors then pressed between two glass slides after that CGF membrane was attained. Fig (2)

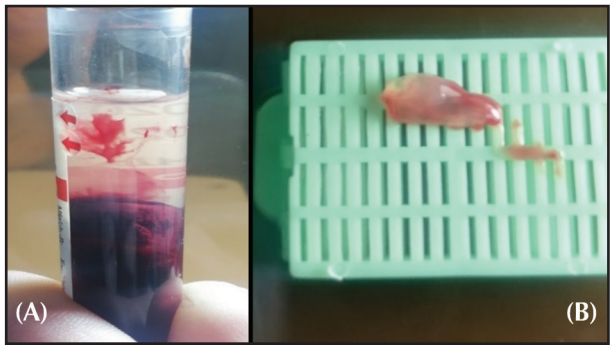

Figure (2) Photomicrograph showing (A) centrifuged blood, (B) CGF membrane.

Sample Grouping:

The animals were distributed randomly into three groups, each group consisted of 12 animals (n=12). Group I: the bony defect was left untreated (control group), Group II: the bony defect was filled with PCL nanofiber membrane (PCL group), Group III: the bony defect was filled with CGF membrane (CGF group).

Surgical procedure:

The surgical procedures were done after intramuscular administration of anesthetic solution of 10mg/kg of xylazine hydrochloride and 100mg/kg of ketamine chloride. Incision sites were cleared of hair using razor. Submandibular skin incision was done to expose the buccal bone of the mandible. A bony defect of 5x5mm size and 1mm depth was created using round bur with high-speed instrumentation. The defect was initiated with No.4 round bur until the roots appeared, then continue with a curette to remove cementum. The treatment was applied into the created defect, the muscle was repositioned by resorbable suture, and the skin was closed with black silk [9]. At day 7,30,45 after surgery, 4 rabbits from each group were euthanized by anesthetic overdose. The mandible of each was fixed for H&E sampling examination using 10% formalin and Cone beam computed tomography for measuring bone density.

RESULTS

Histopathological evaluation:

A- Group I: (Control group)

At day 7: the defect appeared covered by layer of collagen fibers containing numerous blood capillaries followed by thin layer of newly forming bone containing some osteocyte cells. Fig (2.A1)

At day 30: the tooth structure appeared surrounded by PDL fibers that run parallel to the tooth surface followed by trabeculae of new bone enclosing wide marrow spaces and that bone contained osteocytes and was lined by osteoblast cells. Fig (2.A2).

At day 45: the defect appeared covered with PDL fibers arranged perpendicular on the tooth surface, there were blood capillaries and interstitial tissue spaces within the PDL fibers. The bone in the healing site appeared spongy bone containing marrow spaces and osteocyte which appeared oval and some of them appeared round, the periphery of bone near to PDL showed organization of osteons Fig (3.A3, 3.A4).

B- Group II:(PCL group)

At day 7: the defect area was covered by thick layer of collagen fibers characterized by thickening in the fibers facing the tooth structure while the fibers facing PCL membrane showed small foci of mineralized tissue. Fig (3.B1)

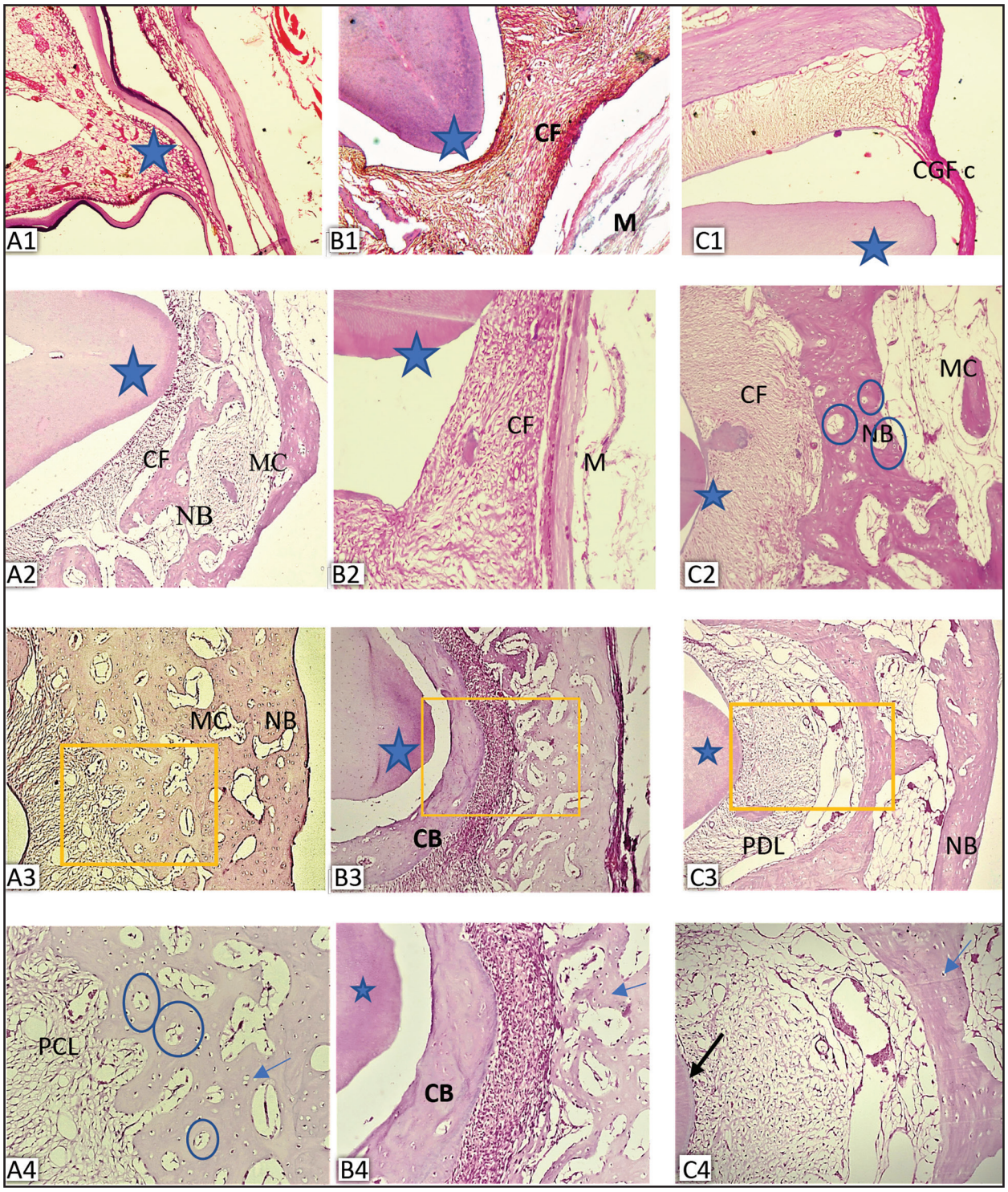

Figure. (3) Micrograph of defect area: **A1**-Control group, **B1**- PCL group, **C1**- CGF group (at day 7), **A2**-Control group**, B2**- PCL group, **C2**- CGF group (at day 30), **A3**-Control group, **B3**- PCL group, **C3**- CGF group (at day 45) (Magnification x 100)- **A4**-Control group, **B4**- PCL group, **C4**- CGF group (at day 45) (Magnification x 200). (star) tooth structure, (NB) new bone, (MC) medullary cavity, (CF) collagen fibers, (CB) compact bone, (M) membrane, (CGF c) CGF clot, (blue arrow) osteocyte, (black arrow) cementum, (blue circle) osteon.

At day 30: The tooth structure appeared surrounded by thick and irregularly arranged PDL fibers, the outer layer of fibers facing PCL membrane showed continuous band of osteoid tissue. Fig (3.B2)

At day 45: the tooth dentin appeared with no cementum regeneration, the tooth structure surrounded by bone which appeared dense and compact, the lower part of that bone had some osteons and containing osteocyte cells followed by collagen fibers which appeared thick and short unlike that present in the control one, these fibers were run parallel to the tooth structure and surrounded by spongy bone with narrow trabeculae and wide marrow spaces. Fig (3.B3, 3.B4)

C- Group III:(CGF group)

At day 7: the defect area was covered by CGF clot consisted of thick fibrillar element. Fig (3.C1)

At day 30: tooth structure was surrounded by wide area of PDL fibers that run perpendicular to the tooth surface, followed by a thickened layer of bone formation containing large number of osteocytes and the bone showed organization of osteons formation. Fig (3.C2)

At day 45: there was marked cementum regeneration followed by PDL formation. The fibers of PDL appeared perpendicular on the tooth surface and on the other side perpendicular on the bone structure. PDL area contained large blood vessel near to bone surface. PDL followed by bundle bone. It characterized by thick and dense bone lamellae containing osteocyte which appeared oval and arranged regularly. The bone showed presence of incremental line. Fig (3.C3, 3.C4)

Statistical analysis

The results of current study showed that when comparing the means and SD of bone density between the groups revealed that there was a statistically significant difference between them ($P < 0.05$) As shown in the table (1), At the baseline there was no statistically significant difference in mean of bone density between all groups. On day 7, there was statistically significant difference in mean of bone density between three groups since $P \leq 0.05$. The highest score on day 7 was for the nanofiber membrane PCL (274.2) group, and the lowest was for the CGF (79.89) group.

On 30 days, there was no statistically significant difference in mean of bone density of all groups. However, on day 45 there was statistically significant difference in mean of bone density of three groups *$P \leq 0.05$*, the highest score was for the CGF (1053.67) group. Fig (4)

Table (1) *Comparison of means and SD of bone density between three groups:*

	Group I		**Group II**		**Group III**		***P*-value**
	Mean	SD	Mean	SD	Mean	SD	
Base line	-315.1	10.4	-298.9	10.4	-323.7	10.4	*0.4*
Day 7	149.69	12.9	274.2	7.7	79.89	4.7	*0.000**
Day 30	406.75	3.2	566.74	5.9	708.52	3.96	*4.6*
Day 45	554.42	5.03	818.65	14.6	1053.67	1.49	0.020*

**: Significant at $P \leq 0.05$, Different superscripts in the same row are statistically significantly different*

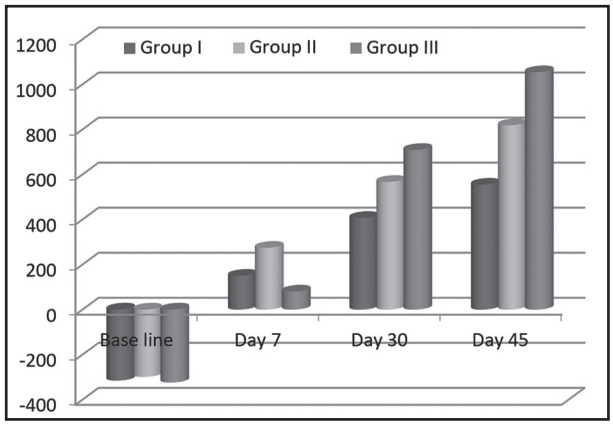

Figure (4) Bar chart representing increase of mean in bone density among three groups

DISCUSSION

Tissue engineering methodology and progress in nanotechnology have triggered the use of nanostructures as scaffold for the purpose of tissue engineering. Nano fibers are common for the biomedical application because, they have fibrous structure which resemble that present in the extracellular matrix (ECM). These characteristics provide a porous structure for the transport of nutrients, the communication with other cells, cell ingrowth, cell attachment, and cell differentiation [10,11].

Consequently, development of electrospinning technique is very important for producing nanostructured material as it is a quick, simple, and cost-effective method [12]. PCL were fabricated from the nanofibrous structures, it was found that, these scaffolds showed improved osteogenic differentiation through activation of BMP 2 signalling and efficient of stimulating osteoblast growth and its functions[13].

Rabbits is represented as a commonly used animal model because, there was similarity in bone density between rabbits and human. Moreover, rabbit has faster skeletal change. oral microorganisms in rabbits showed pathogenic bacteria which are like oral flora in humans [14]. for this reason, rabbits were used for formation of periodontal defect and to study periodontal regeneration [15].

PCL was utilized as a material for guided tissue regeneration that can be applied for bone healing. There was indication of remodeling and improving the compact bone in the PCL group after 45 days[16]. This result return to PCL membrane has osteoinductive effect and increased cellular activity[17].

The result of PCL group regarding PDL regeneration conflicted with another study proved that alignments of nanofiber membrane was considered to improve cellular orientation and consequently regenerate PDL [18].

In the present study addition of CGF membrane to PDL model defect showed regeneration of PDL fibers which run perpendicular on the tooth and bone, there was bone formation which appeared more mature than the control group. These results could be contributed to the fact that CGF is a fibrin matrix containing growth factors and cytokine. It promotes chemotaxis and mitosis of osteoblast and direct synthesis of collagen fiber [17]

On the other hand, CGF potentiate wound healing and decrease the inflammatory response through regulation of PDGF which act as chemoattractant of immune cells and fibroblast, inducing cell proliferation and stimulate collagen formation. These effects explained the regeneration of PDL fibers and increased attachment gain to the tooth. [19]

Furthermore, the defect showed new blood vessels formation at the periphery of the bone that's back to vascular endothelial growth factor (VEGF) which released from CGF that instructs vascular endothelial formation and stimulates endothelial proliferation. [20]

At day 30, there was bone formation in control and CGF group but in CGF group the bone appeared well formed and show organization of osteons formation these explained by in vitro study proved that VEGF and BMP-2 had slow kinetic release and reached its maximum after 13th day [21,22].

CONCLUSIONS

Within limitations of the current study, it was concluded that:

- PCL nanofiber membrane is an effective scaffold for bone regeneration.
- PCL nanofiber membrane is proved to be not suitable for PDL regeneration alone.
- Application of CGF membrane to the PDL model defect regenerate cementum, bone and PDL fibers that attached to the tooth structure like the normal fibers.

RECOMMENDATION

- Further studies are recommended by using different modalities in PCL nanofiber membrane.
- Clinical trials are required to explore the application of CGF in return of PDL.

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No fund received for this study

CONFLICT OF INTEREST:

The authors have no conflicts of interests to declare.

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