Electrospinning of Ascorbic Acid with Collagen/PCL scaffold as coating on CpTi Implant Surface: Preparation, Morphological and Histological Properties

Shanai M. Al-Bayati [a′1], Raghdaa K. Jassim[B′2], Akram R. Jabur[c,3]

[a]PhD student Department of Prosthodontics College of Dentistry University of Baghdad

[B]Professor, Department of Prosthetics College of Dentistry, University of Al-Farahidi

Professor ,Material engineering dept., University of Technology- Iraq,Alsinaa street,10066 Baghdad,Iraq

Abstract

Dental implants inserted into the jawbone, where they fuse to provide a stable, durable foundation for restorations. However, even the best dental implants can fail. Therefore, researchers focus on developing surface coatings for implants to enhance Osseointegration. Objectives of this study are to form a biodegradable scaffold by electrospinning of ascorbic acid, collagen, and polycaprolactone (PCL) fibers onto the surface of commercial pure titanium (CpTi) implants.

Methods three composite fibers (scaffold) created using the electrospinning procedure first scaffold formed from collagen/PCL, the second scaffold formed from ascorbic acid added to the collagen/PCL, and finally the third scaffold formed from PCL polymer alone with ascorbic acid. After that characterization done by SEM, AFM , scaffolds cross cut test, and wettability. Another part of study include examination of histological response for coated and uncoated implant in femur of rabbits statistical analysis of data was performed by SPSS version 22. The results of SEM analysis revealed the formation of nanofiber scaffold with pores. while the results of AFM surface roughness indicate that the ascorbic acid/PCL scaffold had the roughest surface compared to the other two groups .And the collagen /PCL scaffold appears to have a rather smooth topography,. Histological finding after 2 and 6 week of implantation appear show thicker layer of fibrous osteogenic cells in ascorbic acid loaded to collagen/PCL coat on CpTi . The cross-cut test demonstrates that scaffold adhesion to CpTi is robust and strong. within limitation of this study it can be concluded that Ascorbic acid with collagen can be efficiently coat titanium surface by using electrospinning technique. In addition, a scaffold formed with an appropriate properties include improve hydrophicity of surface and increase surface roughness in addition to increase shear bond between implant and bone.

Key word:

Electrospinning scaffold, ascorbic acid, biochemical coating, implant coating, collagen scaffold

1. Introduction

The quality of life for those who are missing teeth has improved because of dental implants, which are growing in popularity. In patients with advanced periodontal disease or those requiring sophisticated implant surgery[1], the process of wound healing following dental implant surgery is a critical aspect affecting the survival and clinical effectiveness of dental implants [2]. Mogan 2006 study was conducted using collagen as a coating material for titanium and its alloys, and it was concluded that collagen promotes surface biological properties by increasing the proliferative capacity of osteoblasts [3]. Hauck etal study in 2021 investigating the effects of collagen coating on tissue vascularization, inflammatory response, macrophage activity, and osteoclast activity have also been done [4], [5]. In an animal model, biochemical alteration of Ti surfaces by collagen could accelerate wound healing [6]. However, collagen coating alone is not sufficient to accelerate the differentiation of osteoblasts [7]. Although Morwood etal in 2023 claimed that collagen coating promotes cell adhesion, differentiation, and mineralization of the extracellular matrix [8], others have not found these stimulatory effect[9] .In one investigation, at 4 weeks, acid-etched Ti surfaces and a collagen-only coating showed no noticeable improvement. [10]. In extracellular matrix (ECMs), collagen type I is the main fibrillary structural component. It is present almost everywhere in the body, with the exception of cartilaginous tissues, where collagen type II predominates [11]. Natural polymers known as collagen types II and I were frequently utilized in cartilage tissue engineering because they provide biological signals that help chondrocytes connect with the scaffold and give the developing tissue the room it needs. This is a result of cellular enzymes recognizing collagen. [12], However, the majority of investigations have concentrated on using collagen type I scaffolds as cell carriers for MSCs or chondrocytes. In full-thickness defects in rabbit articular cartilage, allograft chondrocytes have been implanted within collagen type I gels; 24 weeks following transplantation, the defects were filled with hyaline cartilage as opposed to those lacking seeded gels, which were filled with fibrocartilage [13]

Ascorbic acid is a crucial component for many enzymatic processes in the body and can advance anti-inflammatory processes in macrophages through a variety of methods. [14], As a result, vitamin C aids in the healing of wounds by encouraging matrix deposition and neovascularization and inhibiting the release of inflammatory mediators. [15]. Vitamin C is a cofactor for a large family of metallic enzymes involved in the synthesis of collagen, neurotransmitters, and peptide hormones, as well as the regulation of transcription factors such as hypoxia inducible factor-1 [16]. Vitamin C especially promotes keratinocyte proliferation and fibroblast migration, enhances synthesis collagen, and stabilizes the collagen molecule tertiary structures; it appears crucial for the healing/regeneration process and wound healing.[17] ,[18]. In addition, vitamin C is an effective antioxidant that relieves oxidative stress and participates in a variety of biochemical reactions. Therefore, vitamin C promotes wound healing through pleiotropic mechanisms, including promoting matrix deposition and neovascularization in healing wounds and reducing the secretion of inflammatory mediators [19]. Ascorbic acid, or vitamin C, aids in the production of collagen, which is a crucial element of the extracellular matrix of bone. Recent research has looked at how ascorbic acid affects the in vitro differentiation and proliferation of primary bovine osteoblasts. Results demonstrated that when ascorbic acid concentrations were increased to 200 g/ml, extracellular matrix proteins such collagen type I, osteonectin, and osteocalcin displayed rapid growth and increased synthesis. [20] In addition to its role in collagen synthesis, vitamin C

has been found to scavenge oxygen-derived free radicals such superoxide anion to prevent them from interacting with vasoactive nitric oxide. This can improve endothelial function over time and advance cardiovascular health in general [18]. Polycaprolactone is biocompatibility, versatility, processability, and suitability for various biomedical applications have cemented its position as a valuable polymer in the field. Its ability to blend with other polymers further enhances its adaptability and allows for the creation of custom materials tailored to specific medical needs. [21]. This polymer has FDA approved and its manufacturing methods are relatively easy and affordable, however, it has a slow degradation rate which makes it difficult to use on its own [22]

One technique that is frequently used to make scaffolds for tissue engineering is electrospinning. A thin polymer solution is stretched during solidification by electrostatic forces [19], [20]. The resultant scaffold is an effective way for manufacturing micro and nanofibers for tissue engineering since the fibers closely mirror the dimensions and composition of the natural extracellular matrix [21], [22], and using solvents and aqueous-based solutions helps in the assimilation of biological substances like collagen. Collagen and elastin combinations are only one of the many uses for electrospinning of collagen [23], scaffolds constructed of polycaprolactone and collagen for the creation of vascular tissue [24], and fibrinogen fibers that allow for natural collagen deposition during cell growth [24]. Due to the fact that many therapeutic applications for scaffolds need for the monitoring of bacterial growth, the future success of human tissue adopting the scaffolds may depend on the addition of chemicals, such as antibiotics, to polymer-collagen blends. One criterion for keeping track of intra-abdominal infection, for instance, is maintaining optimum levels of an antibacterial antibiotic throughout medication delivery [25]. This study aimed to study electrospinning coating technique for coating CpTi with collagen and ascorbic acid scaffold fibers.

2. Materials and methods

Commercial pure titanium CpTi (Baoji Jinsheng Metal Material Co., Ltd.), pure collagen type I (bovine tendon powder, neutral type I collagen, suitable for biomedical research) from Sigma SKU (molecular weight **2,500–4,000 g/mol**.), pure ascorbic acid powder (Melrose Laboratories Pty Ltd., Melrose Health.com.au) (Molecular Weight: **198.11 g/mol**.) **Density 0.6 ~ 0.9 g/ml)**, PCL (C6H10O2) n (molecular weight 45,000 g·mol-1 density 1.146 g/mL at 25 °C) were purchased from Sigma-Aldrich (molecular weight), and finally acetic acid glacial (CH3CO2H) at a 98 percent concentration served as the solvent for polymers. The scaffold was created using an electrospinning system (Nano NC, ESB200, South Korea).

The electrospinning device (figure.1) setup consists of three main parts: a conductive collector, flow rate, and a high-voltage power source. The electrospinning processes method produces nanofibers [26]. At a certain voltage, repulsive forces may overcome the surface tension of the polymeric solution, causing a jet to emerge from the Taylor cone. The jet moves between the needle tip of the syringe and the collector, dividing into a vast number of filaments, which then undergo solvent evaporation along with the distance. [27]; [28].

Figure 1: electrospinning /electrospray system (Nano NC, ESB200, South Korea

2.1 in vitro part of study

2.1.1 Specimen preparation

Disc-shaped CpTi (china) specimen with a diameter of 15mm and width of 2mm was used as a substrate for surface modification. According to the design of study three group were preparedas follow the 1st group consisting of collagen / PCL, the 2nd group having ascorbic acid added to the collagen /PCL, and the 3rd group having ascorbic acid and PCL alone.

A. Preparation of collagen with PCL scaffold.

For this step, two-solutions was prepared as follow, first solution 20% PCL solution mixed with a magnetic stirrer bar for three hours at room temperature. Second, a 20% by weight solution of collagen was prepared by dissolving collagen in glacial acetic acid. The two solutions then stirred with a magnetic bar at a 1:1 volume ratio for three hours. The prepared solution collected using a 10 mL syringe with a voltage of 15 kV, a needle tip diameter of 0.7 mm, and a distance between electrodes of 15cm. electrospinning performed at flow rate of 1 mL / hour. A DC power supply with voltage regulation created the electrically charged collagen and PCL jet. [29]. the electrospun collagen fibers and PCL scaffold sheet left to dry at room temperature[30] .then characterization of coat layer was performed.

B. Preparation of ascorbic acid loaded to mixed polymer solution (PCL & collagen)

Ascorbic acid used as a coated material. Because of large particle size of ascorbic acid which ranging from micrometers (μm) to a few millimeters (mm). The particle size of ascorbic acid reduced to nanoscale by grinding using planetary boll mill Machen. The milling done twice each duration was a half hour. Particle size measured by particle size analyzer. The final particle size was 142.8nm measured using 90 plus particles size software ver.5.3. At this step Ascorbic acid Nano-particle size can pass through the electrospinning needle during electrospinning procedure. In addition to, Nano size particles can make the polymer emulsion and precipitation less [31]. After complete preparation of collagen and PCL solution as mentioned previously in A as 1st solution .2nd solution of Ascorbic acid with the concentration of 0.1% w/v stirred for one hour to ensure that the Nano-sized vitamin C particles were evenly dispersed in the solution and prevent aggregation .After that the emlusion were stirred by using an ultrasonic homogenizer for 1minute to produce a drug-polymer suspension. The resulting mixture were loaded to 10ml needle and fixed to electrospinning aperture using the same parameters applied in collagen /PCL scaffold

electrospinning . namely a voltage of 15kV, a distance between electrodes of 15cm, and a flow rate of 1ml/h. [32] .

C. Preparing PCL polymer loaded by ascorbic acid

PCL particles dissolved in 98% glacial acetic acid in concentration 20% and stirred for three hour by magnetic bar at room temperature. Then ascorbic acid added at concentration 0.1% and stirred by magnetic bar for one hour .later on the suspension mixed by ultrasonic homogenizer for one minute and electrospinned with same parameter of electrospinning device used in this study.

2.1.2 Characterization of electrospinning scaffold:

This was done by using first FESEM examination done at a 15 KV accelerating voltage to evaluate fiber diameter and the distribution of ascorbic acid nanoparticles inside the fibers scaffold, the specimens were first coated in gold using sputtering. [33]. Scaffold analysis utilizing ImageJ software and SEM characterization were performed. Second, the wettability test conducted to determine the contact angle, which indicates hydrophilicity of the surface. Third analysis for the surface morphology and roughness by using AFM 2D and 3D imaging with particle size distribution. Fourth examination done by using Atomic force microscopy (AFM) to provide high-resolution three-dimensional (3D) topographic data, making it an excellent tool for topographic investigation of relatively flat surfaces.[34].

2.2 In vivo part of the study,

A CpTi implant was prepared with a 3mm diameter and 8mm length. Electrospinning coating performed for three different composition; collagen/PCl, collagen/PCL and Ascorbic acid, Ascorbic acid/PCL. White, healthy male 10–12-month-old New Zealand rabbits weighing 2.2–2.5 kilograms were employed as test subjects. The rabbits were submerged in an anti-parasite agent (Newcidol) to avoid any external skin infections. To ensure that the animals were parasite-free, Ivome intramuscular injection was administered, and oxy-tetracycline intramuscular injection was used as an antibiotic to cover the infection [35]. The surgical operation was performed under gentle highly sterile aseptic surgical technique, all instruments and towels were autoclaved at 134 C˚ and 15 bars for 90 minutes [36]. The coating samples are organic material so sterilization done under UV radiation for 24h [37]. Four groups of implants were subjected to in vivo investigation: the first group was non-coated CpTi as a base group; the second group was coated with a scaffold of collagen / PCL; the third group was CpTi coated with ascorbic acid loaded to collagen /pcl ; the fourth group was CpTi coated with ascorbic acid loaded to PCL. The study involved the evaluation of implants at two distinct time intervals, namely 2 weeks and 6 weeks post-implantation, in animal subjects. After the completion of the designated time periods, the animals were humanely sacrificed to retrieve the implant sites. To assess the bone response to the implants, histological specimens were meticulously prepared from bone blocks extracted at these intervals. Subsequently, a series of well-defined laboratory procedures were executed to prepare slides for examination under a light microscope. This comprehensive histological analysis allowed for the in-depth examination of tissue and bone interactions with the implanted material over time, shedding light on the biocompatibility and long-term effects of the implants within the biological system. [38]

2.2.1 **Biomechanical Push out bond strength**

Rabbits were sacrificed after 2 and 6 weeks time intervals. Right and left femurs with implant specimens were dissected and all flesh was removed as shown in (figure 2A, C). On the same day

as euthanasia, push out failure test was done by using Instron universal testing machine 5kN load at a rate of 1 mm/min with a working head 2mm in diameter. The clearance hole was made below the implant specimen at least 3.5mm in diameter to record the pure force required for the implant/bone bond breakage (figure 2.B) [39]**.** Powder and liquid of self-cure acrylic resin had mixed according to the manufacturer's instructions. The femur bone block had embedded in a dough mixture of self-cure acrylic resin at the base and the sides of the bone to facilitate the push-out test (figure2.D). The acrylic block had set at room temperature. The femur was fixed in a clamp for support the bone, and the clamp was fixed in the universal testing machine. The specimen was loaded at a rate of 1 mm/min; load was applied to the implant specimen through a specially designed plunger, with cylindrical working head 3 mm in diameter, connected to the crosshead of the universal testing machine, (figure2 .F). The maximum load of failure was recorded in Newton (N), the apparent shear stress was obtained from dividing the maximum load on the contact area which was the periphery of cylindrical implant specimen. [40] All data were analyzed by using SPSS version 22

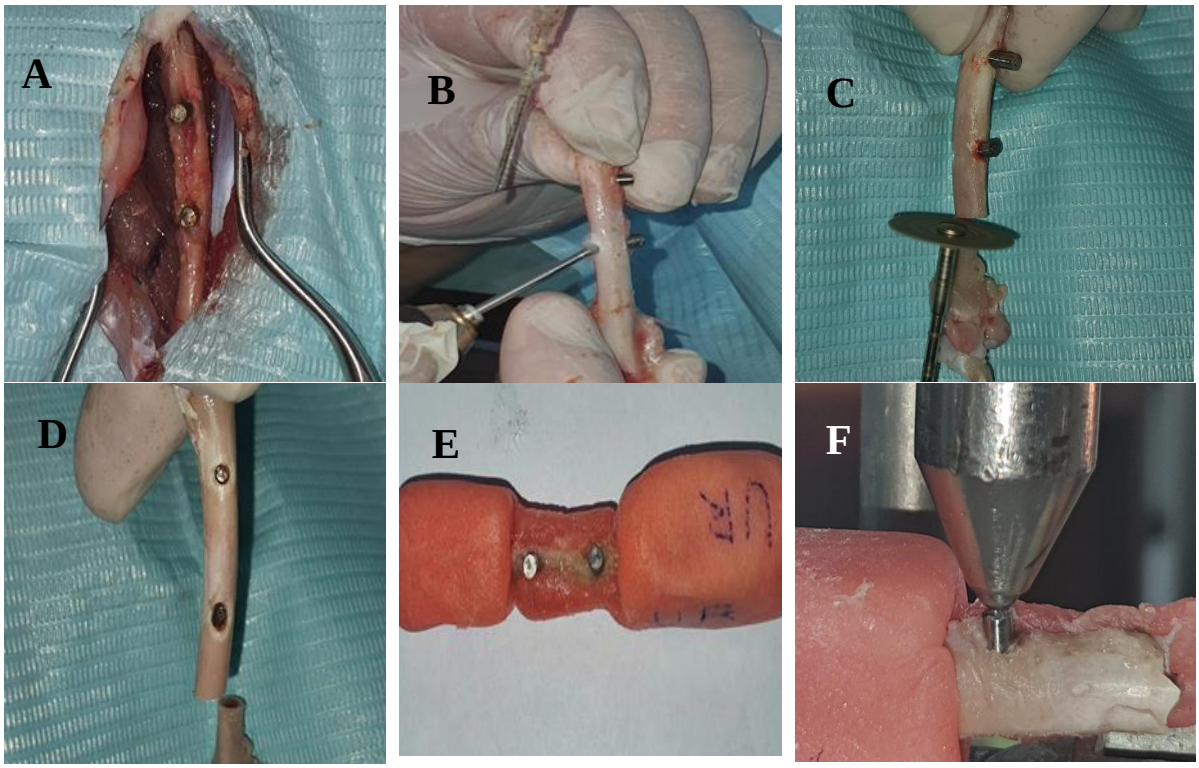

Figure 2: pushout bone strength test specimen preparation and measurements. open the surgical side and uncover the implants. remove the bone from basal side ;C,discting the bone ;D. The bone have been cute as block; E. use cold cure acrylic to support the specimen to be fixed in clamp E. The specimen was loaded

3. Result

3.1 Morphology characterization by field emission scanning electron

Three distinct scaffold shown in SEM (figure 3) with different fiber diameter and porosity. Image j conducted to measure the mean fibers diameter and porosity. The addition of 0.1w/v ascorbic acid to the collagen/PCL scaffold (Figure 4.B) resulted in a significantly smaller mean fiber width

compared to the scaffold prepared without ascorbic acid (Figure 4.A). Meanwhile, when ascorbic acid added to the PCL scaffold alone (Figure 4.C), there was no significant change in the mean fiber width compared to the collagen/PCL scaffold without ascorbic acid (Figure 1). These results suggest that the presence of ascorbic acid has a more pronounced effect on reducing fiber width in the collagen/PCL scaffold compared to the PCL scaffold alone.

The provided SEM images were able to provide valuable information regarding the porosity of different scaffold structures. The average porosity diameter for ascorbic acid/PCL scaffold was found to be 7767 nm. This suggests that the scaffold with only ascorbic acid and PCL has larger gaps or voids compared to the other scaffold types. The average porosity diameter for collagen/PCL scaffold was found to be 5700 nm. When ascorbic acid was added to collagen/PCL scaffold, the average porosity diameter increase to 6357nm.

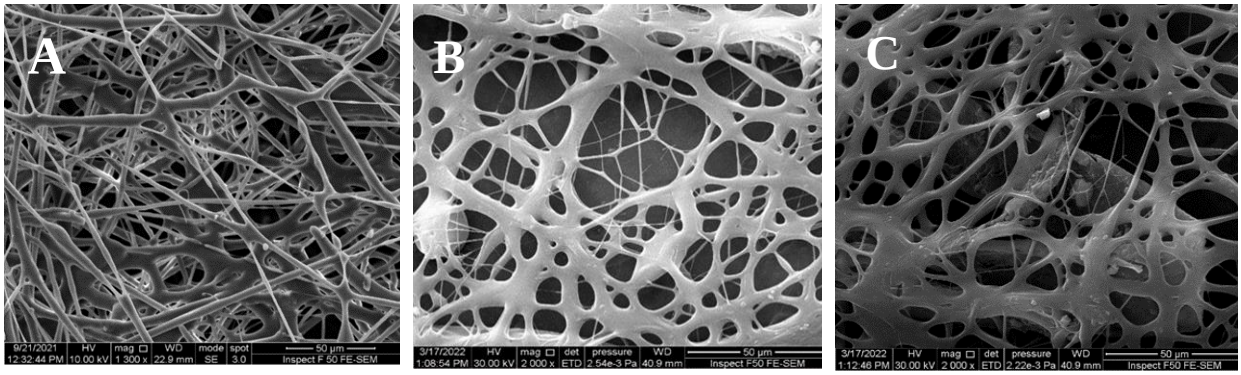

Figure 3: fibrous scaffold formed by electrospinning technique; .A. Collagen/PCL scaffold; B. Ascorbic acid loaded to collagen /PCL scaffold; and C. Ascorbic acid loaded to PCL

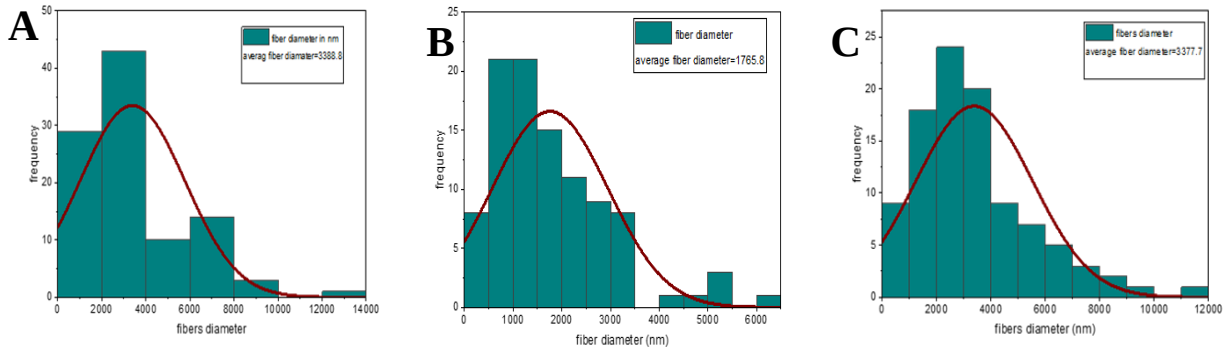

Figure 4: diagram show mean of fibers diameter with SD; A. collagen/PCL; B. ascorbic acid loaded to collagen/PCL; C ascorbic acid loaded to pcl alone

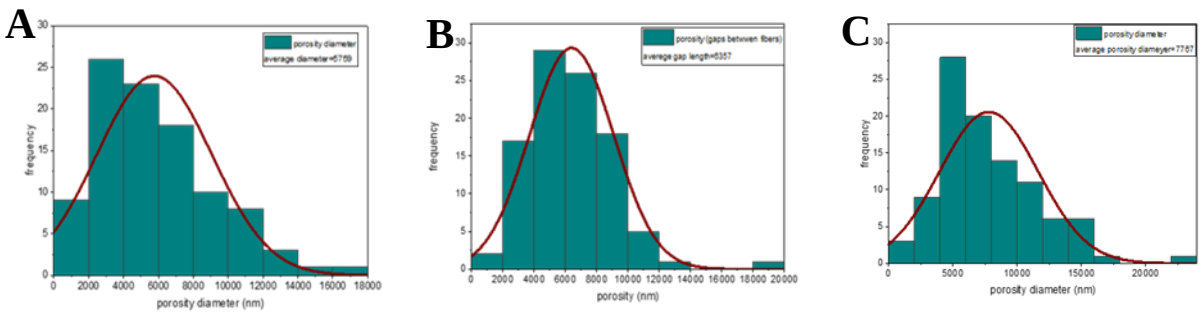

Figure 5: diagram show mean of purosity with SD;A. collagen/PCL; B. ascorbic acid loaded to collagen/PCL; C ascorbic acid loaded to pcl alone

3.2 AFM results

The height parameter "Sq" (root-mean-square roughness) was utilized to assess surface roughness.the highest value was found in ascorbic acid/PCL group 148.1nm, while the lowest sq value was found in collagen/pcl group which was 19.30nm, finally the ascorbic acid loaded to collagen/PCL was in midlle 82.96nm but its roughness closer to third group than the first .

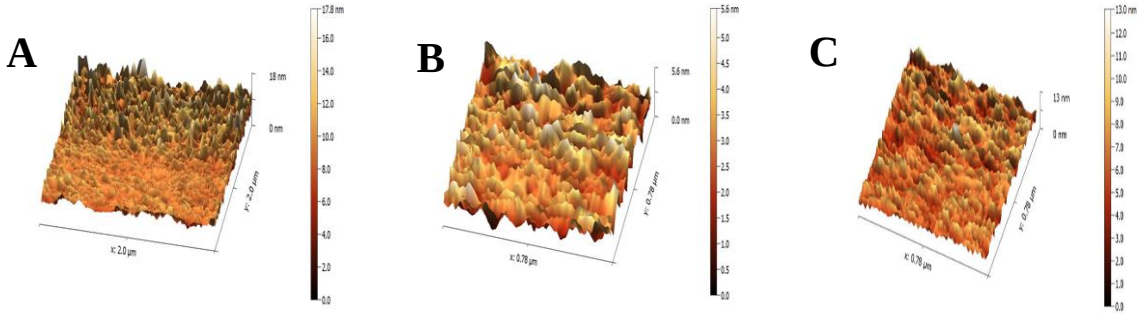

Figure 6: AFM TEST; A Group one; B. Group two ; C. Group three

3 .3Wettability and Contact Angle

Wetting is commonly characterized by the contact angle, which is defined as the angle between the tangent to the liquid vapor interface and the solid surface at the three-phase contact line [41]. In this study, an investigation of the wettability for different type of scaffolds. A mean contact angle of 25.24 degrees for collagen/PCL scaffold suggests moderate hydrophilicity; Ascorbic Acid loaded to Collagen/PCL Implant Surface has a mean contact angle of 12.49 degrees indicates a relatively high level of hydrophilicity; Ascorbic Acid & PCL Implant Surface a mean value of contact angle of 82.4030 degrees suggests significant hydrophobicity.

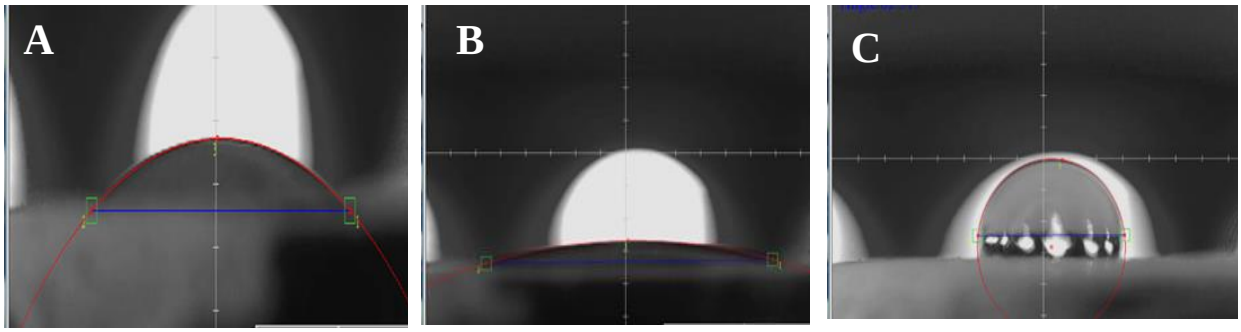

Figure 7: wettability test contact angle for three groups; A. collagen/PCL scaffold; B. ascorbic acid loaded to collagen/PCL; C. ascorbic acid loaded to PCL

3.4 Crosscut test

To make the judgment, an ISO rating scale was used to determine performance depending on ISO 2409:2013. Group 1 (collagen/PCL) has the largest proportion of adhesion level at 5B%16.7 (the edges of the cuts are completely smooth; none of the squares of the lattice are detached), whereas Group 2 (vitamin C added to collagen/PCL) has the highest percentage of adhesion level at 4B (detachment of small flakes of the coating at the intersections of the cuts). A cross-cut area not significantly greater than 5% is affected.

Table 1: ***Description for frequency, percentages and Chi-Square Tests of cross hatch adhesion test***

Groups		**Data**				**Total**
		2B	**3B**	**4B**	**5B**	
Group 1	Fr	0	1	4	5	10
	%	0.0%	3.3%	13.3%	16.7%	33.3%
Group 2	Fr	2	1	7	0	10
	%	6.7%	3.3%	23%	0%	33.3%
Group 3	Fr	0	4	3	3	10
	%	0.0%	13.3%	10.0%	10.0%	33.3%
Total	Fr	2	6	7	15	30
	%	6.7%	20.0%	23.3%	50.0%	100.0%
Chi-Square Tests	**Fisher's Exact Test**		**Value**	**Exact Sig. (2-sided)**		
			10.572	**0.049**		
Symmetric Measures	**Symmetric Measures**		**Phi**	**0.641**		

3.5 Histological features of bone/implant at two week intervals after implantation

Histological figures of bone after implantation for 2week (figure.8) showed the cortical bone revealed normal appearance and fibrous osteogenic tissue in all groups but it show thicker layer in third group that formed from CpTi coated with ascorbic acid and collagen/PCL ,then the collagen/PCL group and nan coated CpTi group , the thinner osteogenic fibrous layer shown in ascorbic acid/PCL group. Figure.9 show the response of bone after 6-week implantation and in all groups there is layer of new bone formation around the implants but the ascorbic acid /PCL group show the thinner bone formation in comparison to the other groups and ascorbic acid with collagen/PCL show better response by formation thicker new bone layer.

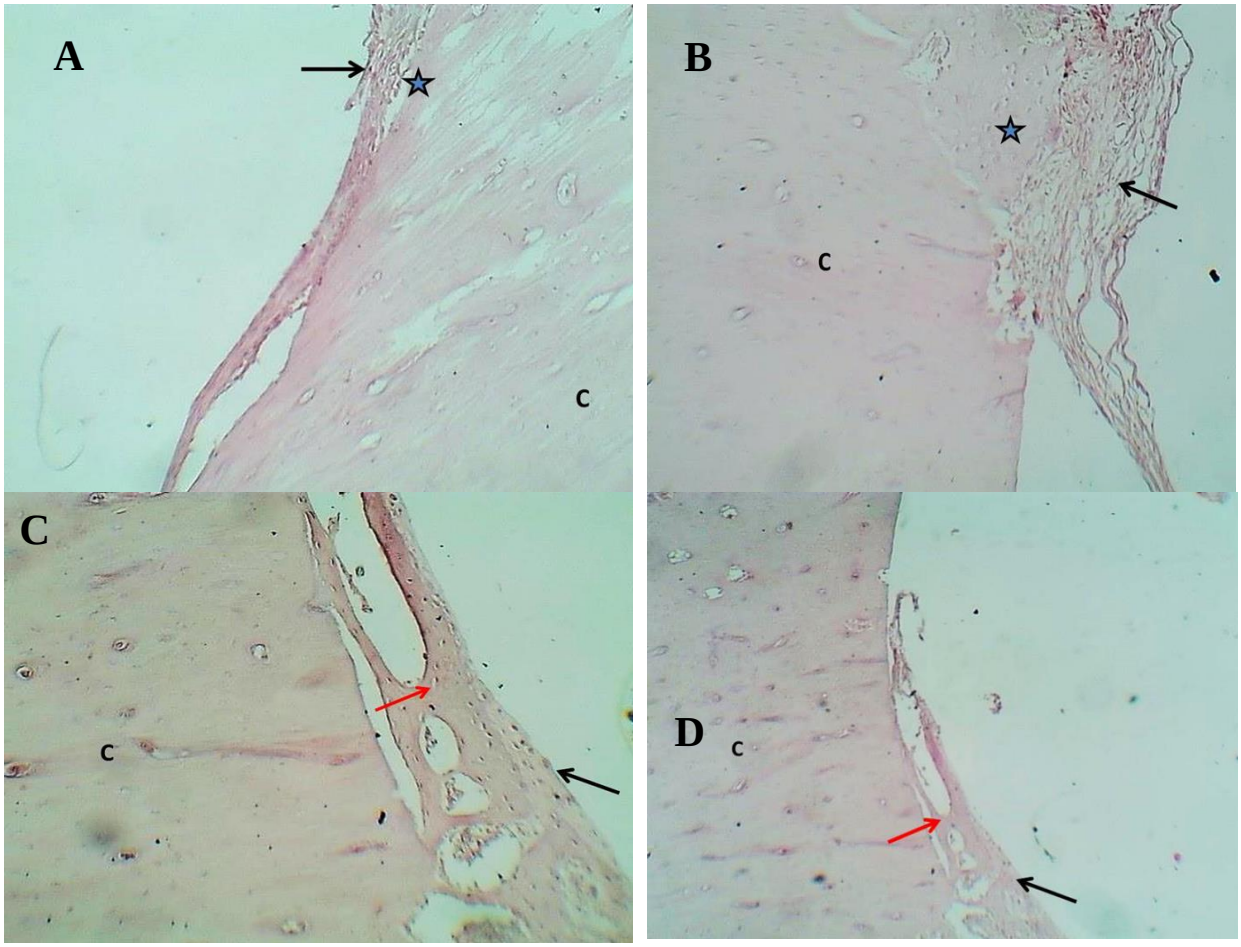

Figure 8; histological figure of four groups at 100x magnification, A. non-coated CpTi; B. coated with collagen/PCL;C. coated with ascorbic acid loaded to collagen/PCL ;D. Coated with ascorbic acid/PCL

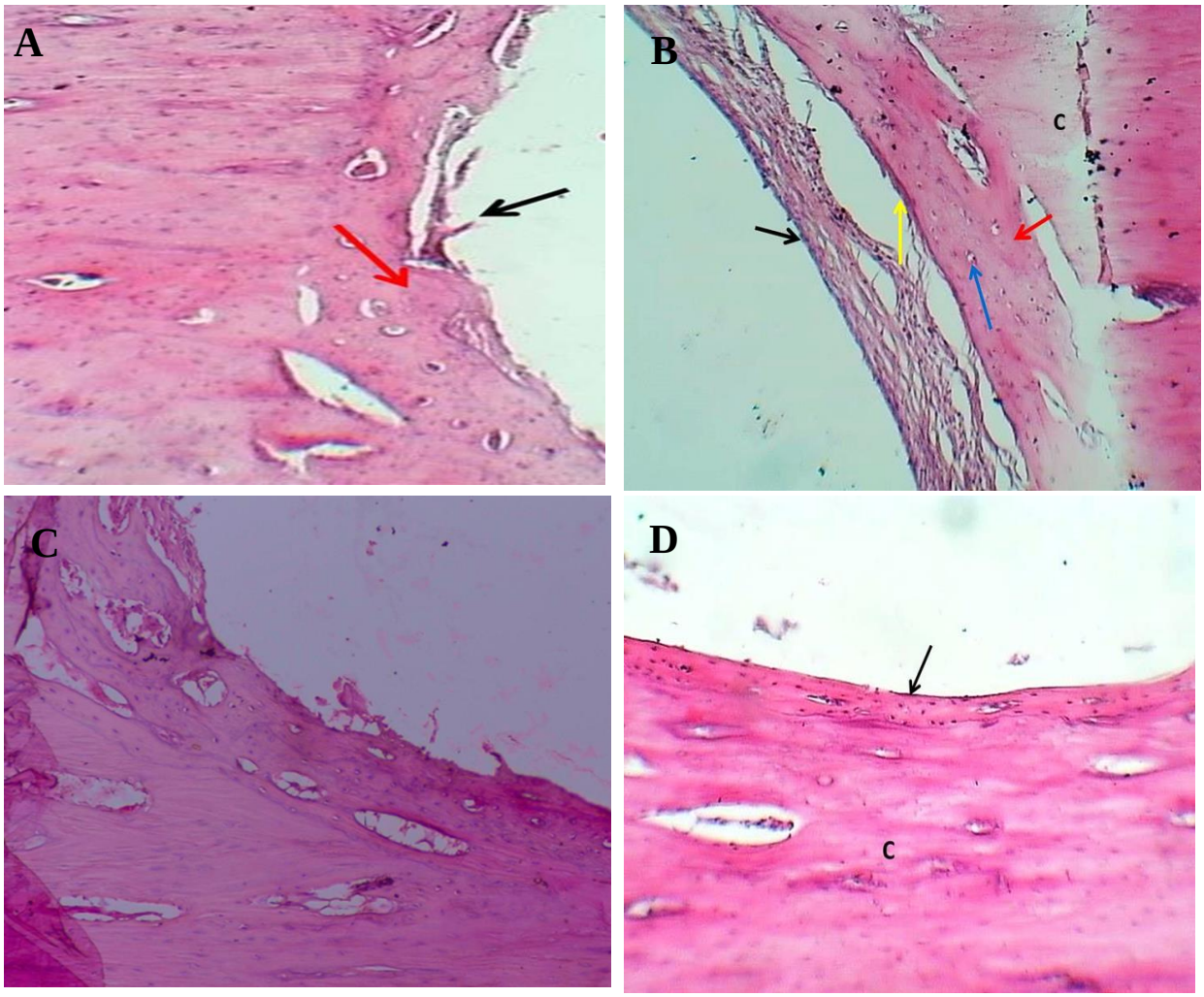

Figure 9: histological figures show bone after 6 week implantation Magnification 100xx; A. bone of non-coated CpTi ;B. for collagen/PCL scaffold;C.for ascorbic acid loaded to collagen /PCL ;D. for coated ascorbic acid /PCL

3.6 Pushout bone strength test

The results show that the push-out bond strength tends to increase over the 2- to 6-week period in all groups, indicating potential improvement in the integration of the implants with the surrounding tissue. Group 3 has the highest mean bond strength at 2 weeks (22.2520), followed by Group 2 (20.3270), Group 1 (18.1750), and Group 4 (16.7510). Group 3 continues to have the highest mean bond strength at 6 weeks (24.4560), followed by Group 2 (22.8150), Group 1 (21.8880), and Group 4 (20.1980).

Table 2: mean values and standered deviation of pushout bone strength for each studied group after 2 and 6 weeks implantation

groups	time	means	std.	F	sig
group 1	2weeks	18.1750	0.65997	74.141	0.000
	6weeks	21.8880	1.19328		
group 2	2weeks	20.3270	0.64902	74.780	0.000
	6weeks	22.8150	0.63762		
group 3	2weeks	22.2520	0.92067	32.842	0.000
	6weeks	24.4560	0.79462		
group4	2weeks	16.7510	1.57370	40.993	0.000
	6weeks	20.1980	0.64959		

3. Discussion

In the present study, we prepared and characterized scaffold for coating dental implants. To enhance bone-healing efficiency of a scaffold, different composite of scaffolds prepared and various in vitro and in vivo tests were used to evaluate the role of combination of collagen and ascorbic acid in bone healing. According to the literature, the morphology and diameter of fibers in the electrospinning process are dependent on the intrinsic properties of the polymer solution such as the type of polymer, the conformation of polymer chain or molecular weight, viscosity or concentration, elasticity, electrical conductivity, and the polarity and surface tension of the solvent[42], in addition to Processing parameters as the applied voltage, the injection rate, and the collector distance can also affect the properties of the fibers[23] . The molecular weight of the polymer has a significant effect on rheological and electrical properties such as viscosity, surface tension, conductivity and dielectric strength[42]

The SEM analysis showed that the electrospinning of the first group with collagen and PCL produced a fibrous scaffold had a uniform structure with smooth morphology and they were randomly distributed. In contrast, the addition of ascorbic acid to the collagen and PCL solution resulted in a scaffold with smaller fiber diameter it is possible to see that when the injection rate decreases because of the ascorbic acid Nano particles hamper the flow of polymer, the diameter of the fibers decreased and cluster that filled with ascorbic acid nanoparticles appear, and this is the same with third group when adding ascorbic acid to PCL solution , also the increase in viscosity of solution as a result of adding ascorbic acid effect the porosity and fibers diameter. The viscosity of solution varies with the various compositions. The higher the electrospinnability, the lower the viscosity, It indicated why electrospinnability was excellent collagen/ PCL alone [43], Therefore, the injection rate was the factor that had the most impact on the shape and diameter of the fibers. Colmenares-Roldán 2017 found that when the injection rate was low, more nanofibers were formed during electrospinning.

The surface roughness of Collagen/PCL Scaffold (group1 indicates that this group has a relatively smooth surface at the analyzed length scale and this shown in SEM analysis the fibers are smooth and clear because there is no particles add to electrospinning polymer solution . In case of Collagen/PCL Loaded with Ascorbic Acid (group2): suggests that the addition of ascorbic acid to the collagen/PCL scaffold has led to an increase in surface roughness as the presence of undissolved ascorbic acid particles has great role in increase surface roughness. The results of PCL scaffold loaded with ascorbic acid indicates a relatively rough surface also this related to incorporation of ascorbic acid, which distributed on the fibers surface in addition to formation of many clusters and nodules that filled with ascorbic acid this can influence the surface roughness of the scaffolds. This alteration in roughness could be linked to changes in the scaffold's microstructure due to the presence of ascorbic acid.

Any implant material wettability has a major impact on how well cells adhere to and grow on the surface, making it a critical component of success[44]. It is characterized as the attraction of a liquid phase to a solid surface, and it may be measured by determining the liquid contact angle with the surface [45] . Hydrophilicity or hydrophobicity of an implant surface can influence its interaction with body fluids and tissues. A more hydrophilic surface might facilitate better cell adhesion and integration, which can be beneficial for certain implant applications. Conversely, a hydrophobic surface might reduce the adhesion of biological materials, potentially preventing undesirable interactions[46]. In other words, the surface of the Collagen/PCL implant appears to have moderate affinity for liquids. It is neither strongly hydrophilic nor strongly hydrophobic meaning it has a strong tendency to attract and interact with liquids, this medium hydrophilicity is because the scaffold formed from 1:1 of collagen which is hydrophilic and PCL which is hydrophobic, while the in the second group the hydrophilicity increase because of adding ascorbic acid which is hydrophilic in addition the hydrophilicity can be increase as the surface roughness increase which is conducted by AFM test[47] [48] , finally ascorbic Acid & PCL Implant scaffold Surface has the highest significant hydrophobicity And this will lead to appears to repel liquids and is less likely to interact with them. This could be due to the hydrophobic nature of the PCL material which is the main polymer in scaffold.

Finally, ascorbic acid and PCL implant scaffold the surface has the highest significant hydrophobicity. The ascorbic acid and PCL implant surface appear to repel liquids and are less likely to interact with them. This could be due to the hydrophobic nature of the PCL material. In addition, adding ascorbic acid to collagen increases surface roughness, which also has an appositive effect on hydrophilicity.

The histological results reveal that the scaffold formed from ascorbic acid loaded to collagen/PCL have thick osteoginc fibrous tissue in 2week implant and formation of thick new bone when investigate the 6 week implant, this finding improved by bush out bone strength test that show the shear strength for both intervals 2 and 6 week is greater for vitamin C loaded collagen/PCL group this improvement can be related to multiple factors, first the morphological factor which this type has increased pores size which will give more permeability for body fluid and cells, second the surface roughness increase which improve cell attachment , and the increase of surface hydrophilicity will increase cell attraction and body fluid, finally which is the more important is

the composition of the scaffold from ascorbic acid and collagen as they both have great impact on bone healing and osseointegration, as ascorbic acid plays a great role in the expression of osteocalcin and Runx2, which are key genes for osteoblast differentiation. Ascorbic acid deficiency can lead to bone pain and incomplete wound healing; many studies have shown that ascorbic acid modulates various cellular responses, such as proliferation, differentiation and extracellular matrix synthesis. Ascorbic acid at 50 mg/mL promotes cell proliferation, which is evident in malignant cells and normal cells [49]. In a separate study using mesenchymal stromal cells of bone marrow, supplementation of ascorbic acid enhanced proliferation of mesenchymal stromal cells without compromising the differentiation potency [50]. Conductor to these observations, induction by ascorbic acid improve proliferation of suspension MNCs, and this effect marked the beginning of a differentiation process induced by ascorbic acid [51], in addition the combination of ascorbic acid with collagen which has reported its beneficial effect on enhancing bone formation [52], collagen-based scaffolds can be surface modified by attaching bioactive substances to promote bone regeneration. Collagen based hydrogel is made up of collagen and other materials, which makes up for the problem of excessive collagen degradation and swelling. The degradation time of collagen-based hydrogel can be prolonged for several months according to different materials. Bioactive sub stances can be directly loaded into hydrogels and released at appropriate concentrations to promote bone regeneration locally [53]. All these researches and finding improve this study which will have great impact on dental implant.

4. Conclusion

within limitation of this study it can be concluded that electrospinning coating of collagen and ascorbic acid have adequate surface feature such as surface roughness hydrophilicity that improve the bond strength of implants with bone in rabbit models and enhance osseointegration.

Further research may be needed to understand the mechanisms behind these effects and their implications for tissue engineering or other applications. In addition, studies could investigate into the relationship between surface roughness, cellular response, and the structural characteristics of these scaffolds to inform their suitability for different applications.

4. Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not for- profit sectors

5. Conflict of interest

The authors have no conflicts of interest to declare.

6. Ethical approval

The study were approved by Ethics Committee of the College of Dentistry, University of Baghdad

Project No.	830223

7. Authors contributions

Conceptualization, SH.M. and R.K.; Data Collection, SH.M.; Methodology, SH.M.;R.K.;A.R. ; Writing-Original Draft Preparation, SH.M.; Writing- Review & Editing, SH.M.; R. K.;A.R.;

Supervision, R.K.;A.R.; Project Administration, SH.M.; R.K.;; Funding Acquisition, SH.M.;R.K.;A.R.. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

8. Reference

[1] L. Ibraheem, B. Ahmmad, A. Dhafer, and J. Dhafer, "Effect of diabetes mellitus on periodontal health status, salivary flow rate and salivary pH in patients with chronic periodontitis," *J. Baghdad Coll. Dent.*, vol. 32, no. 2, pp. 12–16, 2020.

[2] K. Gulati, D. Chopra, N. A. Kocak-Oztug, and E. Verron, "Fit and forget: The future of dental implant therapy via nanotechnology," *Adv. Drug Deliv. Rev.*, vol. 199, p. 114900, 2023.

[3] M. Morra, "Biochemical modification of titanium surfaces: Peptides and ECM proteins," *Eur. Cells Mater.*, vol. 12, pp. 1–15, 2006.

[4] S. Hauck *et al.*, "Collagen/hyaluronan based hydrogels releasing sulfated hyaluronan improve dermal wound healing in diabetic mice via reducing inflammatory macrophage activity," *Bioact. Mater.*, vol. 6, no. 12, pp. 4342–4359, 2021.

[5] H. H. Afify, M. E. Hassan, A. M.badr, and H. A. Elsheikh, "Effect of hydrogen on pristine amorphous v 2 O 5 thin film," *J. baghdad Eng. Technol.*, vol. 7, no. 1, pp. 1–5, 2018.

[6] S. M. Lupi, M. Torchia, and S. Rizzo, "Biochemical modification of titanium oral implants: Evidence from in vivo studies," *Materials (Basel).*, vol. 14, no. 11, 2021.

[7] N. T. B. Linh, C. D. G. Abueva, D. W. Jang, and B. T. Lee, "Collagen and bone morphogenetic protein-2 functionalized hydroxyapatite scaffolds induce osteogenic differentiation in human adipose-derived stem cells," *J. Biomed. Mater. Res. - Part B Appl. Biomater.*, vol. 108, no. 4, pp. 1363–1371, 2020.

[8] A. J. Morwood, I. A. El-Karim, S. A. Clarke, and F. T. Lundy, "The Role of Extracellular Matrix (ECM) Adhesion Motifs in Functionalised Hydrogels," *Molecules*, vol. 28, no. 12, p. 4616, 2023.

[9] M. S. Carvalho and J. M. S. Cabral, "Bone Matrix Non-Collagenous Proteins in Tissue Engineering : Creating New Bone by Mimicking the Extracellular Matrix," pp. 1–33, 2021.

[10] C. Behrens, P. Kauffmann, N. von Hahn, U. Schirmer, K. Liefeith, and H. Schliephake, "Collagen-Based Osteogenic Nanocoating of Microrough Titanium Surfaces," *Int. J. Mol. Sci.*, vol. 23, no. 14, 2022.

[11] V. Pankova, K. Thway, R. L. Jones, and P. H. Huang, "The Extracellular Matrix in Soft Tissue Sarcomas : Pathobiology and Cellular Signalling," vol. 9, no. December, pp. 1–17, 2021.

[12] R. Naomi, P. M. Ridzuan, and H. Bahari, "Current Insights into Collagen Type I," *Polym. MDPI*, pp. 1–19, 2021.

[13] V. Horbert *et al.*, "In Vitro Analysis of Cartilage Regeneration Using a Collagen Type I Hydrogel (CaReS) in the Bovine Cartilage Punch Model," *Cartilage*, vol. 10, no. 3, pp. 346–363, 2019.

[14] B. M. Mohammed *et al.*, "Resolution of Sterile Inflammation : Role for Vitamin C," vol. 2014, 2014.

[15] X. X. Yu *et al.*, "Ascorbic acid induces global epigenetic reprogramming to promote meiotic maturation and developmental competence of porcine oocytes," *Sci. Rep.*, vol. 8, no. 1, pp. 1–12, 2018.

[16] A. Ahuja, R. Staffy, M. Minz, V. Ahuja, A. Mishra, and S. Kumari, "Evaluation of Regenerative Potential of Locally Delivered Vitamin C along with Microneedling in the Treatment of Deficient Interdental Papilla : A Clinical Study," pp. 2–6, 2022.

[17] J. M. Pullar, A. C. Carr, and M. C. M. Vissers, "The roles of vitamin C in skin health," *Nutrients*, vol. 9, no. 8, 2017.

[18] A. M. Nimer and R. K. Jassim, "Studying the effect of ascorbic acid on some properties of autoclave and heat cure denture base material," *Pakistan J. Med. Heal. Sci.*, vol. 14, no. 4, pp. 1714–1718, 2021.

[19] B. M. Mohammed *et al.*, "Vitamin C promotes wound healing through novel pleiotropic mechanisms," *Int. Wound J.*, vol. 13, no. 4, pp. 572–584, 2016.

[20] Z. Łukasik, J. Makowska, K. Walczak, and O. Brzezi, "Role of Vitamin C in Osteoporosis Development and Treatment — A Literature Review," pp. 1–22, 2020.

[21] B. Sowmya, A. B. Hemavathi, and P. K. Panda, "Poly (ε-caprolactone)-based electrospun nano-featured substrate for tissue engineering applications: a review," *Prog. Biomater.*, vol. 10, no. 2, pp. 91–117, 2021.

[22] E. Margerrison, M. Argentieri, D. Kommala, and C. N. Schabowsky, "Polycaprolactone (PCL) Safety Profile Report Details Date of Submission ECRI Corporate Governance Project Manager," no. 540, 2021.

[23] A. Al-Khateeb, E. S. Al-Hassani, and A. R. Jabur, "Metallic Implant Surface Activation through Electrospinning Coating of Nanocomposite Fiber for Bone Regeneration," *Int. J. Biomater.*, vol. 2023, 2023.

[24] M. Li, M. J. Mondrinos, X. Chen, M. R. Gandhi, F. K. Ko, and P. I. Lelkes, "Elastin Blends for Tissue Engineering Scaffolds," *J. Biomed. Mater. Res. Part A*, vol. 79, no. 4, pp. 963–73, 2006.

[25] A. Zielińska *et al.*, "Scaffolds for drug delivery and tissue engineering: The role of genetics," *J. Control. Release*, vol. 359, pp. 207–223, 2023.

[26] X. Gao, S. Han, R. Zhang, G. Liu, and J. Wu, "Progress in electrospun composite nanofibers: Composition, performance and applications for tissue engineering," *J. Mater. Chem. B*, vol. 7, no. 45, pp. 7075–7089, 2019.

[27] B. N. Blackstone, S. C. Gallentine, and H. M. Powell, "Review collagen-based

electrospun materials for tissue engineering: A systematic review," *Bioengineering*, vol. 8, no. 3, pp. 1–16, 2021.

[28] H. A. Owida *et al.*, "Recent Applications of Electrospun Nanofibrous Scaffold in Tissue Engineering," *Appl. Bionics Biomech.*, vol. 2022, pp. 1–15, 2022.

[29] S. A. Moosa, A. R. Jabur, and E. S. Al- Hassani, "Preparation and physical properties of Pcl-metoprolol tartrate electospun nanofibers as drug delivery system," *Key Eng. Mater.*, vol. 886, pp. 183–188, 2021.

[30] J. R. Dias, A. Sousa, A. Augusto, P. J. Bártolo, and P. L. Granja, "Electrospun Polycaprolactone (PCL) Degradation: An In Vitro and In Vivo Study," *Polymers (Basel).*, vol. 14, no. 16, pp. 1–15, 2022.

[31] A. Luraghi, F. Peri, and L. Moroni, "Electrospinning for drug delivery applications: A review," *J. Control. Release*, vol. 334, pp. 463–484, 2021.

[32] A. R. Jabur, L. K. Abbas, and S. A. Moosa, "Fabrication of Electrospun Chitosan / Nylon 6 Nanofibrous Membrane toward Metal Ions Removal and Antibacterial Effect," vol. 2016, 2016.

[33] N. Brodusch, H. Demers, and R. Gauvin, *Field emission scanning electron microscopy: New perspectives for materials characterization*. 2018.

[34] Z. Chen, J. Luo, I. Doudevski, S. Erten, and S. H. Kim, "Atomic Force Microscopy (AFM) Analysis of an Object Larger and Sharper than the AFM Tip," *Microsc. Microanal.*, vol. 25, no. 5, pp. 1106–1111, 2019.

[35] B. Thomas, K. Bhat, and M. Mapara, "Rabbit as an animal model for experimental research," *Dent. Res. J. (Isfahan).*, vol. 9, no. 1, p. 111, 2012.

[36] B. Skiles, N. A. Johnston, G. K. Hendrix, and D. L. Hickman, "Effectiveness of the Glass Bead Sterilizer for Sterilizing Surgical Instruments," *J. Am. Assoc. Lab. Anim. Sci.*, vol. 61, no. 3, pp. 252–255, 2022.

[37] F. A. and A. and M. AL-Askar, "Does Ultraviolet Radiation Exhibit Antimicrobial Effect against Oral Pathogens Attached on Various Dental Implant Surfaces ?," *Dent. J.*, 2022.

[38] Z. S. Abdullah, M. S. Mahmood, F. M. A. Abdul-Ameer, and A. A. Fatalla, "Effect of commercially pure titanium implant coated with calcium carbonate and nanohydroxyapatite mixture on osseointegration," *J. Med. Life*, vol. 2023, no. 1, pp. 52–61, 2023.

[39] Z. T. Lawson, J. Han, W. B. Saunders, M. A. Grunlan, M. R. Moreno, and A. B. Robbins, "Methodology for performing biomechanical push-out tests for evaluating the osseointegration of calvarial defect repair in small animal models," *MethodsX*, vol. 8, p. 101541, 2021.

[40] M. A. Alkhodary and A. Alkhodary, "Axial Push-out Resistance of Dental Implant Different Thread Designs," vol. 7, no. 6, pp. 21–26, 2023.

[41] D. Katyal, A. K. Subramanian, A. Venugopal, and A. Marya, "Assessment of Wettability

and Contact Angle of Bonding Agent with Enamel Surface Etched by Five Commercially Available Etchants: An in Vitro Study," *Int. J. Dent.*, vol. 2021, 2021.

[42] G. J. Colmenares-Roldán, Y. Quintero-Martínez, L. M. Agudelo-Gómez, L. F. Rodríguez-Vinasco, and L. M. Hoyos-Palacio, "Influence of the molecular weight of polymer, solvents and operational condition in the electrospinning of polycaprolactone," *Rev. Fac. Ing.*, vol. 2017, no. 84, pp. 35–45, 2017.

[43] A. Haider, S. Haider, and I. K. Kang, "A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology," *Arab. J. Chem.*, vol. 11, no. 8, pp. 1165–1188, 2018.

[44] A. T. Noaman and Y. Salwan, "The relationship of implant stability quotient and insertion torque in dental implant stability," *J. Baghdad Coll. Dent.*, vol. 34, no. 1, pp. 29–35, 2022.

[45] R. A. Gittens *et al.*, "A review on the wettability of dental implant surfaces II: Biological and clinical aspects," *Acta Biomater.*, vol. 10, no. 7, pp. 2907–2918, 2014.

[46] S. Kligman *et al.*, "The impact of dental implant surface modifications on osseointegration and biofilm formation," *J. Clin. Med.*, vol. 10, no. 8, 2021.

[47] N. Yi and Z. Chiang, "Topical Vitamin C and the Skin," *Jcad J. Clin. Aesthetic Dermatology*, vol. 14, no. 7, pp. 14–17, 2017.

[48] S. Zhou *et al.*, "Effects of surface roughness on the hydrophilic particles-air bubble attachment," *J. Mater. Res. Technol.*, vol. 18, pp. 3884–3893, 2022.

[49] S. N. Hadzir *et al.*, "Ascorbic acid induces osteoblast differentiation of human suspension mononuclear cells," *Cytotherapy*, vol. 16, no. 5, pp. 674–682, 2014.

[50] T. Maekawa, T. Miyake, M. Tani, and S. Uemoto, "Diverse antitumor effects of ascorbic acid on cancer cells and the tumor microenvironment," *Front. Oncol.*, vol. 12, no. September, pp. 1–16, 2022.

[51] H. K. Choi *et al.*, "Vitamin C activates osteoblastogenesis and inhibits osteoclastogenesis via Wnt/β-catenin/ATF4 signaling pathways," *Nutrients*, vol. 11, no. 3, 2019.

[52] S. Santhakumar, A. Oyane, M. Nakamura, Y. Yoshino, M. K. Alruwaili, and H. Miyaji, "Bone tissue regeneration by collagen scaffolds with different calcium phosphate coatings: Amorphous calcium phosphate and low-crystalline apatite," *Materials (Basel).*, vol. 14, no. 19, 2021.

[53] Y. Li *et al.*, "Collagen-based biomaterials for bone tissue engineering," *Mater. Des.*, vol. 210, p. 110049, 2021.