Histological evaluation of (Nano-chitosan / calcium carbonate paste) as injectable pulp capping material In vivo study on animal s

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INTRODUCTION

OVERVIEW

Dental pulp is a highly specialized connective tissue occupy the centre of the tooth and surrounded by mineralized tissue, it preform vital function for the tooth and periodontium

VITAL PULP is essential for tooth viability, development ,nutrition, protection, and sensory functions

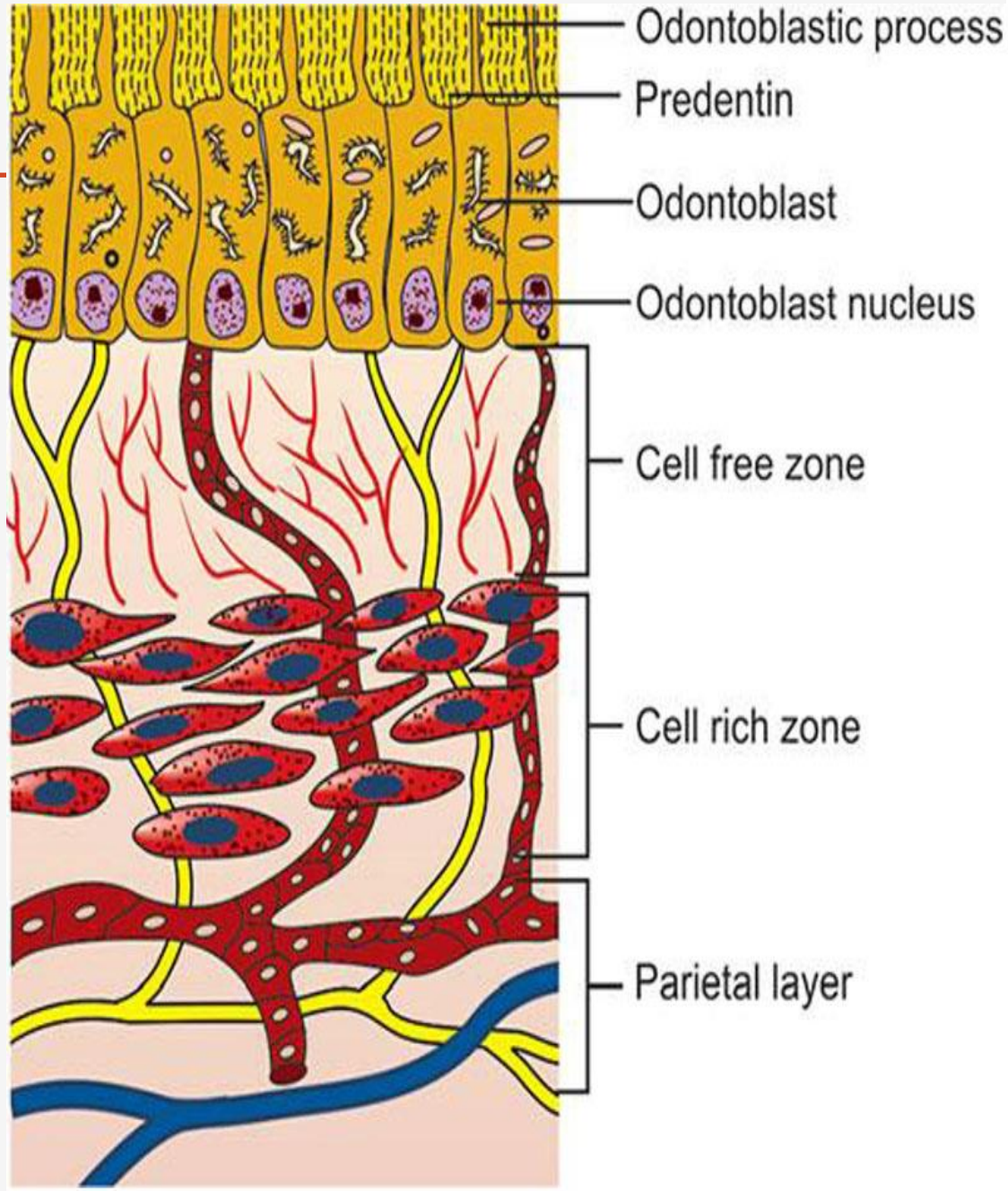
MAINTAINING PULP VITALITY AND FUNCTIONS are of paramount importance in different branches of modern dentistry like (Conservative,Endodontics, Pedodontics ,Traumatology and Orthodontics)

- Vital pulp is delicate soft tissue with unique histological structure composed of four distinct anatomical zone

(odontoblastic ,cell free zone ,cell rich zone and pulp trunck) .

- Vital pulp posses the ability to produce mineralized tissue (dentine) throughout the life under normal and pathological conditions

- Dentine is a calcified organic matrix produce by pulpal cell ,act as hard protective shield against external irritant factor that may be compromise pulp vitality and provide cushion effect reduce the incidence of fracture of the tooth due to force of occlusion.



❑ Vital pulp produce three type of dentine

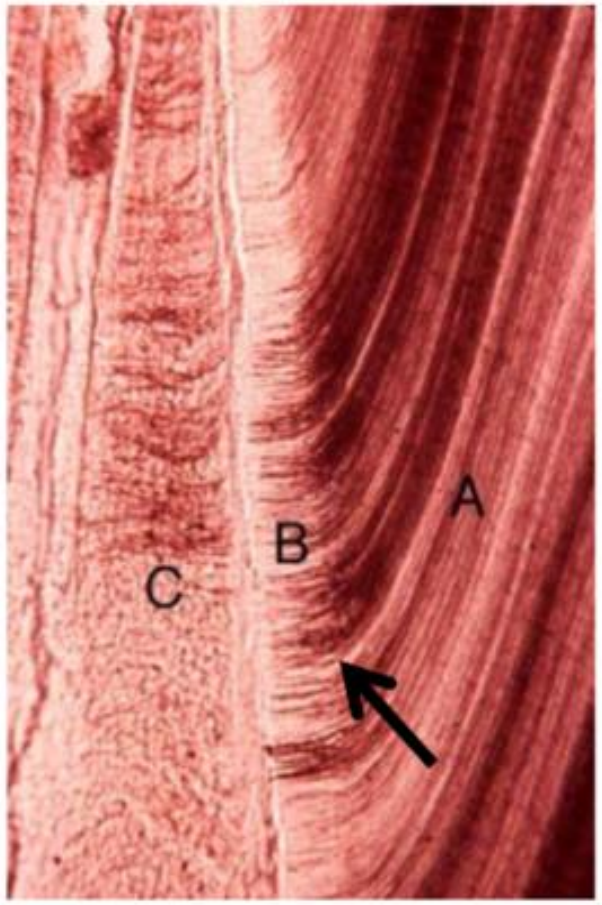
✓ Primary dentine :which have regular tubular calcified structure and produced by pulpal cell (odontoblast) during tooth development till reaching to occlusion

✓ Secondary dentine: which is produced by pulpal cell after reaching the tooth to the occlusion ,having continuous regular tubular structure with primary dentine

✓ Tertiary dentine:type of dentine produced by pulpal cell in response to irritation ,have mostly A tubular or irregular tubular structure and soldemly having regular tubular structure and separated by calciotraumatic line from secondary dentine

✓ Its of two type (reactionary produced by viable odontoblast cell)or (reparative produced by pulpal cell other than odontoblast cell)

✓ despite the process of tertiary dentine formation is unpredictable and may associated with the many defect (tunnel defect,hypomineralized area) but it represent the most reliable protective assets of the vital pulp against irritant factor and their quality and quantity mostly used by invivo studies to characterise the pathophysiological response of vital pulp to irritation or pulp capping material



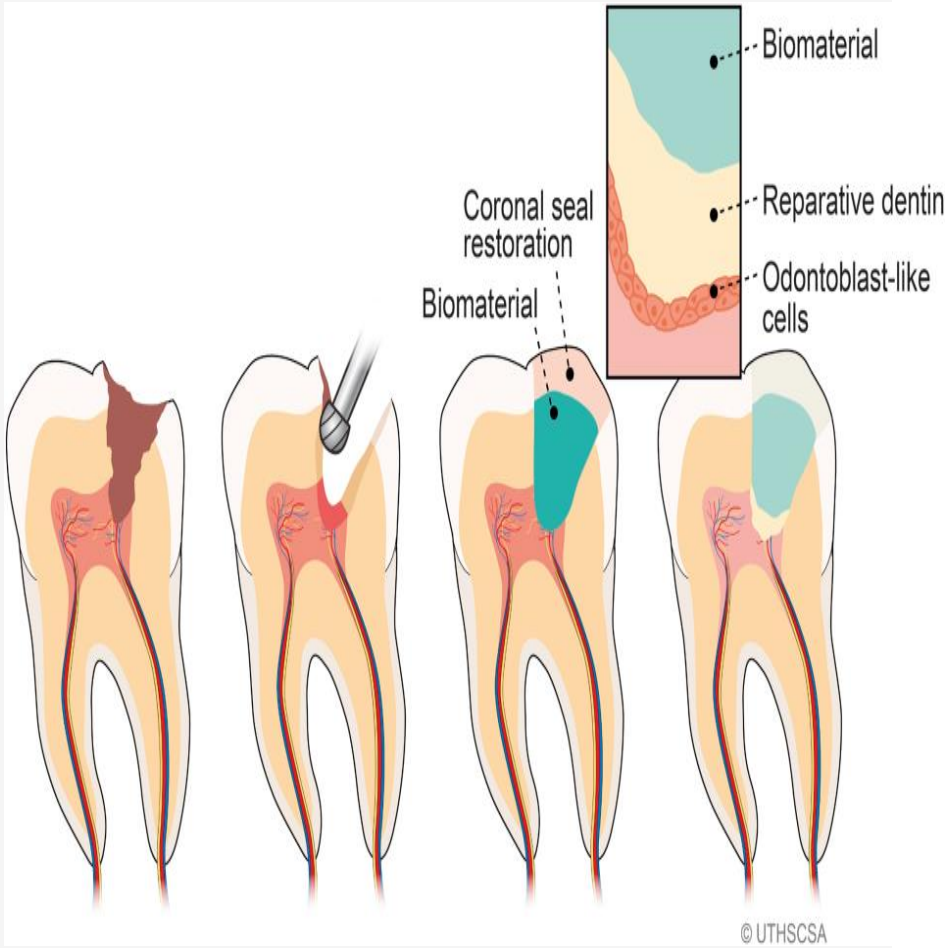
A. Primary dentin
B. Secondary (regular) dentin
C. Tertiary (reactive) dentin

BACKGROUND

- **If the vital pulp is not severely damaged, it has great potential to survive by application of Vital Pulp Therapy with the aid of pulp capping material**

- **Vital pulp therapy (VPT) is minimally invasive endodontic treatment involves the selective and conservative surgical removal of infected or inflamed tissue (pulp or dentine)and replace it by capping material ,where the main functions of pulp capping material are:**
- **Protect the pulp against irritant factors especially microbial factor**
- **Create conducive environment for pulp healing process**

- **Increasing the demand on VPT as minimum invasive endodontic treatment, driven by the necessity to address and treat the drawbacks of current pulp capping material have stimulate the efforts to find innovative pulp capping material or adopting new therapeutic concepts (nano-technology or tissue engineering) to optimize the clinical outcomes in more predictable fashion.**



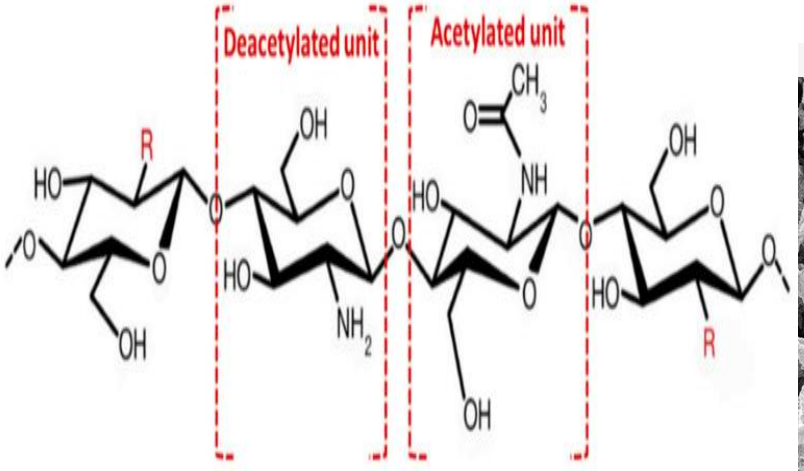
Nano-chitosan

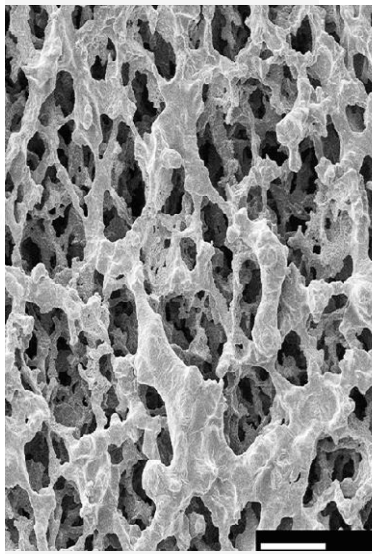
Chitosan is a linear polysaccharide derived from chitin and has a chemical structure similar to glycosaminoglycans of ECM

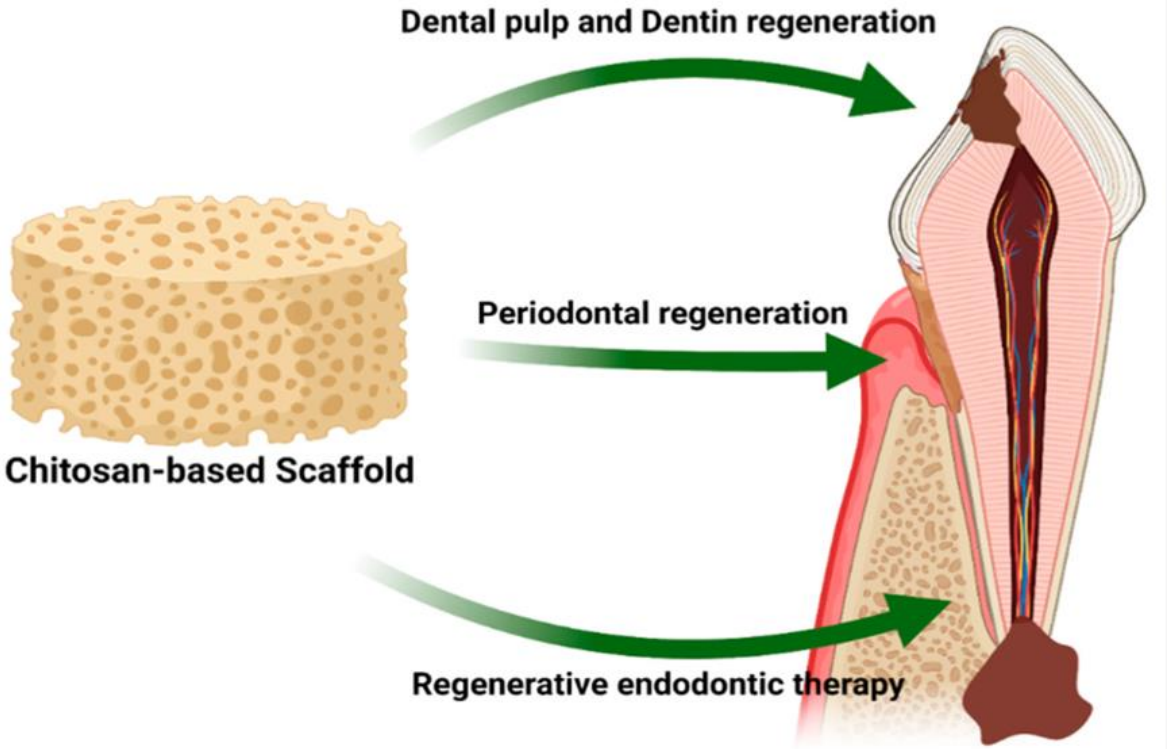
Upon acidic dissolution it convert from polysaccharide to polyelectrolyte gel having 3D structure and express many biological properties like non-cytotoxicity ,hemostasis antimicrobial and safe biodegradable behaviour. Make it suitable candidate to be used for dentine-pulp regeneration

Studies concerning the using of Nano-chitosan paste alone as PULP CAPPING MATERIAL are very limited with conflict results and lacks of standardization.

In the Present research we investigate the pulp response to pure acidic nano-chitosan paste of 50 nm particle size ,161 MW and more than 90% DD







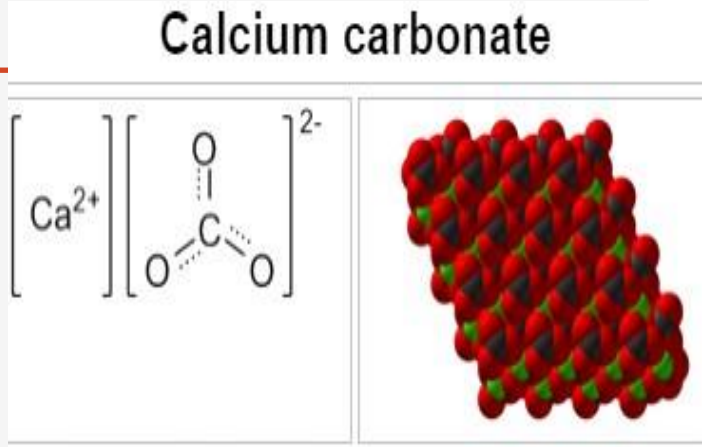
Calcium Carbonate /Calcium Acetate (CCA)

Nowadays **CaCO3** is a ceramic material of high scientific interest as it **is naturally occurring cheap calcium releasing biomaterial**, has excellence **biocompatibility** ,**porosity and pH sensitivity**

CaCO3 has been **added** to some sorts of hydraulic cement (Biodentin,Micro Mega MTA and ProRoot MTA) as filler to enhance the **mechanical properties and hydration reaction** ,it has been found the biological performance of these capping material is greatly enhanced furthermore, In vitro **CaCO3** pure paste have been tried as pulp capping material which showed intimate involvement of dentinogenesis.

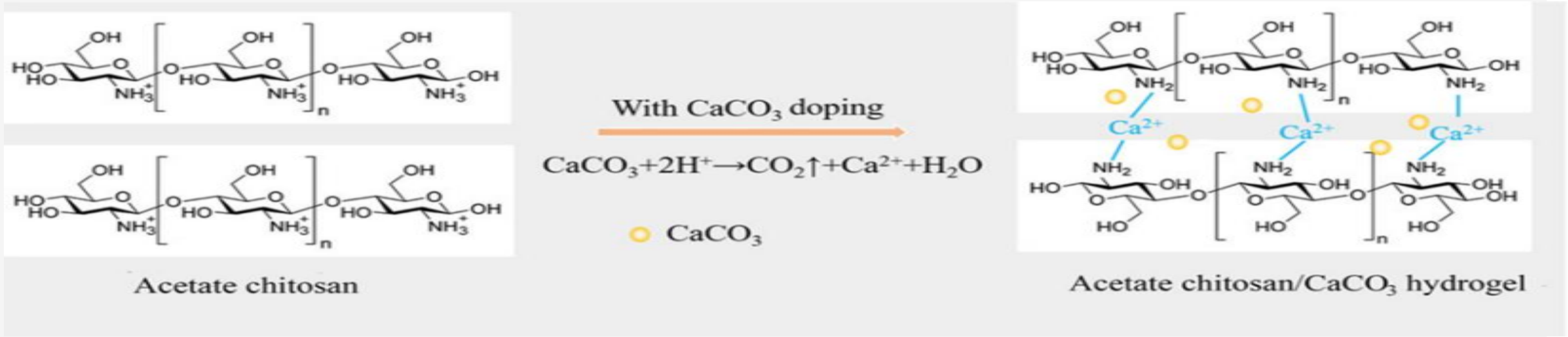
In present study we develop novel cement by mixing CaCO3 with Acetic acid

According to the best of our knowledge it is the first time to use this material(calcium carbonate/calcium acetate) as pulp capping agent



Nano- chitosan/CaCO3 (NCsC) composite

By the presence of weak acid (acetic acid) the polymeric chitosan intereact with the $CaCO_3$ elaborating biomimetic composite of 3D structure and organic and inorganic component similar to microenvironment of dentine or bone with improvements of injectability, setting time, biomechanical properties and ensure sustained calcium ion release upon degradation



According to our best of our knowledge it is first time to use NCsC composite of (40% Nan-chitosan/60% CaCO3 wt ratio) as injectable pulp capping material after partial pulp amputation of rabbit incisors.

Aims of the study

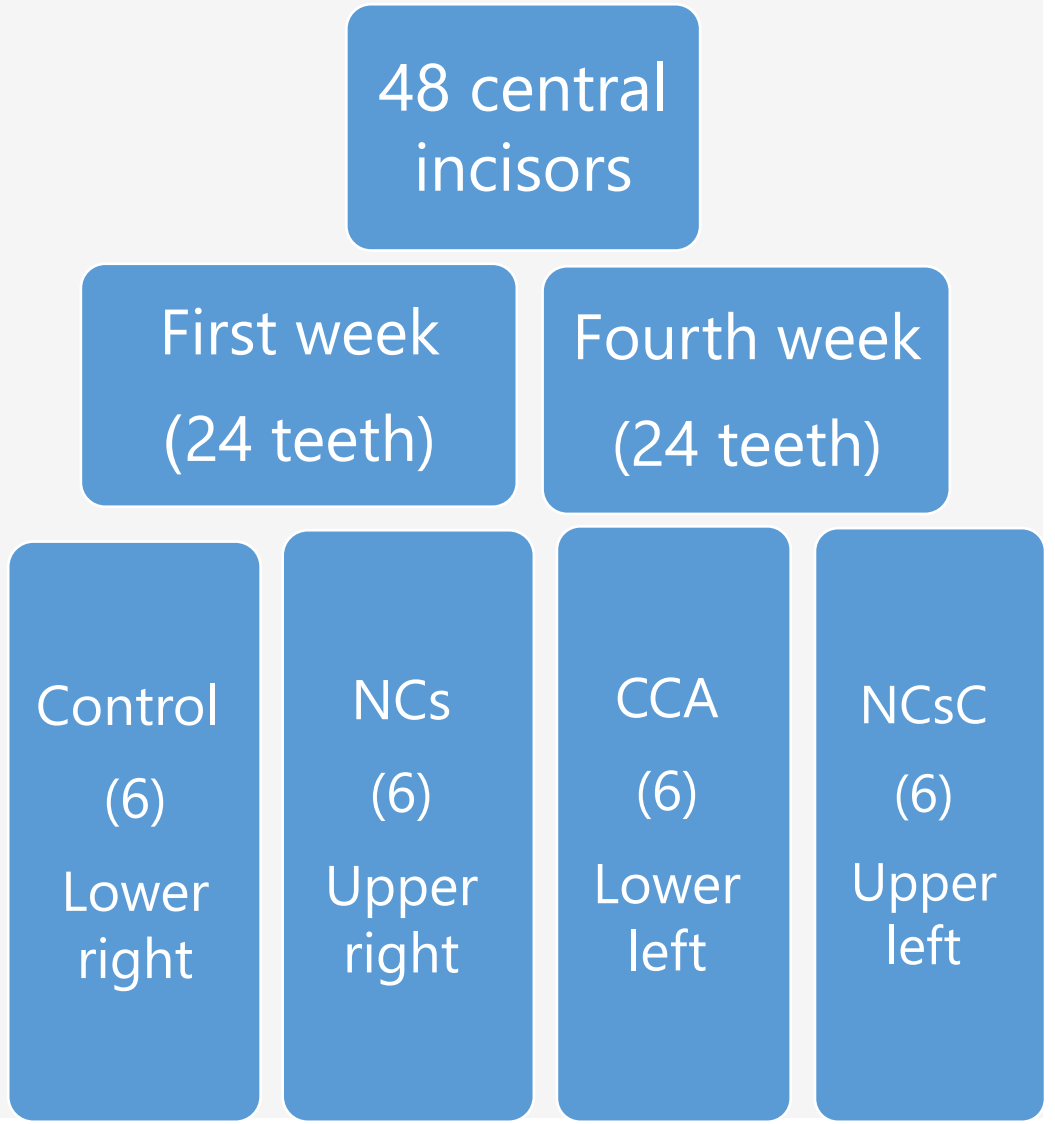
- **present study was conducted to develop and evaluate the biological efficacy of either Nano-chitosan paste, Calcium Carbonate/Calcium Acetate or combination of them as a novel injectable pulp capping material.**

Material and method

Material and methods

Study design: 48 central incisors of 12 .

New Zealand rabbits were used, subdivided into two group of 24 teeth according to sacrificing time (1 and 4 week), the 24 teeth assigned into four group of six teeth (control, nano-chitosan, calcium carbonate/calcium acetate and nano-chitosan /calcium carbonate group), in control the pulp is partially amputated left free of capping material just sealed with RMGIC in all group



Paste preparation

To achieve the best handling properties of the pastes as injectable pulp capping material (putty or creamy consistency) , spoon of powder to drops of liquid ratio was used, and determined by trial

liquid phase (0.5% acetic acide) solution prepared by adding 0.5 ml of 99.8% Glacial acetic acid to 99.5 ml of distilled water

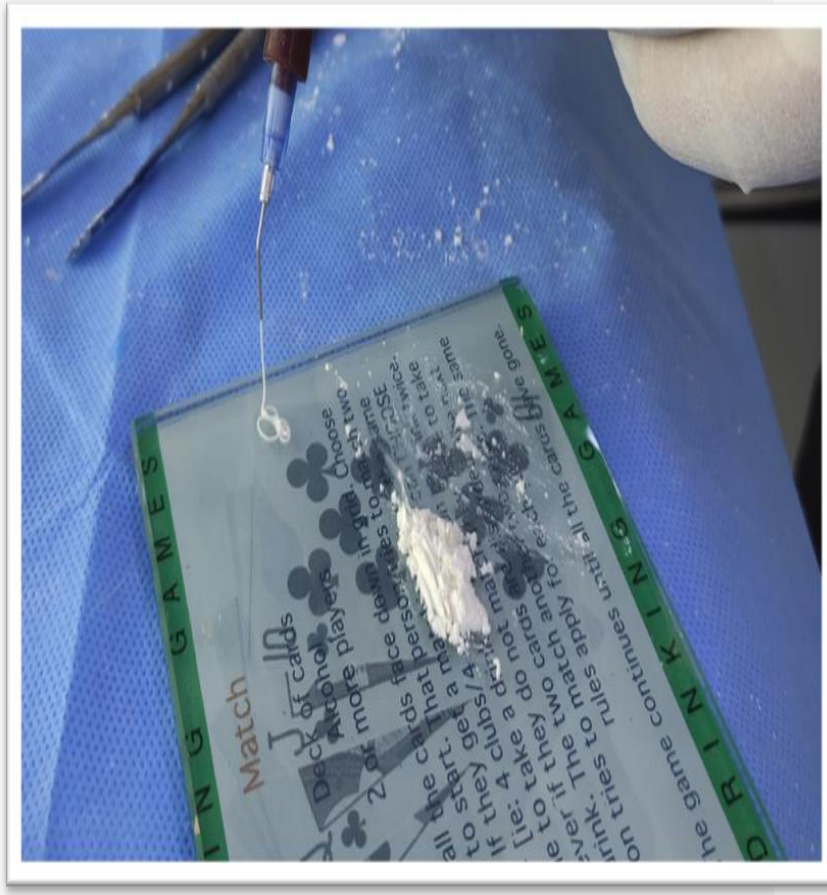
Nano- chitosan paste preparation(NCs)

By in situ preparation (manual and incremental mixing) 5 Drops of diluted glacial acetic acid (0.5%) was mixed with 3 full spoons of Nano-chitosan powder, each full spoon weighing **10 mg of Nano-chitosan** powder. After mixing and by using spatula, the paste apply into Sterile disposable syringe (1ml) (ultradent-USA)

Paste preparation

Nano- chitosan/calcium carbonate paste preparation(NCsC)

First we prepare the powder by **admixing** of 1g of pure Nano-chitosan powder with 1.5g of pure calcium carbonate powder to achieve (40/60% ratio of mixture) each full spoon of this admixture **weighing 15 mg** .To prepare the paste we mix 3 drops of diluted glacial acetic acid (0.5%) with one spoon of the admixture mixed for 1 minute to achieve creamy consistency. Then applied into Sterile disposable syringe (1ml) (ultra-dent.USA)



Paste preparation

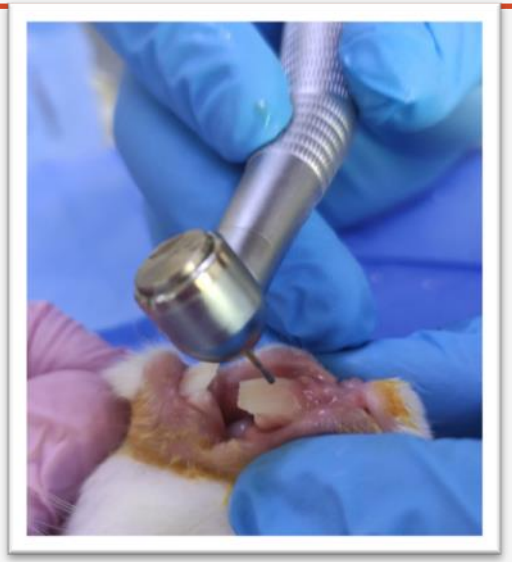
Calcium Carbonate/Calcium Acetate paste preparation(CCA)

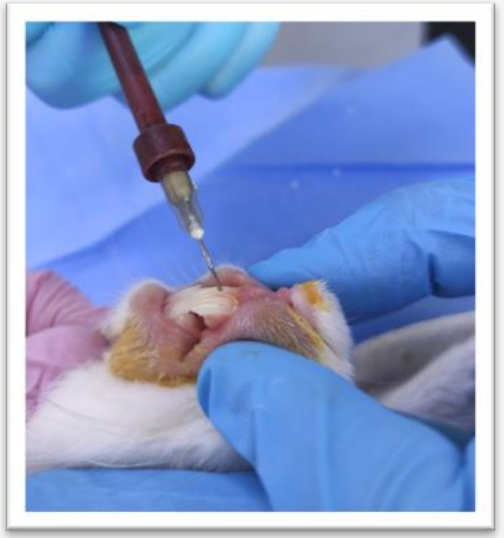
3 Drops of diluted glacial acetic acid (0.5%) was mixed with 1 full spoon of calcium carbonate powder, each full spoon **weighing 25 mg** of pure calcium carbonate**,** Then apply into Sterile disposable syringe (1ml) (ultra dent-USA)

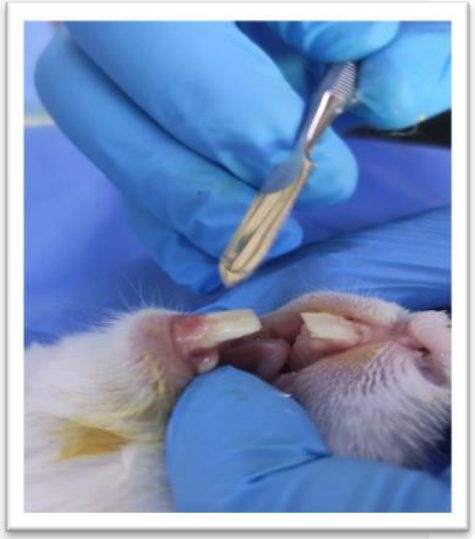
Surgical procedures

After achieving general anesthesia. **Class V cavities** were made at the cervical buccal surface of the lower two and the upper two central incisorsand 1 mm above the marginal gingiva.the drilling made at 45 degrees inclination to the marginal gingiva and extended apically toward the pulp chamber

the pulp exposure was done by using small spoon excavator, removing part of the pulp tissue, the cavity is approximately 2mm depth Immediately after pulp exposure, the cavity were irrigated with distilled water to Followed by applying of **5.25% concentration of NaOCl for 30 sc** to achieve the disinfection and hemostasis using needle brush, then the cavity washed with distilled water to remove the excess of NaOCl and dried by brief air blot The capping materials were applied according to study design







Histological evaluation and statistical analysis

All the tissue sections were prepared for routine **Hematoxyline-Eosin** stain and **immunohistochemical** staining

they are blindly examined by two experienced investigators, and evaluated for

- The extent of the inflammatory reaction.(inflammatory cell infiltration,dilated or congested blood vessel ,tissue disorganization and necrosis)

(Mild,moderate,sever,complete necrosis)

- Dentine bridge formation.

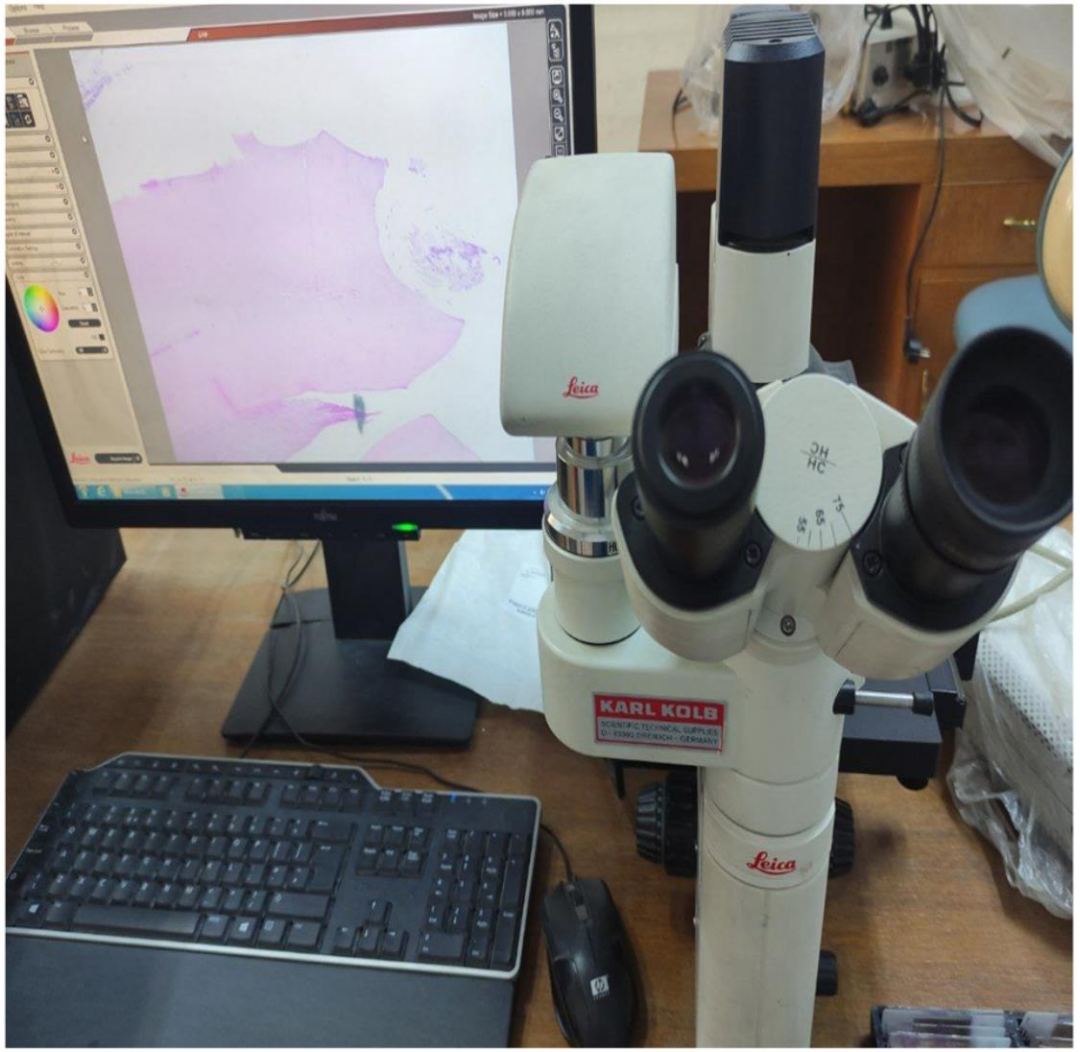
(CompleteDB,partial,scanty thin, absence of DB)

- Dentine bridge morphology.

(Tubular regular,mixed tubular and atubular irregular,atubular,absenceof DB)

- Anti-DSPP marker expression intensity

(Sever(dark brown),moderate(yellowish brown),mild (lightbrown), absence of color)



Histological evaluation and statistical analysis

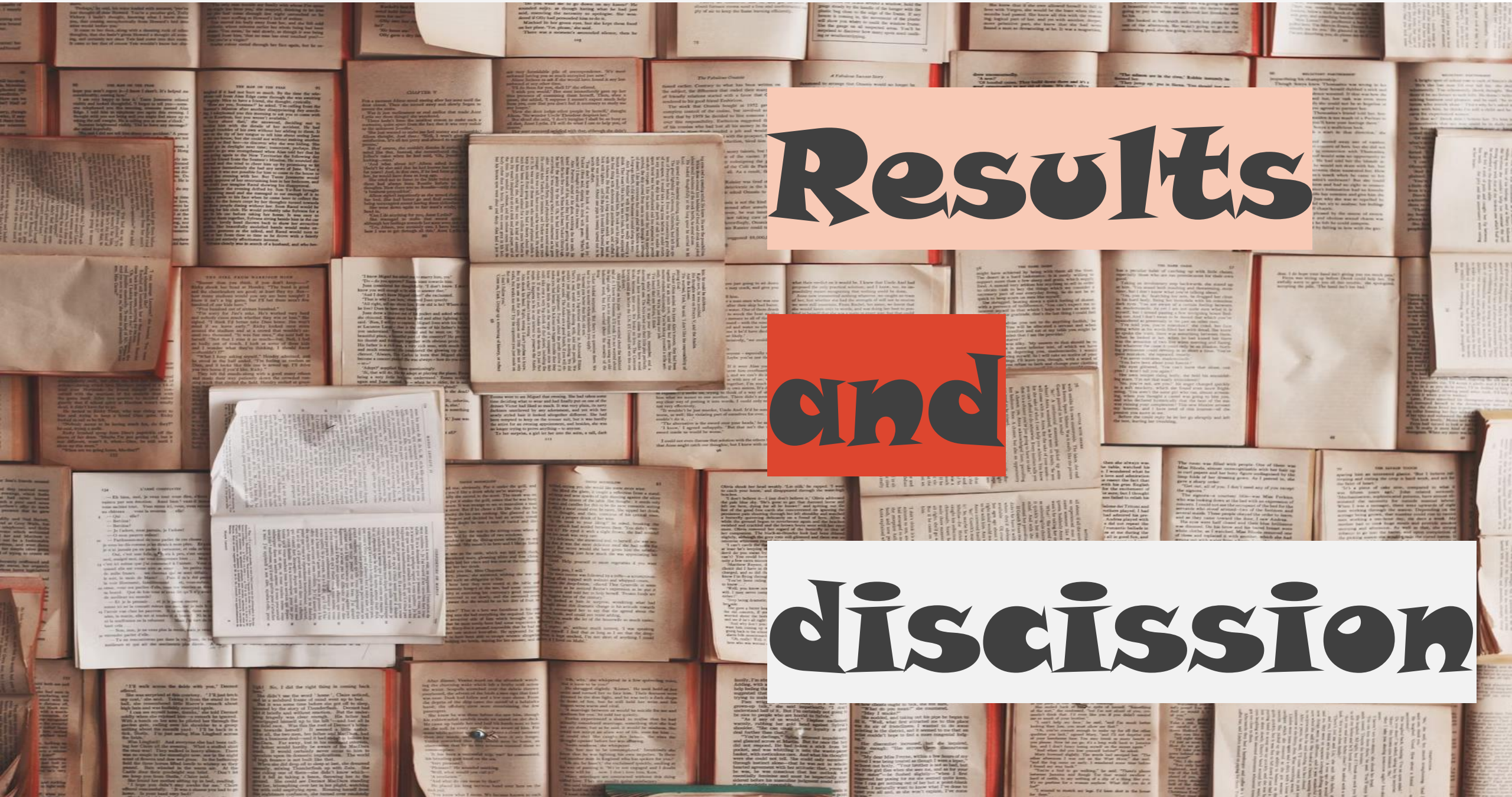
- Angiogenesis: blood vessel counting that positively express CD31 marker was done manually .

two field area (coronal and middle portion of the pulp)at magnification power 20X were surveyed by using microscope grid

Statistical analysis

The collected data was analysed using the following :

- Fisher's exact tests
- Descriptive analysis
- Kraskal-Wallis test


Results
and
discission

Results and discussion

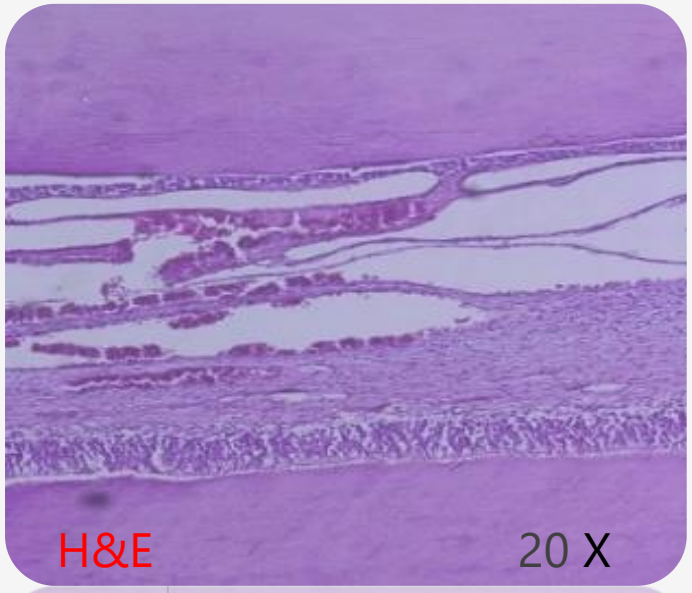
- Histological evaluation represents the golden standard above the other evaluation measures(clinical and radiographic) to assess the pulpal state after capping procedure and estimate the prospective clinical outcomes

- In present study Rabbits incisor was selected because it represent a valid analog to human immature tooth in term of large crown size suitable for restorative procedure, open apex , high regenerative capacity , being free of age changes ,histological resemblance of coronal pulp anatomy to that of human being and the effect of capping material can extend in most apical part of the pulp

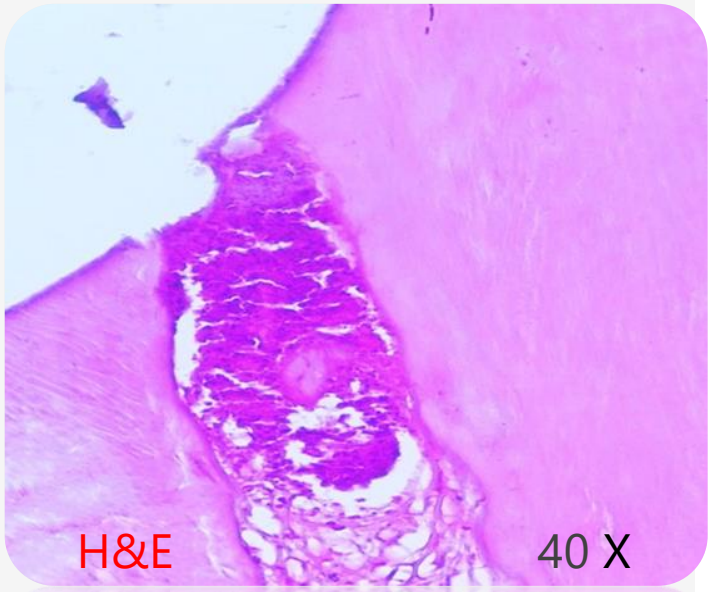
- **In the present study the control group (free of capping material) used for evaluation and comparison between self healing and material induced healing and used as abase line to maintain consistent methodology within the research**

Histological Results Control group (1ST Week)

H&E 10 X



H&E 20 X



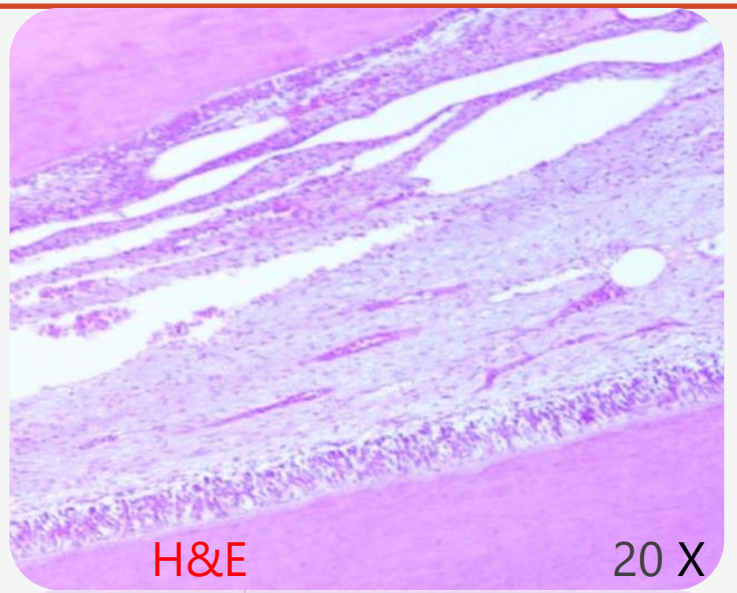
H&E 40 X

DSPP 40 X

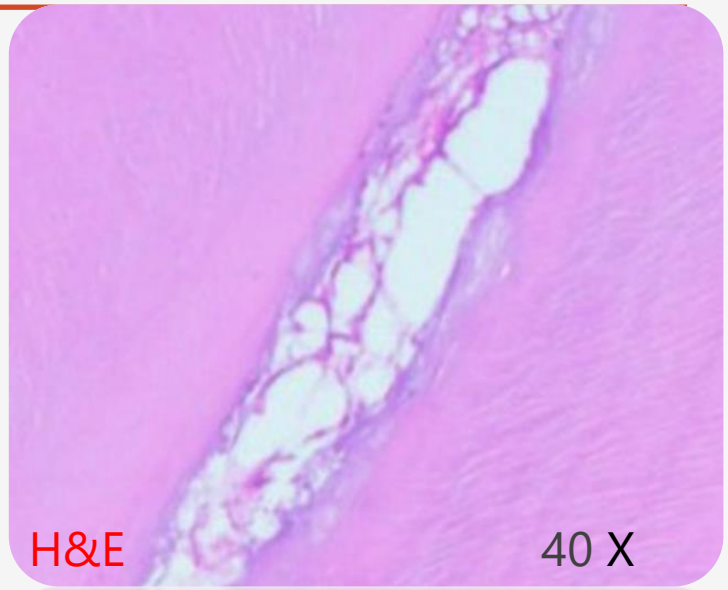
CD31 40 X

Histological and NCs group (1st Week)

H&E 10 X



H&E 20 X

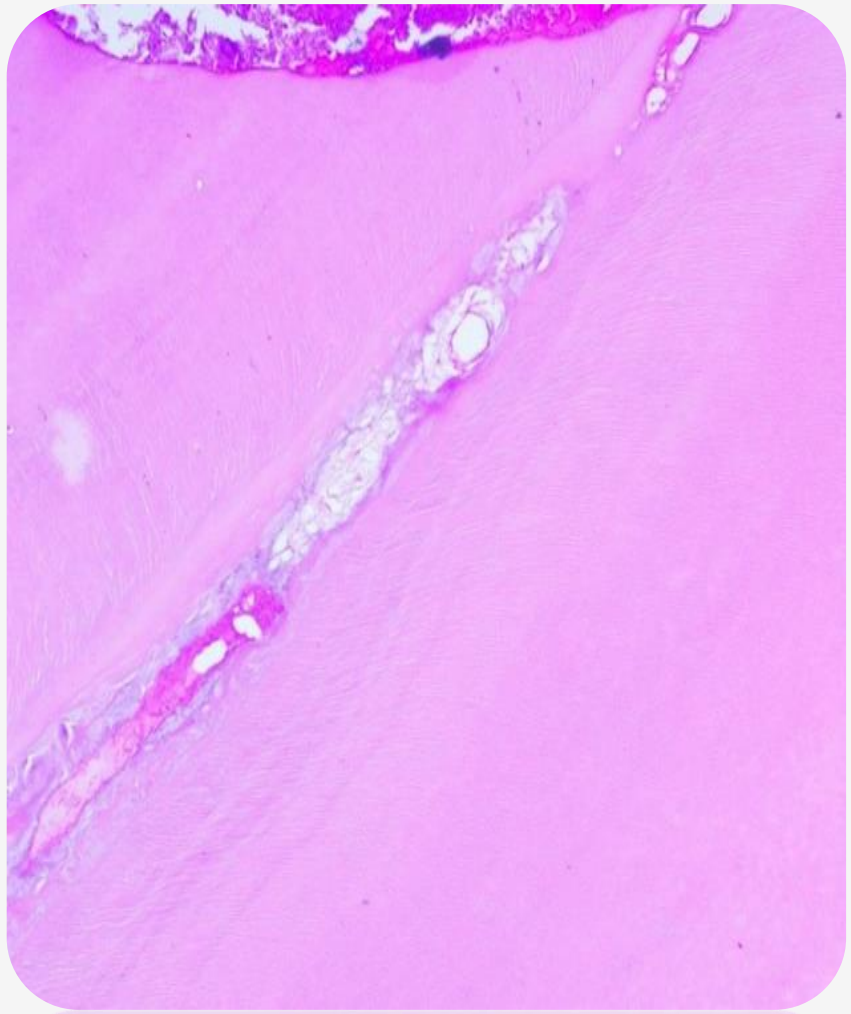


H&E 40 X

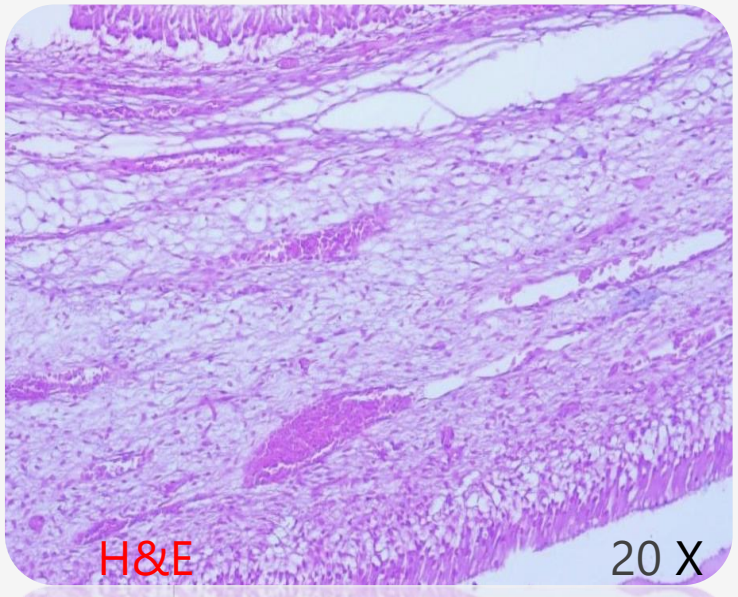
DSPP 40X

CD31 40 X

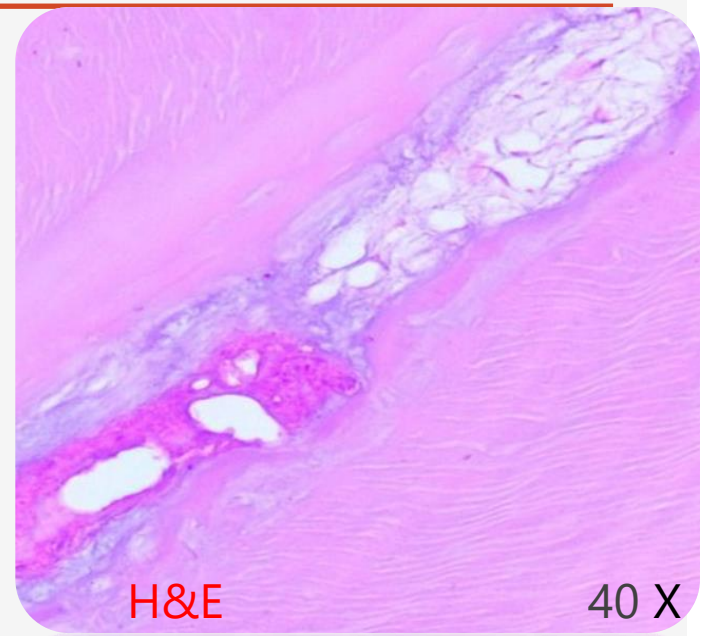
Histological Results (NCsC) group (1st Week)



H&E 10 X



H&E 20 X

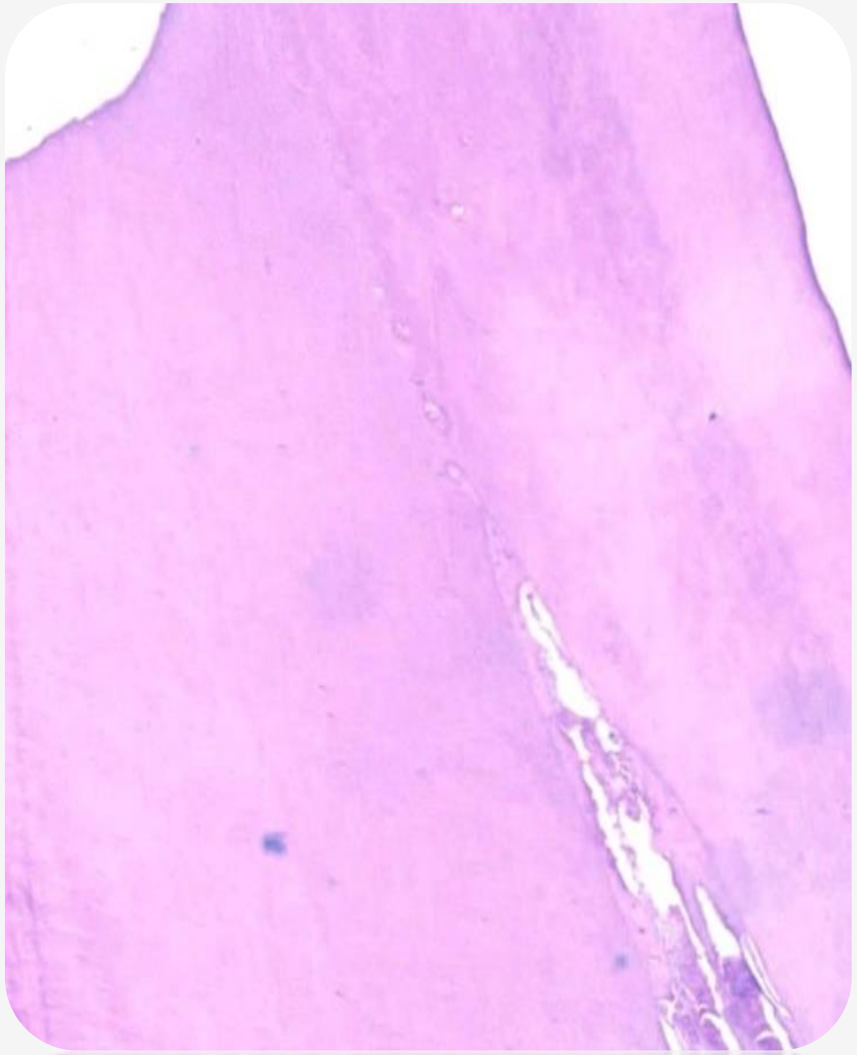


H&E 40 X

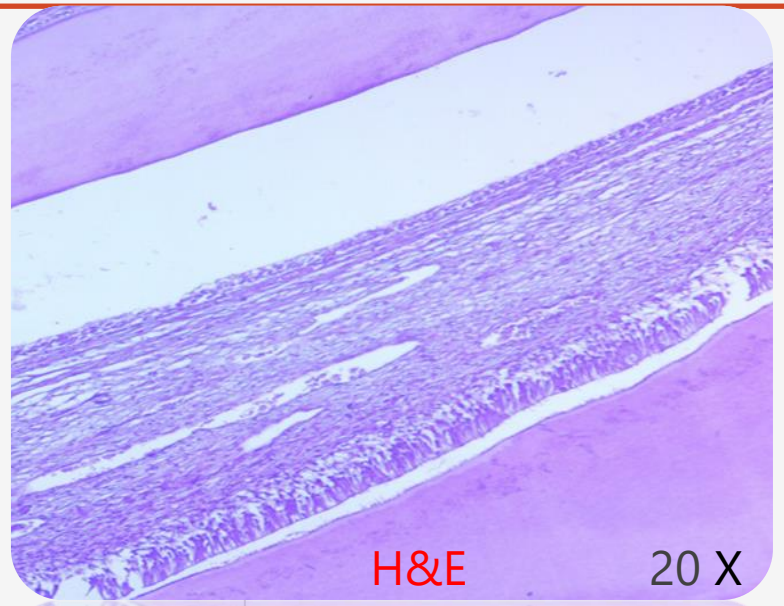
DSPP 40 X

CD31 40 X

Histological Results CCA group (1st Week)

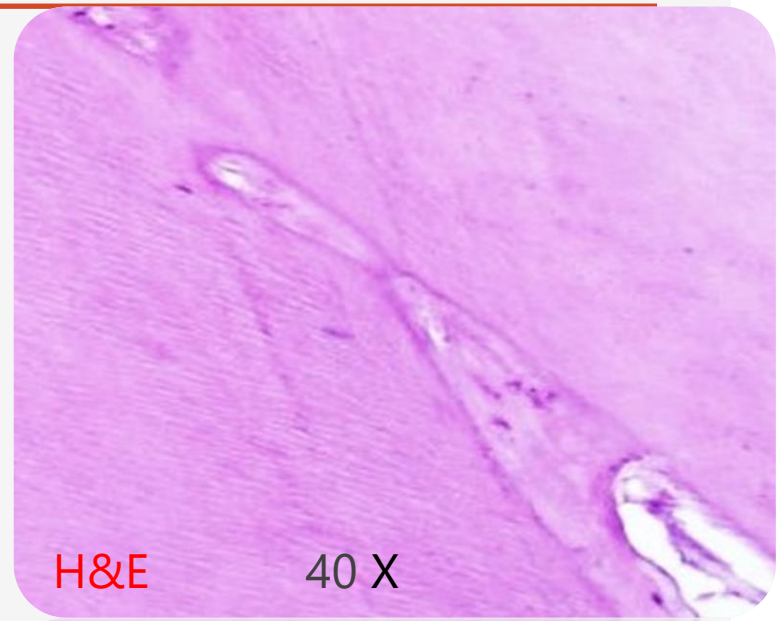


H&E 10 X



H&E 20 X

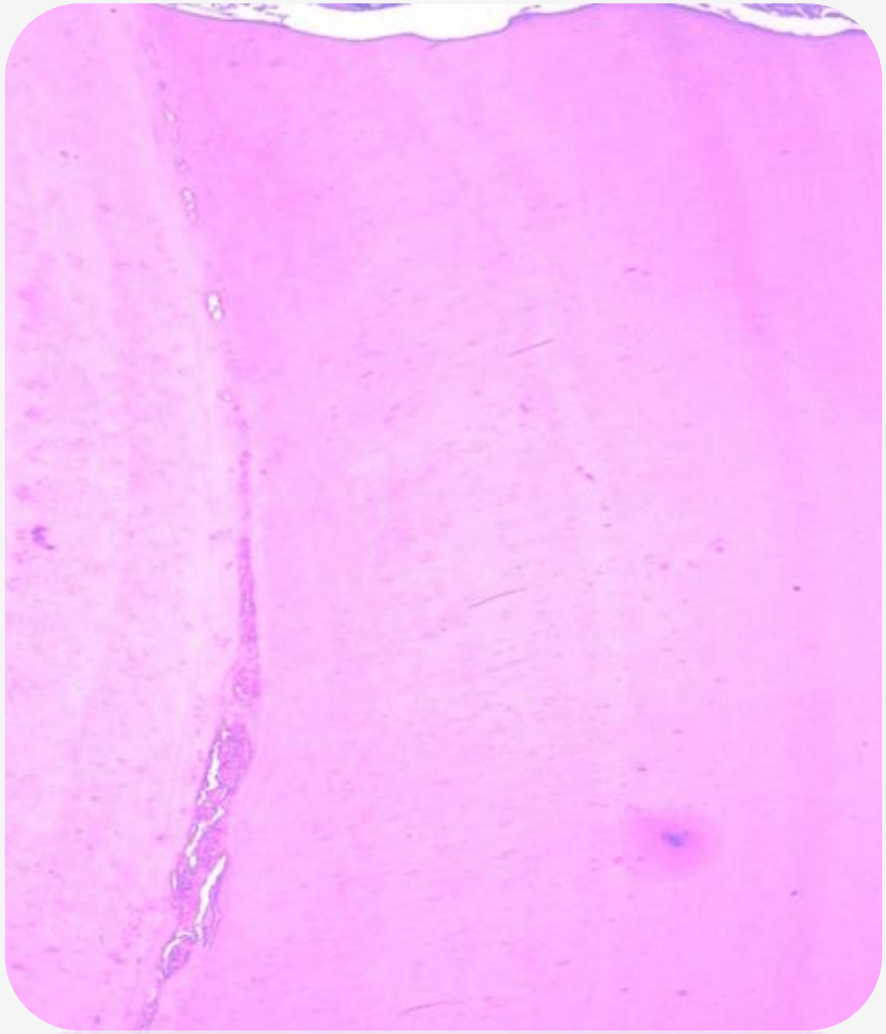
DSPP 40 X



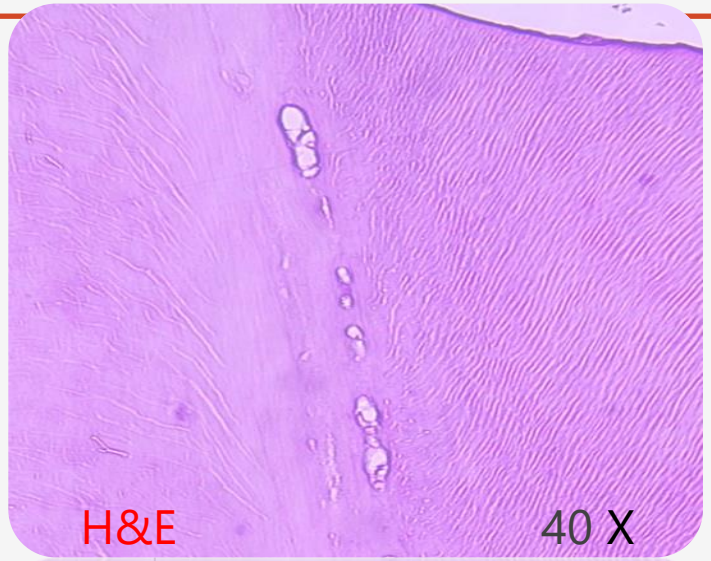
H&E 40 X

CD31 40 X

Histological Results Control group (4th Week)

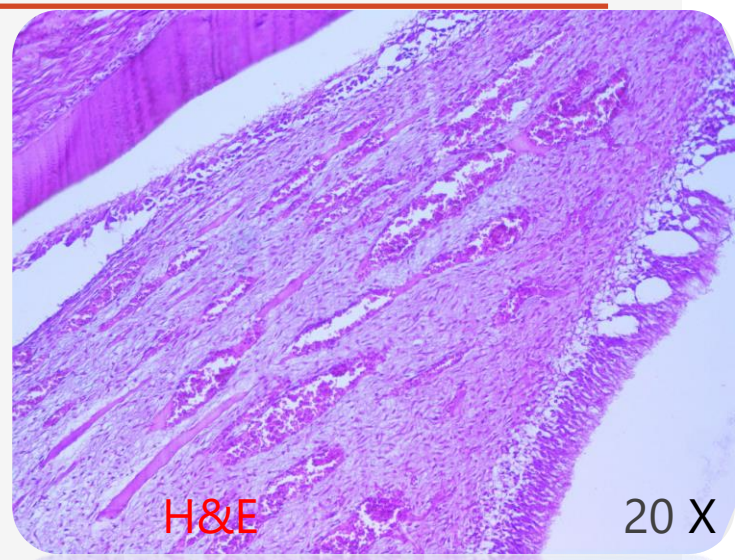


H&E 10 X



H&E 40 X

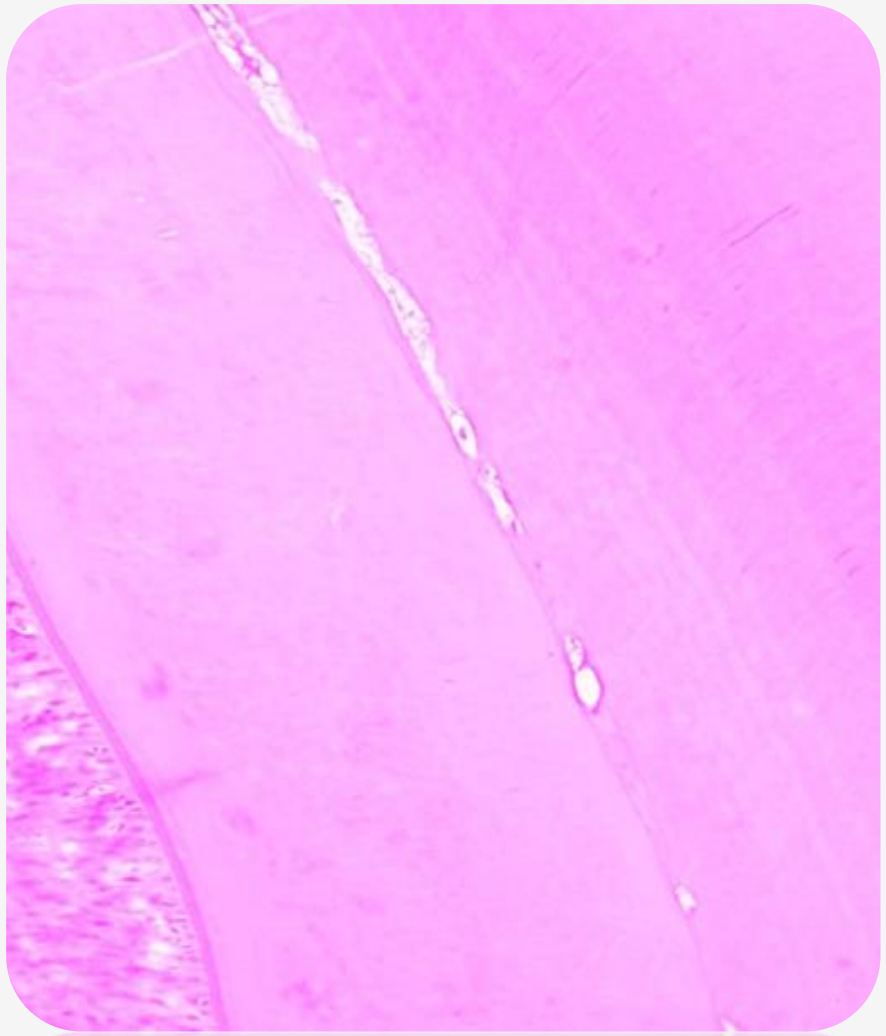
DSPP 40 X



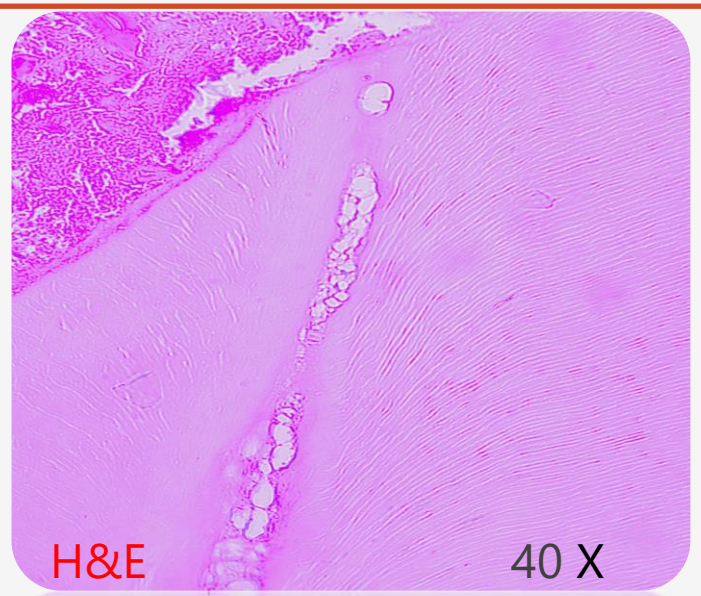
H&E 20 X

CD31 40 X

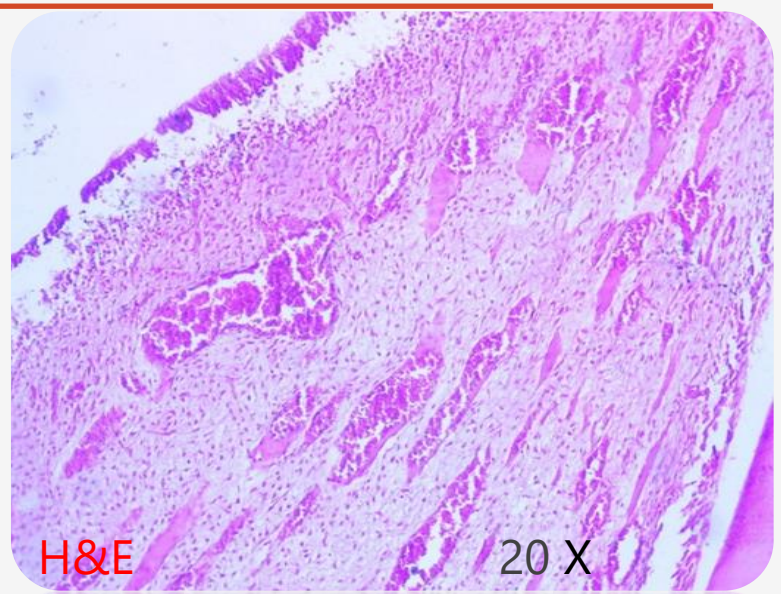
Histological Results NCs group (4th Week)



H&E 10 X



H&E 40 X



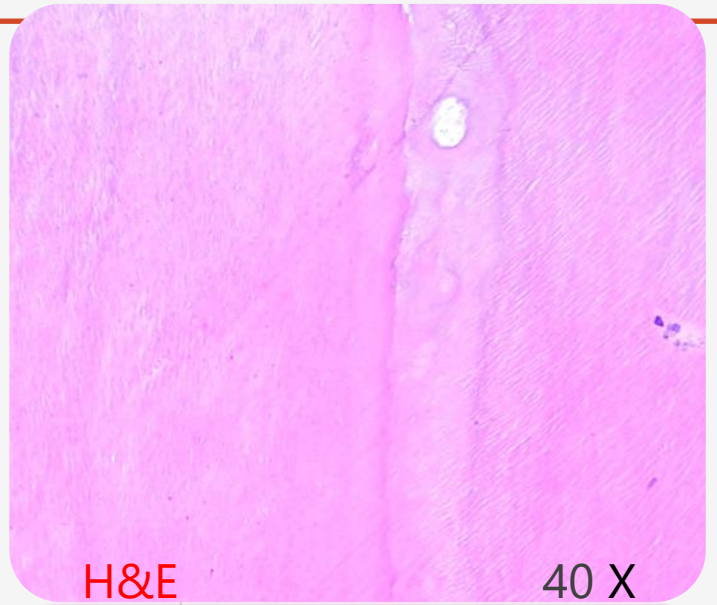
H&E 20 X

DSPP **40 X**

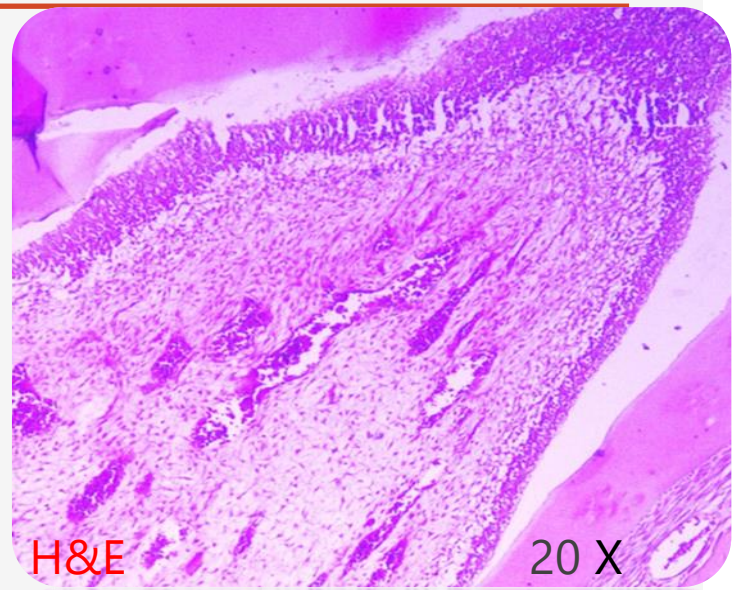
CD31 **40 X**

Histologica Results NCsC group (4th Week)

H&E 10 X



H&E 40 X

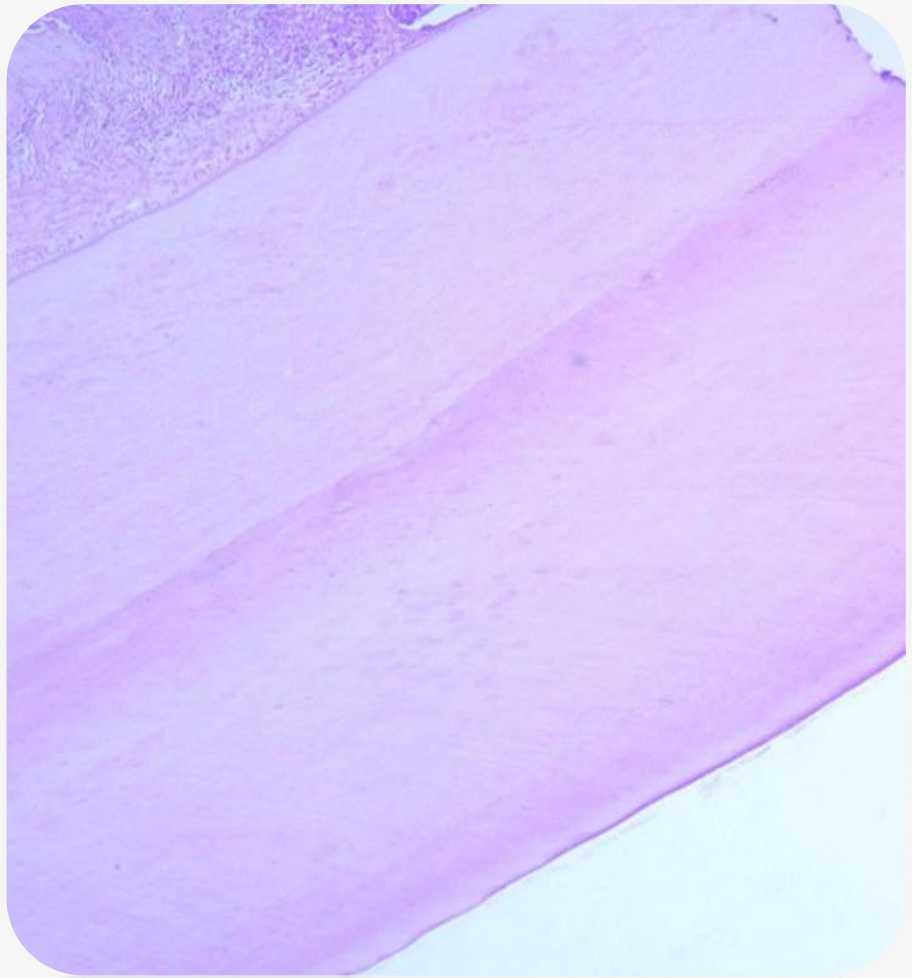


H&E 20 X

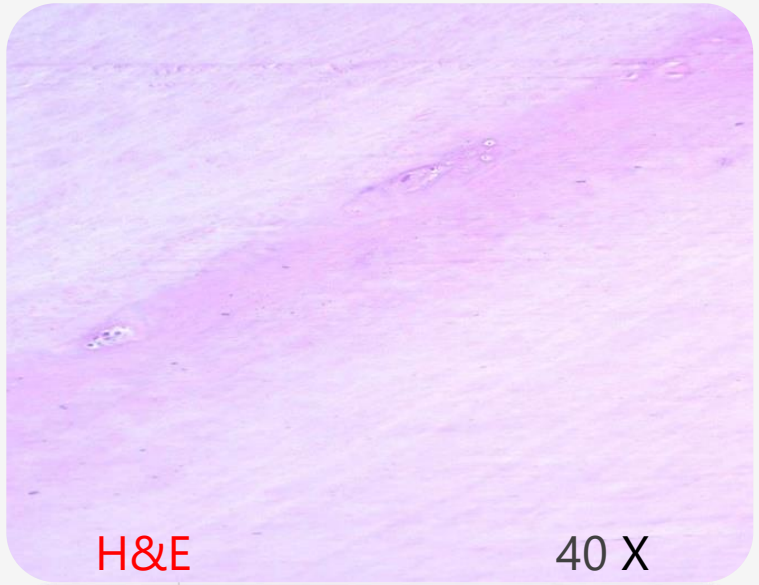
DSPP 40 X

CD31 40 X

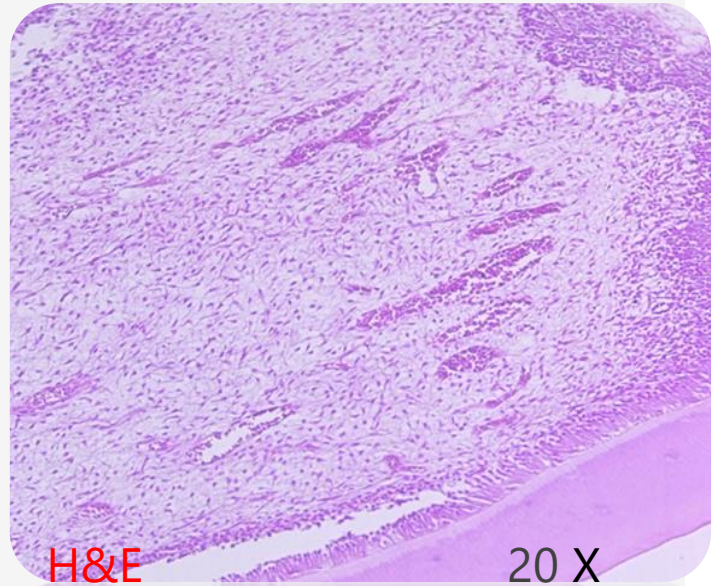
Histological Results CCA group (4th Week)



H&E 10 X



H&E 40 X



H&E 20 X

DSPP 40 X

CD31 40 X

Statistical analysis of inflammatory extent scores using Fisher's exact tests

Difference among groups regarding inflammatory extent at (1 week)

Variable	Score 1		Score2		score 3		score 4		P value
	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	
Inflammatory extent									
Control	1	16.7	5	83.3	0	0.0	0	0.0	0.180
Nano-Chitosan	3	50	3	50	0	0.0	0	0.0	
NCsC	4	66.7	2	33.3	0	0.0	0	0.0	
CCA	5	83.3	1	16.7	0	0.0	0	0.0	

Difference among groups regarding inflammatory extent at (4week)

Variable	Score 1		Score2		score 3		score 4		P value
	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	
Inflammatory extent									
Control	4	66.7	2	33.3	0	0.0	0	0.0	0.573
Nano-Chitosan	5	83.3	1	16.7	0	0.0	0	0.0	
NCsC	6	100	0	0	0	0.0	0	0.0	
CCA	6	100	0	0	0	0.0	0	0.0	

Statistical analysis of inflammatory extent scores using Fisher's exact tests showing that:

- Non-significant differences between groups at both period with the mildest inflammatory extent in CCA group following by NCsC, NCs,and control group respectively.
- All the used material and applied procedures are compatible with pulp tissue since there is absence of partial or complete necrosis in all group at both period
- Good sealing ability and the biocompatibility of RMGIC as final restoration if not come in direct contact with exposed pulp

Agree with (sulyiman et al.,2023; Aanchal et al.,2021)

✓ **Disagree with (Likitpongpipat et al.,2021)**

Statistical analysis of DB formation scores using Fisher's exact tests

Difference among groups regarding Dentine bridge formation at (1week)

Variable	Score 1		Score2		Score 3		Score 4		P value
	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	
Dentine bridge formation									
Control	0	0.0	0	0.0	6	100	0	0.0	0.000*
Chitosan	0	0.0	0	0.0	5	83.3	1	16.7	
NCsC	0	0.0	6	100	0	0.0	0	0.0	
CCA	1	16.7	5	83.3	0	0.0	0	0.0	

Difference among groups regarding Dentine bridge formation at(4weeks)

Variable	Score 1		Score2		Score 3		Score 4		P value
	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	
Dentine bridge formation									
Control	0	0.0	6	100	0	0.0	0	0.0	0.000*
Chitosan	0	0.0	6	100	0	0.0	0	0.·	
NCsC	6	100	0	0.0	0	0.0	0	0.0	
CCA	6	100	0	·0.	0	0.0	0	0.0	

Statistical analysis of DB formation scores using Fisher's exact tests showing that

- **high-significant difference between groups at both period**

- In comparison between control group with chitosan group there is non-significant difference of DB formation at both period but in comparison between control group with each of CCA group or NCsC group there is a high –significant difference .which means that the nano-chitosan paste doesn't support dentine bridge formation when used as pulp capping material which is agree with **(Kim et al .2016;Paula et al.,2019)**

- **Faster formation of dentine bridge in CCA group and NCsC group at both period may be attributed to chemical properties of these material (slightly alkaline ,sustained release of calcium and acetate ions) and physical properties (surface porosity, 3D structure and stiffness) which is agree with (Soares et al.,2019; Liu & Wang.2023)**

Statistical analysis of DB morphology scores using Fisher's exact tests

Difference among groups regarding Dentine bridge morphology at (1week)

Variable	Score 1		Score2		Score 3		Score 4		P value
	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	
Dentine bridge morphology									
Control	0	0.0	0	0.0	6	100	0	0.0	0.000*
Chitosan	0	0.0	0	0.0	5	83.3	1	16.7	
NCsC	0	0.0	6	100	0	0.0	0	0.0	
CCA	0	0.0	6	100	0	0.0	0	0.0	

Difference among groups regarding Dentine bridge morphology at (4week)

Variable	Score 1		Score2		Score 3		Score 4		P value
	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	Frequency	Percent %	
Dentine bridge morphology									
Control	0	0.0	6	100	0	0.0	0	0.0	0.002*
Chitosan	0	0.0	6	100	0	0.0	0	0.0	
NCsC	4	66.7	2	33.3	0	0.0	0	0.0	
CCA	6	100	0	0.0	0	0.0	0	0.0	

Statistical analysis of DB morphology scores using Fisher's exact tests howing that:

- Statistical analysis of the results shows high –significant difference between group at both period

- Statistical analysis of the results of control group compare to that of nano-chitosan group shows there is non-significant difference in DB morphpology at both period

- Statistical analysis of the result of control group compare to that of NCsC group shows there is high-significant difference in DB morphology at first week period but there is non-significant difference after the fourth week

- Statistical analysis of the result of control group compare to that of CCA group shows there is high-significant difference in DB morphology at both period

- The significance of difference in DB morphology can be explained as ,the early damping of inflammatory response with sustained calcium ion release accelerate the deposition of good quality dentine bridge (regular tubular dentine) meanwhile the (sustained irritation even it is mild) may postpone the deposition of good quality dentine as in control group ,nano-chitosan and NCsC this result agree with **(salah et al.,2018;Sulyiman et al.,2023)**

Conclusion and suggestion

Conclusions

- **Nano-Chitosan paste** is biocompatible and pro-angiogenic material but not suitable to used as pulp capping material
- **Both of calcium carbonatelcalcium acetate paste (CCA) and nano-chitosanlcalcium carbonate paste (NCsC)** are biocompatible and bioactive material .itis practical to be considered as potential pulp capping material


thank you