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UV- Spectrophotometric Measurement of Sustained Release of Strontium Renelate Loaded on Platelet Rich Fibrin Membrane: An In-vitro Study

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Abstract: Objective: To study the suitability of the platelet rich fibrin membrane (PRF) as suitable scaffold and delivery system to carry and allow sustained-release of strontium ranelate (SR) and whether the SR can decrease degradation process of PRF. **Study Design:**4 membranes of PRF and 4 membranes of strontium renelate loaded on PRF (SR/PRF) were prepared. 2 membranes of PRF and 2 SR/PRF were examined under electron microscope. 2 membranes of PRF and 2 SR/PRF were investigated to determine the degradation time each one. The concentrations of SR released from the SR/PRF were calculated at 1, 2, 4, 6, 8, 10, 24, 48, 60 and 72 hours by spectrophotometric methods. **Results:** Electron microscope scan showed that SR was carried on PRF membrane. SR/PRF was found to be degraded slower than PRF membrane. Sustained-release of SR from PRF was found to be continuous from the first hour to the end of 72 hours. Peak concentrations of SR in the degradation solution was reported on the first hour then decreased along 72 hours. **Conclusion:** SR could be loaded on PRF and it increase the life time of the membrane. SR is released in a sustained manner from PRF membrane.
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1. Introduction:

In human beings periodontitis is one of the most inflammatory diseases spread widely and the principle etiology for causing tooth loss in adults, by means of destructing the supportive teeth components, which comprise bone and gingival tissues and the periodontal ligament. [1]

The definitive aim of periodontal therapy is to stop periodontal interruption and to initiate restoring of the degenerated periodontal tissues involving periodontal ligament (PDL), gingival connective tissue, cementum and alveolar bone.[2]Conservative therapy for periodontitis chiefly comprises of oral hygiene measures, root planning and scaling. This regimen of treatment is generally effective in stopping additional disease development, but still the regeneration of destructive tissues representing clinical tasks. Advanced treatments involved using of local delivery system,[3] bone grafts, different growth factor-based treatments and guided tissue regeneration (GTR).[4-6]

The introduction of a therapeutic materials in the body by using advanced techniques as drug delivery system (DDS), where DDS is defined as a tool or a formulation that improves and enables the efficiency of drugs, in addition to the safety through controlling the rate of release, site of discharge of therapies in the body and the period of exposure to drugs.[7]

The second generation which introduced recently in field of platelet concentrate is called Choukroun's platelet rich fibrin (PRF) in which a high quantities of growth factors, platelets, fibrin membrane, leukocyte and cytokines are added. The capability of PRF was proven to increase collagen synthesis and proliferate fibroblasts [8] and are applied extensively to repair and improve the regeneration of both soft tissues and hard tissues post different periodontal surgical operations. [9,10]

One of the therapies which are used in the regeneration of soft and hard tissues is the strontium ranelate (SR), it acts by two mechanisms, one through reducing bone resorption via suppressing osteoclastic action and the other mode of action via initiating formation of bone through motivating replication of preosteoblast.[11] Moreover, SR has the ability for increasing the structural and biomechanical characters of bone like density of minerals.[12] In addition, strontium ranelate was established to conjugate with hydroxyapatite crystal surfaces for instance calcium

and has a significant role in the process of bone mineralization.[13]

In the current work, not only we explored the availability of PRF for delivering strontium renelate, but also if strontium renelate loaded on PRF can decrease the degradation and increase life time of PRF membrane.

2. Material and Methods:

Blood samples were collected from four non-smoking, healthy, male volunteers with ages ranging from 25 to 60 years. All patients free from infection, with no history of anticoagulation drug intake or any medication during the previously three months. The study complied with the Helsinki Declaration of 1964, as revised in 2004, and the protocol was approved by the relevant research ethics committee. An informed consent was obtained from all participants.

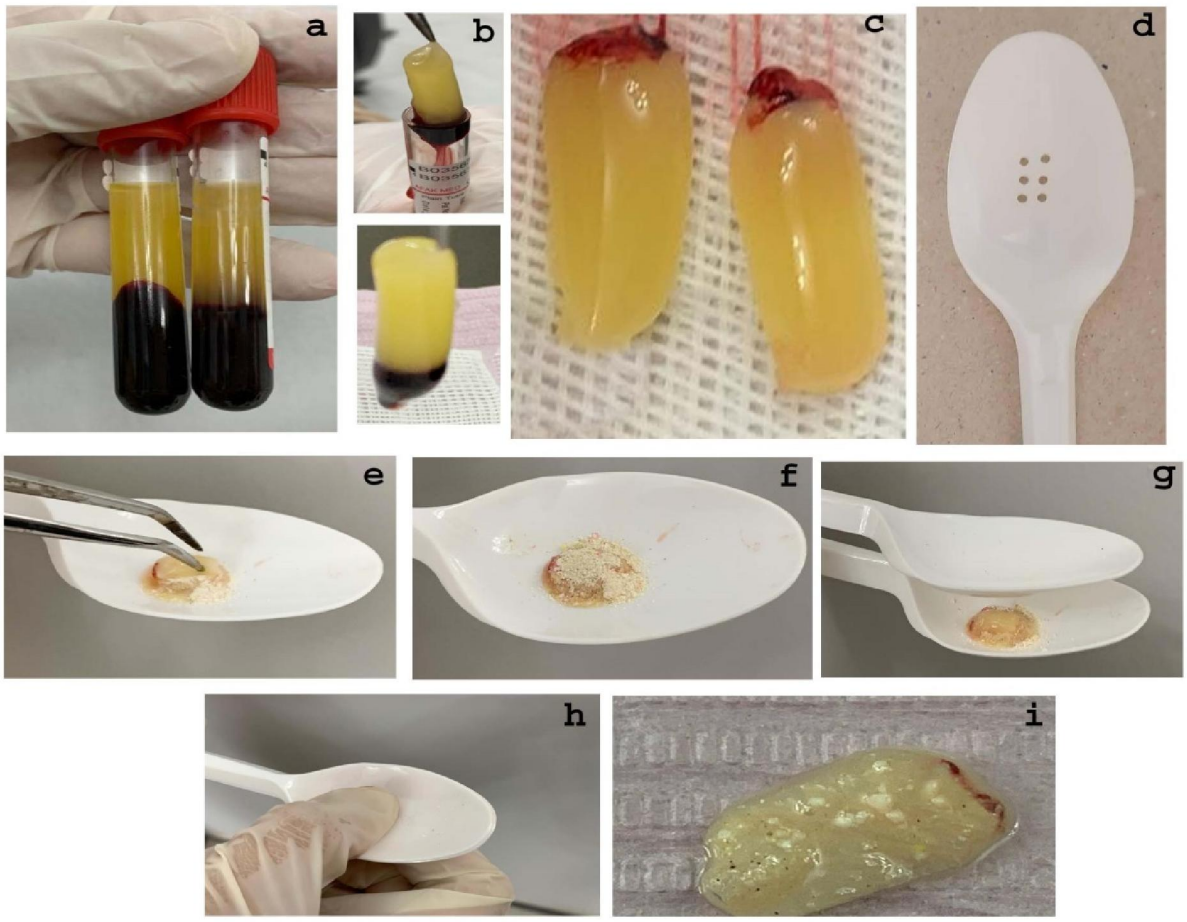

Figure 1: a, Blood centrifugation immediately after collection, three layers were formed: acellular plasma (PPP) at the top, platelet rich fibrin (PRF) in the middle of the tube and the red corpuscles at the bottom. b, the PRF clot was removed from the tube using sterile tweezers, separated from the RBC c, Two similar samples prepared. d, Spoon possesses many pinholes. e,f, PRF mixed with strontium renelate powder. g,h, The PRF clot is placed on the lower spoon, which possesses many pinholes and is compressed by another spoon. i, the resulting strontium renelate loaded on platelet rich fibrin membrane.

8 ml of venous blood was drawn and collected without anticoagulants using conventional vacuum plain glass tubes (Kuwaiti Egyptian for Medical Industries – KEMICO, Plot 111a- Al Mahager Block Industrial area (B & C)- El Obour- Egypt). 8 ml collected blood from each patient was distributed evenly into 2 glass tubes (4 ml each) and centrifuged at 400 x g at room temperature for 12 min to generate PRF in a layer sandwiched between plasma and red blood cells. The plasma was removed and PRF preparations were picked up with forceps, and the red thrombus (the fraction of red blood cells) was eliminated with scissors. After elimination of the red blood cell fractions, one of PRF preparation was compressed between two plastic spoons to develop PRF membrane and placed there for 10 min to let

squeezing out the fluids. The other PRF preparation was mixed with 1ml gram of strontium renelate powder (osteoStatine, Made in A.R.E,) then compressed between two plastic spoons to develop PRF membrane loaded with strontium renelate. The two plastic spoons, used for compressing PRF preparation, one plastic spoon has pinholes made with high speed rose head bur to drain excess fluid from the serum during the compression (Figure 1). Finally, one pure PRF membrane and one SR/PRF membrane obtained from the same sample. This procedure was done to minimalize the differences in samples.

We have prepared eight membranes which are 4 PRF membranes and 4 SR/PRF membranes. The 4 PRF membranes were divided into 2 groups in which 2 membranes were investigated under electron microscope while the other 2 membranes were used to investigate the degradation properties of the membranes. Also, the same procedures were applied to 4theSR/PRF membranes.

We have tried to mix SR with PRF by mixing it with the patient blood before centrifugation. After centrifugation of this mixed blood, we found that SR precipitated in the bottom of the tube and not mixed with PRF (Figure 2).

Figure 2: After centrifugation of SR with blood, SR precipitated in the bottom of the tube and not mixed with PRF.

Scanning electron microscopy (SEM)

The PRF membranes were fixed in 2.5% buffered gluteraldehyde in 0.1 M PBS pH 7.4 at 4C for 2h then, washing three times with PBS (10 min. each). Post fixation in 1% Osmic acid for (30min) and washing three times with PBS (10 min. each), then dehydrated with ascending series of ethyl alcohol (30, 50, 70, 90% and absolute alcohol) infiltrated with acetone, each concentration for 30 min. In SEM, samples were dried in SPI supplies®, critical point drying machine using liquid CO2.

Mounted on aluminum stubs, coated with gold in a SPI- Module ™ Vac/ Sputter. photography using JEOL, JSM- 5200 LV scanning electron microscope, Japan, electron microscope unit- Tanta university.

Preparation of calibration standards

SR was added to 0.05% trypsin plus 0.53 mM EDTA solution to form different concentration of SR (10, 20, 30, 40, 50 ppm). SR was not completely dissolved in this solution so, we prepared another solvent solution to ensure complete dissolution of SR. 10 ml phosphoric acid were added to one liter of distilled water and this solution used as a solvent of SR. Then, the new solvent solution was used to prepared the following concentration of (0.2, 0.4, 0.6, 0.8, 1 ppm) and the absorbance was measured at 323nm.[14]The calibration curve was plotted and data presented in Table 1, Figure 3.

Table 1: Calibration curve data for strontium renelate

Concentration in ppm	Absorbance at 323 nm
0.2	0.247
0.4	0.44
0.6	0.628
0.8	0.82
1	1.00

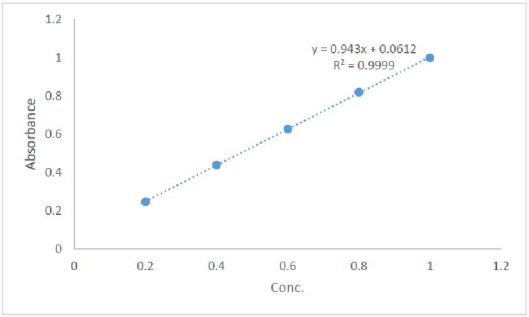

Figure 3: Calibration curve of strontium ranelate (SR) at 323 nm

PRF membrane degradation

The 2 PRF and one SR/PRF membranes were immersed into 4 mL of 0.05% trypsin plus 0.53 mM EDTA solution (digestion solution) (Invitrogen, Carlsbad, CA, USA) in a 35-mm dish inside a CO2 incubator to measure degradation properties of platelet-rich fibrin membrane by regular inspection of the membrane every day.[15]One SR/PRF membrane was inserted into 4 mL of the digestion solution. This digestive solution was completely collected after one hour and replaced by another 4ml of digestive

solution. This process was repeated at 1, 2, 4, 6, 8, 10, 24, 48, 60 and 72 hours. The collected solutions we restored at −20 °C until SR concentration measure by spectrophotometer device (AG lab American Germany laboratory, 84 Southaven Ave Medford NY 11763 USA). Before measurement, 1 ml solvent prepared solution was added to 4 mL digestion solution.

3. Results:

Scanning electron microscope evaluation:

Platelet-rich fibrin membrane was evaluated under SEM to visualize its surface morphology. Under magnification X500, X1000, X3500, SEM revealed that there was a dense fiber-like appearance of the PRF (Figure 4). For strontium renelate PRF membranes, scanning electron microscope, using different magnifications X500, X1000, X3500, showed the incorporation of strontium renelate particles within fibrin matrix. The size of strontium renelate particles within the fibrin matrix ranged from 500 nm-900 nm (Figure 5).

PRF membrane degradation results:

The PRF membrane started to degrade after 3 days from the periphery while SR/PRF membrane started degradation after 7 days. The PRF membrane degraded by more than 50% within 7 days, while the SR/PRF membrane maintained its original shape to the day 7 (Figure 6).

Measurement of strontium renelate released from platelet-rich fibrin:

The concentrations of SR in the solutions collected within the 72 hours were estimated (Table 2). The peak concentration was in the first hour (35.81µg/ml). The concentration of strontium renelate decreased over the 72 hours to reach 0.00µg/ml at 72 hours. The total volume of the released strontium renelate was 540.16 (0.54016 ml gram) µg.

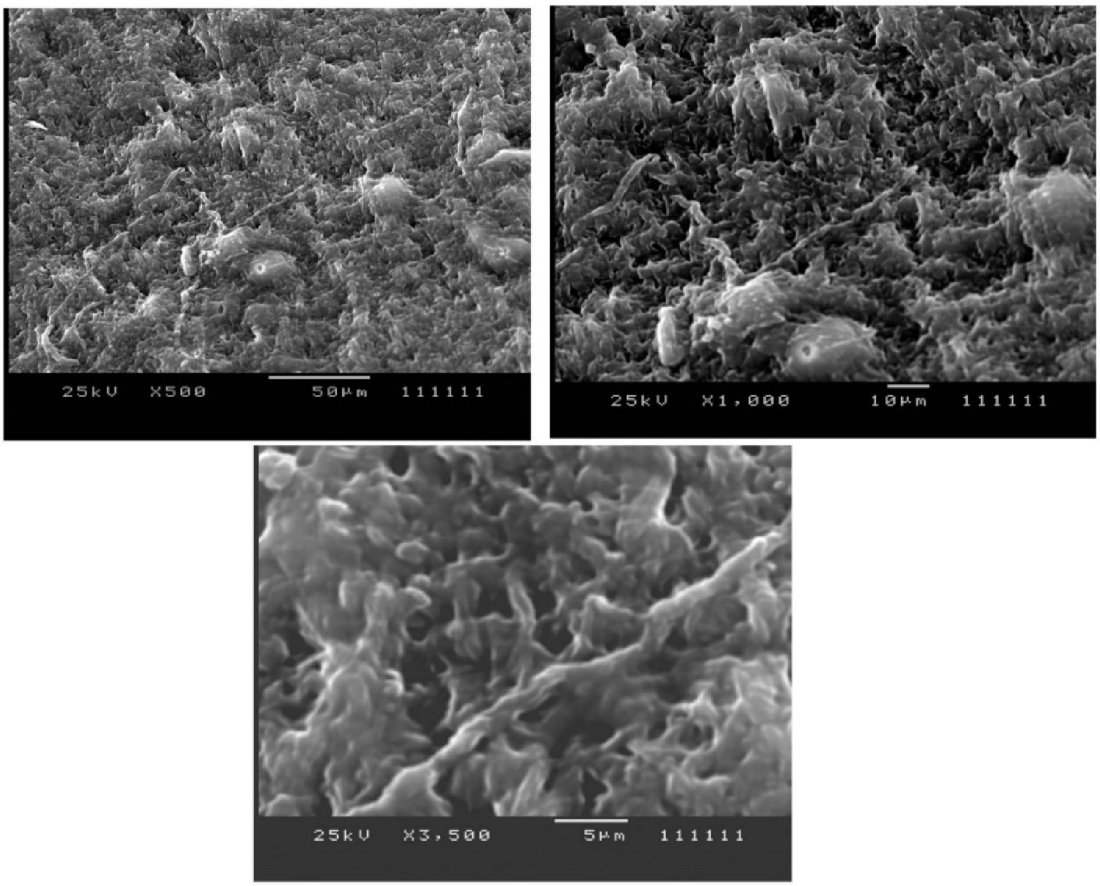

Figure 4: Surface microstructures of PRF at magnification x500, x1000, x3500 shows well organized dense and mature fibrin matrix.

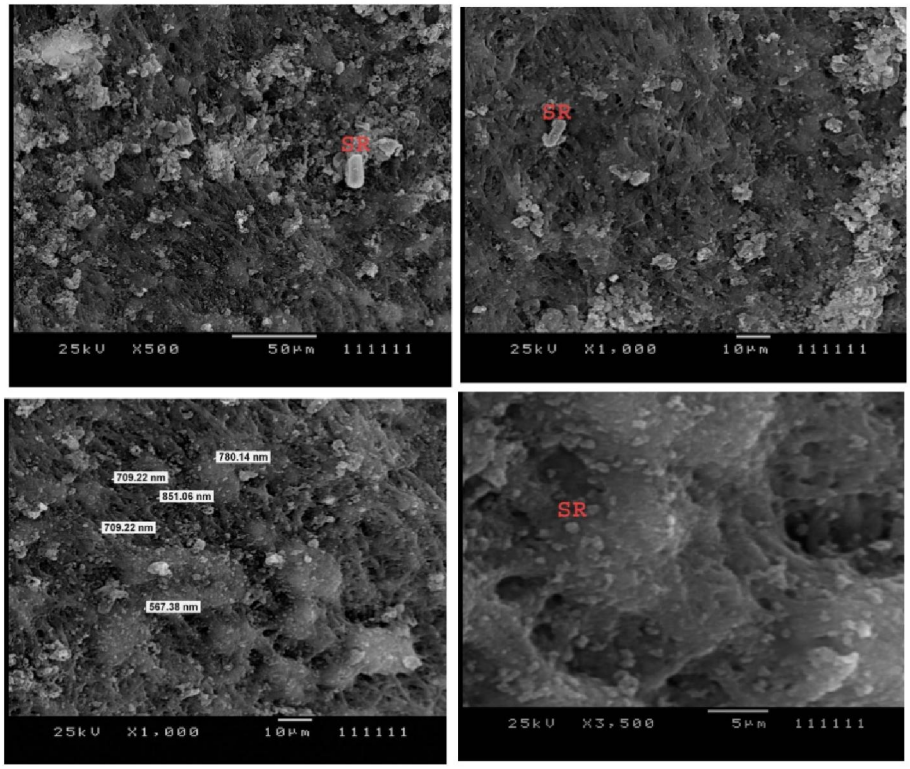

Figure 5: another Scanning electron microscope (SEM) at magnification x500, x1000, x3500 showing strontium renelate (ranged from 500 nm-900 nm) loaded on well dense and mature fibrin matrix.

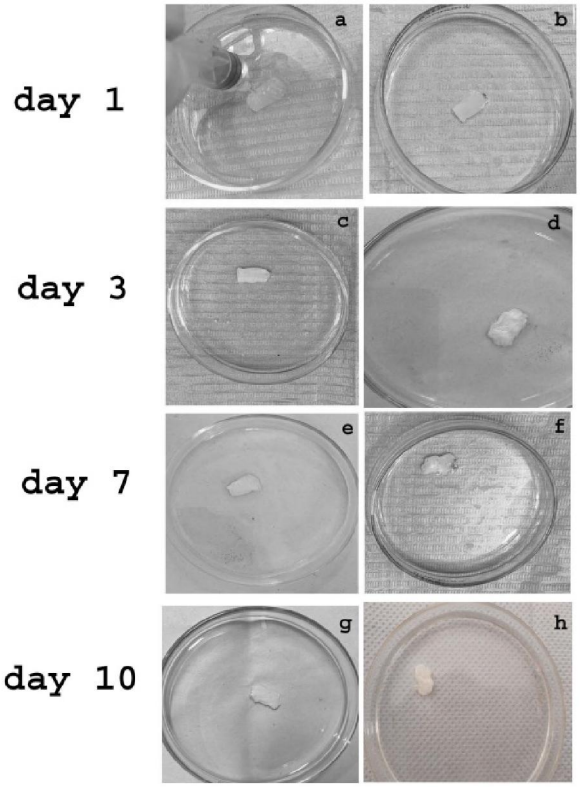

Figure 6: Degradation of PRF and strontium renelate loaded PRF membranes among 1, 3, 7, 10 days.

Table 2: Measurement of strontium renelate released from platelet-rich fibrin at 1, 2, 4, 6, 8, 10, 24, 48, 60 and 72 hours.

hours	Dilute concentration **(µg/ml)**	Absorbances	Original concentration **(µg/ml)**
1	0.81	0.821	35.81
2	0.51	0.546	25.71
4	0.42	0.439	20.91
6	0.31	0.374	15.71
8	0.26	0.305	12.93
10	0.22	0.267	10.91
24	0.13	0.185	6.56
48	0.08	0.136	3.97
60	0.05	0.109	2.53
72	-0.02	0.0442	0.00

4. Discussion:

PRF is an inexpensive pure natural matrix easy produce from a patient's blood by one time centrifugation without anticoagulant or chemical agent and acts as a biodegradable scaffold. [16] So, I have used the PRF for this study.

SR is a new antiosteoporotic treatment which is used generally for osteoporosis treatment and has a two mechanisms of action, both increasing bone formation and decreasing resorption of bones, where it re-equilibrates bone yield in support of formation of bone and augments potency of bone. SR also, play an important role in augmenting osteoblastic cell replication and enhance synthesis of collagen, whereas, in vitro, it suppresses differentiation of preosteoclast and bone-resorbing action of mature osteoclasts.[17,18]

SR level was determined by using UV spectrophotometric method. In the current study, I used this method because it is accurate and precise for the determination of SR.[19]

The PRF membrane EMS showed dense fiber due to the transformation of soluble fibrinogen into insoluble fibrin with the help of fibrin stabilization factor XIIIa. PRF formed a tetramolecular structure containing cytokines, platelets, and stem cells.[16]

Our result was in accordance with Isobe et al.,[20], Aktaş et al., [21], Kardos et al.,[22] who state that PRF formed from well□organized dense and mature fibrin matrix.

Our results were in accordance with **Du** et al.,[23]who loaded aspirin on PRF and Aktaş et al.,[21] who loaded ankaferd blood stopper on PRF. Both studies "**Du** et al.,[23] and Aktaş et al.,[21] also show connection between particles used (asprin and blood stopper particles) and fibrin matrix. In our study strontium renelateisloaded on PRF and the electron microscopy scans show connections between SR particles and fibrin matrix in SR loaded membrane.

The results of present study revealed that SR/PRF membrane less degraded than PRF membrane. This phenomenon may be referred to present of SR on PRF that takes a time to release and then the membrane begging to degradation.

The release of SR was the high concentration in the first hour then decrease with the time and this result in agree with Du et al.,[23] who state that the aspirin level in solution was gradually elevated for the period of the first 2 hours, and subsequently dropped.

The data in the present study might offer a simple clinical therapeutic approach as a new safe and to endorse periodontal bone formation through use of SR/PRF which not acts as guided tissue regeneration (GTR) only but also as sustain release of SR for periodontal regeneration.

However more studies are needed to study the use of different concentrations of SR/PRF membrane. In addition clinical studies are needed to assess the use of the SR/PRF membrane in animal and human to evaluate its beneficial effect on regeneration. Lastly, more studies are needed to evaluate other materials such as nano-material on PRF.

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