# The use of Platelet Rich Plasmain combination of guided tissue regeneration (chitosan) for bone defects: homogeneity analysis

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**Background and objective** Guided bone regeneration is an accepted surgical procedure intended to increase the quantity and quality of host bone in localized defects of the alveolar bone. In this investigation, a novel combination by mixing of Platelet Rich Plasma (PRP) with the chitosan solution has been prepared.

**Method** In method Platelet-rich plasma was prepared and mixed with chitosan in three different ratios (80:20, 90:10 and 95:5) the homogeneity status of the utilized samples has been studied using scanning electron microscopy (SEM), energy dispersive X-ray (EDS) analysis and FT-IR spectroscopy techniques.

**Results** The result showed The homogeneity status of the utilized samples has been studied scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analysis and FT-IR spectroscopy techniques SEM Bigger cracks and obvious gaps can be seen clearly in the 20:80 ratio sample which shows the adhesion failure between PRP and chitosan in this mixture. By energy dispersive X-ray (EDX) It can be seen, that carbon percentages, which belong to chitosan, is a vital parameter that shows the optimum percentage for the formation of a homogeneous PRP-chitosan compound and FTIR show the homogenous mixing without chemical changing of the mixture and showed the preservation of the chemical properties of each content.

**Conclusion** The study concluded The homogeneity status of different ratios of novel material (mixture of PRP / Chitosan) revealed that 95:5 ratio was preferable to test for biocompatibility and bone regeneration

**Keywords:** PRP, chitosan, scanning electron microscope (SEM)**,** energy dispersive X-ray (EDS), FTIR

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## Introduction

In this investigation, a novel combination by mixing of Platelet Rich Plasma (PRP) with the chitosan solution has been prepared. PRP represents a relatively new approach in reformative medicine. It contains more than 1100 different proteins, with numerous post-translational modifications. Similarly, PRP comprises diverse growth factors and other essential biomolecules for wound healing.[1] The description of the chitosan solution has been mentioned intensively in the experimental part. It can be stated that acetic acid has a vital role in chitosan size reduction. This is, most likely, due to that acetic acid provides the hydrogen ions, $H^+$, that will protonate the amine groups of chitosan.[2]

Additionally, if the amount of hydrogen ions is enough to balance the number of amine groups, it will be entirely liquefied. On the other hand, dissolving chitosan in the acetic acid solution would cause a unimodal and nanoparticle size distribution.[3] This is a good indicator that the acetic acid solution enhances the degree of protonation of chitosan, which, basically, increases the potential capacity of chitosan to form cross-linking with PRP. Accordingly, the inspection of the optimum concentration is a key parameter that affect directly the homogeneity status of the chitosan-PRP mixture.

Chitosan is an important ingredient in medicine and food. It's a polysaccharide comprising copolymers of glucosamine and bb

N-acetylglucosamine and can be derived by partial deacetylation of chitin from crustacean shells.[4]

Another easy, cost-effective way to obtain tissue healing and regeneration is Platelet-rich plasma (PRP). Platelet-rich plasma is an autologous concentration of human platelets in a small volume of plasma. It is also a concentration of the 7 fundamental protein growth factors including platelet-derived growth factor (PDGF) aa, PDGF bb, PDGF ab, transforming growth factor (TGF) b1, TGF b2, vascular endothelial growth factor, and epithelial growth factor. It also contains cell adhesion molecules such as fibrin, fibronectin, and vitronectin.[5]

Chitosan may be an effective carrier system for growth factors. Yong-Moo Lee and coworkers showed effective therapeutic concentrations of PDGF-BB released from chitosan sponges up to 21 days after the initial burst.[6]

It has been shown that chitosan is a useful tool to deliver other growth factors including basic fibroblast growth factor16 and TGF b1.[7] Reports regarding the effects of the combination of chitosan with PRP showed an increase in the release of growth factors from PRP, and also increased glycoprotein IIIa expression in platelets.[8]

Because of the Effects of PRP and Chitosan on Bone Regeneration observations of growth factors released from activated human platelets after chitosan stimulation, it has been suggested that chitosan can be an appropriate substitute for thrombin in PRP preparation.[8]

researchers' knowledge; the novel material of PRP modified by the addition of biopolymer chitosan had not been assessed previously for general oral bone defect treatment especially for combined periodontal-endodontic defects.

## METHOD

### Experimental Preparation

The chitosan CH powder {75-85% deacetylated chitin, poly (D-glucosamine)} will be purchased from Sigma-Aldrich, USA. The powder is white flaky and has a medium molecular weight (190,000-310,000 Dalton). The chitosan solution will be made by dissolving 1 g of chitosan powder in 1000 ml of acetic acid (0.1 Mol/L) followed by stirring and heating at about 55 c by keeping in an oven overnight to form a clear homogeneous 10 g L −1 chitosan solution.[9]

The solution will then be filtered through a paper filter to get rid of dust and insoluble impurities and will be used as a stock solution. The stock solution will then be diluted with the acid to three ranges of concentrations. Freshly prepared solutions will be used for each test due to the polymer degradation by storage. To prepare varieties of chitosan-modified PRP solutions, Aliquots of the above concentrations of chitosan solution will be added to PRP in three different volume/volume ratios. As follows (5:95, 10:90, 20:80).

### Preparation Of PRP

The blood sample was drawn into a citrated tube. The sample tube was then spun in a standard centrifuge for 10 minutes at 2400 rpm to produce platelet-poor plasma. The platelet-poor plasma was taken up into a syringe with a long cannula and an additional air-intake cannula. Second centrifugation (15 minutes at 3600 rpm) was performed to concentrate the platelets. The second supernatant was also taken up by a long cannula and an air-intake cannula. For each 10 mL of blood, the volume of supernatant was about 0.6–0.7 mL: this was the PRP.[10]

### Mixture Preparation

The mixture was prepared by adding a chitosan solution to the PRP solution in three different volume/ volume ratios (5:95, 10:90, 20:80).

### Homogeneity Test

The homogeneity status of the utilized samples has been studied using scanning electron microscopy (SEM), energy dispersive X-ray (EDS) analysis and FT-IR spectroscopy techniques

## RESULTS

The homogeneity status of the utilized samples has been studied using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analysis and FT-IR spectroscopy techniques. Figure 1 shows the SEM images of three different concentrations, chitosan: PRP namely; 5:95, 10:90 and 20:80 respectively.

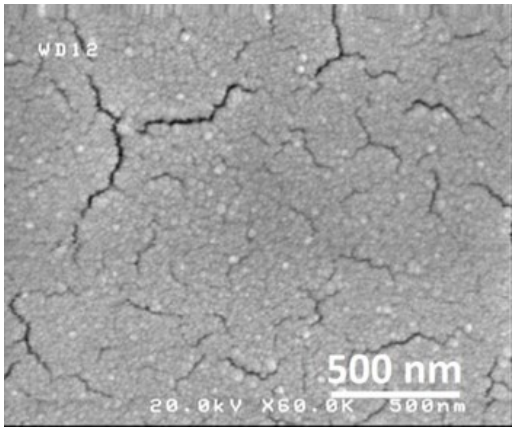
A closer inspection of the SEM images (**Figure 1**) indicates that chitosan possesses a nanoparticle's size and is distributed in-

side the PRP solution homogenously. The common and dominant feature that can be seen among the utilized concentration is that all the samples are not free from the Nano cracks. The resultant data depicted that the surface morphology of these compositions was formed in crumble grains with a porous surface and a high density of Nano cracks. Moreover, with increasing the percentage of chitosan, the amount and size of the Nano cracks increased dramatically (**Figure 1**). In this manner, further increase of chitosan did not validate substantial enhancement in a chitosan-PRP composite**.** It can be stated that, this study was originally conducted to assess the observation of Nano cracks and homogeneity of mixing of mixing of two compounds (PRP-chitosan biodegradable film). Since, Nano cracks are considered as failure in the PRP-chitosan biodegradable film.[11] hence, the minimum Nano cracks combination, 5:95 ratio, is recommended. Bigger cracks and obvious gaps can be seen clearly in the 20:80 ratio sample which shows the adhesion failure between PRP and chitosan in this mixture

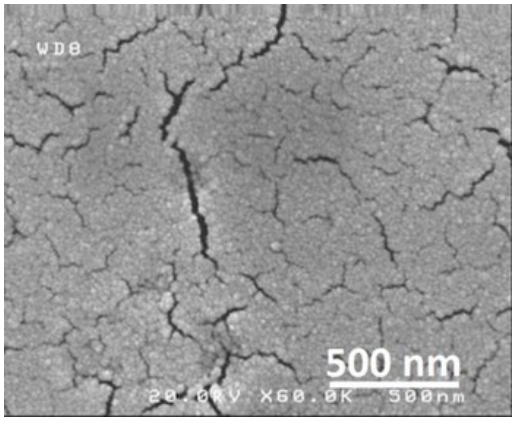
The elemental analysis of the PRP-chitosan compound has been conducted using energy dispersive X-ray analysis (Figure 2). Bigger cracks and obvious gaps can be seen clearly in the 20:80 ratio sample which shows the adhesion failure between PRP and chitosan in this mixture

The chemical structure and feature of available bonding can be explained using FTIR analysis, another important measurement to know whether the two mixed compounds were physically mixed or chemically changed, the FTIR is used to monitor any functional group appearance or disappearance.

a




c



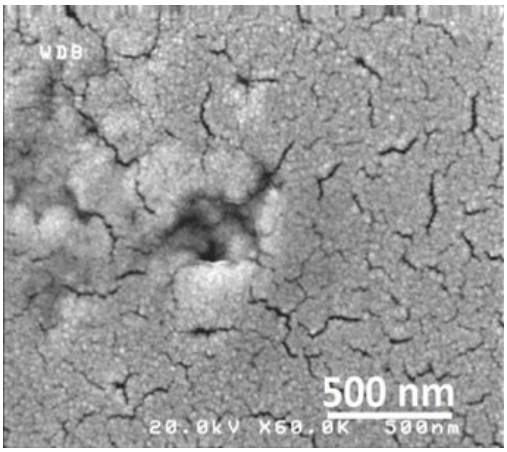
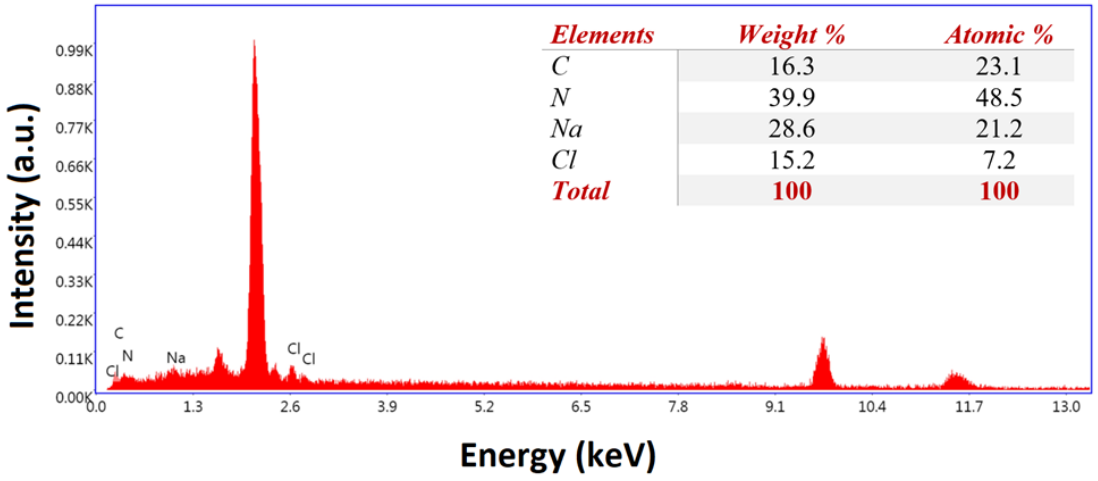



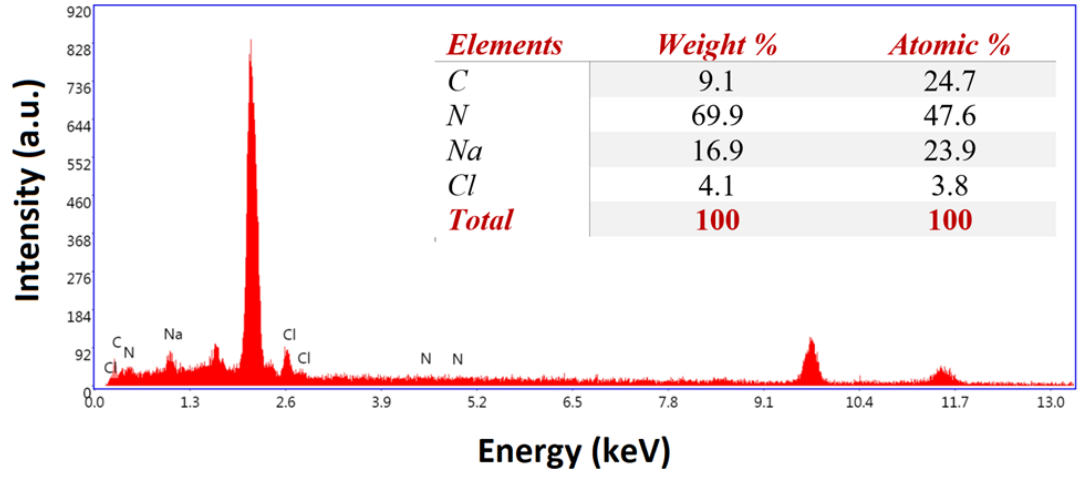

Figure 1: SEM image of (a) 5:95 (b) 10:90 and (c) 20:80 chitosan: PRP solution concentration

a



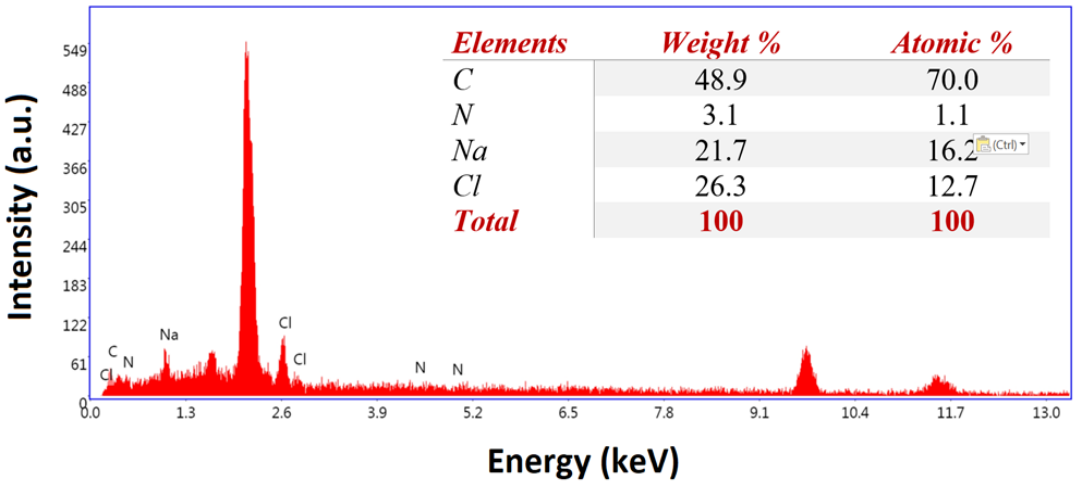

| Elements | Weight % | Atomic % |
|---|---|---|
| C | 16.3 | 23.1 |
| N | 39.9 | 48.5 |
| Na | 28.6 | 21.2 |
| Cl | 15.2 | 7.2 |
| Total | 100 | 100 |

a




| Elements | Weight % | Atomic % |
|---|---|---|
| C | 9.1 | 24.7 |
| N | 69.9 | 47.6 |
| Na | 16.9 | 23.9 |
| Cl | 4.1 | 3.8 |
| Total | 100 | 100 |

b




| Elements | Weight % | Atomic % |
|---|---|---|
| C | 48.9 | 70.0 |
| N | 3.1 | 1.1 |
| Na | 21.7 | 16.2 |
| Cl | 26.3 | 12.7 |
| Total | 100 | 100 |

c

**Figure 2:** EDX analysis of (a) 5:95 (b) 10:90 and (c) 20:80 chitosan: PRP solution concentration

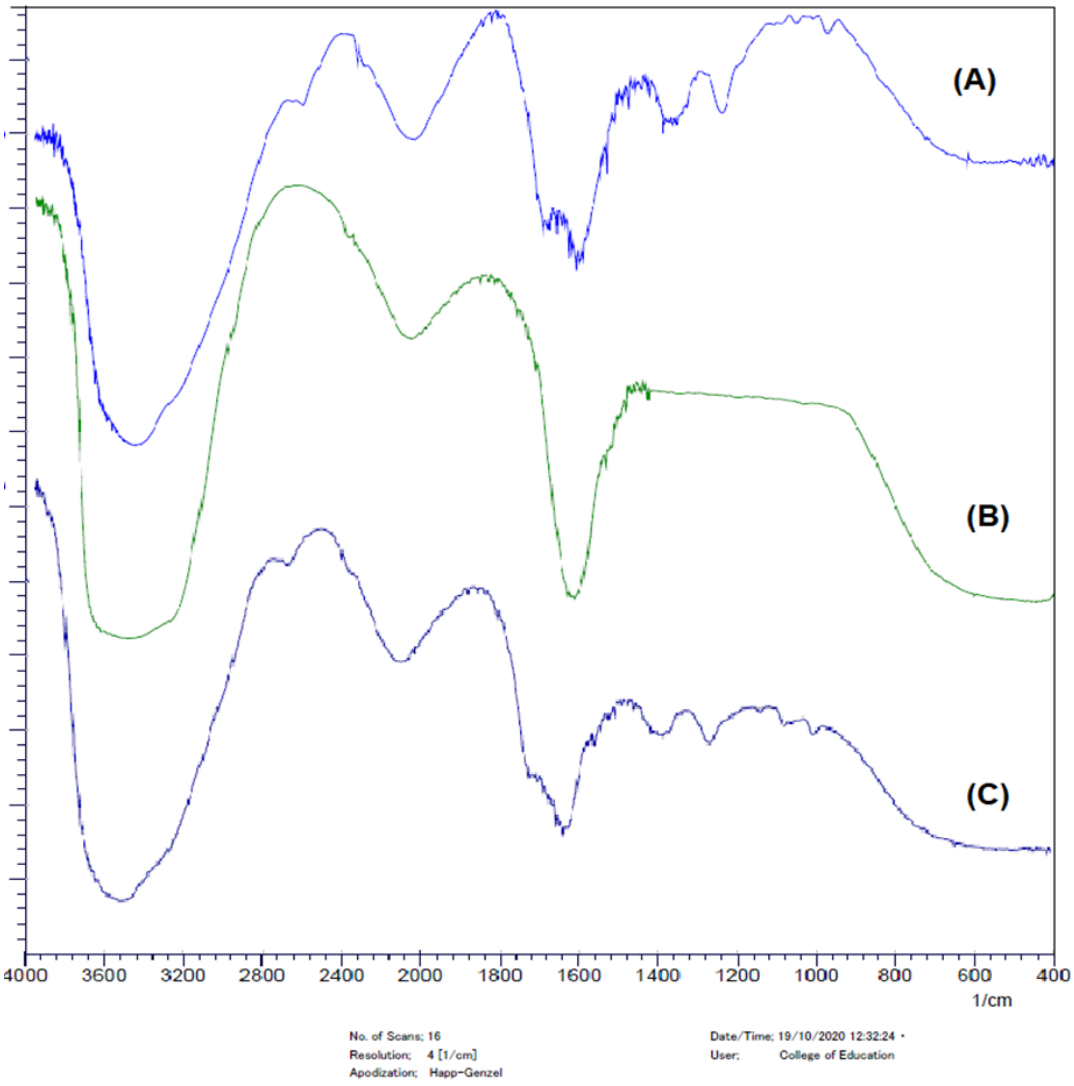



**Figure 3:** showed the FTIR spectrum of : A- chitosan, B- Blood plasma and C-mixture of chitosan and blood plasma.

## Discussion

The common and dominant feature that can be seen among the utilized concentration is that all the samples are not free from the nano-cracks. The resultant data depicted that the surface morphology of these compositions was formed in crumble grains with a porous surface and a high density of nanocracks. Moreover, with increasing the percentage of chitosan, the amount and size of the nanocracks increased dramatically (Figure 1). In this manner, further increase of chitosan did not validate substantial enhancement in the chitosan-PRP composite.

It can be stated that this study was originally conducted to assess the effects of PRP-chitosan biodegradable film on full-thickness wound healing in rabbits. Since nano cracks are considered as failure in the PRP-chitosan biodegradable film.[11] hence, the minimum nanocracks combination, 5:95 ratio, is recommended. Bigger cracks and obvious gaps can be seen clearly in the 20:80 ratio sample which shows the adhesion failure between PRP and chitosan in this mixture.

According to the literature; the weight percentage of carbon in pure chitosan is about 48% .[12] The dominance of the carbon weight percentage is clear evidence that explains the failures in the 20:80 ratio sample. It is clear that chitosan is a polysaccharide

which contains poly hydroxyl groups and acetyl groups, from FTIR spectrum of chitosan the appearance of a broad peak at 3400 cm-1 confirms the presence of hydroxyl group and 1712 cm-1 due to the presence of C=O bond as in Fig. (3A), on the other hand, also the FTIR spectrum of plasma (Fig. 3B) shows an abroad peak in 3400 cm-1 for water molecules. When mixing in the equivalent ratio of chitosan and blood plasma, each of the components retains its bonds and this confirms that the mixing is just physically far away from any chemical changes as shown in (Fig. 3C).

## CONCLUSION

The homogeneity status of different ratios of novel material (mixture of PRP / Chitosan) revealed that a 5: 95 ratio was preferable to test for biocompatibility and bone regeneration.

## Conflict of interest

The author reported no conflict of interests.

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