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# The effects of titanium on blood derived biomaterials pertaining to regeneration: A comparative study

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## ABSTRACT

**Aim** Ever since Brånemark's discovery of osseointegration, developments of titanium and its applications are numerously leading to recent studies, focusing on the evaluation of its hemocompatibility with reference to regeneration. The aim of the present work is to compare and evaluate effects of titanium on different platelet rich products.

**Materials and methods** Titanium tubes were manufactured from raw titanium. Platelet concentrates were prepared using titanium and platelet rich product. Study groups were divided into six groups. Titanium platelet preparations were denoted as TPRFX , TPRFY, TPRFZ which are titanium and fibrin preparations. TPRPX, TPRPY, TPRPZ are titanium and plasma preparations. TPRFZ and TPRPZ were novel products, that are platelet preparations activated with titanium. Each of the formulations prepared, were measured according to their influence on growth of cell which was analyzed following tissue culture.

**Results** Minimum regenerative potential was seen in TPRPy. Maximum regenerative potential was seen in TPRFz which was followed by TPRPz.

**Conclusion** This is a novel study discovering better regeneration when platelets undergo shear stress with hemocompatible biomaterials like titanium, thereby proving that thus obtained platelet concentrates TPRFz and TPRPz have better efficiency. A combination of titanium and platelets need to be further studied from all aspects to promote these novel products and advance in their benefits on healing.

## INTRODUCTION

In 1791, William Gregor, a Cornish amateur chemist, used magnets to extract the ore, that we now know as ilmenite, from a local river. He extracted iron from this black powder with hydrochloric acid, which left a residue that was the impure oxide of titanium. After 1932, a process developed by William Kroll permitted commercial extraction of titanium from mineral sources. At the end of World War II, titanium materials made their way from military application to peacetime uses. Titanium alloys were developed in mid-1940s for aviation industry and were utilized in orthopedics at round the same time. Major breakthrough in the use of titanium for bony tissue implants, was Brånemark's discovery of osseointegration. Post–World War II alloys, commercially pure titanium (CPTi) and Ti-6Al-4V, remained the dual dominant titanium alloys for implants. Commercially pure titanium (CPTi, ASTM F67) is 98–99.6% pure titanium. Although CPTi is preferred in dental applications, stability of oxide layer formed on CPTi, its high corrosion resistance and its relatively higher ductility compared to Ti-6Al-4V, has led to the use of CPTi in porous coatings. A passive oxide film (primarily of TiO2) protects both Ti-6Al-4V and CPTi alloys. Torsional and axial stiffness (moduli) of Ti alloys are closer to those of bone and supply less stress shielding than do Co alloys and steel. Titanium alloys are particularly sensitive to geometrical factors, especially notch sensitivity. Consequently, quest for new metal alloys with improved biocompatibility and mechanical properties remains an ongoing one (1).

Among the great challenges facing clinical research, is the development of bioactive surgical additives regulating inflammation and healing (2). Titanium was studied as a biomaterials due to its bioactive surface at Chichester, UK in 1998 (3). Kinetic mechanisms for biomaterials components are in part with those of xenobiotics which are subject to oxidation, reduction, hydrolysis followed by conjugation mechanisms. Their transport could also be facilitated by reversible binding to plasma proteins, globulins (metal compounds), and chylomicrons

(lipophilic substances). Storage and later release, take place for certain components in tissues such as fat and bone (4).

Passivating oxide films spontaneously grow on metals surface, having few primary physical characteristics which limit further oxidation. They are required to fully cover metal surface, have atomic structures limiting ions or electrons migration across metal oxide solution interface and must be able to remain on surfaces even with mechanical stressing and abrasion, expected with orthopedic devices. Oxide film growth depends on the electrical field across them. If potential across metal oxide-solution interface is decreased (made closer to electromotive series potential), then film thickness will decrease by reductive dissolution processes at the oxide. If interfacial potential is sufficiently negative or pH of the solution is low enough, then oxide films will not be thermodynamically stable and can undergo reductive dissolution, without which corrosion will increase (5). Oxide films share the characteristics of semiconductors with an atomic defect structure, determining the ability for ionic and electronic transport across films.

Metal cations and oxygen anions require presence of cationic or anionic vacancies (respectively) within the oxide, for transportation across films. If there's deficit of metal ions in oxide films (cationic vacancies), for instance, then there is metal ion transport. These oxides are referred to as p-type semiconductors but if there are more metal ions in oxides (deficit of anions) then cation transport is restricted and anion transport can occur. These oxides having excess electrons are referred to as n-type semiconductors. TiO2 spontaneously formed on titanium alloy implant (Ti-6Al-4V) surfaces is one such semiconductor. The greater the number of defects (vacancies or other valence species), the lesser the oxide film is able to prevent migration of ionic species and lower is kinetic barrier to corrosion. TiO2 is very close to being stoichiometric (chemically homogeneous), hence does not have many ionic defects, resulting in an increased resistance to ionic transport. Other defects may be present in these passive oxide films that may alter their ability to limit corrosion. For instance, addition of other metal ions with valence states, which are different from native metal ions, can alter both electronic and ionic transport of charge across interfaces (5). Discoloration typically ascertained on titanium implants after autoclaving was found to be associated with accelerated oxides growth and thicknesses of 650Å containing good amount of fluorine beside alkali metals and silicon. Since fluorine strongly affects oxide growth, authors advise avoiding fluorinated materials during sterilization (6).

Ratio of 'oxide specific volume' to 'metal alloy specific volume' (Pilling Bedworth ratio) will determine oxide adherence to metal. If there is a great difference between metal and oxide lattice parameters then eventful stresses will be generated between them (5). Morphology of these films is not a continuous, flat, smooth sheet of adherent oxide covering metals. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) have shown that oxides of titanium consist of needle or dome shapes. Size and shape of these oxide domes change with applied potential when immersed in oxalic and other acids.

Mechanical factors like fretting, micromotion, or applied stresses might abrade or fracture oxide films. When an oxide film is detached from metal substrate, fresh unoxidized metal is exposed to solution. Once these films reform or repassivate, magnitude of currents that are generated may be great as massive driving forces exist for oxidation. When kinetic barrier is removed, these large driving forces can operate to cause oxidation. However, extent and duration of oxidation currents will depend on repassivation kinetics for oxide film formation. Hence, mechanical stability of oxide films, as well as nature of their repassivation process, is central to the performance of these films in orthopedic applications (5).

Clinical success of implanted materials is dependent not only upon osseointegration but also on neovascularization (7). Hemocompatibility tests evaluate effects on blood components by blood-contacting medical devices or materials. *In vivo* hemocompatibility tests are usually designed to simulate geometry, contact conditions, and flow dynamics of devices or materials in its clinical application. From the ISO standards perspective, 5 test categories are indicated for hemocompatibility evaluation: thrombosis, coagulation, platelets, hematology, and immunology. In the selection of tests for hemocompatibility of medical devices or biomaterials, several issues are important. Hemocompatibility depends not only on materials characteristics, but also on fluid mechanics (stasis promotes thrombus formation), and coagulatability of blood (1).

Platelet activation is fundamental to initiate and support hemostasis because of aggregation on injured site and interaction with coagulation mechanisms. Platelet concentrate releasate promotes angiogenesis. Enhanced proliferation and migration of endothelial cells towards platelet-derived growth factors are driven by plasma component of these preparations (8). Platelet rich fibrins' slow blood activation process could induce an increased leucocyte degranulation (9). This fact represents one of the most important differences between swift polymerization of PRP and slow gelation of PRF. Previous conclusion from platelet cytokine quantifications imply that tested cytokines (IL-1beta, IL-6, TNF alpha, IL-4) and VEGF would be also progressively trapped in fibrin networks during polymerization and slowly released (11). Release of these crucial angiogenic factors in platelet derived fraction preparations could be useful in tissue regeneration and wound healing (10).

To understand the biological effects of this fibrin matrix, their clinical observations were grouped into 4 highly specific aspects of healing: angiogenesis, immune control, harnessing circulating stem cells and wound protection by epithelial cover (13). Main angiogenesic soluble

factors such as fibroblast growth factor (FGFb), vascular endothelial growth factor (VEGF), angiopoietin and platelet-derived growth factor are included in fibrin gels. Therefore, direct fibrin angiogenesis induction could be explained by fibrin binding of numerous different growth factors. Rigidity of the matrix considerably influences capillary formation by endothelial cells in response to fibroblast growth factor or vascular endothelial growth factor stimulation (14).

Titanium prepared platelet rich fibrin was recently developed. It was observed that titanium activates platelets and had superior qualities than PRF (15). TPRP was introduced and observed that it has better angiogenic potential than PRP (16).

In this study we have introduced novel concentrates of titanium prepared platelet rich products (TPRPZ and TPRFz) to evaluate their effects. In this study, we have attempted to mechanically activate platelets using titanium to promote cell growth and study the most efficient titanium prepared platelet rich product among them.

## MATERIALS AND METHODS

The samples to be prepared were TPRFX and TPRFY which are titanium prepared platelet rich fibrin prepared in titanium tubes. TPRPX and TPRPY are titanium prepared platelet rich plasma prepared in titanium tubes. Novel products TPRFZ is titanium prepared platelet rich fibrin activated with titanium spirals and TPRPZ is titanium prepared platelet rich plasma activated with titanium spirals (Fig. 1).

### Titanium

Raw titanium bars were cut with power hacksaw machine into rectangular rods. Titanium tubes were manufactured to simulate vacuette anticoagulant tubes, so as to facilitate centrifugation in heavy centrifuge (Eppendorf). Lathe (Kirloskar) was used to cut titanium rods, to design the external and internal diameter of tubes according to the drawn dimensions obtained from vacuette tubes. Inner length and outer diameters were 74 mm and 9.9 mm respectively. Rims were made with 12.89 mm as diameter, on to which corks of the vacuette tubes were used to keep them air tight. Texture of inner surface of the titanium tubes were made smooth to prepare (TPRFx, TPRPX) and (TPRFy, TPRPY) samples. Rough titanium spirals were used in centrifuge tubes to prepare (TPRFZ, TPRPZ) samples. These rough spirals were used to study their effects in platelets activation. Grade five titanium used in manufacturing of medical/dental purposes contains titanium, nitrogen, carbon, hydrogen, iron and oxygen. They were used for preparing (TPRFx, TPRPX) samples. Titanium tubes and rough titanium spirals were manufactured at Jayons Implants Pvt.Ltd, Palakkad. Grade five titanium used for aerospace purposes has aluminium


FIG. 1 Titanium used in the preparation of platelet rich products.

and vanadium as added contents and were used for preparing (TPRFy, TPRPY) samples. These tubes were manufactured at S.G. Precision Engineering Industries, TVM (India), using raw titanium rods which were cut using the lathe. They were manufactured using same measurements and design as the tubes manufactured to prepare (TPRFx & TPRPX) samples.

### Sample preparation

Blood samples were collected from 6 healthy young adult volunteers, who were not under any medication and healthy. They were explained the nature and objective of the study. Volunteers gave their informed consent.

The study protocol was approved by the institutional committee of ethics. Study was conducted as per the Helsinki declaration. Aseptic measures were adopted for all procedures. Blood products were TPRFx, TPRFy; TPRPX, TPRPY; TPRFZ, TPRPZ.

Titanium prepared platelet rich plasma (TPRPx, TPRPy): 8 ml of blood was drawn from the antecubital vein and transferred into 4 vacuum glass tubes (2 ml vacuette) containing 3.2% of sodium citrate and centrifuged for a speed of 1500 rpm/6 min. The supernatant was carefully pipetted and subjected to a second spin for which titanium tubes were used. Thus TPRPx and TPRPy were obtained. The same was repeated for all volunteers.

Preparation of titanium prepared platelet rich plasma activated with titanium spirals (TPRPz): The preparation is similar to that of plasma but prior to second centrifugation, titanium spirals of 3.5mg, weighed digitally, were added to six vacuette tubes. During second

centrifugation, titanium spirals with sharp edges subjects platelets to a certain amount of shear stress (speed of centrifugation will itself activate platelets) (15). Thus (TPRPz) was obtained.

Preparation of titanium prepared platelet rich fibrin (TPRFX), (TPRFy),(TPRFz): Titanium tubes were used to centrifuge blood to obtain (TPRFX) and (TPRFy). 5ml of blood was drawn, quickly transferred to titanium tubes and centrifuged immediately at a speed of 2000 rpm for 7min. For the preparation of titanium prepared platelet rich fibrin activated with titanium spirals (TPRFz); centrifuge tubes were used, into which titanium spirals weighing 3.5mg, was added to each of the six tubes and subject to centrifugation at a speed 2000 rpm for 7min. Fibrins were separated from the RBCs and titanium spirals. All samples obtained were subject to tissue culture.

### Tissue culture

Cultures (Fig. 2) were prepared with samples of platelet rich products. Cultures were endothelial cells and endothelial growth medium-2 (EGM-2) from Lonza. Cells were fed with growth medium and incubated. Cells were transferred on to well plates at a density of 1x103 cells. EGM-2 was added to each well. The samples TPRFx, TPRFy, TPRFZ, TPRPX, TPRPY and TPRPZ were grouped into 6 groups respectively. Obtained samples of platelet concentrates were then added to them. For positive controls, bovine serum was added and for negative controls, growth medium was not added. Wells were incubated at 37°C. Change of medium was given each day and cells were observed for confluence, after which their observation and growth analysis under microscope followed.

### Statistical analysis

Data was arranged and analyzed using statistical package for social sciences (SPSS) 22 version by IBM Corp, New York, USA program. Data analyzed follows normal distribution. Comparison of quantitative variables were analyzed by one way-Anova. Post hoc test was done for multiple and pairwise comparison between and within groups (P value < 0.05). The study was considered as statistically significant.

## RESULTS

Results were expressed in means obtained from culture wells viewed under phase contrast microscopy. Values obtained were subject to statistical analysis. Post hoc shows a statistical significance when following variables were compared; Post hoc shows a statistical significance at 1% level (P<0.01) when following variables were compared; TPRFz when compared with TPRFx (P=0.000), TPRFy (P=0.000) differs significantly on cell growth; Group three shows a higher cell growth. TPRFz when compared with TPRPx (P=0.000), TPRPy(P=0.000) differs

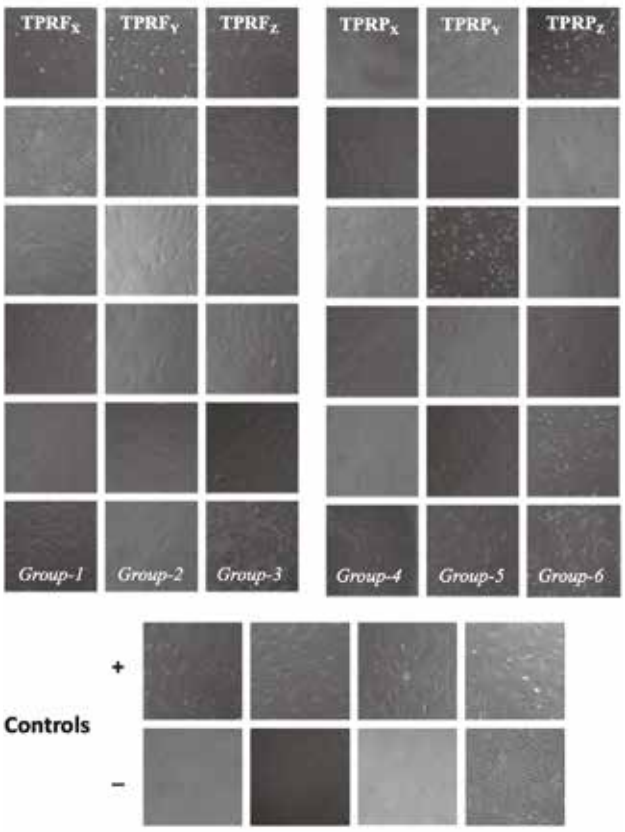


FIG. 2 Tissue culture viewed under phase contrast microscope.

significantly on cell growth; Group three shows a higher cell growth. TPRPz when compared with TPRPx (P=0.009), TPRPy (P=0.001), TPRFy (P=0.002) differs significantly on cell growth; Group six shows a higher cell growth. Post hoc (Table 1) shows a statistical significance at 5% level (P<0.05) when following variables were compared; TPRPz when compared with TPRFx (P=0.013) differs significantly on cell growth. Group six shows a higher cell growth. Post hoc shows no statistical significance P>0.05 when following variables were compared; TPRFx, and TPRFy (P=0.439), TPRFx and TPRPx (P=0. 885), TPRFx and TPRPy (P=0.317), TPRFy and TPRPx (P=0.527), TPRPy and TPRFy (P=0. 818), TPRFz and TPRPz (P=0.061), TPRPx and TPRPy (P=0.390). Web diagram shows TPRFz (Group 3) with highest cell growth followed by TPRPZ (Group 6). Percentage of growth detected were as follows (Fig. 3): Group 1 = 35.17, Group 2 = 30.67, Group 3 = 61.50, Group 4 = 34.33, Group 5 =29.33 and Group 6 = 50.33.

## DISCUSSION

Surface modifications have shown great potential for improving hemocompatibility of biomaterials and devices (17). Contact guidance is the phenomenon that cells adapt and orient to substrate surface microtopography (18). Early studies on contact guidance describe alignment of cells and focal adhesions to microgrooves

with dimensions 1.65–8.96 µm in width and 0.69 µm in depth. This cellular behavior was suggested to be due to mechanical properties of cytoskeleton (19). Relative inflexibility of cytoskeletal components was considered to prevent bending of cell protrusions over surface configurations with too large an angle. Later studies and hypotheses, focused on relationships among cell contact site, deposited extracellular matrix, surface microtexture, and cell response. Microtextured surface was supposed to possess local differences in surface free energy resulting in a specific deposition pattern of substratum bound attachment proteins (20).

Spatial arrangement of adsorbed proteins and their conformational state were hypothesized to be affected. In addition to wettability properties, specific geometric dimensions of cell adhesion sites were suggested to induce a cell orientational effect (21).

A recent hypothesis suggests that contact guidance on microtextured surfaces is a part of cellular efforts in achieving a biomechanical equilibrium condition with a resulting minimal net sum of forces. Anisotropic geometry of substratum surface features, establishes stress and shearfree planes that influence direction of cytoskeletal elements, in order to create a force economic situation (22). Further, a mechanical model to explain contact guidance suggests that "surface feature stimulus" is transduced to the cytoskeleton via cell contact sites and cell surface receptors. In this model, cytoskeleton is considered as a static structure. Contrarily, Lackie et al. studied that cytoskeleton is a highly dynamic system, which is constantly broken down and elongated in living cells (23). Breakdown and formation of fibrous cellular components, especially in the filopodium, is influenced by microgrooves. Microgrooves create patterns of mechanical stress, which affects cell spreading and causes alignment of cells (24). Although these studies varied in cell type used, substrate surface feature dimensions and substrate bulk chemical composition, results clearly confirmed that very fine microgrooves (≤ 2 µm) have an orientational effect on both cell body and cytoskeletal elements. Cells were also observed to possess

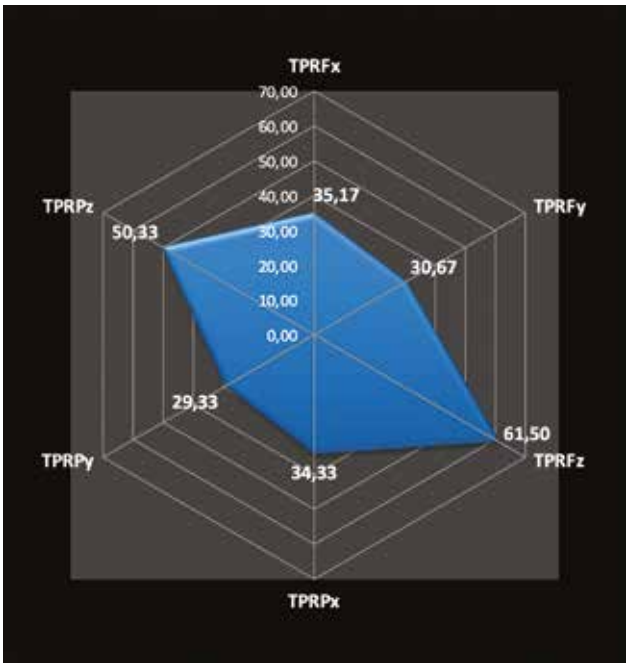


FIG. 3 Web diagram showing comparison of cell growth among titanium prepared platelet rich products.
Minimum cell growth was seen in TPRPy. TPRFZ is double TPRFy. Maximum cell growth (61.50%) was seen in TPRFz followed by TPRPz. Cell growth between 29%-36% seen in TPRFx, TPRFy, TPRPx and TPRPy.

| Multiple comparison Groups | | Mean difference | P | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| 1-TPRFx | TPRFy | 4. 500 | . 439 | -7. 21 | 16. 21 |
| | TPRFz | -26. 333 | . 000 | -38. 04 | -14. 62 |
| | TPRPx | . 833 | . 885 | -10. 88 | 12. 54 |
| | TPRPy | 5. 833 | . 317 | -5. 88 | 17. 54 |
| | TPRPz | -15. 167 | . 013 | -26. 88 | -3. 46 |
| 2-TPRFy | TPRFx | -4. 500 | . 439 | -16. 21 | 7. 21 |
| | TPRFz | -30. 833 | . 000 | -42. 54 | -19. 12 |
| | TPRPx | -3. 667 | . 527 | -15. 38 | 8. 04 |
| | TPRPy | 1. 333 | . 818 | -10. 38 | 13. 04 |
| | TPRPz | -19. 667 | . 002 | -31. 38 | -7. 96 |
| 3-TPRFz | TPRFx | 26. 333 | . 000 | 14. 62 | 38. 04 |
| | TPRFy | 30. 833 | . 000 | 19. 12 | 42. 54 |
| | TPRPx | 27. 167 | . 000 | 15. 46 | 38. 88 |
| | TPRPy | 32. 167 | . 000 | 20. 46 | 43. 88 |
| | TPRPz | 11. 167 | . 061 | -. 54 | 22. 88 |
| 4-TPRPx | TPRFx | -. 833 | . 885 | -12. 54 | 10. 88 |
| | TPRFy | 3. 667 | . 527 | -8. 04 | 15. 38 |
| | TPRFz | -27. 167 | . 000 | -38. 88 | -15. 46 |
| | TPRPy | 5. 000 | . 390 | -6. 71 | 16. 71 |
| | TPRPz | -16. 000 | . 009 | -27. 71 | -4. 29 |
| 5-TPRPy | TPRFx | -5. 833 | . 317 | -17. 54 | 5. 88 |
| | TPRFy | -1. 333 | . 818 | -13. 04 | 10. 38 |
| | TPRFz | -32. 167 | . 000 | -43. 88 | -20. 46 |
| | TPRPx | -5. 000 | . 390 | -16. 71 | 6. 71 |
| | TPRPz | -21. 000 | . 001 | -32. 71 | -9. 29 |
| 6-TPRPz | TPRFx | 15. 167 | . 013 | 3. 46 | 26. 88 |
| | TPRFy | 19. 667 | . 002 | 7. 96 | 31. 38 |
| | TPRFz | -11. 167 | . 061 | -22. 88 | . 54 |
| | TPRPx | 16. 000 | . 009 | 4. 29 | 27. 71 |
| | TPRPy | 21. 000 | . 001 | 9. 29 | 32. 71 |

*Post hoc showing significance at 1% level (P< 0.01) and significance at 5% level (P< 0.05).*

TABLE 1 Multiple comparison of cell growth among Titanium prepared platelet rich products.

cell adhesion structures that were wrapped around edges of a ridge or attached to walls of the ridge (25). On the other hand; we must also notice that ECM (extracellular matrix) possesses mechanical properties. ECM is not a rigid structure, but a dynamic mass of molecules. In vitro studies indicate that cell-generated forces of tension and traction can reorganize ECM into structures that regulate single cells behavior (26).

Apart from changes in cell size, shape, and orientation, surface microtopography has been reported to influence other cell processes. Evidences describe changes in cellular differentiation, DNA/RNA transcription, cellular metabolism, and cellular protein production of cells cultured on microtextured surfaces (27). A study using μCP (microcontact printing) surfaces, having square cell adhesive and nonadhesive domains has shown that in small surface adhesive domains (< 75 μm), apoptosis levels in endothelial cells is high (particularly so for 5 μm × 5 μm domains). When cells are placed on larger domains, cell spreading and growth occurs (28). According to Hong and Brunette, surface microtopography can enhance production of specific favorable proteins. On the other hand, production or secretion of less favorable metabolic products can also be enhanced. If this occurs, this might have a deleterious effect on overall cell response. Rise in production or release of proteinases may not be beneficial for connective tissue cell response reflecting that, at least at molecular levels, regulation of cell function by substrate surface microtexture may be a complex affair (29). Modifications to surface microarchitecture, chemistry or energy can alter cell adhesion, proliferation and gene expression. By designing materials to present specific surface properties, there is potential to control cell responses to achieve desired application (7).

The examination of surface oxidation effect on platelet adhesion found that composition and thickness of oxide layers influences platelet adhesion. Thick titanium oxide layers formed on Ti substrates by heating displayed minor platelet adhesion than thin oxide layers on untreated Ti substrates (17). Following vessel damage, circulating platelets immediately interact with subendothelial components such as collagen (ECM). Platelets are thereby stimulated by secreting substances such as ADP, thromboxane A2 and serotonin (5-ht) (platelet activation). These secreted compounds are proaggregatory, and so stimulates other platelets in the microenvironment (platelet recruitment) (30).

Once release of biomolecules from platelets are activated, a network is formed to establish a fibrin clot that acts as scaffold for growth factors over a limited period of time (12). Weed et al along with other authors have proposed alternative methods to liberate growth factors from platelets, such as lysing platelets by freezing them or using sonication or ultrasound (31).

Chaotropic agents, such as chlorine, interact with proteins significantly causing deleterious effects on fibrin polymerization (32). Another factor that influences growth factors release is pH of membranes. Factors such as transient alkalization to pH 12, acidification to pH 2, heat and many chaotropic agents activate this stable fibrin complex (33). An acidic environment of pH 6 can slow down clot formation. Hence, the structure of fibrin matrix varies with changes in local pH, thus influencing its biological function. Variation in fibrin structure is described by fiber thickness, fiber length, number of branch points and porosity (34).

Juana Valles et al proved that erythrocytes promoted platelet reactivity in plasma medium, as demonstrated in an in vitro system that independently evaluated biochemistry of platelet activation and recruitment ,suggesting that erythrocytes and collagen activates platelets (35). Sensitivity of platelet responses was compared to shear stress stimulation of human and bovine blood using multiple platelet activation markers. Results indicated that exposure to shear stresses above 20 Pa caused significant changes in all three platelet markers for human blood (36). Mechanically, platelet activation has been shown to be a function of elevated shear stress and exposure time (37). Govindarajan et al. made models solely based on shear stress time integral experienced by platelets, that passes through gap widths of the hinge region. Studies have suggested that a specific relationship exists for activation of platelets in arterial flows. Platelets get activated beyond a minimum shear stress as in the shear stress time integral model (33). Joel L. Moake et al. constructed a Lagrangian particle tracking method used to model and track platelets, to compute magnitude of shear stress on platelets as they pass through hinge regions. Results showed a boundary layer separation in gaps between leaflet ear and the constricted hinge geometry. Separated shear layers roll up into vortical structures that lead to high residence times combined with exposure to high shear stresses for particles in hinge regions. Particles are preferentially entrained into this re-circulation zone, presenting possibility of platelet activation, aggregation, and initiation of thrombi (38). A fluid shear stress of 180dyn/cm2 was applied for 0.5 and 5 min to platelets in citrated plasma or blood in a cone and plate viscometer with minimal platelet-surface interactions. Platelets aggregated in shear field (39).

Platelets get activated beyond a minimum shear stress from rough and sharp titanium spirals, rather than smooth surfaces of titanium tubes. Thereby, samples of platelet rich fibrin activated with titanium spirals showing increased endothelial cell growth (groups three, six) may be due to forces on surfaces of spirals, as exerted on the platelets. This condition may be due to activation resulting in an enhanced reorganization of deposited proteins. Consequently, contact guidance and other cell behaviors are induced in case of in-vivo situations. Cell surface receptors and inside-outside cell signaling phenomena play important roles. Conversely, samples of platelet rich plasma and fibrin prepared in smooth titanium tubes showed decreased cell growth

(groups two, five) may be due to decrease activation of platelets or due to addition of other metal ions with valence states (Grade five titanium containing aluminium and vanadium) which are different from native metal ions, can alter both electronic and ionic transport of charge across interfaces. More studies need to be done on *in-vivo* applications of stress on platelets, particularly differences in surface topography, microtexturing and hemocompatible materials and their relationships.
Hypothesis TPRFz and TPRPz can be used to promote better healing in branches of medicine and dentistry. With reference to applications in the field of Endodontics, titanium may be used as a root canal filling material, which is not harmful even if obturation extends beyond apex, due to its hemocompatibility, nevertheless regeneration at the apex would be primarily odontogenic or osteogenic has yet to be studied. Hence, breach of the apical foramen would not be a violation as titanium is hemocompatible unlike gutta percha which is only biocompatible.

## CONCLUSION

Comparison among the titanium prepared platelet rich products was done inferring about a new method of activation of platelets. This is a novel study comparing titanium prepared platelet rich plasma/fibrin in titanium tubes and titanium prepared platelet rich plasma/fibrin activated with titanium spirals, subjecting them to tissue culture. Group three shows highest cell growth followed by group six. These groups contained platelet concentrates, prepared by subjecting platelets to an increased amount of shear stress unlike other groups. To simplify, in the present study, TPRFz overrides TPRPZ, TPRFx, TPRPx, TPRFy and TPRPy. Web diagram points the highest cell growth at TPRFz and lowest at TPRPy. The titanium prepared platelet rich fibrin 'TPRFz' activated by using titanium spirals has proved to be most efficient. Therefore, we can infere that when platelets are mechanically activated with rough hemocompatible materials like titanium, they undergo a shear stress which activates them, leading to increased revascularization and thus regeneration. This concludes that an elevated amount of growth factors may be present in cell cultures, when platelets are subjected to certain amount of stress, discovering an alternative and better method of wound repair using these platelet concentrates. Thus we can develop a new generation of platelet rich products by inducing platelet activation with rough hemocompatible biomaterials like titanium promoting faster regeneration and wound healing. A combination of titanium and platelets need to be further studied from all aspects to promote these novel products and advance in their benefits on healing.

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## Institutional Ethical Committee
No. NICDS/IEC/2014/03

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