1. INTRODUCTION
Platelet rich plasma (PRP) is the cell-free plasma containing a higher than normal concentration of human platelets per volume. Treatment with PRP has become an increasingly popular clinical tool utilized in soft tissue augmentation as well as bone regeneration. Soft tissue augmentation includes: (i) facial rejuvenation therapy for wrinkles and solar damaged skin1, (ii) surgery-free repair of sports injuries3, (iii) cutaneous wound remodelling1, 2.

In essence, as the concentrated platelets in the PRP are activated, it mimics the wound healing cascade and accelerates recovery by releasing a variety of cytokines and growth factors leading to new formation of extracellular matrix proteins such as collagen. In practice, treatment with PRP is made possible by easily obtaining autologous PRP with the aid of commercially available kits.

After 4 years of extensive research the celluVance™ PRP kit was designed to achieve optimal results while ensuring a practical easy-to-use extraction and activation process for physicians. The celluVance™ PRP kit contains special citrate containing pyrogen- and endotoxin free vacutainers for separation of platelet poor and platelet rich plasma during a once-off centrifugation step, which is followed by a safe and effective, thrombin-free activation method, thereby eliminating the risk of immunological reactions. The activation steps ensure the release of the relevant growth factors from the platelet rich plasma.

The celluVance™ PRP kit can be classified as a leukocyte-containing PRP (L-PRP) kit (Figure 1) that also contains fibrinogen. This type of kit is advantageous due to the fact that leucocytes possess anti-infectious actions and immune regulation properties. They also release vast amounts of a growth factor known as VEGF. Added VEGF in the PRP might aid in the promotion of angiogenesis. It has also been indicated that the leucocyte content does not seem to induce negative effects or impair the potentially beneficial effects of PRP, even when used in joints. Furthermore, it is important to not only focus on the role of growth factors in wound healing but also on the circulating cytokines. The fibrin matrix acts as a reservoir for the surrounding cytokines, supporting their release. The fibrinogen concentration originates from the platelet α-granules after activation. If only the PRP (“buffy coat”) is collected the final fibrin concentration is low, whereas collection of circulating fibrinogen (found in the PPP fraction) supports the final fibrin network and will improve healing.

2. BACKGROUND

2.1 celluVance™ PRP kit
The celluVance PRP kit medical device is the first of its kind to be manufactured in South Africa in a Good Manufacturing Practice (GMP) accredited laboratory. The manufacturing process complies with the strictest of standards to ensure sterility and safety.

The in vitro properties of and in vivo results obtained via a series of case studies demonstrating the effect of platelet rich plasma (PRP) extracted and activated with the celluVance™ PRP kit for improvement of different aesthetic and injury-related cases.

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Figure 1. Schematic illustration of the matrix and cell architecture of the four categories of platelet concentrates. Two key parameters are important: leucocyte content (blue circles) and density of fibrin (yellow/light-brown fibres). Platelet aggregates (light-grey shapes) are always assembled on the fibrin fibres.

2.2 Applications
Traditionally PRP has been used in surgical procedures but has become an increasingly popular clinical tool utilized in soft tissue augmentation as well as bone regeneration. Soft tissue augmentation includes facial rejuvenation therapy for wrinkles and solar damaged skin, surgery free repair of sports injuries and cutaneous wound remodelling such as diabetic foot ulcers. PRP has been shown to be successfully used in injected form for the treatment of tendonitis as well as for the treatment of long bone delayed healing. Furthermore PRP has been shown to diminish pain and inflammation, in
2.3 Safety and contra-indications
The use of PRP is a safe alternative for soft tissue augmentation as the patient’s own blood component are used in the preparation of PRP. The celluVance™ PRP kit is manufactured in a GMP accredited facility and makes use of non-toxic substances in the blood collection and activation process of PRP. The celluVance PRP kit does not contain non-autologous thrombin. The use of non-autologous thrombin in some protocols is worrisome as it can result in an allergic reaction in some individuals. There are no indications in the literature that PRP is carcinogenic, but patients with the presence of a tumour or metastatic disease should not receive PRP treatment. Other contra-indications include: an active infection at the site of injection, individuals with low platelet counts or individuals who are treated with anti-platelet- or anti-coagulation therapy and pregnant or breastfeeding patients. The patient’s age or extent of the disease might affect the outcome. It should be noted that some patients might experience worsening of symptoms for the first few days as the body’s natural healing response is enhanced and inflammation induced.

As with any injection, sterility should be maintained at all times to avoid the possibility of an infection at the injection site.

This paper presents the in vitro results that demonstrate the effect of the celluVance™ PRP kit on cell proliferation and growth factor release as well as in vivo results that were obtained on a case study basis as part of routine practice.

3. MATERIALS AND METHODS

3.1 In vitro

3.1.1 Platelet counts
Venous blood (±16ml) was collected from 30 volunteers under strict aseptic conditions with the aid of two 8ml citraplate™ tubes and one BD Vacutainer® CPT™ K2E tube (purple EDTA tube). The citraplate™ tubes were inverted 8 times and centrifuged within 2 hours of collection. The citraplate™ tubes were centrifuged at 1800g for 20min. The now separated plasma layers were collected in the following order: firstly up to the mononuclear cell layer (labeled as PPP), after which the PRP layer was collected and labeled as such (Figure 2). A 500µl sample of each volunteer’s PRP and PPP was collected. The 3ml EDTA tube, PRP and PPP samples were sent to AMPATH (Drs Du Buisson, Kramer, Swart, Bouwer Inc) National Laboratories for a platelet count. The volume of the obtained PRP and PPP was measured.

3.1.2 Growth factor quantification
After extraction and activation of PRP by using the celluVance™ PRP kit (please refer to attached instruction leaflet for activation procedure), the PRP suspension was left at room temperature until a solid platelet gel (fibrin clot) had formed. The fibrin clot was transferred in 15ml polypropylene containers and stored at -70°C until completely frozen. Thereafter the platelet gels were freeze dried in a Freezone® 6 freeze dry system (LABCONCO, Kansas City, US). Once the platelet gels were completely dry, the gel powder was re-suspended to the corresponding PRP volume obtained for that particular sample with sterile PBS. The tubes were gently inverted to dissolve the gel powder. The dissolved gel powder was divided into 1.5ml aliquots and stored at -70°C until use. TGF-β1-specific ELISA (Quantikine® Human PDGF-AB Immunoassay, R&D Systems, Minneapolis, US) was used for the quantitative determination of activated human TGF-β1 concentrations released from activated platelets as described above. All reagents were prepared according to the manufacturer’s instructions. All samples, standards and controls were assayed in duplicate. Briefly, the suspension was centrifuged 10000g (Microfuge® 16 centrifuge, Beckmann Coulter, Berea, US) for 10min, the supernatant collected. The samples were diluted 20-fold in Calibrator Diluent RDS-53 before acid treatment (suggested by manufacturer). To activate the samples, 20µl 1N HCl was added to 40µl of sample. The samples were incubated for 10min at room temperature and neutralized with 20µl 1N NaOH. Thereafter 50µL of the standard, control (Calibrator diluents RDS-53) or
sample was added per well. The optical density was determined using a microplate reader set to a wavelength of 450nm and reference wavelength of 570nm. The concentrations of the TGF-β₁ standards were plotted against the corresponding absorbance to give a standard curve used to determine the total TGF-β₁ in the unknown samples.

3.1.3 Cell proliferation assay

Human dermal fibroblasts were kindly donated by Southern Biotech (PTY) Ltd. (1 Albert road, Centurion, ZA). The cells were maintained in DMEM supplemented with 10% heat inactivated foetal calf serum (HI FCS). The cells were harvested by adding 5ml Trypsin / Versene solution to the 75cm² culture flask and incubated for 5-10min until the cell detached from the bottom of the flask. The cells were re-suspended in the 15ml polypropylene centrifuge tubes at 50 000 cells/ml. The negative control groups received a volume of 20µl of the appropriate serum free medium. The positive control groups received a volume of 20µl of the 10% HI FCS to give a final volume of 200µl per well, whereas the “treated” groups received 20µl of PRP from 3 randomly selected volunteers resulting in a final concentration of up to 10% activated PRP. These cell cultures were transferred to separate wells of a 96-well microtitre plate and incubated for 1 or 3 days at 37°C in a 5% CO₂ atmosphere in a closed container with sterile deionized water. At the end of the incubation period, the viable cells were detected with the crystal violet assay.

3.2 In vivo case studies

Physicians recommended PRP treatment obtained and activated with the celluVance™ PRP kit to suitable candidates as part of their normal routine practice. Approval from the South African Medical Association Research Ethics Committee was obtained in order to document and publish results obtained from these treatment procedures. Physicians were asked to diagnose the condition from which the patient was suffering as well as the injection technique that was used during treatment. Activated PRP was obtained and prepared by using the celluVance™ PRP kit (refer to attached instruction leaflet). Physicians documented the severity of each condition before and after 4-8 weeks of treatment on a visual analogue scale (VAS) (Figure 3). Informed consent was obtained from patients, after which they indicated the severity of their condition before and 4-8 weeks after treatment on an identical but separate visual analogue scale (Figure 3).

Pharmacists and patients were asked to indicate their satisfaction with the product by means of marking the appropriate comment on a satisfaction rating scale (Figure 4). Cases were classified as either being of aesthetic nature or injury and osteoarthritis related.

4. RESULTS

4.1 In vitro

4.1.1 Platelet counts

Platelet counts were obtained from both the separated PRP and PPP plasma layers and it was found that the platelet count present in the PRP fraction was significantly higher when compared to that of the PPP fraction (Table 1).

4.1.2 Growth factor quantification

The quantification of TGF-β₁ was done via an ELISA assay in order to confirm the release of this important growth factor after activation of the PRP with the celluVance™ PRP kit. A concentration of 45.5 ± 3.8ng/ml was detected (Table 1).

Table 1. Quantification of platelets and TGF-β₁ concentration in PRP and/or PPP extracted with the celluVance PRP kit from 30 volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Whole blood</th>
<th>PRP</th>
<th>PPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet counts</td>
<td>313 ± 11 x 10⁹/L</td>
<td>657 ± 28 x 10⁹/L*</td>
<td>14 ± 2 x 10⁹/L</td>
</tr>
<tr>
<td>Growth factor (TGFβ₁)</td>
<td>N/A</td>
<td>PRP</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>45.5 ± 3.8 ng/ml</td>
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*Statistically significant increase when compared to the PPP and whole blood (p<0.05); SEM: Standard error of the mean; PRP: Platelet rich plasma; PPP: Platelet poor plasma; TGFβ₁: Transforming growth factor beta-1
4.1.3 Cell proliferation assay
It was tested whether PRP that was activated and extracted with the celluVance™ PRP kit was able to stimulate cell proliferation and initiate extracellular matrix production formation. By adding various concentrations of activated PRP (up to 10%), it was clearly demonstrated that it induced human dermal fibroblast proliferation in a concentration dependant manner (Figure 5). Furthermore, it is shown in Figure 6 that the activated PRP, even at the low concentration of 10%, resulted in a significantly higher cell proliferation rate when compared to the cells treated with the positive control and negative control.

Figure 5. Human dermal fibroblasts were incubated in serum free medium and in varying concentrations of PRP or 10% FCS. Cellular proliferation was determined with the crystal violet-assay. PC: positive control; NC: negative control; PRP: platelet rich plasma

Figure 6. Human dermal fibroblasts were incubated in serum free medium and in 10% FCS, 10% PRP. Cellular proliferation was determined with the crystal violet-assay. PC: positive control; NC: negative control; PRP: platelet rich plasma; a: statistically less than positive control (p<0.05); b: statistically more than positive control (p<0.05)

4.2 In vivo case studies
All the treatments that are mentioned in this section were conducted by injection of the activated PRP that was obtained by using the celluVance™ PRP kit. Percentage (%) symptom improvement from baseline was calculated with the formula:

\[
\frac{(\text{Pre-treatment score} - \text{Post-treatment score})}{\text{Pre-treatment score}} \times 100 = \% \text{ improvement}
\]

The results of this section are graphically summarised in Figures 7 and 8.

Figure 7. Percentage improvement from baseline as indicated by physicians and patients by marking the appropriate symptom severity score on a visual analogue scale. PZ: patient.

Figure 8. Product satisfaction score as indicated on a satisfaction rating scale by physicians and patients.

4.2.1 Aesthetic cases
Patient 1
The patient presented with severe acne of the face and erythema associated with oral isotretinoin use. After 2 treatments with activated PRP administered via mesotherapy injection technique, the physician reported excellent results (60% improvement) on post-acne scars and skin healing (Figure 9). Results were visible from 6 weeks after treatment.

Figure 9. Improvement in acne scars and redness associated with oral isotretinoin use. Two treatments with activated PRP were administered. (a: pre-treatment; b: post-treatment).
Patient 2
The patient obtained burn wounds to both legs which left severe scarring. Scars were infiltrated with PRP by means of injection. Both the physician and patient recorded an initial symptom severity of 9/10 on the VAS. After a period of 4 weeks, the physician and patient recorded a 33% and 22% improvement respectively. It was noted by the physician that the indentations of the scars have filled up, the colour improved a lot and that an overall very good result was achieved. A follow-up treatment was done 2 weeks later by using the same technique. A total improvement of 56% and a satisfaction score of 3/4 were documented by both physician and patient after 4 weeks.

Patient 3
The patient was diagnosed with hypotrophic skin lesions and hypopigmentation thereof. Activated PRP was injected into the skin lesions with a 30G needle (subcutaneous and intradermal). After a period of 12 weeks, both the physician and patient documented an improvement of 11% stating that plumping out of hypotrophic skin lesions was observed. A second treatment was conducted. It was noted (Figure 10) that there was very good improvement of scars and an overall improvement in skin appearance (44% and 56% by the physician and patient respectively). A product satisfaction score of 3/4 was documented.

Patient 4
This patient was treated for pigmentation and scaring of the face (severity of 6/10 and 7/10 according to the physician and patient respectively) by injecting the activated PRP with a standard mesotherapy technique. After a period of 6 weeks, an improvement of 67% and a product satisfaction score of 4/4 was documented by the physician, whereas the patient noted an improvement of 71% and a recorded a product satisfaction score of 4/4.

Patient 5 (treatment area 1 and 2)
Primarily, the patient was treated for rejuvenation and pigmentation of the neck and fine lines and wrinkles of around the peri-orbital area. Previous laser and needling treatments showed no decrease in pigmentation, therefore treatment with activated PRP was performed by using a standard mesotherapy technique. A 50% improvement was documented after a period of 8 weeks. Furthermore, the patient was treated for fine lines and wrinkles in the peri-orbital region with a small volume of PRP (due to the sensitivity of this area). A 20% improvement was documented after a period of 8 weeks.

Patient 6
Facial rejuvenation therapy was done by injection of activated PRP with a standard mesotherapy technique in the forehead, peri-orbital region, naso-labial folds and cheeks. After a period of approximately 4 weeks a 60% improvement in skin texture and radiance was marked by the physician and patient. It was stated that the skin appears more radiant and youthful.  

Patient 7
The patient was treated for fine lines and wrinkles in the neck area and the left forehead with a severity of 2/10 and 4/10 according to the physician and patient respectively. Nappage, intradermal injection and a point-point injection technique was used with a 12mm x 32G needle and 4mm x 32G needle + SIAdaptor. No lignocaine or Emla® was used. After a period of 6.5 weeks a 50% improvement in skin texture, tightness and smoothness was reported by the physician who noted that wrinkles were also shallower or even disappeared. A 50%
improvement was recorded by the patient as well and a product satisfaction score of 4/4 was given by both the physician and patient. (Please note that the patient underwent dermarolling/needling a month before treatment with activated PRP).

### 4.2.2 Accelerated healing and assisted symptom management of various injuries and osteoarthritis

Results of this section are graphically summarised in Figures 13 and 14.

**Figure 12.** Improvement in skin texture, tightness and smoothness. Neck lines are less visible post-treatment. One treatment with activated PRP was administered. (a: pre-treatment; b: post-treatment)

**Patient 8**

The patient was diagnosed with chronic knee pain due to meniscal degeneration. Activated PRP was injected around the right knee area after which an improvement in pain and flexibility of 29% was noted by the physician and 14% by the patient after a period of 4 weeks. After 2 months the symptoms returned after which a follow-up treatment was done by intra-articular injection of the activated. After 4 weeks, the patient documented a total of 29% improvement in pain. Both the physician and patient recorded a product satisfaction score of 3/4.

**Patient 9**

The patient was diagnosed with right lower leg injury, associated with poor healing of soft tissue contusion, which was obtained 1 year previously. Activated PRP was injected around & over injury area with a 4mm needle and 12mm needle. A 67% and 45% improvement of chronic pain and swelling was documented by the physician and patient respectively after a period of 7 weeks. A product satisfaction score of 4/4 was recorded by both parties.

**Patient 10**

The patient has been suffering from osteoarthritis in both knees and an initial symptom severity score of 7.5/10 and 6/10 was recorded by the physician and patient respectively. After a period of 8 weeks an improvement of 13% was noted by the physician, however, no improvement was experienced by the patient. Post injection pain and swelling was experienced 3-4days after treatment administration. A product satisfaction score of 2/4 and 1/4 was documented by the physician patient respectively. The physician stated that he suspected that the patient should be treated for a different indication and that osteoarthritis might not be the primary cause of symptoms.

**Patient 11**

Severe osteoarthritis in both knees was diagnosed. However, the right knee was documented to be more severe and was therefore selected for treatment with activated PRP via intra-articular injection. Initially, a symptom severity score of 7/10 and 6/10 was recorded by the physician and patient respectively. An improvement of 43% was documented by the physician whereas the patient experienced a 33% improvement in symptoms after 9 weeks. Product satisfaction scores of 3/4 and 2/4 were recorded by the physician and patient respectively.
Patient 12
The bone fracture present in the arm of the patient displayed typical non-attachment characteristics with clear absence of cortical bone (Figure 15a). The patient was injected with activated PRP in the fracture area. It was seen that, after a period of 16 weeks, new cortical bone deposition occurred and bone density has increased dramatically (Figure 15b).

Patient 13
The patient experienced chronic pain due to plantar fasciitis with associated calcification (Figure 16a) for a period of 6-9 months before being treated with activated PRP. Injection with a 23G needle in the plantar fasciitis implant on the calcaneus was performed. After a period of 8 weeks it was reported that the chronic pain has disappeared and an overall improvement of 86% was reported. Furthermore, it was seen that the calcification has decreased dramatically, which correlated with the clinical symptom improvement (Figure 16b).

5. DISCUSSION AND CONCLUSION
PRP is obtained by concentrating the platelets from the patient’s whole blood mainly by centrifugation. The high concentration of platelets contains a rich source of growth factors and cytokines located in their α-granules. Platelet activation results in the release of growth factors from the α-granule stores that are capable of soft tissue augmentation. It is well established that growth factors are able to enhance connective tissue healing. A growth factor of particular importance is transforming growth factor β (TGF-β). TGF-β contributes to a number of developmental, physiological and immunological processes and is able to stimulate proliferation in cultured fibroblasts.

The celluVance™ PRP kit employs a simple protocol for the collection and activation of a patient’s PRP. A previous study by, Anitua et al., indicated that the aim of a PRP protocol is to prepare PRP with a platelet count in excess of $300 \times 10^9$ platelets / L. The results of the present in vitro study indicated that the volume of platelets concentrated in the PRP fraction extracted with the celluVance™ PRP kit is $657 \times 10^9 \pm 28$ (SEM) platelets / L. The volume of platelets extracted from the PRP fraction is significantly higher than that in the whole blood and PPP fraction respectively. It was found that TGF-β1 concentrations obtained in the PRP that was extracted and activated from 16ml whole blood with the celluVance™ PRP kit, were comparable to that of similar PRP kits when compensating for different blood volumes obtained for different kits. From the cell culture proliferation studies (Figures 5 and 6) it is clear that even a concentration of 10% of the activated PRP was sufficient to induce significant cell proliferation in a human dermal fibroblast culture in comparison to the positive control (HI FCS, which is routinely used in cell culture experiments to induce cell proliferation in a primary cell cultures.). This proves that the extraction and activation method used by the celluVance™ PRP kit induces growth factor release that stimulates primary cell proliferation and extracellular matrix deposition.

From the data reported by physicians and patients in a series of case studies it is evident that activated PRP obtained with the celluVance™ PRP kit enhances skin texture and smoothness while decreasing scars, fine lines and wrinkles. This resulted in a more radiant and youthful skin appearance. Furthermore, treatment with the activated PRP resulted in symptom reduction and accelerated healing of various injuries and symptom management in most of the patients diagnosed with osteoarthritis.
Figure 16. A decrease in calcification was detected post treatment as indicated by the arrow in b when compared to a (pre-treatment). One treatment with activated PRP was administered.

Although improvement is clearly visible after only 1 treatment with the activated PRP, more prominent results might be achieved after 2-3 treatments. In general, results are visible from week 4 onwards.

The patients that were treated with the activated PRP in the present report did not report any product related side effects. However, two patients (one with osteoarthritis of the knee and one with plantar fasciitis) reported severe pain at the injection site 3-4 days post treatment. This can be managed in future by oral administration of opioids.

In conclusion, PRP extracted and activated with the celluVance™ PRP kit has shown to initiate soft tissue augmentation by cell proliferation and extracellular matrix production. These results correlate with clinical findings of skin rejuvenation and accelerated healing. This is a safe and effective treatment for improvement of scars, fine lines and wrinkles, pain and symptom management of chronic injuries and osteoarthritis, and overall skin revitalisation.

6. ACKNOWLEDGEMENTS
Thank you to the physicians who reported the results obtained with the celluVance™ PRP kit. In alphabetical order of surnames thanks to:

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- Dr A.B. de Villiers
- Dr N. Morrow
- Dr A.D. Ramagole
- Dr M. Smit
- Dr J.M. vd Merwe

7. REFERENCES

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