The objective evaluation of the effect of autologous platelet concentrate on post-surgery scarring in deep burns

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Abstract
Introduction: The healing of grafted areas after surgical treatment of deep burns frequently generates mutilating scars and rises the risk of subsequent scar hypertrophy. Scar assessment based on clinical evaluation is inherently subjective, which stimulates search for objective means of evaluation.
Objective: The aim of this study was to objectively evaluate the effect of using autologous platelet concentrate (APC) in combination with split thickness skin grafting (STSG) on scarring processes following surgery of deep burns as compared with application of sole STSG.
Method: Selected viscoelastic properties of 38 scars on 23 patients in total were examined using the Cutometer MPA 580 under controlled conditions for long-term outcomes 1, 3, 6 and 12 months after the surgery following deep burns.
Results: The findings of this study suggest that the STSG+APC combination reduces the time of scar viscoelastic properties recovery as compared with application of the sole STSG. This was statistically significant for viscoelastic parameters $R_2$ and $Q_1$.
Conclusion: APC has been advocated to enhance scarring after surgery of deep dermal and full thickness burns. We objectively demonstrated that the viscoelastic properties of scars treated with STSG+APC combination return more rapidly to the plateau state than areas treated with STSG only.
Keywords: Burns assessment, scar, surgical treatment of deep burns, split thickness skin graft (STSG), autologous platelet concentrate (APC), skin viscoelasticity, cutometer

1. Introduction

Increasing emphasis is placed on the functional and cosmetic outcomes following treatment of non-healing deep dermal and particularly full thickness burns which are subject to surgical treatment in the form of sharp tangential excision followed by skin grafting. Such a practice is a golden rule in burns treatment [1]. Meshed Split Thickness Skin Grafting (referred to as STSG hereinafter) represents one of the independent risk factors for postburn pathologic scarring [2]. The healing of grafted areas frequently generates widespread and mutilating scars, thus giving rise the risk of subsequent scar hypertrophy at the same time. The prevalence of postburn pathologic scarring has not yet been well documented [2] and remains in fact unknown [3].

Several scales were proposed and have been routinely used in clinical evaluation of postburn scarring and scars therapy response. The main representatives include the Vancouver Scar Scale (VSS), Patient and Observer Scar Assessment Scale (POSAS), Visual Analog Scale (VAS), and Manchester Scar Scale (MSS), among which the VSS is the most frequently used one [4]. Scar assessment routinely based on the above mentioned scales is inherently subjective and thus highly observer dependent [5]. This lack of objectivity stimulates the search for methods of objective evaluation based on more reliable assessment techniques. Non-invasive suction method has been found to be a reliable for single-observer measurement of scar viscoelastic properties [6].

Autologous Platelet Concentrate (referred to as APC hereinafter) has been used for about 20 years in diverse surgical fields of medicine to improve wound healing and tissue repair. Platelet growth factors have shown its potential to improve healing in many human clinical studies, particularly in oral and periodontal surgery [7–11], orthopaedics and trauma surgery [12–14], and plastic surgery [15, 16]. On the other hand, there is a lack of clinical experiences and long-term outcomes of deep burns surgical treatment using APC simultaneously with STSG.

The aim of this retrospective study was to analyze and evaluate the long-term effect of APC in combination with STSG on scarring processes following skin grafting of deep burns, as compared to applying sole STSG by using the objective method. The alternate hypothesis was that using APC in com-
bination with STSG leads to better viscoelastic scar properties and, consequently, to better functional and cosmetic outcomes, against the null hypothesis that no effect is produced by using APC in combination with STSG.

2. Methods

The study protocol was reviewed and approved by the Institutional Review Board of the University Hospital Ostrava. All patients were fully informed about the study and signed the informed consent with the treatment and subsequent measurements.

The study presented is a retrospective data analysis obtained by the objective testing of long-term outcomes of scarring after deep burns surgery. The assessments were carried out under controlled conditions by objective measuring the viscoelastic properties of skin using the suction method.

2.1. Subjects

From March 2011 till October 2012, a total of 23 patients (10 men and 13 women) underwent measurements of scar and normal skin viscoelastic parameters one, three, six and twelve months after the surgical treatment of full thickness and non-healing deep dermal burns. Total of 38 scars were subjected to those measurements. Both scars healed using STSG+APC (total of 24 scars) and those healed using STSG without APC (total of 14 scars), as well as normal skin sites for comparison (total of 42 sites) were involved. Five patients were treated with the use of STSG only, 5 patients underwent surgery using both STSG with the APC in one area and sole STSG in another area, and 13 patients whose burns were treated with the use of STSG in combination with the APC.

The distributions of age, burns extent, surgically treated (necrectomed) area extent (equal to the STSG treated total area extent), STSG in combination with APC treated area extent, STSG only treated area extent (all in percents of TBSA), and surgery post-traumatic day are given in Table 1 and graphically represented in Figs 1 and 2. The statistics of scar numbers are graphically represented in Fig. 3. The burn injuries were in most cases caused by flame and by hot liquid (43.5%), contact (8.7%), and electricity (4.3%). The surgery was performed on 11th (±3.7) day after injury (see Table 1).

### Table 1: Basic demographic statistics.

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>Mean</th>
<th>Median</th>
<th>Max</th>
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<tr>
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<td>48.6</td>
<td>40</td>
<td>81</td>
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<tr>
<td>Surgery PTD</td>
<td>6</td>
<td>10.9</td>
<td>11</td>
<td>22</td>
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<tr>
<td>% TBSA of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>burns</td>
<td>1.25</td>
<td>7.0</td>
<td>6.0</td>
<td>17.0</td>
<td>5.4</td>
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<tr>
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<td>3.7</td>
<td>3.0</td>
<td>15.5</td>
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<tr>
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<td>3.7</td>
<td>3.0</td>
<td>15.5</td>
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</tr>
<tr>
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<td>2.5</td>
<td>2.5</td>
<td>9.0</td>
<td>2.2</td>
</tr>
<tr>
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<td>0.0</td>
<td>1.2</td>
<td>0.0</td>
<td>8.0</td>
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</tr>
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</table>

Figure 1: (a) Distribution of burned patients by age. The patients aged 20–40 years were 50% of all patients. (b) Distribution of burns etiology. (c) Distribution of post-traumatic day of the surgery.
autologous thrombin for activation of platelets was prepared, was collected in two consecutive steps – from the first batch the APC was produced. The blood was centrifuged in accord with the manufacturer’s recommendations at 2,400 rpm for 14 min at room temperature. 9 ml of blood volume enabled to produce 3 ml of autologous thrombin, 60 ml of blood volume enabled to produce 10 ml of the APC. To get an idea about the quantity needed, the rule of thumb is that 10 ml of APC can cover about 4% of TBSA.

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The APC and autologous thrombin were applied to the skin grafted areas using a special SmartJet Applicator (Harvest Technologies Corporation, Plymouth, MA, USA). APC is a fluid which jellifies within 20–30 seconds. Gelation is an important factor for graft adherence to the recipient area that prevents grafts displacement and creates favourable input conditions for graft take. After local transplantation of APC and platelet activation by autologous thrombin, the selective sequential release of platelet growth factors improves healing, accelerates scars maturation and reduces subsequent hypertrophic scarring [17]. Since APC is a topically applied autologous material, the risk of blood transfer disease is virtually nonexistent, and no systemic undesirable effects have been identified.

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2.3. Material and instrumentation

The APC and autologous thrombin were prepared by density gradient centrifugation using the Harvest SmartPReP Platelet Concentrate System (manufactured by Harvest Technologies Corporation, Plymouth, MA, USA). The whole process was carried out under strictly sterile conditions right in the operating room simultaneously with the surgery. Hence, the surgery time was not increased at all. The patient’s peripheral venous blood was collected in two consecutive steps – from the first batch the autologous thrombin for activation of platelets was prepared, from the second one, about 30 minutes later, the APC was produced. The blood was centrifuged in accord with the manufacturer’s recommendations at 2,400 rpm for 14 min at room temperature. 9 ml of blood volume enabled to produce 3 ml of autologous thrombin, 60 ml of blood volume enabled to produce...
side the probe, and a connecting tube between the probe and the main unit (see Fig. 4). The whole system is connected to and controlled by a computer equipped with dedicated software Cutometer MPA Q supplied with the hardware [18].

Four measuring modes differing in the time dependence of underpressure are currently implemented for the cutometer. However, only Mode 1, characterized by a constant underpressure value during a predefined suction time (also referred to as on-time) immediately followed by an interval of switched off underpressure (referred to as relaxation time or off-time), is almost exclusively used for results published in current literature [18]. During the suction period the skin is drawn into the probe opening, and the depth of the skin penetration (skin deformation) is measured by a precise non-contact optical system with a sampling interval of 0.01 seconds. The sampled values of skin deformation are then stored for future processing on the attached computer system in the form of digitalized strain-vs-time cutometric curves.

The typical shape of a strain-vs-time curve measured in Mode 1 is shown in Fig. 5. Each suction-relaxation cycle has a rising part during the suction followed by a decreasing part during relaxation. The ascending part consists of two segments. In the first segment which is dominated by the elastic component of the skin distensibility, the slope of the curve is very steep, and the maximum deformation reached is usually denoted in literature as \(U_e\). Within the second segment the curve gradually flattens more and more which is typical for the viscoelastic component of the skin behaviour [18, 22, 23]. During this viscoelastic segment the curve increases by \(U_v\), reaching its full extension of \(U_f = U_e + U_v\) (see Fig. 5). In highly elastic materials (like a rubber balloon) the value of \(U_v\) is negligible in comparison with \(U_e\).

The descending part (during the relaxation when the underpressure is released) consists of two segments as well. Similarly to the suction part, the (negative) slope of the strain-vs-time curve in the first segment (which is dominated by the elastic component of the skin retractability) is very steep, and the extension quickly drops by a value referred to as \(U_r\), leaving total extension \(U_f - U_r\). The values of \(U_e\) and \(U_r\) are related to the function of elastin fibres [24–26]. Within the second segment of the decreasing part the curve gradually flattens more and more which is again typical for viscoelastic component of
the skin relaxation behaviour. During the second segment the curve continues in drop by $U_2 - U_1$, reaching its total drop of $U_2$ (relative to the maximum $U_1$), leaving a residual strain of $R = U_1 - U_a$ as shown in Fig. 5. In highly elastic materials the value of $U_a$ is very close to $U_1$, in other words, the material almost completely restore its original shape when it went out of the probe opening.

The parameters $U_1$, $U_e$, $U_v$, $U_c$ and $U_d$, and related parameters used in literature ($R_0 \equiv U_1$, $R_1 \equiv R = U_1 - U_a$, and $R_2 \equiv U_a$) are all dimensional (usually expressed in millimetres), thus dependent on the skin thickness. In other words, these parameters mix both intrinsic viscoelastic properties of the skin and the probed area spatial dimensions. To get rid of the skin thickness contribution, it is advantageous to introduce intrinsic tissue characteristics $U_1^*$, $U_e^*$, $U_v^*$, $U_c^*$ and $U_d^*$ which take into account the corresponding skin thickness $r$ in the form of product

$$U_1^* = U_1 r$$

(and analogously for the remaining parameters), as utilized, e.g., in paper [27]. However, such approach requires time consuming measurement of skin thickness. Another possibility which we finally decided to adopt in this study is to restrict only to dimensionless parameters defined as

$$R_2 = \frac{U_a}{U_1}, \quad R_3 = \frac{U_1}{U_e}, \quad R_6 = \frac{U_v}{U_e}, \quad R_7 = \frac{U_c}{U_1}. \quad (1)$$

Due to the fractional nature these parameters do not depend on whether the starred form or the plain form of the $U$-parameters is used, since the skin thickness is eliminated from their definition.\(^1\) An additional advantage of these parameters lies in the fact that to the first approximation they should be independent on the probe aperture size.

There is another suitable choice in introducing area $Q$-parameters [18]. They are defined as follows. Let $Q_0$ is the area of the suction part bounding box (grey filled area in Fig. 5), let $Q_e$ is the elastic recovery area (marbled area in Fig. 5), and let $Q_r$ is the viscous recovery area (paper texture filled area in Fig. 5). Then we define

$$Q_2 = \frac{Q_e}{Q_0}, \quad Q_3 = \frac{Q_1}{Q_0}, \quad Q_1 = Q_2 + Q_3 = \frac{Q_e + Q_r}{Q_0}. \quad (2)$$

The $Q$-family parameters are dimensionless and more robust than the $R$-parameters, since they take into account not only values of final strain but they also depend on the path of the strain-vs-time curve within the relaxation period.

At this point it is worth noting that the standard algorithm engaged in determining the inflection points separating the elastic and viscoelastic segments of the strain-vs-time curve relies heavily on the experimental fact that the time $T_{inf}$ elapsed from the instant of underpressure switch-on to attaining the value of $U_e$ approximately equals to 0.1 second; the same value is used for separation the elastic and viscoelastic segment of the relaxation part. These inflection points are denoted in Fig. 5 as $I_1$ and $I_2$, respectively. The above empirical value of time $T_{inf}$ (which corresponds to 10 sampling intervals) is hardwired in the configuration file of the cutometer software. Although an experienced user is given an opportunity to change the value by editing the configuration file, the basic principle of a constant elastic segment duration still survives. This setup leads to a strong dependence of all inflection point based dimensional parameters (i.e., $U_e$, $U_v$, and $U_c$), as well as all derived dimensionless linear parameters ($R_3$, $R_6$, $R_7$) on the fixed time $T_{inf}$. Indeed, a subtle perturbation in the slope of the elastic segment leads either to mutually compensating underestimation or overestimation of these parameters. As a consequence, the parameter $R_2$ remains the most reliable and robust one. Similar considerations apply also for the $Q$-parameters. While the values of $Q_2$ and $Q_3$ strongly depend on the position of the inflection point $I_1$ (hence, on the value of $T_{inf}$), the value of $Q_1$ is independent on the empirical value of $T_{inf}$. Similarly, the $Q$ parameter is the most reliable one among the $Q$-parameter family provided the constant elastic segment duration is used.

In the literature, the parameter $R_3$ is referred to as the gross-elasticity of the skin (it includes the skin viscous deformation), and represents the ability of total redeformation of the skin. The parameter $R_5$ is referred to as net-elasticity of the skin (with the viscous deformation excluded), and it represents the ratio of ability of immediate retraction to the ability of immediate distortion. The parameter $R_6$ represents the portion of viscoelasticity in the elastic part of the curve. Finally, the parameter $R_7$ is referred to as the biological elasticity of the skin, and it represents the ratio of the ability of immediate retraction to the ability of final distention. For more detailed analysis, see [22] and papers cited therein.

To conclude the selection of cutometric parameters to be analyzed, we preferentially take the dimensionless parameters $R_2$ and $Q_1$ defined by Eqs (1) and (2) into account for their independence on the position of the visco-elastic transition point. In order to assess the elastic and viscous part separately, we adopt the area parameters $Q_2$ and $Q_3$ defined by Eq. (2) because they are superior to the $R_5$, $R_6$, $R_7$ parameters in the sense of their greater robustness.

2.4. Data acquisition

The cutometer was operated in Mode 1 in all patients under the following controlled conditions: the probe aperture of 2 mm, constant suction underpressure of 450 mbar, equal suction and relaxation phases of 2 seconds each. Until July 2012, the default number of ten cycle repetitions have been used, which resulted in total measurement duration 40 s. As of August 2012 the number of repetitions has been reduced to 4 because none repetition-dependent parameters (such as $R_5$, $R_6$, and $R_0$ or area $F$-parameters) have been taken into account. This adjustment shortened a single site measurement down to 16 s without having effect on the results. The measurements were

\(^1\)Definitions of other cutometric parameters can be found in the literature; worth mentioning are namely $R_3$ and $R_4$ related to the last cycle maximum and minimum amplitude, respectively, then parameter $R_0 = R_3 - R_4$, and so called area $F$-parameters $F_0$ through $F_4$ [18]. All these parameters require repetitions of the suction-relaxation cycle (conventionally 10 repetitions) and are not considered in this paper.
performed on each patient at the same daytime, in the same examination room with controlled room temperature within 22–24 °C and air relative humidity of 50% ± 10%. Measured sites were shaved to prevent penetration of hairs into the probe aperture. All patients were accustomed to the ambient temperature, had been inactive for period of 15 minutes, and were always examined in the same position with limbs rested. The cutometer was regularly calibrated against the etalon supplied along with the device. The aperture was cleaned if necessary following the manufacturer instructions [18].

The examined areas were (1) scars following surgical treatment using STSG in combination with APC, (2) scars following surgical treatment using STSG only, and (3) normal skin areas in the contralateral or adjacent location (or otherwise best-matched site) to the scars, which acted as the control. All measurements were always carried out by the same physician to avoid introducing various kinds of errors caused the probe operator. Prior to examination, a digital photo of the measured site was taken.

Since the burn scars are planar, usually have an irregular shape and inhomogeneous surface, the measurements were performed in four different representative points of a scar in each patient in order to collect sufficient amount of data characterizing the scar viscoelastic properties in its entirety. For this purpose, as the first step, a “site map” was prepared in the form of a transparent foil placed on the scar area, while the scar borders were marked using indelible marker as shown in Fig. 6. Consequently, four quadrants were identified in the site map, and one measuring point was chosen in the centre of each quadrant. These site maps were filed and served as a guarantee for exact match of sites and reproducibility of measurements.

Total number of 418 curves were measured, of which 24 were excluded because of poor quality of measurement. Although the reasons of such flaws have not been explicitly examined, we are inclined to ascribe them to the most plausible sources – mostly to air leaks around the probe aperture and to soaking up a hair remnants that escaped being shaved into the probe aperture, which resulted in confusion of the measuring optical system. Thus, a net total of 394 curves entered processing and statistical analysis. Examples of both eligible and flawed curves are shown in Fig. 7.

2.5. Data processing and statistical analysis

All measured strain-vs-time curves acquired in the course of study were in the form of DBF and XLS files transferred from the control PC to another computer. Consequently, they were categorized by patient identification, by post-surgery month, and by treatment (STSG only scars, STSG+APC scars, and normal skin areas). The algorithm utilized by the original software to compute cutometric parameters from the curves was reimplemented under system Mathematica® (see, e.g., [28]).

Measured values of cutometric parameters \( R_2, Q_1, Q_2 \) and \( Q_3 \) were grouped as follows.\(^2\) Values for each parameter were placed into a sample labelled with a specific post-surgery month (PSM 1, 3, 6 and 12) and treatment type/control (STSG+APC, STSG only, and Normal skin) regardless of the patient identification. Thus, total of 12 samples reflecting all possible combinations of post surgery month and type of surgery were formed. These 12 samples were subjected to statistical treatment under the Mathematica® system. However, before any statistical calculations were commenced, an unabridged catalogue of all cutometric strain-vs-time curves had been generated for the purpose of exclusion of all corrupted curves as described in the previous section (see also Fig. 7), having left net total of 394 curves to be subject to statistics.

\(^2\)See the discussion on parameter selection in the section 2.3.
The former which tested the hypotheses concerning the equal-
ity of all 12 means was one-way ANOVA followed by post
hoc analysis in the form of Tukey test, the latter which tested
the hypotheses concerning the equality of all 12 medians was
Kruskal–Wallis test followed by post hoc analysis in the form
of Bonferroni test [29]. Significance level of 5% was adopted.
Finally, best fits to cutometric data as a function of time
were calculated using nonlinear regression (gradient descend
algorithm) for parameters $R_2$, $Q_1$, $Q_2$ and $Q_3$ in both treatment
modes STSG+APC and STSG only, as well as normal skin.
The model dependence was considered in the form
\[ p(t) = p_s - p_d e^{-kt}, \]  
where $p(t)$ stands for the value of any cutometric parameter at
time $t$, $p_s$ is its limit steady-state value (to which the parameter
value converges for large $t$), $p_d$ is the difference between the

For the purposes of descriptive statistics, box-and-whiskers
chart was chosen for its ability to provide comprehensive graph-
ical overview of the basic statistical quantities superior to tabu-
lar form (description of basic box-and-whiskers chart anatomy
is given in the caption of Fig. 8).

The inferential statistical analysis was carried out using
multiple comparison tests, both parametric and non-parametric.
The former which tested the hypotheses concerning the equality
of parametric $R_2$ and $Q_1$ were carried out on three groups of
scars: those treated with combination STSG only, STSG+APC, and control
group with normal skin sites. For all resulting 12 samples the box-and-whisker
charts were constructed as shown. The upper and lower sides of black boxes
mark the upper and lower quartiles, respectively. The upper and lower fences
ending the whiskers mark maximum and minimum values, respectively (out-
liers, if any, are marked by grey dots). White horizontal lines in the narrowest
places of the boxes mark median, the light grey notches mark the median con-
fidence intervals. White circled crosshairs mark the location of means, and ver-
tical dimension of the dark grey diamonds centred on crosshairs mark the mean
confidence interval. The respective sample sizes are marked by the numbers
above the time axis ticks.

Figure 7: (a) Example of eligible cutometric curve with 10 repetitions. (b) Ex-
ample of eligible cutometric curve with 4 repetitions. (c) Example of cutometric
curve corrupted presumably by air leakage around the probe aperture. (d) Ex-
ample of cutometric curve corrupted presumably by inadvertent penetration of
hair remnants into the probe aperture. All 24 cases similar to those in the bot-
tom row were expunged from the set of eligible curves.

Figure 8: Top: Box-and-whisker chart for cutometric parameter $R_2$. Bottom:
Box-and-whisker chart for cutometric parameter $Q_1$. In each of four post-
surgery months 1, 3, 6 and 12, cutometric measurements of viscoelastic prop-
erties (including parameters $R_2$ and $Q_1$) were carried out on three groups of
scars: those treated with combination STSG only, STSG+APC, and control
group with normal skin sites. For all resulting 12 samples the box-and-whisker
charts were constructed as shown. The upper and lower sides of black boxes
mark the upper and lower quartiles, respectively. The upper and lower fences
ending the whiskers mark maximum and minimum values, respectively (out-
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places of the boxes mark median, the light grey notches mark the median con-
fidence intervals. White circled crosshairs mark the location of means, and ver-
tical dimension of the dark grey diamonds centred on crosshairs mark the mean
confidence interval. The respective sample sizes are marked by the numbers
above the time axis ticks.
steady-state and initial \((t = 0)\) values, and \(k\) can be referred to as a “recovery constant” related to a more suitable characteristics of the decay – the recovery halftime \(T_{1/2}\) – by the formula
\[
T_{1/2} = \frac{0.693}{k}.
\]
The meaning of the halftime is that its every elapse reduces the difference between the steady state value and an immediate value in half.\(^3\) Naturally, the model cannot be interpreted literally for time between \(t = 0\) (surgery day) and \(t = 1\) (the first post-surgery month). On the other hand, within the interval of measuring (starting from the first post-surgery month) it is capable to reflect the real progress of healing process reasonably. Typical behaviour of the model function is depicted in Fig. 11.

3. Results

The results of descriptive statistics are diagrammatically shown in Figs 8 to 12. In Fig. 8, three types of behaviour can be observed for both parameters \(R_2\) and \(Q_1\): (1) In the post-surgery month 1 both kinds of treatment and the normal skin boxes differ from each other. The means and medians of STSG+APC and STSG only treated scars are relatively close, but very distinct from the mean and median of the control box constructed from normal skin data. While the STSG+APC treated scars have the mean greater than the median (the distribution has some excessive higher values that drag the mean up above the median), the opposite applies for the remaining two distributions. (2) In the post-surgery month 3 the STSG+APC treated scars and control normal skin show similar means and medians, but the STSG only treated scars seems to be in a somewhat lower position. All means are below medians, suggesting occurrence of some isolated distinctly lower values. (3) In the post-surgery months 6 and 12, a steady state seems to be achieved, neither means nor medians markedly differ from each other.

Cutometric parameters \(Q_2\) and \(Q_3\) charted in Fig. 9 show similar, but more chaotic behaviour. The main distinction lies in completely different behaviour in the first post-surgery month. While the parameter \(Q_2\) has all three medians unequal and rising in the sequence STSG+APC, STSG only, and normal skin, the parameter \(Q_3\) has medians (and means) equal for STSG+APC and normal skin, but with distinctly different STSG only median and mean, similarly to \(R_2\) and \(Q_1\) cases. The \(Q_2\) means of STSG+APC and STSG only are very close but distinctly lower than normal skin mean.

Inferential statistical analysis, both parametric (one-way ANOVA applied to 12 samples combining various combinations of 4 post-surgery months and 3 treatments including control normal skin) and nonparametric (Kruskal–Wallis test applied to the same samples), rejected at the 5% significance level the null hypothesis that the means (medians) are equal. This was obtained for all parameters with the exception of \(Q_2\), where only median comparison test (Kruskal–Wallis test) rejected the null hypothesis. The respective \(p\)-values are listed in Table 2. Subsequent post hoc analyses detected 5, 2, none, and 5 significantly different pairs using the Tukey post ANOVA test, and 6, 3, none, and 5 significantly different pairs using the Bonferroni post Kruskal–Wallis test for parameters \(R_2\), \(Q_1\), \(Q_2\) and \(Q_3\), respectively. And most importantly, the "worst offenders" of rejection were detected: for parameter \(R_2\), all 11 pairs (5 coming from ANOVA, 6 from Kruskal–Wallis test) had the sample STSG only in PSM 1 as a member. Similarly for parameter \(Q_1\), all 5 pairs (2 coming from ANOVA, 3 from Kruskal–Wallis test) had the same sample STSG only in PSM 1 as a member. In parameter \(Q_2\), no significantly differing pairs were detected by post hoc tests despite the fact that the Kruskal–Wallis test rejected the null hypothesis that the medians are equal. Finally, for parameter \(Q_3\), all 10 pairs (5 coming from ANOVA, 5 from Kruskal–Wallis test) had the same sample STSG only in PSM 1 as a member. All these figures are diagrammatically presented in Fig. 10.

The above statistical results suggest that the parameters \(R_2\), \(Q_1\), and \(Q_3\) can be convicted from being significantly worse for the scars treated with STSG only in the first post-surgery month than the remaining ones.
Figure 10: Results of post hoc analysis for parametric testing for cutometric parameters (a) $R_2$, (b) $Q_1$, and (c) $Q_3$. Solid lines connect samples that significantly differ in ANOVA post hoc test (Tukey test), dashed lines connect samples that significantly differ in Kruskal–Wallis post hoc test (Bonferroni test).

The results of the exponential recovery model regression are depicted in Fig. 11 for parameters $R_2$, $Q_1$ and in Fig. 12 for parameters $Q_2$, $Q_3$. Each plot contains distributions of cutometric parameters (in dark grey), the model curve (in bold black), confidence interval (in light grey) for the mean calculated from post-surgery months that belong to the steady-state portion of

Table 2: $p$-values for multiple comparison statistical tests. The sole value above the adopted significance level 0.05 is highlighted.

<table>
<thead>
<tr>
<th>Parameter</th>
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<td>0.0021</td>
</tr>
<tr>
<td>$Q_1$</td>
<td>0.0073</td>
<td>0.0034</td>
</tr>
<tr>
<td>$Q_2$</td>
<td>0.14</td>
<td>0.038</td>
</tr>
<tr>
<td>$Q_3$</td>
<td>0.00063</td>
<td>0.00019</td>
</tr>
</tbody>
</table>

Figure 11: Left column from top (a, c, e): Exponential recovery model fits of the cutometric parameter $R_2$ time dependence for STSG+APC, STSG only, and normal skin, respectively. Right column from top (b, d, f): Exponential recovery model fits of the cutometric parameter $Q_1$ time dependence for STSG+APC, STSG only, and normal skin, respectively. The light grey bar defines the confidence interval at confidence level 95%. The initial part of the model curve between zero and the first post-surgery month is removed due to unacceptable (or at least poor descriptive ability) of the model function within this interval (cf. end of subsection 2.5).

The plots presented in Fig. 11 for parameters $R_2$ and $Q_1$ are alike and convincingly demonstrate fast onset of the APC healing effect in the initial phase shortly after the surgery in comparison with STSG only treatment, in addition to the standard statistical approach elaborated above in this section. While the confidence interval strip is reached by the STSG only curve between roughly 4th and 5th PSM, the same is achieved for the STSG+APC, STSG only, and normal skin, respectively. The light grey bar defines the confidence interval at confidence level 95%. The initial part of the model curve between zero and the first post-surgery month is removed due to unacceptable (or at least poor descriptive ability) of the model function within this interval (cf. end of subsection 2.5).

The results presented in Fig. 12 for parameters $Q_2$, $Q_3$ do not sound so convincing, but at least for the parameter $Q_3$, a
behaviour similar to that observed for the gross-elasticity type parameters occurs, i.e., STSG+APC healed scars recover to the steady-state value about $\bar{Q}_2 \approx 0.28$ much more rapidly than STSG only healed scars (with halftime about 3-4× smaller). The parameter $Q_2$ does not fit in the remaining parameters behaviour, displaying virtually the same recovery halftimes in both for STSG+APC and STSG only treatment. Nevertheless, the exception of common steady-state value about $\bar{Q}_2 = 0.42$ can also be observed.

We can generalize that APC accelerates reaching a steady plateau for gross elasticity cutometric parameters $R_2$, $Q_1$, and for pure viscous portion reflecting parameter $Q_3$ in the early stages after surgery. On the contrary, the APC-induced improvement of parameter $Q_3$ (closely related to biological elasticity $R_2$) remains negligible and insignificant.

4. Discussion

Continual advances in bioengineering methods has increasingly allowed modulation of wound healing and the resultant scars. Autologous platelet concentrate has been used for about two decades in diverse surgical fields to improve wound healing and tissue repair. In this study, autologous platelet concentrate has been advocated to enhance scarring after surgical treatment of non-healing deep dermal and full thickness burns. For objective evaluation of the development of scar viscoelasticity in the course of two year period, the Cutometer MPA 580 device was engaged. This simple but useful bioengineering method enabled fast and reliable routine assessment of the skin viscoelastic properties. Using this method the presented study has objectively demonstrated that the viscoelastic properties of skin areas treated with STSG+APC combination return more rapidly to the viscoelastic properties of normal skin than those treated with STSG only, particularly in the early phases shortly after the APC application.

Despite of the small size of experimental material the statistically significant results were obtained for the parameters $R_2$, $Q_1$ (skin gross elasticity) and $Q_3$ expressing the pure viscous portion of skin retractability. This significance was not seen with the $Q_2$ parameter (pure elastic portion of skin retractability). As already mentioned in subsection 2.3, we are inclined to blame such incompatible behaviour on presumptively incorrect definition of the inflection point on the strain-vs-time curves, which can consequently spoil the values of parameters dependent on its correct position. However, it is too early for such a complex issue to be judged.

We are aware of limitations of this study, namely small number of patients, lack of experiences with applications of autologous platelet concentrate in deep burns and still insufficient number of publications dealing with the scarring processes after APC application. Also the way of determining the cutometric parameters from the strain-vs-time curves should be revised in the sense described in the previous paragraph.

The factor behind the slight decrease in time of normal skin parameters $R_2$, $Q_1$ and $Q_2$, and slight increase of normal skin parameter $Q_3$ in time remains unknown to the authors. For now we will content ourselves with ascription it to statistical fluctuations. However, a plausible explanation could be based on the changing age of the patients entering respective post-surgery months [27, 30]. The factors may be subject to investigation in future studies.

5. Conclusion

The findings of this study objectively indicate that the combination of STSG+APC in the surgery of full-thickness and non-healing deep dermal burns reduces the time of scar viscoelastic properties recovery to those of normal skin, as compared with application of the sole STSG. This was statistically significant for cutometric parameters $R_2$ and $Q_1$ expressing the gross elasticity of the skin, and parameter $Q_3$ expressing the pure viscous portion of skin retractability. Based on the results of the statistical analysis, parameters $R_2$ and $Q_1$ showed statistically significantly improved viscoelastic properties of scars in early phases (up to a few months) after the surgery. An alternate wording of the results is that using the autologous platelet concentrate reduces the gross-elasticity and pure viscous recovery halftime roughly by one order.

Figure 12: Left column from top (a, c, e): Exponential recovery model fits of the cutometric parameter $Q_1$ time dependence for STSG+APC, STSG only, and normal skin, respectively. Right column from top (b, d, f): Exponential recovery model fits of the cutometric parameter $Q_2$ time dependence for STSG+APC, STSG only, and normal skin, respectively. The light grey bar defines the confidence interval at confidence level 95%. The initial part of the model curve (cf. end of subsection 2.5).
However, future studies are necessary to complete understanding of the role played by APC in acceleration of recovery in deep burns scars healing.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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