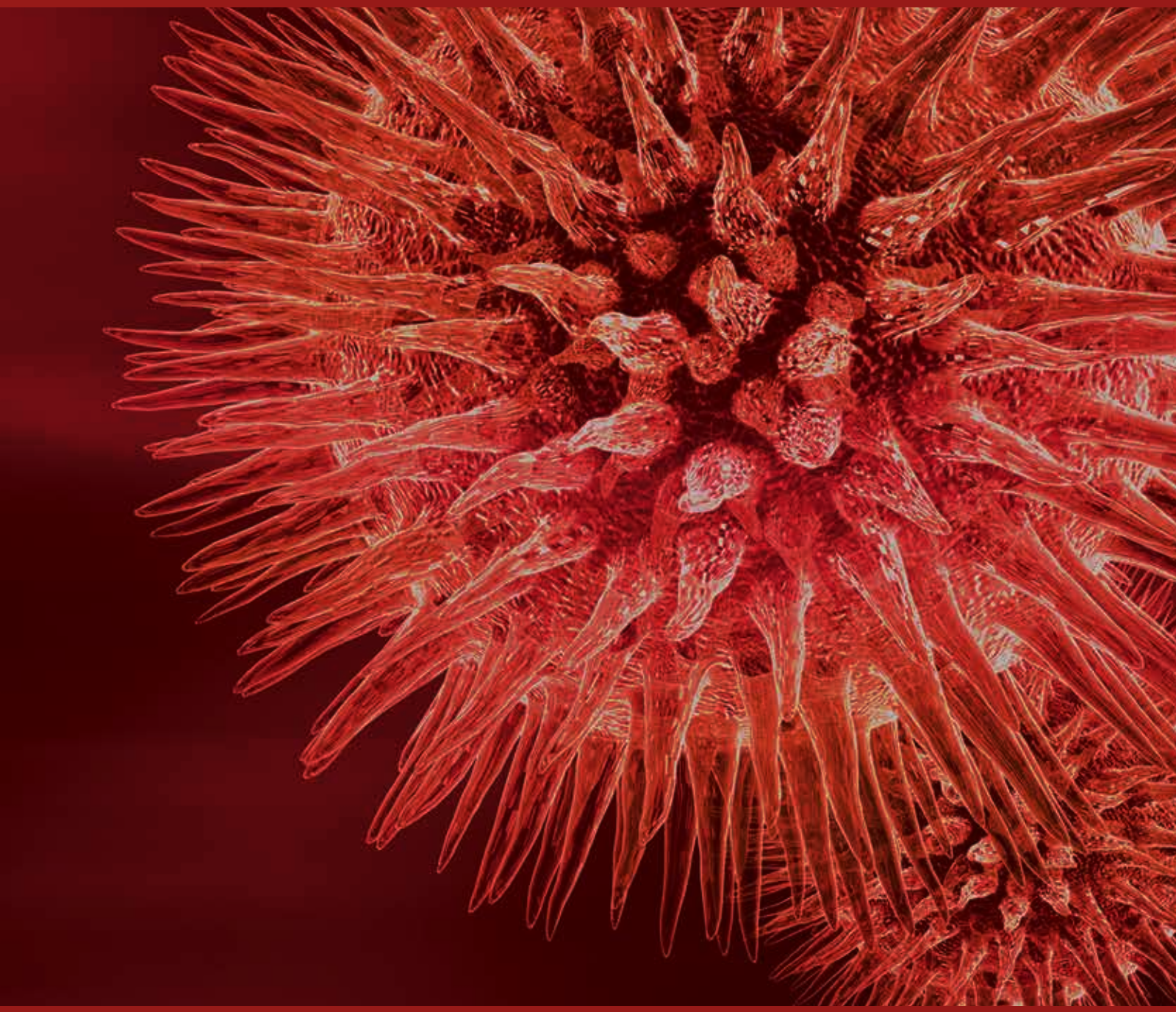
BioMed Research International

Physical Therapy in Wound Healing, Edema, and Urinary Incontinence

Guest Editors: Jakub Taradaj, Tomasz Urbanek, Luther C. Kloth, and Marco Romanelli



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Editorial

Physical Therapy in Wound Healing, Edema, and Urinary Incontinence

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The development of civilization leads to many chronic diseases. Of the health problems specific to frail both young and older people, unhealed chronic wounds (venous and pressure ulcers and diabetic foot), cancer-related lymphedema, and urinary incontinence are the major health disorders, and the establishment and spread of effective treatment methods for the following health problems are a pressing issue. The described disorders are a common and costly problem in nursing home settings, with the prevalence of estimates varying widely from 17 to even 53%.

Care and management can have significant economic consequences. Staff time for ongoing assessment, documentation, and dressing changes and expensive pharmaceuticals drain the available resources. Well-documented, promising, and inexpensive methods for physical therapy are necessary.

This special issue includes eight interesting papers. It has to be mentioned that this issue contains, among others, the following main topics: new promising methods in wound healing, prevalence, diagnostics, surgery, and physical therapy of urinary incontinence, electromyography and biofeedback in rehabilitation of pelvic floor muscles, and kinesiology taping in lymphedema.

Each manuscript submitted to the issue underwent during the course of the peer-review process by three independent researchers. The peer-review process was single blinded; that is, the reviewers knew who the authors of the manuscript are, but the authors did not have access to the information of who the peer reviewers are.

We believe that published articles will be interesting for readers.

Jakub Taradaj
Tomasz Urbanek
Luther C. Kloth
Marco Romanelli

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Clinical Study

Efficacy of Physiotherapy for Urinary Incontinence following Prostate Cancer Surgery

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The study enrolled 81 with urinary incontinence following radical prostate-only prostatectomy for prostatic carcinoma. The patients were divided into two groups. The patients in Group I were additionally subdivided into two subgroups with respect to the physiotherapeutic method used. The patients of subgroup IA received a rehabilitation program consisting of three parts. The patients of subgroup IB rehabilitation program consist of two parts. Group II, a control group, had reported for therapy for persistent urinary incontinence following radical prostatectomy but had not entered therapy for personal reasons. For estimating the level of incontinence, a 1-hour and 24-hour urinary pad tests, the miction diary, and incontinence questionnaire were used, and for recording the measurements of pelvic floor muscles tension, the sEMG (surface electromyography) was applied. The therapy duration depended on the level of incontinence and it continued for not longer than 12 months. Superior continence outcomes were obtained in Group I versus Group II and the difference was statistically significant. The odds ratio for regaining continence was greater in the rehabilitated Group I and smaller in the group II without the rehabilitation. A comparison of continence outcomes revealed a statistically significant difference between Subgroups IA versus IB. The physiotherapeutic procedures applied on patients with urine incontinence after prostatectomy, for most of them, proved to be an effective way of acting, which is supported by the obtained results.

1. Introduction

The International Continence Society defines urinary incontinence as involuntary leakage of urine that has been diagnosed objectively and is associated with additional hygienic and social problems [1]. The most common types of urinary incontinence following radical prostatectomy include stress incontinence, urge incontinence, and mixed incontinence. The European Association of Urology recommends two approaches to the management of these dysfunctions: noninvasive and invasive (surgical) [2]. Noninvasive modalities are considered first-line treatment during the first 6–12 months following prostatectomy [3]. Physiotherapy is administered especially early on after the onset of symptoms and in incontinence of moderate severity. At the same time, the approach to men with urinary incontinence following prostatectomy is not unified. An optimal rehabilitation plan requires prior determination of the type of urinary incontinence and of factors that may influence volitional control of micturition (motor control, musculo-fascio-skeletal relations, and behavioral factors) [4]. The choice of invasive versus noninvasive treatment depends on the severity and duration of symptoms and the type of urinary incontinence. Conservative modalities include physiotherapy techniques, the most common of which, used in male incontinence following prostatectomy, include pelvic floor muscle training (PFMT) with or without biofeedback (BFB), noninvasive pelvic floor electrical stimulation, extracorporeal magnetic innervation (ExMI), behavioral modification, and external penile compression devices [5–8]. A standardized program

of pelvic floor muscle reeducation that would guarantee complete therapeutic success is yet to be developed. However, it is quite clear that a plan of pelvic floor muscle training should determine the level of exercise intensity with regard to exercise duration, the number and frequency of repetitions, and the type of load to induce permanent changes in the muscles [9–11]. Of no less importance for the final outcome of therapy are the patient's motivation, the correct execution of exercises, and compliance with the physiotherapist's orders [12].

The aim of the present study was to evaluate continence outcomes in a group of postprostatectomy males who underwent physiotherapy (Group I) as compared to a control group of males not undergoing physiotherapy (Group II) and to compare outcomes between Subgroup IA and IB, treated using different physiotherapy methods. Outcomes were also evaluated with regard to time between surgery and rehabilitation in Subgroup A (<3 months) and B (>3 months). The odds ratio for regaining continence in Group I versus (control) Group II was also evaluated.

2. Materials and Methods

The study enrolled 81 males aged 53–82 years (mean age 68 ± 6.65 years) with urinary incontinence following radical prostate-only prostatectomy for prostatic carcinoma.

The exclusion criteria included patients with an artificial pacemaker, musculoskeletal deformities making rehabilitation impossible, bleeding from the urinary bladder or the digestive tract, urinary tract infection, polyuria, uncontrolled diabetes mellitus, neurologic conditions affecting coordination and balance, lack of consent of the patient to commence therapy, and previous rehabilitation for urinary incontinence.

The patients were divided into two groups. Group I comprised $n = 49$ males aged 54–80 years (mean age 67.9 ± 6.81 years). The patients in Group I were additionally subdivided into two subgroups with respect to the physiotherapeutic method used.

Subgroup IA ($n = 23$) was made up of patients aged 54–77 (mean age 66.9 ± 7.07 years). These patients received a rehabilitation program consisting of three parts as follows:

(a) pelvic floor muscle training (PFMT) with biofeedback (PFMT + BFB) (once weekly for 20–30 min),

(b) pelvic floor muscle training according to spinal segmental stabilization principles (PFMT + SSS) (once weekly for 30 min),

(c) a set of exercises for the patient to perform on his own at home (3 times daily for 15–20 min).

The set of exercises comprised isolated contractions of PFMT and the urethral sphincter and particular pelvic floor muscle exercises according to the principles of spinal segmental stabilization training, performed in the lying, sitting, and standing positions.

The efficacy of PFMT with BFB was recorded graphically in a chart and numerically (in seconds and microvolts) using sEMG with a dual channel software-assisted Neuro Trac ETS device from Verity Medical Ltd. All patients from Subgroup IA had an individual anal probe mounted. A self-adherent reference electrode was positioned onto previously defatted akin at an electrically inactive site on the anterior superior iliac spine. The electrodes were FAD- and CE-certified.

Subgroup IB comprised a total of $n = 26$ patients aged 57–80 (mean age 68.8 ± 6.59 years) subjected to a rehabilitation program involving the following:

(a) PFMT without BFB according to the principles of segmental spinal stabilization training (twice weekly for 30 min),

(b) A program of home-based exercises identical to that for Subgroup IA.

Subgroup IB included those patients who had not agreed to have an anal probe mounted for exercises.

All patients followed a standardized and reproducible exercise program.

Therapeutic sessions in both subgroups were one-on-one meetings between patients and one physiotherapist at the Department of Rehabilitation, Gdańsk Medical University. Patients commuted for the sessions from their place of residence. The rehabilitation program was administered to patients who had developed urinary incontinence following a prostate-only prostatectomy and had been referred for rehabilitation by a urologist. The rehabilitation program in Subgroups IA and IB was divided into a number of stages.

Stage I, the same in both groups, began with educating the patient about the anatomy and physiology of the lower urinary tract and the respiratory system, the physiology of micturition, and muscle synergies between the musculoligamentous system of the pelvic complex and pelvic floor muscles.

In Stage II, also the same in both groups, the therapist carried out exercises with the patients according to the principles of segmental spinal stabilization training. Muscle activation involved kinesthetic awareness in tensing muscles while the patient maintained a neutral position of the spine. The exercises were performed at a slow pace, with a loading threshold of 20–25% of maximum MVC (maximum voluntary contraction strength), duration of muscle tensing of 5–10 seconds, and 7–10 repetitions. Training progression consisted of stimulating muscles of the external group at isolated positions while maintaining control of tensing, changes in starting positions for exercises, exercises in open and closed kinematic chains, sensorimotor exercises on unstable bases (mattress, theraband ball, and disks), and the addition of resistance and functional exercises. The duration of physiotherapy in both subgroups depended on the severity of incontinence. The therapy ended when continence was regained but did not last longer than one year.

The third stage was only implemented in Subgroup IA and involved the incorporation of biofeedback to monitor the parameters of pelvic floor muscle training.

The severity of incontinence was determined with the 1-hour and 24-hour pad test, where the number of pads used before and after therapy was recorded by patients in micturition diaries. Pelvic floor muscle tension was recorded and measured by sEMG (surface electromyography).

The continence thresholds were 2 g in the 1-hour pad test and 4 g in the 24-hour pad test. Continence parameters were assessed at the beginning (baseline) and on completion of the therapy. Patients were divided into subgroups with regard to the amount of urine leaked to determine whether baseline urine loss would influence continence outcomes of the physiotherapy.

Group II, a control group, comprised 32 men aged 53–82 years (mean age 68.3 ± 6.49 years) who had reported for therapy for persistent urinary incontinence following radical prostatectomy but had not entered therapy for personal reasons. 1-hour and 24-hour pad tests were conducted when the patients reported for therapy and repeated at one year postsurgery. Information on continence outcomes was collected by phone on the basis of patients' self-reported subjective assessment.

Consent for conducting the study was given by the Independent Ethical Review Board for Scientific Research at Gdańsk Medical University (NKEBN 208/2011).

The study data were analyzed in STATISTICA 9.0. The Kolmogorov-Smirnov test was used to determine data distribution patterns. Quantitative characteristics were compared between the two populations with the Mann-Whitney U test. A statistical test for dependent samples was used to compare parameters for the same population (group) before and after treatment. The Chi^2 test was used to define correlations between two qualitative variables. A test for differences between two components of a structure served to verify the significance of particular frequency distributions (of patient numbers and percentages) of specific variable categories. Regression analysis was used additionally to assess odds ratios. A P value of $P < 0.05$ was regarded as statistically significant in all analyses.

3. Results

The 1-hour and 24-hour pad test were conducted in patients from Groups I and II before rehabilitation was started in order to test for homogeneity of the groups with regard to the amount of urine leaked. The tests did not reveal statistically significant differences between the groups ($P = 0.329$ for the 1-hour test and $P = 0.105$ for the 24-hour test).

The 1-hour pad test was also used to provide an objective measure of the amount of urine leaked before and after rehabilitation in Group I patients ($n = 49$). The results showed a statistically significant difference ($P < 0.001$) in reducing the loss of urine. 24-hour pad test data also showed a statistically significant difference between measurements before versus after rehabilitation therapy ($P < 0.001$). The number of pads used per day before and after therapy (185 versus 58) was analyzed based on data from micturition diaries. A significant reduction in the number of pads used was observed following the therapy compared to baseline data ($P < 0.001$). Differences in demand for pads for the entire study group are presented in Figure 1.

sEMG traces obtained during biofeedback exercises for pelvic floor muscles were analysed statistically with regard to the following parameters: arithmetic mean of contraction voltages (μV), mean deviation of contraction voltages (%), mean response time (s), arithmetic mean of relaxation voltages (μV), mean deviation of relaxation voltages (%), and mean relaxation time (s). These indices were measured before and after therapy.

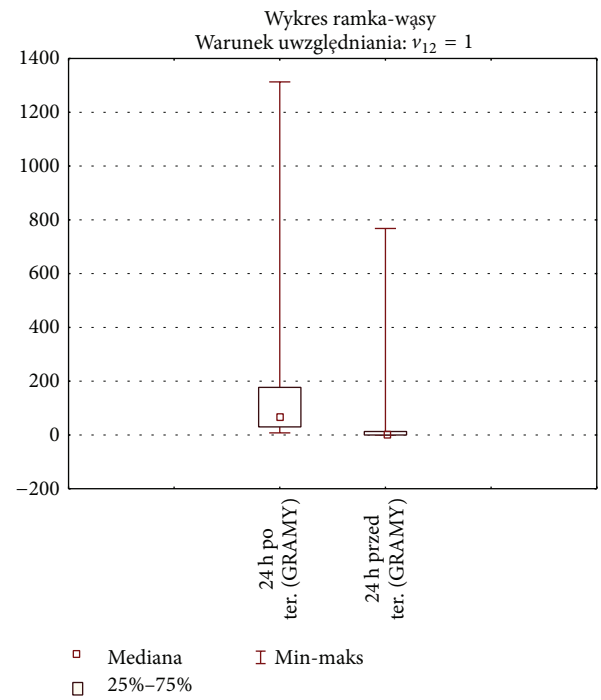

Figure 1: Differences in the amount of urine leaked before and after therapy for Group I ($n = 49$). Box-and-whiskers diagram. Case selection condition. 24-h test baseline (grams). 24 h test posttreatment (grams). Median. Min-max.

The only statistically significant ($P = 0.03$) difference among these values was in response times before and after treatment for continent versus incontinent patients. No significant differences were revealed with regard to the remaining indices.

On completion of the therapy, continence (C) had been restored in 9/23 (39.1%) patients in Subgroup IA, while 14/23 (60.9%) patients remained incontinent (INC) at various degrees of severity. In Subgroup IB ($n = 26$), continence had been restored in 24/26 (92.3%) patients, and 2/26 (7.7%) were still not fully continent. These outcomes of different models of rehabilitation for urinary incontinence (PFMT + BFB versus PFMT + SSS) appear to be more favourable for Subgroup IB (Figure 2).

Analysis for the entire Group I shows a significant correlation between therapeutic outcome and the physiotherapeutic method used (Chi^2, $P = 0.00007$). There were considerably more incontinent patients in Subgroup IA (61%) than in Subgroup IB (8%) on completion of the therapy. The study did not demonstrate statistically significant differences with respect to incontinence between Subgroup IA versus IB ($P = 0.08$). A comparison of continence outcomes revealed a statistically significant difference between Subgroups IA versus IB ($P = 0.007$). The highest percentage of continent

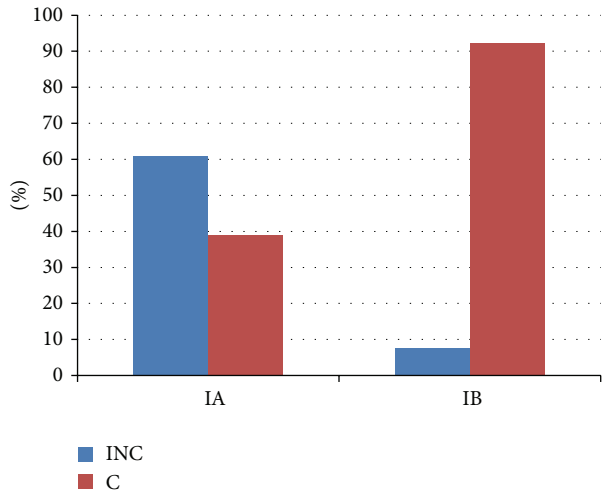

Figure 2: Comparison of outcomes of incontinence therapy (C versus INC) for Subgroup IA (PFMT + BFB) versus Subgroup IB (PFMT + SSS).

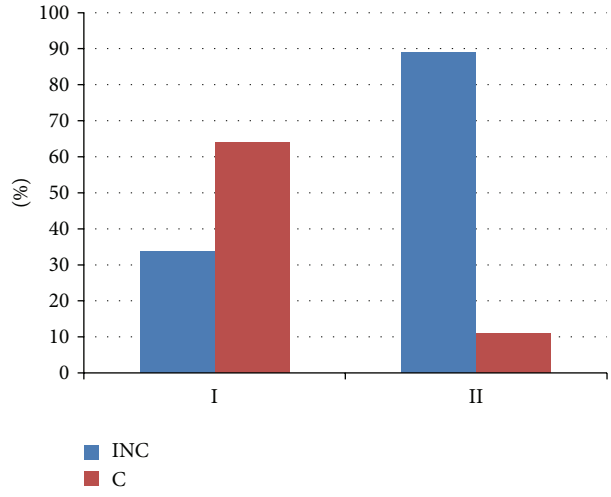

Figure 3: Therapy outcomes (C versus INC) in Group I versus Group II.

patients following physiotherapy was in Subgroup IB, at 24/26 (92%).

We also studied the effect of time between prostatectomy surgery and commencement of physiotherapy on continence outcomes. The patients were accordingly divided into two subgroups. Subgroup A comprised patients who began rehabilitation within 3 months of surgery and Subgroup B included those who reported for rehabilitation three months or more after surgery. The patients were additionally classified as continent (C) and incontinent (INC). The analysis showed that the best continence outcomes were obtained in patients who entered rehabilitation within three months of surgery. Differences in the odds ratio for continence between Subgroup A (<3 months) versus B (>3 months) were in favor of those who entered rehabilitation earlier. The odds ratio for the recovery of continence in Subgroup A amounted to 4.88 while in the group B 0.29. These results emphasize that the chances for regaining full continence decrease considerably with increasing time between prostatectomy surgery and entering rehabilitation.

Analysis of data for the entire study population ($n = 81$) revealed differences in continence/incontinence between males from Group I versus Group II (33/49 versus 4/32; 89% versus 11%). Superior continence outcomes were obtained in Group I versus Group II (33 versus 4) and the difference was statistically significant ($P = 0.0001$). The odds ratio for regaining continence was greater in rehabilitated Group I and smaller in the group II without the rehabilitation. The odds ratio for the recovery of continence in Group I amounted to 2.06 while in the Group II 0.15.

The results are presented in Figures 1, 2, and 3. 1-hour and 24-hour pad testing did not reveal significant differences in the amount of urine loss between Group I (rehabilitated) versus Group II (not rehabilitated) before rehabilitation began. The consumption of pads decreased significantly ($P < 0.001$) on completion of the therapy compared to baseline. On completion of the therapy, 9/23 patients in Subgroup IA were continent compared to 24/26 in Subgroup IB. BFB produced statistically significant changes ($P = 0.03$) only in response time as measured by sEMG. Incontinence following therapy was still present in 14/23 patients in Subgroup IA and 2/26 patients in Subgroup IB. Superior continence outcomes were noted in patients who had entered rehabilitation within 3 months of prostatectomy surgery compared to those who had taken longer to begin rehabilitation (Subgroup A versus Subgroup B; OR 4.88 versus 0.29). Analysis of the entire study population ($n = 91$) revealed superior continence outcomes in Group I versus Group II (33/4), representing better odds for regaining continence in rehabilitated patients versus nonrehabilitated controls (OR 2.06 versus 0.15).

4. Discussion

The management algorithm for urinary incontinence in men following radical prostatectomy is not fully specified with regard to both the choice of physiotherapy methods, timing, and duration of therapy and exercise intensity [13–20]. The risk of incontinence may be influenced not only by the surgical method and operative technique but also by other attendant factors [18, 21], such as age, prostate volume, tumor stage, history of TURP (transurethral resection of the prostate), and volume leaked following catheter removal [22–24]. Porru et al. described a much higher rate of resolution of incontinence and dribbling symptoms in a rehabilitated group versus a control group [25]. The authors recommend physiotherapy exercises on account of their efficacy and ease of execution [25]. Guidelines regarding the timing of physiotherapy vary between authors. The timing of therapy is important for continence outcomes [17, 23]. In our study, patients reported for physiotherapy at various times following prostatectomy surgery. They were admitted for rehabilitation on the basis of a referral note for physiotherapy from their urologists. The data show that Subgroup A (<3 months between surgery

and rehabilitation) had greater odds for regaining continence than Subgroup B (>3 months). Physiotherapy for continence was more beneficial in patients who started treatment up to 3 months following prostatectomy versus those who entered rehabilitation after a longer interval (Subgroup A versus Subgroup B, OR 4.88 versus 0.29). The chances for complete recovery of continence decreased considerably with increasing interval between surgery and beginning of rehabilitation. A review of the literature showed that both the duration of supervised therapy and session frequency are not uniform across published papers [7, 14, 16, 22, 26, 27]. In the present study, as in van Kampen et al.'s, the therapy continued until micturition control had been regained and also did not exceed 12 months [22]. All patients from the group undergoing rehabilitation had one-on-one sessions with the same physiotherapist. Similar to other studies, two sessions a week were held and each lasted on average 30 minutes [3, 23]. Patients were instructed to repeat a set of exercises at home 3 times a day in sessions not exceeding 20 min. Overgård et al. demonstrated that superior increments in muscle diameter and strength could be obtained with twice-daily versus once-daily exercise sessions [7]. The 1-hour and 24-hour pad tests were used to compare urine loss between Group I and II at baseline (before rehabilitation commenced), but did not reveal statistically significant differences ($P = 0.329$ for 1-hour pad test; $P = 0.105$ for 24-hour pad test), which means that the two groups were comparable with respect to the amount of urine leaked. van Kampen et al. observed that low urine loss immediately following catheter removal may be prognostic for rapid recovery of continence following prostatectomy [22]. Published studies have used the 1-hour pad test to assess the amount of urine leaked. This tool has been recommended for that purpose by ICS [28, 29]. Börgermann et al. point out that a variety of criteria are used to classify a postprostatectomy patient as "dry" [30]. For some researchers, a patient is continent when he does not use any protection while for others a continent patient does not use one and/or more pads [19, 30]. The present study relied on micturition diaries to obtain data on pad consumption in Group I ($n = 49$). Pad consumption decreased after therapy in a statistically significant manner compared to baseline ($P < 0.001$). Micturition diaries are another effective and noninvasive tool for assessing incontinence. Researchers vary in their opinions about the optimal number of records. Some recommend keeping a diary for 7 days [31], while others make do with 4-day records [32]. In the present study, patients kept micturition diaries for 4 days. Methods of treatment of iatrogenic urinary incontinence following prostatectomy in men include biofeedback [16, 17]. However, this is not a method in itself. It is only a tool helping patients achieve exercise goals. The sound and vision make it easier to monitor exercise tasks. Our study used sEMG to measure and monitor pelvic floor muscle contractions. Various outcomes have been reported with this kind of therapy. Ribeiro et al. used biofeedback combined with sEMG monitoring of muscle activity to train pelvic floor muscles. The biofeedback group registered much better continence outcomes compared to the control group [19]. Some reports have stated no effect of BF on the efficacy of continence recovery [13, 33]. In our study, PFM training combined with biofeedback was also used in Subgroup I. Performance of exercises was monitored by the registration of action potentials from muscles during contraction and relaxation. Of all indices monitored, a statistically significant difference ($P = 0.03$) was only observed with regard to response time at baseline versus on completion of therapy, which may indicate improved timing in neuromuscular coordination. However, the additional incorporation of biofeedback in Subgroup I did not increase the efficacy of continence recovery, similar to the findings of other studies [13, 33]. Some authors believe that patients with incontinence following prostatectomy may regain continence spontaneously within one or even two years following the surgery [22, 30, 34]. In order to ensure an objective assessment of our therapy, we followed up both rehabilitated and nonrehabilitated patients. More patients regained continence in the physiotherapy group, with 33/49 (67.3%) continent patients in Group I, compared to 16/49 (32.7%) incontinent patients. In Group II (nonrehabilitated control group), continence was confirmed in 4/32 (12.5%) patients and 28/32 (87.5%) remained incontinent. In our opinion, these comparative data encourage the use of physiotherapy in men with this dysfunction. Studies of incontinence present results of single-modality therapies [14, 17, 20, 35, 36]. The present study compared continence outcomes in patients treated with a combination of two methods. Patients in Subgroup I entered a biofeedback-enhanced exercise program for pelvic floor muscles based on spinal segmental stabilization training, while those in Subgroup II only practiced pelvic floor muscle exercises based on segmental spinal stabilization training. The addition of biofeedback in Subgroup I did not bring about a dramatic increase in the efficacy of continence recovery.

Pelvic floor muscles are morphologically classified as skeletal muscles and so they adapt to exertion in the same way as do other muscles from this group. Regular training leads to an increase in muscle bulk, which can also be observed in pelvic floor muscles. Endurance and strength training for incontinent patients aims to change muscle morphology by increasing their diameter, improve neurological indices by increasing the numbers of active motor neurons and the number of excitatory stimuli, and improve muscle tone [11, 37, 38]. Factors that slow down muscle responses to exertion include age, which is also associated with slower response times. Older (>70) men were found to have grossly limited ability for migration of the urethra against the symphysis pubis and had blurred anatomical borders of the urethral sphincter system [39]. Increased diameter and endurance of pelvic floor muscles are achieved using the principles of training designed for other skeletal muscles as pelvic floor muscles are made up of striated tissue. It is not completely understood how these training principles translate into changes in the continence muscles. Good neuromuscular coordination appears to be of immense importance for normal pelvic floor muscle function. The muscles of the pelvic floor have different points of origin and attachment in that region. Some attach directly to bones and others to fasciae and ligaments. In describing the functions of pelvic floor muscles, Shafik points out that the external anal sphincter (EAS),

external urethral sphincter (EUS), and the bulbospongiosus muscle (BC) have their origins on the puborectalis muscle (PR) and act as a mutually dependent muscle complex. His study demonstrated that pelvic floor muscles behave like one muscle, which does not preclude the possibility of them working independently from one another [40]. A number of strategies for activating pelvic floor muscles are available. These strategies are based on isolated muscle tensing, functional training and motor control, the use of muscle synergies involving the transversus abdominis muscle, and the use of appropriate breathing patterns [41]. Some researchers believe that the pelvic floor muscles are part of a structural complex that forms a lumbopelvic cylinder comprised of the pelvic floor, respiratory diaphragm, transversus abdominis muscle, and lower spine, including the multifidus muscle [42]. Mutual coordinated relationships between these components result in increased intraabdominal pressure, which has a stabilizing role. A rise in intraabdominal pressure precedes an increase in intraurethral pressure [43]. A relationship between PFM activity and intraabdominal pressure contributing to urethral elevation was demonstrated by Junginger et al. [44]. To prevent urine leakage, the actively contracting pelvic floor muscles increase urethral closing pressures and are accordingly viewed not only as stabilizers but also as promoters of abstinence. Other researchers emphasize the importance of the transversus abdominis (TrA) as a significant structure taken advantage of in exercises for incontinent patients. The function of the transversus abdominis in generating pressures transmitted onto the pelvic floor muscles has been extensively studied. The hypothesis that the roles depend on the site of origin/attachment of a muscle is yet to be fully confirmed [45]. Junginger et al. demonstrated that elevation of the bladder neck is consistently seen when pelvic floor muscles are tensed in combination with mild tensing of TrA. Inhibition of urethral elevation was seen when abdominal muscles were slightly tensed in combination with a slight Valsalva maneuver and raising of the head [44]. The authors suggested that their findings should be incorporated into plans for the rehabilitation of patients with urinary incontinence. They indicated that PFM and TrA were jointly activated only during submaximal tensing, while 100% tensing caused an excessive rise in intraabdominal pressure as a result of the activation of all abdominal muscles, which lowered the bladder neck. Thus, the authors concluded that only submaximal PF tensing should be achieved therapeutically [44]. Sapsford suggested that a contraction of the transversus abdominis may facilitate tensing the pubococcygeal part of the levator ani [46].

Other researchers suggest, on the basis of their findings, that tensing abdominal muscles facilitates the tensing of PF muscles. Jones believes that viewing the role of pelvic floor muscles in a broader perspective paves the way for other rehabilitation modalities that can now be used for this purpose [42]. She notes that physiotherapy methods used for the selection of exercises for incontinent patients and those employed in the treatment of back pain and spinal instability have a lot in common as both take advantage of connections between muscle groups in the lumbar region, abdominal muscles, and pelvic floor muscles.

The role of this muscle complex is to produce and control tensions in response to changes in intraabdominal pressure. Richardsoan et al. point out that good control of pelvic floor muscle tension is an important element of the restoration of abdominal stabilization in incontinent patients [47]. In their work with patients, physiotherapists do not "train" only one muscle. Approaching the dysfunction as a local problem manifesting only at one site in the body is not sufficient in view of the current knowledge of physiology, biomechanics, and neurology. One has to agree with Dr. Lee, who notes that "understanding musculoskeletal disorders requires an awareness of how loads are transferred by the body and how a disturbance in one part may affect the functioning of the entire system" [48]. Many authors have tried, first theoretically and subsequently in practice, to explain the mechanisms underlying mutual effects that muscular and fascial structures exert on one another. Researchers who have worked to unravel the integrity of these structures include F. Meziera and K. Tittel. They have presented certain connections related to muscle chains whose mutual relations stemmed from functional links [49]. A similar view was presented by Myers in "Anatomy Trains," where he pointed to musculofascial connections as the bearers of mutual links. Kassolik and Andrzejewski similarly sought links in musculofascial connections in their tensegration massage theory [50]. All these authors based their proposals on the assumptions of tensegration formulated by Buckminster Fuller. With this approach, the human body, particularly the musculoskeletal system, is viewed as a whole subject to continuous shifts between stability and mobility, which is associated with constantly shifting tensions and the emergence of compressions in the human body both in movement and at rest. These aspects are explained by D. Ingber [51]. Approaching the musculoskeletal system as a spatial musculonervoskeletal structure is conducive to finding muscle connections that can be used in working with patients and whose role can be understood solely by reference to their structural and functional relations. Similar assumptions were presented by D. Lee based on earlier work by Vleeming. D. Lee introduced an easily understood division of muscle trains into an external and internal group. Connections between muscle trains as presented by her are used in restoring stability of the lumbar spine and pelvic girdle, but have also been employed in the treatment of urinary incontinence [48]. The conception of stabilization and appropriate choice of exercises has been presented by other authors, including Dr. Richardsoan et al. [47]. Urinary incontinence is associated, among others, with functional weakening of the pelvic floor muscles, which are regarded as deep (central) stabilization structures. Jones emphasizes that, when the identification of pelvic floor muscles is disturbed, the use of other muscles forming the central stabilization cylinder results in tensions being automatically transferred onto the muscles of this region [42]. The pelvic and sacral bone are the sites of attachments of approximately 35 muscles; therefore, their mutual relations play a role in the movement and stabilization of this region of the body as well as other segments. Our approach to working with incontinent males following prostatectomy is based on principles used in central

stabilization training, especially with regard to training motor functions, proposed for that purpose by Richardsoan et al. Their training principle involves a system of three successive stages of rehabilitation offering progressively increasing loads and always taking into account local segmental control. In line with Richardson's recommendation, tensions used during training sessions with patients did not exceed 30% of the MVC. Movements were performed at a slow pace in order to activate tonic muscles [42, 48, 52]. The duration of sustained tensing was 10 s with 5–10 repetitions, in accordance with Libenson's instructions for this type of training [52]. The rehabilitation also involved "functional respiratory patterns" as proposed by Sapsford for strengthening pelvic floor muscles in incontinent women [46]. The author refers to mutual relations in the inspiratory and expiratory phase between the abdominal muscles, respiratory diaphragm, and pelvic floor muscles. Exercises during the respiratory phase were also used during sessions with patients by Ribeiro et al. [19]. Published strategies of management of urinary incontinence have been changing, from local approaches to the problem [53–57] towards concepts linking pelvic floor muscles with other muscle groups forming a functional whole [46–48]. State-of-the-art knowledge affords a better understanding of relationships within the musculofascioligamentous system in the space of the pelvic floor and the cylinder formed by the respiratory diaphragm, abdominal muscles, and lower back. Imbalance between these structures produces functional changes. The ability to grasp mutual relationships in the locomotor system enables for the therapeutic use of the influence of particular elements on other components that are often not directly related.

5. Conclusion

The findings of the present study show that a physiotherapy program can improve or fully restore continence. Data for the entire Group I suggest that early institution of physiotherapy after a prostatectomy procedure contributed to regaining continence. Continence outcomes were better in the rehabilitated group compared to nonrehabilitated controls. The study tools (pad testing, micturition diaries, and sEMG) proved useful for our analyses and presentation of the results of the study.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Role of the Extracellular Matrix Components in Cutaneous Wound Healing

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Wound healing is the physiologic response to tissue trauma proceeding as a complex pathway of biochemical reactions and cellular events, secreted growth factors, and cytokines. Extracellular matrix constituents are essential components of the wound repair phenomenon. Firstly, they create a provisional matrix, providing a structural integrity of matrix during each stage of healing process. Secondly, matrix molecules regulate cellular functions, mediate the cell-cell and cell-matrix interactions, and serve as a reservoir and modulator of cytokines and growth factors' action. Currently known mechanisms, by which extracellular matrix components modulate each stage of the process of soft tissue remodeling after injury, have been discussed.

1. Introduction

Wound healing is a complex, biological process which concerns replacing damaged tissue by a living one [1–3]. The restoration of tissue integrity is the result of the interaction of platelets, cells, such as neutrophils, monocytes/macrophages, fibroblasts, endothelial cells, and keratinocytes as well as extracellular matrix (ECM) components, such as fibronectin, glycosaminoglycans, proteoglycans, thrombospondins, tenascin, vitronectin, or collagens [4, 5]. The mentioned cell interaction with ECM components is subject to a regulation of biochemical mediators, numerous cytokines, and growth factors, such as arachidonic acid derivatives (prostaglandins and leukotrienes), interleukins, interferons, TNF-α, PDGF, FGF, TGF, or EGF [6]. First of the mentioned compounds participates in creating the inflammatory response, while the others, that is, growth factors, take part in controlling proliferation, differentiation, and metabolism of cells involved in the healing process. The latter mediators assist in regulating inflammatory processes and play a chemotactic role for neutrophils, monocytes/macrophages, fibroblasts, and epithelial cells (keratinocytes) stimulating the angiogenesis and formation of ECM [7, 8].

The delicate balance between the above mentioned processes—proliferation and differentiation—is regulated by stem cells capable of enhancing the repair via secretion of paracrine factors [9, 10]. Endothelial progenitor cells, derived from hematopoietic stem cell lineage, play a key role in the neovascularization [3]. It was also observed that conditioned media obtained from mesenchymal stem cells promote wound healing through activation of host cells. Furthermore, topical application of mesenchymal stem cells enhances chronic wound healing and implementation of recombinant cytokines secreted by stem cells could be beneficial for recalcitrant wounds [10]. Moreover, bone-marrow mesenchymal stem cells may differentiate into fibroblasts and keratinocytes—cells responsible for synthesis of ECM constituents [3, 11–13].

ECM components play a significant role in each stage of the healing process. It concerns, on one hand, the structural biomechanical aspect of the process in question because the ECM components create "scaffolding" (a temporary matrix, granulation tissue, and scar), which is indispensable in the repairing process, providing in this way a structural integrity of the matrix during each stage of the healing process [5, 12–14]. On the other hand, however, the role of ECM components

is connected with the action aspect of the healing processes since the mentioned compounds also fulfill a function of signal transduction in this dynamic, interactive sequence of biological reactions [15–20]. The latter functions are connected with stimulating the adhesion and migration of cells during the healing process as well as with mediating the interactions among cells, between cells and the matrix, or between ECM proteins [12, 13, 17, 21]. ECM components serve also as a reservoir and a modulator of cytokines and growth factors' action, thus regulating wound repair activity [5, 15, 22–24].

Dermatan sulfate enhances endothelial leukocyte adhesion by the stimulation of ICAM-1 or fibroblast growth factor-2 as well as it participates in the interaction with hepatocyte growth factor/scatter factor heparin cofactor II, platelet factor 4, fibronectin, or protein C inhibitor [25–28]. Chondroitin sulfate is able to induce FGF-2-mediated cell proliferation, control cell adhesion, and stimulate cell spreading and migration by activating focal adhesion of growth factor [28, 29]. Heparan sulfate/heparin participates in regulation of angiogenesis, cell growth, migration, and differentiation [30–32]. Hyaluronic acid determines tissue hydration, functions as a signaling molecule, interacts with cell surface receptors, and stimulates cell proliferation, migration, differentiation, and gene expression [28, 33].

The wound healing of skin is different from the process of damaged bone repair [34, 35]. Skin wounds heal by first intention or granulation [6]. So, a surgically sewn cut, which is not accompanied by tissue loss, leads to healing by first intention. Greater wounds, including postburn injuries, in which tissue loss and infection of the damage take place, heal by granulation. In the mentioned case, the final effect of healing is a scar.

Wound healing proceeds through four, but overlapping, phases, such as hemostasis, inflammation, proliferation (also known as replication and synthesis stage), and remodeling [3, 36]. 4 stages were created because of practical reasons, while the division itself has an arbitrary character because subsequent stages overlap as before one stage finishes, the next one starts [37, 38].

Healing skin wounds proceeds in accordance with the mentioned below stages (Figure 1).

2. Healing Stages

2.1. Hemostasis. The first stage of wound healing starts immediately after an injury appears [36]. It begins with narrowing the damaged vessels, which is caused by the activity of vasoconstriction factors, such as serotonin, thromboxane $A_{2,}$ or adrenaline being, on the other hand, connected with adhesion, aggregation, and platelets' activation in the damaged place [37].

The platelets are early modulators of the healing process [15]. They undergo adhesion, aggregation, and activation as a result of their contact with collagen of the damaged vessels, which leads to ADP and adhesion glycoprotein release from them which in turn supports further platelet aggregation [37]. The key glycoproteins, which are released from α granules of platelets, are fibrinogen, fibronectin, vitronectin, thrombospondin, and von Willebrand's factor [39, 40]. The surface of the activated platelets simultaneously becomes the place of prothrombin activation, which leads to creation of active thrombin—the key factor of the coagulation process catalyzing the transformation of fibrinogen into fibrin and as a result of that it forms a blood clot [35, 39, 40].

The blood clot protects the structural integrity of vessels and provides a provisional "scaffolding" which enables formation of a temporary matrix in the wound bed. Besides fibrin molecules, the main component of this temporary, hyaluronan-rich matrix is also plasma fibronectin, which is accumulated in the wound during the first 24 hours after the injury [41]. The polymerized fibronectin shows highly adhesive properties entering the interaction with numerous cells by integrin receptors and stimulates the migration and adhesion of fibroblasts, keratinocytes, and endothelial cells. Being one of the ligands for platelet integrin, it supports further adhesion and aggregation of these morphotic elements [42]. The aggregated platelets, "trapped" in the provisional matrix, release, from α granules, numerous growth factors, such as PDGF, TGF-α, TGF-β, bFGF, IGF-1, and VEGF [3, 35, 36, 43]. These mediators influence neutrophils, monocytes/macrophages, smooth muscle cells, endothelial cells, and fibroblasts [3]. So, neutrophils and monocytes are recruited into the wound environment by PDGF and TGF-β, which is to initiate the inflammatory response [15, 44]. Additional, chemotactic stimuli intensifying the recruitment of neutrophils are products of C5a complement degradation as well as the products of bacteria decomposition. Endothelial cells are activated by TGF-α, Bfgf, and VEGF in order to initiate angiogenesis. Fibroblasts, in turn, are activated and recruited by PDGF and IGF-1 in order to initiate the migration of these cells into the wound environment and their proliferation as well as biosynthesis of glycosaminoglycans and collagen [36, 44].

Summing up, the healing process is initiated by the hemostasis stage, which is connected with forming a temporary matrix, secreting cytokines and other growth factors, and interaction of the latter ones with ECM, which initiates the repairing process, preparing the wound bed to the next stage of the healing process—the inflammatory stage [36, 37].

2.2. Inflammatory Stage. Inflammatory phase of the healing process develops during 24 hours from the moment when an injury occurred and lasts for up to 48 hours on average [34]. This phase is accompanied by characteristic inflammatory symptoms, such as redness, body heat, swelling, and pain around the wounded place [34]. The early inflammatory phase of the wound healing cascade is characterized by subsiding of the initial vessel contraction followed by widening their lumen with accompanying increased vascular permeability of walls, which promotes "leaking" of plasma to the wounded tissue area [2, 6, 45]. The changes are supported by histamine, kinins, and prostaglandins and, moreover, leukotrienes, proteases, acid hydrolases, nitrogen oxide, and reactive oxygen species [3, 37]. The latter one is a major stimulus of VEGF synthesis and provides a substantial role

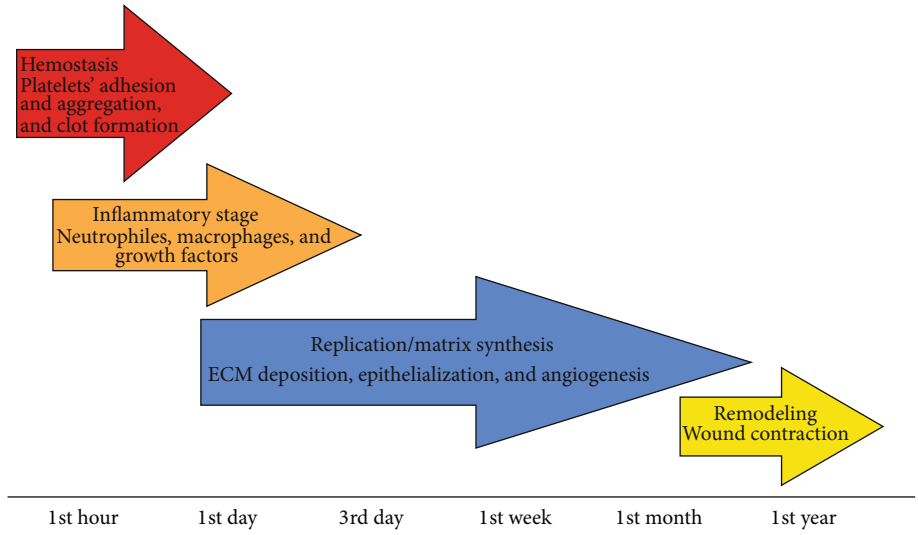

Figure 1: Healing stages.

in the immune defence in the wound [46]. In our previous works, different types of free radicals were found in the burn wounds samples by application of an innovatory numerical procedure of spectroscopic skin analysis such as continuous microwave saturation of multicomponent electron paramagnetic resonance spectra. The effect of microwave power on the asymmetry parameters of the spectra indicated the complex character of free radical system in the tested samples and allowed to obtain the major information about multicomponent structure of free radical system in burn wounds [47, 48].

The key cells of the inflammatory phase are neutrophils and monocytes/macrophages [2, 6, 45]. These cells in addition to keeping the wound aseptic by active phagocytosis and debridement, they simultaneously release a large number of active mediators (cytokines and growth factors), the action of which is crucial to initiate the next phase of the healing process [3, 37, 38, 49, 50].

Neutrophils are the first inflammatory cells which appear in the wound area. The recruitment of these cells takes place a few minutes after the injury [35, 39, 51]. Under the influence of chemotactic factors, such as thrombin, products of fibrin decomposition, bacteria, complement (C5a) components, histamine, PGE_2, leukotrienes, TGF-β, and PDGF, neutrophils are "attracted" to the place of damage [3, 39, 50]. These cells create the first line of defence against infections phagocytising and killing the bacteria by generating reactive oxygen and nitrogen species and digesting, by released proteases (elastase, collagenase, and cathepsin G) the damaged, during the injury, connective tissue components [52–54].

The cells in question intensify the inflammatory reaction by releasing proinflammatory cytokines—IL-1 and TNF-α [37]. After two- or three-day presence in the wound area, the neutrophils are depleted in the process of apoptosis and are replaced by monocytes [36, 37].

Monocytes migrate from capillary to ECM where, under the influence of inflammatory mediators, such as TGF- β and products of fibrin and fibronectin degradation coming from the "temporary" wound matrix, they undergo a transformation into macrophages [3]. The chemotactic and mitogenic factor for monocytes/macrophages is, furthermore, thrombin [39]. The influx of the inflammatory cells in question to the wound area begins on the first day after the tissue injury, while, after 48 hours, they become the dominating inflammatory cells in the wound bed [34, 39].

Macrophages are cells of a great importance for the process of healing [3, 49, 55, 56]. Similar to neutrophils, macrophages play a double role in the healing process [3, 49, 57, 58]. On one hand, they participate in phagocytosis and process of killing bacteria or removing debris, by secreting matrix metalloproteinases, for example, collagenase, or elastase; on the other hand, however, they are the main source of cytokines and growth factors stimulating the proliferation of fibroblasts and collagen biosynthesis [3, 36, 57–59]. Releasing the plasminogen activator, they cause the removal of fibrin cloth. Moreover, they are the source of TGF-β also secreting PDGF, TGF-α, bFGF, HB-EGF, IL-1, IL-6, and TGF-α [15, 50]. The mentioned mediators do not only control the inflammatory process but also modulate the epithelialization, collagen accumulation, and angiogenesis [35, 37, 39, 60].

In the late inflammatory phase, lymphocytes also infiltrate the wound environment influencing fibroblast proliferation and collagen biosynthesis [3].

Summing up, the inflammatory phase, which is initiated by neutrophils and developed under the influence of macrophages, is connected with cleansing the bacteria and debris remains from the wound area as well as with releasing from the mentioned inflammatory cells soluble mediators, such as proinflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α) and growth factors (PDGF, TGF-α, TGF-β, IGF-1,

and FGF) responsible for recruitment and activation of fibroblasts and epithelial cells creating in this way conditions for initiating the next phase of the healing process [49, 55, 61–63].

The absence of neutrophils as well as the reduced amount of macrophages in the wound environment indicates that the inflammatory phase comes to an end and the proliferation phase starts [15].

2.3. Proliferation Phase. After hemostasis and inflammatory phases, which have lasted from 2 to 3 days, the process of rebuilding the damaged tissue is intensified [3]. During this time, the number of cells in the wound bed increases, which is connected with migration and proliferation of fibroblasts and endothelial cells as well as keratinocytes. The first of them—fibroblasts—secretes IGF-1, bFGF, TGF-β, PDGF, and EGF. Endothelial cells synthesize VEGF, bFGF, and PDGF, while keratinocytes synthesize TGF-α, TGF-β, and KDAF (an autocrine factor that derives from keratinocytes). The mentioned mediators stimulate and modulate (a) ECM biosynthesis, (b) epithelialization, and (c) angiogenesis [6].

(a) ECM Biosynthesis. A temporary matrix, formed mainly from fibrin and fibronectin network is replaced by collagen matrix, enriched in proteoglycans, glycosaminoglycans, and noncollagenous glycoproteins, which further lead to restoring the structure and function of the proper tissue [4].

The key cells of the discussed phase are fibroblasts. They are formed mainly from nondifferentiated mesenchymal cells, residing in the dermis, which, under the influence of cytokines and growth factors, released from blood platelets, neutrophils, and macrophages, undergoes a transformation into fibroblasts [3, 64, 65]. These cells migrate to the place of damage during 48–72 hours from the moment when the injury appears [34]. The cells in question are "attracted" to the wound area according to the chemotactic PDGF, EGF, IGF-1, and TGF-β gradient where the proliferation of these cells takes place (stimulated by growth factors) and, after that the synthesis of ECM components and formation of "granulation" tissue starts [24, 39, 66, 67]. The term "granulation" comes from a specific, granulated look of the newly formed connective tissue framework which is "intertwined" by many capillaries [68]. This tissue appears around the fourth day after the injury [69].

The granulation tissue is created by collagen (mainly types I and III), elastin, proteoglycans, glycosaminoglycans, and noncollagenous proteins synthesized mainly by fibroblasts whose activity is regulated by PDGF and TGF-β [70–72]. The first of the mentioned growth factors, originating mainly from blood platelets and macrophages, stimulates also the expression of collagenase, while the second one, which is also secreted by blood platelets and macrophages, regulates the accumulation of ECM components [15]. The matrix of the early granulation tissue (up to the third day after the injury) contains great amount of hyaluronic acid and fibronectin. The hyaluronic acid molecules, which are characterized by an ability to swelling, create a woven structure which enables the coming cells to penetrate the wound area [70]. Fibronectin, however, creates "scaffolding" facilitating the fibrogenesis of collagen [34]. Starting with the third day after the injury, the concentration of hyaluronic acid within the wound area quickly decreases, while collagen takes the place of this glycosaminoglycan. The collagen content in the granulation tissue increases up to the third week, from the moment when the wound appeared, which is accompanied by a gradual decrease of the fibroblast amount up to the moment when they disappear in the process of apoptosis [73]. In the dermis, the dominating types of collagens are types I and III which occur in a proportion of 4 : 1. During the initial phases of healing, however, collagen type III predominates. This protein "toughens" the newly created tissue giving it the feature of tensile strength [39]. The matrix of granulation tissue is also enriched in heparan sulfate proteoglycans, which appear in the wound area after a few hours from the injury [74], as well as chondroitin/dermatan proteoglycans, which appear in the wound area significantly later in the second week of the healing process [28, 70]. The granulation tissue, temporarily substituting the dermis, ultimately matures to a scar during the remodeling phase. It has a thick network of vessels and capillaries, a significant amount of cells—macrophages and fibroblasts as well as collagen fibers of an accidental spatial orientation. It is characterized by a faster metabolism, compared with the dermis, which indicates that the cell in question has an increased energy demand, which, in turn, is connected with cell migration, division, and with an intensified protein biosynthesis [70].

(b) Epithelialization. Epithelialization is a multiphase process which is about reconstructing the epithelium after the injury [15]. Epithelial cells, participating in closing the wound surface, originate both from the wound edges and epithelial appendages, such as hair follicles, sweat glands, or sebaceous glands.

The process in question comprises cellular detachment, their migration to the wound area, proliferation, and differentiation [15]. The mediators which stimulate the migration and proliferation of the mentioned cells are the growth factors, such as EGF, KGF, and TGF-α. The properties of proliferation are demonstrated only by cells lying directly on a basement membrane [35]. They also "deliver" new cells to the epithelial layer which is being created. The cell migration lasts up to the moment when the epithelial cells are connected and create a uniform layer. TGF-β is the only growth factor which accelerates "maturation" of epithelial cell layers [37]. A significant role in the process of keratinocytes separation from their basement membranes is played by the matrix metalloproteinase—MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) which degrade collagen type IV of the basement membrane and collagen type VII which creates anchoring fibrils. MMP-1 (interstitial collagenase) supports the migration of keratinocytes by a network of collagens types I and III, while stromelysin-1 and stromelysin-2 support the migration of these cells by a network of fibronectin, laminines and glycosaminoglycans.

Epithelialization is a clinical symptom of healing; however, it is not a sign of the end of this process. The final phase of the above-mentioned process is the remodeling of the granulation tissue [15, 69].

(c) Angiogenesis. Angiogenesis is a process of creating new blood vessels [7, 34, 75–77]. This process restores blood circulation in the place of damage and prevents the development of ischemic necrosis simultaneously stimulating the tissue repair process. It is stimulated by microenvironment local factors, such as low oxygen tension, low pH, or high lactic acid concentration [38, 78]. Moreover, many soluble mediators, such as bFGF, TGF-β, TNF-α, VEGF, angiogenin, and angiotropin secreted by epithelial cells, fibroblasts, endothelial cells, and macrophages demonstrate a strong proangiogenic activity [2, 34]. The phenomenon of angiogenesis activation or suppression via hypoxia inducible factor was also described [15].

The regulation of angiogenesis, besides stimulating factors, comprises also factors hindering the process in question. The latter ones are angiostatin and thrombospondin [4, 75]. Proangiogenic activity is exerted by hyaluronic acid molecules of a low molecular mass, while the ones of a big molecular mass exert the opposite activity [79, 80].

Angiogenesis is a key phase of the healing process. In the course of this process, endothelial cells migrate to the temporary matrix of the wound after which, they proliferate and subsequently they create a network branching into a form of tubular structures [13, 35]. The migration of endothelial cells requires a local secretion of matrix metalloproteinases digesting basement membranes and releasing growth factors sequestrated in the ECM [35]. Joining of independent "budding" branches of endothelial cells creates a structure which gives the beginning for a new blood vessel loop. This process lasts until the essential restoration of the capillary system and up to the moment of providing proper oxygen influx and nutrients to the wound environment because of that [35]. Visible capillary tuffs give the wound surface a granular appearance, which is the reason for the expression "granulation tissue." When the tissue is replaced by collagen matrix and in the last phase by a scar, its "requirements" concerning oxygen influx and nutrients are significantly lower. Angiogenesis is stopped, while a part of capillaries disintegrates during the process of apoptosis. It is a sluggish process and paling of the scar takes many years.

Summing up, the proliferation phase is connected with the activity of fibroblasts which, in the presence of newly formed blood vessels, proliferate and synthesize ECM components. Endothelial cells proliferate and migrate above the granulation tissue "closing" the wound surface.

2.4. Remodeling Phase. Remodeling is the last phase of the healing process [35]. In its course, the wound surface is contracted [81]. The key phenomenon of wound contracture is phenotypic differentiation of the preexisting fibroblasts into myofibroblasts [82–84]. The latter ones contain fibrils of alpha smooth muscle actin (α-sma) microfilaments, which give the cells the property of contracting [85]. In turn, the integrin receptors $\alpha_1\beta_1$ and $\alpha_2\beta_1$ react with specific places on collagen and mediate in contracting the granulation tissue [82]. The mentioned transformation takes place in the second week of healing, which is why myofibroblasts become the most numerous populations of cells in the granulation tissue [35, 38, 86].

During this phase of the healing process, the granulation tissue "matures" to the form of a scar, which is accompanied by the increase of mechanic strength of the formed tissue. The maturation process of the granulation tissue comprises the reduction of capillary amounts, by aggregating into bigger blood vessels, lowering the content of glycosaminoglycans and proteoglycans as well as the water content connected with glycosaminoglycans and proteoglycans [15, 39]. Cell density and metabolic activity of the tissue are also lowered. The mutual proportion of collagen types changes (type I collagen content increases in favor of collagen type III), the total collagen content increases, its spatial organization becomes arranged, and the number of covalent cross-links increases, which leads to increased tensile strength of the tissue. The tensile strength, in the case of the wound freshly covered with epithelium, equals 25% related to the dermis, while, after many months of reconstruction, the strength equals 80% related to the unchanged tissue [15, 38, 87].

Summing up, during the remodeling phase, the amount of fibroblasts decreases and the vascular density is lowered. The initial scar tissue, characterized by delicate, accidentally organized collagen fibers, typical for proliferation phase, is replaced by a matrix which resembles the dermis in which mature, cross-linked collagen fibers, of the proper diameter, construct a framework of the newly formed tissue [1, 35, 88].

The functions of cells participating in the healing process are regulated by cytokines and growth factors as well as by interactions with ECM components, mediated by integrin receptors and adhesive molecules. Matrix metalloproteinases, which are released by endothelial cells and fibroblasts, enable these cells to migrate, while the neutrophil and macrophage proteases remove degraded matrix components assisting in the remodeling of the initial scar tissue [35].

The fundamental role in the healing process is played by extracellular matrix components. In our previous experimental studies, we proved that ECM constituents, including collagen, glycosaminoglycans, vitronectin, and laminin, turned out to be better effectors of natural therapeutic agent such as propolis than silver sulfadiazine (so-called "gold standard" in topical wound management) in experimental burn wound healing [28, 32, 89]. Estimating the expression of mentioned GAGs during burn treatment with propolis, we observed that the apitherapeutic agent we used accelerates the burned tissue repair by stimulation of the wound bed glycosaminoglycan accumulation needed for granulation, tissue growth, and wound closure. Moreover, propolis accelerates chondroitin/dermatan sulfates structure modification responsible for binding growth factors playing a crucial role in the tissue repair [28, 32]. The role of ECM components in repairing tissue damages is the subject of the in-depth, overview studies [70, 90–93].

In conclusion, ECM components, particularly glycosaminoglycans and proteoglycans, play a fundamental role in wound healing process. Understanding biochemical mechanisms by which ECM components modulate each stage of the process of soft tissue remodeling after injury is of great importance in the description (implementation) of new

therapeutic strategies connected with generating a favorable biochemical environment supporting wound healing process.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Tension-Free Vaginal Tape, Transobturator Tape, and Own Modification of Transobturator Tape in the Treatment of Female Stress Urinary Incontinence: Comparative Analysis

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Introduction. This study is a comparative evaluation of the TVT, TOT, and our own modification of TOT (mTOT) in the treatment of female stress urinary incontinence from a single center experience. *Material and Methods*. The study was conducted on 527 patients with SUI diagnosed on the basis of urodynamic studies. They were divided into three groups—TVT: $n = 142$, (TOT): $n = 129$, and mTOT: $n = 256$. All of the patients underwent evaluation at 1, 3, and 6 months after surgery. Results were statistically analysed and compared. *Results*. Objective and subjective effectiveness after the surgery were not significantly different in the study groups and ranged from 90.1% to 96.4%. Mean surgery time was 32.3, 28.2, and 26.4 in the TVT, TOT, and mTOT, respectively. Mean hospitalization time was 2.51 days. Mean catheter maintenance time was significantly higher in the TVT than in other groups. In the TVT group total incidence of complications was 13.4%, and it was significantly higher than that in TOT and mTOT (9.3% and 8.6%, resp.). *Conclusions*. TVT, TOT, and mTOT are highly effective and safe methods in the treatment of SUI. There are no differences in the efficacy between the methods with a little higher percentage of complications in the TVT group.

1. Introduction

Stress urinary incontinence (SUI) in women is a widespread disease all over the world. It causes many psychosocial problems and generates significant costs to the budget of health in many countries. In 1993 DeLancey as one of the first researchers concluded that its pathophysiology is associated with a defect in bladder neck and urethra due to the laxity of surrounding tissues and the insufficiency of the internal sphincter of urethra [1]. Various factors may affect the development of SUI. The most well known are vaginal births, overweight and obesity, hormonal disorders, and muscle weakness of pelvic diaphragm. First choice of the treatment for SUI is conservative treatment, whose main elements are lifestyle modifications (physical activity, dietary habits, and weight loss), bladder control exercises, and pelvic floor muscle training (PFMT). In the absence of effects in the conservative treatment including medications and physiotherapy, surgical treatment is necessary. In addition to open techniques such as Burch colposuspension, currently the most often used are minimally invasive methods. Their aim is the suspension of the bladder neck and urethra using synthetic materials, the so-called sling. Abnormal positioning of the urethra and the bladder neck implied the possibility of introducing the method of correcting this condition and in 1996 Ulmsten and colleagues have published the report describing the TVT (tension-free vaginal tape) technique in the treatment of SUI [2]. A few years later, TOT (transobturator tape) method was described in which the tape is carried out between the obturator holes [3]. Both methods are now widely accepted methods of surgical treatment of SUI. The transobturator variant, however, has become more popular nowadays due to similar cure rate with relatively less complications.

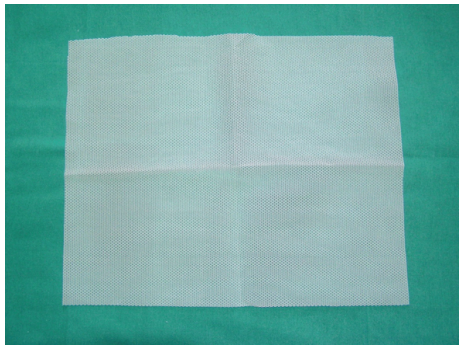

Figure 1: Polypropylene mesh used to prepare TOT tape.

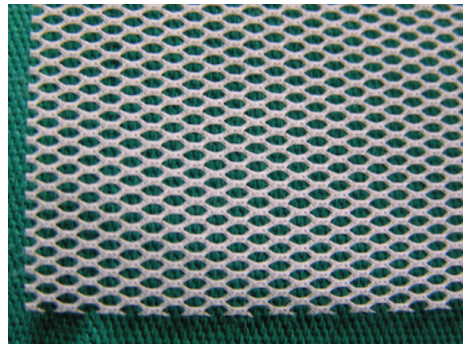

Figure 2: Close-up of polypropylene mesh shown in Figure 1.

2. Aim of the Study

The aim of the study is to evaluate the comparative results of surgical management of the stress urinary incontinence in women using tension-free vaginal tape (TVT), tension-free transobturator tape (TOT), and own modification of TOT (mTOT). Study is based on over 10 years of experience from a single center.

3. Materials and Methods

In the years 2001–2012 in Department of Urology in Zabrze, 527 women with SUI were treated using midurethral slings. The age of patients ranged between 45 and 64 years, mean 55.1 years. All patients before treatment were carefully examined and the diagnosis of SUI and qualification for surgical treatment were established on the basis of physical examination, urinalysis and urine culture, abdominal ultrasound, and urodynamic studies in the form of pressure-flow study. 456 patients (86.5%) had pure form of stress urinary incontinence. The remaining 71 (13.5%) presented mixed urinary incontinence (MUI) with urge incontinence component. The treatment option was chosen for the patients according to the routine method used in department at the time when the patients were admitted and treated. Patients were divided into three study groups depending on the type of surgical procedure (Table 1). Group I accounted for 142 patients (26.9%) operated with classical TVT technique. They were treated in the years 2001–2005. Group II consisted of 129 patients (24.5%) who have undergone TOT procedure in the years 2002–2009, using original sets dedicated to this type of treatment. Group III included 256 women (48.6%) who underwent TOT in the years 2004–2012 with our own modification. In that group instead of the original tape dedicated to TOT, self-prepared tape from a polypropylene mesh destinated for abdominal hernias repair was used. It was prepared just before the procedure by the operator from Dallop PP TDM KTM mesh (Figures 1, 2, and 3). All TOT procedures were performed using Stamey needle. The procedures were performed by three different operators with similar experience in the surgical treatment of stress urinary incontinence. Most procedures were carried out under spinal anesthesia (502, 95.3%) and the other in a short general anesthesia due to anaesthesiological indications. Woman

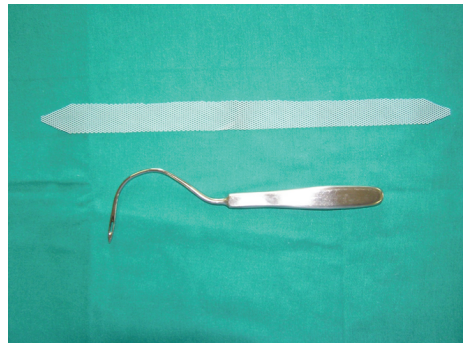

Figure 3: Tape prepared for the mTOT procedure with Stamey needle.

with MUI after surgery were subjected to pharmacological treatment with antimuscarinic drugs. Postoperative evaluation was performed after one, three months, and then every 6 months after surgery. The subjective cure rate was evaluated by patients' satisfaction with surgery interpreted as a patient-reported success rate. Patients answered a short questionnaire consisting of three possible answers: (a) I'm very satisfied with treatment results, (b) I'm rather satisfied with treatment results, and (c) I'm not satisfied with treatment results. Answers (a) and (b) were interpreted as patients' related success. Objective cure rate was evaluated on the basis of cough test and one-hour pad test. Completely dry pad after 1 hour of normal day activity was interpreted as a negative result. During the follow-up all of the patients underwent also physical examination, urinalysis, and ultrasound evaluation of postvoiding residual volume. Duration of follow-up was 6–130 months. All data were statistically analysed with Kolmogorov-Smirnov test. For analysis of continuous variables without normal distribution nonparametric U-Mann-Whitney test was used. For analysis of categorical variables χ^2 was used. Statistical examination was conducted with the aid of Statistica Statsoft v 9.0. P values <0.05 were considered as statistically significant.

4. Results

First evaluation of the cure rates was obtained in 1 month after surgery (Table 2). Objective (cough test and 1-hour pad

Table 1: Comparison of the study groups.

	Group I (TVT)	Group II (TOT)	Group III (mTOT)	*P* value
Number of patients	142 (26,9%)	129 (24,5%)	256 (48,6%)	
Age	55,2 ± 8,3	54,7 ± 7,8	55,9 ± 8,6	0,115
Complications	19 (13,4%)	12 (9,3%)	22 (8,6%)	0,04
Operation time (min)	32,3	28,2	26,4	0,33
Hospitalization time	2,54	2,48	2,50	0,76
Catheter maintenance	1,84	1,58	1,52	0,04

Table 2: Cure rates in 1 month after surgery.

	Group I (TVT)	Group II (TOT)	Group III (mTOT)	*P* value
Cough test negative	128 (90,1%)	118 (91,5%)	236 (92,2%)	0,09
Pad test negative	133 (93,7%)	122 (94,6%)	241 (94,1%)	0,88
Satisfied with surgery	135 (95,1%)	123 (95,3%)	244 (95,3%)	0,91

Table 3: Cure rates in 3 months after surgery.

	Group I (TVT)	Group II (TOT)	Group III (mTOT)	*P* value
Cough test negative	129 (90,8%)	120 (93,0%)	238 (92,9%)	0,07
Pad test negative	133 (93,7%)	121 (93,8%)	241 (94,1%)	0,95
Satisfied with surgery	137 (96,4%)	123 (95,3%)	246 (96,1%)	0,88

Table 4: Cure rates in 6 months after surgery.

	Group I (TVT)	Group II (TOT)	Group III (mTOT)	*P* value
Cough test negative	130 (91,5%)	120 (93,0%)	239 (93,3%)	0,08
Pad test negative	134 (94,4%)	120 (93,0%)	243 (94,9%)	0,12
Satisfied with surgery	137 (96,4%)	124 (96,1%)	247 (96,4%)	0,98

test) and subjective (patient's satisfaction) cure rates were analysed. There were no statistically significant differences in the efficacy of the surgery between the study groups. Depending on the cure criteria, efficacy ranged from 90.1% to 95.3%, with the highest values in the patients' satisfaction.

Three months after surgery second evaluation was performed (Table 3). Both subjective and objective cure rates were a little higher than 2 months earlier and ranged from 90.8% to 96.4%. No significant differences between the study groups were observed.

Last evaluation of the efficacy was performed 6 months after surgery and it contained the same methods as earlier (Table 4). Subjective and objective cure rates ranged from 91.5% to 96.4% without any differences between the study groups.

Surgery time ranged from 20 to 42 minutes, with means 32.3, 28.2, and 26.4 in the TVT, TOT, and mTOT, respectively. There were no statistical differences between the study groups. Hospitalization time ranged from 2 to 5 days, mean 2.51 days with no significant differences between the groups.

Mean catheter maintenance time in the Group I (TVT) was 1.84 days and it was significantly higher than in TOT and mTOT groups (1.58 and 1.52, resp.). It occurred probably due to higher percentage of bladder injuries in the TVT group.

There were no significant intraoperative and postoperative complications observed in the study groups that required reoperation. Bladder injury, de novo OAB (overactive bladder symptoms), postvoiding residual urine >100 mL, and tape extrusion were analysed as intra- and postoperative complications (Table 5). In the TVT group total incidence of complications was 13.4%, and it was significantly higher than that in TOT and mTOT (9.3% and 8.6%, resp.). The analysis of individual complications showed that the only statistical difference was observed in the frequency of bladder injuries during the surgery and it was highest in the TVT group (4.2%); it occurred only in few cases in TOT and mTOT (0.7% both). De novo OAB occurred in 3.5–4.7% without differences between the groups and was treated pharmacologically after the surgery. Postvoiding residual urine above 100 mL was discovered in the abdominal ultrasound during follow-up in 2.3–4.9%. Tape extrusion was discovered in 5 cases in the whole study group without statistical significance between the groups.

Analysis between TVT and TOT in total (both TOT and mTOT) was also performed in the 6th month after surgery (Table 6). It showed no statistical difference between TVT and TOT in total in both subjective and objective cure rates but it proved higher percentage of complications in the TVT group with statistical significance.

Comparison between TOT and mTOT was also made in the 6th month after the treatment (Table 7). It revealed no differences in cure rates and complications between

Table 5: Complications.

	Group I (TVT)	Group II (TOT)	Group III (mTOT)	*P* value
Bladder injury	6 (4,2%)	1 (0,7%)	2 (0,7%)	0,01
De novo OAB	5 (3,5%)	6 (4,7%)	11 (4,3%)	0,15
Postvoid residual	7 (4,9%)	3 (2,3%)	7 (2,7%)	0,09
Tape extrusion	1 (0,7%)	2 (1,6%)	2 (0,7%)	0,22

Table 6: Comparison between TVT and overall TOT in 6 months after surgery.

	TVT ($n = 142$)	Overall TOT ($n = 385$)	*P* value
Cough test negative	130 (91,5%)	359 (93,2%)	0,07
Pad test negative	134 (94,4%)	363 (94,3%)	0,98
Satisfied with surgery	137 (96,4%)	371 (96,4%)	0,99
Complications	19 (13,4%)	34 (8,8%)	0,01

Table 7: Comparison between TOT and TOT with our own modification (mTOT).

	TOT ($n = 129$)	mTOT ($n = 256$)	*P* value
Cough test negative	120 (93,0%)	239 (93,3%)	0,09
Pad test negative	120 (93,0%)	243 (94,9%)	0,88
Satisfied with surgery	124 (96,1%)	247 (96,4%)	0,08
Complications	12 (9,3%)	22 (8,6%)	0,07

females operated with classic TOT and TOT with our own modification described above.

5. Discussion

World literature reports that TOT has now become a bit more popular, mainly due to the similar efficacy and a slightly lower rate of complications [4, 5]. Published studies show about 80–85% success rate in the efficacy of the TVT method [6, 7]. The most frequently reported complications of this method are bladder perforation, bleeding disorders, and de novo micturition urgency [8]. Published efficacy of the TOT technique is similar, with a slightly lower percentage of complications [9, 10]. Due to the preferences of centers performing such procedures and preferences of individual operators, the number of scientific comparisons of the two methods is low. There are also no standardized uniform methods of follow-up in analysed groups of patients. Depending on study, the level of patients' satisfaction, the results of standardized questionnaires, pad tests, or results of urodynamic studies undergone analysis in the efficacy evaluation. Hence, there are few meta-analyses comparing the efficacy of both methods. In a prospective randomised study comparing the effectiveness of TOT and TVT, based on an analysis of 70 cases, a comparable efficacy of both surgical techniques was achieved, with shorter surgery time and the risk of bladder injury in favor of TOT [11]. Italian researchers, based on a comparative analysis of 148 patients, showed no difference of statistical significance in both—the effectiveness and complication rate between the two groups [12]. In the latest publications from 2013 by Darabi et al., no significant differences in efficacy and safety in both groups were shown, except the bladder catheter maintenance time after surgery [13]. There are a few analyses based on patient-related success rate, same as subjective cure rate in the above article. For example, British analysis from 2012 in prospective randomised controlled trial proved 73% patient-reported success rate for TOT [14]. Large comparative analysis of 1000 cases of SUI treated with TOT and TVT revealed subjective cure rates ranging from 85 to 96% and objective efficacy ranging from 86–91% [15]. Based on 5-year-follow-up evaluation of TOT a study was published in 2013 that showed subjective and objective cure rates at about 90% [16].

The phenomenon of de novo OAB symptoms is a largely debated postoperative complication of midurethral slings. Some studies reported de novo urgency symptoms in 4–33% of operated patients [17, 18]. Confounding role can also play spontaneous development of age-related OAB in a certain percentage of women. In our study de novo OAB was observed in less than 5% of cases and reduced in time during pharmacological treatment.

There have also been some assessments of the results in the treatment of patients with mixed urinary incontinence using midurethral slings. Korean authors concluded in 2003 that treatment with TVT and TOT reduced the percentage of daily incontinence from urgency in these patients, with the higher efficacy in the TVT group [19]. This confirmed the Finnish study from 2013, in which 70% of patients with symptoms of detrusor overactivity declared improvement after the surgery; there was no significant difference between the two operating techniques [20]. A meta-analysis of American scientists in 2007, based on 492 cases, showed no significant differences in the efficacy between the two methods. The conclusion was made that a small number of reports are not clear in showing whether any of these methods are effective

in the treatment of urinary incontinence with mixed etiology [4].

The main economical differences between all those three methods were the cost of materials used in the procedure. In mTOT we used a tape made from original mesh used in hernias repair. One tape for mTOT made from it costs around 10 EUR. Original TOT tape in Poland costs about 170 EUR.

6. Conclusions

(i) All sling procedures are effective in the treatment of stress urinary incontinence and in 6th month after surgery achieved cure rates range from 91.5% to 96.4% in subjective and objective parameters of efficacy.

(ii) There are no differences in the efficacy of the treatment of SUI between TVT, TOT, and self-modification of TOT.

(iii) There is a little higher risk of bladder injury during the TVT procedure than in the TOT and mTOT.

(iv) Self-modification of TOT, which consists of self-prepared polypropylene tape instead of original tape, is as effective and safe as original TOT with a lower cost of the procedure.

(v) Sling procedures in the treatment of SUI are safe and do not cause serious complications.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Propolis Modulates Fibronectin Expression in the Matrix of Thermal Injury

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The aim of the study was to assess the propolis effect on fibronectin metabolism in the course of burn wounds healing process. A model of burn wound healing of pig skin was applied. The amount of the released glycoprotein was assessed by a surface plasmon resonance. The profile of extracted fibronectin components was also assessed by an electrophoresis in polyacrylamide gel, with a subsequent immunodetection by Western Blotting. Propolis burn treatment decreased the release of fibronectin components from healing wounds in relation to damages treated with silver sulfadiazine. The main reason of decreased extraction of fibronectin components from wounds treated with propolis was a substantial decrease of degradation product release of the mentioned glycoprotein, which was observed particularly from the 3rd to 5th day of the repair. Wounds treatment with propolis demonstrated, especially in relation to damages treated with silver sulfadiazine, the decreased release of synthesized fibronectin molecules. The obtained results suggest that propolis modifies fibronectin metabolism in the course of wound healing process. The influence of propolis is reflected in prevention of fibronectin biosynthesis as well as its degradation in the wound area. The above-mentioned metabolic changes may decrease the risk of complications in the repair wounds process.

1. Introduction

Fibronectin (FN) is a high molecular weight, multifunctional glycoprotein that determines the structural integrity and various functions of different organs and tissues [1, 2]. Fibronectin is a dimeric molecule [3], built of two similar but not always identical polypeptide chains [2], consisting of three repeating amino acid motifs, named type I, type II, and type III modules [1]. In the course of wound healing, FN modified from inactive soluble molecule into biologically active form [3] participates in every phase of wound healing [4]—hemostasis, inflammation, proliferation, and tissue remodeling [5]. Fibronectin controls bleeding and limits the extent of tissue damage through clot formation [6]. Moreover, the mentioned glycoprotein is important for platelet activity, for example, adhesion, migration, and proliferation [4]. During the second stage of wound repair—inflammation—FN participates in opsonizing ECM debris and activates macrophages for phagocytizing the destroyed tissue residues [1, 4]. The accumulation of fibronectin in wound matrix also impacts on the next phases of the repair process, since the mentioned glycoprotein stimulates theangiogenesis, collagen biosynthesis, granulation tissue formation, and reepithelialisation [1, 4]. In the course of the remodeling phase FN polymerization determines the composition, stabilization, and turnover of the wound extracellular matrix molecules as well as cell-matrix adhesion [4]. However, little is known about the influence of propolis—one of the most promising candidates for burn wound management [7]—on fibronectin expression in the course of thermal damage regeneration. Propolis is a natural resinous material, collected and used by honey bees for the construction of hives, which presents various biological activities such as immunostimulatory, antioxidant, antimicrobial, antiviral, antifungal, antiulcer, anti-inflammatory, and radioprotective as well as properties responsible for augmenting the effects of certain antibiotics

[7–12]. Active compounds of Polish propolis used in experiments underlying this paper are flavonoids, aromatic acids, aromatic esters, terpenes, sesquiterpenes, steroids, bioelements, and enzymes [12, 13]. The most important ingredients of Polish propolis are flavonoids, that is, chrysin, tectochrysin, apigenin, pinocembrin, pinostrobin, pinobanksin, galangin, kaempferol, kaempferide, and quercetin as well as phenolic acids such as cinnamic, p-coumaric, ferulic, caffeic acid, and caffeic acid phenylethyl ester (CAPE) [14, 15]. Nowadays, silver sulfadiazine (SSD) is used as an agent of choice in topical burns treatment; however, it exerts serious adverse reactions leading to neutropenia, erythema multiforme, crystalluria, and methaemoglobinemia [9, 16] as well as cytotoxic effect toward fibroblasts and keratinocytes which may retard wound healing process [17]. In addition to injuries treated with silver sulfadiazine, novel methods of wound management such as electrotherapy, laser irradiation, and ultrasound therapy should be paid attention to [18–20]. The first mentioned method—electrotherapy—based on high voltage stimulation, exerts a bactericidal effect, increases cutaneous perfusion, promotes granulation tissue formation, stimulates migration of epidermal cells, restores natural electric potentials, and improves the healing rates [18, 21–23]. Unfortunately, other results suggest that electrotherapy does not influence the acceleration of total therapy of skin damages and the final phase of wound healing [18, 24]. Another method—laser irradiation—is reported to accelerate the healing process, effectively facilitate wound contraction as well as to modulate the inflammation by reducing the levels of proinflammatory cytokines and increase the levels of anti-inflammatory growth factors [25, 26]. On the other hand, there are critical results suggesting that laser irradiation does not enhance the wound healing process [19]. The last listed novel method—ultrasound therapy—prepares the wound bed for healing by reducing bioburden, enhancing angiogenesis, assisting in debridement of necrotic and devitalized tissues, and stimulating cellular activity [27]. However, the results of examination conducted by Dolibog et al. [20] and by Taradaj et al. [28] may suggest that ultrasound therapy does not influence the repair process acceleration. Taking into account the controversies associated with application of SSD and novel methods, such as electrotherapy, laser irradiation, and ultrasound therapy, in wound healing, the introduction of alternative therapeutic agents/methods, such as propolis application, is needed.

Therefore, given that fibronectin is the key component of the interstitial matrices [3], playing structural and functional roles during wound repair process [2, 4], the aim of the present study was to compare the propolis and silver sulfadiazine therapeutic efficiency using the quantitative and qualitative evaluation of the mentioned glycoprotein expression in the matrix of thermal injuries.

2. Materials and Methods

2.1. Reagents. The following antibodies were used: monoclonal mouse anti-human fibronectin antibody number 42042, purchased from QED Bioscience Inc., San Diego, California, USA, and goat anti-rabbit immunoglobulin G number A5420, conjugated with horseradish peroxidase, obtained from Sigma-Aldrich, Germany. The following reagents were applied: sodium metaperiodate and hydrazide LC-biotin obtained from Thermo Scientific, USA; standard fibronectin from human plasma, DMSO (dimethyl sulfoxide), sodium dodecyl sulfate, Triton X-100, Brilliant blue R250, glycine, Immobilon P membranes, dithiothreitol, Tween 20 (polyoxyethylene sorbitan monolaurate), and TMB (3.3′,5,5′-tetramethylbenzidine), all supplied by Sigma-Aldrich, Germany; Sephadex G-25 obtained from Pharmacia, Sweden; HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid) supplied by Fluka, Germany; BLOT-QuickBlocker purchased from Millipore, USA; TEMED purchased from ICN Biomedicals, USA; streptavidin-coated sensor chip (SAP) obtained from XanTec Bioanalytics, Germany. All of the remaining, applied reagents were supplied by POCh Gliwice, Poland.

2.2. Therapeutic Agents. Apitherapeutic ointment containing the ethanolic extract of Polish propolis (prepared according to the method described by Szliszka et al. [14]) was accepted by the National Institute of Hygiene (certificate number: HZ/06107/00; date: November 4, 2000). 1% (0.01 g/mL) silver sulfadiazine (AgSD) cream, obtained from Lek Poland was used.

2.3. Tissue Materials. The study protocol was approved by the Ethics Committee of the Medical University of Silesia, Poland (Nr 6/2004). Four domestic four-month-old, pigs were used for the evaluation of wound repair because of many similarities between pig and human skin. Seventy-two contact burn wounds were inflicted on the right and left flanks of the pigs' body, according to the methods of Hoekstra et al. [29] and Brans et al. [30]. Pigs were housed according to the Good Laboratory Practice (GLP) Standards of Polish Veterinary Law. Animals were divided into control (two pigs) and experimental (two pigs) groups. In the control group, wounds were treated with physiologic saline (NaCl) (one animal) or with a propolis vehicle (another animal) twice a day, for 21 days. Wounds treated with NaCl allowed us to observe the healing process occurring without management. Wounds treated with the vehicle alone allowed us, in turn, to assess its possible impact on the propolis therapeutic effect. In the experimental groups, burns were treated with propolis (one animal) or AgSD (another animal), twice a day, for 21 days. Biopsies, in three replications, were taken from healthy skin on day "0" and from the different wound beds on post-burn days 3rd, 5th, 10th, 15th, and 21st. Analgesics given were ketamine hydrochloride and thiopental sodium. Following the thermal damage, tissues were rinsed with an antiseptic solution and treated with apitherapeutic agent, AgSD, apitherapeutic agent vehicle, and physiologic saline. In the case of burns treated with the apitherapeutic agent, AgSD, and apitherapeutic agent vehicle, the wound beds were covered with 55–75 mm layer of used experimental agents. Then, the injuries were protected with a woven cotton material. The thermal injuries left by the biopsy were protected with the collagen dressing.

2.4. Extraction of Tissue Fibronectin. Fibronectin was extracted from dehydrated, degreased, and homogenized tissue material with Tris-HCl buffer, pH 7.2, containing 2 M urea, 4% sodium dodecyl sulfate (SDS), and protease inhibitors (0.005 M EDTA, 0.005 M ε-amino-n-caproic acid, and 0.001 M PMSF) for 2 h, at 21°C. Then, samples were centrifuged (21000 ×g, 25 min, 21°C), and the tissue pellet was repeatedly submitted to urea/SDS extraction. Fibronectin was precipitated from combined supernatants by an addition of 100% solution of TCA to its final concentration of 10% and incubation for 12 h, at 4°C. The pellets containing fibronectin were separated by centrifugation (21000 ×g, 25 min, 21°C) and repeatedly washed with 80% ethanol to remove TCA [31]. Then, fibronectin samples were stored at −75°C prior to analyses.

2.5. Biotinylation of Antibody against Fibronectin. Antibody against fibronectin was dissolved in 0.1 M acetate buffer (pH = 5.5). The obtained solutions were cooled and protected from light and then mixed with an equal volume of 0.02 M sodium metaperiodate in 0.1 M sodium acetate buffer (pH = 5.5). Subsequently, the samples were incubated for 0.5 h at 4°C and then subjected to gel filtration on Sephadex G-25 equilibrated in PBS buffer, pH = 7.2. 1 mL fraction eluting at column void volume and containing anti-fibronectin antibody was collected and mixed with 111 μL of 0.05 M solution of hydrazide LC-biotin in DMSO. The biotinylation of antibodies was being conducted for 2 h, at room temperature. Next, free biotin was removed by dialysis against distilled water and modified anti-fibronectin antibody was lyophilized [32]. The efficiency of antibody biotinylation was controlled with special EZ biotin quantitation kit (Thermo Scientific).

2.6. Quantification of Fibronectin in the Hydrolyzates of Burn Wounds. The assessment of fibronectin content in the tissue material derived from healing postburn wounds was made by surface plasmon resonance (SPR) measurement in SPRINGLE instrument (Autolab, the Netherlands) [33]. For this purpose, the biotinylated anti-fibronectin antibodies were immobilized onto streptavidin-coated sensor chip (SAP from XanTec, Germany) and exposed at 21°C to components extracted from tissue with urea/SDS solution. The binding was conducted in 0.01 M HEPES buffer, pH 7.4, containing 0.0034 M EDTA, 0.15 M NaCl, and 0.05% (v/v) Triton X-100. The formation of complexes between the antibody and fibronectin was detected as changes in the SPR signals which were proportional to the amount of bound fibronectin molecules. After binding, the disc surface was regenerated through the dissociation of immune complexes with 0.01 M glycine solution, pH 2.0. The calibration curves were done using various concentrations of standard fibronectin.

2.7. The Assessment of Fibronectin in Burn Wounds. Samples of porcine skin extracts were subjected to electrophoresis in a 6% polyacrylamide gel in the presence of SDS according to the method of Laemmli [34]. Prior to electrophoresis, the extract components were treated with 0.04 M dithiothreitol as an agent reducing disulfide bonds. After the electrophoresis, some gels were stained with Brilliant blue R250 and others submitted to electrotransfer on Immobilon P membranes, followed by Western blotting with anti-fibronectin antibodies. Then, the obtained gels and blots were analyzed densitometrically.

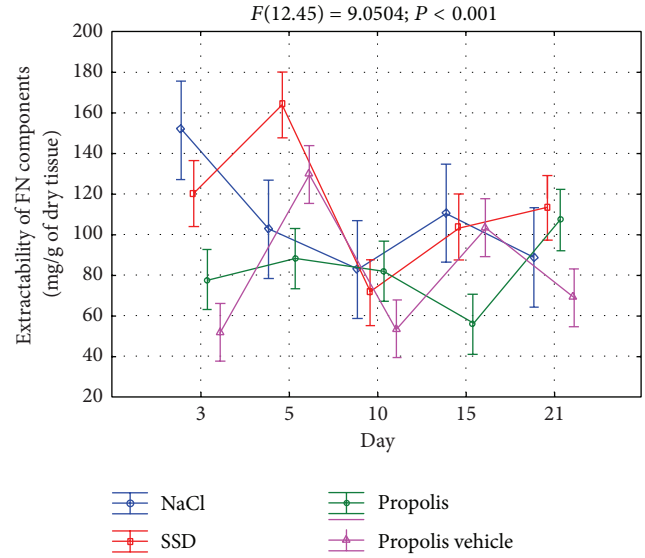

Figure 1: Extractability of FN components isolated from wounds treated with NaCl, SSD, propolis, and propolis vehicle and reacting with reagents against fibronectin.

2.8. Statistical Analysis. Repeated measures analysis of variances (ANOVA) was applied to test the significance of univariate measures of factors with more than two levels (in our research—six levels: day "0" and postburn days—3rd, 5th, 10th, 15th, and 21st) followed by Tukey's post hoc tests, accepting $P < 0.05$ as significant. The special assumption of sphericity (which is a necessary and sufficient condition for the F-test to be valid) was verified and held for fibronectin content [35].

3. Results

In order to assess the FN metabolism in the course of differently-treated burn wounds, the extracts of tissue material were collected, which, by using the surface plasmon resonance method, allowed us to assess the content of components reacting with the reagents of this glycoprotein. The obtained data are shown in Figure 1.

As it results from Figure 1, the FN extractability from the place of injury in the course of 21 days of the repair process, regardless of the applied treatment, was significantly diversified demonstrating two maxima. In the case of wounds treated with NaCl, which depict the physiological healing process, the first and particularly abundant release of fibronectin components from the place of damage appeared on the 3rd day of the repair, while the second one, which was significantly lower, appeared on the 15th day (Figure 1). Contrary to damages treated with NaCl, the application of SSD and propolis for wound treatment caused a characteristic

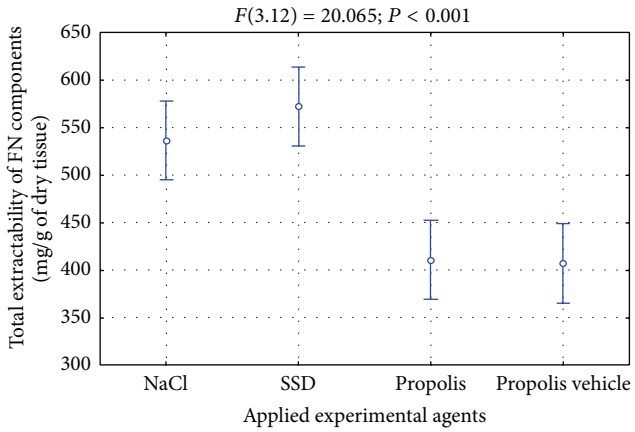

Figure 2: Total extractability of FN components isolated from wounds treated with NaCl, SSD, propolis, and propolis vehicle in the course of healing.

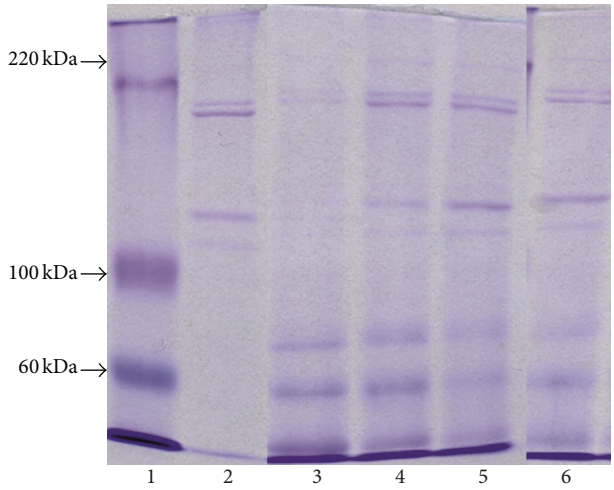

Figure 3: The characteristics of molecular profile of extracts differently treated burn wounds buffered by urea solution and sodium dodecyl sulfate (SDS). Extract components, subjected to dithiothreitol as a factor reducing the disulfide bonds, were subjected to the electrophoresis in a 6% polyacrylamide gel in the presence of SDS. Lane 1, molecular mass markers; lane 2, standard of collagen type I; lanes 3, 4, 5, and 6, components extracted, on the 5th day of healing, from wounds treated by NaCl, SSD, propolis, and propolis vehicle, respectively. The arrows indicate the migration position of standards of known molecular weights (60 kDa, 100 kDa, and 220 kDa).

delay in the maximal FN extractability with the first apex falling on the 5th day of healing and the second one appearing on the 21st day (Figure 1). However, only in the case of silver sulfadiazine, similarly to the application of NaCl, the first maximum of the fibronectin components' release was significantly higher than the second one (Figure 1), whereas in the case of wounds treated with propolis, the first increase of FN extractability was gentle and considerably lower than the second one (Figure 1). Moreover, the above dynamics of FN release from the burn wounds seems to be caused by a characteristic effect of propolis on the metabolism of this glycoprotein. This conclusion results from a different course of the curves of the mentioned glycoprotein extractability from damages treated with both propolis and the vehicle of apitherapeutic agent (Figure 1).

The data concerning the overall number of components reacting with the antibodies for FN, which are released from skin burn wounds during initial 3 weeks of the repair, are shown in Figure 2.

As it results from Figure 2, the FN extractability from the place of damage during initial weeks of healing was not big. Furthermore, characteristically similar amounts of the mentioned glycoprotein were released from wounds treated with NaCl and SSD (Figure 2), whereas wounds treated with propolis and its vehicle were characterized by a lower FN extractability (Figure 2). However, it is worth pointing out that the character of FN metabolism changes in the place of damages is important for the correct course of the healing process. The intensification of proteolysis process of the mentioned glycoprotein in the wound area may lead to releasing biologically active degradation products, for instance, capable of amplifying the inflammatory condition or inhibiting the reconstruction of fibrous weave of the extracellular matrix. The stimulation of FN biosynthesis with a subsequent fibrogenesis of this glycoprotein should intensify the granulation process by stimulating the proliferation, migration, and adhesion of cells and also by restoring the framework of the extracellular matrix.

The character of metabolic processes concerning FN in the area of differently treated skin burn wounds should be displayed in the profile of fibronectin components extracting from the wound beds. The assessment of molecular composition of extracts was conducted using polyacrylamide gel electrophoresis in the presence of SDS and dithiothreitol as a factor reducing the disulfide bonds. The identification of components released from the wounds was done by assessing the immunoreactivity of these molecules with antibodies for FN by Western blotting method. The electropherograms obtained for material extracts taken from differently treated wounds in several different time points of healing of these damages are presented in Figure 3.

As it can be concluded from Figure 3, all extracts display the similar electrophoretic profiles. Particularly strongly marked components were those which migrated in the front of the separation, as well as bands of three components which are characterized by great electrophoretic mobility and weights equaling about 40, 70, and 100 kDa, respectively, as it can be concluded from the comparison between migration mobility of these components and migrations of reference markers (Figure 3). Despite the above-mentioned molecules, there were also other molecules released from the wound place creating, during electrophoresis, grouped bands in three characteristic doublets (Figure 3). Two of them, created by components with molecular weights of about 110 and 120 kDa as well as of about 190 and 200 kDa, could be clearly seen. Furthermore, the molecules forming the mentioned doublets demonstrated electrophoretic mobilities similar to those characteristic for α chains and β chains of standard of collagen type I. However, the third doublet was formed by band of components with molecular weights of about 220 and 240 kDa (Figure 3) which were hardly seen on

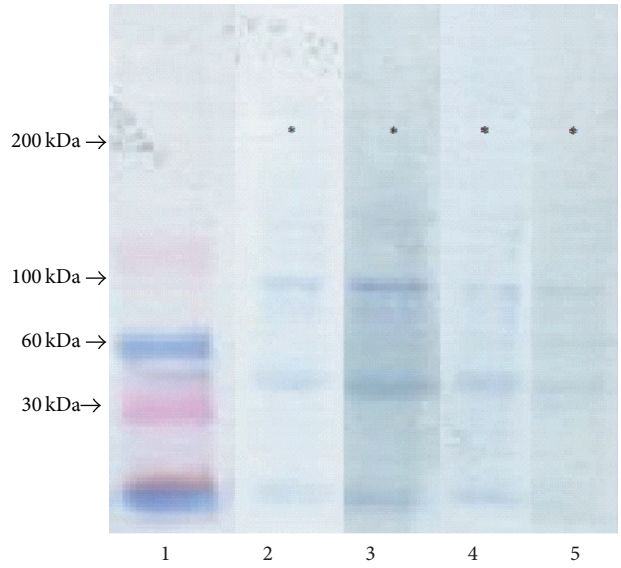

Figure 4: Immunoreactivity assessment of extract components of burn wounds with antibodies against FN. The extract components after electrophoretic separations in gradient polyacrylamide gel 4–15% were subjected to electrotransfer on the membrane Immobilon P and next to the reaction with the antibodies by Western blotting method; lane 1, molecular mass markers, lanes 2, 3, 4, and 5, components released on the 5th day of healing from wounds treated with NaCl, SSD, propolis, and propolis vehicle. The arrows indicate the migration position of molecules of known molecular masses, while the stars indicate the position of fibronectin monomers.

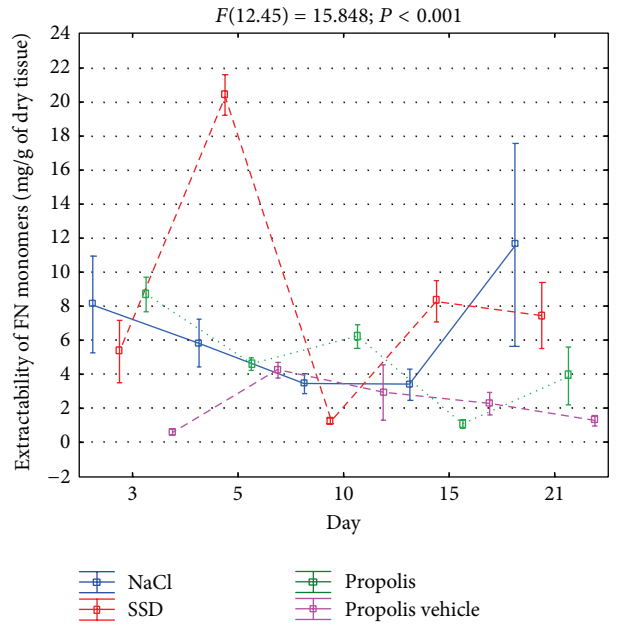

Figure 5: Dynamics of extractability of FN monomers isolated from wounds treated with NaCl, SSD, propolis, and propolis vehicle on the 3rd, 5th, 10th, 15th, and 21st days of healing.

electropherograms. Examination of the influence of extract components with the antibodies against FN by Western immunoblotting method (Figure 4) allowed us to identify the reactivity in the case of elements migrating from the front of resolution components with molecular weights of 40, 70, and 100 kDa and molecules creating the slowest migrating molecular doublet.

The above-mentioned results together with the presented data about molecular weights, characterizing particular components of extracts, suggest that among molecules, released from all tested wounds, there were FN degradation products and native molecules of this glycoprotein which, during electrophoresis in reducing conditions, disintegrated into 2 subunits (monomers). The quantitative assessment of the amount of fibronectin components extracting from differently treated wounds was conducted only on the basis of the densitometric analysis of electropherograms not blots. The reason for doing such an action was significant differences in molecular weights; fibronectin components displayed significant diversity in the velocity of electrotransfer on the membrane which preceded the immunoblotting. Therefore, we suggest that the proportions among fibronectin components on the blots did not reflect the actual quantitative proportions among the molecules in extracts.

The densitometric analysis of electropherograms confirmed that the native FN molecules constitute only a small percentage of components reacting with antibodies for this glycoprotein in material extracts taken from the area of all tested wounds. Moreover, the presence of native FN molecules in all extracts proves that this glycoprotein is produced in each wound regardless of the way of treating it; only newly secreted molecules from cells are available for extraction which, then, quickly polymerized creating insoluble fibers of the extracellular matrix. As is shown in Figure 5, regardless of the way of treating the wounds, during the healing process of these damages, a diversified release of native fibronectin molecules can be observed.

The release of such components, from wounds rinsed with NaCl, was decreasing between 3rd and 15th days of the repair and then increased twofold on the 21st day. The extractability of native fibronectin molecules from wounds treated with SSD displayed a more strongly marked two-phase character (Figure 5). Particularly intensified release of fibronectin was observed on the 5th day of healing, after which the second, less intense, period of eluting the mentioned glycoprotein came, extending from 15th and 21st days of healing (Figure 5). However, the extractability of fibronectin molecules from wounds treated with propolis had a similar character to that observed in the case of the mentioned glycoprotein on the 21st day of healing. Furthermore, it appears that such dynamics of releasing FN molecule from the wound may be caused by a characteristic influence of propolis on the synthesis of this glycoprotein. This suggestion comes from the fact that there are two different courses of curves of FN molecule extractability from wounds treated with propolis and propolis vehicle (Figure 5).

The total extractability of native FN molecules from burn wounds in the monitored period of healing was determined as a sum of the mentioned time points. This value reflects the accumulation of fibronectin synthesized in the wound area

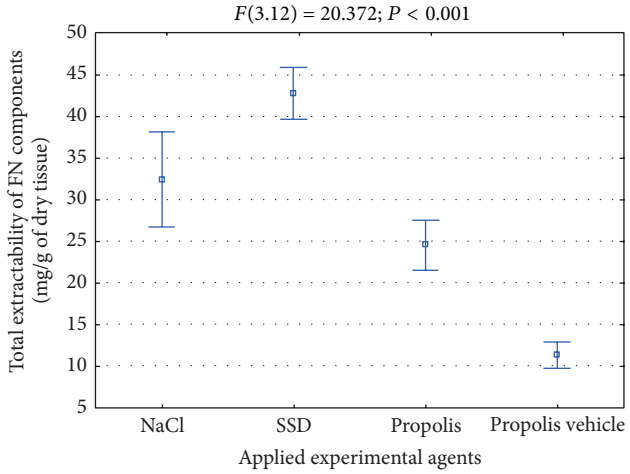

FIGURE 6: Total extractability of FN monomers isolated from wounds treated with NaCl, SSD, propolis, and propolis vehicle during healing.

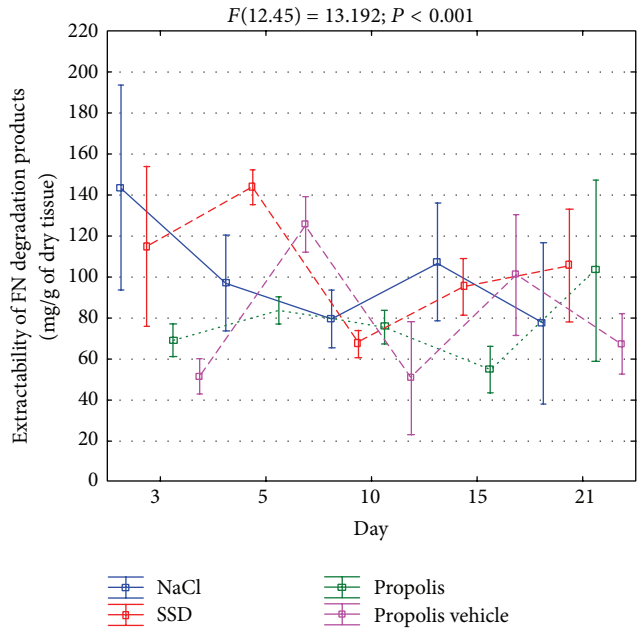

FIGURE 7: Dynamics of extractability of FN macromolecular degradation products isolated from wounds treated with NaCl, SSD, propolis, and propolis vehicle on the 3rd, 5th, 10th, 15th, and 21st days of healing.

in the monitored time of healing of tested wounds. As it can be concluded from the data presented in Figure 6, from the wounds treated with propolis, a significantly smaller amount of fibronectin molecules was released in relation to wounds treated with both NaCl and SSD.

The enzymatic FN degradation in the extracellular space leads to releasing a few, well characterized types of degradation products, among which those whose molecular weight equals 40, 70, and 100 kDa demonstrate a biologic activity of an antagonistic character towards native molecules of this glycoprotein. The extractability of assessed macromolecular products of FN degradation, from differently treated burn wounds in the initial period of healing, is presented in Figure 7.

As can be concluded from Figure 7, irrespective of the way of treating the wounds, the release of FN degradation products during the process of healing was diversified, which suggests the intensity changes of catabolic processes of the glycoprotein in question. The increased release of FN macromolecular degradation products from wounds treated with NaCl and silver sulfadiazine had a clear, two-phase character (Figure 7). First particularly high increase of extractability of the components in question appeared rather early in the course of the repair process which fell on the 3rd day of healing in damages rinsed with NaCl and on the 5th day of the repair in wounds treated with SSD (Figure 7). The second, smaller increase of released products of fibronectin degradation was observed on the 15th day of the repair of damages treated with NaCl and on the 21st day of healing of wounds treated with SSD (Figure 7). However, the curve of extractability of FN degradation products of wounds treated with propolis had a different character. Particularly characteristic was a slight release of these components in the first phase of healing (days 3 and 5), two times lower than that observed in the case of wounds treated with the other agents (Figure 7). A quite significant increase of FN macromolecular degradation products from wounds treated with propolis was seen not until the 21st day of the repair process reaching the values similar to those observed in wounds treated with silver sulfadiazine at the same time of the healing period (Figure 7). It should be also emphasized that the above-presented dynamics of the release of FN degradation products from wounds treated with propolis was most probably a characteristic effect of the apitherapeutic agentactivity on the catabolism of the mentioned glycoprotein. The presented suggestion results from a different course of extractability curves of FN degradation products from wounds treated with propolis and its vehicle (Figure 7).

The total amount of FN macromolecular degradation products, released from wounds in the course of healing, is very important for the correct course of the repair process. The mentioned amount proves the intensity of FN degradation in the course of the repair process. On the other hand, the presence of the FN degradation products in the healing tissues, taking into consideration the biological action of the latter ones, may upset the course of the repair process. As the presented data in Figure 8 suggest, in the course of healing of wounds treated with propolis, a significantly smaller amount of FN macromolecular degradation products was released than in the case of damages treated with SSD or NaCl.

4. Discussion

Wound healing is a complex process which comprises 4 partially overlapping phases: hemostasis, inflammatory phase, proliferation phase, and remodeling. All mentioned phases are indispensable for the repair process; the second and third phase seem to be particularly important for the modification

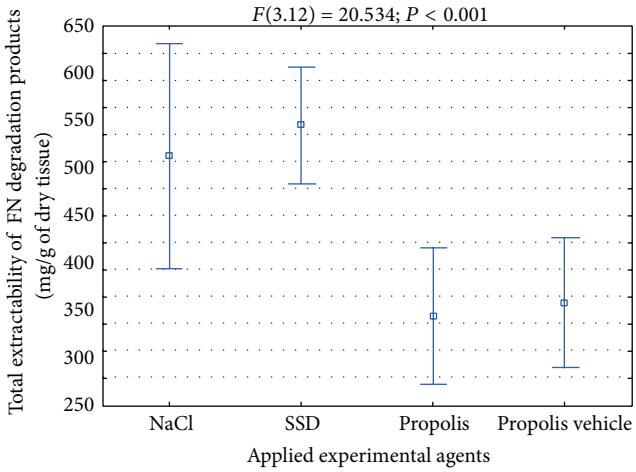

Figure 8: Total extractability of FN macromolecular degradation products isolated from wounds treated with NaCl, SSD, propolis, and propolis vehicle in the process of healing.

of the process in question [36]. The inflammatory condition, which is already induced during hemostasis, develops during the first day after the injury and remains especially intense until the third day in the case of the correctly proceeding repair process [37, 38].

Around the third day after the injury, the transition of the inflammatory phase to the process of rebuilding the damaged tissue takes place [39]. The transition is caused by the influence of numerous growth factors, secreted by macrophages, which activate the adjacent cells stimulating their proliferation and directed migration. Furthermore, the growth factors, such as PDGF or TGFβ, stimulate the fibroblasts entering the wound place in order to produce and secrete the extracellular matrix components including fibronectin and different types of collagen. Restoring the extracellular matrix of a specified composition in the wound area is fundamental for the migration of cells as well as for their adhesion and differentiation [38, 39]. All mentioned phenomena, taking place during the proliferation phase of the healing process, lead to formation of a new tissue in the wound area—the granulation tissue. According to the current results, it has been assumed that, in the correctly proceeding healing process, a significantly intense proliferation of cells in the wound place takes place between the third, fourth, and tenth days after the injury, while the fully formed granulation tissue appears in about 15th–20th days of the repair [37]. Thus, applied in the present study time of monitoring the burn wounds, lasting from 3rd to 21st day after the injury, should include the end of the inflammatory phase and the whole proliferation phase of the healing process until the granulation tissue appears. The course of the mentioned phases determines the effectiveness of the repair process. The inflammatory condition is particularly important for the course of healing. On the other hand, the chronic character of the inflammatory condition in the injury place leads to a significant inhibition of the healing process or even to stopping it, which is clinically manifested by incorrectly healing wounds or nonhealing wounds [38–40].

In order to avoid the mentioned complications in the healing process, the damages are subjected to various medical procedures. The classic way of treating such wounds is based on applying silver sulfadiazine [41–43]. However, this compound also demonstrates a series of undesirable local actions such as cytotoxicity against fibroblasts and keratinocytes [44]. The systemic disorders following the silver sulfadiazine application have also been described [45–47]. Beside the application of SSD in the course of injury management, other novel, promising methods, such as electrotherapy, laser irradiation, and ultrasound therapy, of possible application in wound treatment exist. However, SSD application and implementation of novel techniques are not free from certain disadvantages [18–20]. This is the reason why the alternative agents for burn wound treatment are being sought for. One of the examined agents is propolis, a complex, resin-like substance, accumulated and processed by bees. Propolis demonstrates a series of properties which may be favorable for the course of wound healing. Among these properties there are the antibacterial, antivirus, and antifungal activity [48–51]. Moreover, propolis significantly decreases the activity of free radicals in the healing wound beds which favors the repair process itself. This phenomenon was confirmed in our previous studies [51–53]. It was proven that in the tissue samples, taken from the burn wound areas, treated with propolis and silver sulfadiazine, respectively, the expression of free radicals was significantly lower in the case of the first medical substance. Propolis also had a positive effect on collagen metabolism in the area of burn wounds during the healing process, increasing the tissue content of collagen of both type I and type III, which is known to lead to restoring the extracellular matrix and stimulating in this way the granulation tissue [50]. The results obtained in the present study for the first time reveal that the mentioned bee product also modifies the metabolism of fibronectin, which creates the fibrous weave of the extracellular matrix. The influence could be seen in significant differences concerning the extractability of components reacting with the antibodies for fibronectin from the wounds treated with propolis to damages treated with silver sulfadiazine or the wounds treated with NaCl which represent the control and the physiological course of the healing process. The differences in releasing the fibronectin components from the wound area were particularly visible in the initial period of healing (third and fifth days of the repair, resp., related to the control wounds and to those treated with silver sulfadiazine) and also on the fifteenth day after the injury. In the mentioned time points, the release of fibronectin from wounds treated with propolis was significantly lower than in damages treated with the other agents. An important reason of the decrease of extractability of fibronectin components from wounds treated with propolis to the other damages was a significant (about one a half time) reduction in releasing the macromolecule degradation products of fibronectin. Particularly clear differences in releasing such components among wounds treated with propolis and control wounds fell on the third and fifteenth days of healing, while among those treated with propolis and SSD on fifth and fifteenth days of the repair. The observed decrease of extractability of fibronectin

degradation products for the healing wounds, treated with propolis, suggests that this bee product may inhibit the disintegration of the fibronectin in the course of the repair process. Fibronectin is catabolized by metalloproteinases (MMPs), particularly by MMP-3 (stromelysin) [38, 50]. These enzymes with zinc ions in the active centre, during the repair of tissue damages, are secreted at first by macrophages and later, under the influence of FGF and PDGF, by cells migrating into the wounds, particularly by fibroblasts which are responsible for the reconstruction of the extracellular matrix in the wound area [37, 40]. Thus, it can be concluded that a particularly high activity of MMPs in the wound area should fall on the third-fifth and fifteenth to twenty-first day of healing. The mentioned dynamics of MMPs activity corresponds with the location of maxima of the extractability of fibronectin macromolecular degradation products from control wounds and those treated with silver sulfadiazine. By contrast, the lack of increased release of the degradation products of the glycoprotein in question, which falls on the third-fifth day of healing in wounds treated with propolis ointment, suggests that this therapeutic agent may regulate the expression and/or activity of MMPs produced by macrophages. It seems that the mechanism of the observed propolis effect may lie in stimulating the production of TGFβ [36, 37], which is a well-known inhibitory factor of the MMPs expression, as well as in stimulating the production of tissue inhibitors of these enzymes [36, 37, 40].

Our results point out that during healing of propolis treated wounds, especially when related to those treated with SSD, the fibronectin content in wound bed is being decreased. This phenomenon was reflected in the differences of the amount of fibronectin monomers extracted from both types of wounds. These differences were particularly strongly manifested on the fifth, fifteenth, and twenty-first days of the repair. However, in the case of the control wounds and those treated with propolis, the differences in the accumulation of synthesized fibronectin in the wound bed concerned the twenty-first day of healing. Taking into account that propolis significantly decreased the fibronectin degradation during the healing process, the observed changes suggest that this apitherapeutic agent has an inhibiting effect also on the fibronectin biosynthesis. The source of this glycoprotein, synthesized in the damage area mainly during the proliferation phase, is the endothelial cells, mainly fibroblasts [38, 54, 55]. It has been proven that the latter cells, originating from the keloid samples, inhibit the fibronectin biosynthesis in the presence of quercetin, being one of the propolis components [56]. It was also shown that another propolis component, that is, resveratrol, possesses the ability to inhibit the TGFβ-dependent production of fibronectin in C2C12 myoblasts [57]. The mechanism responsible for the mentioned effect is connected with the activating action of resveratrol on the NAD^+-dependent SIRT1 histone-protein deacetylase [57]. It is not known, however, if a similar phenomenon is fundamental for the observed propolis effect on fibronectin metabolism in cells (most probably fibroblasts) which are present in the area of healing skin wounds. In fibroblasts, in the conditions of tissue damage repair, the regulation of fibronectin expression takes place not only by the path induced by TGFβ but also by that stimulated by a group of Wnt ligands which involves β-catenin as an intracellular signal transmitter [38]. The presented results pointed out the propolis influence on the fibronectin metabolism in the course of wound healing process poses a question about possible consequences of such an action on the repair itself. In the process of healing, fibronectin fulfills many key functions regulating the cell behavior and being responsible for the formation of the fibrous weave of the extracellular matrix. The three-dimensional fibronectin matrix is the necessary environment for cell migration, proliferation, and differentiation as well as adhesion and apoptosis [54]. The influence of cell surface integrin receptors with fibronectin molecules launching a complex intracellular signalization and determining the expression of proper genes [54] is fundamental for the mentioned activities of cells. The extremely important role of fibronectin in the repair process is visible in the conditions of the glycoprotein intensified degradation, leading to its lack in the cell microenvironment, followed by the disorder in forming the granulation tissue, which, in turn, causes the complications in the healing process or even its total inhibition [54]. It was found that there are close, however not well-known, relations between the fibronectin content in the cell environment and their activity. Hamill at al. [58] found that the decreased fibronectin content in the extracellular matrix stimulates the mobility of skin cells. Similarly, Inoue at al. [59] showed that migrating epithelial cells are characterized by a lower fibronectin expression than those in the resting state. Therefore, the lower content of the glycoprotein in the propolis treated wounds compared to those treated with SSD suggests that the application of the apitherapeutic agent for burn wound treatment may be more beneficial as far as the pace of forming the granulation tissue is concerned. Moreover, this phenomenon may be also supported by the inhibitory effect of propolis on the fibronectin degradation. In fact, it is known that some macromolecular products of fibronectin degradation not only interfere in the cell migration or adhesion to native fibronectin molecules but also have chemotactic properties for inflammatory cells, making the inflammatory phase longer [54, 60, 61].

Despite its effect on the cells, fibronectin also influences the composition and structure of the extracellular matrix. It has been proven that the accumulation of the mentioned glycoprotein in the extracellular space regulates the secretion of other components of this matrix such as collagen type I and type III, tenascin, laminin, and fibrillin [54]. The last mentioned glycoprotein forms fibrillar components of elastin fibers. Furthermore, fibronectin is one of the main factors determining the collagen fibrogenesis [54]. The excessive production and secretion of collagen in fibromatous tissues are preceded by the increased fibronectin accumulation in the extracellular matrix [38, 54]. Thus, the reduced *de novo* fibronectin synthesis in propolis treated burn wounds related to control ones (NaCl treated), which was observed on the twenty-first day of the repair process, may be the factor decreasing the risk of keloid development, being a frequent reason for complications in the burn wound healing [38]. The obtained results indicate that the propolis influence on fibronectin metabolism may be one of the mechanisms

because of which this apitherapeutic agent exerts beneficial effect on wound healing process.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Evaluation of Bioelectrical Activity of Pelvic Floor Muscles and Synergistic Muscles Depending on Orientation of Pelvis in Menopausal Women with Symptoms of Stress Urinary Incontinence: A Preliminary Observational Study

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Objectives. Evaluation of resting and functional bioelectrical activity of the pelvic floor muscles (PFM) and the synergistic muscles, depending on the orientation of the pelvis, in anterior (P1) and posterior (P2) pelvic tilt. *Design*. Preliminary, prospective observational study. *Setting*. Department and Clinic of Urology, University Hospital in Wroclaw, Poland. *Participants*. Thirty-two menopausal and postmenopausal women with stress urinary incontinence were recruited. Based on inclusion and exclusion criteria, sixteen women aged 55 to 70 years were enrolled in the study. *Primary Outcome Measures*. Evaluation of resting and functional bioelectrical activity of the pelvic floor muscles by electromyography (sEMG) and vaginal probe. *Secondary Outcome Measures*. Evaluation of activity of the synergistic muscles by sEMG and surface electrodes. *Results*. No significant differences between orientations P1 and P2 were found in functional and resting sEMG activity of the PFM. During resting and functional PFM activity, higher electrical activity in P2 than in P1 has been recorded in some of the synergistic muscles. *Conclusions*. This preliminary study does not provide initial evidence that pelvic tilt influences PFM activation. Although different activity of synergistic muscles occurs in various orientations of the pelvic tilt, it does not have to affect the sEMG activity of the PFM.

1. Background

Normal ageing of the reproductive system in women can be divided into periods as follows: reproductive (premenopausal), menopausal transition (perimenopausal), and postmenopausal. In the perimenopausal period characteristic symptoms of menopause begin to appear [1–4]. Many studies [1–6] of prevalence of menopause have reported symptoms of menstruation dysfunctions, symptoms of menopausal syndrome (hot flushes, profuse sweating, sleep disorders, irritability, depression, dizziness, headache, articular and muscular pain, and general weakness), and libido dysfunctions.

Menopause may also be an etiological factor in the development or progress of urinary incontinence (UI) [1, 7–10]. In the postmenopausal period, stress urinary incontinence (SUI) appears often. It is probably connected with hormonal disturbances leading to muscle and fascial flaccidity and their decreased tone [6, 7]. Prevalence of UI in women ranges from 5% to 62% [11–18] and the incidence of UI changes with age [9]. Studies of Minassian et al. [9] show that the median of prevalence of all types of UI in women clearly increases in the 35–44 age group and is reaching about 30%. Maximum occurrence of the UI is in the 45–54 age group and is about 70% and in the age groups: 35–44 (nearly 60%), 55–64 (more than 60%) and 65–74 (about 50%). According to other studies [7–11], the estimated percentage of women suffering from UI is up to 73% during the peri- and postmenopausal periods.

To reduce the occurrence of the UI and in particular the incidence of SUI, some form of effective therapy should be applied. It is well known that a form of conservative treatment is recommended as the first-line procedure but such still needs to be improved [19–26]. The physiotherapeutic treatment of SUI is mainly focused on achieving increased resting and functional activity of the pelvic floor muscles (PFM) [21, 27–33]. Activation of isolated contractions of the PFM as well as the activation of synergistic muscles should increase the effectiveness of the treatment. In the literature [21, 32–36], muscles considered as important in the treatment of SUI are gluteus maximus; medial femoral; rectus abdominis; and oblique, external, and internal abdominal muscles. These trunk and hip muscles that attach to the pelvis or sacrum have an influence on the motion of the pelvis as a whole. Pelvic orientation depends on the contraction or extension of these muscles. Increased anterior pelvic tilt can result from weak hamstrings or abdominals, hypertonicity of the lumbar extensors or hip flexors, or contractures of the rectus femoris. While the posterior pelvic tilt could be a result of shortened hamstrings, hypertonic abdominals, weakened lumbar flexors or hip extensors [37–42]. Additionally, these muscles are functionally and morphologically connected with the pelvis and indirectly with the PFM [36, 38, 40, 43]. Taking into account these interdependencies, the authors decided to assess the PFM and synergistic muscle function in various positions of the pelvis. To evaluate bioelectrical activity of these muscles surface electromyography (sEMG) was used [44].

2. Objective and Hypothesis

The primary aim is the evaluation of resting and functional bioelectrical activity of the PFM, depending on the orientation of the pelvis, in forward and backward inclination. We assume that higher bioelectrical activity of the PFM will be observed in the posterior pelvic tilt than in the anterior pelvic tilt.

The secondary aim is the evaluation of the activity of synergistic muscles at different orientations of the pelvis. Higher muscle activity is expected in the posterior pelvic tilt. The higher activity of these synergistic muscles can lead to an increase of PFM activity.

3. Material and Method

3.1. Design. Preliminary, prospective, cross-sectional observational study evaluating resting and functional PFM activity depending on pelvic orientation in menopausal and postmenopausal women with SUI.

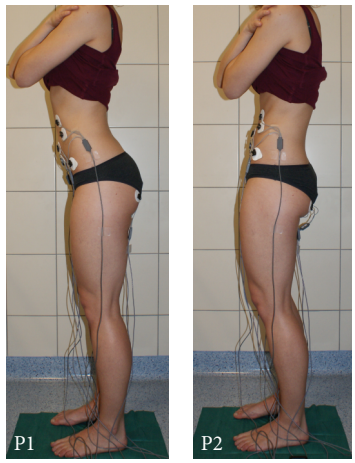
3.2. Participants. Thirty-two menopausal and postmenopausal women with SUI were recruited from volunteers and patients at the Department and Clinic of Urology, University Hospital in Wroclaw, Poland. The study was approved by the Bioethics Committee of the Wroclaw Medical University (KB-611/2012) and was registered at the Australian New Zealand Clinical Trials Registry (ACTRN12613001144707). The project was funded by the National Science Centre allocated on the basis of the decision number DEC-2011/03/N/NZ7/00505.

Included were women of ages 55 to 70 years with good general well-being on the day of the examination and who were able to contract the PFM correctly. All patients reported symptoms of menopause (hot flushes, sweating, heart discomfort, sleep problems, depressive mood, irritability, anxiety, physical and mental exhaustion, sexual problems, bladder problems, dryness of vagina, and joint and muscular discomfort), which were verified by the Menopause Rating Scale (MRS) [45] and symptoms of SUI (especially leaks when coughing or sneezing, leaks during physical activity/exercising, or leaks when lifting heavy objects) which were evaluated by the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF) [46]. All included women had a history of stress urinary incontinence and gave written consent to participation in the study.

Subjects were excluded if they could not comprehend Polish instructions; had a previous history of gynaecological and abdominal surgery; had a malaise on the examination day (the participants of the study were not able to perform the test procedures); had a neurological condition; had contraindications to measurements such as infection, menstruation, and allergy to nickel; had a previous history of injuries within the pelvis, hip joint, or spine; or had a vaginal prolapse.

3.3. Experimental Protocol. Functional and resting electrical activity was recorded from the PFM (with an electromyographic instrument and vaginal probe) in a standing position when the patients had the pelvis rotated forward (Position 1—P1) and backward (Position 2—P2) around the transverse axis (Figure 1) (primary outcome).

In P1 and P2, the participants made five, 5-second maximal isolated contractions of the PFM (functional sEMG activity) with a 5-second rest (resting sEMG activity) between each contraction. A random integer generator (http://www.random.org/nform.html) was used to randomly select the order of the positions in which the PFM were tested. Subjects were given 60 seconds of rest between trials. Before the measurements, the physiotherapist taught the patients how to accomplish a correct PFM contraction. Some preliminary contractions were effected to check that the



(a) (b)

Figure 1: Two positions during the examination (P1—active, the maximum orientation of the pelvis in anteversion performed by patients; P2—active, the maximum orientation of the pelvis in retroversion performed by patients).

Figure 2: The probe used in the study.

probe was in the correct placement and that the contractions were being performed properly.

Electrical activity was bilaterally recorded (with an electromyographic instrument and surface electrodes) from the synergistic muscles of the PFM: lower rectus abdominis (RA), the gluteus maximus (GM), the adductor magnus (AM), and only from the left side of the external oblique (EO) (secondary outcome).

3.4. Electromyography. Electromyographic measurements were conducted using the Myosystem 1400 (Noraxon, Scottsdale, Arizona, USA). Technical specifications include the following: analog output gain—x1000 standard (5000 selected units); common mode rejection ratio (CMRR)—min 100 dB at 50–60 Hz; input impedance > 100 MΩ on sEMG channels (isolated to >3000 Volts); outputs—analog ±5 Volts all sEMG channels, digital 12-bit resolution per channel from USB port; inputs—8 sEMG channels at ±10 mV max, 8 sensor channels at ±5 Volts max, and power 100–240 VAC at 50/60 Hz (0.9 A max); sEMG amplifier performance—1 uV sensitivity and <1 uV RMS baseline noise; data acquisition—12-bit resolution 8 channels, and USB update to PC every millisecond; high pass cutoff—10 Hz first order on sEMG channels; low pass cutoff-selectable 500 or 1000 Hz on sEMG channels; and physical—width: 28 × 19.7 cm, height: 10.2 cm, and weight: 1400 g. MyoSystem 1400L components are as follows: MyoSystem 1400L instrument, power cord, EMG active cable, and preamplified electrode lead (one per channel)—one channel: 3 snaps, seven channels: 2 snaps; USB cable.

3.5. sEMG Data Analysis. sEMG recordings were analysed using Noraxon MyoResearch XP Master Edition Version 1.07 software (Noraxon, Scottsdale, Arizona, USA). Electromyographic data were bandpass-filtered between 50 and 1000 Hz (FIR filter—finite impulse response filter), rectified and smoothed using 50 ms RMS (root mean square), and were expressed in microvolts (μV).

3.6. Probe. To record sEMG signals from the PFM, a Lifecare Vaginal Probe PR-02 (Everyway Medical Instruments Co., Ltd., Taiwan) was used (Figure 2). The probe has a total length of 7.6 cm and a maximal circumference of 2.8 cm. This pear-shaped probe has two longitudinal recording plates (stainless steel, and containing nickel) embedded on the right and left sides and has been reported to record PFM activity with minimal crosstalk during tasks [47–49]. The length of the recording plate is 4.5 cm and the active surface area is 7.68 cm^2/band. The distance between the two recording plates is 2 cm. The distance of the recording plate to the top of the probe is 0.7 cm and 1.2 cm to the base. This probe is inserted up to the handle at the introitus of the vagina.

The probe was for a single user only. It was cleaned with a pad soaked in surgical spirit, rinsed in clean running water, and dried with a paper towel, before and after each measurement.

3.7. Electrodes. The single electrodes are disposable, self-adhesive Ag/AgCl snap electrodes for surface EMG applications only. Diameter of the circular adhesive area is 3.8 cm; diameter of the circular conductive area is 1 cm. Electrodes are hypoallergenic gel and adhesive. The interelectrode spacing between the recording electrodes was 2 cm [50, 51]. The skin was prepared by shaving excess hair and wiping the skin with alcohol (Skinsept pur, Ecolab) to reduce impedance [50–55].

The electrodes were attached parallel to the muscle fibre orientation over the following muscles: RA—the electrodes were below the umbilicus, on the lower rectus abdominis [51, 52]; GM—the electrodes were placed at 50% on a line between the sacral vertebrae and the greater trochanter [56]; AM—the electrodes were positioned midway between the posterior edge of the gracilis and the longitudinal fascial plane, this being between the adductor magnus and the medial hamstrings [57, 58]; EO—the electrodes were just

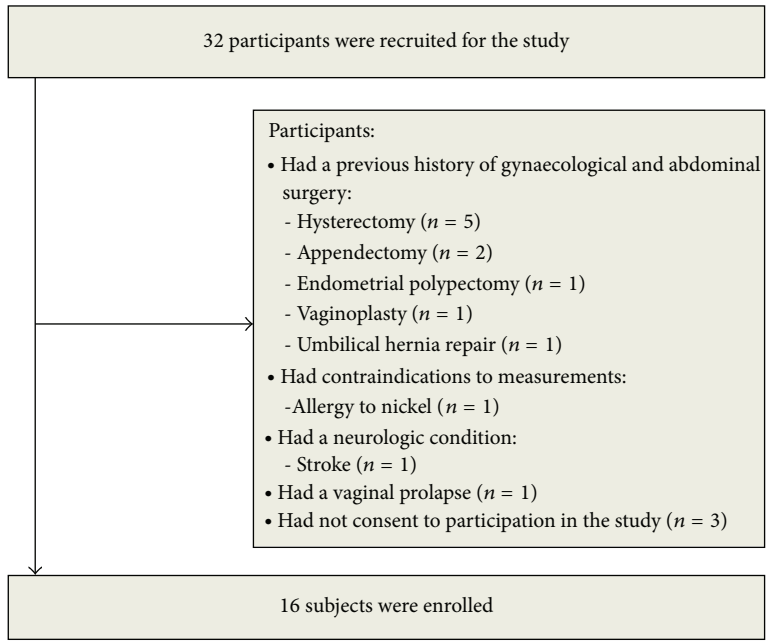


Figure 3: Flow diagram includes detailed information on the excluded participants.

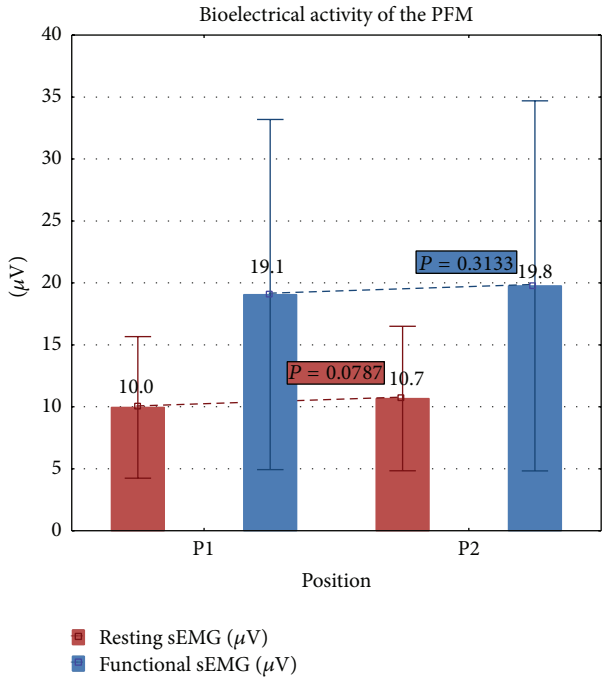


Figure 4: Comparison of resting and functional bioelectrical activity of the PFM in P1 and P2.

inferior to the 8th rib, in the line of the middle of clavicle and on a 45° angle [48]. The monopolar, reference electrode was placed on the anterior superior iliac spine.

3.8. Statistical Analysis. Statistical analyses were performed using Statistica 10 (Stat Soft Inc., USA). For all variables, were calculated, the mean, minimum and maximum values and standard deviation. Differences between the two positions in bioelectrical activity of all muscles were compared using the Wilcoxon signed-rank test. Alpha level was set at 0.05.

4. Results

Sixteen women (age: $\overline{x}$ = 63.1 year, min = 55 year, max = 70 year, and SD = 4.07 year) were enrolled in the study. The group characteristics are shown in Table 1. Sixteen were excluded: ten because they had a previous history of gynaecological and abdominal surgery; five because they had not consented to participation in the study; and three because they had a stroke (n = 1), were allergic to nickel (n = 1), and had vaginal prolapse (n = 1) (Figure 3).

4.1. Primary Outcomes. No significant differences between P1 and P2 were found in functional and resting sEMG activity of the PFM (Figure 4). In P1, the average resting activity was $\overline{x}$ = 10.0 μV (min–max: 4.0–22.1 μV; SD = 5.71 μV) and in P2 it was $\overline{x}$ = 10.7 μV (min–max: 3.9–23.8 μV; SD = 5.83 μV). The average functional activity was $\overline{x}$ = 19.1 μV (min–max: 5.1–60.9 μV; SD = 14.13 μV) in P1 and $\overline{x}$ = 19.8 μV (min–max: 5.3–63.7 μV; SD = 14.94 μV) in P2.

4.2. Secondary Outcomes. The secondary outcomes are listed in Tables 2 and 3. Significant differences were found between some synergistic muscle activity in orientations P1 and P2.

During resting PFM activity, higher electrical activity in P2 than in P1 has been recorded in the synergistic muscles: GM (left side, P = 0.0097) and RA (left side, P = 0.0146 and right side, P = 0.0386) (Table 2).

Table 1: Characteristics of group.

	Patients (women, $n = 16$)		
	Range	Mean	SD
Age (year)	55–70	63.1	4.07
Weight (kg)	52–101	75.2	11.35
Height (m)	1.50–1.70	1.61	0.05
ICIQ-SF	1–19	8.3	5.42
MRS	6–22	12.7	4.95

During functional PFM activity, higher electrical activity in P2 than in P1 has been recorded in the synergistic muscles: GM (left side, $P = 0.0061$; right side, $P = 0.0494$) and RA (left side, $P = 0.0113$; right side, $P = 0.0131$) (Table 3). Amongst other muscles, no statistically significant differences were registered.

5. Discussion

In the present preliminary, prospective study, we evaluated sEMG activity of the PFM in two positions of the pelvis. The results of the present study showed that bioelectrical activity of the PFM does not depend on the orientation of the pelvis. Work by Capson et al. [48] demonstrated the effect of changing the standing lumbopelvic posture on PFM activation amplitude. Amongst other things, they assessed bioelectrical activity of the PFM with sEMG and vaginal probe in three different standing postures (normal lumbopelvic posture, hyperlordosis, and hypolordosis). They did not measure the pelvic tilt, but hypolordotic posture is related to the posterior pelvic tilt. They observed higher resting PFM activity in the hypolordotic posture as compared to the normal and hyperlordotic postures. Chen et al. [59] have reached different conclusions. They examined 39 women with SUI (aged from 38 to 72 years) in order to determine changes in PFM activity triggered by various feet positions. Positioning of the feet was achieved by using a special platform changing the inclination angle of the base on which the examined person was standing. The investigators performed PFM electromyographic measurements by means of an endovaginal probe in three different positions of the feet: plantar flexion, dorsal flexion, and horizontal position. The achieved results showed that during the dorsal flexion of the feet, the anterior pelvic tilt increased and higher PFM activity was observed. Similar studies were carried out by Cerruto et al. [60], who examined 15 women suffering from SUI. Bioelectrical activity of PFM was measured in the standing position with horizontal positioning of the feet and their plantar (5°, 10°, and 15°) and dorsal (5°, 10°, and 15°) flexion. sEMG activity of PFM was significantly higher in the dorsal flexion of the feet, regardless of the flexion angle, in comparison to PFM activity measured for feet in a horizontal position. In the next study, Chen et al. [61] examined 31 healthy women between the ages of 30–56 years. The measurement of PFM tone was performed by means of EMG biofeedback and endovaginal probe. In this study, the PFM tone was measured in a total of 9 positions of feet, active and passive. The highest PFM tone values were achieved in the plantar flexion of the feet with raised upper limbs and were the highest of all values achieved for other positions. In this group, muscle tone, including PFM, could be related to increased intra-abdominal pressure. Bioelectrical PFM stimulation in active positions was higher than in passive positions, probably due to activation of both abdominal wall muscles and the PFM. Such a muscle synergy may probably contribute to more effective PFM training.

We determine, how the values of bioelectrical activity of synergistic muscles were changing during two different orientations of the pelvis. We observed a higher activity of the muscles (GM left side and RA both sides) when the position of the pelvis was backwards. Some studies showed [27, 28, 32–38, 59–61] that the increased activity of these muscles has contributed to increased PFM activity. According to muscle synergy rules, the hip joint muscles located in the vicinity of the PFM have an influence on their activity. A concurrent measurement of the activity of the PFM and the above-mentioned muscles at rest and during contraction has demonstrated the correlation between the examined groups of muscles [27, 28, 32–38]. Soljanik et al. [43] evaluated relations between the levator ani, GM muscle, and the fossa ischioanalis in healthy women. The authors demonstrated cooperation of these structures and revealed synchronous activation of the levator ani and GM in 97% of subjects. The study of Sapsford et al. [62] confirms the increased activity of the PFM and two abdominal muscles, obliquus internus abdominis and obliquus externus abdominis, during various sitting postures. Their highest activity was observed in the very tall unsupported postures. They conclude that unsupported sitting postures require greater pelvic floor muscle activity than the supported ones. Smith et al. [63] conducted a comparison of activity of the PFM and the abdominal muscles between continent and incontinent women in response to a postural perturbation. Women with incontinence demonstrated increased PFM and abdominal obliquus externus sEMG activity compared to continent women. By the authors, in women with more severe symptoms, activity of the abdominal muscles was higher and PFM activity could be insufficient to control continence. Therefore, it is important to assess not only the PFM but also muscles influencing the pelvic floor indirectly.

These studies [32–38, 61–65] have contributed to the verification of the opinion that evaluation and training of synergistic muscles are necessary during the conservative treatment of SUI.

Attention should be given to the practical implications of this study. During the treatment of patients with SUI, exercises of synergistic muscles should also be conducted. The position of the pelvis setting backwards increases the activity of these muscles. The authors believe that the physiotherapy exercise procedure should take into account the position in which the pelvis is set, being a posterior pelvic tilt.

5.1. Limitation of the Study. The small number of participants and lack of a control group were limitations of the study. This study will be continued among menopausal women with SUI and without SUI (a control group).

Table 2: sEMG activity during resting PFM activity (μV) in P1 and P2.

Muscles	sEMG activity during resting PFM activity (μV)								P value
	P1				P2				
	Mean	Min	Max	SD	Mean	Min	Max	SD	
Left side									
AM	5.1	2.8	9.9	2.17	6.0	3.4	11.2	2.19	0.1627
GM	5.5	2.8	13.0	3.09	12.8	3.1	68.9	17.75	**0.0097**
RA	4.3	3.3	6.3	0.73	5.1	3.3	9.0	1.53	**0.0146**
EO	13.6	2.5	60.5	13.71	14.1	3.8	59.4	13.26	0.5349
Right side									
AM	5.0	2.7	9.8	2.14	5.5	3.5	11.2	2.24	0.4380
GM	7.7	2.84	22.0	5.86	11.4	2.93	49.0	14.87	0.6417
RA	5.9	3.1	31.0	6.75	6.4	2.7	31.8	6.87	**0.0386**

Table 3: sEMG activity during functional PFM activity (μV) in P1 and P2.

Muscles	sEMG activity during functional PFM activity (μV)								P value
	P1				P2				
	Mean	Min	Max	SD	Mean	Min	Max	SD	
Left side									
AM	7.1	2.9	21.3	5.12	8.2	3.5	18.1	4.79	0.1626
GM	10.9	3.0	69.5	16.20	23.3	3.3	93.8	29.64	**0.0061**
RA	4.6	3.3	6.3	0.85	5.6	3.2	12.4	2.23	**0.0113**
EO	17.4	2.5	70.3	17.65	17.5	3.8	62.8	14.68	0.7564
Right side									
AM	7.3	2.7	17.9	4.68	7.0	3.5	16.8	4.11	0.8361
GM	13.1	3.29	61.6	15.13	19.9	3.36	74.8	24.42	**0.0494**
RA	6.1	3.1	31.1	6.70	6.8	2.7	32.8	7.07	**0.0131**

6. Conclusion

This preliminary study does not provide initial evidence that pelvic tilt influences PFM activation. Although different activity of synergistic muscles occurs in various orientations of the pelvic tilt, it does not have to affect the sEMG activity of the PFM. Further studies with a control group will contribute to a more accurate assessment of dependence between pelvic tilt and PFM activity.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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Clinical Study

The Application of Negative Pressure Wound Therapy in the Treatment of Chronic Venous Leg Ulceration: Authors Experience

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The aim of the study was to use negative pressure wound therapy (NPWT) in patients with chronic venous leg ulceration. The authors present their experience in treatment of 15 patients whose average ulceration surface area was 62.6 cm^2. In 10 patients, the ulcers healed within 6 weeks and in the remaining patients within 20 weeks. Based on the results obtained, the authors imply that NPWT is an effective method in the treatment of chronic venous leg.

1. Introduction

Negative pressure wound therapy (NPWT), also known as vacuum assisted closure (VAC), subatmospheric pressure dressing (SPD), vacuum sealing technique (VST), foam suction dressing, sealed surface wound suction (SSS), vacuum pack therapy, and sealing aspirative therapy, is used in the treatment of acute and chronic wounds. The treatment requires a vacuum source to create a continuous or intermittent form of negative pressure inside the wound. Doing so removes fluid and exudates infectious materials to aid in wound healing and closure [1–3].

There are many documented cases of NPWT in wound healing throughout history. In fact, it is one of the oldest methods used in wound treatment and can be traced back to 400 BC when the Greeks practiced cupping using heated copper bowls. Hippocrates and his followers used "collection vessels" whose openings were heated and applied directly over wounds to draw out and collect blood and fluids. Cupping as a vacuum therapy has been used for centuries; however, the technique and design changed as cupping spread west. By the end of the 19th century, Professor August Bier defined the concept of cupping by a method of igniting alcohol within a glass and placing a rubber tube on the skin prior to application of the heated cupping glass. In 1908, Bier's hyperemic treatment method was described and since then vacuum therapy has been used for the treatment of all types of open wounds (traumatic, chronic, and postoperative) as well as for the treatment of infections [4].

In 1907, Dr. E. Klapp first used a suction pump for removal of infectious materials in tuberculosis lesions in patient with advanced tuberculosis. In 1952, the use of NPWT with natural sponge, rubber sponge, foam rubber, cellulose sponge, gauze, cotton, and other filler materials was patented in Germany.

The descriptions of more contemporary uses of this method come from the former Soviet Union. In the 1970s, NPWT was used for postsurgical tissue repair and for removal of wound fluids. In 1986, the so-called *Kremlin Papers* started to be published in Soviet medical journals. They describe the use of NPWT for removal of wound exudates from postsurgical wounds. Gauze was applied as the dressing medium, a silicone surgical drain was placed under low continuous wall suction and occlusion with secondary dressings [5]. Vacuum sealing was described in Fleischamnn's work [6, 7]. In 1988, Russian authors published an article in which they explored the use of negative pressure for managing suppurative (pus exuding) wounds. The authors treated 338 patients with abscesses, phlegmons, and purulent wounds. 173 patients were treated by traditional incisive-draining methods and 165 patients were treated by using vacuum therapy by the method proposed by the authors. The advantages of vacuum therapy were shown in the acceleration of reparative processes and in shortening the time of treatment [8]. In 1985, Jeter explored a unique combination of products to deliver negative pressure to the wound bed. She pioneered the use of suction to treat wounds utilizing a gauze dressing and wall suction. In cooperation with Chariker, she drew up a clinical study in which they stated that "their closed suction wound drainage system revolutionized the management of enterocutaneous fistulae complicating ventral abdominal wounds." In 1989, Chariker et al. developed a technique utilizing standard surgical dressings and wall suction to create a "vacuum" that aided in wound healing. Moist gauze was placed over the wound surface and a flat drain inserted over the gauze and covered with an occlusive dressing. The drain was then connected to a standard hospital wall suction source with continuous pressure set at approximately –60 to –80 mmHg. This method later became known as the "Chariker-Jeter technique" [9].

In 1986, Kostiuchenok et al. showed that application of NPWT in combination with surgical debridement resulted in improved wound healing by reducing considerably the bacterial load within purulent wounds [10]. In the same year, Davydov et al. discovered that vacuum therapy significantly affected the healing process by reducing the bacterial burden and septic complications. It was shown that the use of vacuum therapy shortened healing time, stabilized the immune process, reduced scar tissue formation, and, in consequence, reduced hospital stays [11].

In 1997, Morykwas and Argenta studied the use of suction applied to polyurethane foam in wounds. In their study, subatmospheric pressure was applied through a closed system to an open wound for periods of 48 hours. The subatmospheric pressure was directed at the surface of the wound through an interface between the wound surface and a polyurethane sponge, allowing distribution of the negative pressure and use of either a constant or intermittent mode. In conclusion, the authors stated that the application of controlled subatmospheric pressure creates an environment that promotes wound healing [12, 13]. In 1999, Philbeck Jr. et al. found that "healing time can be as high as 61% faster and 38% less costly with combination treatment utilizing a controlled-suction drain system" [14].

By 2003, NPWT was a commonly accepted therapy. Its use has recently been reviewed and results have been published for a wide range of wound types including diabetes, foot ulcers, surgical wound infections, traumatic wounds, skin graft fixation, pressure ulcers, and leg ulcers. It is thought that NPWT promotes wound healing through multiple actions, including the removal of exudate from the wounds to help establish fluid balance, provision of a moist wound environment, a potential decrease in wound bacterial load, a reduction in edema and third-space fluids, an increase in the blood flow to the wound, and the promotion of white cells and fibroblasts within the wound [15–18].

Literature data concerning application of this method for treatment of venous leg ulceration are scarce; that is why the aim of this paper is to present our own experience in using NPWT for treatment of chronic leg ulcers.

2. Materials and Methods

2.1. Patients. The study comprised 15 patients (8 women and 7 men) with an age span from 53 to 79 years (mean 62.1 years). The ulcer surface area was from 50.80 cm^2 to 76.20 cm^2 (mean 60.71 cm^2) with persistence time from 60 weeks to 112 weeks (mean 76.3 weeks). In 6 patients, the ulcer was situated on the right leg and in 9 on the left one. Full lower extremity motion was observed in 5 patients and limited motion in 10 patients. The ankle brachial index (ABI) varied from 0.9 to 1.1 (mean 0.98). The body mass index (BMI) varied from 27.8 to 38.2 kg/m^2 (mean 33.3 kg/m^2). All patients had been previously treated in dermatological and surgical clinics without success.

After clinical examination, the venous origin of the ulcer was confirmed by means of the venous duplex Doppler sonography and ABI measurement. The patients with previous or active deep vein thrombosis were excluded from the study. The additional exclusion criteria were chronic or critical leg ischemia, contraindications to compression therapy, immobilization in orthesis or plastic cast, paresis related to stroke or paraplegia, chronic cardiac failure with peripheral swelling, and systemic infection. In all the cases, diabetes was also excluded on the basis of laboratory data.

Each patient presented history of the index lesion, treatment, and other significant medical conditions. All patients had been previously treated by their personal physicians by means of elastic bandage compression stocking with wound antiseptic lavage and local application of traditional dressing such as hydrogel and hydrocolloid dressing. However, none of these methods resulted in complete healing of the wound within prerandomization period. Each ulcer was classified according to wound morphology, severity, and location. A systematic description of wound and limb appearance was recorded, including edema, erythema, exudation, granulation, and presence of fibrin or eschar.

2.2. Methods. In this study negative pressure wound therapy was provided by the Genadyne A4 system (Genadyne Biotechnologies Inc., Hicksville, NY, USA). The system consists of three components: a negative pressure generating unit

Table 1: Characteristics of the patients.

Patient	Sex	Age (years)	Ulcer surface area (cm^2)	Ulcer duration (weeks)	Time to completely heal (weeks)
1	Male	53	50.80	60	6
2	Female	60	52.40	62	6
3	Female	64	64.10	70	6
4	Male	58	58.20	68	6
5	Female	59	53.40	64	6
6	Male	60	64.40	72	11
7	Female	66	70.10	96	14
8	Male	61	72.40	100	16
9	Female	72	59.30	68	6
10	Female	68	65.10	76	12
11	Male	55	66.30	80	10
12	Female	63	51.60	70	6
13	Male	54	51.80	68	6
14	Female	79	76.20	112	20
15	Male	59	54.60	78	6

Table 2: Patients with healed ulcers according to the duration of treatment.

	Duration of treatment (weeks)																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
NPWT						10				1		1		1		1				1

with a disposable canister, a pad with evacuation tube, and a reticulated, open cell sterile polyurethane or a dense open-pore polyvinyl alcohol foam dressing cut to fit the wound. The system unit is programmed to deliver controlled negative pressure ranging from 50 to 200 mmHg. NPWT was applied to the ulcer as specified by manufacturer's guidelines, and treatment was continued until ulcer closure, sufficient granulation tissue formation for healing by secondary intention. [2, 19] NPWT dressing changes were performed every 48–72 h, not less than three times per week.

Prior to the treatment, a bacterial swab was taken from each ulcer. During the wound dressing and compression changes, the area of the ulcers was constantly measured. The procedure was as follows. At the outset, homothetic congruent projections of the ulcers were plotted onto transparent foil, after which planimetric measurements of the wounds were taken with the use of digitizer Mutoh Kurta XGT-1218A3 (USA). The area of the ulcer was determined once a week until the wound healed completely. All patients received micronized flavonoid fraction (450 mg diosmin, 50 mg hesperidin), 2 tablets of 500 mg once daily.

3. Results

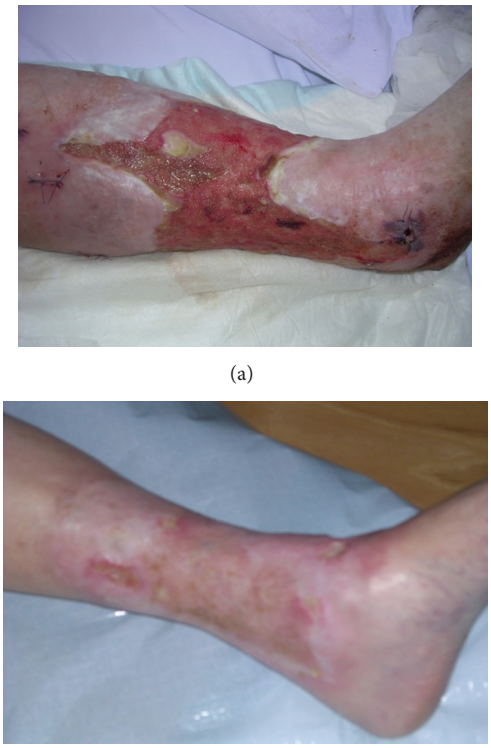
We treated 15 patients (8 women and 7 men) with a mean age of 62.1 years (range 53–79 years). The ulcer surface area was from 50.80 cm^2 to 76.20 cm^2 (mean 60.71 cm^2). The mean ulcer duration prior to the treatment with negative pressure was 76.3 weeks the range of 60–112 weeks (Table 1). The mean treatment time with NPWT was nine weeks. Treatment time for 10 patients was six weeks, and for the remaining five patients, the treatment times were 10, 12, 14, 16, and 20 weeks, respectively (Table 2).

We found that in all patients the fibrin on the wound bed was replaced by granulation tissue after one to two weeks (Figures 1(a) and 1(b)). Mean ulcer size was reduced from 15.2 cm^2 to 13.0 cm^2–10.6 cm^2 in the first three weeks of the treatment. In the following weeks, when NPWT was used, mean venous ulcer size got reduced to 4.6 cm^2–5.7 cm^2.

4. Discussion

Venous leg ulcer is a common ailment, sometimes resulting in disability. Approximately, 2% of the population has a chronic ulcer of the lower limb with female-male ratio 3 : 1. The incidence of venous ulcers increases with age and in the over-65 population it is estimated at the level of 6%. The mean cost of the treatment of leg ulcers in the United States of America is 80 billion dollars per year [20].

Venous leg ulcer is one of the biggest clinical problems in phlebology. Despite epidemiological and pathophysiological knowledge improvement, the number of patients suffering from this complication remains still high stimulating the research focused on the more effective treatment methods. According to the previously performed studies as well as the daily clinical practice, the compression therapy is crucial for the healing of the venous leg ulcers, although the local therapy may also improve the healing rate, if correctly applied to



(a)

(b)

Figure 1: Venous leg ulcer patent number 8 before (a) and after (b) the treatment with NPWT.

the wound. In this respect, the crucial role of the time strategy and proper wound dressing should also be emphasized including many currently available nonocclusive or occlusive dressings such as hydrogels, hydrocolloids, alginates, or foams [20]. In our study, NPWT was used for the treatment of chronic venous leg ulcers of a surface area greater than 50 cm^2.

TNWP promotes wound healing through a number of mechanisms. These include edema reduction, increased wound/dermal perfusion, increased granulation tissue stimulation, decreased bacterial loading, and enhanced wound exudates removal [2, 3].

All patients in our study group had had conventional therapy with a mean of 76 weeks before treatment with NPWT. When negative pressure wound therapy was used, complete healing of ulcers was achieved in all patients. Healing time for 10 patients was six weeks, and in the remaining five patients the ulcers healed after 10, 12, 14, 16, and 20 weeks, respectively. In the first three weeks of treatment, the average ulcer surface area was reduced by 24.28%–27.4% and 53%, respectively. In the next weeks of treatment, the ulcer surface area got reduced by 6.7–10%, on average.

Kieser et al. examined 12 patients with chronic resistant venous ulcers. They used NPWT and compression bandaging for 4 weeks. The wounds were monitored for a total of 12 weeks. The authors found statistically significant reductions in ulcer surface area in the first weeks of NPWT therapy [21]. These results are in accordance with ours.

5. Conclusions

The results of our study show that negative pressure wound therapy improves the healing process of venous ulcer by decreasing its surface area, which significantly reduces the time of wound treatment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Evaluation of Bioelectrical Activity of Pelvic Floor Muscles Depending on Probe Location: A Pilot Study

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Objectives. The main objective was to determine how the depth of probe placement affects functional and resting bioelectrical activity of the PFM and whether the recorded signal might be dependent on the direction in which the probe is rotated. *Participants*. The study comprised of healthy, nulliparous women between the ages of 21 and 25. *Outcome Measures*. Bioelectric activity of the PFM was recorded from four locations of the vagina by surface EMG and vaginal probe. *Results*. There were no statistically significant differences between the results during functional sEMG activity. During resting sEMG activity, the highest bioelectrical activity of the PFM was observed in the L1 and the lowest in the L4 and a statistically significant difference between the highest and the lowest results of resting sEMG activity was observed ($P = 0.0043$). *Conclusion*. Different electrodes placement during functional contraction of PFM does not affect the obtained results in sEMG evaluation. In order to diagnose the highest resting activity of PFM the recording plates should be placed toward the anterior vaginal wall and distally from the introitus. However, all of the PFM have similar bioelectrical activity and it seems that these muscles could be treated as a single muscle.

1. Introduction

A proper assessment of the pelvic floor muscles (PFM) is an important part in the diagnosis and treatment associated with pelvic floor dysfunction, particularly with respect to urinary incontinence, faecal incontinence, or genital prolapse in women [1–9]. Methods for evaluating the strength and the endurance of the PFM are subjective transvaginal digital palpation (e.g., The Oxford Scale or The Modified Oxford Scale) and objective methods such as perineometry and electromyography (EMG) are often indicated [1, 10–18]. In understanding the proper neural control as well as normal and pathological activity of the PFM a needle or surface EMG is proving to be a useful tool [19]. Increasingly common apparatus for the objective assessment of PFM is surface electromyography (sEMG) with a vaginal probe [20–23]. Some studies [24–27] indicate that exact assessment of PFM function with the probe is facilitated by the fact that these muscles can behave as a single muscle during resting and functional activity. However, in accordance with the principles of evidence-based medicine we should seek to standardize measurements in terms of research equipment parameters, time, location of the measurement, and a patients position during the examination [28–35]. Reliable and consistent recording of PFM activity can be difficult, which transpires from the diversity of vaginal probes and their placement [36]. It is known that the shape and size of the probes may influence the results obtained, so it is important to optimize the type of the probe which is used to assess the strength of the PFM [19, 37].

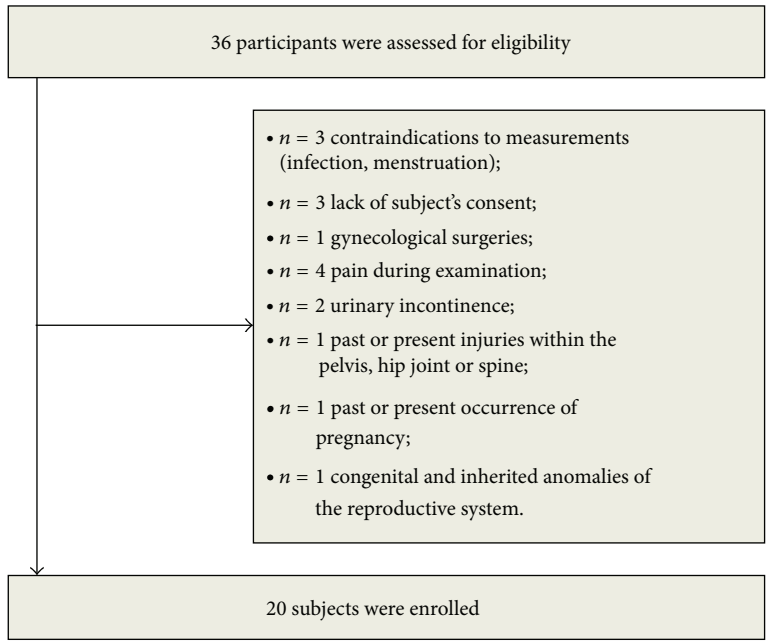

Figure 1: Flow diagram includes detailed information on the excluded participants.

2. Objectives

The wide differentiation in vaginal probes and the lack of clear methodology of their application prompted the authors to perform an evaluation of PFM bioelectrical activity corresponding to probe location. The main objective was to determine how the depth of probe placement affects the functional and resting bioelectrical activity of the PFM and whether the recorded signal might be dependent on the direction in which the probe is rotated. The probe was placed in two different orientations, toward the anterior or posterior wall of the vagina. A secondary objective was to evaluate any correlation between sEMG activities of the PFM which were measured at various areas of the vagina.

3. Materials and Methods

3.1. Subjects. This study was approved by the Bioethics Committee of the Wroclaw Medical University (KB-611/2012, Wroclaw, Poland) and all subjects provided written informed consent. Thirty-six healthy, nulliparous women were recruited from the Public Higher Medical Professional School population to participate. Women with a history of incontinence, gynaecological surgeries, congenital and inherited anomalies of the reproductive system, past or present injuries within the pelvis, hip joint or spine, and pregnancy were excluded, as well as women with contraindications to measurements (such as infection and menstruation) (Figure 1). Finally, the study comprised of twenty volunteers between the ages of 21 and 25 ($\overline{x}$ = 22.3 years, SD = 1.28 years).

3.2. Electromyography. The electromyographic signal was registered by a dual-channel sEMG NeuroTrac ETS device integrated with computer software for digital analysis and report creation (Verity Medical Ltd., UK). This device is characterized by an amplitude range of 0.2–2000 μV RMS continuous in the frequency band of 2–100 Hz and pulse width from 50 to 450 μS for recording signals generated by muscles. Device sensitivity is established at a level 0.1 μV (4% accuracy; readings ±0.3 mV at 200 Hz), with selectable bandpass filter (3 db bandwidth) and 50 Hz notch filter (33 dbs; 0.1% accuracy). The analogue signal recorded by the sEMG electrodes was amplified, filtered, and subsequently transformed into a digital signal. Such signal facilitated statistical analysis of acquired results and allowed for data representation in a graphical form. Mean values of muscle bioelectrical activity were given according to root mean square algorithm (RMS) [28, 38–40]. The monopolar, self-adhesive reference electrode was placed on the anterior superior iliac spine.

3.3. Probe Descriptions. To investigate the pelvic floor muscle activity we used Vaginal Probe Periprobe Optima 3 (Sugar International, France) with 3 independent, hemispherical, nickel-free electrodes (recording plates). The top (electrode A), the middle (electrode B), and the bottom (electrode C) electrodes of the probe are three detection surfaces. The probe has a total length of 12 cm and total weight of 21 g. The circumference of the top electrode is 7.5 cm. The circumference of the middle and the bottom electrode is 6.5 cm. The distance between the electrodes is 3.3 cm. The position of the probe was determined by a mark to be placed in line with the introitus of the vagina. The middle of electrode A is 8.7 cm from the introitus, the middle of electrode B is 5.4 cm from the introitus, and the middle of electrode C is 2.1 cm from the introitus.

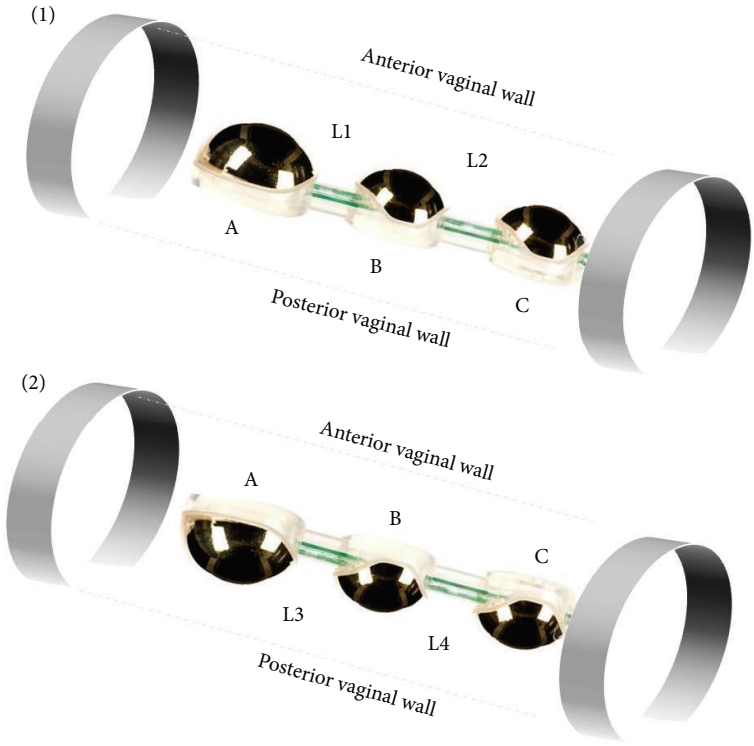

Figure 2: Location of the probe during measuring of PFM activity. (1) The probe was toward the anterior wall of the vagina. (2) The probe was toward the posterior wall of the vagina; L1, L2, L3, and L4: locations 1, 2, 3, and 4; A: the top electrode, B: the middle electrode, and C: the bottom electrode.

3.4. Experimental Protocol. Measurement of electrical activity of the PFM was assessed in a standing position. Prior to measurements, each participant was instructed how to perform an isolated PFM contraction. Resting and functional sEMG activity (in microvolts μV) were recorded. All of the women participating in this study were asked to contract the PFM as hard as possible for five seconds (functional activity). The contractions were repeated five times with five-second break between each contraction (resting activity). The probe was placed in two different orientations and measurement was performed when the probe was toward the anterior wall of the vagina and afterward toward the posterior wall. Bioelectric activity of the PFM was recorded from four locations (Figure 2).

Location 1 (L1) is the circuit between electrodes A and B towards anterior wall of the vagina.

Location 2 (L2) is the circuit between electrodes B and C towards anterior wall of the vagina.

Location 3 (L3) is the circuit between electrodes A and B towards posterior wall of the vagina.

Location 4 (L4) is the circuit between electrodes B and C towards posterior wall of the vagina.

3.5. Statistical Analysis. Statistical analysis was performed using Statistica 10. Analysis of variance (ANOVA) of Kruskal-Wallis was used to examine the difference between the sEMG activity in each location. A value of $P < 0.05$ was considered statistically significant. The differences between measurements obtained during resting and during functional sEMG activity were compared. In addition, Spearman correlation was made to show the relationship between the variables.

4. Results

There were no statistically significant differences between the results during functional sEMG activity (Figure 3). During resting sEMG activity, the highest bioelectrical activity of the PFM was observed in L1 ($\overline{x} = 2.4\,\mu$V, min-max: 1.3–4.0 μV, SD = 0.69 μV) and the lowest in the L4 ($\overline{x} = 1.7\,\mu$V, min-max: 0.9–3.2 μV; SD = 0.63 μV). A statistically significant difference between the highest and the lowest results of resting sEMG activity was observed ($P = 0.0043$) (Figure 4). Among other results, no statistically significant differences were registered.

In the study population, a statistically significant correlation was found for all analyzed variables. The correlations are presented in Tables 1 (for resting sEMG activity) and 2 (for functional sEMG activity). Spearman analysis showed statistical correlation between the results of PFM functional activity of four locations (Table 1): between L1 and L2: $P = 0.0008$, $r = 0.69$; L1 and L3: $P = 0.0000$, $r = 0.85$; L1 and L4: $P = 0.0012$, $r = 0.67$; L2 and L3: $P = 0.0000$, $r = 0.83$; L2 and L4: $P = 0.0000$, $r = 0.89$; L3 and L4: $P = 0.0000$,

Table 1: The correlation between the results of PFM activity of four locations—functional sEMG activity.

	Functional sEMG activity of PFM			
	L1	L2	L3	L4
L1	—	$r = 0.69$ $P = 0.0008$	$r = 0.85$ $P = 0.0000$	$r = 0.67$ $P = 0.0012$
L2	$r = 0.69$ $P = 0.0008$	—	$r = 0.83$ $P = 0.0000$	$r = 0.89$ $P = 0.0000$
L3	$r = 0.85$ $P = 0.0000$	$r = 0.83$ $P = 0.0000$	—	$r = 0.87$ $P = 0.0000$
L4	$r = 0.67$ $P = 0.0012$	$r = 0.89$ $P = 0.0000$	$r = 0.87$ $P = 0.0000$	—

Table 2: The correlation between the results of PFM activity of four locations—resting sEMG activity.

	Resting sEMG activity of PFM			
	L1	L2	L3	L4
L1	—	$r = 0.60$ $P = 0.0052$	$r = 0.64$ $P = 0.0022$	$r = 0.53$ $P = 0.0173$
L2	$r = 0.60$ $P = 0.0052$	—	$r = 0.68$ $P = 0.0010$	$r = 0.81$ $P = 0.0000$
L3	$r = 0.64$ $P = 0.0022$	$r = 0.68$ $P = 0.0010$	—	$r = 0.83$ $P = 0.0000$
L4	$r = 0.53$ $P = 0.0173$	$r = 0.81$ $P = 0.0000$	$r = 0.83$ $P = 0.0000$	—

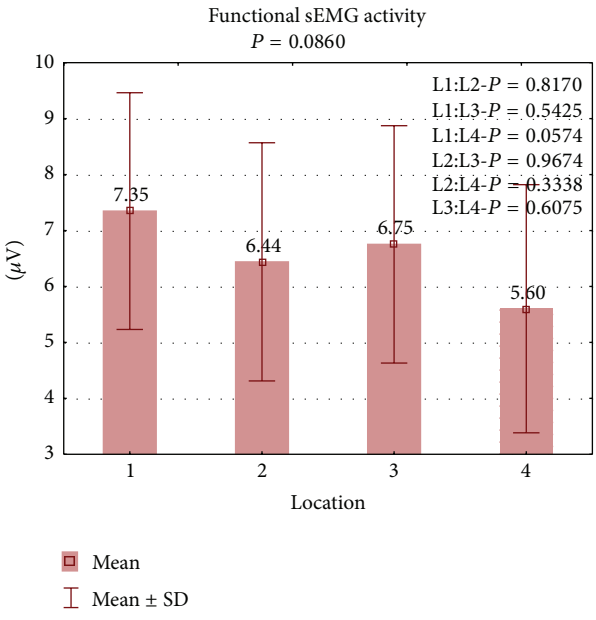

Figure 3: The results of PFM activity during functional sEMG activity in four locations.

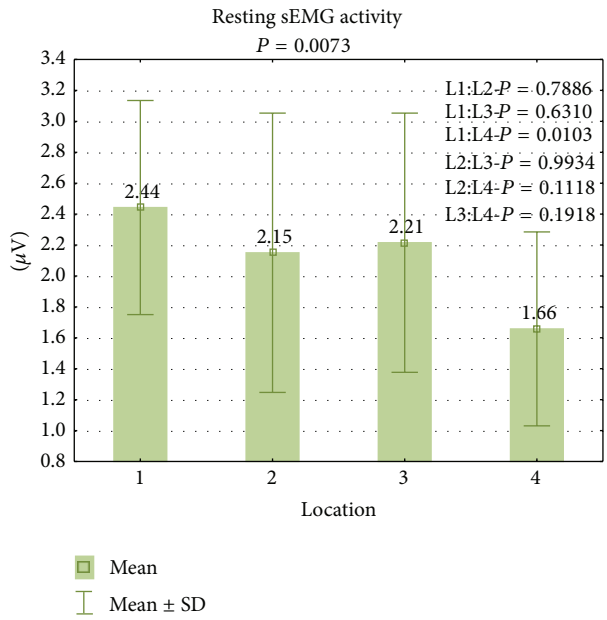

Figure 4: The results of PFM activity during resting sEMG activity in four locations.

$r = 0.87$. We also observed statistical correlation between the results of PFM resting activity (Table 2): between L1 and L2: $P = 0.0052$, $r = 0.60$; L1 and L3: $P = 0.0022$, $r = 0.64$; L1 and L4: $P = 0.0173$, $r = 0.53$; L2 and L3: $P = 0.0010$, $r = 0.68$; L2 and L4: $P = 0.0000$, $r = 0.81$; L3 and L4: $P = 0.0000$, $r = 0.83$.

5. Discussion

This study tries to determine the evaluation of bioelectrical activity of the PFM according to probe location. The factors which were taken into consideration are depth of electrode

placement and their orientation. Functional and resting sEMG activity was assessed with an Optima 3 vaginal probe.

The presented results pertain to the diagnostics of PFM activity and they confirm that the activity depends on the area where recording plates are located. Long probes have recording plates which can record the sEMG signal from areas of vaginal wall located distal to the vaginal introitus. Shorter probes collect the activity from proximal locations.

Voorham van der Zalm et al. [36] conducted similar assessment of the location of different types of electrodes. In their study they used five common probes which differed in shape, length, and width of the recording plates as well as in circumference, length of the probe, and place of insertion of the probe. The position of recording plates was evaluated in relation to puborectal muscles and examined by ultrasound. Although the study did not have a representative research group, on the basis of the results the authors recognize the value in conducting further studies in order to optimize the probes used.

Bø et al. [37] also noticed that the size and location of vaginal probes have an impact on the obtained results. In the assessment of the PFM they used two types of vaginal probes: the Camtech Squeez meter (length: 6.7 cm, diameter: 1.7 cm, location: the middle of the balloon was 3.5 cm from the introitus) and the Peritron (length: 10.8 cm, diameter: 2.8 cm, location: 0.5–1 cm of the probe was visible outside the introitus). The results of vaginal squeeze pressure varied depending on the type of vaginal probe used in the study. Therefore, the use of various electrodes in the studies does not allow for effective comparison of results.

A clinician should be able to match the appropriate type of probe depending on the therapeutic purpose (specifically in electrical stimulation). This is due to another feature of vaginal probes. In addition to their usage in the evaluation of PFM activity, they can be applied in the treatment of urinary incontinence. For example, in therapy for urge incontinence, the stimulation should include afferent nerve fibres of the plexus pelvicus and the pudendal nerve. In cases where the patient suffers from stress incontinence, electrical stimulation should influence the external sphincter and pelvic floor muscles. Thus, size, shape, length, width, and circumference play a significant role both in the diagnostics and in the therapy of urinary incontinence [36, 41, 42].

The distribution of forces acting on the vagina following pelvic floor contractions is varied which was confirmed by our results. Although, most of the results are not statistically significant, higher bioelectric activity was observed more distally from the introitus and on the anterior wall of the vagina. Constantinous and Omata's study [43] is another investigation into the distribution of forces acting on the vagina. They evaluated the distribution of anisotropic forces on the vagina following voluntary and reflex pelvic floor contractions. The probe with four pairs of force and displacement sensors was used to measure the pelvic floor closure force. The researchers observed significantly higher maximum forces of contraction in the anterior aspects of the vagina during reflex pelvic floor contractions. The unequal distribution of forces in the vaginal walls is the subject of other similar studies [44–47].

However Shafik's study [24] demonstrates that all of the pelvic floor muscles behave as one muscle since they contract or relax collectively, which was also noticed in this study in strong correlation between measurements from particular localizations. He explains this phenomenon by referring to the origin of pelvic floor muscles. External anal (EAS) and urethral sphincters (EUS) as well as the bulbocavernosus muscle (BC) arise from the puborectalis muscle (PR). Though the levator ani (pubococcygeus) is not descended from the puborectalis muscle, it shares with it its innervations through the pudendal nerve. Stimulation of sensory fibres of the pudendal nerve activates reflex contractions of the stimulated muscle and of all of the muscles supplied by this nerve. Nonetheless, he also confirmed a voluntary selective muscle activity and that each individual pelvic floor muscle can act independently of the others.

The results prompt for further studies, in order to find research tools for more accurate assessment of pelvic floor muscles, in addition to the evaluation by sEMG or perineometer. It may be very meaningful to diagnose the PFM using ultrasound or MRI. Furthermore, there is a need to strictly determine the methodology of measurements and the type of equipment used.

Attention should be given to the practical implications of this study. The clinical reliability and accuracy of PFM measurements are indeterminate and should be reevaluated. The study is highlighting aspects of the objectification of both the measurement and the measurement tools. In this pilot study the authors used a probe which has not yet been the subject of a randomized trial and which assessed the bioelectrical activity from various localizations of the vagina.

6. Limitation of the Study

Some limitations of the study were the small number of participants, no measurements in patients with pelvic floor dysfunction, and the lack of more sensitive multichannel sEMG. This study will be continued among patients with the pelvic floor dysfunction and complemented by measurements using different types of probes.

7. Conclusion

Different electrodes placement during functional contraction of the PFM does not affect the obtained results in sEMG evaluation. In order to diagnose the highest resting activity of the PFM the recording plates could be placed toward the anterior vaginal wall and distally from the introitus. However, all of the PFM have similar bioelectrical activity and it seems that these muscles could be treated as a single muscle. Therefore, it is appropriate to continue to conduct measurements of bioelectrical activity of the PFM, depending on the placement and the type of probes. Further experimental research should include a larger number of participants as well as individuals with lower urinary tract symptoms.

Conflict of Interests

There is no conflict of interests.

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Clinical Study

Effect of Kinesiology Taping on Breast Cancer-Related Lymphedema: A Randomized Single-Blind Controlled Pilot Study

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The aim of the study was to assess the efficacy of Kinesiology Taping (KT) for treating breast cancer-related lymphedema. Sixty-five women with unilateral stage II and III lymphedema were randomly grouped into the KT group (K-tapes, $n = 20$), the Quasi KT group (quasi K-tapes, $n = 22$), or the MCT group (multilayered compression therapy group, $n = 23$). Skin care, 45 min pneumatic compression therapy, 1 h manual lymphatic drainage, and application of K-tape/Quasi K-tapes/multilayered short-stretch bandages were given every treatment session, 3 times per week for 1 month. Patient evaluation items included limb size and percentage edema. Comparing the changes in K-tapes with quasi K-tapes changes, there were no significant differences ($P > 0.05$). The edema reduction of multilayered bandages was much better than in results observed in taping groups. The KT appeared to be ineffective at secondary lymphedema after breast cancer treatment. The single-blind, controlled pilot study results suggest that K-tape could not replace the bandage, and at this moment it must not be an alternative choice for the breast cancer-related lymphedema patient. The trial is registered with ACTRN12613001173785.

1. Introduction

Lymphedema is a chronic and progressive condition resulting from an abnormality or damage to the lymphatic system. It is marked by an abnormal increase of tissue proteins, edema, chronic inflammation, and fibrosis. Secondary lymphedema is caused by multiple factors related with lymphatic stasis, such as tumor lymph node infiltration, lymph node dissection, radiotherapy, trauma, and infection. Upper limb lymphedema occurs in 24–49% of the cases with total mastectomy and in 2.4–49% of the cases with axillary lymph node dissection [1, 2].

In Western Europe [3], upper limb secondary lymphedema has been reported in 22% of patients after breast cancer therapy. Lymphedema occurs when there is an imbalance due to reduced lymph transport capacity which leads to interstitial fluid and protein accumulation. It further leads to chronic inflammation and fibrosis caused by the secondary proliferation of neutrophils, macrophages, and fibroblasts and accumulation of collagen.

Physical therapy is a common management for lymphedema. A program combining skin care, manual lymphatic drainage, exercise, and compression therapy (multilayered bandage, intermittent pneumatic compression) is recognized as the best practice in lymphedema management. There have been numerous prospective investigations with different treatment frequency and duration showing the effect of physical therapy, which has been accepted as a standard "gold" therapy for many years [4–8].

However, standard care and management can have significant economic consequences. Bandage changes and expensive compression hosiery drain the available resources. Well-documented, promising, and inexpensive methods from alternative medicine are still needed [9–13].

Kinesiology Taping (KT) for lymphatic drainage is a new choice in the field of physical and alternative therapy. The material used for the Kinesio tape and the original concept of the taping technique were introduced by Dr. Kenso Kase in 1973. K-tape had been designed to allow 30–40% longitudinal stretch. It is composed of 100% cotton fibers and acrylic heat sensitive glue. Development of the technique for its administration is still ongoing. Dr. Kase claimed that applying K-tape would have physiological effects including decreasing pain or abnormal sensation, supporting the movement of muscles, removing congestion of lymphatic fluid or hemorrhages under the skin, and correcting misalignment of joints. After applying K-tape, the taped area will form convolutions to increase the space between the skin and muscles. Once the skin is lifted, the flow of blood and lymphatic fluid is promoted. Other advantages are that a patient can take a shower without taking the tape off since it is waterproof. Patients can wear it from 1 to 4 days and even longer if it is applied on the back or buttock area [14, 15].

Many practitioners use it in clinical practice in European countries, and it has a beneficial effect. However, there is insufficient evidence for its clinical effects on lymphedematous limbs. The aim of the study was to assess the efficacy of Kinesiology Taping (KT) for treating breast cancer-related lymphedema. The endpoints were the reduction of limb volume and percentage edema size after a month's therapy.

2. Materials and Methods

The Research Ethics Committee from the Academy of Physical Education in Katowice, Poland, approved this study (national registration number 1605/12/2012). The trial is registered in the Australian New Zealand Clinical Trials Registry with ID number ACTRN12613001173785.

2.1. Settings and Participants. The study was performed at the Provita Clinic in Zory and Limf-Med Hospital in Chorzow, Poland, from December 2012 to August 2013. Participating women met the following inclusion criteria: (1) unilateral breast cancer-related lymphedema for at least one year, (2) moderate-to-severe lymphedema (stages II and III of upper limb edema, the volume difference between affected and healthy extremity with being more than 20%), (3) lack of chemo- or radiation therapy for at least 6 months, and (4) good compliance and willingness to sign the written consent form. Subjects with the following conditions were not allowed to participate or were excluded from the study: (1) active cancer or disease that might lead to swelling and presently taking diuretic therapy or other lymphedema-influencing drugs, (2) skin disease, (3) irremovable bracelet or ring, (4) marked restriction of active range of motion in the affected upper extremity, (5) the presence of a pacemaker, heart disease, pregnancy, metallic devices in the limb to be treated, infectious disease, epilepsy, cartilage growth, thrombophlebitis, arterial hypertension, or metastases, which are the treatment contraindications, and (6) the presence of mental, sensorial, or language problems, which could make cooperation difficult (more details in Figure 1).

2.2. Randomization and Intervention. Participants were randomly allocated to the groups. Computer-generated random numbers were sealed in sequentially numbered envelopes, and the group allocation was independent of the time and person delivering the treatment. The physician (main coordinator) who allocated the patients to groups had 75 envelopes, each containing a piece of paper marked with either group KT, Quasi KT, or MCT. The physician would select and open an envelope in the presence of a physiotherapist to see the symbol and would then direct the patient to the corresponding group. A clinical nurse collected the data and coded them into an Excel database. The "blinded" results were transferred to a STATISTICA version 10.0 (StatSoft Inc., Poland) database by a technician. The research coordinators had no contact with and could not identify the patients.

Subjects from all groups received a routine treatment, including skin care, 45 min pneumatic compression therapy in use of the DL1200 device (at pressure 90 mmHg, 12 chambers arm overlapping cuff, hold time 3 seconds with no interval), 1 h manual lymphatic drainage, and application of multilayered short-stretch bandages (50–60 mmHg). The tape groups (KT and Quasi KT groups also received standard therapy, but K-tapes were used instead of bandages). Each of the groups was treated 3 times weekly (bandages or K-tapes were applied and changed on Mondays, Wednesdays, and Fridays) for in the 4-week intervention period. One physical therapist (PT) provided treatment. The program was standardized, following the same protocol for lymphatic drainage to the anterior trunk, posterior trunk, and affected arm, always mowing fluid from the affected side toward the unimpaired side, after lymphatic drainage and before either the short-stretch bandages (Figure 2) or the Kinesiology Taping application (Figure 3). Both bandages and K-tapes (Figure 4) were applied by the by the physical therapist.

In KT group, the fan tape anchor started at the anterior aspect of the hand with no tension. The tails of the tape were applied to the anterior, medial, and posterior aspects of the forearm and arm with 5–15% tension and then on anterior part of chest. The tapes were left on the patient's skin for the next three days. In Quasi KT group, we used tapes without therapeutic effects-common surgical plasters stuck using the same methodology as in KT group.

In MCT group, we used 4-layered compression bandaging. The first layer was applied to the skin directly with Tubula

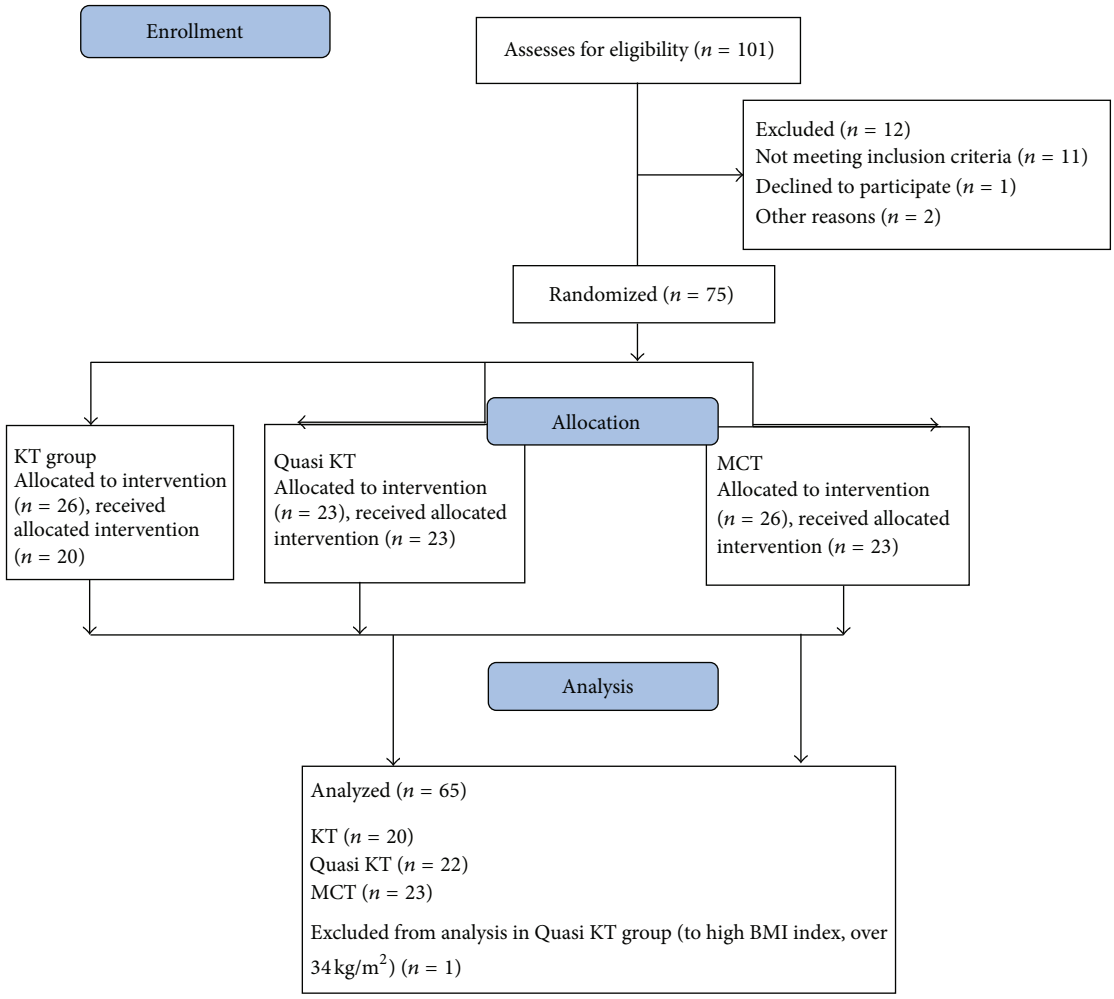

Figure 1: Flow diagram of the study.

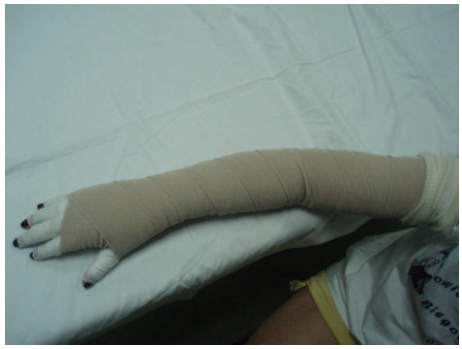

Figure 2: Multilayered compression bandaging.

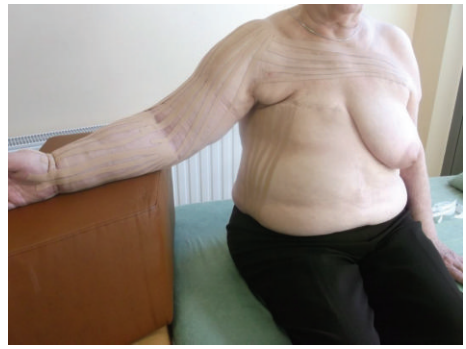

Figure 3: Kinesiology Taping application.

orthopedic sleeve. Then a supporting bandage Matoplast was applied to the fingers and on hand. Another layer was cotton Rolta-Soft covering the whole limb. The external layer consisted of short-stretch Hartmann bandages.

The following research was a single-blind, controlled, randomized clinical study. The experiment design, methodology, and treatment parameters were programmed by coordinators (physiotherapist, general surgeon, oncologist, and an internist). Standard care, optoelectronic measurements, and data collection were provided by a nurse. The KT/Quasi KT was performed by a physiotherapist. The final statistical analysis was performed by a technician.

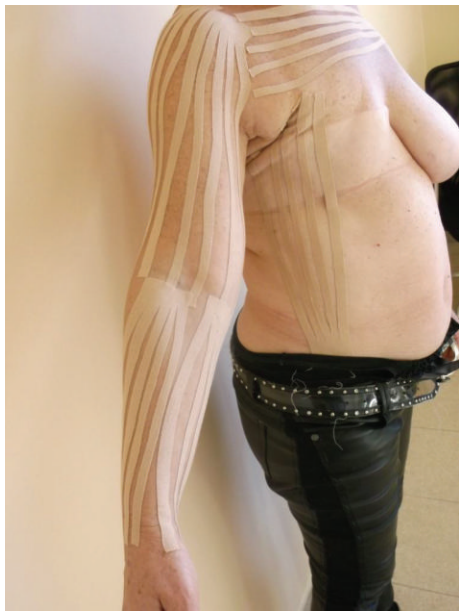

Figure 4: K-tapes technique.

Figure 5: Optoelectronic limb volume measurement.

2.3. Outcomes Assessment. To assess the volume of limb, we used an optoelectronic Perometer 40 T, cooperating with a personal computer. This method allowed us to estimate the volume of the measuring error for only 0.5%. The assessment technique was based on a special ring, equipped with a system of 378 LED diodes (emitting the infrared radiation). Within the ring were also the optical sensors that receive electromagnetic stimuli. In the course of measuring the limb was located inside the ring on the diode-sensor lines. The registered light pulses on the detectors were turned into electronic signals. The ring was moved during measurement to cover the entire limb (Figure 5). Measurements of the limb volume (both affected and healthy upper limb) were made for all three groups of patients before and after therapy (Figure 6).

2.4. Statistical Analysis. To compare the individual parameters that characterized the study groups, the nonparametric Kruskal-Wallis test for countable variables and the chi-squared test (χ^2) for categorical variables were used. The nonparametric matched pair Wilcoxon test was used to compare the within-group results before and after therapy. The Kruskal-Wallis analysis of variance (*post hoc* Tukey's test) was used to evaluate differences in the changes between the groups in the limb volume and edema values. Two-sided results ($P < 0.05$) were considered to be statistically significant.

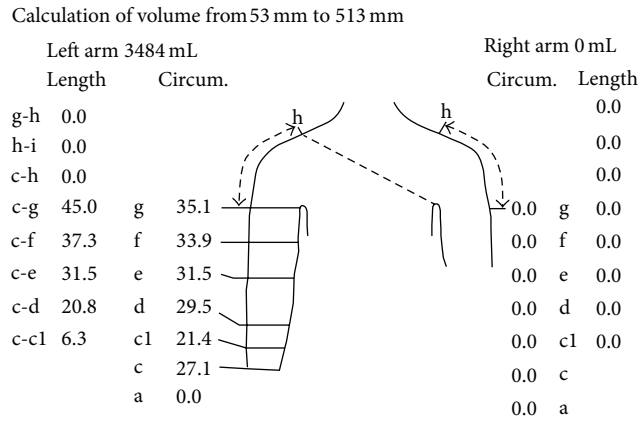

Figure 6: Graphical presentation of optoelectronic measurement.

3. Results

In total, 75 individuals were qualified to participate in the treatment. Six patients dropped out from the study during therapy in the KT group (one patient chose to discontinue treatment and withdrew from the study for personal reasons—taking care in the home of her daughter suffering from scarlet fever—four women had skin allergy after K-tapes, and one woman had a heart attack). Three patients from MCT group had complications unrelated to the treatment and were directed to other hospitals (one patient died of brain stroke) before the final observation. One patient in the Quasi KT (placebo group) was excluded from the analysis (BMI over 34 kg/m^2, which was too high and significantly increased the SD; this increase could have seriously affected the reliability of both the nonparametric Kruskal-Wallis analysis of variance and the final conclusions).

Of the 65 patients who completed the study protocol (and were analyzed) and had stage II and stage III of secondary lymphedema of upper limb (Table 1), the average volume of the affected extremities in women from group KT was 9414.01 cm^3 and decreased after treatment to 8051.15 cm^3 ($P = 0.002$). The average volume of affected limb in women from Quasi KT group was 9621.33 cm^3 and decreased after treatment to 8041.02 cm^3 ($P = 0.002$).

In turn, the average volume of the affected limbs in women from MCT group was 10089.41 cm^3 and after treatment it was 5021.22 cm^3 ($P = 0.000001$).

In the following study, we observed that the most significant decrease of edema was in patients undergoing multilayered compression bandaging. Results in patients undergoing K-tapes were similar to those obtained in the single-blind placebo (Quasi KT) group (Table 2 and Figure 7).

Table 1: Characteristics of patients.

	Group KT	Group Quasi KT	Group MCT	P
Number of women**	20	22	23	0.784
Age (years)**				
Range	44–80	39–81	42–81	
Average	67.34	65.43	66.45	0.835
Median	66.11	63.89	67.81	
SD	12.03	13.13	11.99	
Total mastectomy*	20	22	23	0.784
Number of patients with adipositas* (BMI > 30 kg/m^2)	7	7	6	0.812
Smokers*	7	8	7	0.812
Chemotherapy*	12	10	11	0.812
Radiation therapy*	15	13	13	0.788
Side of lymphedema*				
Right	8	10	10	0.679
Left	12	12	13	
Duration of lymphedema (months)**				
Range	12.2–63.6	12.3–46.6	15.3–33.8	
Average	22.12	22.78	20.03	0.621
Median	22.02	22.52	21.67	
SD	12.56	13.01	13.02	
Lymphedema severity** (% compared to healthy limb)				
II stage (20–40%)	15	16	16	0.788
III stage (40–60%)	5	6	7	

*χ^2 test.
**Kruskal-Wallis test.

Table 2: Results in percentage edema (affected upper limb compared to healthy limb volume and expressed in percent).

	Group	Average ± SD		P
		Before therapy	After therapy	
Decrease of edema (%)	KT	31.03 ± 28.17	25.03 ± 23.08	**0.005**
	Quasi KT	30.28 ± 30.12	24.47 ± 23.55	**0.005**
	MCT	31.07 ± 29.30	14.02 ± 10.03	**0.000003**

Wilcoxon test.

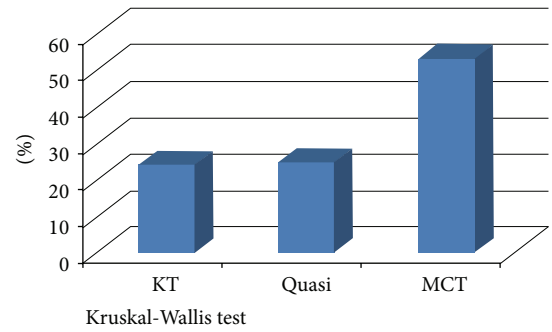

Figure 7: Comparing percentage edema reduction between groups. KT versus MCT group (24.45% versus 53.21%, $P = 0.02$). Quasi KT versus MCT group (24.78% versus 53.21%, $P = 0.02$). KT versus Quasi KT group (24.45% versus 24.78%, $P = 0.455$).

4. Discussion

The KT has been suggested as a promising treatment option for acute sport injuries [16], musculoskeletal disorders [17, 18], and also edema like venous and lymphedema [19–21], but there are still many controversies connected with methodology, application technique, and pressure values.

For example, Olszewski [22] maintains that lymphatics contract rhythmically with a frequency depending on the volume of inflowing tissue fluid. In regions with high capillary filtration rate and tissue fluid formation the frequency is high. The recorded pressures at rest, irrespective of whether in the lying or upright position, with free proximal flow (lateral pressure) range between 7 and 30 mmHg and during finger flexing between 10 and 30 mmHg. The pulse amplitude is from 3 to 20 mmHg and 5–17 mmHg, respectively. The pulse frequency is from 0.6 to 6/min and from 2 to 8/min, respectively. The resting end pressures with obstructed flow (e.g., corresponding to lymphatic obstruction in postsurgical lymphedema) range between 15 and 55 mmHg and during foot flexing from 15 to 50 mmHg. The pulse amplitude is from 3 to 35 mmHg and from 3 to 14 mmHg, respectively. The pulse frequency is from 2.5 to 10/min and from 3 to 12/min, respectively.

It means that external low pressure value during compression procedures (under 50 mmHg) has no effect on lymph pressures. In obstructive lymphedema only few lymphatic collectors remain patent. The recorded pressures during rest

range from 5 to 45 mmHg depending on the remaining contractility force of the damaged lymphatic musculature. During calf muscular contractions pressures are generally low ranging from 10 to 25 mmHg, although well-conducted compression may in some cases generate pressures of above 60 mmHg. The author [22] recommends only high pressure range: 50–60 mmHg. In his opinion, lower values are useless, which is similar to our view of the results arising from the study, because K-tapes cannot induce higher external pressure than 15–20 mmHg.

We could find only one reliable meta-analysis presented by researchers from Israel [20], whose systematic review article tried to assessed the effects of therapeutic Kinesiology Taping on pain and disability in participants suffering from musculoskeletal, neurological, and lymphatic pathologies. Four online databases (CINAHL, Cochrane Library, MEDLINE, and PEDro) were comprehensively searched from their inception through March 2012. The initial literature search found 91 controlled trials. Following elimination procedures, 26 studies were fully screened. Subsequently, 12 met our inclusion criteria. The final 12 articles were subdivided according to the basic pathological disorders of the participants' musculoskeletal ($n = 9$), neurological ($n = 1$), and lymphatic ($n = 2$) systems. As to the effect on musculoskeletal disorders, moderate evidence was found supporting an immediate reduction in pain while wearing the KT. In 3 out of 6 studies, reduction of pain was superior to that of the comparison group. However, there is no support indicating any long-term effect. Additionally, no evidence was found connecting the KT application to elevated muscle strength or long-term improved range of movement. No evidence was found to support the effectiveness of KT for neurological conditions. As to lymphatic disorders, inconclusive evidence was reported. Although KT has been shown to be effective in aiding short-term pain, there is no firm evidence-based conclusion of the effectiveness of this application on the majority of movement disorders within a wide range of pathologic disabilities. In the authors' opinion, more research is clearly needed.

However (in only one found clinical trial in Pubmed and MEDLINE), Tsai et al. [21] presented a study about the positive effects of KT. The purpose of this experiment was to compare the treatment and retention effects between standard physical therapy combined with pneumatic compression and modified physical activity, in which the use of a short-stretch bandage was replaced by the use of Kinesiology Taping combined with pneumatic compression. The study results suggest that K-tapes could replace the bandage in therapy and could be a good alternative for patients with poor short-stretch bandage compliance. In our opinion, the mean weakness of this cited study are as follows: the Korean authors applied only single-layered compression therapy (15–20 mmHg, which is not enough to treat any kind of lymphedema) and there was a lack of estimation of the placebo effect in this article.

In the literature, there is a lack of well-conducted, randomized, controlled studies with KT and breast cancer-related lymphedema. It means that we will have to conduct our study. To this moment, we analyzed only a pilot group of women with secondary lymphedema after breast cancer treatment; further studies will be provided.

5. Conclusion

The KT appeared to be ineffective at secondary lymphedema after breast cancer treatment. The single blind, controlled pilot study results suggest that K-tape could not replace the bandage, and at this moment it must not be an alternative choice for the breast cancer-related lymphedema patient.

Ethical Approval

The Research Ethics Committee from the Academy of Physical Education in Katowice, Poland, approved this study (national registration no. 1605/12/2012).

Conflict of Interests

The authors would like to certify that they have no commercial associations with the manufacturers of the equipment described in the paper and other conflict of interests.

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