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Research article

A novel approach for knee osteoarthritis using high molecular weight hyaluronic acid conjugated to plasma fibrinogen – interim findings of a double-blind clinical study

Leonid Kandel [a,1], Gabriel Agar [b,1], Ori Elkayam [c,1], Aybek Sharipov [d], Omer Slevin [e], Gurion Rivkin [a], Moshe Dahan [b], Valerie Aloush [c], Amos B. Pyeser [d], Yaron Brin [e], Yiftah Beer [b], Avner Yayon [f,*]

[a] Joint Replacement and Reconstruction Unit, Department of Orthopedic Surgery, Hadassah Mount Scopus Hospital, Jerusalem, Israel
[b] Department of Orthopedic Surgery, Shamir Medical Center, Beer Yaakov, Israel
[c] Rheumatology Institute, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Israel
[d] Department of Orthopedic Surgery, Shaarei Zedek Medical Center, Jerusalem, Israel
[e] Department of Orthopedic Surgery, Meir Medical Center, Kfar Saba, Israel
[f] Procore Ltd., Ness Ziona, Israel

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ABSTRACT

Objective: Osteoarthritis (OA) is a widespread degenerative joint disease leading to progressive loss of function and pain. Available treatments do not provide long-term relief or improvement. This study aimed to assess the safety and efficacy of a novel intra articular supplement, made of high molecular-weight hyaluronic acid (HA) uniquely conjugated to either purified (RegenoGel) or autologous plasma-derived fibrinogen (RegenoGel-OSP), as a long-term treatment for knee OA.

Methods: Sixty-seven consecutive participants (mean age 67.26 ± 7 years) with symptomatic OA were randomly assigned to receive intraarticular injections of either RegenoGel, RegenoGel-OSP or saline solution (placebo). The active treatment groups received a second, repeat injection of the corresponding treatment at the 3-month evaluation, at which time, the placebo group was divided into two subgroups, one receiving RegenoGel and the other receiving RegenoGel-OSP. The OA symptoms were assessed by VAS, WOMAC, and IKDC questionnaires at baseline and at 1, 3, 4, and 6 months following the first injection. OA-related quality of life was evaluated by the SF-12 survey.

Results: Our preliminary data suggests that both fibrin-HA formulations have positive effects on OA symptoms for all assessed parameters with the most prominent trend for reduction in OA-associated pain. Pooled data analysis of RegenoGel and RegenoGel-OSP shows significantly improved VAS scores compared to placebo at three months after the first injection, and sustained for another three months after the second injection. Both RegenoGel, RegenoGel-OSP had an excellent safety profile.

Conclusions: Interim analysis results indicate that RegenoGel and RegenoGel-OSP are safe and are potentially effective for at least six months in alleviating pain and symptoms of knee OA.

1. Introduction

Osteoarthritis (OA) is a widespread degenerative joint disease with the knee being a principal peripheral target that results in progressive loss of function, pain, and stiffness [1, 2]. Its etiology and pathogenesis are not fully understood [3], and currently there is no cure. Biochemical changes in the subchondral bone, the articular cartilage, and the synovial membrane reportedly play important roles, and changes in extra-cartilaginous tissues may also serve as primary disease initiators, preceding cartilage damage [4, 37]. As of today, knee OA diagnosis is primarily based on the patient's history and clinical examination verified by radiography [5, 6]. Among non-surgical OA treatments are physical

* Corresponding author.
E-mail address: yayon@procore-bio.com (A. Yayon).
[1] Leonid Kandel, Gabriel Agar and Ori Elkayam equally contributed to this paper.

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exercises, weight control, physiotherapy [7, 8, 9] and pharmacotherapy [10]. These options are not disease-modifying, and provide only short-term symptomatic effects.

A common palliative treatment is a local injection of viscous solutions consisting primarily of hyaluronic acid (HA) [11, 12, 13, 14]. Naturally occurring HA is a high-molecular-weight linear polysaccharide (glycosaminoglycan), highly concentrated in synovial fluid, vitreous humor, cartilage, and skin. Although the potential of this treatment is encouraging [11, 14], current HA-based viscosupplements provide only short-term relief in most cases, due to instability and relatively fast decomposition of the injected components [13, 15]. To further stabilize HA and prolong its effect, two novel, self-settling viscosupplements were developed: RegenoGel and RegenoGel-OSP in which high molecular weight HA is uniquely conjugated to purified or autologous plasma derived fibrinogen respectively.

Fibrinogen is a major plasma protein that upon conversion to fibrin naturally acts as the major blood clotting component and as the primary extracellular fibrous matrix promoting wound repair. As such, in situ stabilized fibrin may also contribute to OA symptoms alleviation by promoting stem and progenitor cell attachment, migration and focal tissue regeneration [16, 17, 18].

HA and fibrin have been previously combined into a hybrid network. The polymers were either mixed or attached to the protein of interest [19]. These strategies however, provided only limited control over the gel's microstructure and physical properties. HaProlink™, a novel, non-destructive chemical conjugation strategy, overcame this limitation by conrolled coupling of pre-activated HA carboxilic functions to amine groups in the coiled-coil regions of fibrinogen [20]. Upon the addition of thrombin, the conjugate molecules assemble into a network of fibrin fibers with pendant HA chains filling the voids between the fibers. This strategy allows better control over the hybrid material's nanostructure through targeted conjugation and fine tuning of the HA/fibrinogen grafting ratio [20]. In contrast to most HA formulations, this fibrinogen-HA conjugate is non-viscous and therefore can be injected via a fine needle and freely distribute throughout joint compartments, filling small defects in damaged cartilage. Upon activation by thrombin and tissue factors, the conjugate assembles into a solid, insoluble fibrin-HA hydrogel, stable within cartilage defects and the synovial space [21].

Preclinical studies in murine, caprine and canine [22] models of damage-induced OA, comparing the efficacy of fibrin-HA to HA alone, showed a substantial decrease in cartilage degeneration with significant overall joint preservation only in animals treated with the fibrin-HA conjugate. In all of these models, a single injection of either Regenogel or Regenogel-OSP was enough to confer significant cartilage preservation that was not observed even following multiple HA injections suggesting a potential shift in the balance from an actively catabolic environment to an anabolic, potentially regenerative microenvironment [23]. A proof-of-concept, pilot, open label, human clinical trial revealed that fibrin-HA hydrogels had lasting benefits for at least twelve months in individuals with severe OA. Results from phase 1 proof of concept pilot study that were the basis for this study are presnted in Figure 6.

Here, we report the results of an interim analysis of an ongoing prospective, multicenter, randomized, double blind, placebo-controlled clinical trial for assessing the efficacy and safety of the two fibrin-HA conjugates, RegenoGel and RegenoGel-OSP, for treating OA of the knee.

2. Methods

From January 2017 to December of 2018, 67 consecutive participants (mean age 67.26 ± 7 years) with symptomatic OA were randomly assigned to RegenoGel, RegenoGel-OSP, and saline solution (placebo) groups. The placebo group was later subdivided between those who received either RegenoGel or RegenoGel-OSP treatments. Ethical approval was obtained from the ethics committees of the participating medical centers (Hadassah Medical Center, Tel Aviv Sourasky Medical Center, Shaare Zedek Medical Center, Meir Medical Center, Shamir Medical Center). All procedures involving human participants were in accordance with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all participants.

2.1. Participants and settings

Participants with symptomatic OA with Kellgren-Lawrence (KL) grades II, III, and IV (mild, moderate, and severe OA, respectively) [5, 6] were recruited. KL grade IV OA was used as a randomization stratification factor. Eligible participants (Table 1) were randomly assigned to one of the four study arms (Table 2) in a 2:2:1:1 ratio. During the first three months of the study period, the two placebo arms (3 and 4 in Table 2) were considered and treated as a single group.

Prior to treatment, 15–20 ml of blood were drawn to produce autologous RegenoGel-OSP and for blood tests. The participants then received the first intraarticular injection according to their study arm. The same procedure was followed for the second injection that was scheduled to take place three months later. The participants were assessed at 1, 3, 4, and 6 months after treatment initiation and monitored for safety and response to treatment through questionnaires.

2.2. Study objectives

The study's primary aim was to assess the treatment benefit in terms of knee pain reduction at three months after the first injection. In addition, the safety profile of the treatment was assessed, and the treatment efficacy was evaluated over an additional three-month period following the second injection.

2.3. Study treatment

2.3.1. RegenoGel

RegenoGel is a chemical conjugate of HA and pooled human plasma derived fibrinogen (Omrix, Israel). It comes as a concentrated solution containing 21–27 mg/mL fibrinogen after removing plasminogen. HA (LifeCore, USA) is a bacterial-cell derived, high-molecular weight (average moecular weight: $1.6*10^6$ Da) hyaluronate lyophilized material.

2.3.2. RegenoGel-OSP

RegenoGel-OSP is very similar to RegenoGel, but made with autologous plasma fibrinogen instead of a commercial source. Between 10-12 ml of blood per patient was collected into citrate-containing tubes to obtain 4–5 ml plasma by centrifugation (at 600 RCF, 8 min). The fibrinogen available for conjugation was at a known plasma concentration of 2–3 mg/ml. RegenoGel-OSP was prepared utilizing a freeze-dried active ester of HA, mixed on-site with the patients' plasma for 30 min.

2.3.3. Placebo

The placebo administered in this study was a 0.9% NaCl saline solution. The administered volume and route of administration were identical to those of the treatment arms.

2.3.4. Product administration

Treatment products or placebo (4ml) were administered via a single intraarticular injection into the target knee. The choice of the injection volume was based on the amount of standard sodium hyaluronate products that is commonly injected several times at weekly intervals.

The target knee was prepared for sterile injection using routine aseptic procedures. A 21-gauge needle loaded onto an empty syringe was inserted into the joint in an inferolateral position relative to the patella. Then, 0.5–1.0 ml of lidocaine was administered, unless there was known hypersensitivity to phenobarbitals, to verify location by loss of resistance. The lidocaine syringe was replaced with an empty syringe to remove as much of the synovial fluid as possible from the knee. This syringe was

Table 1. Inclusion/exclusion criteria.

Inclusion criteria	Exclusion criteria
1 Subject has signed and dated the informed consent form	1 History of significant knee trauma or previous arthroscopic surgery of the intended study knee within the last 3 months preceding screening
2 Age – 50≤ and ≤85 years old	2 Pain in both knees with a VAS score of ≥5
3 Pain in the intended study knee with an average VAS score (active) of ≥5 over the last week prior to screening.	3 Intra-articular injection to the intended study knee within 3 months prior to Screening
4 Degenerative changes in the intended study knee that can be categorized as grade II-IV Kellgren Lawrence based upon standing posterior-anterior and lateral radiographs of the knee	4 Life expectancy of less than 12 month
5 Body Mass Index (BMI) between 18.5 and 35	5 Intake of chronic pain medications (especially opioid pain relievers) without an option to pause for the period of the study
	6 History of Psoriatic Arthritis, Rheumatoid Arthritis or any other inflammatory condition associated with arthritis
	7 Wound in the area of the intended study knee
	8 Fever signs or symptoms of systemic infection or infection of the intended study knee, on the day before or the day of administration of treatment or placebo
	9 Known sensitivity to any of the treatment components, egg, rubber or latex
	10 History of anaphylactic shock or other severe systemic response or other adverse event to human blood products
	11 Known Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS), Hepatitis B or C viral infections, or acute or chronic liver disease
	12 History of cellulitis of the lower extremities, a peripheral vascular disease, or acute or chronic liver disease
	13 Cancer in the past 3 years or surgery involving the chest, abdomen, pelvis, or lower extremities in the past year
	14 History of treatment with investigational device or product within 30 days prior to Visit 1 of the study.

Table 2. Study arms.

Arm	First injection	Second injection (three months after first injection)
1. Treatment with RegenoGel	RegenoGel	RegenoGel
2. Treatment with RegenoGel-OSP	RegenoGel-OSP	RegenoGel-OSP
3. Placebo followed by treatment with RegenoGel	Placebo	RegenoGel
4. Placebo followed by treatment with RegenoGel-OSP	Placebo	RegenoGel-OSP

later replaced with a treatment product/placebo syringe and its contents were injected into the knee. Following injection, ten rounds of knee bending and straightening were performed to ensure efficient dispersion of the injected material throughout the joint space.

2.3.5. Blinding

Since the packaging was different for the three injected products, the association between the specific product and the study arm was unavoidable. To achieve blinding, the screening processes and the first injections were carried out by one group of physicians, and the follow-up procedures were carried out by another group of physicians who were blinded to the first round of injections.

2.4. Measurements

The effect of the administered treatments and placebo on the OA status was assessed using a visual analog scale (VAS) [24], the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [25] and International Knee Documentation Committee (IKDC) questionnaires [26]. The quality of life impact was assessed by the 12-item short form (SF-12) survey [27].

2.4.1. VAS

The VAS consisted of a 10 cm line with the endpoints defining extreme limits, such as “no pain at all” and “pain as bad as possible” [24]. In the current study, a numerical scale and a descriptive term “moderate” were added to mark the middle of the line. The individual VAS score was defined as the numeric scale value, corresponding to the individual evaluation of pain severity.

2.4.2. WOMAC

WOMAC is widely used to specifically evaluate osteoarthritis of the hip and knee. It consists of 24 items divided into the three subscales of pain, stiffness, and physical function [25]. The scores for the individual subscales are summed and result in a possible score range of 0–20 for pain, 0–8 for stiffness, and 0–68 for physical function. Higher WOMAC scores indicate worse pain, stiffness, and functional limitations.

2.4.3. IKDC

IKDC is a knee-specific, patient-reported outcome measure [26]. The IKDC questionnaire subjectively evaluates overall function according to three categories: symptoms, sports activity, and knee function. The scores are obtained by summing the individual items. The total score is calculated as (sum of items)/(maximum possible score)*100 to obtain a scaled number that ranges from 0 to 100, with higher scores representing higher functionality levels.

2.4.4. SF-12

SF-12 is widely used to assess self-reported health-related quality of life [27, 28]. It covers four physical and four mental health domains. The individual physical and mental scores were obtained utilizing the scoring method described by Ware et al. [27].

2.5. Statistical analyses

Sample size calculations were conducted on VAS under active and passive positions, with the following assumptions: 1. Placebo-induced change after three months is -2.83 under active and -0.98 under passive positions [28, 29]; 2. Treatment-induced change is -4.5 under active and -2.5 under passive positions (based on RegenoGel pilot studies); 3. Standard deviations are 1.9 and 1.6, respectfully, for changes between the findings at baseline and at three months.

A minimal sample size per group of 29 subjects (87 total) was sufficient for a significance level of 5% and a power of 80%. A Bonferroni correction for multiple comparisons was applied. To account for lost to follow-up subjects and the lack of direct comparison between RegenoGel and placebo, a sample size increase of 50% was set, yielding 44 subjects per group. Interim analysis was performed when the recruitment reached ~50% of the aimed sample. Analyses of variance (ANOVA) and planned pairwise comparisons with Bonferroni corrections were used to compare the scores separately for each parameter between the treatment and the placebo arms. Paired two-tailed *t*-tests were used to evaluate the difference separately for each parameter at baseline and at three months.

3. Results

From January 2017 to December 2018, 67 participants were randomly assigned to the treatment or placebo groups (Table 2), and 59 completed the

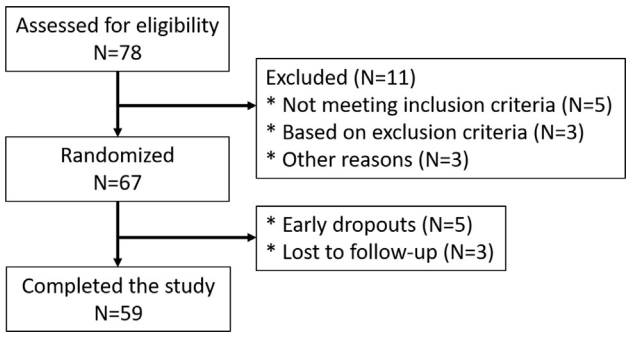

Figure 1. Participants flow diagram.

study. Participants' Flow Diagram is shown in Figure 1. Their mean age was 67.26 ± 7, with no significant difference between the study arms. There was a preponderance of female participants (74.24%), and the ~3:1 ratio was consistent across study arms 1, 2, and 4. The gender distribution was closer to 1:1 (54.55% females) in study arm 3. The results for treatment arms 1 and 2 are reported both separately and pooled.

3.1. Pain symptoms 1 and 3 months after the first injection

VAS and WOMAC pain assessments were obtained at baseline and at 1 and 3 months following the first injection. The mean baseline VAS pain score under active position and the mean WOMAC pain score of the placebo group were 7.36 ± 1.18 (indicating "worse-than-moderate" pain level) and 9.32 ± 3.24 (indicating "moderate" pain level), respectively. The matching values of the pooled treatment groups were 7.69 ± 1.18 and 10.02 ± 3, respectively. The dynamics of these scores following treatment for the placebo group and for each treatment group separately are presented in Figure 2. A marked improvement in pain status was already recorded at one month after the first injection. There was a significant reduction in the VAS and WOMAC pain scores compared to baseline across all groups (Table 3). Importantly, pain scores in the treatment groups continued to decrease at the 3-month evaluation, whereas the placebo group's pain scores remained unchanged (WOMAC) or even worsened (VAS) (Figure 2).

Applying ANOVA to the placebo and pooled treatment pain scores yielded no significant effect at one month following the first injection. Pairwise comparisons revealed that although the VAS score for the treatment groups did not differ significantly from that of the placebo group at one month after the first injection, it was significantly lower at the 3-month evaluation (pooled treatment diff = -2.38 ± 2.44 vs placebo diff = -0.9 ± 2.07, $p < 0.002$), indicating a substantial treatment-specific improvement. WOMAC pain scores showed a similar trend. However, they were not significant and remained similar to the placebo group scores at the 3-month evaluation (pairwise comparison, $p = 0.11$).

The VAS score under passive position showed similar trends as under active position. The mean baseline values were 4.91 ± 2.74 for the placebo group, 5.04 ± 2.29 for the RegenoGel group, and 4.95 ± 2.17 for the RegenoGel-OSP group, all corresponding to "moderate" pain levels. At one month following the first injection, there was a reduction in VAS values in all groups. However, the reduction was significant only in the RegenoGel-OSP group (RegenoGel-OSP, diff = -1.14 ± 1.96, $p < 0.015$). There was nevertheless, a significant reduction in both treatment groups at the 3-month evaluation (paired, two-tailed *t*-tests: RegenoGel, diff = -1.76 ± 2.21, $p < 0.002$; RegenoGel-OSP, diff = -1.37 ± 2.34, $p < 0.02$), indicating marked treatment-induced improvement, with pain reduction to "below moderate" levels. In contrast, the placebo group's 3-month VAS scores remained similar to the baseline scores (paired, two-tailed *t*-test: placebo, diff = 0.71 ± 3.51, $p < 0.36$).

3.2. OA-associated function limitation from baseline to the 3-month evaluation

Functional limitations were assessed by the WOMAC stiffness and function scores as well as by IKDC. The dynamics of these scores from baseline to the 3-month evaluation for the placebo group and for each treatment group separately are presented in Figure 3. At baseline, the WOMAC evaluation indicated that the mean stiffness (~4) and functional limitation (~34) levels were moderate in all groups. A significant improvement, represented by reduced WOMAC scores, was observed at the 1-month evaluation in both the placebo group (stiffness, diff = -0.71 ± 1.52, $p < 0.04$; function, diff = -4.25 ± 8.96; $p < 0.04$) and the combined treatment groups (stiffness, diff = -1.12 ± 1.57; $p < 0.001$; function, diff = -5.30 ± 9.00; $p < 0.001$). There were no significant differences in WOMAC stiffness between the treatment and the placebo groups at one-month post-injection. The WOMAC stiffness scores remained similar at the 3-month evaluation to those at the 1-month evaluation in the combined treatment groups, indicating preservation of the gained improvement. In contrast, the stiffness scores of the placebo group increased, indicating deterioration and loss of the previously gained improvement.

IKDC scores at baseline were low, indicating a substantial level of functional limitation across the study groups. At the 1-month evaluation, there was a significant increase in the IKDC scores in the combined treatment group (diff = 10.8 ± 12.58, $p < 0.001$), reflecting treatment-induced improvement, but not in the placebo group (diff = 5.4 ± 13.22, $p < 0.08$). The between-group difference did not reach a level of significance. At the 3-month evaluation, there was no additional change in IKDC scores in either the placebo group or the RegenoGel arm. The RegenoGel-OSP treatment showed additional improvements, as indicated by increases in the IKDC score. However, the difference did not reach a level of significance between the placebo and the combined treatment groups (pairwise comparison, $p < 0.098$).

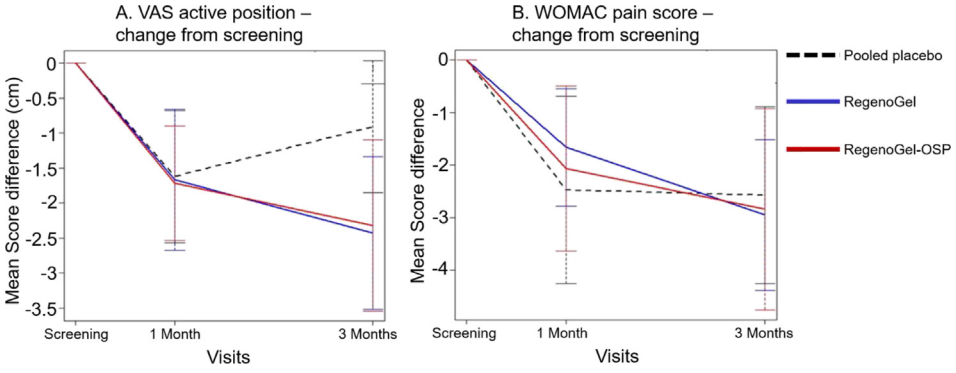

Figure 2. Changes in pain scores from baseline to the 3-month evaluation. A. Differences in the mean VAS scores under active position between the values obtained at baseline and those obtained one and three months after the first injection in the placebo (dashed line), RegenoGel (blue), and RegenoGel-OSP (red) groups. **B.** Differences in the mean WOMAC pain scores between the values obtained at baseline and those obtained 1 and 3 months after the first injection in the placebo (dashed line), RegenoGel (blue), and RegenoGel-OSP (red) groups. Vertical lines denote standard deviations.

Table 3. Changes in the pain scores as registered at one month after the first injection.

Group	VAS under active position		WOMAC pain	
	Difference from baseline*	*p* value (paired, two-tailed *t*-test)	Difference from baseline*	*p* value (paired, two-tailed *t*-test)
Placebo	-1.62 ± 2.09	<0.002	-2.48 ± 3.9	<0.01
RegenoGel	-1.67 ± 2.22	<0.003	-1.67 ± 2.46	<0.01
RegenoGel-OSP	-1.71 ± 1.79	<0.001	-2.07 ± 3.45	<0.01

* Mean ± standard deviation.

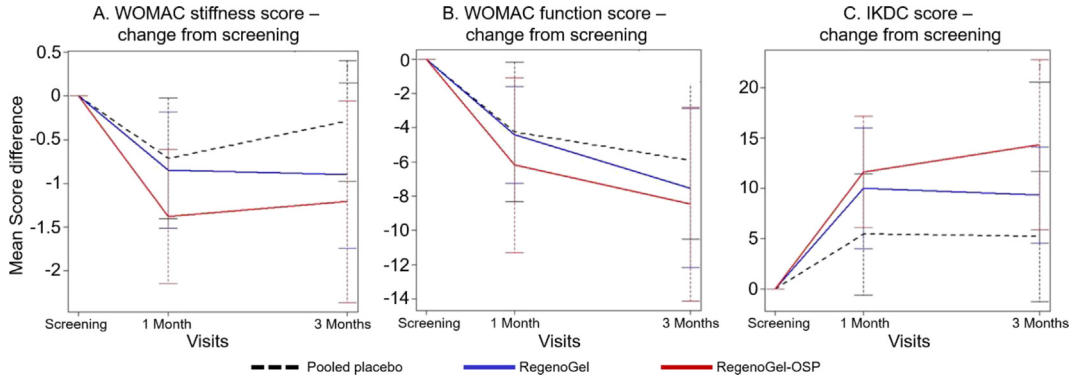

Figure 3. Changes in function limitation scores from baseline to the 3-month evaluation. A-C. Differences in the mean WOMAC stiffness (A), WOMAC function (B), and IKDC (C) scores between the baseline values and those obtained one and three months after the first injection in the placebo (dashed line), RegenoGel (blue), and RegenoGel-OSP (red) groups. Note; positive IKDC values indicate improvement. Vertical lines denote standard deviations.

3.3. Quality of life

The SF-12 mental and physical normalized scores obtained at baseline and at the 1- and 3-month evaluations are presented in Figure 4. The mean SF-12 mental scores at baseline were similar in all groups (~49), indicating overall satisfactory mental status of the participants (US national normalized mean score = 50). Moreover, no significant changes were observed in the mean SF-12 mental scores at the 1- and 3-months evaluation compared to the baseline evaluation, nor were there any significant between-group differences.

The mean physical scores were ~32 in all groups, indicating a difference of ~2 standard deviations below the American national normalized mean score. At the 1-month evaluation, the SF-12 mean physical score in the combined treatment group did not differ from that obtained at baseline and was similar to the mean score obtained in the placebo group. When evaluated at three months after the first injection, the combined treatment group score became significantly higher (indicating improvement) compared to baseline (paired, two tailed *t*-test, diff = 4.08 ± 9.92, $p < 0.01$). The differences in physical scores between the combined treatment group and placebo group were not significant.

3.4. Results at the 6-month evaluation

The participants received the second injection at their 3-month visit according to their assigned arm (Table 2), and were followed for three additional months. Importantly, participants who received a placebo injection were now reassigned to receive one of the two treatment injections. The dynamics of the assessment parameters following the second injection are summarized in Table 4. Figure 5 displays the active state and functional changes in OA symptoms. These results demonstrate that the participants who received two treatment injections exhibited consistent improvements compared to baseline levels in all parameters. As expected, SF-12 mental scores were not affected by the treatment and yielded similar values at baseline and at all post-injection evaluations.

3.5. Safety reports

The reported adverse events (AEs) are presented in Table 5. No serious adverse events (SAEs) were reported. Thirty-four adverse events (AEs) were reported of which twenty were classified as treatment-unrelated and fourteen as possibly related. Based on a physician's

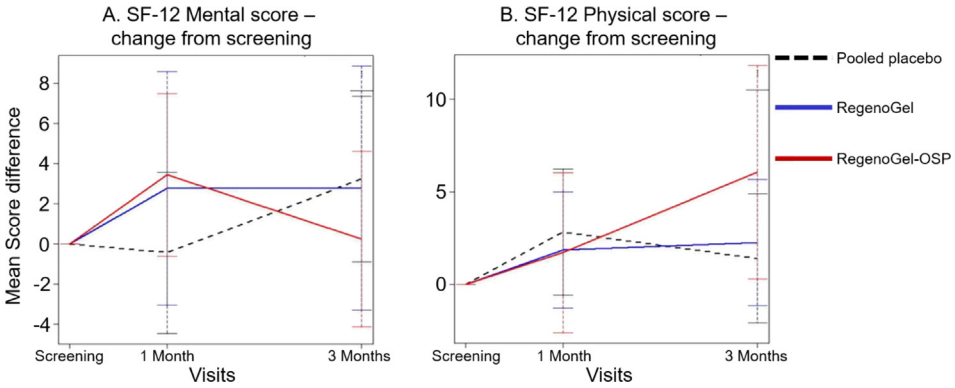

Figure 4. Changes in mean SF-12 physical and mental scores from baseline to the 3-month evaluation. A. Differences in the mean SF-12 mental scores between the baseline values and those obtained one and three months after the first injection in the placebo group (dashed line), RegenoGel arm (blue), and RegenoGel-OSP arm (red). **B.** Differences in the mean SF-12 physical scores between the values obtained at baseline and those obtained one and three months after the first injection in the placebo group (dashed line), RegenoGel arm (blue), and RegenoGel-OSP arm (red). Vertical lines denote standard deviations.

Table 4. Dynamics of the assessment parameters during the follow-up period after receiving the second injection.

Parameters	Groups*	4-month follow-up scores *n*, mean (std	Compared to baseline *p* value	6-month follow-up scores *n*, mean (std	Compared to baseline *p* value
VAS active position	A	N = 9; 5.67 (1.87)	n.s	4.70 (2.41)	0.04
	B	5.43 (2.79)	0.002	5.38 (2.69)	0.001
	C	5.2 (2.97)	0.02	5.70 (2.58)	0.03
	D	4.67 (2.5)	<0.001	4.67 (2.50)	<0.001
VAS passive position	A	N = 9; 3.89 (2.15)	n.s	2.70 (2.54)	n.s
	B	3.57 (2.48)	0.04	4.48 (2.82)	n.s
	C	4.40 (2.84)	n.s	4.00 (2.26)	n.s
	D	2.00 (2.28)	<0.001	3.17 (2.90)	n.s
WOMAC pain	A	N = 9; 7.00 (3.77)	n.s	6.90 (3.54)	0.03
	B	7.71 (3.52)	0.008	8.33 (3.64)	0.01
	C	6.80 (3.49)	n.s	5.50 (3.47)	0.01
	D	4.35 (2.87)	<0.001	5.06 (3.75)	0.003
WOMAC stiffness	A	N = 9; 3.00 (1.80)	0.03	3.80 (1.55)	n.s
	B	3.1 (1.64)	0.03	3.00 (1.7)	0.03
	C	3.00 (1.70)	n.s	2.7 (1.25)	n.s
	D	2.33 (1.53)	p < 0.001	2.94 (1.76)	0.03
WOMAC functional limitation	A	N = 9; 26.11 (14.77)	0.04	30.10 (14.65)	0.04
	B	30.05 (12.28)	n.s	29.62 (13.76)	0.04
	C	24.64 (12.67)	0.05	21.91 (11.01)	0.01
	D	17.72 (10.10)	<0.001	20.94 (14.16)	0.002
IKDC	A	N = 9; 38.38 (16.52)	n.s	41.56 (19.71)	n.s
	B	39.89 (16.62)	0.01	40.57 (15.50)	0.006
	C	44.57 (16.37)	0.05	47.95 (17.52)	0.01
	D	51.61 (15.20)	<0.001	48.68 (20.81)	<0.001
SF-12 mental	A	N = 9; 47.22 (11.22)	n.s	49.89 (19.42)	n.s
	B	49.84 (8.97)	n.s	48.96 (9.17)	n.s
	C	47.01 (9.75)	n.s	49.84 (9.44)	n.s
	D	53.9 (7.53)	n.s	52.76 (8.59)	n.s
SF-12 physical	A	N = 9; 34.66 (9.02)	n.s	38.39 (9.37)	0.05
	B	35.87 (8.06)	n.s	35.90 (9.58)	0.01
	C	34.41 (9.71)	n.s	38.85 (10.60)	n.s
	D	41.25 (7.84)	<0.001	38.41 (11.07)	0.02

* A = Placebo-RegenoGel ($n = 10$); B = RegenoGel x2 ($n = 21$); C – Placebo-RegenoGel-OSP ($n = 10$); D = RegenoGel-OSP x2 ($n = 18$).

evaluation, five AEs (three mild and two moderate) that were classified as treatment-unrelated were managed with supportive care. Twenty-three AEs were resolved during the course of the study and one remained ongoing. A single participant was excluded from the study. Overall, the safety profile was good and local inflammatory signs frequently observed following some HA products were not observed upon either Regenogel or Regenogel-OSP treatments. It is important to note that the association between the AEs and the active treatments was not evaluated at this point to maintain blinding.

4. Discussion

Our main findings are that both RegenoGel and RegenoGel-OSP positively impact on OA symptoms and have good safety profiles. The most prominent improvement was registered for OA-associated pain, with significant decreases in VAS (active) and WOMAC pain scores, already observed at one month following the first injection. A further decrease in pain at three months after the first injection, was evidenced by the difference in VAS compared to the pretreatment score, reaching significance compared to the placebo. Unlike the placebo, the extent of treatment induced improvement in OA-associated pain levels were consistent and can be considered clinically relevant [30]. Following the second injection, both treatments produced a substantial and consistent improvement compared to baseline levels. This effect seemed to be somewhat stronger in the group that received two injections of the autologous plasma-based product, RegenoGel-OSP.

The VAS scores under passive position demonstrated similar albeit weaker results. Lower pain scores are typically reported when no active movement is required which reduces the range of potential changes and makes treatment effects harder to detect.

A trend towards treatment-induced improvement was also seen in OA-associated stiffness and functional limitation, as assessed by the WOMAC-stiffness, WOMAC-function, and IKDC scores. A significant difference from baseline levels was demonstrated in all of these scores at one- and three-months after the first injection, while the placebo group demonstrated short-term improvement that was subsequently lost. Similar trends were preserved during the follow-up period after the second injection, demonstrating that the treatment-induced improvement was stable and consistent, with somewhat stronger results recorded for participants who received RegenoGel-OSP. Importantly, the consistency of the treatment-related effects was maintained despite the highly variable and subjective measures.

Quality of life assessment (SF-12) showed an improved physical state following treatment (first injection) and no change compared to baseline following placebo. As expected, the SF-12 mental scores were not sensitive to the treatment. This is in line with OA characteristics, which at

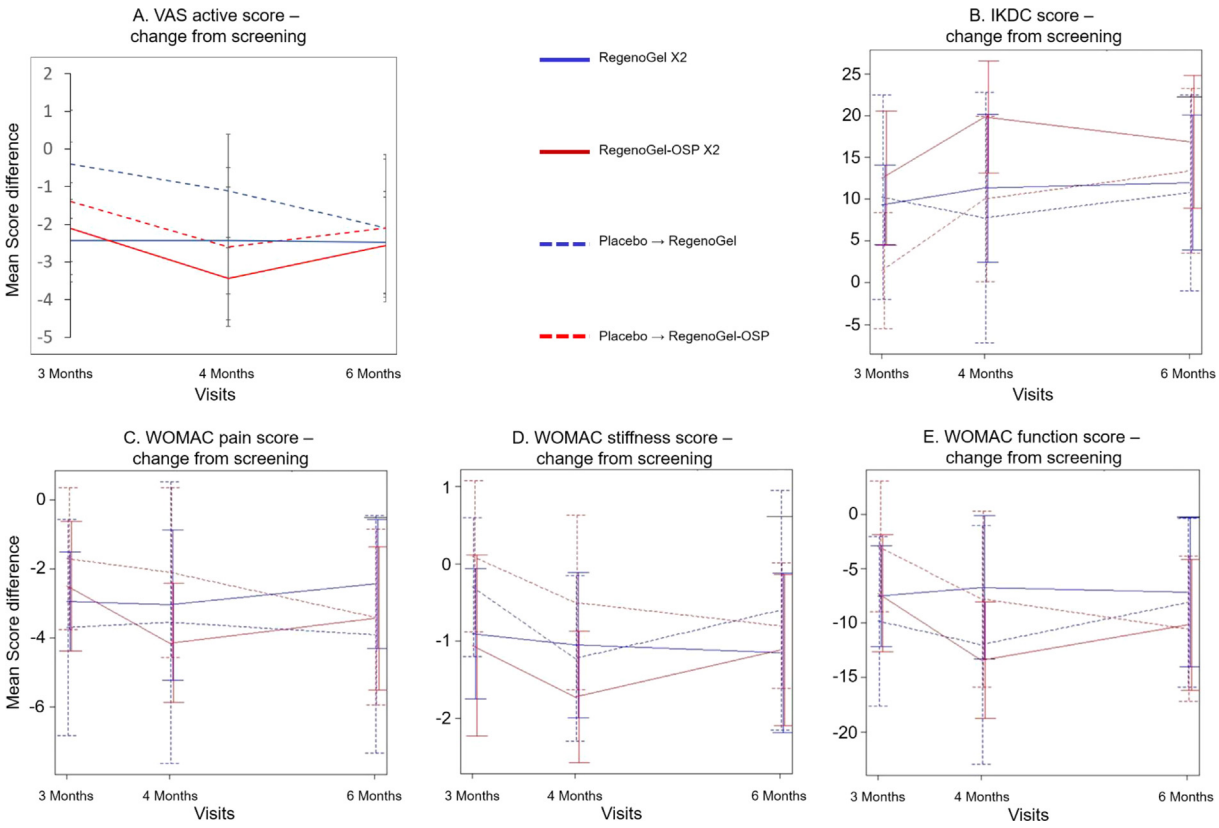

Figure 5. Changes in VAS, IKDC, and WOMAC scores between baseline and 3, 4, and 6 months following the first injection. A-B. Differences in the mean VAS scores under active position (A) and IKDC scores (B) between the values obtained at baseline and those obtained at 3, 4, and 6 months after the first injection in the two groups whose second injection (administered three months after the first) was the same as the first (Regenogel x2 in solid blue, RegenoGel-OSP x2 in solid red), and the two groups who switched from placebo to treatment (Placebo→RegenoGel in dashed blue, Placebo→RegenoGel-OSP in dashed red). Note, positive IKDC scores indicate improvement. **C-E.** Differences in the mean WOMAC scores for pain (C), stiffness (D), and functional limitation (E) between values obtained at baseline and those obtained at 3, 4, and 6 months after the first injection in the two groups whose second injection (administered three months after the first) was the same as the first (Regenogel x2 in solid blue, RegenoGel-OSP x2 in solid red), and the two groups who switched from placebo to treatment (Placebo→RegenoGel in dashed blue, Placebo→RegenoGel-OSP in dashed red). Vertical lines denote standard deviations.

Table 5. Adverse events.

	Severity	Unrelated	Possibly Related
Adverse Events Total = 34	Mild	13	5
		Ongoing = 6 Resolved = 7 Unknown = 0	Ongoing = 2 Resolved = 2 Unknown = 1
	Moderate	7	6
		Ongoing = 4 Resolved = 3 Unknown = 0	Ongoing = 4 Resolved = 2 Unknown = 0
	Severe	0	3
			Ongoing = 1 Resolved = 1 Unknown = 0 Discontinued = 1

the assessed severity levels do not include a mental component, further suggesting that the obtained improvements are treatment-specific rather than holistic effects generally associated with receiving attentive care.

Significant placebo effects were registered in all parameters, and most of the treatment-placebo comparisons failed to reach levels of significance as a result. It is well-recognized that demonstrating significant differences between intraarticular treatment and intraarticular placebo in OA patients is extremely challenging [31, 32]. Saline injections can have actual positive impacts on osteoarthritic knees [32], especially for pain, stiffness, and self-reported function. Taking into account the extent of an expected placebo effect, the significant reduction in VAS scores in the treatment groups compared to the placebo group three months after the first injection further supports the potential effectiveness of RegenoGel treatments.

The benefits of one RegenoGel treatment over the other are inconclusive. There are inherent differences between the two conjugates, autologous vs. purified fibrinogen, in terms of the presence of platelets, and quality following extraction and purification. Platelets and their products play an important role in tissue regeneration [31]. Platelet-rich plasma injections have been used to treat knee cartilage degeneration with results being inconclusive in some studies and effective in others [31, 33]. RegenoGel-OSP includes less processed plasma, thereby retaining viable platelets, active platelet products and potentially other plasma components. This may be potentially advantageous for RegenoGel-OSP over RegenoGel. Fibrin is a major blood component responsible for hemostasis, in addition to its numerous beneficial properties as a versatile scaffold [16], and being critically involved in stimulating tissue regeneration [17, 18]. Industrially purified fibrin enriched preparations constitute highly concentrated fibrin, thus providing more stable fibrin-HA conjugates which are potentially beneficial to the RegenoGel conjugate. Based on the current results, it would appear that both treatments are similarly effective. An attractive alternative being considered is to enhance the autologous, plasma-based product with purified fibrinogen to achieve optimal mechanical and biological properties, thereby having the best of both worlds.

Most regenerative-medicine approaches employ single-polymer-

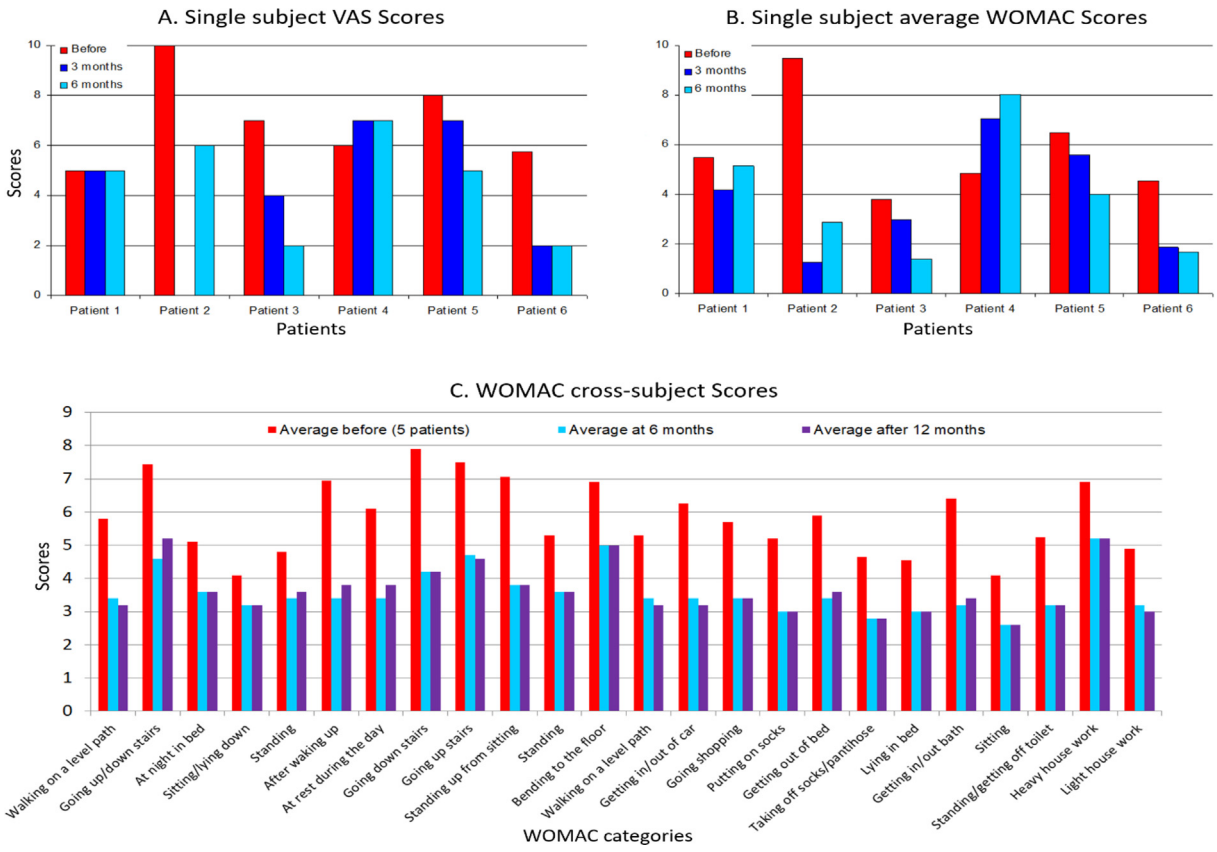

Figure 6. Results of a phase I, prospective, open-label, clinical trial for safety evaluation of intra-articular injection of RegenoGel for the treatment of moderate to severe osteoarthritis. A single intra-articular injection of RegenoGel was administered to six patients diagnosed with grade IV (Kellgren-Lawrence) OA. No adverse effects of any kind were reported. Blood chemistry was normal and was not affected by the treatment. A. Single patient VAS scores before treatment (red) and at three (dark blue) and six (light blue) months post treatment. B. Single patient cross-categories WOMAC scores before treatment (red) and at three (dark blue) and six (light blue) months post treatment. C. Cross-patient (n = 5) WOMAC scores separately presented for each WOMAC category before treatment (red) and at six (light blue) and 12 (purple) months post treatment. * Patient 4 was determined as an outlier, as it was discovered that a detached piece of cartilage was freely floating in his knee space prior to treatment. It is reasonable to assume that this cartilage particle was pinned to a confined area by the RegenoGel injection, potentially causing increased damage thus leading to "treatment-induced worsening" of the measured scores.

based scaffolds that are inherently incapable of mimicking the complexity and authentic properties of the multifactorial and diverse tissue extracellular matrices [34]. The fibrinogen component in RegenoGel provides cell-adhesion sites and self-assembly properties, and at the same time acts as an ancillary agent to enhance the stability and mechanical properties of HA. The resulting hybrid hydrogel demonstrated favorable mechanical properties under multiaxial loading, retaining water and shape, being highly elastic, and less brittle [23]. Thus, it is expected to withstand significant shear stress compared to viscous liquids such as HA or to fibrin alone.

The effects of RegenoGel on pain and function demonstrated by the present analysis may be attributed to its protection and support of cartilage integrity, as observed in animal models of damage-induced OA [22]. Such effects were not observed following multiple injections of either low or high-molecular-weight HA. It is therefore suggested that RegenoGel's beneficial effects may extend beyond mechanical protection from friction, although the exact mechanism has not yet been clarified. One such mechanism can be attributed to their biocompatibility as well as their capacity to retain and deliver bioactive agents [35, 36]. Embedded with chondrocytes and mesenchymal stem cells the Regenogel matrix supports cell survival, proliferation and differentiation which may also promote focal cartilage repair [20, 21, 23]. A limited open label, clinical study (Figure 6) has also demonstrated long-term improvement (12 months) following treatment with RegenoGel, implying possible long-term joint tissue preservation. Larger, long-term follow-up studies with objective assessments of cartilage in OA are required to evaluate and better understand the potential long-term preservation properties of RegenoGel.

The presented interim analysis is limited by the small sample size, and substantial between-participant variability that is evident mostly when between-group comparisons are performed, masking potential clinically relevant, treatment-specific benefits. In addition, the follow-up period was relatively short, not allowing sufficiently extended evaluations of the treatment-affected stability. Although six months follow-up is generally accepted, longer follow-up periods are indicated in this context.

5. Conclusions

Our preliminary results demonstrate a potentially consistent benefit of RegenoGel and RegenoGel-OSP in treating the symptoms of OA of the knee, as reflected by VAS, WOMAC, IKDC, and SF-12 survey scores. Taken together with their excellent safety profile, this evidence supports the continued testing and development of both Regenogel and Regenogel-OSP for treating this ubiquitous and insidious condition.

Declarations

Author contribution statement

A. Yayon: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

L. Kandel, G. Agar, O. Elkayam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

O. Slevin, A. Sharipov, M. Dahan, G. Rivkin, V. Aloush, Y. Brin, A. Pyeser and Y. Beer: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare the following conflict of interests: A. Yayon is the CEO of Procore Ltd.

Additional information

The clinical trial described in this paper was registered at ClinicalTrials.gov under the registration number NCT03479749.

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