Double Blind Placebo Controlled Study Confirms Rapid 10-Day Results Seen in Previous Human Trials:

Natural Eggshell Membrane (NEM®) is a Natural Therapeutic Choice for Joint & Connective Tissue Disorders

Abstract

Introduction: Natural Eggshell Membrane (NEM®) is a new novel dietary supplement that contains naturally occurring glycosaminoglycans and proteins essential for maintaining healthy joint and connective tissues. Two single center, open label pilot clinical studies were conducted to evaluate the efficacy and safety of NEM® as a treatment for pain and inflexibility associated with joint and connective tissue disorders. The follow-up randomized, multicenter, double blind, placebo controlled Osteoarthritis Pain Treatment IncorpOrating NEM® (OPTION) clinical study was conducted to evaluate the efficacy and safety of NEM® as a treatment for pain and stiffness associated with osteoarthritis of the knee.

Methods: Patients received oral NEM® 500 mg once daily for four weeks (open label) or eight weeks (placebo controlled). The primary outcome measure for the open label trials was to evaluate the change in general pain associated with the treatment joints/areas at 7 and 30 days. In the Single-Arm Pilot Trial, range of motion (ROM) and related ROM-associated pain was also evaluated. The primary endpoint for the OPTION trial was the change in overall Western Ontario and McMasters Universities (WOMAC) Osteoarthritis Index, as well as pain, stiffness, and function WOMAC subscales measured at 10, 30, and 60 days.

Results: Single-Arm Pilot Trial: Supplementation with NEM® produced a significant treatment response at 7 days for flexibility (27.8% increase, P = 0.038) and at 30 days for general pain (72.5% reduction, P = 0.007), flexibility (43.7% increase, P = 0.006), and ROM-associated pain (75.9% reduction, P = 0.021). Double-Arm Pilot Trial: Supplementation with NEM® produced a significant treatment response for pain at 7 days for both treatment arms (X: 18.4% reduction, P = 0.021, Y: 31.3% reduction, P = 0.014). There was no clinically meaningful difference between treatment arms at 7 days, so the Y arm crossed over to the X formulation for the remainder of the study. The significant treatment response continued through 30 days for pain (30.2% reduction, P = 0.0001). Placebo Controlled OPTION Trial: Supplementation with NEM® produced an absolute rate of response that was statistically significant (up to 26.6%) versus placebo at all time points for both pain and stiffness, and trended toward improvement for function and overall WOMAC scores. Rapid responses were seen for mean pain subscores (15.9% reduction, P = 0.036) and mean stiffness subscores (12.8% reduction, P = 0.024) occurring after only 10 days of supplementation. At 60 days, pain response was maintained (15.4%, P = 0.038), while stiffness had improved further to 26.6% reduction (P = 0.005). Mean function subscores showed a 15.5% (P = 0.084) absolute improvement versus placebo at 10 days, which fell slightly to 13.5% (P = 0.076) by day 60. Overall mean WOMAC scores resulted in a 15.2% (P = 0.059) absolute improvement versus placebo at 10 days, which was maintained at 60 days (15.1%, P = 0.052). There were no serious adverse events reported during any of the studies and the treatment was reported to be extremely well tolerated by study participants.

Conclusions: Natural Eggshell Membrane (NEM®) is a possible new effective and safe therapeutic option for the treatment of pain and inflexibility associated with joint and connective tissue (JCT) disorders, particularly osteoarthritis (OA). Supplementation with NEM®, 500 mg taken once daily, significantly reduced pain and stiffness, both rapidly (7-10 days) and continuously (60 days). It also showed clinically meaningful results from a brief responder analysis,

demonstrating that significant proportions of treated patients will be helped considerably with NEM® supplementation. Subjects taking NEM® did not report any gastric or cardiac side effects associated with long-term use of other JCT or OA treatments, such as NSAIDs. The Clinical Trial Registration numbers for these trials are: NCT00750230, NCT00750854, and NCT00750477.

Introduction

It is estimated that 140 million adults in the U.S. suffer from some form of joint or connective tissue (JCT) disorder (i.e. arthritis, lupus, gout, fibromyalgia, neck or back pain, etc.) with arthritis being the most prevalent (1; 2). Osteoarthritis (OA) is by far the most common form of arthritis and is estimated to affect nearly 27 million adults in the U.S., with one third of those 65 and older having been diagnosed with OA (2). As the population ages, this estimate is expected to grow rapidly. Traditional treatments for most of these disorders attempt to address only the symptoms (pain, inflammation, and discomfort) associated with the diseases. This usually involves the use of analgesics (i.e. acetaminophen, oxycodone, propoxyphene) or non-steroidal anti-inflammatory drugs (NSAIDs) (i.e. ibuprofen, diclofenac, celecoxib), alone or in combination. Most of these treatments have shown limited effectiveness in randomized controlled clinical trials (RCTs) (3; 4; 5; 6) or are known to have significant and sometimes severe side effects. To avoid the cardiac risks (7; 8), gastrointestinal issues (9; 10), and dependency issues (11; 12) associated with traditional treatments (particularly with long-term use), many patients have turned to complementary and alternative medicines (CAMs) such as dietary supplements.

Glucosamine and chondroitin alone and in combination, are widely marketed as dietary supplements to treat joint pain due to osteoarthritis. There have been two major human clinical trials that have investigated the role of these two dietary supplements in the treatment of OA symptoms. The Glucosamine/chondroitin Arthritis Intervention Trial (GAIT), a 1583 patient, 6 month trial sponsored by the National Institutes of Health (NIH), failed to show significant improvement in the Western Ontario and McMasters Universities (WOMAC) Osteoarthritis Index in the overall patient population for glucosamine, chondroitin, or their combination (13). The Glucosamine Unum In Die (once-a-day) Efficacy (GUIDE) trial, a 318 patient, 6 month European trial sponsored by industry, showed a small, 5-6% improvement in total WOMAC Index score over placebo for glucosamine sulfate (14). Because of their limited effectiveness, the search for additional CAMs to treat OA continues.

Other vitamins, minerals, and botanicals such as methylsulfonylmethane (MSM), *S*-adenosylmethionine (SAMe), kava, pine bark extract, capsicum, boswellia root extract, turmeric/curcumin, etc. are also marketed for various JCT pain maladies. We present here the use of eggshell membrane as a possible new natural therapeutic for JCT disorders. In the U.S. alone, an estimated 600,000 tons of eggshells are produced annually as a by-product of the poultry industry (15). Disposal of these eggshells creates an environmental and financial burden and, therefore, alternative uses for these materials would be of obvious benefit. Eggshell membrane is primarily composed of fibrous proteins such as Collagen Type I (16). However, eggshell membranes have also been shown to contain glycosaminoglycans, such as dermatan sulfate and chondroitin sulfate (17), sulfated glycoproteins including hexosamines, such as glucosamine (18), hyaluronic acid (19), sialic acid (20), desmosine and isodesmosine (21), ovotransferrin (22), lysyl oxidase (23), and lysozyme (24). The discovery of eggshell membrane as a natural source of combined collagen, glucosamine, chondroitin, and hyaluronic acid has prompted the evaluation of this material as a potential treatment for joint and connective tissue pain. ESM Technologies, LLC (Carthage, MO) has developed methods to efficiently and effectively separate eggshell membrane from eggshells to create an essentially shell-free eggshell membrane. The isolated

membrane is then partially hydrolyzed using a proprietary process and dry-blended to produce 100% pure Natural Eggshell Membrane (NEM®). Compositional analysis of NEM® conducted by ESM has identified a high content of protein and moderate quantities of glucosamine, chondroitin sulfate, hyaluronic acid, and collagen (primarily Type I).

Initially, two 1-month pilot clinical studies were conducted to evaluate the efficacy and safety of NEM® for the relief of the pain and discomfort associated with joint and connective tissue disorders. Based on the preliminary positive results from these pilot studies, a follow-up eight week randomized, multicenter, double blind, placebo controlled supplementation trial was conducted to evaluate the efficacy and safety of NEM® for the relief of the pain and stiffness associated with moderate OA of the knee – the Osteoarthritis Pain Treatment IncorpOrating NEM® (OPTION) trial. The results of these trials are presented herein. To review the study design, patient eligibility, or statistical analysis parameters for the studies please see the **Patients and Methods** sections of the full published references: Pilot Studies (*Clinical Interventions in Aging*) (25) or the OPTION Study (*Clinical Rheumatology*) (26).

Treatment Response

Single-Arm Pilot Trial: The primary outcome measure of this study was to evaluate the mean effectiveness of NEM® (500 mg, once daily) in relieving general pain associated with moderate JCT disorders. Additional primary outcome measures were to evaluate flexibility, as well as the pain associated with the range of motion (ROM) evaluation. The primary treatment response endpoints were the 7 & 30 day clinic assessments utilizing a zero to 10 analog Likert-scale, with zero equating to no pain and 10 equating to most severe pain. Patients were asked to record a number equating to the perceived pain from the treatment joint/area. Endpoints were then compared to pretreatment assessments.

Double-Arm Pilot Trial: The primary outcome measure of this study was to evaluate the mean effectiveness of NEM® (500 mg, once daily) in relieving general pain associated with moderate JCT disorders. Subjects were allowed to evaluate multiple treatment joints/areas. The primary treatment response endpoints were the 7 & 30 day clinic assessments utilizing a zero to 10 analog Likert-scale, with zero equating to no pain and 10 equating to most severe pain. Patients were asked to record a number equating to the perceived pain from the treatment joints/areas. Endpoints were then compared to pretreatment assessments.

Placebo-controlled OPTION Trial: The primary endpoint of this study was measurement of the effectiveness of NEM® (500 mg, once daily) in relieving pain, stiffness, and discomfort associated with moderate OA of the knee and to compare its effectiveness to placebo. The primary treatment response endpoints were the 10, 30, & 60 day clinic assessments utilizing the Western Ontario and McMasters Universities (WOMAC) Osteoarthritis Index – Visual-Analog Scale (100 mm) version (VA 3.1) (27). This version of the WOMAC questionnaire consists of five questions addressing the severity of joint pain, two questions addressing joint stiffness, and seventeen questions addressing limitations in performing physical activities (function). Endpoints were compared to pretreatment assessments and to placebo controls.

Results

Single-Arm Pilot Trial: A total of eleven (11) subjects were enrolled with various joint and connective tissue conditions. The treatment joints/areas consisted of knees (3), hips (1), elbows (1), neck (1), shoulders (1), & lower back (4). All eleven subjects completed baseline evaluations and were therefore used as the intent to treat (ITT) population. Ten (91%) of the eleven ITT subjects completed the one month study per the protocol. Compliance with the study treatment regimen was good in the treatment group. In those subjects that completed the study, the rate of compliance was >98% (as judged by capsule count at clinic visits).

Single Arm Trial

Mean values for NEM® supplemented treatment group at baseline and 7 & 30 days post treatment

	Days post-treatment	Mean ± SD	Percent Improvement	*P*-value
General Pain	Baseline (n = 11)	3.6 ± 1.8	-	-
	7 (n = 11)	2.7 ± 1.7	25.8%	0.515
	30 (n = 11)	1.0 ± 1.2	72.5%	*0.007
Flexion (ROM)	Baseline (n = 11)	64.2° ± 36.5°	-	-
	7 (n = 11)	82.0° ± 41.4°	27.8%	*0.038
	30 (n = 11)	92.2° ± 38.4°	43.7%	*0.006
ROM Pain	Baseline (n = 11)	2.9 ± 2.6	-	-
	7 (n = 11)	1.7 ± 2.1	43.3%	0.112
	30 (n = 11)	0.7 ± 1.3	75.9%	*0.021

P-values were determined by pairwise, two-sided, t-test comparison, and represent treatment versus baseline. *$P < 0.05$

A clinical comparison of valid subjects was carried out to obtain a mean baseline pain value for the study population of 3.6 ± 1.8 (mean ± standard deviation (SD)), a mean flexion range of motion (ROM) of 64.2° ± 36.5 °, and a mean ROM-associated pain value of 2.9 ± 2.6. Statistical analysis of the primary outcome measures revealed that supplementation with NEM® produced a significant treatment response at 7 days for flexibility (27.8% increase, P = 0.038) and at 30 days for general pain (72.5% reduction, P = 0.007), flexibility (43.7% increase, P = 0.006), and ROM-associated pain (75.9% reduction, P = 0.021).

Double Arm Trial

Mean pain values for NEM® supplemented treatment group at baseline and 7 & 30 days post treatment

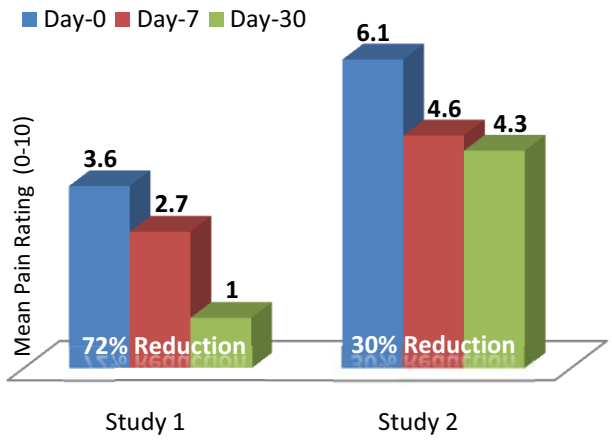
Days post-treatment	X Mean ± SD	Y Mean ± SD	Percent Improvement	*P*-value
Baseline (n = 12, 14)	6.8 ± 1.9	5.6 ± 1.9	-	-
7 (n = 12, 14)	5.5 ± 2.0	3.9 ± 2.5	18.4% 31.3%	*0.021 *0.014
30 (n = 26)	4.3 ± 2.3		72.5%	30.2%

P-values were determined by pairwise, two-sided, t-test comparison, and represent treatment versus baseline. *$P < 0.05$

Double-Arm Pilot Trial: A total of twenty-six (26) subjects were enrolled with various joint and connective tissue conditions, some with multiple treatment joints/areas. The treatment joints/areas consisted of knees (6), hips (8), neck (1), shoulders (8), hands (2), legs (1), feet (1), lower back (4), & non-specific (3). All 26 subjects completed baseline evaluations and were therefore used as the intent to treat (ITT) population. Twenty (77%) of the 26 ITT subjects completed the one month study per the protocol. Compliance with the study treatment regimen was good in the treatment group. In those subjects that completed the study, the rate of compliance was >96% (as judged by capsule count at clinic visits).

Effect of NEM® on Joint Pain

In Open-label Clinical Trials



Single Arm Trial

Approx. 1/3 of patients experienced >30% reduction in pain @ 7 Days.

Approx. 1/3 of patients experienced >50% reduction in pain @ 30 Days.

Double Arm Trial

Approx. 2/3 of patients experienced >50% reduction in pain @ 30 Days.

Approx. 1/2 of patients reported that they were Pain-Free @ 30 Days.

Approx. 1/2 of patients experienced >50% improvement in flexibility @ 30 Days.

A clinical comparison of valid subjects was carried out to obtain a mean baseline pain value (mean ± SD) for each arm (X & Y) of the study (X: 6.8 ± 1.9, Y: 5.6 ± 1.9). Patient data was initially evaluated to ensure randomization between groups (P = 0.097). Statistical analysis of the primary outcome measures revealed that supplementation with NEM® produced a significant rapid treatment response for pain at 7 days for both treatment arms (X: 18.4% reduction, P = 0.021, Y: 31.3% reduction, P = 0.014). There was no clinically meaningful difference between treatment arms at 7 days, so the Y arm crossed over to the X formulation for the remainder of the study. The significant treatment response continued through 30 days for pain (30.2% reduction, P = 0.0001.

Placebo-controlled OPTION Trial: A total of 67 subjects were enrolled in the trial and underwent randomization. Of these subjects, 61.1% were from site 1, 29.9% from site 2, and 9.0% from site 3. In terms of OA functional grades, 20.9% were Grade I, 28.4% were Grade II, 20.9% were Grade III, and 29.9% were unassigned. Seven subjects did not complete baseline evaluations, resulting in a total of 60 subjects in the intent to treat (ITT) population. Thirty-one subjects (51.6%) were randomized to the placebo group and 29 subjects (48.3%) were randomized to the NEM® treatment group. Thirty-one percent (31%) of the ITT subjects assigned to NEM® did not complete the 2-month study per the protocol, compared with 42% of the ITT subjects assigned to placebo. Of the 60 subjects in the ITT population, 6 subjects assigned to placebo and 2 subjects assigned to NEM® either violated the protocol or did not begin treatment and, therefore, were not available for further analysis. Those patients lost to follow-up before the first evaluation time point in both the placebo (4 patients) and treatment (3 patients) groups had symptomatically mild OA (mean WOMAC 39.7 and 45.6, respectively). Those patients lost to follow-up (primarily withdrawals) in the remainder of the study in both the placebo (3 patients) and the treatment (4 patients) groups had symptomatically more severe OA (mean WOMAC 76.6 and 63.7, respectively) compared to those patients that completed the study (mean WOMAC at baseline of 52.6 and 45.3, respectively). Five (5) patients in the placebo group and 4 patients in the treatment group officially withdrew from the study due to lack of efficacy. There were no obvious differences in the reason for withdrawal between the study groups. Compliance with the study treatment regimen was good in both treatment groups. In those subjects that completed the study, the rate of compliance was >97% (as judged by capsule count at clinic visits).

Double Blind, Placebo Controlled Trial

Mean WOMAC Scores by category.
NEM® supplemented and control groups at baseline, 10, 30, & 60 days post treatment.

		TREATMENT		
	Days post-treatment	Placebo	NEM®	*P*-value
Pain	Baseline (n = 25, 25)	50.6 ± 19.4	44.0 ± 16.8	0.204
	10 (n = 21, 24)	52.7 ± 24.1	39.0 ± 19.4	*0.036
	30 (n = 21, 24)	53.7 ± 21.0	42.3 ± 26.2	*0.040
	60 (n = 21, 24)	50.7 ± 22.2	37.5 ± 25.2	*0.038
Stiffness	Baseline (n = 25, 25)	59.3 ± 24.0	50.5 ± 20.3	0.167
	10 (n = 21, 24)	57.0 ± 25.6	42.5 ± 25.0	*0.024
	30 (n = 21, 24)	60.6 ± 23.0	43.5 ± 23.5	*0.009
	60 (n = 21, 24)	56.5 ± 24.3	35.0 ± 25.8	*0.005
Function	Baseline (n = 25, 25)	55.2 ± 21.3	48.1 ± 19.5	0.227
	10 (n = 21, 24)	57.3 ± 24.6	43.3 ± 23.0	0.084
	30 (n = 21, 24)	55.6 ± 21.8	45.1 ± 25.5	0.079
	60 (n = 21, 24)	53.1 ± 24.9	40.5 ± 27.1	0.076
Overall	Baseline (n = 25, 25)	54.6 ± 20.4	47.5 ± 17.5	0.191
	10 (n = 21, 24)	56.2 ± 24.1	42.3 ± 21.6	0.059
	30 (n = 21, 24)	55.5 ± 21.4	44.4 ± 25.1	0.055
	60 (n = 21, 24)	52.9 ± 23.9	39.4 ± 26.1	0.052

P-values were determined by pairwise, two-sided, t-test comparison, and represent treatment versus baseline. *$P < 0.05$

Patient data was initially evaluated to ensure randomization within each site. Additionally, patient data was evaluated between sites to exclude site bias. As there were no abnormalities in these evaluations, the data were pooled for all subsequent analyses. A clinical comparison of valid (excluding non-compliance) subjects was carried out to obtain mean baseline values. In all cases, the treatment group values were slightly lower than those of the control group, but were not statistically different. Analysis of the primary outcome measure revealed that supplementation with NEM® produced an absolute rate of response that was significantly better (ranging from 10.3% to 26.6% improvement) than placebo at all time points for both

pain and stiffness, but fell short of significance for function and overall WOMAC, despite improving by 8.8% to 15.5%. There were rapid responses seen for mean pain subscores (15.9% reduction, P = 0.036) and mean stiffness subscores (12.8% reduction, P = 0.024) occurring after only 10 days of supplementation. At 60 days, pain response was maintained (15.4%, P = 0.036), while stiffness had improved further to 26.6% reduction (P = 0.005). Mean function subscores showed a 15.5% (P = 0.084) absolute improvement versus placebo at 10 days, which fell slightly to 13.5% (P = 0.076) by day 60. Overall mean WOMAC scores resulted in a 15.2% (P = 0.059) absolute improvement versus placebo at 10 days, which was maintained at 60 days (15.1%, P = 0.052).

Absolute Treatment Effect (%) in WOMAC Scores From Baseline.

NEM® supplemented and control groups at 10, 30, and 60 days post treatment.

	Days post-treatment	Placebo	NEM®	Absolute Treatment Effect
Pain	10 (n = 21, 24)	+4.2%	-11.7%	-15.9%
	30 (n = 21, 24)	+6.0%	-4.3%	-10.3%
	60 (n = 21, 24)	+0.1%	-15.3%	-15.4%
Stiffness	10 (n = 21, 24)	-3.9%	-16.7%	-12.8%
	30 (n = 21, 24)	+2.2%	-14.6%	-16.8%
	60 (n = 21, 24)	-4.7%	-31.3%	-26.6%
Function	10 (n = 21, 24)	+3.9%	-11.6%	-15.5%
	30 (n = 21, 24)	+0.8%	-8.0%	-8.8%
	60 (n = 21, 24)	-3.8%	-17.3%	-13.5%
Overall	10 (n = 21, 24)	+2.9%	-12.3%	-15.2%
	30 (n = 21, 24)	+1.7%	-7.9%	-9.6%
	60 (n = 21, 24)	-3.1%	-18.2%	-15.1%

P-values were determined by pairwise, two-sided, t-test comparison, and represent treatment versus baseline. $*P < 0.05$

All study populations were too small to stratify the patients according to covariates, such as gender, treatment joint/area, or baseline pain level to obtain statistically relevant data. There were no serious adverse events reported during any of the studies. Of particular note is that there were no allergy-associated adverse events during the studies, although those with known egg allergies were excluded from participating during screening. In general, the treatment was reported to be extremely well tolerated by study participants.

Discussion

Joint and connective tissue disorders are extremely common in the United States and result in significant costs, both financial and quality-of-life, for those that suffer from the debilitating diseases. These human clinical trials were designed to evaluate the efficacy and safety of Natural Eggshell Membrane as a treatment option for JCT disorders, particularly osteoarthritis. Results from these studies indeed indicate that NEM® is both effective and safe for treating pain associated with JCT disorders and considerably improves flexibility and reduces stiffness in the affected joints/areas. NEM® has the added benefit of avoiding the concerning side effects associated with long-term use of other JCT treatments, such as narcotics or NSAIDs.

Single-Arm & Double-Arm Pilot Trials: Patients experienced relatively rapid (7 days) responses for pain (Double-Arm) with a mean response of approximately 25% (X: 18.4% & Y: 31.3%) and flexibility (Single-Arm) with a mean response of approximately 28%. By the end of the follow-up period (30 days) the mean response for pain had improved to 30% (Double-Arm) and 73% (Single-Arm). At the same time, flexibility improved to a mean response of approximately 44% and the ROM-associated pain had a mean response of approximately 76% (Single-Arm). A brief responder analysis of the data provides a number of clinically relevant highlights. In both the Single-Arm Pilot Trial and the Double-Arm Pilot Trial, a significant proportion of the study populations (64% & 35%, respectively) experienced a greater than 50% reduction in pain by 30 days. Of particular note is that nearly half (45%) of the patients in the Single-Arm Pilot Trial reported that they were pain-free (reported a score of zero) by 30 days of supplementation. All patients in the Single-Arm Pilot Trial experienced at least some improvement in flexibility or ROM-associated pain, with more than half (55%) of the subjects experiencing a greater than 50% improvement in flexibility and more than one-third (36%) of the subjects reporting that they were pain free during ROM evaluation.

Percent of patients experiencing reduction in pain from Baseline at 10, 30, & 60 days post treatment.

% Reduction	10 Days		30 Days		60 Days	
	Placebo (n = 21)	Treatment (n = 24)	Placebo (n = 20)	Treatment (n = 22)	Placebo (n = 18)	Treatment (n = 19)
≥ 20	24%	54%	35%	32%	39%	67%
≥ 30	14%	33%	20%	23%	33%	42%
≥ 40	10%	17%	10%	23%	22%	42%
≥ 50	5%	8%	5%	23%	12%	32%

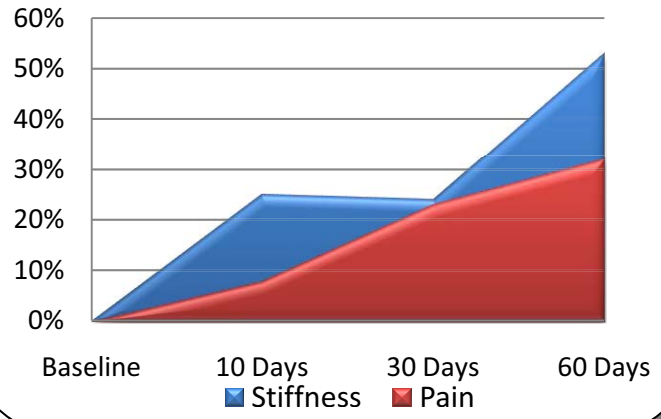
Placebo-controlled OPTION Trial: Patients experienced a relatively rapid (10 days) response for all WOMAC scores with a mean response of approximately 15% (12.8% to 15.9%). By the end of the follow-up period (60 days) the mean response remained approximately 15% (13.5% to 15.4%) for all WOMAC scores except stiffness which was 26.6%. This is superior to the response shown for glucosamine and chondroitin in previous clinical investigations (13; 14).

Although it is important to demonstrate significant results in an overall population, many clinicians believe that the "average" outcome reported in clinical trials fails to adequately describe the potential benefits to the individual patient (28; 29; 30). This is particularly relevant to arthritis-related clinical investigations. The measure of subjective symptoms (i.e. pain, stiffness, etc.) of arthritis and the wide variation in individual patient's perception of these symptoms results in complex relationships that can be difficult to elucidate from the reporting of mean treatment effects in clinical trials. Patients often show large variances in response to pain treatment with NSAIDs and other analgesics – some reporting high levels of pain relief while others report practically none (31; 32).

Number Needed to Treat (NNT) is a form of responder analysis and is a widely accepted and statistically valid measure of treatment effect (33). Perhaps more importantly, it is also clinically relevant (34; 35; 36). Knowing the NNT for different treatment interventions for the same disorder or disease can help guide treatment decisions, allowing physicians and patients to choose the best treatment intervention (on a comparative basis) for therapeutic success. NNT is the reciprocal of the Absolute Risk Reduction (ARR), NNT = 1/ARR (37). For arthritis clinical trials, ARR is calculated as the difference between the positive response rate in the treatment group (TR) and the positive response rate in the placebo group, or baseline response (BR), that is ARR = TR - BR. NNTs of 5 or below are generally accepted as equating to an effective treatment for pain-related conditions (30) and the lower the value the more effective the treatment.

In order to perform an NNT evaluation of the OPTION data, a treatment response rate table was prepared for the treatment and placebo groups at all time points for the pain and stiffness (not shown) WOMAC subscales (as these were statistically relevant). It becomes evident that there are response rates that are quite likely to be clinically relevant (≥ 30% reduction from baseline), as well as response rates that are most assuredly clinically relevant (≥ 50% reduction from baseline). For example, approximately one-third (33%) of study subjects experienced greater than 30% reduction in pain at 10 days, with a similar number of subjects (32%) having experienced greater than 50% reduction in pain at 60 days. In both instances, this rate was more than two times (~2.5x) that of the placebo group. Approximately one-quarter (25%) of study subjects experienced greater than 50% reduction in stiffness at 10 days, with the number of patients increasing to more than one-half (53%) having experienced greater than 50% reduction in stiffness at 60 days. The 10-day result was more than two times (~2.5x) that of the placebo group and the 60-day result was nearly five times (~4.8x) that of placebo.

Percent of NEM® Treated Patients Experiencing >50% Improvement.



- ✓ One-third of patients experienced >30% reduction in Pain @ 10 Days.
- ✓ Nearly one-third of patients experienced >50% reduction in Pain @ 60 Days.
- ✓ One-fourth of patients experienced >50% reduction in Stiffness @ 10 Days.
- ✓ More than half of patients experienced >50% reduction in Stiffness @ 60 Days.

These various responder rates were then converted to NNT values which include 95% confidence intervals (95% CI) according to the method described by Wen, et al. (37). NNT values were determined for each level of improvement for both pain and stiffness. These NNT values can then be plotted for a visual comparison of clinically meaningful treatment response at all time points for both pain and stiffness (not shown).

It becomes evident that although there was a statistically significant change in mean WOMAC pain scores in the overall study population at 10 days, it may be clinically difficult to evaluate this effect in populations smaller than 30 patients. However, by 30 and 60 days, NNTs for at least 50% reduction in pain were 5.6 (95% CI, 3.9 to 7.4) and 5.0 (3.1 to 6.9), respectively. In clinical practice, one out of every five patients should experience at least a 50% reduction in pain within 30-60 days. By comparison, we determined an NNT of 23.8 (95% CI, 15.2 to 32.4) from the GAIT data for a 50% reduction in WOMAC pain scores for the overall study population (13). A similar 50% reduction in rheumatoid arthritis pain was reported as 4 in a review of three clinical trials for adalimumab, etenercept, and double-dose infliximab (38). Similarly, results can be found for painful diabetic neuropathy in which NNTs range from 3.6 to 6.2 for 50% pain relief (39). Although the last two examples are not direct comparisons to OA pain treatment, they serve to demonstrate clinically effective treatment NNT values for pain-associated conditions.

NEM® is almost **5X more clinically effective** than Glucosamine or Chondroitin (alone or in combination)*

Number Needed to Treat Pain Comparison for NEM®

NEM® NNT (> 50%) Pain	Other NNTs (> 50%) Pain
5.0 60 days	**23.8** (Glucosamine / Chondroitin) 6 months
	14.9 (celecoxib) 13 weeks
	4.0 (adalimumab, etanercept, & double-dose infliximab) 12 months

Number Needed to Treat (NNT): the number of patients needed to treat to see a clinically significant treatment effect versus placebo.

*NNT for glucosamine & chondroitin calculated from the GAIT Study (*N Engl J Med* 2006, 345(8):795-808.)

NNT values were also determined for 50% reduction in stiffness at each time point. We obtained NNTs of 6.5 (95% CI, 4.6 to 8.4), 7.9 (6.1 to 9.7), and 2.4 (0.5 to 4.3) at 10, 30, & 60 days, respectively. This demonstrates that there is a clinically relevant reduction in stiffness at all time points during the study. This is particularly true at 60 days where nearly one out of every two patients would experience a 50% reduction in stiffness.

The safety profile for NEM® is also of significance as there are no known side effects, excluding the obvious egg allergy concern. This is of obvious importance in a condition that requires long-term treatment such as JCT disorders. Significant and sometimes serious side effects associated with other treatments can force patients to have to make the difficult decision between living with the disease symptoms or living with the side effect symptoms.

With so many people suffering from joint and connective tissue disorders, and that number expected to grow immensely as the overall U.S. population ages, it is important for patients to have treatment options that are both effective and safe. The reporting of the results from these three human clinical trials demonstrates that Natural Eggshell Membrane (NEM®) is a viable treatment option for the management of JCT disorders, particularly osteoarthritis. In these clinical studies, NEM®, 500 mg taken once daily, significantly reduced pain, both rapidly (7-10 days) and continuously (60 days). It also showed clinically meaningful results from a responder analysis, yielding reasonable Number Needed to Treat values compared to other pain-related treatments. This demonstrates that a significant proportion of treated patients will benefit from NEM® supplementation.

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