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Research Article

A Randomized Study Of The Efficacy And Safety Of Dermal Filler Injection (Formaderm® Young) In The Treatment Of Nasolabial Folds Wrinkle

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Abstract

Introduction: Addressing the challenges of aging skin and collagen loss, hyaluronic acid (HA)-based injectable fillers prove valuable in treating facial areas such as wrinkles as soft tissue augmentation options expand. This study assesses the safety and efficacy of HA filler from Maxigen Biotech Inc. (MBI-FDY) in correcting nasolabial folds (NLFs) wrinkles. **Methods:** MBI-FDY was analyzed for 1,4-butanediol diglycidyl ether (BDDE) residues, injection force test, viscoelastic properties, particle size and observed the protein content. We also cultured MBI-FDY in L929 cells and examined for cytotoxicity. In this multi-center study, 320 subjects were recruited to evaluate effectiveness and safety of MBI-FDY at 24 weeks post-injection. **Results:** The minimal BDDE residues in MBI-FDY (<0.125 μg/mL), non-cytotoxicity, and long-term tissue compatibility aligned with safety guidelines, potentially enhancing patient safety. The protein content is 10.51 μg/g, and the stable injection force by using 27 G injection needle was observed. MBI-FDY demonstrated effectiveness and safety in improving moderate to severe NLFs with no serious adverse events after injecting for 24 weeks. **Conclusion:** Safety assessments indicated mild expected adverse events, with occurrence rates comparable to the control group. The study suggests MBI-FDY as a promising and safe option for nasolabial fold correction.

Keywords: Hyaluronic acid filler; Nasolabial folds; Skin.

Introduction

Nasolabial folds, commonly known as smile lines or laugh lines, are the lines that run from the sides of the nose to the corners of the mouth [1]. As we age, the skin loses collagen and elasticity, leading to the development of wrinkles and folds, including nasolabial folds [2]. During aging, the skin undergoes significant changes, collagen becomes fragmented, and its amount decreases; this hinders the interaction between extracellular matrix and fibroblasts, which leads to further deterioration [3]. Currently, there are numerous strategies available for the prevention and treatment of premature aging, such as cosmetic care, topical medications, invasive surgeries (e.g., skin replacement, wrinkle correction, laser resurfacing), and systemic medications (antioxidants and hormone replacement therapy)[4]. However, the use of dermal fillers remains one of the most widely utilized non-surgical cosmetic procedures worldwide. Dermal fillers are commonly used in facial areas such as wrinkles, lip augmentation, depressed scars, neck, shoulders, and hands [5]. Nasolabial folds, originating at the junction of the nose, cheeks, and upper lip, extend in linear, convex, or concave shapes and terminate below and to the side of the mouth[6]. Currently, dermal fillers, such as hyaluronic acid (HA), are commonly employed for the treatment of wrinkles in the nasolabial fold area [7]. Therefore, choosing the appropriate HA filler is crucial for achieving sustainable results.

HA is a polysaccharide composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine [8]. It is abundantly distributed in the pericellular coating and extracellular matrix (ECM) of connective tissues and is involved in various biological processes, including cell signaling, cell proliferation and wound healing [9]. Due to its biocompatibility, biodegradability and high capability of water retention, HA is developed rapidly as an injection filler material in the medical beauty market [7]. However free HA, also known as uncrosslinked HA polymers, are easily degraded by naturally-presenting enzymes such as hyaluronidase and free radicals in the skin, resulting in a very short half-life in tissue [10]. Since persistency is required for a dermal filler, manufacturers apply crosslinkers to bind HA polymer chains and create a polymer network that is difficult for enzymes to break down, thus extending the longevity [11]. Currently 1,4-butanediol diglycidal ether (BDDE) is the most commonly used crosslinker. Several factors will affect characteristics and performance of HA fillers, including degree of crosslinking, gel consistency, gel hardness, viscosity and total HA concentration [12]. The higher the degree of crosslinking, the greater the viscosity and the harder the gel will be. In the manufacturing process, residual crosslinkers are another issue that needs to be concerned about since they can be harmful at high concentration [13]. Manufacturers take various methods to remove as much of the residual crosslinkers as they can. Formaderm® Young from Maxigen Biotech, Inc. (MBI-FDY) is an HA filler that can be injected into the facial dermis for the correction of nasolabial fold wrinkles. The application of ECHA™ balanced crosslinking technology allows the entangled HA molecular chains to be flattened, then BDDE is evenly distributed around HA polymers, resulting in evenly-crosslinked HA fillers with great viscosity and extrusion force [7]. Special steps are taken to wash free BDDE molecules, leading to the concentration of residual crosslinkers lower than 0.125 ppm [14].

This study was mainly to explore the safety and efficacy of MBI-FDY in the treatment of nasolabial fold wrinkles. First, using MBI-FDY, we conducted a three-stage study to analyze BDDE residues, injection force test, viscoelastic properties, particle size and subsequently observed the protein content. Second, MBI-FDY was cultured in L929 cells and examined for cytotoxicity and *in vivo* degradation test. Third, subjects were recruited to evaluate the efficacy and safety of MBI-FDY at 24 week post-injection.

Materials and Methods

Formaderm® Young Dermal Filler Injection

The HA solution was prepared by dissolving sodium hyaluronate (bacterial fermentation in 0.5-N NaOH and then cross-linked with BDDE to prepare the cross-linked HA (cHA). After crosslinking, the cHA was mixed with uncross-linked sodium hyaluronate solution with a volume ratio of 80:20 to prepare the product formulation with 2% sodium hyaluronate content. The product formulation was then filled into a syringe by using the aseptic filling process in accordance with ISO 13408. Finally, the pre-filled syringe was sterilized by using moist heat in accordance with ISO 17665.

BDDE residues

BDDE residues can be tested by detecting the fluorescence intensity of the substance produced by BDDE and nicotinamide, which have strong fluorescent and excitation, and emission wavelengths located at 360 and 460 nm, respectively. The 0.5 ± 0.05g sample was pretreated by mixed with 0.5 mL 500 U/mL of hyaluronidase enzyme solution and placed in the 37 ± 1˚C oven for hydrolysis and reaction time was 72-75 h. Then, 50 µL of 0.125 M nicotinamide solution were respectively added into test tube which contain 100 µL standard solution (16.0, 8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, and 0.0625 µg/mL) and sample solution and reacted at 80˚C with water bath for 1h.

After reaction, the solution was cooled to room temperature, add 500 µL acetophenon and 500 µL 0.1 M potassium hydroxide at ice-cooled bath for 10 minutes. And then formic acid 2.5 mL was added and the reaction was placed at 100˚C water bath for 5 minutes. The fluorescence values were determined using a multifunctional

microplate reader SpectraMax M5 (Thermo Fisher Science, CA, USA) with excitation and emission wavelengths located at Excitation: 360 nm, Emission: 460 nm. BDDE concentration and fluorescence were used as the abscissa and ordinate to make the standard curve.

Injection force determination

The physical and mechanical aspects of the relevant powers and forces were assessed, and one combinations of HA fillers and 27G needles was selected. The machine (Instron 5543) was programmed to push and displace the syringe plunger at a constant speed. The injection force was determined by analyzing the results corresponding to the speed (30 mm/min). Plunger displacement and injection force measurements were recorded at 0.1-s intervals using a data acquisition system.

Viscoelastic properties analysis

The physical and mechanical aspects of the relevant elastic and viscous were assessed. Rheometer measurements were made using the machine (TA Instruments Discovery HR-1) with a peltier plate cartridge and using a 20 mm parallel plate measuring system. All rheology measurements were performed at 25°C. Load 0.5 g of the sample on the bottom plate of the Rheometer. When the spindle reaches the specified height, use the plastic sheet to scrape off the overflow sample and measure the viscoelastic properties of the sample with Rheometer. The elastic (G') and viscous (G") moduli were determined using the oscillation mode at the frequency of 0.5 Hz.

Particle size analysis

The physical and mechanical aspects of the particle size was assessed. Place 40 μg sample into test tube which contain 1 mL 0.9% normal saline and mix evenly. The particle size was measured by particle size analyzer (HORIBA LA-960).

Protein content determination

Two grams of cross-linked sodium hyaluronate gel was weighed and put in a 20 mL headspace bottle to avoid contacting the bottle wall and affecting acidolysis. Next, 3 mL 0.5 mol/L sulfuric acid solution was added without shaking, and the bottle cap was pressed tightly and placed in a constant temperature drying oven at (95 ± 5)°C for 45 min to completely dissolve it. After cooling at room temperature, the solution was transferred in the headspace vial to a 10 mL quantitative bottle, the headspace vial was rinsed three times using 3 mL of 1 mol/L sodium hydroxide solution, the rinse solution was transferred to a volumetric flask, and finally fixed with water. From the 10 mL solution, 1 mL was aspirated and placed in a test tube. The repeat test number for the protein content of the product was n = 10. The standard protein solution was prepared by using bovine serum albumin with 2, 4, 6, and 10 μg/mL, and the water for injection was used as the blank control. After serial dilution, 5.0 mL of Coomassie brilliant blue G-250 was added to the test tube and mixed well. After 5 min at room temperature, the absorbance was measured at a wavelength of 595 nm. The regression equation was calculated with the concentration of bovine albumin on the corresponding absorbance, and the protein content of the test solution was calculated from the regression equation. Calculate the protein content in sodium hyaluronate according to the following formula:

$$E=(\rho a \times V)/m$$

E = The protein content in sodium hyaluronate, (μg/g).

ρa = The concentration of protein in the test sample solution which is determined from the standard curve, (μg/g).

V = The total volume of the test sample solution, (10 mL)

m = The mass of the test sample, (g)

MTT assay for cell viability

Based on the ISO 10993-5: 2009, an in vitro cytotoxicity study was conducted on the test articles Formaderm® Young to determine the potential for cytotoxicity of the test article. The cytotoxicity of the test article was evaluated according to cell viability percentage of MTT cytotoxicity test. Mouse fibroblast cells (NCTC Clone 929, L cell, L-929, derivate of strain L) were proliferated at 37°C, 5% CO_2 in petro dishes (Ø 100 mm) containing MEM (HyCloneTM) supplemented with 10% fetal bovine serum and antibiotics (100U/mL penicillin, 100μg/mL streptomycin). After grew nearly confluent, the cells were trypsinized, collected and adjusted to 1 x 10^5 cells/mL for following experiment. The extract liquid of the test article was diluted by MEM 100%, 50%, 25% and 12.5% of the extract liquid were prepared for following steps: Stirred cell suspension was pipetted into 96-well plate with 1x10^4 cells/well, incubated at 37°C, 5% CO_2 for 24 hours. Then the supernatant was discarded, and 100 μL of the extract liquid (containing 10% FBS) with different dosage was added into each well with 5 parallel wells; other 5 wells adding MEM (containing 10% FBS) were used as blanks control, the extract liquid of polystyrene as negative control and 5 wells with 10% DMSO as positive control. After cultured at 37°C, 5% CO_2 for 24 hours, the supernatant was discarded, and 50 μL MTT (VETECTM, V900888-1G, Lot: WXBC2166V) solution (1 mg/mL, dissolved in MEM medium) was added into each well and incubated another 2 hours. At the end of incubation, the supernatant in the 96-well was discarded, 100 μL of isopropanol was added and vortexed for 5 minues to allow total color released from the bottom of 96-well plate, the absorbency was measured at 570 nm by a microplate reader (reference wavelength 650 nm). The cell viability percentage was calculated by comparing with the blanks (The cell viability percentage of the blanks represented as 100%).

In vivo degradation test

The test sample identified below was evaluated for subcutaneous implantation of rats in accordance with the guidelines of the ISO 10993-1: 2009, ISO 10993-6: 2016, ISO 10993-9: 2009, and ISO 10993-13: 2010. The purpose of this study was to evaluate the degradation of the test sample after subcutaneous implantation of rats. The animals were prepared for conventional injection. After the anesthesia of pentobarbital sodium by peritoneal injection, the inject area was sterilized, and the test samples were injected into the left and right back subcutaneous of rats. Six sites were made at 2-3 cm interval at the back of animals. Each site was injected 0.5mL test sample. All the animals were intensive cared until the implanting terminals were reached. The animals were sacrificed by pentobarbital sodium overdose at each termination of the experiment period (26 and 52 weeks). The implants and the around tissue were retrieved for macroscopic and histopathological evaluation.

H&E staining

The rat dorsal subcutaneous tissues from each group were soaked in 10% formalin, dehydrated through graded alcohols, and embedded in paraffin wax. The 4-μm–thick paraffin sections were subsequently cut into slices from these paraffin-embedded tissue blocks. The tissue sections were deparaffinized by immersing in xylene and rehydrated. All slices were dyed using H&E and then rinsed with water. Each slide was dehydrated through graded alcohols. The tissue sections were finally soaked in xylene twice.

Clinical trial design

A prospective, randomized, parallel, double-blind (evaluator and subject blind), non-inferiority study (Clinical Trial.gov ID: NCT05935501) was conducted using Q-Med "Restylane® as a comparator (control group) to evaluate the efficacy and safety of Formaderm® Young (test group) in the correction of moderate to severe nasolabial folds (NLFs). The study included subjects aged between 18-65 years, who had bilaterally symmetrical nasolabial folds of moderate (3) or severe (4) score, as determined by Wrinkle Severity Rating Scale (WSRS). Those who were pregnant, had severe skin diseases, scars in the nasolabial fold (NFL) area, connective tissue related diseases, diabetes, uncontrolled systemic diseases, scar-prone skin, a history of allergies to any hyaluronic acid implants, had undergone facial aesthetic treatment or surgery within 6-12 months, or had previously received permanent facial fillers were excluded. The study was conducted in Beijing Anzhen Hospital (IRB Number: 2017-0431) and Tongren Hospital (IRB Number: TREC2018-17) in compliance with the ethical guidelines of the Declaration of Helsinki. Subjects signed informed consent forms before any procedure could take place. Upon inclusion in the trial, subjects were assigned to either the test or control group based on their randomized number sequence. Subjects received the same group treatment on both NFL sides. The selection of the injection dose was determined by the depth of wrinkles and the physician's judgment in the area to be corrected, limited to 2 mL per injection site. The subjects returned for follow-up visits at 1, 3, 6, 9, and 12 months post-injection. The assessment of the effectiveness endpoints was conducted by independent and blinded evaluators who, not being associated with the sponsor, remained unaware of the treatment allocation for the subjects to minimize bias.

Clinical trial endpoints

The primary effectiveness endpoint was the percentage of subjects showing an improvement of at least one point in the Wrinkle Severity Rating Scale (WSRS) at 6 months after injection compared to the baseline, as assessed by evaluators. Secondary effectiveness endpoints included WSRS scores at 1, 3, and 6 months post-injection, as well as improvement rates at 1 and 3 months post-injection based on assessments by blinded evaluators. Additionally, aesthetic improvement at 1, 3, and 6 months post-injection was evaluated using the Global Aesthetic Improvement Scale (GAIS) system, as reported by either subjects or blinded evaluators. Safety assessment involved monitoring adverse reactions/events and incidence rates of symptoms.

Statistical analysis

Data were expressed as means ± SEM. Statistical significance among multiple groups was assessed using one-way ANOVA. P values <0.05 were considered statistically significant. For categorical endpoints in this clinical study, the description included the number and percentage of cases in each category. McNemar's test or Fisher's exact test (if McNemar's test is not applicable) would be used. For ordinal data, Wilcoxon rank-sum test or Cochran-Mantel-Haenszel (CMH) test would be employed. Difference comparisons were subject to two-tailed tests with a 5% significance level.

Results

MBI-FDY had very low BDDE residues and reduced protein content

According to safety data sheets provided for BDDE as a raw material, exposure to BDDE may induce irritation and allergic reactions at greater concentrations than the safety threshold of 2 ppm. Table 1 shows that BDDE residues in MBI-FDY was <0.125 μg/mL. The results indicated that MBI-FDY uses the crosslinking agent BDDE with a low residual content, which can effectively reduce the risk of adverse reactions in patients postoperatively. Moreover, the average protein content in MBI-FDY was 10.51 ± 3.95 μg/g, which complies with the requirement of YYT 0962-

2021 that the protein content of cross-linked sodium hyaluronate gel for plastic surgery should be <20 μg/g.

	Average	Standard deviation
BDDE residues (ppm)	0.016	0.006
Content of protein in sodium hyaluronate gel (μg/g)	10.51	3.95

Table 1: The residues of BDDE and protein in MBI-FDY.

MBI-FDY had stable injection force

By applying force to the push rod of the product syringe, the operation of the product in clinical use was simulated at a speed of 30 mm/min. The results showed that MBI-FDY was fitted with a 27 G injection needle, with an average pushing force of 11.69 ± 0.53 N. which had stable injection force compared to Restylane® LYFT (29.85 ± 13.89 N) (Figure 1). During the extrusion process of the sample, the feedback stress was stable, indicating that the HA gel in the product was uniformly dispersed without aggregation and concentration.

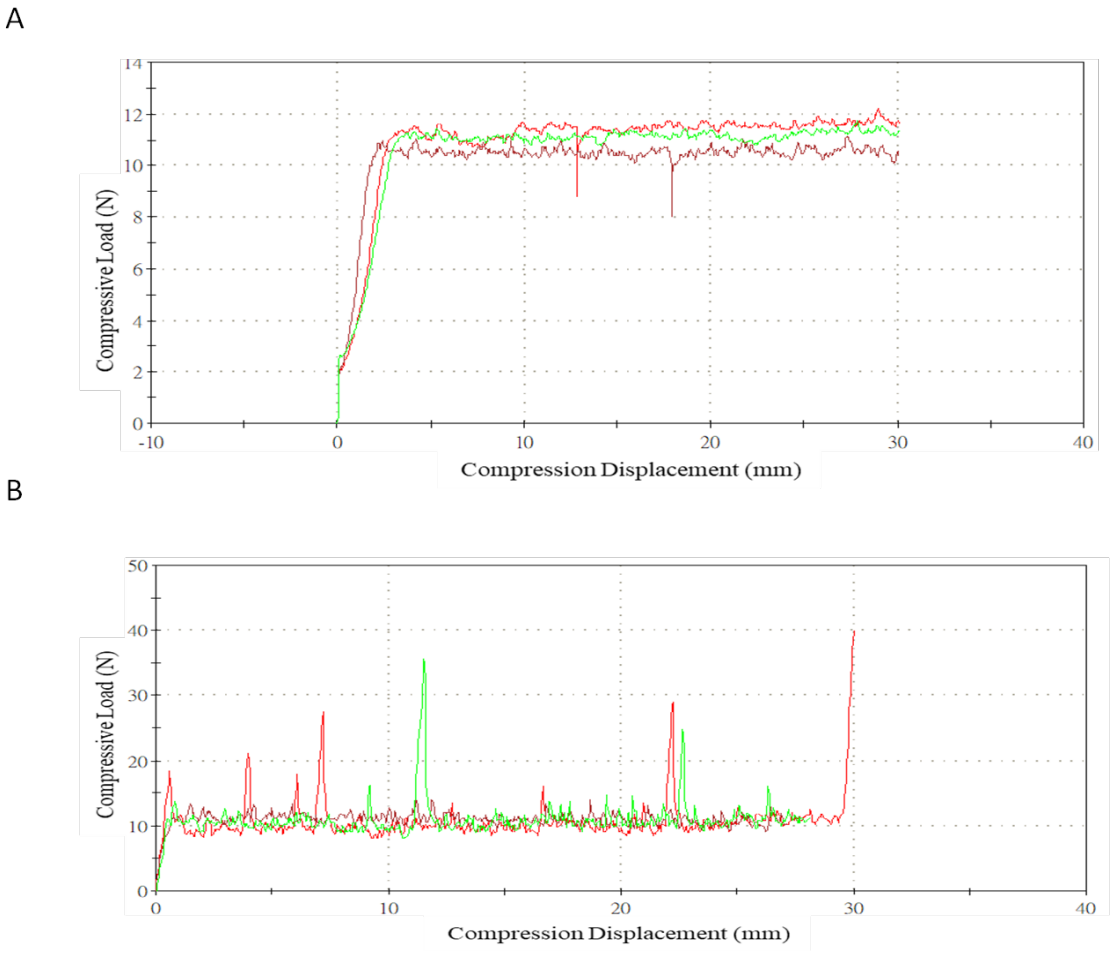

Figure 1: The injection forces achieved using (A) MBI-FDY exhibits an injection force of 11.69 ± 0.53 N corresponding to the 27 G needle; (B) Restylane® LYFT exhibits an injection force of 29.85 ± 13.89 N corresponding to the 27 G needle.

MBI-FDY has better viscoelastic properties and particle size

The study rheological properties of HA are elastic modulus (G'), viscous modulus (G"), the ratio between G" and G' (Tanδ). The higher the G', the greater the strength of the product [15]. G" refers to the inability of HA to recover its original state after deformation, making the product more liquid. A ratio between G" and G', known as Tanδ, determines its viscoelastic properties [15].

Table 2 showed that Tanδ in MBI-FDY is 0.21, which is a filler with a gel-like appearance that has a value below 1 and can be considered elastic enough to be implanted in different areas of the face. Particle size of the gel particles needs to be controlled in order to reduce the extrusion force and associated side effects like pain and bleeding when gels are injected. Therefore, the gels need to be engineered to pass through needles at the appropriate rate with the desired extrusion force. Table 2 showed that particle size in MBI-FDY is 480.96 ± 16.19 (μm), suggested MBI-FDY had the largest particle size and can easily pass through a needle.

	Viscoelastic Properties (Pa)	Tanδ (G"/G')	Particle size (μm)
Formaderm® Young	G'= 562.00 ± 48.30	0.21	480.96 ± 16.19
	G"=118.31 ± 6.02		

Table 2: The viscoelastic Properties and particle size in MBI-FDY.

MBI-FDY had no cytotoxicity *in vitro*

Next, the cytotoxicity of MBI-FDY was examined. We used 100%, 50%, 25% and 12.5% of MBI-FDY to culture with L929 cells for 24 h. Table 3 showed that the cell viability percentages of 100%, 50%, 25% and 12.5% of MBI-FDY were 113.7%, 106.6%, 111.9% and 105.0% respectively, while the cell viability percentages of the positive and negative control groups were 12.6% and 95.0%. The cell viability percentage of the 100% MBI-FDY group was above 70%. Therefore, MBI-FDY exhibited non-cytotoxicity.

Group/Treatments	Viability (%)
Negative control	95
Positive control	12.6
100% MBI-FDY	113.7
50% MBI-FDY	106.6
25% MBI-FDY	111.9
12.5% MBI-FDY	105

Table 3: The cytotoxicity of MBI-FDY by MTT assay.

In vivo degradation of MBI-FDY in rat

Next, to examine the degradation of the MBI-FDY in the rat subcutaneous tissue, MBI-FDY was implanted into the subcutaneous tissues for 26 and 52 weeks, and the rats were subsequently sacrificed. The tissues were evaluated microscopically by a veterinarian. The results indicated that the MBI-FDY can maintain the gel volume at injection site at 26 and 52 week post-implantation in accordance with the gross examination. Additionally, the H&E stain also indicated that the injected MBI-FDY still can fulfill tissue void at 26 and 52 week post-implantation (Figure 2). Most importantly, there is no significant signs of infection, encapsulation, hemorrhage, necrosis, and discoloration in accordance with the histological examination at 26 and 52 week post-implantation.

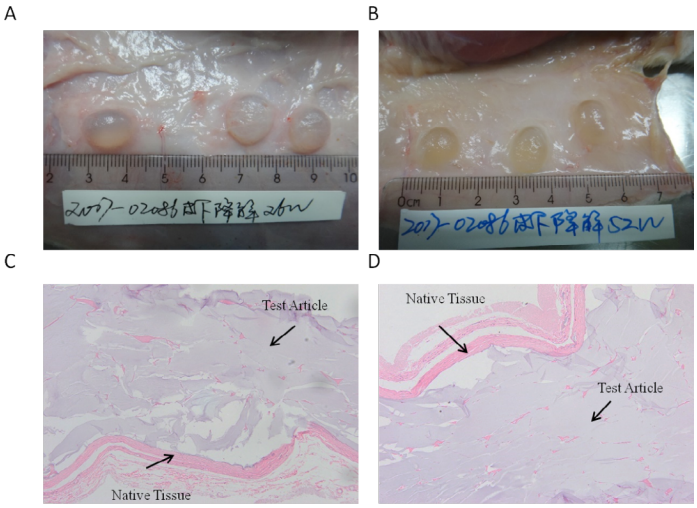

Figure 2: Observations of the MBI-FDY in rat subcutaneous implantation; (A) gross examination at 26 week post-implantation; (B) gross examination at 52 week post-implantation; (C) histologic examination with H&E stain at 26 week post-implantation, 40x; (D) histologic examination with H&E stain at 52 week post-implantation, 40x.

Clinical effectiveness of MBI-FDY in improving moderate to severe NLFs

A total of 358 subjects were screened and 320 completed the injections and were included in the assessment, in which 314 subjects completed the trial, 5 subjects withdrew due to personal factors and 1 subject dropped out the trial due to adverse event. Additionally, 1 subject from each of the hospital seriously violated the protocol and 1 subject didn't complete the assessment of primary effectiveness endpoint. Consequently, out of the 320 subjects, 311 were considered Per Protocol Set (PPS) analysis for effectiveness and 320 were included in Safety Set (SS) for safety assessment.

Table 4 showed that characteristics of all subjects. 320 subjects consisted of 12 males (3.75%) and 308 females (96.25%), with a

mean age of 42.62 ± 9.63 years and a total age range of 21 to 64 years. All the subjects met the WSRS inclusion criteria set for this trial, with wrinkle grading of 3 to 4 scores on both sides of the nasolabial folds and presenting bilateral symmetry. Table 5 showed that the baseline WSRS mean scores for both sides of the nasolabial folds in the MBI-FDY group were 3.54 ± 0.5, 3.52 ± 0.5 and the mean WSRS scores for the control group (Restylane®) were 3.49 ± 0.5, 3.52 ± 0.5 in full analysis set and per protocol set, respectively. The primary effectiveness endpoint was the improvement rate of WSRS assessed by evaluators at week 24 after injection compared to the baseline. Table 6 indicated that PPS population showed that the WSRS improvement rates of the two groups were 89.03% in the MBI-FDY group and 87.82% in the control group. The WSRS improvement rate in the MBI-FDY group was slightly higher than that in the control group, but there was no statistically significant difference between the two groups. Furthermore, the non-inferiority analysis indicated that the lower limit of the 95% confidence interval for the difference in improvement rates between the two groups did not exceed the non-inferiority margin of 10% in Table 7, contributing to the establishment of the non-inferiority hypothesis. Therefore, MBI-FDY was not inferior to Restylane® in correcting moderate to severe nasolabial folds.

Variables	**MBI-FDY group (n=160)**	**Control group (n=160)**	**P-value**
Gender, n (%)			0.556
Male	5 (3.13)	7 (4.38)	
Female	155 (96.88)	153 (95.63)	
Age, means (SD)	42.44 (9.63)	42.81 (9.66)	0.607
Number of smoking cigarettes per day, n (%)			0.723
0	155 (96.88)	156 (97.50)	
Jan-15	5 (3.13)	3 (1.88)	
16-25	NA	1 (0.63)	
Over 26	NA	NA	
Exposure condition, n (%)			0.825
Seldom	106 (66.25)	103 (64.38)	
Often	52 (32.50)	55 (34.38)	
Sometimes	1 (0.63)	2 (1.25)	
Usual	1 (0.63)	NA	
History of disease, n (%)	74 (46.25)	64 (40.00)	0.259
Concomitant medication, n (%)	38 (23.75)	36 (22.50)	0.791
WSRS of left nasolabial fold, n (%)			0.371
Extreme	NA	NA	
Severe	83 (51.88)	75 (46.88)	
Moderate	77 (48.13)	85 (53.13)	
Mild	NA	NA	
Absent	NA	NA	
WSRS of right nasolabial fold, n (%)			0.371
Extreme	NA	NA	

Severe	83 (51.88)	75 (46.88)	
Moderate	77 (48.13)	85 (53.13)	
Mild	NA	NA	
Absent	NA	NA	
Dose of injection in left nasolabial fold, means (SD)	1.23 (0.32)	1.21 (0.32)	0.791
Dose of injection in right nasolabial fold, means (SD)	1.22 (0.32)	1.19 (0.33)	0.762

Table 4: Characteristics between subjects in MBI-FDY group and control group.

Variables	**Full analysis set (FAS)**			**Per protocol set (PPS)**		
	MBI-FDY group	**Control group**	**P-value**	**MBI-FDY group**	**Control group**	**P-value**
Left nasolabial fold, means (SD)	3.54 (0.50)	3.49 (0.50)	0.372	3.52 (0.50)	3.49 (0.50)	0.534
Right nasolabial fold, means (SD)	3.54 (0.50)	3.49 (0.50)	0.372	3.52 (0.50)	3.49 (0.50)	0.534

Table 5: The wrinkle severity rating scale (WSRS) of nasolabial fold at baseline.

Variables	**Full analysis set (FAS)**			**Per protocol set (PPS)**		
	MBI-FDY group	**Control group**	**P-value**	**MBI-FDY group**	**Control group**	**P-value**
Left nasolabial fold, n (%)			1			0.738
Valid number	139 (86.88)	139 (86.88)		138 (89.03)	137 (87.82)	
Invalid number	21 (13.13)	21 (13.13)		17 (10.97)	19 (12.18)	
Total number	160	160		155	156	
Right nasolabial fold, n (%)			0.479			0.687
Valid number	140 (87.50)	144 (90.00)		139 (89.68)	142 (91.03)	
Invalid number	20 (12.50)	16 (10.00)		16 (10.32)	14 (8.97)	
Total number	160	160		155	156	
Both sides of nasolabial fold, n (%)			0.87			0.741
Valid number	139 (86.88)	138 (86.25)		138 (89.03)	137 (87.82)	
Invalid number	21 (13.13)	22 (13.75)		17 (10.97)	19 (12.18)	
Total number	160	160		155	156	

Table 6: Improvement rate in the WSRS at week 24 after injection.

Variables	**MBI-FDY group**	**Control group**	**P-value**
Full analysis set (FAS)	139 (86.88)	138 (86.25)	0.003
Per protocol set (PPS)	138 (89.03)	137 (87.82)	<0.001

Table 7: Improvement rate in the WSRS at week 24 after injection on non-inferiority trial at week 24 after injection.

The safety of MBI-FDY in improving moderate to severe NLFs

In terms of safety, Table 8 showed that 96 expected adverse events occurred in the MBI-FDY group. All anticipated adverse events were of mild severity, and most of the events resolved without sequelae within 6 days of observation. Among the anticipated adverse events, the highest occurrence rate was for tenderness (14.38%), followed by pain (13.13%), bruise (5.63%), and swelling (3.75%). In the control group, a total of 102 anticipated adverse events occurred, with tenderness also having the highest occurrence rate (16.88%), followed by pain (14.38%), bruise (6.88%), and swelling (1.88%). The occurrence rates of anticipated adverse events were similar between the two groups, and there was no statistically significant difference. Based on the above results, this trial confirmed that FDY can effectively correct NLFs without raising any safety concerns.

Characteristics	Test group (n=160)			Control group (n=160)			P-value
	Cases	Number of subjects	Incidence (%)	Cases	Number of subjects	Incidence (%)	
Anticipated adverse event	96	43	26.88%	102	53	33.13%	0.272
Erythema	0	0	0.00%	0	0	0.00%	-
Swelling	12	6	3.75%	4	3	1.88%	0.502
Pain	35	21	13.13%	35	23	14.38%	0.871
Bruising	12	9	5.63%	15	11	6.88%	0.818
Nodule	0	0	0.00%	1	1	0.63%	1
Pruritus	1	1	0.63%	2	2	1.25%	1
Tenderness	36	23	14.38%	38	27	16.88%	0.644
Color changes	0	0	0.00%	3	1	0.63%	1
Papules	0	0	0.00%	0	0	0.00%	-
Desquamation	0	0	0.00%	0	0	0.00%	-
Pigmentation changes	0	0	0.00%	0	0	0.00%	-
Bleeding	0	0	0.00%	0	0	0.00%	-
Herpes	0	0	0.00%	0	0	0.00%	-
Contusion	0	0	0.00%	0	0	0.00%	-
Lip blisters	0	0	0.00%	0	0	0.00%	-
Other	0	0	0.00%	4	1	0.63%	1

Table 8: Distribution of MBI-FDY expected adverse events.

Discussion

In this study, we conducted randomized clinical trials at three centers and found that MBI-FDY had very low BDDE residues, reduced protein content, stable injection force, better viscoelastic properties and particle size, and no cytotoxicity *in vitro*. Moreover, there is no significant signs *in vivo* degradation of MBI-FDY in rat. For analysis of efficacy, the non-inferiority analysis supported the hypothesis that MBI-FDY group was on par with control group in correcting NFLs. Safety assessments showed mild and expected adverse events, with similar occurrence rates between MBI-FDY group and the control group, confirming its safety profile.

HA is a hydrophilic macromolecule that can form viscoelastic gels in aqueous solutions, and it has the ability to expand its volume when contacting with water, therefore filling the space between skin cells and softening wrinkles when it is injected as a dermal filler [16]. The hydration of the skin will optimize dermal absorption of active ingredients and increase their retention in the epidermis. During

aging, the renewal of nature HA in the body becomes slow, and the activity of fibroblasts in the epidermal layer and keratinocytes in the dermal layertends to slow down [17]. In addition to its water-binding capacity, HA also has an impact on fibroblasts and keratinocytes with relation to cell proliferation and wound healing [18]. In the early stage of wound repairing, increased HA synthesis takes place, and cell migration is promoted [19]. When new ECM is deposited, keratinocytes proliferation increases. HA, in this process, binds CD44 receptors, leading to differentiation of keratinocytes and increased motility [20]. Studies have shown that HA treatment can increase the proliferation of fibroblasts and protect the skin from aging [21]. In animal studies, HA treatment increased type I collagen expression compared to PLLA-treated group [21]. Another clinical trial study of 28 healthy older participants supports that injecting cross-linked hyaluronic acid into the skin can stimulate fibroblasts to produce type I collagen, and type I procollagen at 4 and 12 weeks [22]. Notably, TGF-β and CTGF/CCN2 genes were also significantly induced in elongating fibroblasts. This suggests that the mechanism underlying the promotion of CTGF/CCN2 and type I procollagen expression may be the association between fibroblast elongation and TGF upregulation [22]. Other studies have showed that HA in cultured fibroblasts can trigger TGF-β signaling and collagen production, with some of these results being mediated by CD44 binding activity [23, 24]. A previous study by Huang et al. showing safety and efficacy of HA for nasolabial folds reported improvement in the WSRS score at the 6-month follow-up by meta-analysis [25]. Regarding side effects, there are most common and usually transient for appearing injection-related adverse events, whereas vascular occlusion is the most severe complication, which is related to hyaluronic acid filler injection [26]. However, there was no adverse event happened about vascular occlusion in this study. Possible mechanisms underlying the comparable efficacy of MBI-FDY could relate to its formulation, potentially involving the crosslinking process or the distribution pattern of HA within the dermal layers.

The HA structure, crosslinking density, and gel cohesivity often influence product performance. However, without direct mechanistic assessments, the precise reasons for the observed clinical similarity would necessitate further investigation, potentially exploring aspects like gel rheology or tissue integration. Overall, the study provides substantial evidence supporting the safety, biocompatibility, and effectiveness of MBI-FDY in cosmetic procedures aimed at correcting nasolabial folds. The findings endorse its potential as a viable option in clinical practice, offering an improved safety profile and comparable efficacy to established products.

Conflict of interest

The authors declare that they have no conflict of interest.

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