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Ovacore™ 2011

hydrolyzed egg membrane

Overview

Ovacore™ is a natural, water-soluble complex of proteins and small peptides, produced by a patented hydrolization process of egg membrane.

This technical paper describes the ingredient and outlines the current state of knowledge involving specific biological mechanisms of action, proof-of-concept in animal studies, and clinical data in humans.

Eggshell-derived nutritional products

Nutritional products made from eggshells have been used historically as a source of calcium [1-4]. More recently it's also been identified as a source nutrient that supports connective tissues, such as those found in joint health applications [5-9].

The commercial use of unfertilized eggs in the food industry results in vast amounts of eggshells as a byproduct. Ovacore provides value from this resource stream.

Eggshell versus egg membrane

Eggshell is the calcified matrix of glucosaminoglycans and other glycoproteins [10-13]. Approximately 2-4% of the weight of eggshell is organic compounds; the rest of the weight is minerals, predominantly calcium. The organic compounds serve as a scaffold onto which the organized mineralization happens during the formation of the eggshell.

Egg membrane is the thin membrane inside the eggshell, enclosing the egg white. This thin skin also has a matrix of organic compounds, however it has a distinctly different chemical composition. The egg membrane is uniquely designed to protect the egg white and yolk.

Articles in the scientific literature may use the abbreviation "ESM" for either shell or membrane. Therefore it is important to clarify definitions when comparing data on chemical composition and mechanisms of biological activity.

Ovacore™ chemical composition

Ovacore is manufactured from egg membrane to provide a unique, all natural source of key bioactive compounds important for tissue, skin, and vascular structure and integrity.

Ovacore is not enriched, nor concentrated. Ovacore provides the natural profile of compounds in egg membrane without contamination from eggshell.

Ovacore is hydrolyzed to produce a water-soluble nutritional product useful as a functional ingredient in food and beverages, as well as nutraceutical and cosmeceutical formulations.

The key compounds and their known biological properties are listed below.

Ovacore bioactive compounds

Active ingredients	Biological activity
Elastin	Joint, tissue, skin, and vascular elasticity
Desmosine/Isodesmosine	Joint, tissue, skin, and vascular elasticity
Collagen	Support of joint and connective tissue health
Total glucosaminoglycans (GAG)	Support of joint and connective tissue health
Transforming growth factor-beta	Immune regulation, cellular proliferation/ differentiation
Calcium*	None*
Antioxidants	ORAC 448µm TE/gram

** The content of calcium in Ovacore™ is negligible, verifying minimal contamination of the product by components from the mineralized eggshell.*

Joint health

Vertebrates rely on calcified bones for strength and rigidity of the body core and extremities. In conjunction, they rely on the anatomy and composition of the joints for mobility. The joints that allow us our mobility are designed for flexibility, elasticity, and strength. Tensile strength, elasticity, and integrity are compromised under conditions such as arthritis, as inflammation breaks down joint composition, and the joint's building blocks are not replenished.

Vascular health

Our circulatory system is another example of soft tissue structures whose function depends on tensile strength and elasticity. When blood vessels lose their elasticity, the venous return of blood from extremities (particularly the legs) is compromised, and varicose veins may develop. Also, swelling of feet and ankles may be a resulting problem.

Similarly, at the microvasculature level (the finest blood vessels), other problems may also arise. One example is lack of proper blood perfusion, which may contribute to accumulation of waste products in tissue, such as skin. Poor circulation may deliver fewer immune cells to clean up waste accumulation in tissue.

Inflammatory conditions, such as acne and inflamed pores, may develop. These conditions are difficult to clear up if the microvascular blood perfusion is sluggish in the area. A healthy microvascular function in tissue (e.g., skin) results in improved oxygen delivery, as well as increased immune surveillance and waste removal.

Skin aging

As we age, connective tissues tend to become less elastic. Wrinkled skin is an example of this condition. There is major cosmeceutical interest in topical and oral delivery of compounds that may help reduce wrinkle depth.

Ovacore mechanisms of action

Based on historical data from product use, knowledge of the ingredients, and data from cell-based *in vitro* work, the following areas of biological activities are of clinical interest:

- Joint flexibility and health;
- Vascular elasticity and health;
- Skin elasticity and health.

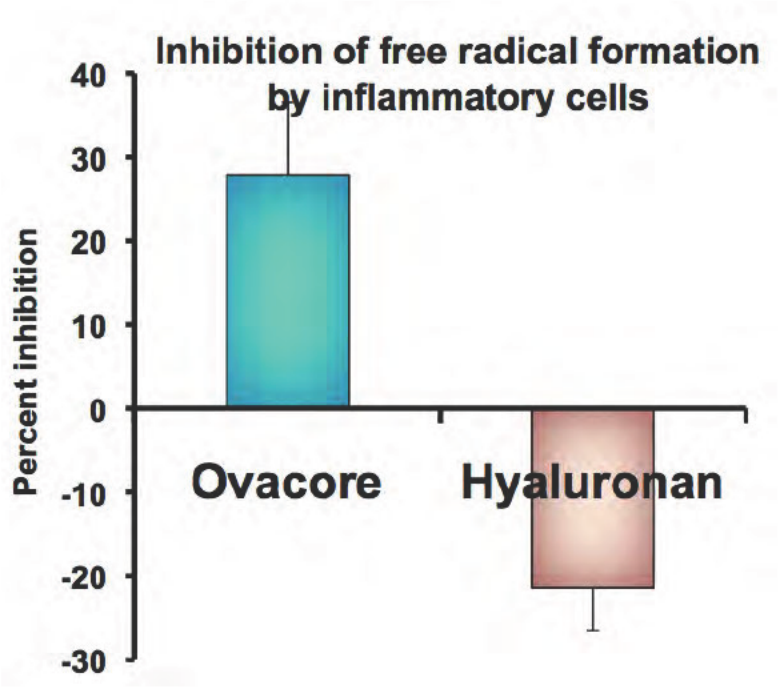
In addition, Ovacore has demonstrated anti-inflammatory activity. This is important in light of the contribution of chronic inflammatory conditions to joint problems, skin problems, and declining cardiovascular health.

Thus, the biological activity of Ovacore reflects a synergy between support of tissue elasticity and strength and reduced inflammatory conditions.

Anti-inflammatory properties of Ovacore

The exposure of inflammatory cells to Ovacore in laboratory tests resulted in a reduced production of damaging free radicals.

As a cellular model, polymorphonuclear cells from healthy human donors were used for the testing. Hydrogen peroxide (H_2O_2) was used to initiate an inflammatory response under oxidative stress conditions.



In parallel, a source of hyaluronan (75%) was tested in the assay. When the inflammatory cells were exposed to hyaluronan, the inflammatory cells responded in an opposite manner, and generated more free radicals in the assay. This is not surprising, due to the CD44 cell surface receptor for hyaluronan can transmit a pro-inflammatory signal in some cell types [14].

Clearly this topic has market significance and additional research is merited. Studies are being developed by Biova to further detail Ovacore's anti-inflammatory response impact.

ANIMAL CLINICAL DATA

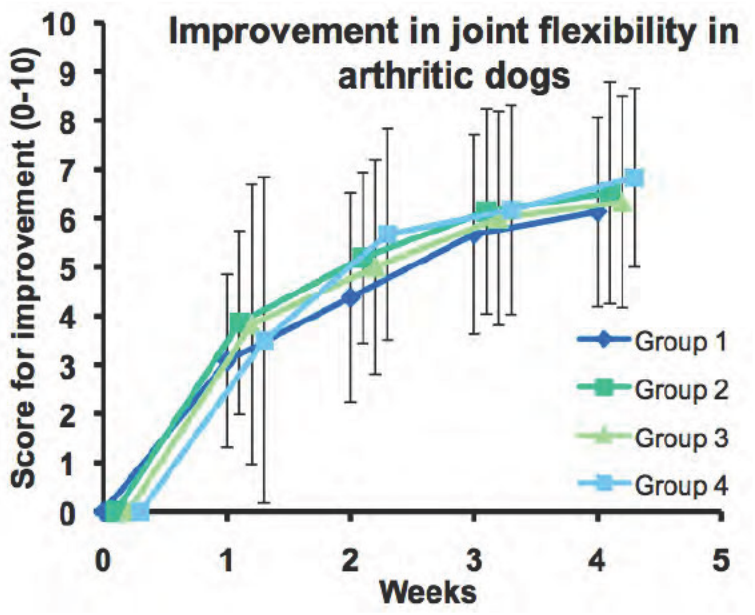
Fifty-seven dogs with age-related arthritis, reduced mobility, and reduced activity levels were studied over a period of 28 days.

The dose of Ovacore was 5 mg/pound body weight of the animal per day, given orally. No placebo group was included; all animals received Ovacore.

Sub-group analysis included analysis of improvements in relation to whether they had previously been given nutritional supplements for joint health.

Group .	Pre-study supplementation	Study supplementation	Number of animals
1	none	Ovacore	24
2	Omega3/ Glucosamine	Omega3/ Glucosamine + Ovacore	21
3	Any supplement	Existing supplement + Ovacore	6
4	Omega3/ Glucosamine	Ovacore	6

Every seven days, a veterinary technician interviewed the owners of the animals and collected joint mobility scores compared to baseline. The scoring was done with "no improvement" as "0," and "complete recovery" was set at "10."



The average improvement across the 57 arthritic dogs was above 6, so equal to a greater than 60% improvement over the four weeks of study.

The improvements were statistically significant already after one week. In the group of dogs (n=24) that were not previously on any nutritional supplement to help alleviate their arthritic symptoms, the animals kept improving throughout the study, reaching high levels of significance at study exit.

HUMAN CLINICAL DATA

Ovacore was studied in several human clinical trials, focused on these health issues:

- Joint mobility;
- Microvascular blood perfusion;
- Skin elasticity, wrinkles.

Joint health study

A Biova sponsored, open-label pilot study was performed where 42 people with knee osteoarthritis were enrolled. Out of this population, 30 people completed the six-week study.

Pain symptoms:

- Reduced 8.25% from baseline to 7 days
- Reduced 16.42% from baseline to 14 days

WOMAC score:

- WOMAC score showed >20% reduction from baseline to study end ($p<0.0001$)

Relative knee functionality:

- Joint function improved 37.8% over the course of the study

The graphs below show data on pain associated with different aspects of daily activities. The top graph shows the data from study start, and the bottom graph shows data at study exit.

Each vertical bar is coded "1" through "5." These numbers refer to the questions asked:

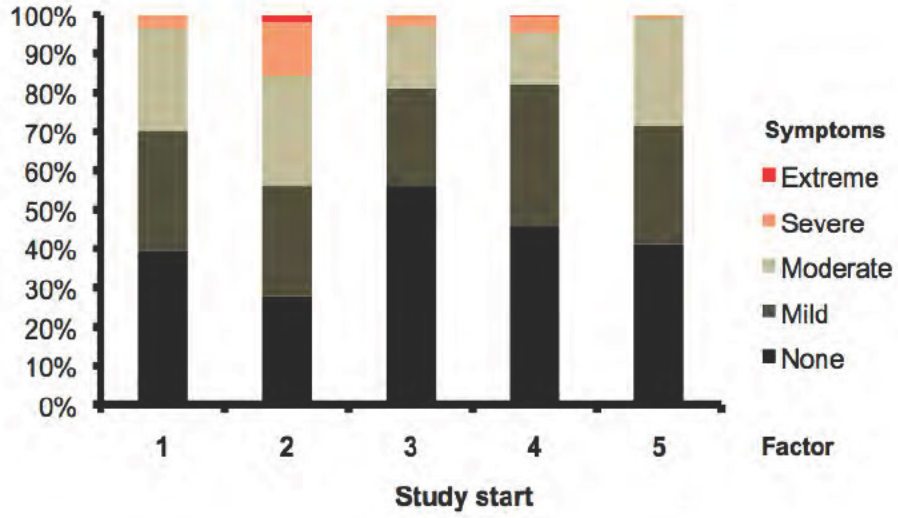
1: Pain while walking on a flat surface;

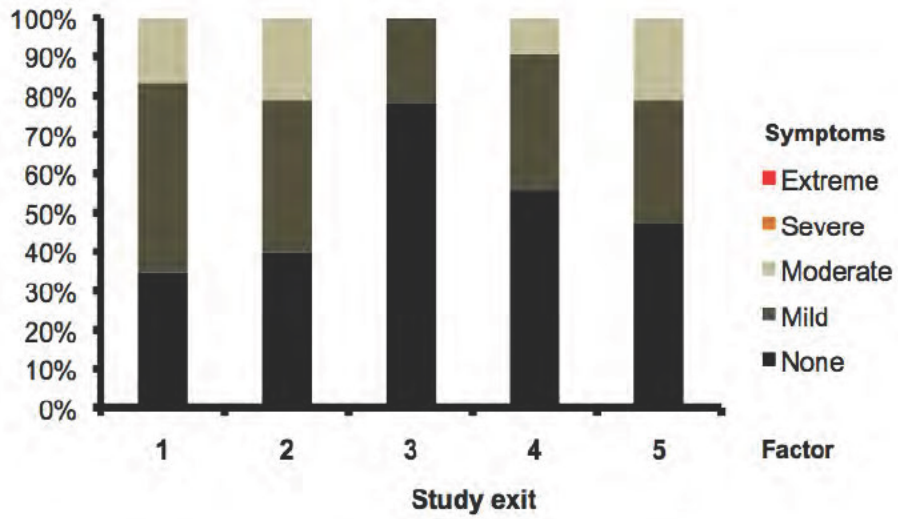
2: Pain going up or down stairs;

3: Pain at night while lying in bed;

4: Pain while sitting or lying;

5: Pain while standing.





Each vertical bar reflects 100% of the study population, and each color-coded portion of the bars reflect whether the answer/score was "none," "mild," "moderate," "severe," or "extreme."

It can be seen that the percentage of study participants in the worst pain groups ("severe," "extreme") dropped to zero at the end of the study.

Skin health study

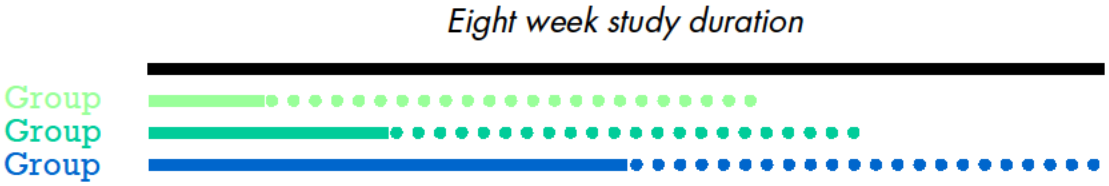
Twenty-three healthy adults with some degree of wrinkles, acne, and pore inflammation were recruited.

The subjects were divided into three groups:

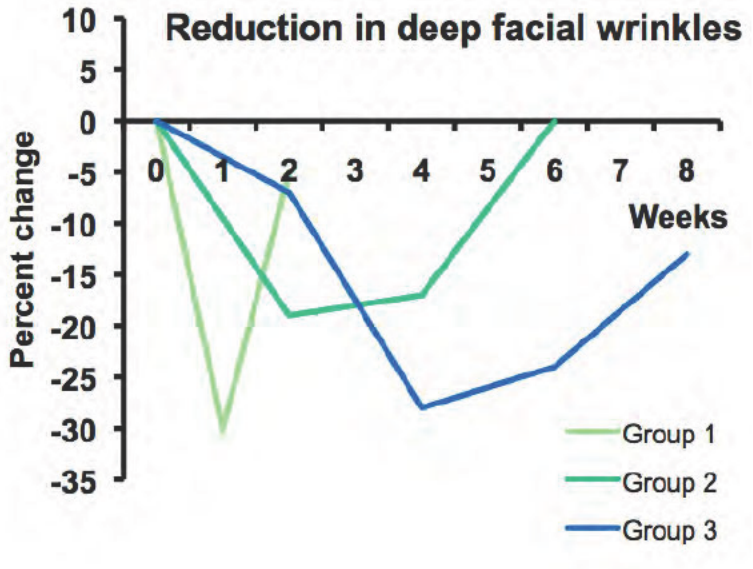
1) Six people applied product for one week;
2) Nine people applied product for two weeks;
3) Eight people applied product for four weeks.

All study participants were followed for four weeks after product application had ceased.

The diagram below shows the overall study design, where the solid lines show duration of product application, and the dashed lines show duration of follow-up after product was no longer applied:



Ovacore was applied to facial skin, and facial scans were performed using a Moritex/BTBP skin analyzer to quantify wrinkle severity and depth, skin complexion health, skin texture and laxity/tone, and UV damage.

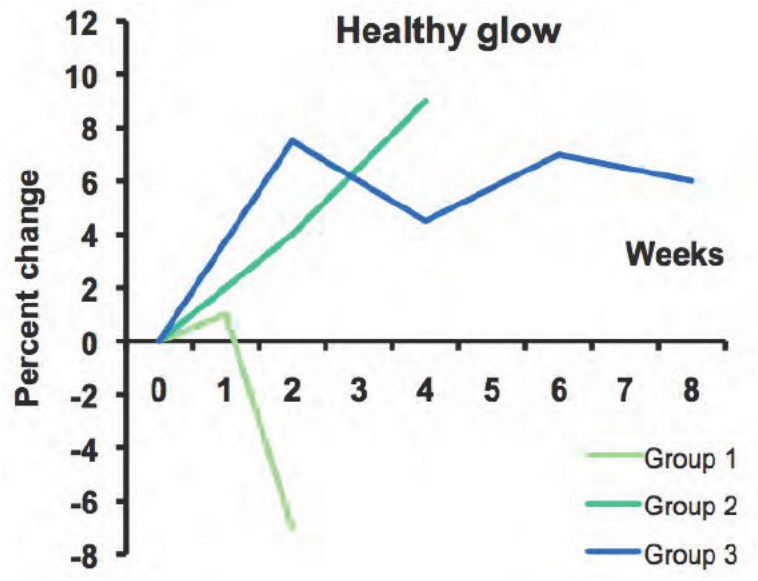


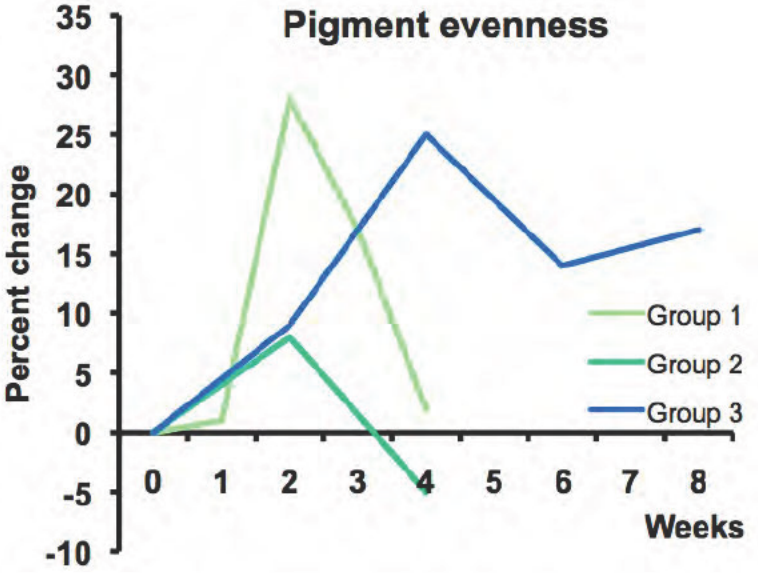
As the data graph above shows, the score (number, severity, depth) of deep wrinkles was reduced in all three groups of study participants.

When Ovacore was applied for only one week, there was a rapid return to baseline the following week.

When Ovacore was applied for two weeks, the return to baseline was slower and only went back to baseline at four weeks after product was no longer applied to the face.

When Ovacore was applied for four weeks, an even better long-term improvement was seen in wrinkle scores. At the eight week study exit time, the average change in wrinkle score was still below 90 percent of the baseline score.





Data from the Moritex skin analyzer generated scores on skin complexion, including healthy rosiness and skin luminescence, which were summarized as a measure of *healthy glow*. It also generates data pertaining to pigment evenness.

The measure of healthy glow may be interpreted in part as an indicator of healthy microvascular blood

perfusion. With respect to healthy glow, improvements were seen in all three groups. It is not clear why there was a delay in improvement in Group 2. The improvement was transient in Groups 1 and 2, but may indicate a long-term improvement in Group 3 who applied product for four weeks, and still showed improved glow and pigment evenness at eight weeks follow-up.

The scores pertaining to overall skin health, excess sebum, acne, inflammation, pigment distribution, and luminescence were more variable. This is likely due to the small population size that could have led to uneven distrubution of subjects with different types of skin problems at study start.

In Group 1, a drastic decrease in acne scores was seen already at one week, and lasted throughout the subsequent four weeks of follow-up.

In Group 2, the scores for acne and for excess sebum were drastically diminished.

In Group 3, a mild reduction in acne scores was seen at study exit.

Further studies on skin inflammation, microvascular function, and pore health are warranted, and it is suggested that each study is targeted at well-defined types of skin problems.

Safety

Throughout all of Biova's animal and human studies, no adverse effects have been reported.

In the joint health study, blood samples were taken at study start and exit, thus including the 30 people who completed the six-week study. Blood was used for the following:

- Blood chemistry;
- Hematology;
- Allergen antibody assays.

With the exception of occasional non-specific results outside the normal reference ranges, such as can be expected if a person transiently has a cold or flu, the results were well within normal ranges.

There were no significant changes observed from baseline to study exit. There were no negative side effects or toxicity observed in blood chemistry analysis. There was no abnormal blood cell morphology.

None of the participants showed any significant increase in IgE antibodies towards egg allergens.

Conclusion

Data from cell-based testing of mechanisms of action suggests strong anti-inflammatory activity of Ovacore™.

This anti-inflammatory activity was confirmed in a study on arthritic dogs, where significant improvements were seen already at one week of adding Ovacore to their food.

Furthermore, a study in humans has confirmed that joint flexibility and function improved with Ovacore consumption in people suffering from arthritis of the knee.

Other aspects of reduction of inflammation were shown in humans suffering from acne and pore inflammation of the facial skin.

In addition, support of skin elasticity and microvascular function was seen in the skin study by wrinkle reduction and improved healthy complexion as a measure of healthy microvascular perfusion of the skin.

In conclusion, Ovacore has anti-inflammatory and tissue-supportive biological activities, both after consumption and with topical use.

To learn more about Ovacore™ and Ovacore-based ingredients contact:

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