RECOMBINANT ENZYMES
A NEW PATHWAY INTO FACIAL RESHAPING AND SKIN REJUVENATION

Jan Balczun, MD, and Nuria Ramirez, MD, highlight the synergistic power of recombinant enzymes in facial reshaping and skin rejuvenation, offering safe, effective, and substrate-specific solutions

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ABSTRACT
Advances in biotechnology have revolutionized aesthetic medicine, introducing recombinant enzymes as safe and effective tools for facial reshaping and skin rejuvenation. These enzymes, including collagenase, lipase, and lyase, act synergistically to address various tissue concerns such as fat deposits, collagen degradation, and extracellular matrix remodeling. Their substrate-specific action ensures minimal side effects and high patient satisfaction. This review highlights the mechanisms, applications, and benefits of enzymatic treatments, underscoring their transformative potential in modern aesthetic practice.

OVER THE PAST FEW DECADES, AN ever-growing number of technological advancements have provided us with a broader range of methods to treat our patients, ensuring optimal results and high patient satisfaction.

One notable advancement over the past 30 years is the research and subsequent development of proteins. These proteins, specifically enzymes, have become valuable tools in a variety of industries, and their medical applications are the focal point of this article[1].

Facial reshaping and skin rejuvenation are primary concerns within the field of facial aesthetics. These issues stem from numerous underlying causes, such as changes in all layers of facial tissues, including bone resorption, subcutaneous fat pad repositioning due to ligament laxity and hypertrophy, and skin atrophy and sagging[2].

The lower third of the face is often targeted for facial reshaping. However, it presents a significant challenge due to the complexity of addressing multiple structures when establishing a treatment plan. The use of recombinant enzymes in this context has proven to be a safe and effective treatment option.

For starters, what is a recombinant enzyme?

In vivo enzymatic production is a complex mechanism closely entwined with gene expression, the process in which the information encoded in the DNA polymer is translated. The resulting proteins are tightly regulated through various mechanisms to meet specific needs[3].

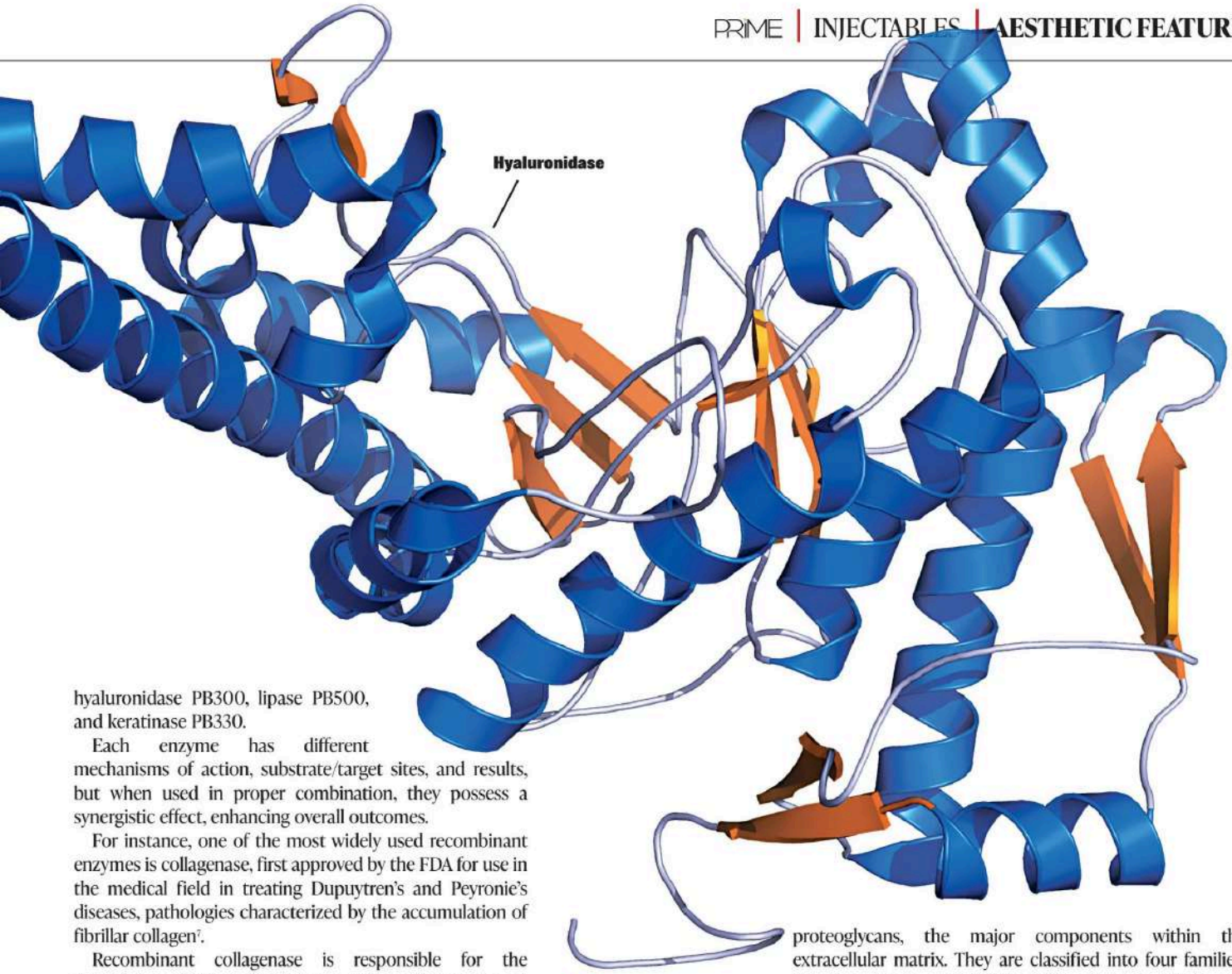
The increasing demand for enzyme availability across different industries for larger quantities, faster production, and lower costs has led to the creation of *in vitro* systems, or so-called heterologous expression, where these proteins can be created on demand: recombinant technology[4].

To achieve a specific, safe, and effective enzyme, the most popular host known so far is gram-negative *E. coli*. However, other types of surrogates are also used depending on the field of application. The popularity of *E. coli* in the genetic engineering field is mainly due to its well-known characterization, complete genome sequence knowledge, and understanding of its biological and metabolic pathways, making it a widely recognized workhorse for genetic manipulation[5].

Mahmoud (2007) best describes the process of recombination in his article[6]: 'To produce a protein heterologously in a living host, the target DNA-coding sequence must be known, and a suitable transformation and selection strategy must exist. Cloning the coding DNA into a suitable vehicle is a prerequisite for introducing it into the selected host. This vehicle is usually called a vector. This vector is typically an independently replicating DNA or at least a DNA segment that can integrate into the expression host genome.'

Enzymes in aesthetics

For as many enzymes that have been developed within the medical field, our focus in this article is the introduction of those entering the field of aesthetics, specifically collagenase G/H PB 220, lyase PB72K,



hyaluronidase PB300, lipase PB500, and keratinase PB330.

Each enzyme has different mechanisms of action, substrate/target sites, and results, but when used in proper combination, they possess a synergistic effect, enhancing overall outcomes.

For instance, one of the most widely used recombinant enzymes is collagenase, first approved by the FDA for use in the medical field in treating Dupuytren's and Peyronie's diseases, pathologies characterized by the accumulation of fibrillar collagen[7].

Recombinant collagenase is responsible for the degradation of the extracellular matrix, particularly type I and III collagen, by breaking down the triple helix and reducing the molecular weight of these fragments[8].

Another significant enzyme is recombinant lipase, a protein that belongs to the triacylglycerol acylhydrolases. Its mechanism of action is to catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids on its ester bonds in the adipose tissue. *E. coli* is the most widely used host for the heterologous expression of this particular enzyme[9].

Lipase's significant safety and strong differentiating element set it apart from other lipolytics, as it keeps the cell membranes intact and healthy, only affecting the fat droplet within. It is also important to note that recombinant lipase is not hormone-dependent.

This results in minimal side-effects, reduced clinical complications, and the achievement of good results in a short period of time.

Finally, recombinant lyases are enzymes that catalyze the breaking of a chemical bond between two parts of a molecule by pathways other than hydrolysis and oxidation, often forming a double bond or adding a new ring to the targeted structure[10].

Their mechanism of action involves directly acting on proteoglycans, the major components within the extracellular matrix. They are classified into four families: heparan sulfate, chondroitin sulfate, keratan sulfate, and hyaluronic acid.

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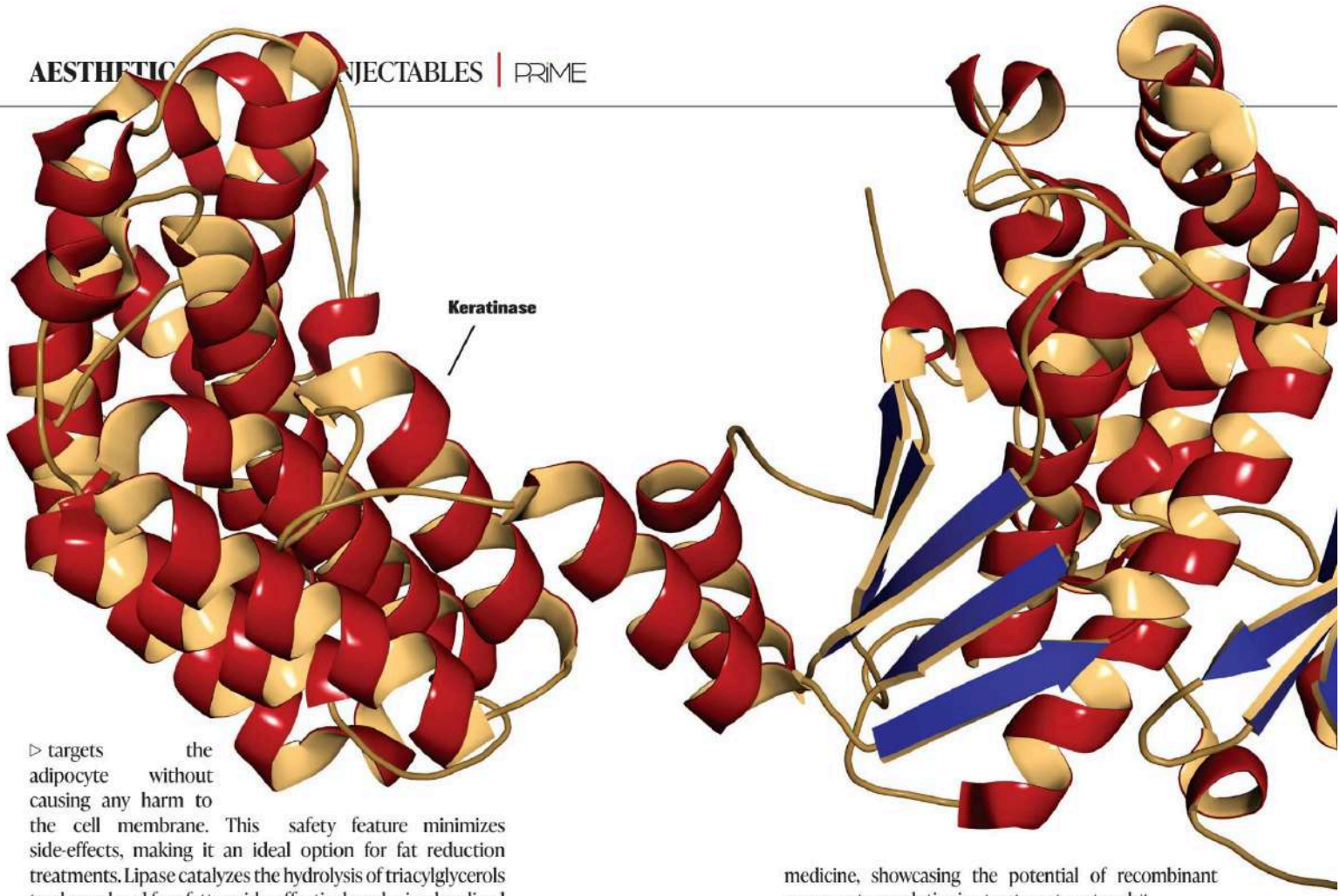
Their breakdown results in two main situations: liquefaction of the matrix, allowing other substances to travel more efficiently in a more physiological environment, and the release of growth factors, cytokines, and chemokines, which act as key modulators for tissue remodeling and development, restoring signaling pathways and cell-to-cell interactions[11].

A synergistic machinery

As described previously, recombinant enzymes have very specific functions based on their substrates. However, their true value within the medical and aesthetic fields lies in the combination of all three enzymes.

The integration of different metabolic pathways provides a synergistic approach to treatment. To expand, collagen degradation through recombinant collagenase is instrumental in breaking down non-functional collagen, which leads to the stimulation of fibroblast proliferation, chemotaxis, and angiogenesis. These processes directly impact skin rejuvenation and tightening, enhancing the skin's overall appearance and texture.

When aiming for fat tissue lysis, recombinant lipase ▷



▷ targets the adipocyte without causing any harm to the cell membrane. This safety feature minimizes side-effects, making it an ideal option for fat reduction treatments. Lipase catalyzes the hydrolysis of triacylglycerols to glycerol and free fatty acids, effectively reducing localized adipose tissue.

Glycosaminoglycan fragmentation, related to recombinant lyase acting on the proteoglycan family, stimulates the repair and remodelling of the extracellular matrix, creating a more physiological intercellular space. This enhanced environment allows other enzymes to perform more effectively at their target sites and strengthens the structural scaffold of the tissue.

The combination of these enzymes forms a synergistic mechanism that addresses some of the main issues in the aesthetic field, such as localized adipose tissue, skin laxity, fibrosis, and scarring. When used together, these enzymes act at different tissue levels, resulting in more harmonious and effective solutions for both patients and doctors[12].

The synergistic effect of combining the three enzymes leads to improved outcomes in aesthetic treatments. For example, the degradation of proteoglycans by lyase within the extracellular matrix not only tightens the skin but also creates an optimal environment for lipase to reduce fat deposits without damaging cell membranes. Meanwhile, collagenase's action ensures that the skin remains healthy and well-structured, promoting overall tissue repair and regeneration.

In summary, the integration of recombinant collagenase, lipase, and lyase in aesthetic treatments offers a comprehensive and effective approach. By targeting different aspects of tissue health and regeneration, this combination provides enhanced results, ensuring higher patient satisfaction and better clinical outcomes. This multifaceted approach represents a significant advancement in the field of aesthetic medicine, showcasing the potential of recombinant enzymes to revolutionize treatment protocols[14].

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Facial reshaping

As a result of the rising demand, the biotech field has developed many practical devices, mostly safe but sometimes carrying side-effects or leaving unwanted aesthetic marks. Recombinant enzymes, with their extensive background and research investment, are fundamental tools in the medical office when devising a treatment plan and making decisions regarding the physician's treatment pathway. These enzymes can be used solo or in combination with other biocompatible agents.

Facial reshaping is a highly demanded yet complex procedure as it involves many structures, from bone resorption and ligament laxity to the descent of facial fat pads and skin sagging. This is where enzymatic usage plays a key role in restoring facial harmony.

Skin sagging can be targeted using a combination of all three enzymes with a higher concentration of collagenase. When applied at a dermal level, lyases will restructure and restore the extracellular matrix, lipase will target any surrounding fat deposits, and collagenase will play a key role by breaking down non-functional collagen and activating the signaling pathway in which fibroblasts undergo replication, consequently starting the neocollagenesis process.

Jowls and double chin can be addressed with a similar enzymatic cocktail but with a higher lipase concentration, targeting the fat tissue by degrading the triglyceride content and thus reducing its volume and weight, resulting in a

more contoured mandibular and/or neckline. Additionally, since collagenase is included in the cocktail, skin laxity due to fat loss will not be evident due to the previously explained process.

This enzymatic combination can be applied to any area of the face, neck, and body where reshaping and skin rejuvenation are desirable.

Regarding safety, enzymatic treatments have proven to be safe, with very few or no side effects, and are easy to use in practice. Their safety is attributed to the fact that enzymes are substrate-dependent.

In the field of aesthetics, there are a variety of feared adverse effects associated with the injector, technique, and product properties. However, if the enzymatic product migrates or is misplaced in a different layer than intended, no action will occur because there is no substrate for the enzyme to work on.

Additionally, the recombinant origin of these enzymes, which produces smaller molecules with fewer glycosylations, significantly reduces the risk of an allergic reaction, making it almost negligible.

In practice

In a clinical, open-label study, 18 patients were treated with a cocktail of recombinant enzymes (collagenase, lipase, and lyase), together with high molecular weight hyaluronic acid (HA), results showed significant improvement after 36 days of treatment, effective against skin flaccidity in 92%, improved firmness in 92% and reduced fat in 84%. Patient satisfaction was 93% and the procedure well tolerated[15].

In another 2024 study, 9 out of 10 patients showed a considerable reduction of 22.8% in the patient-reported sub-mental fat impact scale, while 9 of 10 expressed overall satisfaction with the treatment. Submental fat reduction of more than 10% was observed in 9 out of 10 patients and as the study concluded the enzymatic mixture of lipase, collagenase and hyaluronidase is an effective and safe minimally-invasive method for the reduction of submental fat[16].

Key points

- **Recombinant enzymes, including collagenase, lipase, and lyase, target specific substrates, ensuring minimal side effects and improved clinical outcomes**
- **The combination of enzymes enhances fat reduction, collagen remodeling, and extracellular matrix restoration for comprehensive facial reshaping and skin rejuvenation**
- **Enzymatic treatments effectively address jowls, double chin, skin laxity, and other facial or body concerns, providing safe and harmonious results**

> *"Lipase's significant safety and strong differentiating element set it apart from other lipolytics, as it keeps the cell membranes intact and healthy, only affecting the fat droplet within. It is also important to note that recombinant lipase is not hormone-dependent."*

Conclusion

With many years of research investment in the field of biotechnology, specifically in recombinant enzymes and enzymatic systems, recombinant enzymes have proven fundamental in clinical practice. They demonstrate excellent results in both facial reshaping–targeting jowls, double chin and skin sagging–and overall skin rejuvenation.

This success is attributed to the synergistic effect of combining collagenase, lipase, and lyase, which act on different tissue structures to produce harmonious results without leaving aesthetic marks.

Moreover, this approach allows practitioners to use these enzymes in combination with other devices, ensuring high safety and proven efficacy.

In conclusion, the use of enzymatic treatments has demonstrated both efficacy and safety in skin rejuvenation and facial reshaping, making it a reliable and valuable option for practitioners.

▶ ***Declaration of interest*** *The author of this manuscript has no conflicts of interest to disclose.*

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