Polydeoxyribonucleotide *and Hyaluronic Acid:* two different agents *for skin regeneration.*

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Polydeoxyribonucleotide

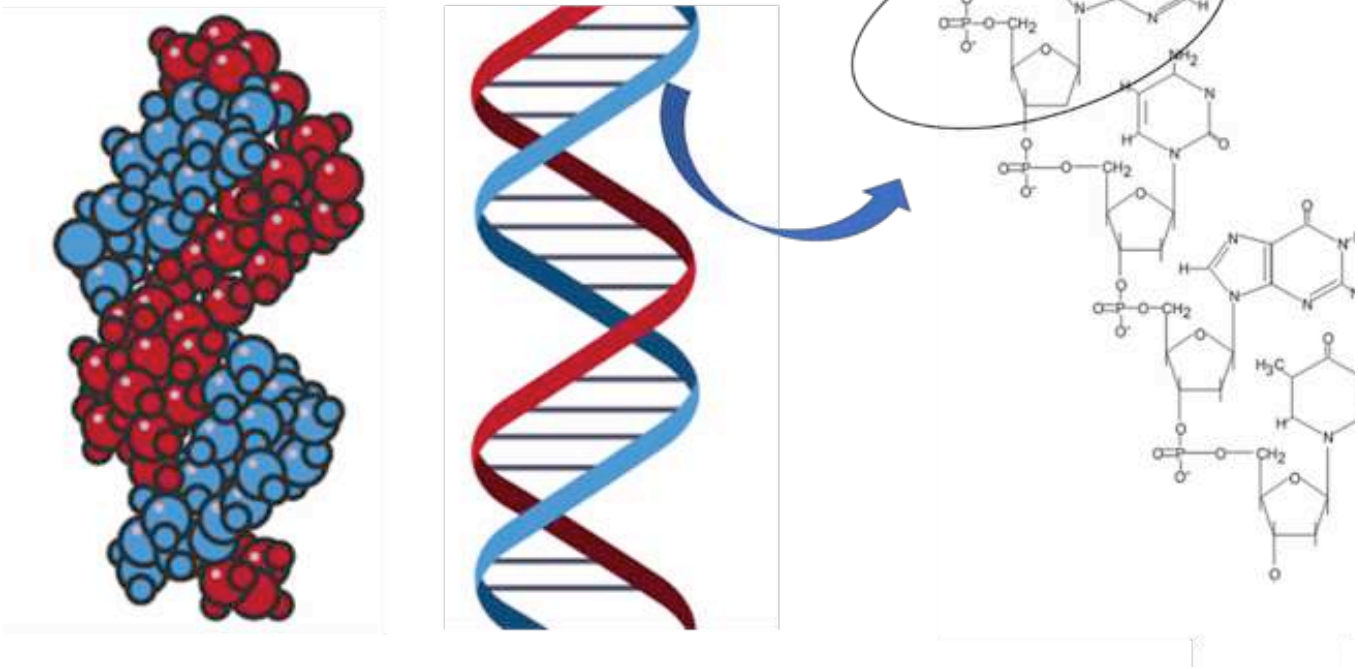
Aging well has become the new target of preventive medicine, and aesthetic dermatology can contribute to this request. Several products and fillers are currently available, and an increasing interest arises in polydeoxyribonucleotides (PDRN). PDRN containing products not only act as fillers, but even improve tissue regeneration. PRDN are widely used in cosmetic medicine, thanking of previous experiences in other clinical applications. Recently, a Korean study on PDRN role on skin rejuvenation has provided the rational for an evidence-based use of PDRN in regenerative dermatology.

Five Korean women received four injections of long-chain PDRN filler in two-week intervals for skin rejuvenation. About 0.05 mL of material was injected in 40 points of one-side cheek.

The pore and skin thickness were markedly improved in patients in their 30s, whereas skin tone, melanin, wrinkles, and sagging were noticeably ameliorated in patients in their 40s. There were no serious side effects.[1]

PNDR is extracted from the sperm of trout and contains deoxyribonucleotide polymers of 50–2000 base pairs. Although the mechanism of action is not completely understood, the mixture of nucleotides exerts its effects via activation of adenosine A_{2A} receptors.[2]

Chemical Structure of Polydeoxyribonucleotide

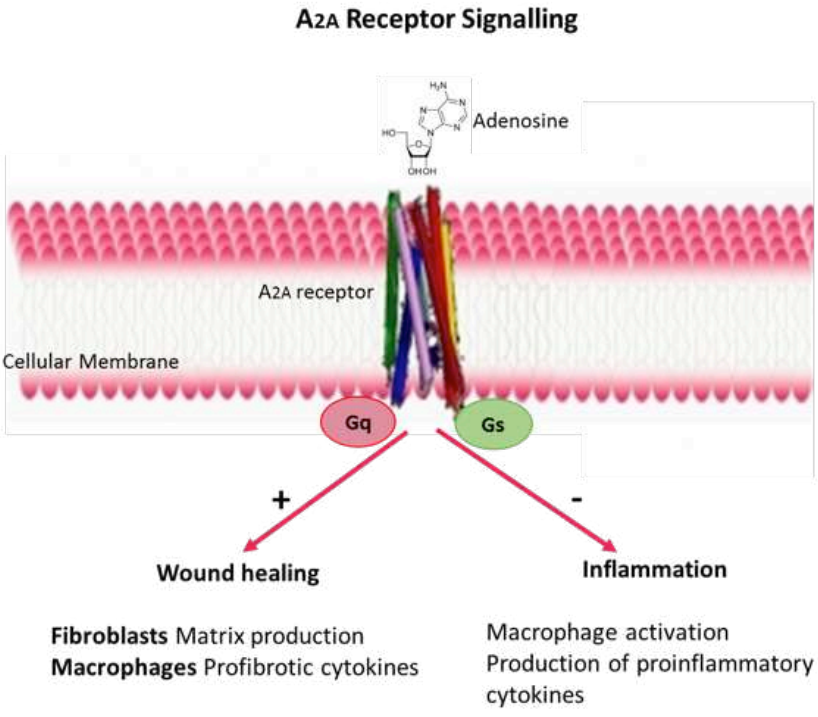


The PDRN use in regenerative medicine started long time ago and PDRN is currently applied to bone, cartilage, tendon, and skin diseases. In 1999, it had been shown that PDRN and adenosine could stimulate the growth rate of human skin fibroblasts in primary cultures and increase cytosolic calcium concentration, in dose-dependent manner and with the involvement of A_{2A} receptor.

It seemed that PDRN operated as a pro-drug, providing cells with an effective amount of mitogenic deoxyribonucleotides.[2] These data were further confirmed by other experiments with cultured human fibroblast, treated with both intact and DNAase-digested PDRN.

In both cases, cell proliferation was induced to a similar extent and specific proteins, such as fibronectin, were expressed, thus suggesting that PDRN could act as signal transducers or, alternatively, could be internalized and utilized to provide purine and pyrimidine rings for the salvage pathways.[3]

An interesting effect of PDRN was observed on pre-adipocytes in primary cultures. These cells are multipotent adult stem cells and can differentiate in vitro into mesenchymal and neurogenic lineages: stem cells have a great potential for tissue repair and regeneration. PDRN promoted proliferation of human pre-adipocytes, even when cell culture was normally senescent and stopped dividing.[4]



Clinical studies on regenerative properties of PDRN are also available.

PDRN (5625 mg/vial) or placebo were concomitantly administered via intramuscular route once daily and subcutaneously (2 vials every 3 days) for 10 consecutive days.

Even after 7 days of treatment, PDRN promoted a more rapid healing of autologous skin graft at the donor sites on 26 patients (both genders), thus suggesting that PDRN favoured re-epithelialization and had a prompter trophic effect than placebo.[5]

PDRN in eye drops was investigated after photorefractive keratectomy for correction of myopic and myopic-astigmatic defects. PDRN stimulated corneal epithelium regeneration and during the re-epithelialization stage eye drops administration four times a day was well tolerated.[6] Taken together, these evidences indicate that PDRN may have a primary role in regenerative medicine and skin anti-aging treatment.

Purinergic A2A receptors are not involved only on cellular proliferation and tissue repair but also regulate the cytokine network and inflammation process, by inhibiting inflammatory cytokines secretion by macrophages in vitro. In experimental model of periodontitis, PNDR gel restored the histological features of gingival tissue and reduced inflammatory and apoptotic proteins expression.[7]

In inflammatory bowel disease rat models, PDRN ameliorated the clinical symptoms and weight loss as well as promoted the histological repair of damaged tissues. Furthermore, PDRN decreased inflammatory cytokines expression, myeloperoxidase activity, and malondialdehyde, thus representing a promising treatment for tissue repair during inflammatory colitis.[8]

cDNA-array gene expression analysis on normal human keratinocytes revealed that PDRN modulated the expression of several genes, including genes involved in hydrous balance, differentiation, and free radical scavenging. Aquaporin and serine palmitoyl-transferase were over-expressed in presence of PDRN (AQP3, +652%; AQP8, +103%; SPTLC1, +777%).
Aquaporin is a membrane water channel that regulates cellular water content and maintains hydrous balance, while serine palmitoyl-transferase is an enzyme which synthesizes ceramides phospholipids which seal the horny layer and avoid dehydration.

PDRN favoured the expression of epithelial late differentiation proteins: cornulin expression increased by 335%, repetin that forms the corneal envelope, increased by 255%, and stratifin, strongly induced by gamma radiation and other DNA-damaging agents increased by 247%.[9]

In addition, PDRN exerts also an antioxidant activity on cells. PDRN regulated the expression of ferritin and transferrin receptor, thus conferring a further protective effect against iron toxicity in scavenging reactions. After UV exposure, ferritin expression increased to complex iron and avoid its catalyst effect on free radicals. When applied very thinly (1 mm), a 1% PDRN solution absorbed all weak intensity UV rays between a 200 nm and 300 nm wavelength. On the skin surface, PDRN could impair UV ray diffusion to the skin.[10]

PDRN addition in a sun care product enhances its photo-protective action by preventing eventual damage caused by UV rays to the skin.

PDRN are hydrophilic compound and are usually formulated in gels or aqueous medium, such as eye drops.

This feature and their steric hindrance require a cutaneous administration directly into epidermis and dermis to overcome the lipophilic barrier that protects the skin. PDRN gels are injected with thin needles and specific procedures have been optimized to gain optimal result in each part of the face. The micro-wheals technique is indicated for all body areas; the retrograde linear injections are used to fill superficial and/or medium dermis, cheek, wrinkles or Langer lines, or nasolabial folds; the cross-link technique forms a net of linear intersecting infiltrations and is recommended for cheek.

Schedule for PDRN treatment depends on patient's age and the clinical outcome which would be achieved.

For young patients, a prevention treatment (one injection every 3 weeks, for three repetition, followed by a maintenance treatment every 2-3 months) is recommended;

for aged skin, a recovery treatment (one injection every 1-2 weeks, for 4 treatment, followed by maintenance sessions every 1-3 months) is preferable.

The injections are usually very well tolerated and do not cause pain. In pain sensitive patients, it is possible to apply a local anaesthetic cream.

After PDRN injections, superficial fine wrinkles are reduced and skin of face, cheek, periocular area and neck appears younger and more tonic. The best clinical results are visible approximately one month after treatment when the regenerative process is almost completed.

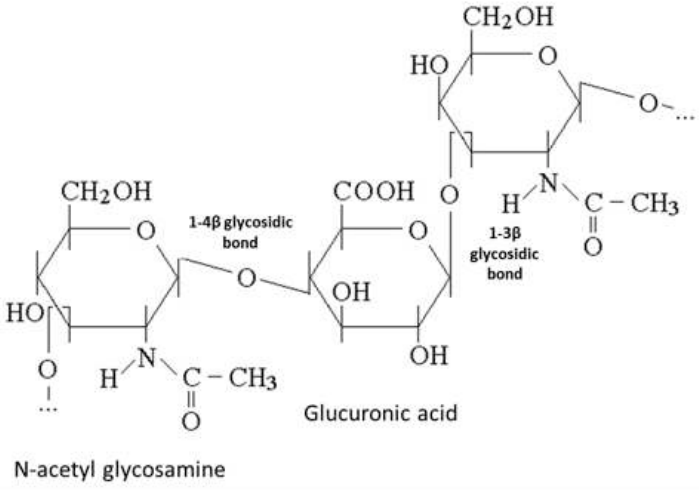
Digital skin examination showed that pH parameters and sebometry did not change, while hydration and elasticity were significantly improved.

Hyaluronic Acid

Hyaluronic acid (HA) is a natural, repetitive disaccharide composed by D -glucuronic acid and N-acetyl- D -glycosamine mono-saccharide.

An average human body contains a total of 15 g of HA, half of which (7–8 g) is in the skin.[11]

Chemical structure of Hyaluronic Acid



As it does not exhibit species and tissue specificity, HA has an excellent tolerance and may be considered as an ideal candidate to create volume in soft tissue such as skin.

However, its half-life is short, especially in the skin where it lasts less than 24 hours.
When injected intradermally, the effect of soft tissue volume and shape enhancement is limited to 6–18 months, depending on type of filler, anatomical site and individual patient's metabolism.

Cutaneous structures remain morphologically intact and no inflammation is induced by HA injection; no granulation or wound healing processes are triggered.

HA injection does not alter even fibroblasts function, collagen and elastin fibrils, as well as other extracellular matrix components.
The mechanism of action is mainly related to high hydrophilicity.

HA attracts water, and this helps it form large concentrations that can occupy a large volume relative to its mass; when water is drawn into the HA matrix, it has been shown to create a swelling pressure or turgor that enables the HA complex to withstand compressive forces.[12]

At the present, HA is produced by biotechnological processes from streptococcus fermentation and cross-linked with a binding agents.
Cross-linked fillers can be classified in two types: cohesive (monophasic) and non-cohesive (biphasic), depending on technological processes used.

Both formulations are directly injected into the dermis around wrinkles or scar in the face and neck.[13]

PDRN and HA: concomitant or consecutive administration?

The role of extracellular matrix components and adenosine A2A receptor mediated signalling has been investigated in several organs with different results.

In lung inflammation, low molecular weight fragments of HA played a critical role in fibrosis by inducing inflammatory gene expression at the injury site, while adenosine signalling via A2A receptor negatively regulated inflammation and protected tissues from immune destruction.[14]

In animal models of myocardial infarction, adenosine stimulated angiogenesis mainly through up-regulation of vascular endothelial growth factor (VEGF).

Human macrophages up-regulated VEGF expression and secretion in adenosine-dependent manner; this effect was exacerbated by hyaluronic acid and other EMC components.

Therefore, it seemed that both adenosine and ECM synergistically acted to stimulate cardiac repair.[15]

In arthritis animal models, the inactivation of hyaluronic acid fragments derived from native HA during inflammation reduced the inflammatory response, as well as the stimulation of adenosine receptors A2A reduced inflammation by inhibiting NF-kB activation.
Two treatment may be combined to obtain a greater clinical outcome in reducing inflammation and apoptotic markers.[16]

Based on our knowledge, data on administration of HA and adenosine signalling activation in the skin have not published yet.

However, some concerns may arise from the high hydrophilicity of both HA and PDNR.

A concomitant administration of two highly hygroscopic compounds, as HA gels and PDRN are, may generate an excessive water attraction, thus altering the ECM hydrous balance.

Consecutive administration of PDRN and HA may overcome this potential limitation and enhance the advantages from regenerative properties of nucleotides and hydrophilic features of HA.

Indeed, cellular proliferation promotes tissue repair, without wound healing or triggering inflammation, while the anti-oxidant action of A2A receptor mediated activity further protects the skin from aging process.
After promoting tissue regeneration, the injection of HA filler may generate volume and achieve an aesthetic favourable result.

Conclusion

Polydeoxyribonucleotide is an important tool for regenerative medicine to promote anti-aging processes.
Cutaneous revitalization is triggered from the main player of dermis development, fibroblasts and stem cells, and it respects the biological timing of tissue formation.
HA injection offers a further aid to improve skin volume and tonicity, especially for patients who would need strong treatments.

BIBLIOGRAFIA

1. Park KY, Seok J, Rho NK, Kim BJ, Kim MN. Long-chain polynucleotide filler for skin rejuvenation: efficacy and complications in five patients. Dermatol Ther. 2016 Jan-Feb;29(1):37-40.

2. Thellung S, Florio T, Maragliano A, Cattarini G, Schettini G. Polydeoxyribonucleotides enhance the proliferation of human skin fibroblasts: involvement of A2 purinergic receptor subtypes. Life Sci. 1999;64(18):1661-74.

3. Sini, P, Denti A, Cattarini G, Daglio M, Tira ME, Balduini C. Effect of polydeoxyribonucleotides on human fibroblasts in primary culture. Cell Biochem Funct. 1999;17:107–114.

4. Raposio E, Guida C, Coradeghini R, Scanarotti C, Parodi A, Baldelli I, Fiocca R, Santi PL. In vitro polydeoxyribonucleotide effects on human pre-adipocytes. Cell Prolif. 2008 Oct; 41(5):739-54.

5. Rubegni P, De Aloe G, Mazzatenta C, Cattarini L, Fimiani M. Clinical evaluation of the trophic effect of polydeoxyribonucleotide (PDRN) in patients undergoing skin explants. A Pilot Study. Curr Med Res Opin. 2001;17(2):128-31.

6. Lazzarotto M, Tomasello EM, Caporossi A. Clinical evaluation of corneal epithelialization after photorefractive keratectomy in patients treated with polydeoxyribonucleotide (PDRN) eye drops: a randomized, double-blind, placebo-controlled trial. Eur J Ophthalmol. 2004 Jul-Aug;14(4):284-9.

7. Bitto A, Oteri G, Pisano M, Polito F, Irrera N, Minutoli L, Squadrito F, Altavilla D. Adenosine receptor stimulation by polynucleotides (PDRN) reduces inflammation in experimental periodontitis. J Clin Periodontol. 2013 Jan;40(1):26-32.

8. Pallio G, Bitto A, Pizzino G, Galfo F, Irrera N, Squadrito F, Squadrito G, Pallio S, Anastasi GP, Cutroneo G, Macrì A, Altavilla D. Adenosine Receptor Stimulation by Polydeoxyribonucleotide Improves Tissue Repair and Symptomology in Experimental Colitis. Front Pharmacol. 2016 Aug 23;7:273.

9. Cavallini M, Papagni M.Long chain polynucleotides gel and skin biorevitalization Journal of Plastic Dermatology 2007; 3, 3

10. Cillard J, Perex S, Cillard P, Sergent O, Morel I, Ranson M. Study of protective effect of highly polymerized deoxyribonucleotides (HPDR) against lipid peroxidation. STP pharma sciences. 1994 4(5): 359-365.

11. Alberts B, Johnson A, Lewis J, et al., editors. The molecular biology of the cell Cell junctions, cell adhesion, and the extracellular matrix 1065. New York: Garland Science; 2002.

12. Gold MH. Use of hyaluronic acid fillers for the treatment of the aging face. Clin Interv Aging. 2007;2(3):369-76.

13. Tran C, Carraux P, Micheels P, Kaya G, Salomon D. In vivo Bio-Integration of Three Hyaluronic Acid Fillers in Human Skin: A Histological Study. Dermatology 2014;228:47–54.

14. Collins SL, Black KE, Chan-Li Y, Ahn YH, Cole PA, Powell JD, Horton MR. Hyaluronan fragments promote inflammation by down-regulating the anti-inflammatory A2a receptor. Am J Respir Cell Mol Biol. 2011 Oct;45(4):675-83.

15. Ernens I, Léonard F, Vausort M, Rolland-Turner M, Devaux Y, Wagner DR. Adenosine up-regulates vascular endothelial growth factor in human macrophages. Biochem Biophys Res Commun. 2010 Feb 12;392(3):351-6.

16. Campo GM1, Micali A, Avenoso A, D'Ascola A, Scuruchi M, Pisani A, Bruschetta A, Calatroni A, Puzzolo D, Campo S. Inhibition of small HA fragment activity and stimulation of A2A adenosine receptor pathway limit apoptosis and reduce cartilage damage in experimental arthritis. Histochem Cell Biol. 2015 May;143(5):531-4

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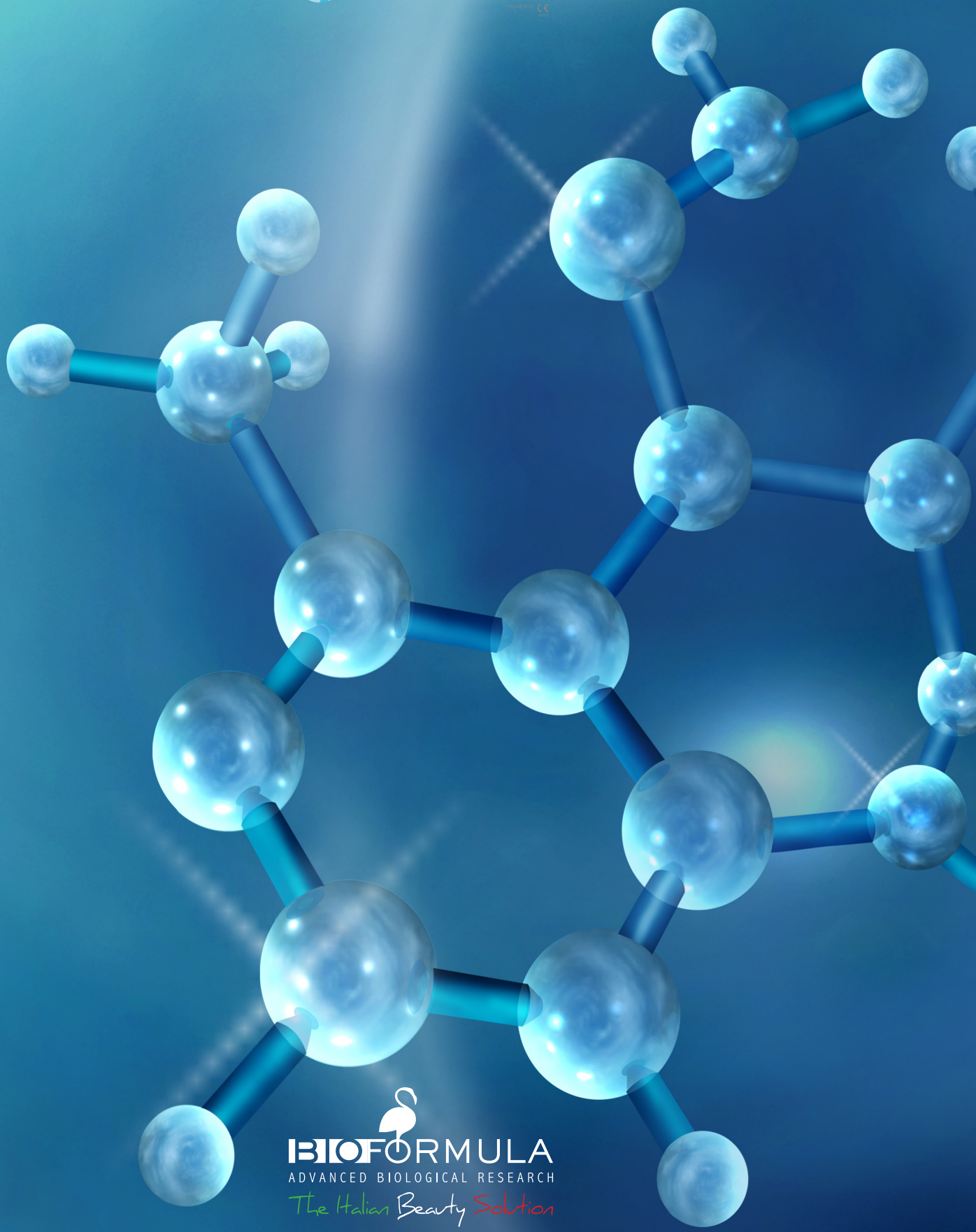

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