

# Neocollagenesis and NeoeLASTinogenesis: From the Laboratory to the Clinic

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## ABSTRACT

An internet search was made looking for articles about chemical and physical modalities that are known to induce collagen and elastin formation. Textbooks, independent articles, journals and books on pathology, biochemistry, aesthetic medicine and cosmetic and plastic surgery were used as references. Here, we take a look at various studies, *in vitro* and *in vivo*, that lend credence to the products and procedures used in clinical practice to induce neocollagenesis and neoeLASTinogenesis.

**KEYWORDS:** Cutaneous ageing, cutaneous scarring, neocollagenesis, neoeLASTinogenesis, wrinkles

## INTRODUCTION

Collagen and elastin are the main fibres that form the extracellular matrix. Both are formed by fibroblasts. Collagen is responsible for tensile strength and elastin provides elasticity to the skin. Production and density of both decreases as a function of age, and results in sagging and wrinkling. Wounding alters the amount and quality of these fibres. Thus, neocollagenesis and neoeLASTinogenesis hold the key to managing aesthetic conditions such as cutaneous ageing and scarring.

## TOPICALS AND NEUTRACEUTICALS

A healthy diet is one of the prerequisites of healthy skin. Proteins, vitamins, minerals, trace elements and antioxidants obtained from our diet are the routinely available basic ingredients for formation and maintenance of skin fibres. Vitamin and protein supplements in the form of neutraceuticals enhance the availability of substrates for synthesis of new fibres. Antioxidants (Vitamins A, C and E, trace elements such as selenium, copper and zinc, polyphenols, flavonoids, glutathione, peroxidases and superoxide desmutases) prevent degradation of tissues caused by free radicals.

## Retinoids

Retinoids are compounds that are chemically related to Vitamin A. Among the retinoids; tretinoin possibly is the most potent and certainly the most widely investigated retinoid for photoaging therapy. Biochemical changes brought about by retinoids include an increase in the expressions of Type 1 and Type 3 procollagen, reticulin fibres, tropoelastin, fibrillin-1 and glycosaminoglycans (GAGs).<sup>[1]</sup> Tretinoin results in complete blockade of interstitial collagenase and gelatinases synthesis and reduces matrix metalloproteinase 1 (MMP1). Metalloproteinases degrade amorphous collagen. Thus, retinoids prevent collagen degradation.<sup>[2]</sup> Histologically, tretinoin results in marked increase in epidermal cell layer thickness and a highly undulating dermo-epidermal junction through the development of rete pegs and produces uniformity in keratinocyte density while it decreases melanocyte vacuolisation. Ultrastructurally, an increase in anchoring fibrils is noted at the level of the dermo-epidermal junction. In the dermis, development of several new

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microvasculatures (angiogenesis) and production of new elastic material and GAGs is observed.<sup>[3]</sup>

Irritant reactions limit the acceptance of retinoids by patients. This problem is more prominent with tretinoin and tazarotene whereas other retinoids mainly represented by retinaldehyde and retinol are considerably less irritating. To minimize these side effects, various novel drug delivery systems have been developed.<sup>[4]</sup> Seletinoid G represents the fourth generation of retinoids that might find an important place in the treatment of intrinsic/photoaging. Topical application of seletinoid G under occlusion induced no skin irritation in contrast to tretinoin which caused severe erythema. Thus, seletinoid G appears to be as effective as tretinoin in the treatment of intrinsic/photoaging with the added advantage of the absence of skin irritation.<sup>[5]</sup> A new drug delivery system, solid lipid nanoparticles for the delivery of retinol based tretinoin gels showed drastic improvement in the tolerability of tretinoin.<sup>[6]</sup>

Retinol [Figure 1] could be as effective as retinoic acid in producing 'retinoid-mediated histological changes' like epidermal thickening and keratinocyte proliferation, but with much less irritancy.<sup>[7]</sup>

**Vitamin C**

Vitamin C (ascorbic acid) is a cofactor in the hydroxylation of lysine and proline required for making the triple helix structure of collagen. It is needed in the cross-linking process that converts soluble tropoelastin to insoluble elastin. Vitamin C induces a dose-dependent increase in collagen Type I deposits by normal human fibroblasts.<sup>[8]</sup>

In a study by Fitzpatrick and Rostan a formulation of Vitamin C - 10% ascorbic acid (water soluble) and 7%

tetrahexyldecyl ascorbate (lipid soluble) in an anhydrous polysilicone gel base resulted in clinically visible and statistically significant improvement in wrinkling when used topically for 12 weeks. Biopsies showed increased Grenz zone collagen, as well as increased staining for microRNA (mRNA) for Type I collagen.<sup>[9]</sup>

**Proteins, peptides and amino acids**

Both collagen and elastin are proteins. Collagen is rich in the amino acids glycine, proline and hydroxyproline. Elastin is mainly composed of glycine, valine and lysine.

Net collagen accumulation depends not only on increased synthesis but also on decreased degradation.<sup>[10]</sup>

Hydrolysed collagen made from native peptides found in animals consists of small peptides with low molecular weight, enriched in specific amino acids: Glycine, proline and hydroxyproline. Due to its low molecular weight, hydrolysed collagen is highly digestible, absorbed and distributed in the different tissues of the human body. In the dermis, hydrolysed collagen has a dual action mechanism - (a) free amino acids provide building blocks for the formation of collagen and elastin fibres, (b) collagen oligopeptides act as ligands, binding to receptors present on the fibroblasts' membrane and stimulate the production of new collagen, elastin and hyaluronic acid (HA).<sup>[11]</sup>

**Oestrogens and geneistein**

Oestrogens have a profound influence on skin. The relative hypoestrogenism that accompanies menopause exacerbates the deleterious effects of both intrinsic and environmental aging. Oestrogens increase collagen content and skin thickness and improve skin moisture. Although systemic hormone replacement therapy (HRT) has been used for many years, recent trials have reported a significant increased risk of breast cancer and other pathologies with this treatment. For this reason, systemic HRT cannot be recommended today to treat skin aging. Currently, intensive research is being conducted to develop new drugs called selective oestrogen receptor modulators (SERMs).<sup>[12]</sup>

Geneistein (a soy isoflavone, known for its phytoestrogenic properties) is the best known natural SERM. As oestrogen receptor agonist, genistein stimulates the production of new collagen. It inhibits tyrosine protein kinase and MMPs, thus stimulating de novo production and inhibiting degradation of collagen and elastin.<sup>[13]</sup> Topically applied genistein cannot exert significant physiological effects on the skin unless encapsulated in liposomes. Liposomal genistein has shown to increase skin thickness and firmness which is principally determined by collagen.<sup>[14]</sup>



Figure 1: (a) Topical retinoids for antiaging- before treatment. (b) Topical retinoids for antiaging - after 2 months of application

## ENERGY BASED TECHNOLOGIES

Various energy based technologies like radio-frequency (RF), infra-red and deep ultrasound are available that trigger neocollagenesis and help to firm, tighten, lift and smoothen skin [Figure 2]. Application of monopolar RF creates an electro-magnetic field (EMF) with alternating polarity which stimulates the movement of charged particles and sets up an electric current. The current conducts most effectively through hydrophilic structures such as dermal collagen framework and underlying fibrous septae and much less effectively through subcutaneous fat. Tissue resistance to the flow of current generates localised heat within the collagen-based structures.<sup>[15]</sup> The threshold of collagen denaturation is approximately 60-65°C. Temperatures below 60° have minimal effect and above 70° show insignificant additional benefits.<sup>[16]</sup>

Monopolar RF leads to neocollagenesis in the papillary dermis, and upper, mid, and deep reticular dermis, and neoelectinogenesis in the papillary dermis, and upper- and mid-reticular dermis. High-intensity focused ultrasound (HIFU) leads to neocollagenesis in the mid and deep reticular dermis and neoelectinogenesis in the deep reticular dermis. Among these treatment methods, HIFU shows the highest level of neocollagenesis and neoelectinogenesis in the deep reticular dermis.<sup>[17]</sup>

Collagen rejuvenates over a month or so after treatment. Increased small collagen fiber formation-evidence of neocollagenesis, has been noted at 30 days post heat treatment. On tracking tissue changes after heating to denaturation range, neocollagenesis, neoelectinogenesis and deposition of new HA at 10 weeks post treatment were found.<sup>[18]</sup>

Nonablative RF is a safe and effective method to achieve tissue tightening of the face to correct excessive sagging from photo aging. Patients had visible results as early as 1 week and generally within 3 months after the procedure without wounding or scarring. Fractional RF (FRF)



Figure 2: (a) Skin tightening with radiofrequency and ultrasound - before treatment. (b) Skin tightening with radiofrequency and ultrasound - after two sittings at 10 days interval

treatment generates an RF thermal zone (RFTZ) pattern in the reticular dermis that consists of zones of denatured collagen separated by zones of spared dermis. A vigorous wound healing response is initiated post-treatment, with a progressive increase in inflammatory cell infiltration from day two through 10 weeks. An active dermal remodelling process driven by the collagen chaperone HSP47 leads to complete replacement of RFTZs with new collagen by 10 weeks post-treatment. RFTZs are observed through day 28 post-treatment but are replaced by new dermal tissue by 10 weeks. Reticular dermal volume, cellularity, HA and elastin content increase. Furthermore, using immunohistochemical and polymerase chain reaction (PCR) studies, evidence of profound neoelectinogenesis following RF treatment of human skin is demonstrated. The combination of neoelectinogenesis and neocollagenesis induced by treatment with the FRF system may provide a reliable treatment option for skin laxity and/or rhytids.<sup>[18,19]</sup>

Broad-spectrum (1100-1800 nm) infrared light in multi-second cycles heats water in the dermis. With this band, bulk heating of the dermis is maximised at one to three mm, and absorption by melanin and haemoglobin is low. The epidermis is protected by a cooling head. Collagen fibril denaturation has been shown by electron microscopy. Multiple passes at low energy levels are associated with bulk dermal heating and collagen contraction.<sup>[20]</sup>

## LASER AND LIGHT THERAPIES

Stimulation of new collagen production by laser treatments has been attributed to the release of inflammatory mediators from vascular epithelial cells. When stimulated by treatment with a combination of 532 nm and 1064 nm laser devices, collagen remodelling continues for 6-12 months and increases with the number and intensity of treatments, as well as elapsed time.<sup>[21,22]</sup>

Cell growth and cellular viability increase in fibroblasts 24 and 48 h after intense pulsed light irradiation. The levels of Type 1 and 3 procollagen mRNA expression in fibroblasts increase.<sup>[23]</sup> There is rearrangement of both collagen and elastin within the stroma.<sup>[24]</sup>

Twenty-two patients completed a 1-year study by Rosenberg *et al.* in which biopsy specimens from the upper lip were taken preoperatively and 6 weeks, 6 months and 1 year after CO<sub>2</sub> laser resurfacing. Trichrome stains and Verhoeff-van Gieson stains were used to demonstrate tissue collagen and tissue elastin. Neocollagenesis beginning at 6 weeks and progressively increasing at 6 months and 1 year was clearly demonstrated. Neoelectinogenesis showed significant increases at 6 months and 1 year. It is hypothesised that the additive



effects of initial collagen shrinkage and the long-term effect of neocollagenesis and neoelastinogenesis are the significant factors contributing to the long-lasting results of CO<sub>2</sub> laser skin resurfacing.<sup>[25]</sup>

### MICRONEEDLING/COLLAGEN INDUCTION THERAPY

In 2006, Dr. Laaff, a dermatopathologist, evaluated twenty blinded biopsies from a study performed by Dr. M. Schwarz, a plastic surgeon, into the effects of the derma roller device on collagen and elastin formation. Biopsies were taken from various parts of the body of ten patients undergoing elective cosmetic surgery. A first 'control' biopsy was taken adjacent to the site that was needled with the derma roller. Six to eight weeks later, another biopsy was taken from the needled skin. New collagen and elastin fibre formation was obvious and quite dramatic. On average, an increase of new fibres of 206% was observed.<sup>[26]</sup> Compared to wounds caused by trauma or surgery, in wounds caused by microneedling, the haemostasis, inflammation, proliferation and remodelling phases are significantly curtailed and healing never ends in a scar formation. Microneedling of normal skin leads to a polarised EMF in the inter-cellular electrolyte. The EMF stimulates DNA-expression of the surrounding cells. This epigenetic DNA-information by electro-taxis leads to an enhanced motility of epithelial and endothelial cells in the wounded area and subsequently to gene expression of growth factors that facilitate healing. After microneedling of a scar, new capillaries and fibroblasts migrate to the scar tissue. Under the influence of transforming growth factor-B3 (TGF-B3),<sup>[10]</sup> new Type 3 collagen fibres integrate into the existing skin matrix without any trace of fibrotic tissue. MMPs degrade excess fibrotic tissue. New collagen is deposited from a depth of 0.6 mm upwards and towards the basal membrane, in the most cases when needles with a length of 1.5 mm are used.<sup>[27]</sup>

Skin improvement is evident 3-4 weeks after a microneedle session. However, collagen maturation needs time, especially to transform into more tensile collagen Type 1.<sup>[28,29]</sup>

### FILLERS

HA fillers were designed to provide temporary correction to enhance static contour defects. It is now theorised that these HA fillers may have more permanent and longer lasting effects.<sup>[30]</sup> Wang *et al.* explored the mechanisms behind potential neocollagenesis from injection of cross-linked HA fillers.<sup>[31]</sup> They were able to prove that fibroblasts take on a more morphologically stretched shape and a more active phenotype as a result of HA fillers. HA injection, however, was not shown to have a direct stimulatory effect on fibroblasts. Instead, it

was demonstrated and theorised that existing collagen fibers are stretched by HA injection, which imposes mechanical tension on surrounding fibroblasts, therefore, stimulating them to increase production of Types 1 and 3 collagen. Procollagen, MMP and MMP tissue inhibitors of metalloproteinase (TIMP-1) were also measured by ELISA and quantitative PCR. They also found a statistically significant increase in procollagen, TIMP-1 and gene expression of procollagen 1 and 3, 1 month after injection. Procollagen levels remained elevated at three and 6 months of subsequent injections.<sup>[32]</sup>

### DERMAL THREADS

Polydioxanone (PDS) is a colourless, crystalline, biodegradable synthetic polymer. It has been used as a suture material for many years. All sutures, regardless of composition, are foreign materials and elicit a foreign body reaction in the body.<sup>[10]</sup> Absorption of suture is by simple hydrolysis within 180 days from implantation day. PDS sutures cause minimal tissue reaction. They stimulate the activity of fibroblasts, thus activating the mechanism of neocollagenesis. It has been histologically confirmed that within the 1<sup>st</sup> month a delicate fibrous capsule forms around the implanted threads. It coats the threads as a small soft connective tissue cover, creating artificial ligaments that strengthen the effect achieved after the thread introduction. As the result, there has been noted a double effect of absorbable threads usage - consistent mechanical lift effect and tissue revitalisation.<sup>[33,34]</sup>

### PLATELET RICH PLASMA

Autologous platelet rich plasma (PRP) has received a lot of attention recently for its healing and rejuvenating properties and is used to treat scars, [Figure 3] wrinkles or hair loss. Platelets on degranulation release growth factors and cytokines like platelet derived growth factor, TGF-B, fibroblast growth factor-2, vascular endothelial growth factor, epidermal growth factor, etc.<sup>[10]</sup> PRP has been investigated for its effects on the remodelling of the extracellular matrix, a process that requires activation of dermal fibroblasts, which is essential for rejuvenation of aged skin.



Figure 3: (a) Acne scars - before platelet rich plasma. (b) Acne scars - after 2 months of single session of platelet rich plasma with microneedling

Levels of procollagen Type 1 carboxy-terminal peptide are high in cells grown in the presence of 5% PRP. In addition, PRP increases the expression of Type 1 collagen, MMP-1 protein, and mRNA in human dermal fibroblasts.<sup>[35]</sup>

In a clinical trial by Redaelli *et al.* involving monthly injections of four ml PRP in 23 volunteers of average age 47 years for facial rejuvenation, almost 30% improvement was seen in deep wrinkles. Furthermore, every patient noted improvement in skin texture, homogeneity and small wrinkles.<sup>[36]</sup> In a split-face trial using PRP after fractional CO<sub>2</sub> resurfacing, PRP enhanced recovery and clinical improvement of acne scars.<sup>[37]</sup>

## DISCUSSION

Skin shows the first visible signs of ageing of the body as the ageing of internal organs are hidden from sight. Scars can cause tremendous psychological distress even in children. In a market that is flooded with products that claim to treat scars and prevent or even reverse ageing, it is important to confirm the bioavailability and efficacy of the ingredients at the level of the dermis and to use formulations that provide maximum benefits with minimal adverse effects. Newer peptides are introduced to us every day in cosmeceuticals, with every new formulation containing new combinations and claiming amazing, almost unrealistic results. Matrikines (Avarta, Dr. Reddy's) are peptide fragments derived from proteolysis of extracellular matrix. They exert feedback control over connective tissue renewal. When combined with palmitic acid, their lipophilicity and hence skin penetration, is enhanced.<sup>[38]</sup> Signal peptides such as Palmitoyl pentapeptides may assist in neocollagenesis. Copper peptides have a dual role as collagen stimulators and antioxidants for wound

healing. Peptides may be synergistic with energy based devices to sustain the neocollagenesis initiated by them. Growth factors are also being used for skin repair and regeneration. They also pose risks because, unregulated, they may mediate carcinogenic transformation of skin cells. This new field of cosmeceuticals is exciting but still in its infancy.<sup>[39]</sup>

Outpatient noninvasive or minimally invasive clinical procedures look promising and effective in the short-term. Neocollagenesis is easily induced, but that does not necessarily translate into mature collagen formation as procollagen has a very short half-life and is easily degraded. Collagen maturation is a process that requires years.<sup>[40]</sup> Long-term studies are needed to know if these energy-based treatments affect the content of mature collagen in the dermis.

The clinician may be faced with a dilemma as to which modality for neocollagenesis and neoelectinogenesis needs to be used in what patient and when [Table 1]. Apart from a healthy lifestyle and neutraceuticals, Table 2 gives a guideline by the author to help select the modality. Different approaches can be used even in the same patient at different times depending on the prevailing skin condition.

Options are growing by the day, so one must carefully screen patients to provide them the therapy ideally suited to their skin which is backed by evidence-based research.

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## Conflicts of interest

There are no conflicts of interest.

**Table 1: Summary of products and procedures currently used in clinical practice to induce neocollagenesis and neoelectinogenesis and their mechanism of action**

Modality	Mechanism of action
Retinoids	Increase in the expressions of Type 1 and Type 3 procollagen, reticulin fibres, tropoelastin, fibrillin-1 and GAGs <sup>[1]</sup> Complete blockade of interstitial collagenase and gelatinases synthesis and reduced MMP1, thus preventing collagen degradation <sup>[2]</sup>
Vitamin C	Cofactor in formation of triple helix of collagen, cross linking of soluble elastin to make it insoluble <sup>[6]</sup>
Hydrolysed collagen	Provides amino acids as building blocks for collagen and binds to fibroblasts stimulating production of collagen and elastin <sup>[11]</sup>
Geneistein/Soy isoflavones	SERM inhibit tyrosine protein kinase and matrix metalloproteinases, thus stimulating de novo production and inhibiting degradation of collagen and elastin <sup>[13]</sup>
Energy based devices- radiofrequency, ultrasound and infrared	Heat induced denaturation followed by new collagen and elastin formation driven by HSP47 <sup>[18,19]</sup>
Laser and light based devices	Release of inflammatory mediators from vasculature, improved growth and viability of fibroblasts <sup>[21-23]</sup>
Microneedling	DNA expression stimulated by electromagnetic field in normal skin, degradation of old fibrotic tissue by MMPs and formation of new collagen Type III in old scar tissue <sup>[26]</sup>
Fillers	Stimulation of fibroblasts due to stretching <sup>[32]</sup>
Threads	Foreign body reaction, stimulation of fibroblasts <sup>[10]</sup>
PRP	Release of growth factors from platelet granules <sup>[10]</sup>

SERM: Selective oestrogen receptor modulator, MMP1: Matrix metalloproteinase 1, GAGs: Glycosaminoglycans, PRP: Platelet rich plasma

**Table 2: Guideline by the author to help select modality to induce neocollagenesis and neoelastinogenesis**

Modality	Indication
Vitamin C (e.g., VC 15 serum (15%) [Cipla], Facemed gel (5%) [Ethicare remedies], Flavo C serum (8%) [Bioderma])	Initiation of antiaging program, age under 30 years, pigmentation, adjuvant with other products and procedures
Retinoids (e.g., Yugard (0.15% retinol) [Sun-Ranbaxy], Ega (0.15% retinol) [Curatio], Ystheal plus antiaging cream and emulsion (0.05% retinaldehyde), Ystheal plus eye and lip contour cream (0.025% retinaldehyde) [Avene], Retin-ox correxion intensive antiwrinkle serum (0.06% retinol) Retin-ox correxion wrinkle smoothing eye cream (0.02%) [RoC, Johnson and Johnson], Vichy Liftactive Advanced Filler (0.2% retinol) and Liftactive Retinol HA eye, (0.1% retinol) Neutrogena Rapid Wrinkle Repair cream [Retinol 0.04%])	Should be part of all antiaging regimens unless poorly tolerated
Soy extracts [e.g., Epilyno (Sun-Ranbaxy), Flavonex (Bioderma) etc.]	Females in perimenopausal age group, intolerance to retinoids
Energy based devices (e.g., RF and ultrasound - Exelis Elite [BTL India], RF - Thermage [Solta Medical, USA], Ultrasound - Ulthera [Merz aesthetics, USA] infrared - Titan, [Cutera, USA], Harmony IR handpiece [Alma, USA])	Initially to prevent sagging and later to provide a lifting effect, for patient on a weight loss programme, patient unwilling to go for neurotoxins/fillers
Laser and light therapy (e.g., IPL, Nd: YAG, CO <sub>2</sub> etc.)	Non ablative lasers and IPL for patients who have pigmentation, telangiectasis; ablative lasers for scars and wrinkles, textural anomalies
Fillers, threads	Static, deep wrinkles and contour correction
Microneedling, platelet rich plasma	Fine lines, scars, textural anomalies

Disclaimer - Names and contents of products and names of devices are provided as a reference for the reader and taken from product inserts, representatives or websites. The author is not responsible for the correctness of this information. IPL: Intense pulsed light, Nd:YAG Neodymium-doped yttrium aluminium garnet, RF: Radiofrequency

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