Perspective | Received 14 August 2024; Accepted 11 November 2024; Published 18 November 2024
https://doi.org/10.55092/bm20240009

Erase the trace: new frontiers in scar prevention and skin repair

Shi Fu[1,†], Niharika Singh[2,†], Shiffoni Sukhlal[1], Huiting Luo[1], Divleen K. Singh[2], Mimi R Borrelli[2], Duc Bui[2], Sami Khan[2], Alexander B. Dagum[2] and Gurtej Singh[2,*]

[1] Department of Materials Science and Chemical Engineering, Stony Brook University, Stony Brook, New York, USA
[2] Department of Surgery, Stony Brook University Hospital, Stony Brook University, Stony Brook, New York, USA

† Joint first authors.

* Correspondence author; E-mail: Gurtej.Singh@stonybrookmedicine.edu.

Abstract: The management of scarring continues to be significant in health care due to their ubiquity and impact on daily life. Scars include immature, mature, atrophic, hypertrophic, and keloid scars, with hypertrophic and keloid scars commonly being targeted for therapeutic interventions. Hypertrophic and keloid scars have dysregulated wound healing phases, involving higher levels of inflammatory markers, such as TGF-β, PDGF, VEGF, as well as increased type 1 collagen. Current treatments for hypertrophic scarring and keloid scars include pressure garments, corticosteroids, laser therapy, scar excision, and radiation. The next steps in therapy involve minimizing scars and eventually eliminating scars by tissue regeneration; current research is exploring the inhibition of Yes-associated protein and harnessing TGF-β3 to support tissue regeneration over scarring in humans.

Keywords: scars; scar prevention; skin regeneration

1. Understanding scars: functional and aesthetic aspects

Scars can pose functional, aesthetic, and psychological concerns. Due to the ubiquity and ramifications of scars, the global scar treatment market continues to grow, expected to reach $32 billion annually by 2027 [1]. An international epidemiological survey in adults found that visible scars had a significant psychological impact on individuals; visible scars detrimentally impact self-esteem and body image and reduce quality of life due to adverse effects on social interactions, personal relationships, and professional opportunities [2]. Scars include immature, mature, atrophic, hypertrophic, and keloid scars. Normal scars are formed under controlled conditions of wound healing; they begin as immature scars and transition to

mature scars over months as inflammatory cells undergo apoptosis. Normal (optimal) scars are thin, skin tone, lay flat, and are within the confines of the initial injury. Abnormal scars include atrophic scars, which are flat, widespread and often depressed. Pathologic scars include hypertrophic scars and keloid scars. Hypertrophic scars are raised lesions that do not extend beyond the boundaries of the original injury. They usually affect areas near joints. Histologically, the collagen fibers are fine, well-organized, and oriented parallel to the epithelium [3,4]. In contrast, keloid scars extend beyond the margins of the injury. They occur most commonly in patients of African or Asian ancestry and usually affect skin areas without hair follicles. Histologically, collagen bundles are thick, large, and oriented randomly [3,4].

2. Phases of wound healing

Scarring is the end product of wound healing and contrasts greatly with the regenerative processes observed in the human fetus. The complex biological process of wound healing progresses through three distinct phases: inflammation, proliferation, and remodeling. Disruptions to any of these phases can result in impaired healing and influence the type and severity of scar formation as detailed below [5]. The different features of normal scars, hypertrophic scars, and keloid scars are described in Table 1.

2.1. Inflammatory phase

Normal Process: When an injury occurs on the skin, the body activates its defense mechanism, the innate immune system, to prevent infection by eliminating pathogens and removing debris from the wound. Neutrophils arrive first to remove bacteria and produce transforming growth factor alpha (TNF-α), IL-1, and IL-6, triggering inflammatory responses and the secretion of vascular endothelial growth factor (VEGF) and IL-8 to repair blood vessels [6]. Recruited monocytes then mature into macrophages, which ensure phagocytosis to clear the wound and subsequently secrete growth factors including TGF-α, TGF-β, FGF, PDGF, and VEGF, thereby promoting cell proliferation, migration, and angiogenesis in preparation for the proliferative phase [6].

Disruptions: In hypertrophic and keloid scarring, TGF-β and PDGF are upregulated, which promotes fibroblast migration and proliferation, as well as collagen production [7]. In keloid scars, the overproduction of TGF-β results in fibroblasts that are hyperresponsive to TGF-β and PDGF [7].

2.2 Proliferative phase

Normal Process: This phase is characterized by the formation of new tissue and the development of the extracellular matrix (ECM). Key processes during this phase include re-epithelialization, angiogenesis, and collagen deposition. During re-epithelialization, keratinocytes, the primary cells in the epidermis, re-form the epidermal layer [5]. Angiogenesis, the formation of new blood vessels, is driven by factors such as VEGF, PDGF, bFGF, and thrombin to ensure the newly formed tissue receives adequate nutrients and oxygen [5].

Fibroblasts also play a critical role in this phase by synthesizing collagen and other ECM components which provide structural support to the newly formed granulation tissue. Fibroblasts become activated myofibroblasts through a process called Fibroblast to Myofibroblast Transition (FMT) that is thought to be triggered by matrix metalloproteinases (MMPs); the activated myofibroblasts aid in replacing the provisional matrix and with wound contraction [5,8]. The newly synthesized ECM acts as a scaffold for cell attachment and tissue organization, filling up the wound gap and preparing the wound for the remodeling phase in normal scars. During the proliferative phase, angiogenesis is primarily promoted by macrophage-released cytokines, such as TGF-β and VEGF. VEGF, which can be upregulated by TGF-β, induces the growth and migration of endothelial cells and stimulates fibroblasts in collagen production and cell proliferation during the healing process [9].

Disruptions: Excessive VEGF activity leads to hypertrophic scars or keloids due to increased collagen synthesis and fibrotic response, influenced by enhanced angiogenesis and inflammation [8]. Additionally, there is less synthesis of MMPs in keloid scars, leading to scars that extend beyond the boundaries of the initial injury [7].

2.3. Remodeling phase

Normal Process: The final phase of wound healing is the remodeling phase. This is the scar formation phase. During this phase, the immature granulation tissue undergoes further reorganization as collagen type I replaces collagen type III of the early wound [8]. Collagen type I has a higher tensile strength but requires a longer time to form [5]. MMPs and its inhibitors, tissue inhibitor of metalloproteinases (TIMPs), slow the synthesis of the ECM, allowing for the remodeling and reshaping of the ECM [7]. This balance gives rise to the features of the scar. The skin elasticity molecule, elastin, also appears, and proliferative cells, such as myofibroblasts, undergo apoptosis, which greatly reduces cellular activity within the wound as it resolves [8].

Disruptions: In hypertrophic scars and keloid scars, collagen synthesis is higher, with a higher ratio of type 1 to type 3 collagen present, thought to be due to poor downregulation of type 1 collagen synthesis [7]. Keloids contain 20 times higher collagen content than normal skin and three times higher collagen content than hypertrophic scars [7]. Keloidal fibroblasts also have a lower rate of apoptosis than normal skin fibroblasts [7].

3. Comprehensive scar management: from prevention to treatment

3.1. Prevention and initial treatments

Hypertrophic scars and keloid scars, while treated with similar strategies, show varying responsiveness; hypertrophic scars typically respond better. Preventing keloid scars and hypertrophic scars is crucial due to limited efficacy of established treatments. Enabling rapid wound healing via moisturization and maintaining a clean environment is significant. Surgical techniques to facilitate appropriate wound healing can be implemented intraoperatively to minimize scarring. Incisions should be made along natural skin tension

lines when possible, to decrease tension and the risk of wound dehiscence and development of atrophic and hypertrophic scars. Gentle tissue handling and wound edge eversion decrease the risk of postoperative abnormal or pathologic scar formation.

Additionally, appropriate surgical field preparation and antibacterial prophylaxis prevent poor wound healing secondary to infection. Maintaining the vascular dermal plexus while undermining skin or maintaining vascular pedicles while elevating flaps, combined with careful hemostasis, allows for appropriate blood supply without hematoma formation.

Multiple treatment options are available and offered as there is no one established treatment algorithm for hypertrophic scars.

3.2. First-line therapeutic options

For hypertrophic scars, common treatments include compression therapy, silicone gel sheeting, and topical or intralesional corticosteroids. Pressure garments are commonly used in the prevention of hypertrophic and keloid scarring but require significant patient compliance and lose effectiveness at about 6 months. Compliance to pressure garment therapy continues to be an ongoing issue. Optimizing the fit of pressure garments, providing social support for people who wear pressure garments, and close follow-up in the outpatient setting may improve patient compliance [10]. The suggested mechanism of action includes reduction of swelling and decreased hypoxia-induced fibroblast apoptosis during the remodeling phase [3,4]. Gel sheeting includes silicone applications, which has been shown to significantly improve pigmentation and vascularity in scars. It is thought to work by inhibiting fibroblast activity during the proliferative phase via occlusion [3,4]. Additionally, the use of corticosteroids has level 1 evidence supporting their use to reduce scar thickness [4]. By inactivating TGF-β1 in the inflammatory phase, collagen degradation is activated, allowing for softening of scars. Laser therapy or surgery can be offered if more conservative management fails. Pulsed-dye laser has been shown to improve the appearance of hypertrophic median sternotomy scars [3,4]. It is most effective for hypertrophic scars that are less than 1 year old and thin and works via destruction of the skin microvasculature, thereby decreasing fibroblast proliferation [4]. Surgical resection of hypertrophic scars to release tension can also be offered for refractory disease in conjunction with adjuvant treatment including silicone sheeting, pressure therapy, and possible intralesional cortisone injection.

Meticulous surgical repair is helpful in the prevention of hypertrophic scarring. Early silicone sheeting has been determined to be the most effective as a non-invasive preventive measure, usually applied after wound epithelialization for at least 1 month [11–13]. It is cost efficient with only minimal side effects including itching and contact dermatitis [11–13]. Silicone sheeting is used with pressure garments, another cost-effective method with minimal side effects, as they have a synergistic effect to prevent hypertrophic scarring [11–13]. Corticosteroids are an invasive treatment for hypertrophic and keloid scars with significant supporting evidence, especially useful in patients with a high risk of scarring [11–13]. Laser therapy is an invasive treatment with less supporting evidence than corticosteroids but can be used for hypertrophic scars that are refractory to steroids [12]. Surgical scar excision

serves as an expensive and invasive treatment for scars refractory to medical treatment and is generally used in conjunction with adjuvant treatments including gel sheeting, pressure dressings, and early corticosteroid injection in cases where the scar is starting to become hypertrophic [11,13].

3.3. Advanced treatments for keloids

Keloid scars, on the other hand, require a more multimodal approach involving surgical excision, intralesional steroids, mitomycin C, and occasionally adjuvant radiation therapy. The addition of intralesional corticosteroids to surgical excision significantly decreases recurrence in keloid scars. Mitomycin C or bleomycin suppresses fibroblast proliferation and is often used in conjunction with intralesional steroids [4,13]. For large recalcitrant keloid scars, radiation after surgical excision provides the most effective treatment to decrease recurrence rates by targeting the proliferative phase of wound healing [14]. While laser treatments have been described in the treatment of keloids, they often lack the penetration to effectively treat keloids, which are usually thicker than their hypertrophic counterparts.

4. Advancing scar treatment: exploring new therapeutic targets

4.1. The role of En1 in scar formation and healing

After injury, Engrailed-1 (En1) is activated via dermal fibroblasts and leads to scarring by generating about 50% of scar fibroblasts [15]. Mascharak *et al.* described that mechanical tension promotes En1 activation via signaling proteins like Yes-associated protein (YAP) [15]. A case study by Aramaki-Hattori et al showed that, compared to normal skin, fibroblasts in keloid tissues have a significantly higher percentage of YAP nuclear localization [16]. By inhibiting YAP with the medication verteporfin, skin regeneration, rather than scarring, occurred in mouse models [15]. Next steps would require in vivo experiments in large animal models such as pigs, followed by studies in humans. Although there are limited clinical trials on YAP inhibitors for keloid treatment, preclinical findings suggest that targeting YAP, along with its associated pathways, could help manage fibrosis [16,17].

4.2. The role of TGF-β in scar formation and healing

Therapeutic interventions targeting VEGF, such as VEGF inhibitors like bevacizumab [18], could potentially reduce scarring. The TGF-β/SMAD signaling pathway is crucial in forming fibrotic scar tissue. The TGF-β family includes three isoforms: TGF-β1, TGF-β2, and TGF-β3, each with distinct roles in the healing process. Immediately after an injury, TGF-β1 is released, prompting the recruitment of inflammatory cells to the injury site. This isoform is rapidly upregulated and secreted by keratinocytes, platelets, monocytes, macrophages, and fibroblasts, promoting fibroblast migration, proliferation, and collagen synthesis and deposition. TGF-β1 is essential for initiating inflammation, forming granulation tissue, stimulating wound contraction, and promoting angiogenesis by upregulating VEGF; it is also an important factor in vascular wall enhancement during vascular development. TGF-β2 is

involved in all wound healing stages, stimulating epithelial regeneration, macrophage and fibroblast recruitment, and fibrous ECM production.

4.3. Harnessing TGF-β3 for regenerative healing

On the other hand, TGF-β3 limits scarring by modulating the inflammatory response, reducing the ECM deposition and enhancing keratinocyte migration [19]. During the remodeling phase, high levels of TGF-β1 and TGF-β2 are associated with increased fibrosis and scarring. Promoting TGF-β3 activity while inhibiting TGF-β1 and TGF-β2 might reduce scarring and enhance regenerative healing. Clinical trials have demonstrated that Avotermin, an anti-scarring agent based on human recombinant TGF-β3, significantly improves the appearance of scars by neutralizing the effects of TGF-β1 and TGF-β2 [20]. From the several clinical trials in an evidence-based medicine study, Avotermin has been shown to be significantly effective in reducing scarring when administered prophylactically at the wound margins during surgery. The evidence from the trials suggests that it can significantly improve scar appearance without increasing the risk of serious side effects [21].

4.4. Balancing scar management

The balance of VEGF and TGF-β activities determines the outcome of wound healing. While VEGF promotes angiogenesis and collagen production, TGF-β1 and TGF-β2 drive fibrosis, and TGF-β3 promotes regenerative healing. Modulating these pathways with VEGF inhibitors and TGF-β administration shows promise for improvement in scarring. Even VEGF and TGF-β are central players in wound healing and scarring, they act within a complex network of other factors like CTGF, IGF-IR, cytokines (IL-4, IL-13, IL-17, IL-10), and EGF. Notably, TGF-β induces high levels of Connective Tissue Growth Factor (CTGF), which amplifies its effects by further stimulating collagen production and ECM remodeling [22]. MicroRNA-143-3p has been identified as a potent inhibitor of hyperplastic scar formation by targeting CTGF/CCN2 via the Akt/mTOR pathway, reducing collagen I and III production and enhancing fibroblast apoptosis, providing a promising therapeutic way for managing hyperplastic scars [23]. And studies have found that insulin-like growth factor 1 receptor (IGF-IR) is upregulated in keloids and hypertrophic scars, promoting fibroblast survival and collagen synthesis [24]. Also, the IL-4/IL-13 cytokine axis enhance VEGF expression, further promoting fibrosis [25], while IL-17 exacerbates scar formation by enhancing the fibrotic effects of TGF-β [26]. In contrast, IL-10 plays an anti-inflammatory role, indirectly reduce VEGF and TGF-β-driven fibrosis, showing its anti-scarring potential [27]. Recombinant human EGF (rhEGF) has shown success in facilitating epithelial healing and preventing fibrosis by suppressing TGF-β1 expression and complement the effects of VEGF [28]. The clinical application of microencapsulated rhEGF with silicone gel significantly reduced scarring compared to silicone gel alone [29]. The balance of these pathways offers a more comprehensive approach to minimizing excessive scarring while promoting effective tissue repair.

Table 1. Various features of normal, hypertrophic, and keloid scars.

Aspect	Normal Scar	Hypertrophic Scar	Keloid Scar
Features of Scars	- Heals with a thin line that becomes less noticeable - Minimal texture change [3,4]	- Raised above skin surface - Remains red or darker than surrounding skin for extended periods - Stays within boundaries of the original injury [3,4]	- Growth outside original wound boundaries - Thick, rubbery texture - Often accompanied with itching or discomfort [3,4]
Proliferation	- Controlled collagen production - Normal fibroblast activity [5]	- Excessive collagen deposition - High fibroblast proliferation [9]	- Excessive collagen beyond wound boundaries [3,7] - Abnormal fibroblast growth [9]
Remodeling	- Collagen fibers neatly align parallel to skin tension lines - Scar matures and fades [3,4]	- Collagen fibers fine, parallel to epithelium - Scar remains raised [3,4]	- Collagen fibers thick and random - Scar continues to grow [3,4]
Cell Types Involved	- Fibroblasts - Macrophages [5][5,6]	- Increased fibroblasts [7,9]	- Increased fibroblasts [7,9]
Proteins and Cytokines	- Normal levels of TGF-β, PDGF [5]	- Elevated TGF- β, PDGF, VEGF [7,9]	- Elevated TGF- β, PDGF, VEGF - Less synthesis of MMPs, less keloidal apoptosis [7,9]
Mechanical Strength	- Regains 80% normal skin strength by 3 months	- Reduced elasticity - Increased stiffness [3,4]	- Poor elasticity - Increased stiffness [3,4]
Collagen Deposition	- Parallel to skin surface [3,4]	- Collagen fibers are fine, well-organized, and oriented parallel to the epithelium [3,4]	- Collagen bundles are thick, large, and oriented randomly [3,4]
Treatment Options	- Basic wound dressings	- Pressure garments - Silicone sheets - Corticosteroid injections [4]	- Surgical excision - Intralesional steroids, mitomycin C, bleomycin - Possible radiation [4]

5. Conclusion

Scars pose cosmetic and functional concerns, and many therapeutic agents in the medical field focus on hypertrophic and keloid scars with varying degrees of success. Common treatments to minimize scars include compression therapy, silicone gel sheeting, topical or intralesional corticosteroids, laser therapy, and radiation. Next steps in scar therapy lie in possible scar elimination via tissue regeneration, through the use of verteporfin to inhibit Yes-associated protein or promotion of TGF-β3 activity.

Conflicts of Interests

The authors declare no conflicts of interest.

Authors' Contribution

Writing—original draft, S.F., N.S. and S.S.; writing—final draft, N.S.; conceptualization—G.S. and A.D.; writing—review and editing, H.L., D.S., M.B., D.B., S.K., A.D. and G.S. All authors have read and agreed to the published version of the manuscript.

References

[1] Sen CK. Human wound and its burden: updated 2020 compendium of estimates. *Adv. Wound Care.* 2021, 10(5):281–292.

[2] Amici JM, Taieb C, Le Floc'h C, Demessant AL, Seité S, *et al.* The impact of visible scars on well-being and quality of life: an international epidemiological survey in adults. *J. Eur. Acad. Dermatol. Venereol.* 2023, 37:3–6.

[3] Shirakami E, Yamakawa S, Hayashida K. Strategies to prevent hypertrophic scar formation: a review of therapeutic interventions based on molecular evidence. *Burns trauma* 2020, 8:tkz003.

[4] Khansa I, Harrison B, Janis JE. Evidence-based scar management: how to improve results with technique and technology. *Plast. Reconstr. Surg.* 2016, 138(3S):165S–178S.

[5] Landén NX, Li D, Stahle M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell. Mol. Life Sci.* 2016, 73:3861–3885.

[6] Ghosh B, Mukhopadhyay M, Bhattacharya D. Biopolymer-based nanofilms for the treatment of burn wounds. In *Biopolymer-Based Nano Films*, Elsevier, 2021, pp. 311–336.

[7] Wolfram D, Tzankov A, Pülzl P,Piza-Katzer H. Hypertrophic scars and keloids—a review of their pathophysiology, risk factors, and therapeutic management. *Dermatol. Surg.* 2009, 35(2):171–181.

[8] Darby IA, Laverdet B, Bonté F,Desmoulière A. Fibroblasts and myofibroblasts in wound healing. *Clin. Cosmet. Investig. Dermatol.* 2014:301–311.

[9] Wilgus TA, Ferreira AM, Oberyszyn TM, Bergdall VK,DiPietro LA. Regulation of scar formation by vascular endothelial growth factor. *Lab. Investig.* 2008, 88(6):579–590.

[10] Atiyeh B, El Khatib A, Dibo S. Pressure garment therapy (PGT) of burn scars: evidence-based efficacy. *Ann. Burns Fire Disasters* 2013, 26(4):205.

[11] Monstrey S, Middelkoop E, Vranckx JJ, Bassetto F, Ziegler UE, *et al.* Updated scar management practical guidelines: non-invasive and invasive measures. *J. Plast. Reconstr. Aesthetic Surg.* 2014, 67(8):1017–1025.

[12] Gold MH, McGuire M, Mustoe TA, Pusic A, Sachdev M, *et al.* Updated international clinical recommendations on scar management: part 2—algorithms for scar prevention and treatment. *Dermatol. Surg.* 2014, 40(8):825–831.

[13] Ogawa R. The most current algorithms for the treatment and prevention of hypertrophic scars and keloids: a 2020 update of the algorithms published 10 years ago. *Plast. Reconstr. Surg.* 2022, 149(1):79e–94e.

[14] Butler PD, Longaker MT, Yang GP. Current progress in keloid research and treatment. *J. Am. Coll. Surg.* 2008, 206(4):731–741.

[15] Mascharak S, DesJardins-Park HE, Davitt MF, Griffin M, Borrelli MR, *et al.* Preventing Engrailed-1 activation in fibroblasts yields wound regeneration without scarring. *Science* 2021, 372(6540):eaba2374.

[16] Aramaki-Hattori N, Okabe K, Hamada M, Takato T,Kishi K. Relationship between keloid formation and YAP/TAZ signaling. *Plast. Reconstr. Surg. Glob. Open* 2017, 5(6):e1357.

[17] Clark RA. To scar or not to scar. *N. Engl. J. Med.* 2021, 385(5):469–471.

[18] Kwak DH, Bae TH, Kim WS,Kim HK. Anti-vascular endothelial growth factor (Bevacizumab) therapy reduces hypertrophic scar formation in a rabbit ear wounding model. *Arch. Plast. Surg.* 2016, 43(06):491–497.

[19] Jagadeesan J, Bayat A. Transforming growth factor beta (TGFβ) and keloid disease. *Int. J. Surg.* 2007, 5(4):278–285.

[20] Durani P, Occleston N, O'Kane S,Ferguson MW. Avotermin: a novel antiscarring agent. *Int. J. Low. Extrem. Wounds* 2008, 7(3):160–168.

[21] Leon MV. Is Avotermin Safe and Effective Treatment for Scar Improvement in Healthy Males and Females? 2015.
[22] Igarashi A, Okochi H, Bradham DM,Grotendorst GR. Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. *Mol. Biol. Cell* 1993, 4(6):637–645.
[23] Mu S, Kang B, Zeng W, Sun Y,Yang F. MicroRNA-143-3p inhibits hyperplastic scar formation by targeting connective tissue growth factor CTGF/CCN2 via the Akt/mTOR pathway. *Mol. Cell. Biochem.* 2016, 416:99–108.
[24] Hu ZC, Tang B, Guo D, Zhang J, Liang YY, *et al.* Expression of insulin-like growth factor-1 receptor in keloid and hypertrophic scar. *Clin. Exp. Dermatol.* 2014, 39(7):822–828.
[25] Nguyen JK, Austin E, Huang A, Mamalis A,Jagdeo J. The IL-4/IL-13 axis in skin fibrosis and scarring: mechanistic concepts and therapeutic targets. *Arch. Dermatol. Res.* 2020, 312:81–92.
[26] Zhang J, Qiao Q, Liu M, He T, Shi J, *et al.* IL-17 promotes scar formation by inducing macrophage infiltration. *Am. J. Pathol.* 2018, 188(7):1693–1702.
[27] Shi J, Shi S, Xie W, Zhao M, Li Y, *et al.* IL-10 alleviates lipopolysaccharide-induced skin scarring via IL-10R/STAT3 axis regulating TLR4/NF-κB pathway in dermal fibroblasts. *J. Cell. Mol. Med.* 2021, 25(3):1554–1567.
[28] Kim YS, Lew DH, Tark KC, Rah DK,Hong JP. Effect of recombinant human epidermal growth factor against cutaneous scar formation in murine full-thickness wound healing. *J. Korean Med. Sci.* 2010, 25(4):589.
[29] Kao CC, Huang SY, Chiang CH, Lin CH,Chang TC. Microencapsulated rhEGF to facilitate epithelial healing and prevent scar formation of cesarean wound: a randomized controlled trial. *Taiwan. J. Obstet. Gynecol.* 2021, 60(3):468–473.