Enzymatic Debridement: A failed promise, or hope for the future?

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Carica Papaya: a source of cysteine proteases for enzymatic wound debridement

Wound Debridement

Wounds can be characterized by the presence of devitalized, necrotic tissue that stalls wound healing in the inflammatory phase primarily through increased inflammatory mediators and bacterial colonisation leading to impaired formation of granulation tissue (2)(3)(4). Removal of this nonviable or contaminated tissue through debridement has been documented since the late 1800s as an essential part of wound bed preparation for healing (4)(5)(6). Since the earliest descriptions of debridement in the treatment of military wounds, techniques have expanded significantly. Currently available methods of debridement include: surgical, bio-surgical, autolytic, mechanical, chemical and enzymatic (2)(6)(7). A particular method may be selected based on evaluation and consideration of factors such as: cost, presence of infection, clinician experience, tissue type & patient preference (2)(6)(7).

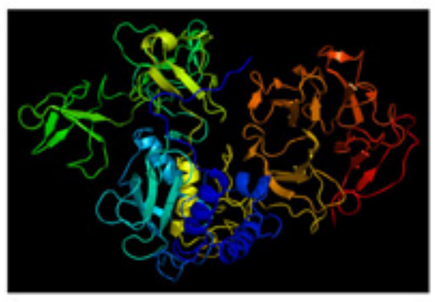
Enzymatic debridement

Enzymatic debridement is the application of exogenous enzymes to the wound bed in order to degrade necrotic tissue whilst minimizing harm to viable granulation tissue (6). A scan of the literature reveals claims that enzymatic debridement has proven itself to be a clinically effective, safe and inexpensive method of debridement (7)(8). It is stated to be an excellent choice of debridement in patients with infected or contaminated wounds requiring the removal of yellow slough or black eschar, as well as in patients who have the option of surgical debridement excluded due to anticoagulant therapy (6)(8). However, a 2013 Cochrane review on wound debridement revealed that enzymatic debridement is relatively slow and requires more frequent dressing changes (2). The review also communicated that there is minimal hardy evidence (level A studies) for the use and efficacy of different enzymatic debriding agents (2). This is customary for most forms of debridement, where practice is based on tradition and anecdote as opposed to evidence based wound care (6).

Ananus Comosus: the source of bromelain from which NexoBrid is synthesized

Current products for enzymatic debridement

From the literature, there appears to be significant promise in the realm of enzymatic wound debridement, however clarity and rigour is lacking. There is a need for rigorous clinical trials comparing the three main potential agents in enzymatic wound debridement: bromelain-derived products, collagenase-based products and cysteine proteases derived from *Carica Papaya* such as papain. There is also a need for comparisons between these products and current standards of care (surgical and non-surgical). This poster illustrates fundamental information on each of these three enzymatic debriding agents.

	BROMELAIN	COLLAGENASE	PAPAIN
What is it and how is it extracted?	• Bromelain belongs to a group of proteolytic enzymes which are extracted from the stem and immature fruit of pineapple (*Ananus comosus*). • These enzymes have been demonstrated in vivo and in vitro to have anti-edematous, anti-inflammatory, anti-platelet and fibrinolytic activities. • It is used in the treatment of multiple inflammatory and haematological diseases as well as in therapy for malignancy. (22) • More recently, it's debriding abilities have been demonstrated, especially in third degree burns and frostbite eschar. • Along with papain, it is a very commonly used meat-tenderizer. • The leading bromelain-derived enzymatic debriding product is *NexoBrid*. • *NexoBrid* (formerly called *Debrase*) is a patented product owned by MediWound. • The patent protects the invention process which involves removing a family of cysteine protease inhibitors; this strongly increases the debriding capacity of the extract. • *NexoBrid* is extracted in an acetic acid and ammonium sulfate solution; the finished product is a powder which is mixed with a gel vehicle before application. • In 2012, NexoBrid received a European Marketing Authorisation but elsewhere in the world the product remains a drug under investigation. (9)	• Collagenase ointment (*Santyl, HealthPoint)* is a selective enzymatic debriding agent derived by fermentation of the bacterial strain *Clostridium Histolyticum.* (3) • The ointment is charged with 250 units of collagenase units per gram of white petroleum. • The use of collagenase for enzymatic wound debridement has been under investigation for a number of decades. • *Santyl* is a collagenase prescription ointment approved by the United States FDA. It has been used in The United States as well as other countries for over 20 years.  Matrix metalloproteinase 2, an endogenous collagenase similar to those extracted from the fermentation product of *Clostridium Histolyticum*	• Papain is one of four cysteine proteases found in the latex of *Carica Papaya*, more commonly known as pawpaw. • The other three cysteine proteases in the papaya latex are: chymopapain, caricain and glycyl endopeptidase (11). • Papain is the most studied and applied protease of the four, and has been employed for decades in the pharmaceutical and food industry, for example as a meat tenderizer (11). • Cysteine proteases (also known as thiol proteases) require the presence of sulfhydryl groups [present in cysteine] to break down peptide bonds (6)(11). They have a molecular mass of about 21-30 kDa and are most active at a pH of 4-6.5; however, papain has a wide pH range of 3-12 (10)(11). • The papaya plant is lactiferous and so contains specialized cells known as lactifers which secrete latex rich in cysteine proteases (11). • Green papaya (unripe papaya) has been evidenced to have the greatest content of papain; this is believed to be due to reduced latex production or increased latex breakdown as the fruit ripens, or both (10)(11). • In the recent study by Anaur et al. in 2008 it was illustrated that the green papaya skin contains the most significant amount of papain, healing wounds on mice at a faster rate compared to extracts from ripe papaya (11). This is believed to be due to the fact that the highest concentration of latex is in the skin of the fruit (11). **Extraction and preparation of papain** • Traditionally papain has been prepared by washing a green papaya, opening it up with a knife, removing the seeds, mashing the flesh into a paste and applying it directly onto the wound (10). • The addition of urea to papain is said to augment the peptolytic activity of the enzyme by altering protein structure through the disruption of hydrogen bond networks; this allows for better exposure of the enzyme's binding site (2)(3)(4)(5)(6).
Molecular activity and Tissue selectivity	• There have been limited studies on the molecular activity and tissue selectivity of NexoBrid. • Rosenberg et al conducted a study in 2012 claiming macroscopic and histological evidence to support the selectivity of bromelain-based debriding gel dressings to non-viable tissue. The study made use of a porcine model. A series of burns were inflicted on each side; one side served as the control which received four hourly application of a hydrating gel while the other side received four hourly application of debriding gel dressing. The paper states that macroscopically it was clear that the test side showed more extensive clearance of burn wound eschar. Furthermore, the authors infer the debriding gel dressing's selectivity to non-viable tissue as histological samples from both sides of the animal showed similar levels of damage within areas of viable tissue. (12)	• There are a number of studies comparing the molecular activity and tissue selectivity of collagenase and papain, however the conclusions are not definitive. • Smith reports that collagenase is selective to denatured collagen in necrotic tissue, thus it is not harmful to healthy tissue around the wound bed. This is an advantage over products containing papain. He also reports that collagenase does not degrade fibrin. (6) • Hebda and Lo published a paper in 2001 reporting that both collagenase and papain-urea digest collagen especially when it is denatured. They also claim that papain-urea digests fibrin more so than elastin but collagenase digests elastin more so than fibrin (13). • In 2008, Kravitz et al stated that papain-urea doesn't digest collagen and that collagenase digests only collagen making it a more suitable product for wound bed maintenance (15).	• Papain is a nonselective cysteine protease (6)(7)(15). • All proteins that contain a cysteine group will be cleaved including proteins in skin, granulation tissue and growth factors (15). • Because of this nonselective proteolytic activity, both nonviable and viable tissue are subject to papain's activity (15). This may induce an inflammatory response that irritates the skin causing pain; it has been documented that the addition of chlorophyllin can reduce pain considerably (15). • It has been reported that papain does not digest collagen because collagen does not contain any cysteine residues (15). • Whether it degrades fibrous tissue in the wound is disputed in the literature (6)(15). However, even if papain doesn't directly degrade fibrous tissue the enzyme at least denudes it by digesting the material that anchors fibrous tissue to the wound bed. Fibrous tissue is digested directly by collagenase, leaving other tissue types intact. (15) • Note that fibrous tissue is not fibrin-containing tissue but rather tissue containing fibers (mostly collagen).
Clinical efficacy	• There is some limited evidence reporting the benefits of NexoBrid as a debriding agent. • Rosenberg et al conducted a study in 2013 and found that patients treated with NexoBrid experienced significantly faster debridement than those under standard of care (surgical and non-surgical); average time to debridement 2.2 days for NexoBrid group and 8.7 days for standard of care. • However, the standard of care group was in no way controlled as it included many different kinds of treatment including: silver sulfadiazine, soaking, bathing, scraping, tangential excision, minor excision, dermabrasion and hydrosurgery. There were no statistically significant differences in healing times between groups (17).	• There is evidence in the literature that papain-urea is a faster debriding agent than collagenase (19). However as alluded to earlier, it is thought that collagenase may be more effective in wound bed maintenance after initial debridement. • The effects of collagenase have been shown to be hindered less than those of papain-urea by various anti-microbial wound dressings (7). • Collagenase ointment is applied daily, although it may be applied more frequent if the dressing becomes soiled in order to provide ongoing debridement. • Adverse side effects tend to be mild and transient, for example a mild stinging on application of the ointment. (6)	• There are limited studies that evaluate the clinical efficacy of papain on wound healing. • The prospective randomized control trial (RCT) by Alvarez and Coworkers compared the use of papain-urea ointment to a petroleum ointment containing collagenase in 28 subjects with pressure ulcers over 4 weeks (20). Papain was shown to be significantly more effective ($P<0.05$) than collagenase at reducing the amount of necrotic tissue in the pressure ulcers. However, there was no significant reduction in wound area between the two treatments. • Wound debridement with papain is recommended to be applied twice daily over a period of 1-3 weeks. A disadvantage of this is that it is both labor intensive and time consuming. Recent studies have demonstrated that continuous streaming of papain may be a clinically superior modality for its application into the wound. (21)

Conclusion

There is a great potential for the use enzymatic debridement on acute/chronic wounds, including burns. However, the current use of these products appears to reflect a failed promise. This may be a result of a number of restricting factors, most prominent being a lack of strong evidence on the clinical efficacy of each product and comparisons between products. The cost of enzymatic debriding agents may be restricting their application and incorporation into wound bed preparation options for wound specialists. Further, staff need to be competent in applying the product correctly. Specific to each product, there are various precautions that have limited their use in most countries around the globe. For example papain is known to cause pain to the patient due to its tissue non-selectivity. It also poses a risk of hypersensitivity reactions in a small number of recipients.

What the future holds for enzymatic debridement is uncertain. However, if the current products were to be refined and their clinical properties and standards of use evidenced through rigorous high level research, there is considerable prospect for their inclusion into best practice wound management in Australia and abroad.

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