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The Influence of Metal Salts, Surfactants, and Wound Care Products on Enzymatic Activity of Collagenase, the Wound Debriding Enzyme

Aleksa Jovanovic, PhD; Ryan Ermis, MPH; Rachel Mewaldt, BS; Lei Shi, PhD; Dennis Carson, PhD, DABT

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From Healthpoint Biotherapeutics, Fort Worth, TX

Address correspondence to:
Lei Shi, PhD
Healthpoint Biotherapeutics
Research and Development
3909 Hulen Street
Fort Worth, TX 76107
lei.shi@healthpoint.com

Abstract: An important part of the wound healing process is the removal of necrotic tissue from a wound to promote healing. Enzymatic debridement is one of the widely used methods to accomplish this goal. *Clostridium* collagenase (*C.* collagenase) containing ointment is frequently used in clinics to debride wounds. In this work, the influence of metal salts and various types of surfactants on the enzymatic activity of *C.* collagenase is tested. The relationship between charge and size of metal ions and surfactant structure is explained in the context of enzyme inhibition. Commonly used wound care products, such as cleansers, dressings, antimicrobial formulations, and silver dressings are tested with *C.* collagenase. The results are discussed in terms of enzyme compatibility with such materials, and recommendations for use of wound care accessories in conjunction with the debriding enzyme are given, with the aim to help wound care providers make more educated choices towards accomplishing optimal therapy outcome.

Effective wound debridement has been widely used to remove necrotic tissue from a wound to promote healing. Necrotic tissue present in a wound bed is undesirable because it prolongs the inflammatory stage and may serve as a reservoir for bacterial growth, thus slowing the tissue regranulation necessary for wound repair.[1] It is increasingly well-recognized that removal of nonviable tissue from a wound[2] is an important step that may facilitate the healing process for a variety of wound types, especially burn wounds and various chronic wounds.[3-5] Wound debridement may be performed in several different ways: surgical, autolytic, enzymatic, and mechanical. Each of these has its own benefits and shortcomings, depending on the wound type and the condition of the patient.[6,7] Enzymatic debridement can provide an effective methodology for various chronic ulcers, especially in patient populations not amenable to surgical debridement.

Currently, Collagenase Santyl® Ointment (Healthpoint Biotherapeutics, Fort Worth, TX) is the only Food and Drug Administration (FDA)-approved enzymatic debriding biological in the United States.[8] The enzyme actively used in this drug is a bacterially derived collagenase from *Clostridium histolyticum* (*C.* collagenase). *C.* collagenase, a metalloproteinase with Zn^{2+} in the

active site, contains 2 principal enzyme species: collagenase I (Col H, 116 kDa, predominantly β-sheet structure) and collagenase II (Col G, 124kDa, predominantly α helix structure). Both enzymes specifically attack collagen in wound necrotic tissue, which contains mostly the denatured collagens.[9]

Frequently, the enzymatic debrider is used in conjunction with various wound dressings[10] to achieve multiple treatment goals simultaneously, including infection control, pain control, and exudate management. Furthermore, wound cleansers are often used before or even alongside debriders to remove loosened tissue debris, bacteria, and other physicochemical contaminants that can seriously impede the wound healing process. Some dressings contain certain levels of metal elements (eg, silver) as principal bactericides, while wound cleansers rely on the cleaning power of various surfactants to remove the debris from the wound bed. The purpose of this work is to evaluate the influence of various metal salts and surfactants on *C.* collagenase enzymatic activity. Moreover, commercially available wound care accessories, such as cleansers, dressings, and antibacterial preparations, will also be tested for compatibility with the enzyme. The outcome of this study should help wound care professionals make appropriate choices with regards to dressings and cleansers (ie, commonly used accessories in the treatment of hard-to-heal wounds) used alongside an enzymatic debrider to ensure the optimal therapy outcome.

Materials and Methods

C. collagenase was manufactured by Healthpoint Biotherapeutics (Fort Worth, TX). N-(3[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala (FALGPA), a chromogenic substrate, was purchased from Bachem Americas, Inc (Torrance, CA). Poloxamers 124, 188, and 407 were gifts from BASF (Floram Park, NJ). Cocamine oxide (Ammonyx®) was a gift from Stepan (Northfield, IL). Cocamidopropyl dimonium chloride phosphate (Arlasilk™ PTC) was a gift from Croda, Inc (Edison, NJ). Collagen FITC was purchased from Elastin products Co, Inc. All other chemicals were purchased from Sigma Aldrich (St. Louis, MO) and used without any further purification.

All of the dressings, cleansers, and anti-bacterial accessories were purchased from the respective manufacturers or specialized stores (Tables 1-6).

Collagenase Activity Assays for Metal Salts and Surfactants. The collagenolytic activity of *C.* collagenase in the presence of metal salts was measured using FALGPA.[11] The concentrations of enzyme, substrate, and salts were the following: *C.* collagenase 0.1 mg/ml, FALGPA substrate 1 mg/ml, and the metal salts at concentrations varying from 100-600 mM (refer to Table 1). The enzyme activity was measured as decreased absorbance at 345 nm for the first 35 minutes at room temperature. The slope of the linear curve is used as the activity rate (Vmax). In the case of an insoluble salt, such as AgCl, the enzyme solution was mixed with the powder for no less than 30 minutes, followed by centrifugation and the analysis of the supernatant.

The activity of *C.* collagenase in the presence of surfactants was measured in a slightly different way. The concentrations of the enzyme, substrate, and surfactant were as follows: *C.* collagenase 0.1 mg/mL, FALGPA 1 mg/ml, and surfactant from 0.1-10 mg/mL (refer to Table 2). The enzyme and surfactant containing solutions were incubated at room temperature for 30 minutes. The 5 millimolar (mM) FALGPA solution was prepared in the assay buffer (400 mM NaCl, 10 mM CaCl2, 50 mM Tricine, pH = 7.4), and mixed well prior to use to ensure complete solubilization. Using a 96-well microplate, 100 L of the enzyme solution was mixed with 150 L of the FALGPA solution. The kinetic reaction was monitored for 35 minutes, and kinetic rates (for enzymatic activity) were determined by recording the absorbance change at 345 nm. Rates were reported as Vmax milli-OD (1/1000 optical density unit) per minute. The results for the metal salts and surfactants are shown as percent enzymatic activity and are given by:

$$\% \text{ Activity} = \frac{\text{Vmax tested article}}{\text{Vmax enzyme control solution}} \times 100$$

The results for influence of wound accessories on enzyme activity are shown as a percent inhibition and are given by:

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Vmax tested article}}{\text{Vmax enzyme control solution}} \times 100 \right)$$

Collagenase activity assays for dressings and other wound care products. The collagenolytic activity of *C.* collagenase in presence of dressings was measured in similar fashion for the metal salts and surfactants. The only difference was that the dressing was soaked in the buffer for at least 3 hours, and this buffer was then used to solubilize the enzyme. The measurement parameters were the same as described above for salts and surfactants. Wound cleansers that are water-based solutions (eg, ALLCLENZ®, Healthpoint, Fort Worth, TX), were used as a solubilization media for the enzyme. Antimicrobial powders (eg, Poly-

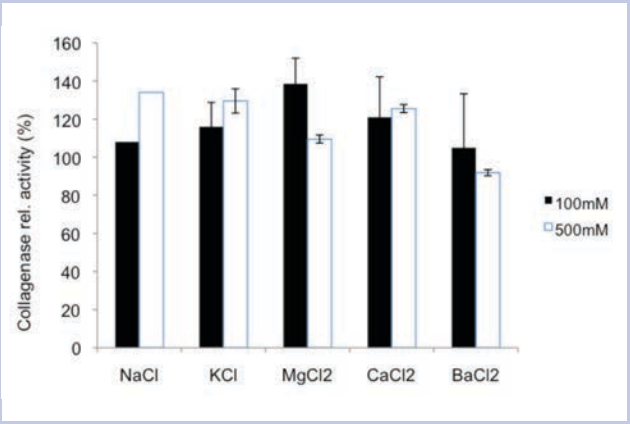

Figure 1. Influence of alkali and earth alkali metal chloride salts on *C.* collagenase enzymatic activity.

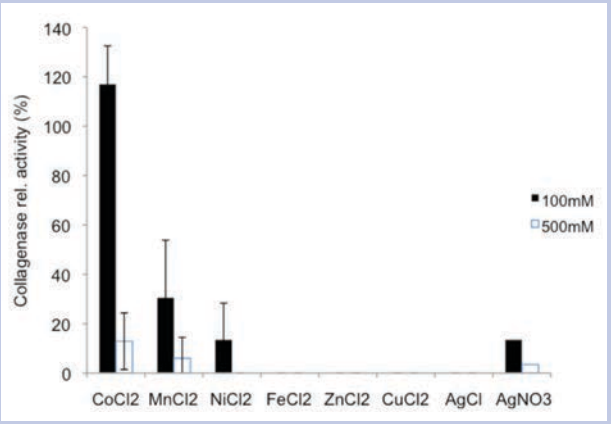

Figure 2. Influence of transition metal salts on *C.* collagenase enzymatic activity.

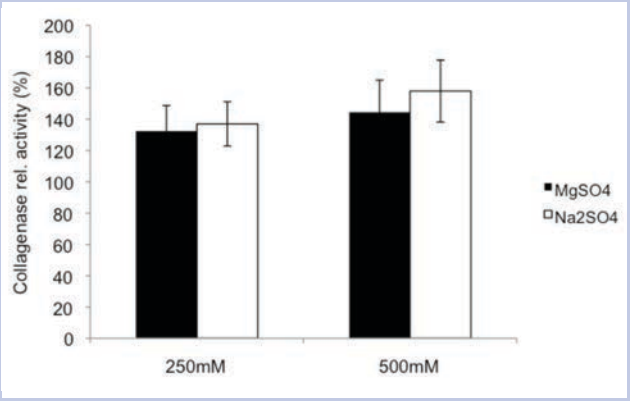

Figure 3. Influence of Na and Mg sulfates on enzymatic activity of *C.* collagenase.

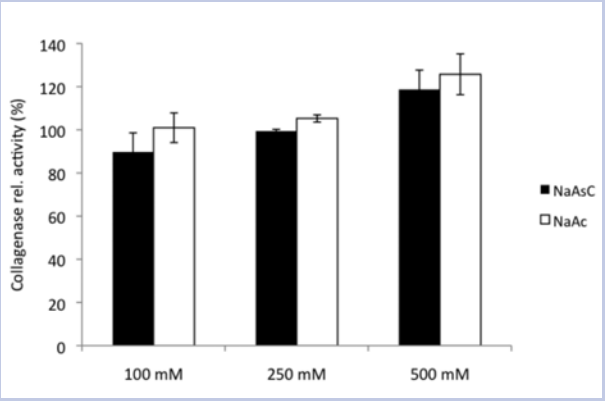

Figure 4. Influence of different concentrations of Na Ascorbate and Acetate on enzymatic activity of *C.* collagenase.

sporin®, Johnson and Johnson, New Brunswick, NJ) were freely soluble in buffered solutions. Ointments, gel dressings, and creams (eg, Bactroban®, GlaxoSmithKline, Research Triangle Park, NC) were mixed with assay-buffered solutions, followed by centrifugation and the analysis of the supernatant. The measurement parameters were the same as already described for salts and surfactants.

Collagenase activity assays for insoluble analytes. Several testing articles (eg, Iodoform) were insoluble in aqueous system, had similar absorption maximum as FALGPA substrate, and therefore could not be tested using the FALGPA method. In this case, an alternative method for determination of *C.* collagenase was employed: 20mg of Collagen-FITC was dispersed in enzyme solution (positive control), buffer (negative control), analyte/enzyme solution, and analyte solution alone. The samples were incubated at RT for 90 minutes, followed by centrifugation and spectrofotometric analysis at 485 nm. The result is shown as percent inhibition and is given by:

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Amax tested article}}{\text{Amax enzyme control solution}} \times 100 \right)$$

Statistical Analysis

Enzyme activity with metal salts and surfactants data were averaged from a group of 3 samples (n=3). Standard deviation was calculated and displayed in the graphs. Enzyme inhibition data with wound care accessories were averaged from a group of 3 samples (n=3) and displayed in tables.

Results and Discussion

Influence of metal salts on enzymatic activity of C. collagenase. It is well documented that certain metal ions exhibit strong influence on the activity of metalloproteins.[12,13] In this work, the influence of metal salts

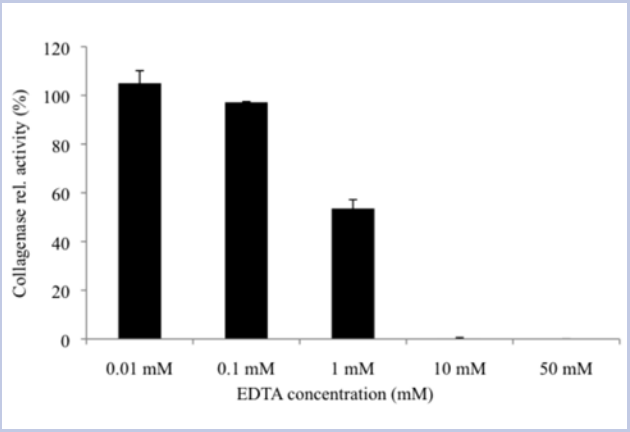

Figure 5. Influence of different concentrations of Na2EDTA on enzymatic activity of *C.* collagenase.

(mostly chlorides) have been investigated on the enzymatic activity of *C.* collagenase. Moreover, salts with a common cation (eg, sodium salts) having different anions (eg, sulfate and acetate) were tested as well. The results are summarized in Figures 1-5.

Chloride salts of alkali and earth alkali metals generally exhibit either no or slightly positive effect on enzymatic activity of *C.* collagenase (Figure 1). There is no apparent difference between 100 mM and 500 mM concentrations. It has been reported that NaCl markedly increases the catalytic activity of thermolysin,[14] a member of the metalloproteinase family. The reason behind this is that Kcat increases due to favorable electrostatic interactions between the protein and the medium.[15] Both of the alkali metals (ie, Na and K) increased the activity of *C.* collagenase in a similar fashion. Therefore, it appears that the ion size and hydration are not the only factors that contribute to enzymatic activity increase.

It is known that enzymes of this family actually have binding sites for divalent cations other than Zn^{2+}, such as Ca^{2+}. These sites are responsible for the stabilization of the enzyme structure. Therefore, as expected, Ca^{2+} enhanced the activity of *C.* collagenase. A somewhat novel finding is that ions smaller and bigger than Ca^{2+}, Mg^{2+}, and Ba^{2+} behave in a similar way. All 3 of the aforementioned metals have unoccupied d orbitals, unlike transition metals.

Transition metals, except Co^{2+} at lower concentrations, exhibit a profound negative influence on enzymatic activity of *C.* collagenase (Figure 2). It is known that the higher a concentration of Zn^{2+}, although present in the active site of the enzyme, diminishes the enzyme activity, probably due to steric hindrance of the active site (ie, more than one Zn^{2+} is in the active site).[16] All other metals,

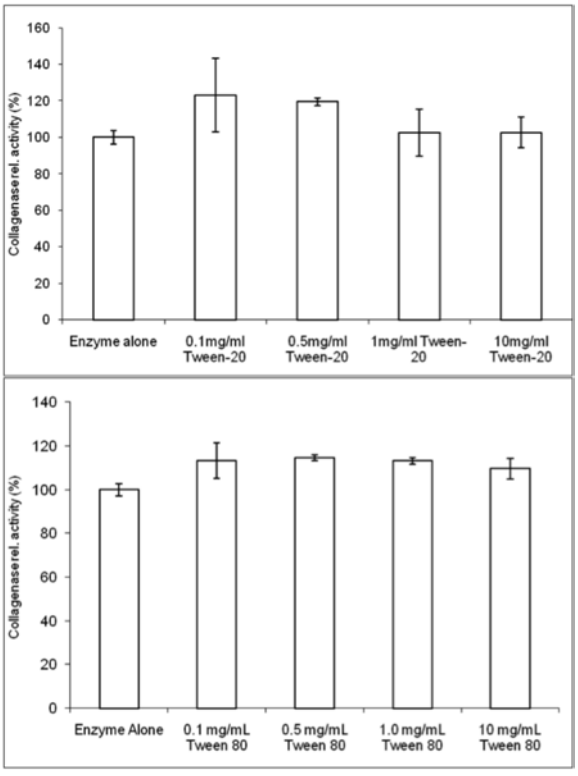

Figure 6. Influence of non-ionic surfactants (Tween 20 and Tween 80) on enzymatic activity of *C.* collagenase.

except for Ag^{+}, were divalent cations and their influence on enzymatic activity was compared to Ca^{2+}. The main difference between the Ca^{2+} and the transition metal ions is the population of the d orbitals. Ca^{2+} has empty d orbitals, while transition metals have between 1 and 10 electrons in these orbitals. The presence of such electrons gives rise to a number of interesting properties such as paramagnetism and color. Moreover, d electrons can aid the complexation between the metal and the ligand (ie, enzyme), which can lead to conformational changes of the latter resulting in the decrease of enzymatic activity.[17,18]

It is of special interest to investigate the influence of Ag^{+} on *C.* collagenase since the Ag-based wound dressings are often applied in conjunction with enzymatic wound debriders in clinics.[19] Ag^{+} is a monovalent cation like Na^{+} or K^{+}; however, the difference between these metals is the presence of d electrons in the case of Ag^{+}. Similarly to divalent cations, it was observed that metallic monovalent ions with d electrons significantly inhibited the enzymatic activity of *C.* collagenase, compared to metals without such electrons (ie, alkali metals). It is interesting to note that silver inhibited the enzyme in the form of a soluble cation ($AgNO_3$), as well as in the form of an insoluble salt

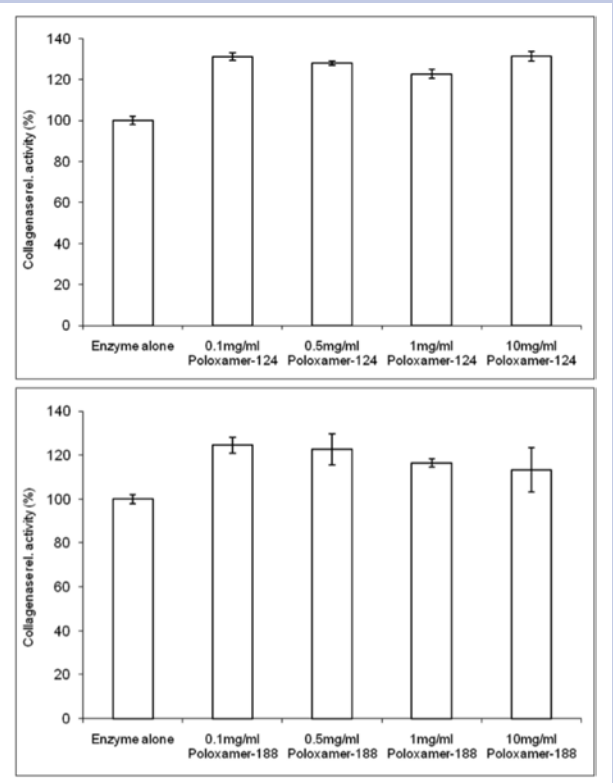

Figure 7. The influence of polymeric non-ionic surfactants (poloxamer 124 and poloxamer 188) on enzymatic activity of *C.* collagenase.

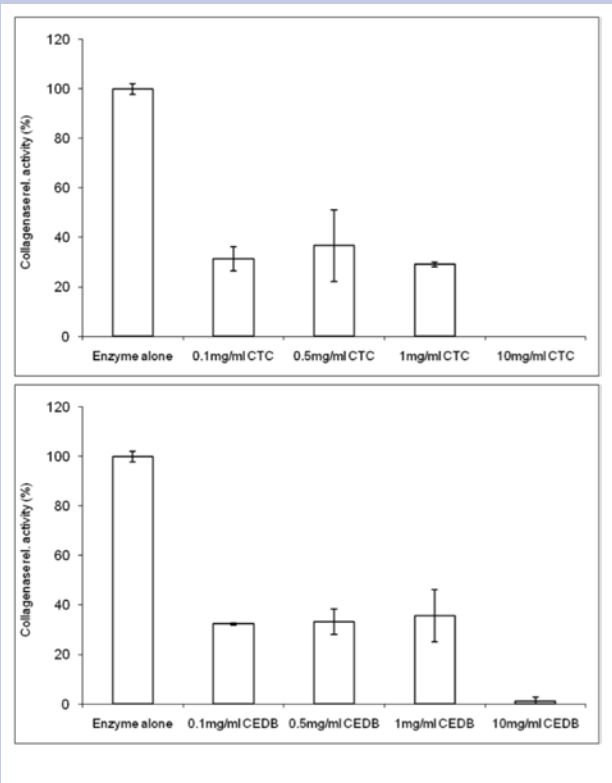

Figure 8. The influence of cationic surfactants (CTC and CEDB) on enzymatic activity of *C.* collagenase.

(AgCl). Various silver dressings will be discussed later.

We also tested the influence of metal salts (eg, Na^+ and Mg^{2+}) with a divalent anion, SO_4^{2-}. The results showed these salts have positive influence on enzymatic activity, regardless of concentration (Figure 3).

Two salts commonly used in physiological buffers, sodium acetate (NaAc), and sodium ascorbate (NaAsc), were tested at 3 different concentrations (Figure 4). A general trend towards an increase of enzymatic activity was observed with the increase in concentration.

Another sodium salt commonly found in many wound care products, disodium ethylenediaminetetraacetic acid (EDTA), a strong metal chelating agent, was tested for enzyme compatibility. Results (Figure 5) suggest that EDTA at concentrations > 0.1 mM (0.0037 w%) inhibits the activity of the enzyme. The reason for this behavior is the complexation of metal ion (Zn^{2+}) from the enzyme active site by EDTA.[20]

Influence of surfactants on enzymatic activity of C. collagenase. Based on the nature of their polar part (ie, "head"), surfactants can be divided into 4 groups: non-ionic, anionic, cationic, and zwitterionic.

Tween 20 and Tween 80 were chosen as representatives of small non-ionic surfactants. As shown in Figure 6, small non-ionic surfactants exhibited none to minimal effect on the enzyme activity up to the concentration of 10 mg/mL.[21] The concentration and physical state of the surfactant, in the form of micelles (at > 0.1 mg/ml) or individual molecules (at I 0.1 mg/ml), seems to have no influence on enzymatic activity.

Poly (ethylene oxide) - poly (propylene oxide) - poly (ethylene oxide) (PEO-PPO-PEO) block copolymer-type surfactant (pluronics or poloxamers), and were used as models for polymeric non-ionic surfactants in this study (Figure 7). It was found that these surfactants actually have a positive effect on *C.* collagenase activity.[22] This is true for both surfactants tested, poloxamer 124 with hydrophilic-lipophilic balance (HLB) of 12-18, and the much more hydrophilic poloxamer 188 with HLB of > 24. Furthermore, the positive influence was independent of concentration, at least in the range tested. This result appears to be in line with the previous findings by Johnston et al[23]

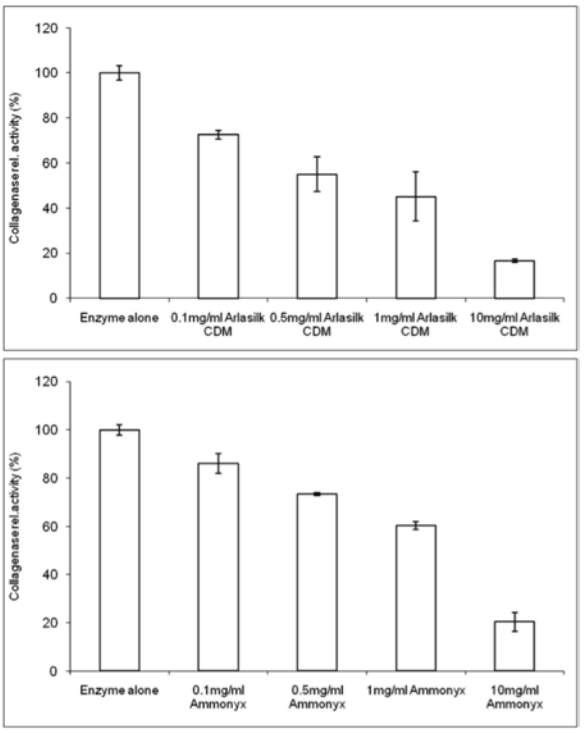

Figure 9. The influence of amphoteric surfactants (Arlasilk CDM and Ammonyx C) on enzymatic activity of *C.* collagenase.

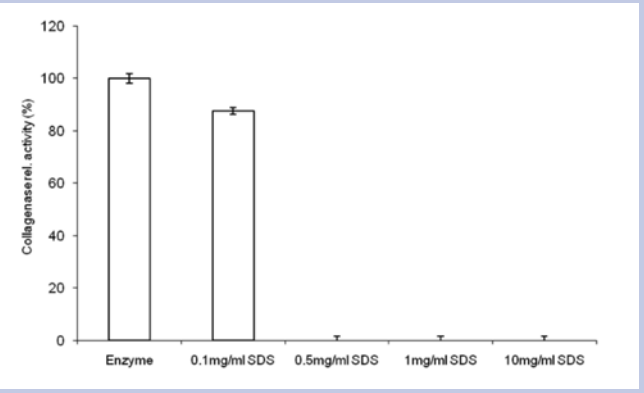

Figure 10. Influence of anionic surfactant (SDS) on enzymatic Activity of *C.* collagenase.

where poloxamer 407 had none to minimal influence on enzymatic activity up to concentrations of 50 mg/ml. It is likely that stabilizing and favorable solubilization effects of a surfactant are responsible for the preservation and enhancement of enzymatic activity.

Cationic surfactants tested in this study were cetyl pyridinium chloride (CPC) and cetyl ethyl dimethyl ammonium bromide (CEDB). Surfactants of this type greatly inhibit the enzyme activity (Figure 8), even at the lowest concentration.[24] Cationic surfactants are able to electrostatically bind to negatively charged amino acid residues (acidic amino acids), those involved in the active site interacting with Zn^{2+} within the protein, and can also disrupt the enzyme native conformation through hydrophobic interaction by their non-polar tail. It is interesting to note that the inhibition happens at concentrations under the critical micelle concentration (CMC) (0.1 mg/ml), persists at the same level through 2 medium concentrations (0.5-1 mg/ml), and finally completely prevails at the highest concentration (10 mg/ml).

Cocamidopropyl dimonium chloride phosphate (Arlasilk™ PTC) and cocamine oxide (Ammonyx®), the zwitterionic surfactants, were the only class of surfactants that displayed linear concentration-dependent inhibition of *C.* collagenase (Figure 9). The linearity probably stems from very low CMC. Therefore, the surfactant structure in water will not change with increasing concentration; only the number of structures (ie, micelles) will change. Furthermore, due to their dual electrostatic nature (ie, both charges on the same molecule), they can easily bind to the enzyme and disrupt the physiological protein conformation.

Anionic surfactant, such as sodium dodecyl sulfate (SDS) had minimal influence on enzymatic activity when it was tested under the CMC (0.1 mg/ml). However, at CMC and above, SDS completely inhibited the enzyme (Figure 10). This behavior is expected since at least 1 *C.* collagenase (Collagenase G) has predominantly an α-helix structure as oppose to an SDS-resistant β-sheet.[25] Moreover, as in the case of cationic surfactants, charge interactions are the primary reason for the inhibition of enzymatic activity.

Influence of dressings and other wound care products on enzymatic activity of C. collagenase. In this work, several classes of drugs and devices used frequently in wound care settings have been tested for compatibility with *C.* collagenase. These include silver, iodine, polymeric (eg, collagen), and other type dressings; wound cleansers; antimicrobial semi-solids (eg, creams and ointments); and antimicrobial actives (eg, gentamicin sulfate). (Refer to Tables 1 through 6).

Silver dressings are considered standard of care for treatment and prevention of infections in clinics today. In this work the authors have tested several products containing various forms of silver with *C.* collagenase. Generally, silver products inhibit the enzymatic activity of *C.* collagenase, as shown in Table 1. However, products that contain ionic

Table 1: Influence of silver dressings on enzymatic activity of *C.* collagenase.

No	Product	Inhibition (%)	Type	Manufacturer	Description
1	Silverlon	44.3	Silver dressing	Argentum Medical, LLC	Elemental Silver
2	Acticoat	52.4	Silver dressing	Smith-Nephew	Nanocrystalline silver
3	Silvadene (10%)	67.0	Silver dressing	Keltman Pharmaceuticals	1% Silver sulfadiazine in cream base
4	Silvasorb	25.0	Silver dressing	Medline	Ionic silver
5	Algidex Ag	3.81	Silver dressing	DeRoyal	Ionic silver alginate wound dressing
6	Maxorb Ag	18.0	Silver dressing	Medline	Ionic silver alginate wound dressing
7	Mepilex Ag	15.7	Silver dressing	Molnlycke	Ionic silver silicone dressing
8	Aquacel Ag	7.7	Silver dressing	ConvaTec	Hydrofiber silver dressing
9	Allevyn Ag	7.6	Silver dressing	Smith & Nephew	Ionic silver urethane

Table 2: Influence of iodine dressings on enzymatic activity of *C. collagenase.*

No	Product	Inhibition (%)	Type	Manufacturer	Description
10	Iodoflex	93.8	Iodine dressing	Smith-Nephew	Iodine dressing
11	Iodosorb	87.0	Iodine dressing	Smith-Nephew	Iodine gel
12	Iodoform	1.6	Iodine dressing	Invacare	Iodoform-impregnated gauze

silver (Products 4-9, Table 1) in the form of water-insoluble chloride salt tend to have a milder effect on the enzyme, while products with elemental or nanocrystalline silver (Products 1 and 2, Table 1) reduce the initial enzyme activity to half. The product that contains silver ionically bound to a sulfadiazine molecule (Product 3, Table 1) inhibits > 60% of the initial activity of the enzyme. It appears the enzyme inhibition of silver-containing products is a function of the silver release potential (ie, available concentration) and chemical reactivity of the various silver forms.[26]

Iodine containing products are also frequently used in clinics to treat wound infections or for sole wound-cleaning applications. In this work, the authors have tested an iodine dressing (Product 10, Table 2), an iodine-containing gel (Product 11, Table 2), and iodine in the form of Iodoform (Product 12, Table 2). Both iodine-containing products almost completely inhibit the activity of the enzyme (Table 2). This finding is in line with previously observed inhibition of matrix metalloproteinases (MMP) in chronic wounds by Povidone-Iodine.[27] However, iodine in the form of Iodoform (Product 12, Table 2.) showed no inhibition of *C.* collagenase, probably due to the fact that it is very insoluble in the aqueous system.

The next set of products tested are various wound dressings made of polymeric materials (Products 13-16, 20-24, and 26-27, Table 3), semi-solid ointments (Products 17, 19, 25, and 28-31, Table 3), and porcine-derived extracellular matrix (ECM) (Product 18, Table 3). The results (Table 3) suggest these types of dressings generally will not cause any inhibition of the enzymatic activity of *C.* collagenase. Few exceptions are Products 25 and Products 30-31, which moderately to severely inhibit the activity of the enzyme. Product 25 inhibits the enzyme due to the presence of poly (hexamethylene) biguanide (PHMB) that has great

Table 3: Influence of various wound dressings on enzymatic Activity of *C.* collagenase.

No	Product	Inhibition (%)	Type	Manufacturer	Description
13	Xeroform	0.0	Wound dressing	Kendall Healthcare	Bismuth in petrolatum gauze
14	Procellera	5.8	Wound dressing	Vomaris	Wound dressing
15	Fibracol	2.7	Wound dressing	Systagenix	Collagen-based Alginate dressing
16	Hydrofera	0.0	Wound dressing	Hydrofera LLC	Dye-containing bacteriostatic dressing
17	Proshield	0.0	Wound dressing	Healthpoint Biotherapeutics	PEG-based semi-solid dressing
18	Oasis	6.1	Wound dressing	Healthpoint Biotherapeutics	SIS product
19	Multidex Gel	< 5.0	Wound dressing	DeRoyal	Maltodextrin gel dressing
20	Aquasorb	0.0	Wound dressing	DeRoyal	Hydrogel-polyurethane dressing
21	Multipad	0.0	Wound dressing	DeRoyal	Non-adherent dressing
22	Covaderm	0.0	Wound dressing	DeRoyal	Absorbent-adhesive dressing
23	Sofsorb	0.0	Wound dressing	DeRoyal	Super Absorbent dressing
24	Transeal	0.0	Wound dressing	DeRoyal	Transparent film dressing (polyurethane)
25	Prontosan Gel (10%)	96.5	Wound dressing	B.Braun Medical	PHMB-containing gel
26	Allevyn	0.0	Wound dressing	Smith & Nephew	Hydrocellular dressing
27	Mepilex	10.4	Wound dressing	Molnlycke	Soft silicone foam dressing
28	Carrasyn Hydrogel	0.0	Wound dressing	Medline	Carbopol-based hydrogel
29	Medihoney	22.3	Wound dressing	Derma Sciences	Leptospermum Honey dressing
30	Glucan Pro Cream 3000	69	Wound dressing	Brennen Medical	Petrolatum-based dressing
31	Bismuth Formic Iodide (BFI)	75.9	Wound dressing	Numark Laboratories	BFI in talc powder base

potential to bind the metal ion at the active site, thus effectively diminishing the enzyme activity. Product 30, thick hydrophobic ointment, contains anionic surfactants (ie, cetyl esters) that can significantly inhibit the enzyme (Figure 10.). Product 31 is a powder that contains iodide, a known *C.* collagenase inhibitor.

Wound cleansers are formulations designated to remove impurities, bacteria, and debris from the wound bed. Several representative examples of such formulations (Products 32-50, Table 4) were tested with *C.* collagenase. Generally these products will lower the enzyme activity due to the presence of cleansing agents (ie, surfactants necessary to clean the wound.) The enzyme is severely inhibited by products that contain strong zwitterionic and/or anionic

Table 4: Influence of various wound cleansers on Eenzymatic Activity of *C.* collagenase.

No	Product	Inhibition (%)	Type	Manufacturer	Description
32	Allclenz	68.8	Wound dressing	Healthpoint Biotherapeutics	Amphotheric surfactant-based cleanser
33	Lactated Ringers Solution pH 6.7	25.0	Wound dressing	Hospira Inc	Sodium lactate based-cleanser
34	Anasept Spray	0.0	Wound dressing	Anacapa T echnologies	Sodium hypochlorite-based cleanser
35	MicrocynRx Spray	0.0	Wound dressing	Oculus	Sodium hypochlorite-based cleanser
36	Dakin's Solution (4X diluted)	0.0	Wound dressing	Century Pharmaceuticals	Sodium hypochlorite-based cleanser
37	Microklenz	100.0	Wound dressing	Medline	Amphotheric surfactant-based cleanser with benzethonium chloride
38	3M Wound Cleanser	76.2	Wound dressing	3M	Pyridoxine- and Zn-salts-based cleanser
39	Biolex	0.0	Wound dressing	BARD	Poloxamine 908 and potassium -sorbate based cleanser
40	Gentell Wound cleanser	63.0	Wound dressing	Gentell	Amphotheric surfactant-based cleanser
41	SAF-Clens AF	77.7	Wound dressing	Convatec	Amphotheric surfactant-based cleanser
42	Seaclens	42.0	Wound dressing	Coloplast	Non-ionic surfactant- and EDTA-based cleanser
43	Restore	35.18	Wound dressing	Hollister	Poloxamer 188 and alkyl paraben-based cleanser
44	Dermal Wound Cleanser	52.0	Wound dressing	Smith & Nephew	Non-ionic surfactant and EDTA-based cleanser with benzethonium chloride
45	Skintegrity	70.0	Wound dressing	Medline	Amphotheric surfactant- and EDTA-based cleanser
46	Shur-Clens	0.0	Wound dressing	Convatec	Poloxamer188-based cleanser
47	CarraKlenz	100.0	Wound dressing	Medline	Anionic surfactant-based cleanser
48	VASHE wound therapy	0.0	Wound dressing	Puricore	Hypochlorite-based cleanser
49	Clorpactin	20.9	Wound dressing	Guardian Laboratories	Hypochlorite-based cleanser
50	Dermaklenz	84.3	Wound dressing	Dermarite Industries LLC	Pyridoxine HCl- and alcohol-based cleanser

surfactants such as those found in Products 32, 37, 40-41, 45, and 47 (Table 4). As discussed earlier (Figure 9), these types of surfactants can alter the structure of enzyme, thus inhibiting its activity. Furthermore, several products (Products 42, 44, and 45; Table 4) contain EDTA, a strong chelating agent that removes metal (ie, Zn^{2+}) from the active site of the enzyme. Wound cleansers that are formulated with antibacterial actives of cationic surfactant structure, such as Products 37 and 44, also inhibit the enzymatic activity as noted earlier (Figure 8). Products 33 and 38, although surfactant free, inhibit enzyme activity due to a lower-than-necessary pH value for optimal enzymatic performance of *C.* collagenase (pH = 6.6 versus pH =7 .2-7.6).[28] Poloxamer-based wound cleansers, (eg, Products 39, 43, and 46, Ta-

Table 5: Influence of various antibacterial formulations on enzymatic activity of *C.* collagenase.

No	Product	Inhibition (%)	Type	Manufacturer	Description
51	Bactroban Cream	0.0	Antibacterial dressing	Glaxo Smith Kline	Antibacterial cream
52	Sulfamylon Cream	0.0	Antibacterial dressing	UDL Laboratories	Antibacterial cream
53	Gentamycin Sulfate Ointment	13.0	Antibacterial dressing	E.Fougera Co.	Antibacterial cream
54	Kerlix Gauze (PHMB)	100.0	Antibacterial dressing	Kendall Healthcare	Bacteriostatic gauze
55	Anasept Gel	83.0	Antibacterial dressing	Anacapa Technologies	Wound Dressing
56	Clindamycin Phosphate Gel	0.0	Antibacterial dressing	Greenstone LLC	Antibacterial hydrogel

Table 6: Influence of various antimicrobial actives on enzymatic activity of *C.* collagenase.

No	Product	Inhibition (%)	Type	Manufacturer	Description
57	Mafenide Acetate	0.0	Antibacterial active	Sigma Aldrich	Sulfonamide-based antimicrobial
58	Polysporine	1.2	Antibacterial active	Johnson and Johnson	Protein antimicrobial mixture
59	Mupirocin	0.0	Antibacterial active	Sigma Aldrich	Carbohydrate-based antimicrobial
60	Gentamycin Sulfate	9.8	Antibacterial active	DPT	Carbohydrate-based antimicrobial
61	Silver sulfadiazine	51	Antibacterial active	Sigma Aldrich	Silver-containing antimicrobial
62	Chlorhexidine Gluconate	0.0	Antibacterial active	Sigma Aldrich	Biguanide-based/ antimicrobial
63	Benzalkonium Chloride	99.0	Antibacterial active	Sigma Aldrich	Cationic surfactant antimicrobial
64	p-DADMAC (from Bioguard gauze)	0.0	Antibacterial active	Sigma Aldrich	Bacteriostatic polymer
65	Neomycin	0.0	Antibacterial active	ICN	Carbohydrate-based antimicrobial
66	Metronidazole	100.0	Antifungal Active	DPT	Antifungal agent

ble 4), are generally very compatible with *C.* collagenase, as noted earlier (Figure 7). The exception is Product 43, which significantly inhibits the enzyme, probably due to the high concentration of alkyl parabens present in the formulation. Finally, Products 34-36 and 48-49, which are based on sodium hypochlorite, are very compatible with *C.* collagenase, with exception of powdered Product 49 that contains unidentified residue after solubilization in water.

Antibacterial formulations (Products 51-56, Table 5) generally exhibited minimal to no influence on the enzymatic activity of *C.* collagenase. Products 51-53 are emulsion creams with small molecule antimicrobials as actives, product 56 is a hydrogel with antimicrobial active. None of these formulations significantly inhibit *C.* collagenase, with the exception of very mild inhibition by Product 53. Product 54 is gauze impregnated with PHMB, a chemical already described as a strong enzyme inhibitor (Product 25,Table 3). Product 55 is a sodium hypochlorite-based an-

timicrobial gel that strongly inhibits the activity of the enzyme. This is somewhat contradictory with the earlier findings for Products 34-36 (Table 4), which contain the same concentration of sodium hypochlorite, yet without any influence on enzyme activity. The difference is the presence of a gelling component in Product 55, sodium magnesium silicate, which is probably responsible for the inhibition of the enzyme activity. Anti-fungal Product 66 (Table 6) completely inhibits the activity of the enzyme, probably due to complexation of the metal by metronidazole (the active molecule in the formula).

Finally, the authors tested drug actives commonly used in antimicrobial formulations (Drug Substances 57-66, Table 6). Drug Substance 58 is a free-flowing white powder, containing active peptide-based antimicrobials bacitracin and polymyxin B in a lactose base. An important finding is that these actives are compatible with *C.* collagenase (Table 6). Products 59, 60, and 65 (Table 6), carbohydrate-based antimicrobials, also have no influence on the enzymatic activity of *C.* collagenase. Drug Substance 61 (Table 6), a silver-based antimicrobial showed significant inhibitory effect (refer to Table 1). Drug Substances 57, 62, and 64 have no negative effect on the enzymatic activity of *C.* collagenase, while cationic surfactant-like Drug Substance 63 strongly inhibited the activity of the enzyme. Product 66, an antifungal, exhibits the total inhibition of the *C.*collagenase.

Conclusions

In summary, our results demonstrated the influence of various metal salts and surfactants on the enzymatic activity of *C.* collagenase. Alkali metal salts are chemical entities that generally have no to slight positive influence on enzymatic activity. It was noted that the positive effect on enzymatic activity is proportional to salt concentration, which implies the electrostatic interactions between the protein and salts are favorable for catalytic transformations. Earth alkali metal salts also exhibited minimal or mild positive influence on enzyme activity. However, divalent transitional metals strongly inhibit the enzymatic activity of *C.* collagenase. The main difference between the 2 populations of divalent cations is the presence of electrons in the d orbitals. Transitional metals have partially filled d orbitals, and thus they are able to make donor-acceptor complexes with the enzyme, change the structure of the latter, and thus decrease its activity. The true nature of this interesting phenomenon was far beyond the scope of this text.

The influence of surfactants on the enzymatic activity of *C.* collagenase is primarily a function of the electrostatic nature of the polar head. Non-charged surfactant molecules generally tend to have minimal or even slightly positive influence on the enzymatic activity of *C.* collagenase. Large non-ionics have a particularly positive effect on enzymatic activity, probably due to favorable solubilization effects and very mild surfactantcy that is unable to unfold the protein structure. Charged surfactants, both cationic and anionic, generally inhibit enzymatic activity even at the lowest concentration tested. This interesting difference was observed in the behavior of these 2 types of surfactants: cationics strongly inhibit the enzyme even at concentrations under the CMC, but do not completely diminish the activity of the enzyme until very high concentrations; anionics (eg, SDS) exhibited only mild inhibition at concentrations under CMC, but completely attenuate the activity of the enzyme at any concentration above CMC. Amphoteric surfactants inhibit the activity of the enzyme proportionally with their concentration.

An important part of this work was to evaluate the compatibility of various wound care products with *C.* collagenase. Most silver-containing products are not compatible with the enzyme, significantly inhibiting its activity. However, certain products containing silver in ionic form, embedded in polymeric matrix or foam, exhibit only mild influence on enzymatic activity. Iodine-containing dressings strongly inhibit enzymatic activity regardless of the form (ie, foam dressing or gel). Commonly used wound dressings are generally compatible with the enzymatic activity of *C.* collagenase, leaving a plethora of choices for clinicians. Wound cleansers tend to inhibit the enzymatic activity of *C.* collagenase, mainly because of the presence of powerful ionic or zwitterionic surfactants. The other reasons for the enzyme inhibition are the presence of EDTA, inadequate pH of the formulations, or presence of cationic surfactant-like structures in the formulas. Wound cleansers containing large block co-polymer surfactants (eg, poloxamers) or formulas with sodium hypochlorite are compatible for use with the collagenase debrider. If wound cleansers, for example, contain any other surfactant type, except non-ionic or sodium hypochlorite salts, it is strongly advised to rinse the cleanser from the wound bed with saline thoroughly before applying the collagenase debrider. Antimicrobial formulations as well as antimicrobial actives are generally compatible with *C.* collagenase.

This work was performed with the intention to help wound care professionals make more educated decisions with respect to what type of supporting products should be used along with a collagenase wound debrider.

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