Structural Formation of the Fibrin Matrix: impact of a biocompatible coal (called Pulcandi) used for epidermal healing

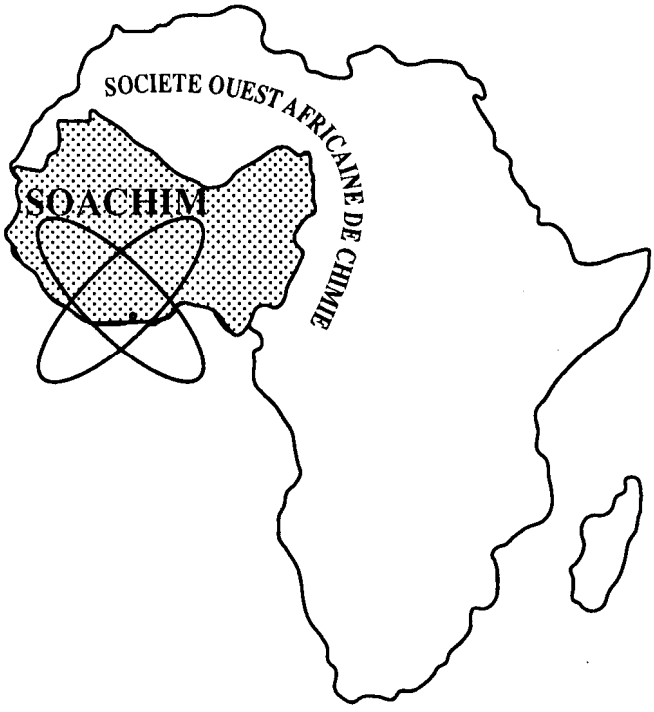
Edefia Akpalo ; Gudrun Aba Eklu; Kwami Lumo Awaga ;

Kwashie Eklu-Gadegbeku ; Abatévi Aba Eklu

Journal de la Société Ouest-Africaine de Chimie

J. Soc. Ouest-Afr. Chim.(2018), 045 : 49 - 53

23ème Année, Juin 2018



ISSN 0796-6687

Code Chemical Abstracts : JSOCF2

Cote INIST (CNRS France) : <27680>

Site Web: **http://www.soachim.org**

Structural Formation of the Fibrin Matrix: impact of a biocompatible coal (called Pulcandi) used for epidermal healing

Edefia Akpalo [a,b] *; Gudrun Aba Eklu[a]; Kwami Lumo Awaga [b]; Kwashie Eklu-Gadegbeku [a,b]; Abatévi Aba Eklu [a]

[a]*COA : Centre Omnithérapeutique Africain ; 1Rue COA Sanguera, 08 BP : 81718 Lomé TOGO*
[b]*Université de Lomé Faculté des Sciences Département de Biochimie ; BP : 1515 Lomé TOGO*

(Reçu le 20/04/2018 – Accepté après corrections le 12/08/ 2018)

Abstract: Fibrin, a key protein of wound healing, plays an important role. It forms a supramolecular dynamic network. Pulcandi powder is indicated to treat external wounds. The aim of this study was to analyse the powder impact on the structural formation of fibrin gel network. Then, we could evidence scientifically the efficacy of this coal in healing really external wounds. We proceed on calorimetric analyses (ATG and DSC) and mineralization on Pulcandi powder. For fibrin gel study we prepared three types of gels which were sprinked with 1 g of Pulcandi powder at different times. Gelation times were determined and electrophoresis analyses were performed. As shown by thermogravimetric and differential scanning calorimetric, Pulcandi powder has a thermal stability. Moreover our results proved that Pulcandi powder can be considered as a natural biocompatible material coming in coal powder form and composed of minerals. These trace minerals (TM) play an important role in skin integrity and wound healing. Electrophoresis analyses showed that Pulcandi powder can be in contact and absorb fibrinogen solutions without any structural modification of the protein or disturbing of the enzymatical gelation process by thrombin an hydrolytic enzyme. Pulcandi powder is then a biocompatible mineral healing coal ancestrally used in Togo.

Keywords: Pulcandi; Fibrinogen; Wound healing; Coal; Biocompatibility;

Formation structurelle de la matrice de Fibrine: impact d'un charbon biocompatible servant à la cicatrisation épidermique et appelé Pulcandi

Résumé: La fibrine, protéine clé de la cicatrisation, y joue un rôle important. Elle forme un réseau supramoléculaire gel. La poudre Pulcandi est conseillée dans le traitement des plaies externes. L'objectif de cette étude a été d'analyser l'effet de cette poudre sur la formation du réseau de fibrine. Des analyses calorimétriques (ATG et DSC) et de minéralisation ont été effectuées sur la poudre Pulcandi. Des analyses électrophorétiques ont été réalisées sur trois types de gel saupoudré chacun de 1 g de Pulcandi à des temps différents. Selon les études calorimétriques, la poudre Pulcandi est thermiquement stable. De plus, nos résultats ont démontré que Pulcandi peut être considéré comme un matériau naturel biocompatible se présentant sous la forme d'un charbon en poudre composé de minéraux. Ces minéraux essentiels jouent un rôle important dans le maintien de l'intégrité de la peau et dans le processus de cicatrisation. Les résultats des analyses électrophorétiques ont montré que la poudre pouvait se trouver en contact et absorber des solutions de fibrinogène sans aucune modification de la structure protéique. Aucune perturbation du processus de gélification enzymatique par la thrombine n'est observée. La poudre Pulcandi est donc un charbon minéral cicatrisant et biocompatible utilisé au Togo.

Mots clés: Pulcandi; Fibrinogène; Cicatrisation; Charbon; Biocompatibilité;

* Corresponding author: edefia@gmail.com

1. Introduction

Wound healing is a whole of physiological phenomena resulting in epidermic or dermo-epidermic wound reparation in the aim to retrieve all or a part of the injured tissue form and function **[1]**. Wound healing is generally subdivided in three consecutive phases: the inflammatory phase, the proliferative phase and the remodeling phase. These three phases could also overlap. Wound detersion occurs during the inflammatory phase. The proliferative phase leads to granulation high red tissue because of new blood vessels appearing; a fibrinous secretions film is formed and colonized by the vascular granulations and dermic cells which allow wound filling. Wound healing ends with the maturation and remodeling phase.

Fibrin plays an important role in wound healing. It forms a supramolecular, tridimensional and dynamic network which is the support of tissular reparation. Fibrin soft gels are at the basis of wound repair and haemostasis. Gel is defined as an elastic solid constituted of a first so-called organized solid in an infinite network which confines a so-called liquid phase and prevents it from flowing. The two phases are continued through all the gel which acts as a soft easy to deform solid. The liquid phase molecules are independent from each other and independent from the solid [2].

Fibrin molecules are formed subsequently to thrombin-hydrolysis of the fibrinogen molecule.Polymerization or gelation of the network occurs due to the supramolecular assembly of fibrin molecules through low-energy bonds **[3] [4]**. Increasing the mechanical strength of those networks may enlarge their potential use **[5]**.

Pulcandi is a phytotherapeutical coal (highly carbonaceous material) used in Togo for the treatment of external wounds. Pulcandi originates from plants and appears as a fine black odorless powder. The raw material contains different salts. These salts, when heated at elevated temperature, can melt and cover the carbonaceous particles and protect it from air oxygen **[6]**. The ashes would then be grey or darkish, which is the case of Pulcandi powder.

Active coal wound dressing is generally used during the phase of detersion and have odor absorption and wound draining properties. The aim of this study is to evidence scientifically the efficiency of Pulcandi in healing external wounds. We particularly focused on the impact of coal on fibrin network formation.

2. Materials and methods

2.1. Chemicals

Thrombin (T-4648), bovin Fibrinogen type IV (F-4753) and human fibrinogen (F3879) obtained from human plasma were purchased from Fisher Reagents. Pulcandi powder was produced by Centre Omnithérapeutique Africain (COA). Experimentations were performed in the biochemistry laboratory of ERRMECE (University of Cergy Pontoise), in the biochemistry laboratory of COA, in CRISMAT laboratory (ENSICAEN, France) and in the GTVD laboratory (Gestion Traitement et Valorisation des Déchets, University of Lomé).

2.2. Thermal Analysis

Thermal properties of Pulcandi powder were evaluated by means of thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA and DSC were performed using NETZSCH STA 449F3 in the temperature range O°C – 1000°C, at a heating rate of 10°C/min and under a nitrogen flow rate of 60 $mL.min^{-1}$. The thermal decomposition of the powder was evaluated by thermogravimetric analysis (TGA). Glass transition temperature (Tg) was evaluated by differential scanning calorimetry (DSC), while the melting temperature (Tm) was evaluated by analyzing the endothermic peaks.

2.3. Minerals composition and concentration

Mineral elements concentrations (Cu, Fe, Zn) were determined by absorption spectrophotometry. For this purpose, Pulcandi powder was mineralized by 1.5 nitric acid. The mix was then heated up until a viscous liquid is obtained and then it was added to distilled water. The solution is filtered and diluted. Determination of mineral elements concentrations was performed using an atomic absorption spectrophotometer (AA Solar, Tehrmo Electron Corporation Germany).

2.4. Gelation procedure

All reactants were solubilized in 50 mmol/L Tris-HCl buffer with pH 7.4 and incubated at 37°C for 15 min prior to mixing. Fibrin gels were prepared by incubation at 37°C of a mixture containing 1 mL of 5mg fibrinogen (bovin or human fibrinogen), 0.04 units of thombin per mg of fibrinogen, 150 mmol/L NaCl and 20 mmol/L $CaCl_2$. This mixture was kept at 37°C for 1h.

Three types of gels prepared each in duplicate with human fibrinogen were sprinked with 1 g of Pulcandi powder at different times: type 1gels at t=0 min (t0), type 2 gels at t=30 min (t30) and type 3 gels at t=60 min (t60). For type 1 gels, a proteic

solution was sprinked with Pulcandi powder just after thrombin addition (t0) means before gelation time (6 minutes). With type 2 gels, Pulcandi powder was sprinked 30 minutes after thrombin addition (t30) means 24 minutes after gelation time. To finish, type 3 gels were sprinked with Pulcandi powder 60 minutes after thrombin addition (t60) means 54 minutes after gelation time.

2.5. SDS-PAGE electrophoresis

Fibrin network in 500 µL gels was disrupted by incubation for 3 h at 37°C in 4.5 mL of a mixture of 8 mol/L urea, 2% w/v sodium dodecyl sulphate (SDS) and 2% v/v β-mercaptoethanol in 0.16 mol/L Tris-HCl buffer with pH 6.8. Sample of the gels corresponding to 5 to 10 µg proteins were analyzed in 8 % polyacrylamide gel. Coomassie blue was used to reveal migration.

3. Results

3.1. Thermal characterization

The thermal properties of Pulcandi powder were investigated by TGA and DSC. TGA evaluation showed that the weight loss curve of the powder was characterized by two main thermal events. The first one centered at around 145°C is relevant to evaporation of atmospheric water and corresponds to a loss of about 8%. The other one centered at around 650°C relates to total decomposition with 10% residue **(Figure 1).**

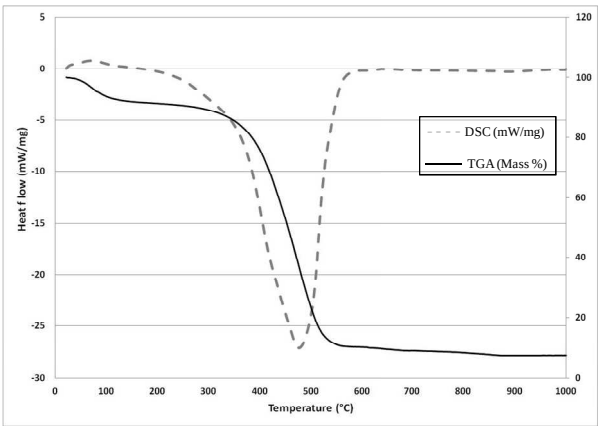


Figure 1: Thermogravimetric analysis (TGA) characterization and representative differential scanning calorimetry (DSC) curves of the analyzed samples.

Representative DSC curve of the characterized samples is reported also in Figure 1. No endothermic peak related to melting or crystalline domains nor glass transition was detectable. Only the broad endothermic peak related to organic materials total decomposition (300°C to 600°C) leaving inorganic elements was present.

3.2. Mineral composition

Pulcandi powder contains oligo-elements and essential minerals. In this study we have just reported the oligo-elements present in the powder. **(Table I)**

Table I: Oligo-elements present in Pulcandi powder

Essentials Oligo-elements	Pulcandi powder contents (mg %)
Copper	0.3
Iron	45
Zinc	4

3.3. Protein solutions and protein gels sprinked with Pulcandi powder

Gelation occured in 8 minutes for bovin fibrinogen and in 6 minutes for human fibrinogen. For type 1 gels, the powder was completely absorbed and we obtained black mixtures. For type 2 gels, the powder was absorbed by the liquid phase of the gels. However, the gels subsisted, not as swelling ones but like fine black proteic networks. Finally, for type 3gels, little liquid phase absorption was observed and gel like materials were retrieved.

3.4. Electrophoresis analysis of protein gel with Pulcandi powder

Investigations on bovin fibrinogen reveal characteristic bands of fibrinogen and fibrin (**Figure 2**).

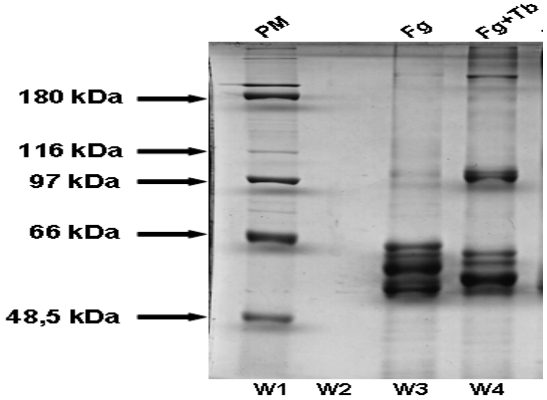


Figure 2: SDS-PAGE electrophoresis of bovin fibrinogen with (Fg + Tb) and without (Fg) thrombin.

The well containing fibrinogen (Fg) showed three characteristic bands: Aα band with 65 kDa molecular weight, Bβ band with 56 kDa molecular weight and γ band with 51 kDa molecular weight. The well

containing hydrolysed fibrinogen (Fg+Tb) exhibited a 59 kDa molecular weight and a 53 kDa molecular weight bands corresponding respectively to α and β chains. The new band appeared at 102 kDa should correspond to γ-γ dimer. This γ-γ dimer is specific to fibrin gel formation. The human protein presented same characteristic bands as bovin fibrinogen (data not shown).

Electrophoresis analyses of human fibrinogen solution added with Pulcandi (**Figure 3**) revealed the presence of the powder did not change the protein structure. We retrieve the three characteristics bands of fibrinogen.

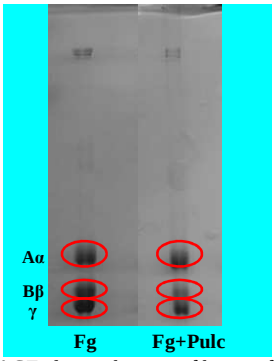


Figure 3: SDS-PAGE electrophoresis of human fibrinogen without Pulcandi powder (Fg) and with Pulcandi powder (Fg + Pulc).

To study gelation process in presence of Pulcandi powder, electrophoresis analyses of human fibrinogen were performed again **(Figure 4).** Bands in well 1 (Fg) and well 2 (Fb) are characteristic to fibrinogen and fibrin bands. Well 3 (Fb+Pulc $t_0$min) contains all fibrinogen characteristic bands and γ-γ band specific to fibrin network formation. Well 4 (Fb+Pulc t_{30}min) reveals exclusively the three characteristic bands of fibrin. They have same molecular weight as bands in well 2 (Fb). As gelling time was 6 minutes, the gel was completely formed before Pulcandi powder addition, and that explains the total absence of γ band even though this γ band is present in well 3 (Fb+Pulc $t_0$min).

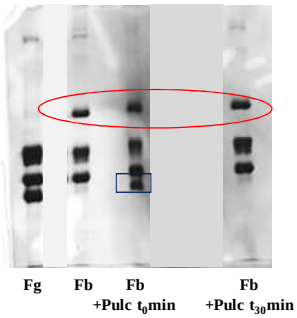


Figure 4: SDS-PAGE electrophoresis of human fibrinogen solution (Fg), fibrin gel (Fb), gel 1 sprinked with Pulcandi powder just after thrombin addition (Fb+ Pulc t_0 min) and gel 2 sprinked with Pulcandi 30 minutes after thrombin addition (Fb+Pulc t_{30} min).

4. Discussion

Thermal properties investigation of Pulcandi powder highlighted the pronounced atmospheric water adsorption. No crystalline or amorphous domains characteristic of polymer materials were detected. Pulcandi powder is a natural macromolecular biocompatible material in coal form. Mineral composition of Pulcandi powder revealed the presence of zinc, copper and iron. Trace minerals (TM) play an important role in skin integrity and wound healing. Their presence increased gene expression of growth factors and enzymes, which promoted collagen synthesis, deposition and organization; cell migration, matrix remodeling, and angiogenesis **[7]**. Pulcandi powder can then be considered as a biomaterial dedicated to wound healing.

As gel is defined as an organized solid in an infinite network which confines a liquid phase, then for type 2 gels, the fine black film observed may result from liquid phase absorption. For type 3 gels, not only they are completely formed, but a little dehydration may occur leading to the shrinkage of the solid phase network. Therefore, the totality of the powder is spread on the surface of the gel.

The presence of migrated bands in well 3 (Fb+Pulc $t_0$min) **(Figure 4)** suggested that Pulcandi powder does not completely prevent fibrinogen hydrolysis and fibrin network formation. The γ and γ-γ bands are both present; this means respectively a characteristic band of fibrinogen and the specific band of fibrin network formation. We consider then that it is a co-existence between non hydrolyzed fibrinogen molecules and hydrolyzed fibrin molecules. The obtained black mixture corresponded then to the proteic solution absorbed by the Pulcandi powder.

Migrated bands present in well 4 (Fb+Pulc t_{30}min) **(Figure 4)** have the same molecular weight as bands in well 2 (Fb) without γ band. We conclude that spreading Pulcandi powder on a preformed gel does not destructure it. Observations of type 2 gels to which Pulcandi powder was added at 30 minutes did not reveal a complete dehydratation. This highlights the fact that Pulcandi powder absorbs exsudate, playing detersion role without damaging fibrinous network.

5. Conclusion

Pulcandi powder is specifically a healing biomaterial issued from plants. This powder could be in contact and absorb fibrin(ogen) proteic solutions

without any modification of the structure of fibrinogen protein or any artefact in gelation process of the initial matrix for neo-dermic synthesis. The reported results herein show that Pulcandi powder heals external wounds by absorbing exudates without interfering in the formation of the tissular reparation support.

Acknowledgements
We sincerely thank CRISMAT Laboratory, UMR 6508 CNRS at Caen (France) for kindly proceeding on calorimetric analyses.

References:

[1] Dallon J. C. Modeling the effects of transforming growth factor-β on extracellular matrix alignment in dermal wound repair. Wound Repair and Regeneration (2001) 9 [4] 270-286.
[2] De Gennes P.G. ,*Soft matter*, Nobel Lecture, 1-11, 9 December 1991.
[3] Mosesson, M. Fibrinogen functions and fibrin assembly. Fibrinolysis and Proteolysis *(*2000) 14,182-186.
[4] Pratt KP, Côté HC, Chung DW, Stenkamp RE, Davie EW. The primary fibrin polymerisation pocket: Three dimensional structure of a 30-kDa C-terminal chain fragment complexed with the peptide Gly-Pro-Arg-Pro. Proc Natl Acad Sci USA (1977) 94,7176-7181.
[5] Akpalo E, Larreta Garde V. Increase of fibrin gel elasticity by enzymes: a kinetical approach. Acta Biomater (2010) 6,396–402.
[6] Masson et Cie Editeurs, Librairies de l'Académie de Médecine, Traité de Biochimie Générale: Tome I, 1959.
[7] Chen J, Tellez G, Vazquez-Anon M. Impact of Trace Minerals on Wound Healing of Footpad Dermatitis in Broilers. Scientific Reports (2017) 7, 1894.