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Pengembangan Sistem Regenerasi Antioksidan Berbasis Enzimatik sebagai Agen Penyembuh Luka Kronis

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BAB I RINGKASAN

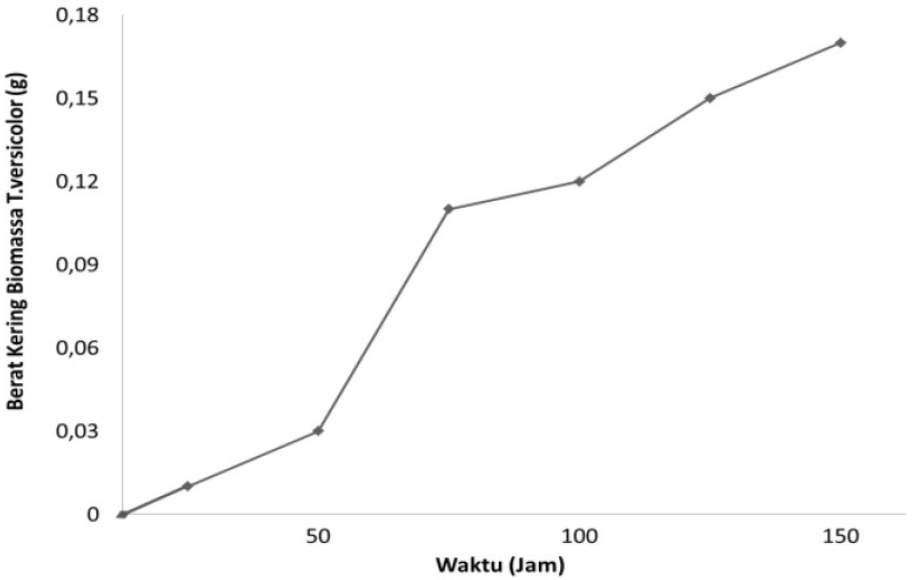
Luka dapat menyebabkan gangguan pada fungsi dan struktur anatomi tubuh. Mencit yang dilukai setelah diinduksi aloksan dapat menyebabkan luka kronis karena mekanisme tersebut. Hidrogel penutup luka berbasis enzimatik cellobiose dehydrogenase (CDH) diusulkan sebagai sistem regenerasi antioksidan yang menangkal reactive oxygen species pada luka dan penyuplai hidrogen peroksida untuk menangkal infeksi mikroba dengan reaksi yang dikatalisasi oleh CDH. Enzim CDH, pada penelitian ini, diproduksi oleh kapang Trametes versicolor dalam kultur produksi dengan Tissue toilet Nice sebagai substrat. Hidrogel penutup luka berbasis enzimatis didukung dengan matriks κ-karaginan dan mengandung CDH, laktosa, dan asam galat. Hasil yang diperoleh menunjukkan Aplikasi hidrogel penutup luka berbasis enzimatis CDH pada luka kronis tidak memberikan pengaruh yang signifikan dalam penutupan luka dibandingkan dengan betadine. Betadine memiliki penutupan luka yang lebih cepat secara signifikan terhadap perlakuan hidrogel. Penilaian penyembuhan epidermal dan dermal pada hari ke-12 pada masing-masing perlakuan tidak menunjukkan perbedaan yang signifikan namun hidrogel penutup luka berbasis enzimatik CDH memiliki kecenderungan penyembuhan dermal dan epidermal yang lebih baik dari perlakuan kontrol.

Kata kunci: Aloksan, Cellobiose dehydrogenase, Hidrogel

BAB II HASIL PENELITIAN

2.1 Produksi *Cellobiose Dehydrogenase* (CDH)

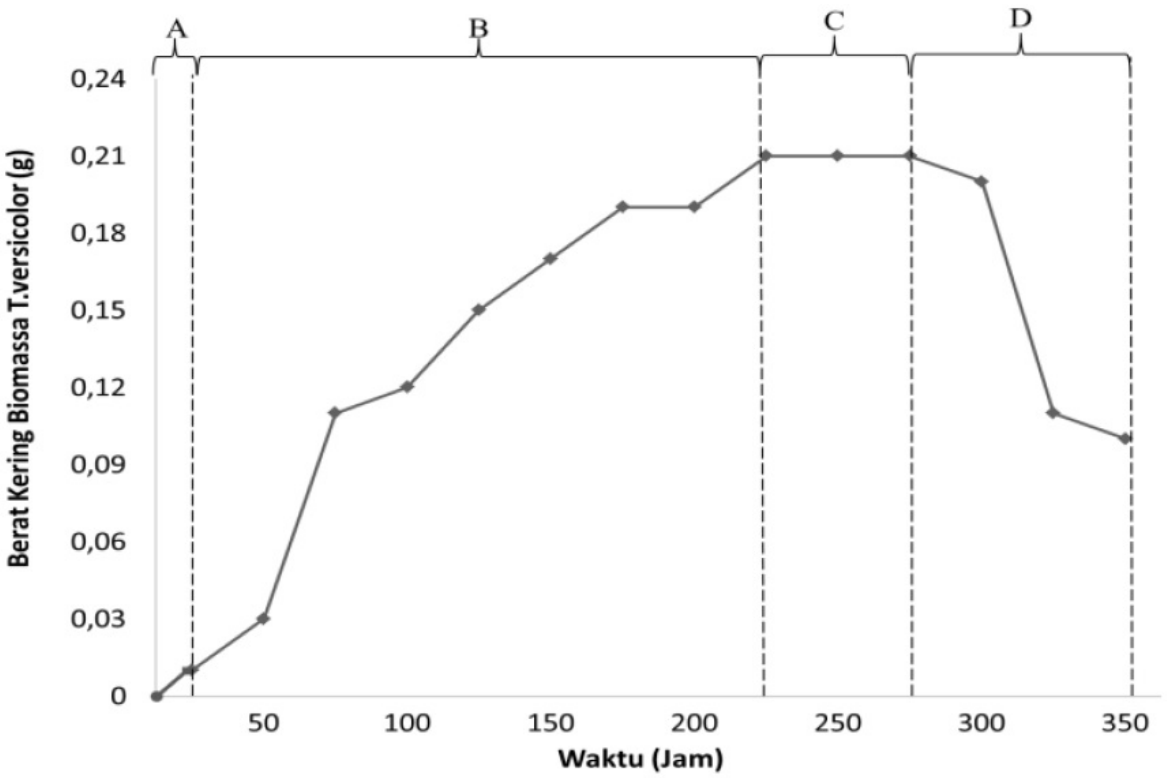
Produksi *Cellobiose dehydrogenase* (CDH) didahului dengan aklimatisasi isolat *T. versicolor* menggunakan medium aklimatisasi dan medium produksi yang mengandung selulosa sebagai sumber karbon. Sumber selulosa yang digunakan berasal dari tisu toilet. Menurut Mahbubillah *et al.*, (2019) bahwa penggunaan substrat turunan dari selulosa tidak memberikan hasil yang optimal untuk memproduksi CDH. Kandungan karbon pada substrat sebagian besar berfungsi sebagai sumber energi dan biomassa jamur. Hasil produksi menghasilkan perubahan pada medium menjadi lebih keruh. Hal tersebut karena terjadi pemecahan senyawa organik kompleks menjadi komponen yang lebih sederhana. Kekeruhan pada medium juga disebabkan oleh bertambahnya jumlah spora kultur *T. versicolor*. Profil fase *logaritmic* selama aklimatisasi tampak pada Gambar 2.1.



Gambar 2.1 Profil fase *logaritmic* pertumbuhan *T. versicolor* selama aklimatisasi.

Aklimatisasi dilakukan sebanyak tiga tahap sampai isolat *T. versicolor* memasuki fase setengah *logaritmic* dalam fase pertumbuhannya yang divisualisasikan pada Gambar 2.1. Kultur yang telah teraklimatisasi digunakan sebagai starter untuk ditumbuhkan dalam medium produksi formulasi Roy *et al.* (1996). Profil pertumbuhan *T. versicolor* dalam media produksi selanjutnya dibuat dalam bentuk grafik kurva pertumbuhan untuk menentukan waktu isolasi (CDH) seperti yang pada Gambar 2.2. Kurva pertumbuhan isolat *T. versicolor* terdiri dari fase *lag*, fase *log*, fase *stationary*,

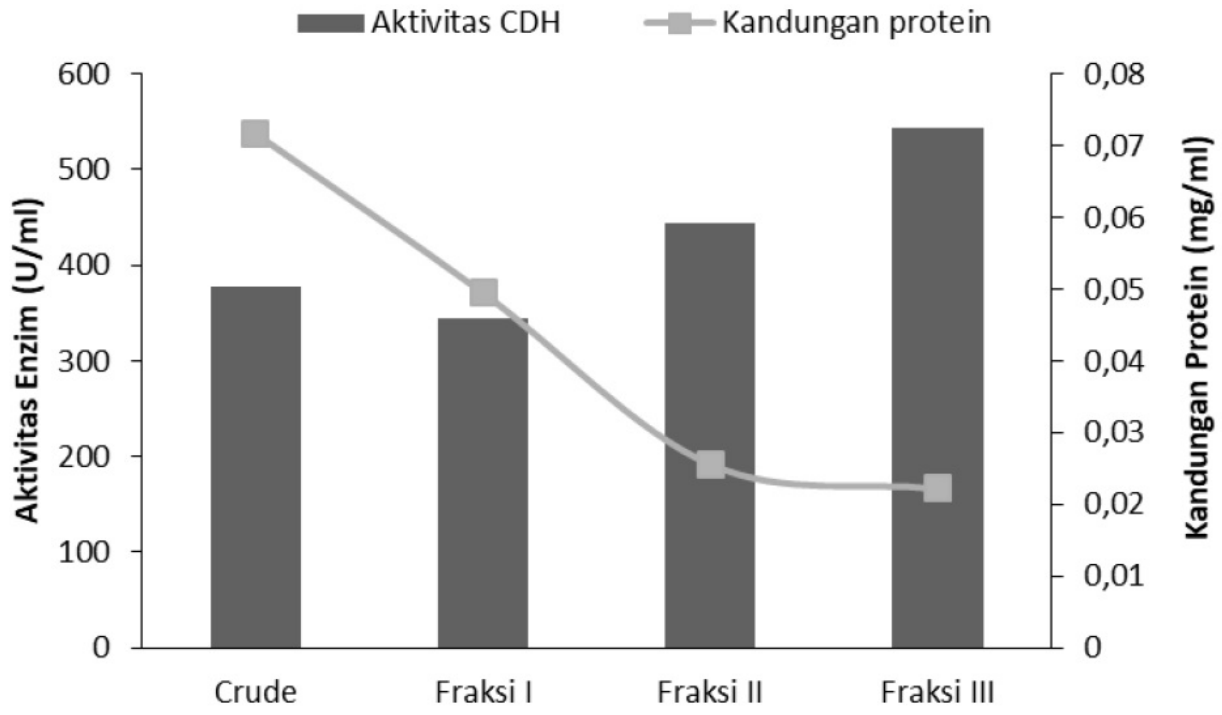
dan fase *death*. Pada fase *lag*, mikroorganisme mulai melakukan persiapan untuk bereproduksi sehingga tampak hampir tidak ada pertumbuhan. Saat sel memasuki fase *log* terjadi peningkatan biomassa seiring berjalannya waktu, dimana sel mikroorganisme melakukan pembelahan tiap satuan waktu. Fase *stationary* dicapai saat laju pertumbuhan sama dengan laju kematian sel. Fase *death* terjadi ketika sel mengalami kerusakan karena adanya akumulasi produk sisa metabolisme (Al-Qadiri *et al.*, 2007).



Gambar 2.2 Kurva pertumbuhan *T. versicolor* pada medium produksi Roy *et al.*, 1996. (A) Fase *Lag*; (B) Fase *Log*; (C) Fase *Stationary*; dan (D) Fase *Death*.

Berdasarkan Gambar 4.2, fase *lag* berlangsung singkat dimulai pada jam ke 0 hingga jam ke 48 yang menandakan bahwa isolat *T. versicolor* telah teraklimatisasi pada medium produksi. Fase *log* dimulai pada jam ke 48 hingga jam ke 240, berlangsung cukup lama dan terjadi penambahan biomassa sel dimana sel memproduksi CDH untuk memanfaatkan selulosa sebagai sumber karbon. Sumber selulosa berasal dari tisu toilet. Pada jam ke 240 hingga jam ke 336 inokulum berada pada fase *stationary* yang ditunjukkan dengan tidak adanya pertumbuhan sel. Fase *Death* terjadi pada jam ke 312 hingga jam ke 336. Isolasi CDH dilakukan ketika kultur *T. versicolor* berada pada akhir fase *log* dan mulai memasuki fase *stationary*. Hal ini didasarkan pada Herreither *et al.*, (2009),

isolasi CDH pada *T.versicolor* dilakukan setelah kultur memasuki *stationary phase* yaitu pada hari ke-9. Ekstrak kasar CDH yang telah diisolasi selanjutnya dipurifikasi melalui presipitasi bertingkat menggunakan garam ammonium sulfat dengan prinsip "*salting in*' dan "*salting out*". Hasil fraksinasi ammonium sulfat tampak pada Gambar 2.3.



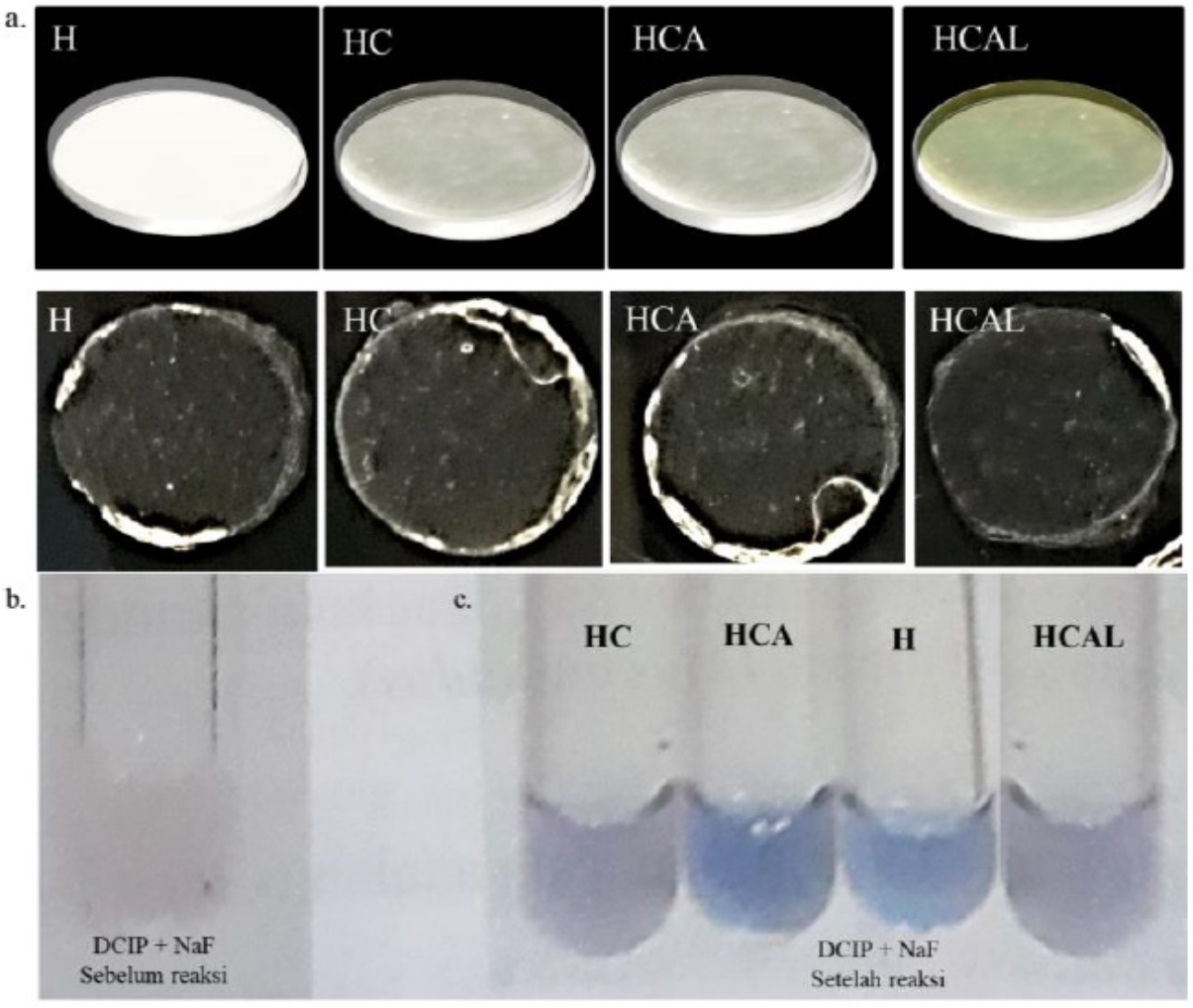
Gambar 2.3 Aktivitas dan kandungan protein pada fraksi pemurnian CDH menggunakan ammonium sulfat.

Fraksinasi amonium silfat dilakukan hingga pemurnian ke 3 seperti yang ditunjukkan pada Gambar 2.3. Aktivitas CDH tertinggi diperoleh pada fraksi pemurnian ke III yaitu 544,44 U/ml dengan kandungan protein sebesar 0,02 mg/ml, sehingga menghasilkan aktivitas spesifik paling tinggi yaitu 24546,43 U/mg. Aktivitas spesifik menunjukkan tingkat kemurnian suatu enzim, semakin tinggi nilainya maka semakin tinggi pula tingkat kemurnian enzim (Bisswanger, 2014).

2.2 Fabrikasi Hidrogel Penutup Luka

Empat jenis hidrogel yang dibuat yaitu hidrogel berbasis enzimatik CDH(HC), hidrogel asam galat (HCA), hidrogel CDH asam galat dan laktosa (HCAL), hidrogel kosong (H) dibuat dengan diameter 1 cm dengan bentuk yang disajikan pada Gambar 2.4 a. Menurut Mahbubillah *et al.*, (2019) bahwa Keempat jenis hidrogel tersebut diuji dengan menggunakan larutan 2,6-dichlorophenol indophenol

(DCIP) đan NaF yang berwarna merah muda yang menunjukkan bahwa larutan dalam suasana asam. (Gambar 2.4 b, Hidrogel berbasis enzimatik CDH pada inkubasi dengan larutan mengandung DCIP dan NaF dapat mengubah warna DCIP yang diteteskan pada hidrogel menjadi biru pudar yang menandakan bahwa DCIP telah tereduksi.Sedangkan hidrogel asam galat dan hidrogel kosong berubah warna menjadi biru yang menandakan pH larutan meniadi netral atau basa (Gambar 2.4 c) (Horwitz, 1992). Hasil tersebut menunjukkan bahwa hidrogel berbasis enzimatik CDH dapat mereduksi akseptor elektron DCIP yang menunjukkan adanya aktivitas dari enzim CDH. Sedangkan pada hidrogel asam galat dan hidrogel kosong tidak menunjukkan aktivitas enzim reduksi DCIP pada sistem.

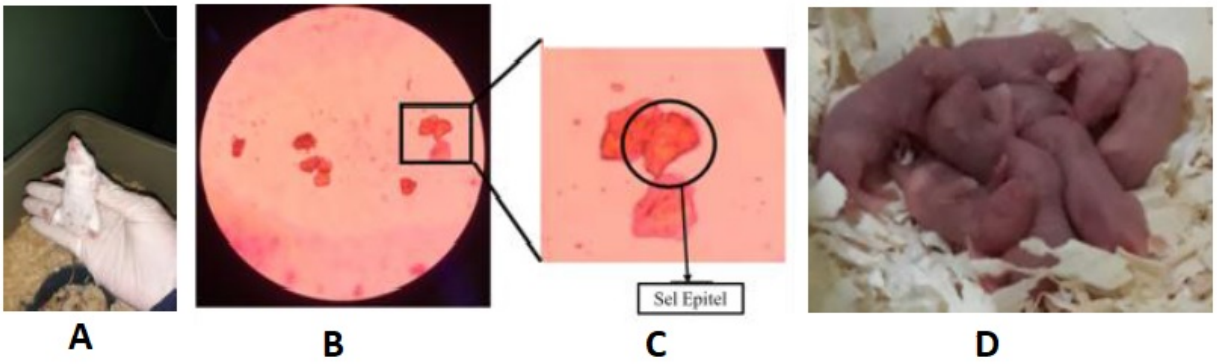


Gambar 2.4 Fabrikasi hidrogel penutup luka a. H : hidrogel kosong, HC : Hidrogel Enzim CDH, HCA : Hidrogel Asam Galat, HCAL : Hidrogel CDH+asam galat+laktosa, b. larutan DCIP + NaF sebelum reaksi, c. larutan DCIP+ NaF setelah reaksi dengan masing-masing hidrogel penutup luka.

2.3 Hasil Pengamatan Hewan Uji *Mus musculus*

2.3.1 Hasil *Breeding* mencit

Sebelum dilakukan pengawinan mencit dilakukan cek vaginal smear. Hasil olesan dinding vagina mencit pada kaca objek kemudian ditetesi pewarna eosin. Eosin yang digunakan yaitu eosin Y (*eosin yellowish*) atau secara kimiawi dikenal sebagai disodium 2-(2, 4, 5, 7-tetrabromo-6-oxido-3-oxo-3Hxanthen-9-yl) benzoate, merupakan pewarna bersifat asam yang tersertifikasi dalam pewarnaan histopatologi mewarnai sel dan sitoplasma, kolagen, dan jaringan otot (Rahman, 2017).



Gambar 2.5 Perbanyakan Mencit Percobaan. A. handling mencit, B.Apusan vaginal smear *Mus musculus* perbesaran 100x (estrus). C. Sel epitel terkornifikasi, D. hasil *breeding* .

Berdasarkan hasil Gambar 2.5 c didapatkan, sel epitel yang yang diberi eosin akan berwarna merah muda karena struktur asidofilik eosin akan mewarnai mitokondria, butir-butir sekresi, kolagen, bagian sitoplasma yang banyak RNA, dan matriks kartilago dengan warna merah muda (Subowo, 2009). Pengamatan menunjukkan sel-sel epitel yang terkornifikasi dimana mencit sedang dalam fase estrus (Kiani,*et al.*, 2018). Setelah dilakukan pengawinan mencit didapatkan hasil keseluruhan anakan dari 3 induk betina yang diuji menghasilkan 10 anak per induk.

2.3.2. Hasil pengamatan mencit selama aklimatisasi

Perawatan hewan uji mencit (*Mus musculus*) dilakukan dengan melakukan penyesuaian terhadap kondisi lingkungan pada kandang uji. Hal tersebut sesuai dengan Conour *et al.*,(2006) yang menyatakan bahwa aklimatisasi diperlukan untuk mempersiapkan hewan uji pada penelitian biomedis. Kebersihan kandang uji terjaga selama aklimatisasi maupun pengujian dengan blower yang selalu menyala untuk membuang bau serta debu yang menumpuk dalam kandang (Gambar 2.6 b). Nutrisi yang diberikan kepada mencit memiliki tekstur yang tidak terlalu keras dan tidak terlalu

lembek sehingga ideal bagi hewan uji (Suckow *et al.*,2000). Faktor-faktor yang dapat mempengaruhi pada penyembuhan luka meliputi oksigenasi, infeksi, umur, jenis kelamin dan hormon sex, stress, obesitas, medikasi, dan nutrisi (Guo *et al.*, 2010). Semua faktor tersebut telah diseragamkan pada hewan uji yang akan digunakan pada penelitian ini. Hasil pengukuran berat badan mencit tergolong stabil dengan menunjukkan peningkatan pada setiap minggunya. Pertambahan berat badan merupakan indikator bahwa mencit uji dalam kondisi sehat atau tidak stress, sehigga layak digunakan dalam penelitian (Conour *et al.*,2006;Capdevila *et al.*,2007.

Gambar 2.6. Kondisi ruang pemeliharaan hewan uji. A. Kotak kandang mencit, dan b. kondisi ruangan pemeliharaan mencit (*Mus musculus*).

2.4 Hasil Induksi Hiperglikemia Menggunakan Aloksan

Mencit uji yang telah diaklimatisasi selama 30 hari, kemudian pada hari ke 31 dilakukan pengukuran kadar glukosa darah awal sebelum induksi hiperglikemia. Hasil pengujian gula darah awal menunjukkan bahwa mencit memiliki rata-rata kadar gula darah yaitu 109,4 mg/dL. Kadar gula darah awal tersebut dikategorikan normal karena <200 mg/dL (Wang *et al.*,2010). Berdasarkan hal tersebut, dapat dikatakan bahwa semua mencit uji yang akan digunakan dalam penelitian ini dalam kondisi sehat dan homogen.

Salah satu metode induksi hiperglikemia adalah menggunakan aloksan. (Shajeela *et al.*, 2012; Chauhan *et al.*, 2008). Pengukuran kadar gula darah terhadap mencit uji yang telah dinduksi aloksan, dilakukan dua hari setelah induksi (H-33). Hasil pengukuran menunjukkan bahwa gula darah rata-rata mencit uji yang telah diinduksi aloksan adalah sebesar 338,5 mg/dL (nilai >200 mg/dL) sehingga dikategorikan hiperglikemia. Hal tersebut sesuai dengan Wang *et al.*,(2010).

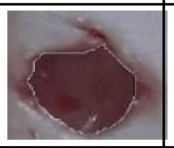
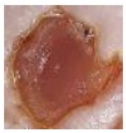
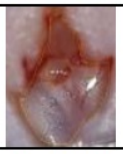
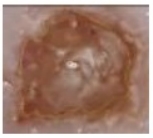
Kondisi hiperglikemia pada mencit uji yang diinduksi aloksan diakibatkan karena aloksan bersifat menghambat sekresi insulin (Tiedge *et al.*, 2000). Menurut Rohilla dan Ali (2012) menyatakan bahwa mekanisme penghambatan sekresi insulin oleh senyawa aloksan sebagai berikut : Aloksan bereaksi dengan dua gugus -SH pada enzim glukokinase di sel-β pankreas, sehingga menghasilkan pembentukan ikatan disulfida dan inaktivasi enzim. Inaktivasi glukokinase tersebut dapat menghambat sekresi insulin karena glukokinase memiliki peran penting dalam memulai serangkaian aktivitas selular untuk sekresi insulin (Lang & Hussain, 2014). Selain itu, aloksan dapat meningkatkan ROS dan radikal bebas sehingga bersifat sitotoksik terhadap sel-β pankreas yan diikuti hiperglikemia (Rohilla & Ali, 2012;Yadav *et al.*, 2002. dalam Shah & Khan, 2014). Pembentukan ROS dan radikal bebas oleh aloksan dikarenakan aloksan dapat membentuk asam dialurat yang dapat menghasilkan H2O2, dan radikal bebas (HO·, O2·-) (Favier, 1994).

Berdasarkan hal tersebut, mencit uji yang diinduksi aloksan dapat mengalami penghambatan sekresi insulin. Hal ini mengakibatkan gula dalam plasma tidak dapat ditranspor ke dalam sel tubuh karena insulin berfungsi menginduksi translokasi GLUT-4 ke membran sel tubuh. Jika GLUT-4 tidak ada yang bertranslokasi ke membran sel, menyebabkan gula dalam plasma tidak dapat ditransport ke dalam sel tubuh (Huang & Czch, 2007). Kondisi demikian menyebabkan mencit yang diinduksi aloksan mengalami peningkatan kadar gula dalam plasma darah (hiperglikemia) (Lenzen, 2008).

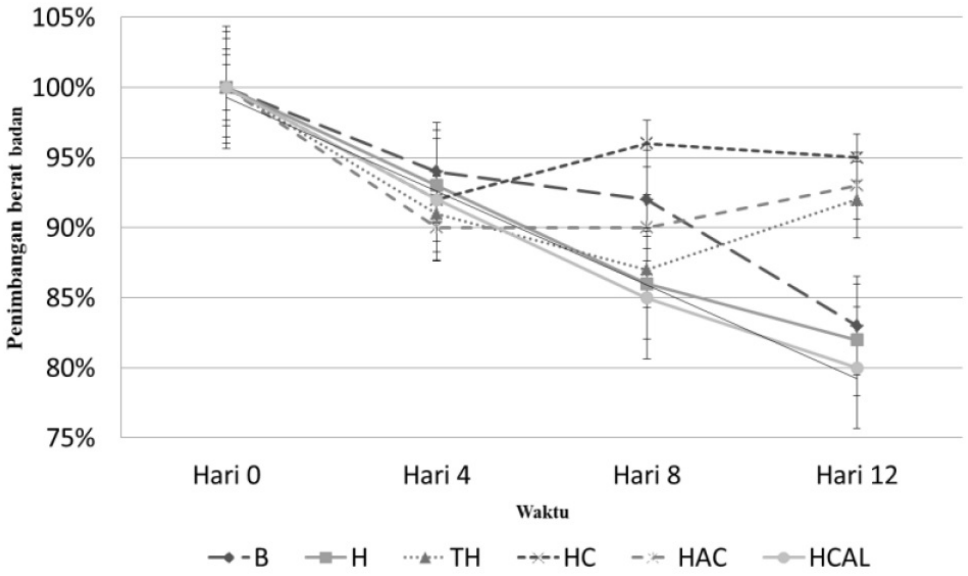
2.5. Luka Kronis pada Hewan Uji *Mus musculus*

Luas luka pada hewan uji yang dikehendaki adalah berdiameter sebesar 0,5 cm, sesuai dengan ukuran biopsy punch yang dipakai. Namun demikian, luas luka yang dibuat mempunyai variasi yang disebabkan oleh pengguntingan setelah penusukan dengan biopsy punch pada jaringan kulit. Rata-rata luasan luka kronis pada hari 0 (Tabel 2.1) adalah pada perlakuan hidrogel berbasis enzimatik CDH 0,359 cm2+ 0,033 cm2, pada perlakuan hidrogel asam galat 0,118 cm^2+ 0,023 cm2, pada perlakuan hidrogel kosong 0,292 cm^2 ± 0,055 cm^2, pada perlakuan tanpa hidrogel 0,340 cm^2 ± 0,007 cm^2, dan pada perlakuan betadine 0,292 cm^2 ± 0,036 cm^2. Semua luka menunjukkan rata-rata luasan luka yang tidak berbeda signifikan antar perlakuan ($p<0,05$).

Tabel 2.1 Luas Luka dalam Percobaan Mencit. Gambar dan luas luka (cm^2) hari ke-0 pada luka kronis gambar diambil dari satu ulangan secara acak pada setiap perlakuan, namun tidak mewakili bentuk luka pada masingmasing ulangan.

Ulangan	H	HC	HCA	HCAL	TH	B
						
1	0,292	0,291	0,118	0,359	0.340	0,292
2	0,213	0,261	0,151	0,312	0,330	0,240
Rata-rata	**0,252**	**0,276**	**0,134**	**0,335**	**0,335**	**0,266**
Standar deviasi	**0,055**	**0,021**	**0,023**	**0,033**	**0,007**	**0,036**

Keterangan = H : hidrogel kosong, HC : Hidrogel Enzim CDH, HCA : Hidrogel Asam Galat, HCAL : Hidrogel CDH+asam galat+laktosa,TH; Tanpa Hidrogel, dan B : Betadine (data disajikan dalam cm2)

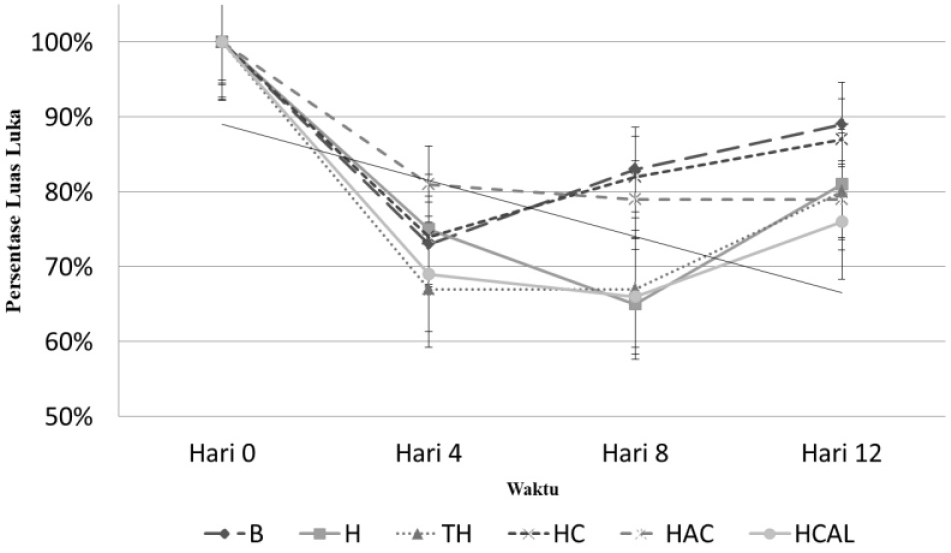


Gambar 2.7. Grafik pengukuran berat badan pada masing-masing perlakuan Luka kronis. H : hidrogel kosong, HC : Hidrogel Enzim CDH, HCA : Hidrogel Asam Galat, HCAL : Hidrogel CDH+asam galat+laktosa,TH; Tanpa Hidrogel, dan B : Betadine. Data disajikan dalam mean = SEM dengan nilai tidak ada signifikansi antar perlakuan.

Kondisi pemerbanan mencit membuat pergerakan mencit mengalami kesulitan, termasuk dalam perilaku makan dan minum. Pengaruh-pengaruh tersebut dapat dilihat jelas dari pengukuran berat badan (Gambar 2.7). Penurunan berat badan terjadi pada hari ke-4 perlakuan. Hal ini disebabkan oleh proses pembiasaan hewan uji dengan perban yang dipasangkan. Terganggunya proses makan dan minum hewan uji membuat penuruan drastis pada seluruh hewan uji pada semua perlakuan. Kondisi berat badan yang stabil pada hari ke-8 dan ke-12 pada semua perlakuan dengan tidak ada perbedaan yang signifikan antar perlakuan ($p < 0,05$).

2.6. Penutupan Luka pada Luka Kronis

Luka kronis adalah luka diabetes yang diberikan induksi aloksan sebelum dilakukan pelukaan pada mencit uji. luka dapat terinfeksi dari dari lingkungan sekitar luka meliputi kulit, bulu, dan udara luar maupun sumber endogen dalam tubuh (Kingsley, 2003). Luka ditunjukkan Lampiran 9. Luka kronis pada hari ke-4 tanpa hidrogel memiliki persentase luasan yang berbeda signifikan ($p < 0,01$) terhadap luka dengan hidrogel kosong dengan persentase luasan 75% ± 4% (Gambar 2.8). 1.uka tanpa hidrogel juga berbeda signifikan ($p < 0,05$) terhadap luka dengan betadine dengan persentase luasan 73% ± 3%. Luka dengan hidrogel CDH berbeda signifikan ($p < 0,01$) dengan luka dengan perlakuan hidrogel asam galat (81% ± 5%) maupun luka dengan perlakuan HCAL memiliki perbedaan signifikan ($p < 0,01$) dengan persentase luasan 69% ± 2%.



Gambar 2.8. Persentase Luasan Luka Kronis pada Hewan Uji. Diamati pada hari ke-0, 4, 8, dan 12. H : hidrogel kosong, HC : Hidrogel Enzim CDH, HCA : Hidrogel Asam Galat, HCAL : Hidrogel CDH+asam galat+laktosa,TH; Tanpa Hidrogel, dan B :Betadine. Data disajikan dalam mean SEM dengan nilai signifikansi $p < 0,001$ = ***, $p < 0,01$, $p < 0,05$ = *

Pada hari ke-8, luka hidrogel kosong mengalami penurunan persentase luasan luka menjadi 65% ± 23% tidak berbeda signifikan dengan perlakuan hidrogel HCAL (66% ± 2%) maupun luka dengan perlakuan betadine (83% ± 4%). Namun berbeda signifikan ($p<0,01$) terhadap luka dengan perlakuan Hidrogel CDH dengan persentase luasan 82% ± 4%.

Pada hari ke-12, persentase luasan luka pada masingmasing perlakuan tidak memiliki perbedaan yang signifikan. Hidrogel CDH memiliki persentase luasan luka sebesar 87% ± 2%, hidrogel asam galat mempunyai persentese luasan luka sebesar 79% ± 6%, hidrogel kosong mempunyai persentase luasan luka sebesar 81% ± 9%, tanpa hidrogel mempunyai persentase luasan luka sebesar 80% ± 3%, dan betadine mempunyai persentase luasan luka sebesar 89% ± 0%.

Luka memasuki fase inflamasi dan terjadi proliferasi dan migrasi fibroblast pada hari ke-4 (Clark, 1993). Peran hidrogel berbasis enzimatik CDH sangat penting pada fase inflamasi. Pada fase tersebut, sel-sel imun akan direkrut dengan pensinyalan H_2O_2 yang dilepaskan oleh neutrofil (Falanga *et al.*, 2005). H_2O_2 yang persentase luasan luka sebesar 87% ± 2% dihasilkan oleh hidrogel berbasis enzimatis CDH pada luka memberikan banyak pengaruh dalam penutupan luas luka pada luka. Sedangkan kandungan ROS yang rendah pada luka (Schreml *et al.*, 2010) tidak memberikan dampak pada pengaisan ROS oleh hidrogel asam galat. Hidrogel kosong mengalami penambahan luas luka yang dimungkinkan karena kondisi luka yang masih dalam fase inflamasi (Pramono *et al.*, 2016). Hidrogel penutup luka dapat menahan air untuk menjaga kondisi luka tetap lembab atau basah (Kamoun *et al.*, 2017). Pada luka kronis tanpa hidrogel memiliki lingkungan luka yang berbeda dengan hidrogel penutup luka. Perlakuan tanpa hidrogel tidak memiliki penahan untuk menjaga kelembaban pada luka yang menjadikan luka menjadi kering. Luka yang kering dapat menyebabkan terbentuknya scab atau kerak luka yang merupakan penutup luka yang alami. Scab merupakan kerak yang dibentuk oleh serum yang kening dengan eritrosit yang terperangkap di dalamnya. Scab dapat memberikan fungsi seperti pada penutup luka yang meliputi perlindungan terhadap benda asing, mengurangi rasa sakit, menahan tepian luka, memfasilitasi kontraksi luka, dan meminimalisasi kehilangan cairan dan protein (Lionelli *et al.*, 2003), Luka kronis betadine mengalami penutupan luka yang cepat dan berbeda signifikan dibandingkan dengan kelima perlakuan hidrogel penutup luka. Hal tersebut disebabkan oleh kandungan povidon iodine dan kontraksi luka yang difasilitasi oleh scab yang dapat mempercepat penutupan luka (Swim *et al.*, 2001). Meskipun scab memiliki keunggulan dalam penutupan luka, namun hal tersebut bukanlah merupakan suatu hal yang ideal. Scab dapat memperlambat epitelialisasi dan dapat menahan bakteri

pada permukaan luka (Lionelli *et al.*,2003). Selain itu pembentukan scab dapat menyebabkan terjadinya bekas luka (Fonder *et al.*, 2007).

Pada hari ke 12, perlakuan dengan hidrogel penutup luka memberikan perbedaan yang signifikan satu sama lain. Hidrogel penutup luka berbasis enzimatik CDH tidak disebutkan dapat membantu dalam proses proliferasi Nyanhongo *et al.*, 2013a). Pada fase proliferatif ini luka mengeluarkan eksudat yang dapat masuk pada sistem regenerasi antioksidan hidrogel berbasis enzimatik CDH maupun pengaisan ROS oleh hidrogel asam galat. Namun support hidrogel karaginan akan mampu memberikan kompatibilitas jaringan dan mampu memberikan kondisi luka yang lembab dan menghindarkan dari iritasi yang menjamin sel-sel untuk berproliferasi dengan baik (Roy *et al.*, 2010). Sedangkan perlakuan betadine mempunyai luasan luka yang lebih kecil dan berbeda signifikan dengan perlakuan-perlakuan dengan hidrogel penutup luka. Secara Penutupan yang cepat ini disebabkan karena pembentukan keropeng yang mempercepat proses penyembuhan luka.

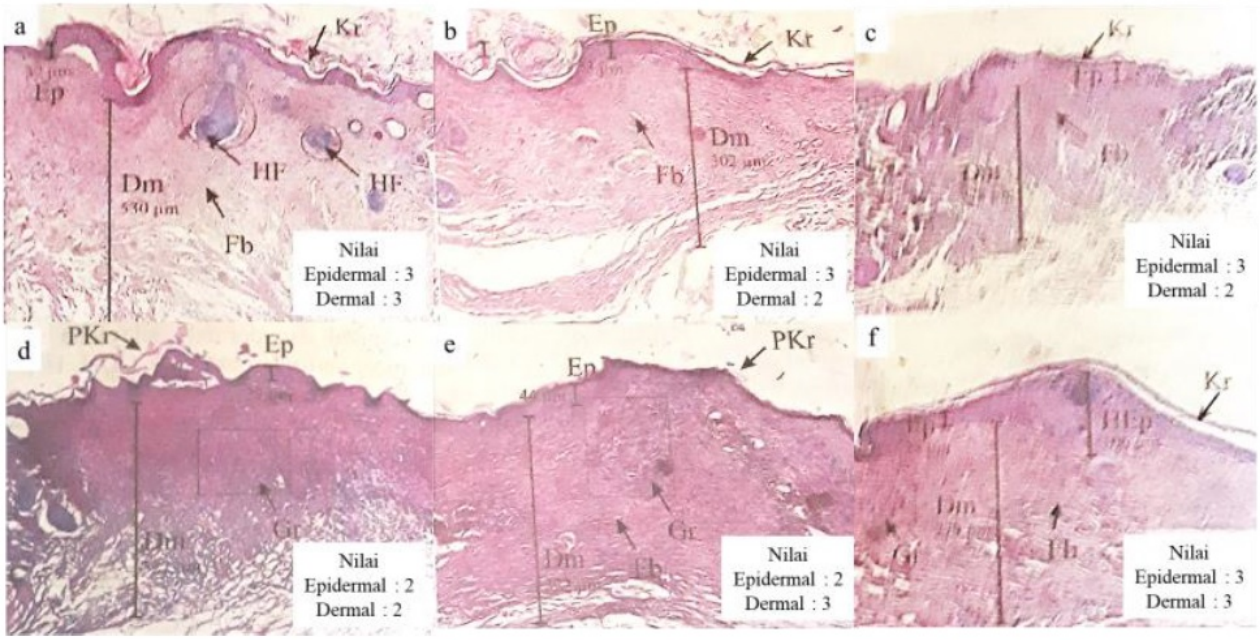
2.7. Hasil Analisis Antioksidan DPPH

Darah yang telah diambil kemudian dimasukan kedalam *microtube* yang berisi larutan EDTA kemudian dilakukan sentrifugasi dengan kecepatan 3000 rpm selama 15 menit untuk memisahkan antara lapisan plasma dan lapisan sel darah untuk sampel pengujian antioksidan dilakukan berdasarkan teknik yang dijelaskan oleh Genovese dan Lannes (2009), dimana sampel dengan berbagai konsentrasi sebanyak 0,5 mL ditambahkan 3,5 mL larutan peraksi DPPH (2,2-diphenyl-1-picrylhydrazyl), dan dihomogenkan menggunakan vortex. Selanjutnya larutan diinkubasi selama 30 menit pada tempat gelap. Absorbansi diukur menggunakan spektrofotometer UV-VIS pada panjang gelombang 517 nm. Sebanyak 300pl methanol murni ditambah 900pl DPPH digunakan sebagai blanko. Asam galat digunakan sebagai baku pembanding. Persentase absorbansi DPPH didapatkan 111,10%. Dari 112 absorbansi DPPH dan 100 absorbansi sampel.

2.8. Penilaian Epidermal dan Dermal Luka Kronis

Pengamatan histologis jaringan epidermis dari luka kronis pada hari ke-12 menunjukkan bahwa seluruh perlakuan yaitu hidrogel berbasis enzimatik CDH, hidrogel asam galat, hidrogel kosong, dan tanpa hidrogel, dan betadine tidak menunjukkan adanya perbedaan antar perlakuan. Jaringan epidermis telah mengalami migrasi lengkap dengan keratinisasi yang lengkap dengan penilaian epidermal 3,00 (Gambar 4.9) (Yates *et al.*, 2007). Perlakuan dengan hidrogel dapat mempercepat proses epitelisasi yang ditujukkan pada Gambar 4.9 a,b,c, dan d. Perlakuan hidrogel penutup luka dapat memberikan kompatibilitas yang baik untuk proliferasi jaringan epidermis (Roy *et al.*,2010).

Sedangkan perlakuan tanpa hidrogel dan betadine menyebabkan adanya hiperplasia pada jaringan epidermis yang merupakan tanda terbentuknya bekas luka yang ditunjukkan pada Gambar 2.9 e dan f (Galili, 2017).



Dm : dermis, **Ep**: epidermis, **Fb**: fibroblast, **Gr**:jaringan granulasi, **HEp:** hiperplasia epidermis, **Hf**: folikel rambut, **Kr**: keratin, **PKr**: keratinisasi parsial

Gambar 2.9. Gambaran jaringan histologis luka.pada hari ke-12., a. Hidrogel Enzim CDH, b. Hidrogel Asam Galat, c. hidrogel kosong, d. Hidrogel CDH+asam galat+laktosa, e. Tanpa Hidrogel, dan f. Betadine. Jaringan diwarnai dengan pewarnaan Hematoxilin & Eosin dan diamati dengan mikroskop perbesaran 100x.

Pengamatan jaringan dermis luka pada hari ke-12 menunjukkan bahwa pada hidrogel CDH memiliki penilaian dermal 2,75±0,25, hidrogel asam galat dan hidrogel kosong memiliki penilaian epidermal 2,67±0,33, Hidrogel CDH+asam galat+laktosa memiliki penilaian epidermal 2,00, tanpa hidrogel memiliki penilaian 3,00 dan betadine memiliki penilaian 2,25±0,25. Penilaian dermal pada hidrogel berbasis enzimatik CDH menunjukkan nilai yang lebih rendah namun terlihat pendewasaan pada jaringan dermis luka dengan adanya folikel rambut (Gambar 2.9 a), Tumbuhnya folikel rambut dapat mereduksi pembentukan bekas luka (Jahoda *et al.*, 2001), Pada hidrogel asam galat tidak ditemukan pertumbuhan folikel rambut (Gambar 2.9 b). Penilaian yang lebih rendah ada pada penggunaan hidrogel kosong yang tidak menunjukkan adanya deposisi kolagen pada jaringan granulasi (Gambar 2.9 c). Pada perlakuan tanpa hidrogel memiliki penilaian penyembuhan dermal

yang paling tinggi dibanding semua perlakuan dengan adanya deposisi kolagen namun masih menujukkan adanya kumpulan dari jaringan granulasi (Gambar 2.9 d). Jaringan granulasi yang lebih lama ada pada jaringan menunjukkan pelambatan dalam penyembuhan dermal (Mann *et al.*, 2006).

BAB III STATUS LUARAN

BAB IV KENDALA PELAKSANAAN PENELITIAN

Kendala-kendala di penelitian ini adalah:

1. Peralatan yang harus bergantian dengan peneliti lain di lab yang sama, menyebabkan tertundanya analisis data.
2. Mencit yang dipelihara memerlukan tempat atau kendang yang tenang, di lokasi penelitian masih tidak sesuai
3. Peralatan analasis histologi yang masih sangat terbatas, sehingga sampel jaringan kulit perlu dikirim ke Fakultas Kedokteran Hewan Unair.

BAB V RENCANA TAHAPAN SELANJUTNYA

1. Penelitian ini perlu dilakukan dengan hewan uji yang lebih besar untuk kondisi uji yang lebih representatif. Penggunaan hewan uji mencit sangat sulit dilakukan karena ukurannya yang kecil dan kesulitan dalam proses pemerbanan mengakibatkan dampak negative bagi pergerakan sehingga pengaruh berat badan.

2. Pada aplikasi di bidang kesehatan seharusnya menggunakan enzim hasil dipurifikasi sehingga diharapkan tidak ada pengotor yang dapat mempengaruhi sistem regenerasi antioksidan dalam penyembuhan luka.

3. Perlu adanya pengujian kadar H2O2 yang mampu dihasilkan oleh sistem regeneraasi antioksidan dalam hidrogel..

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BAB VII LAMPIRAN

LAMPIRAN 1 Tabel Daftar Luaran

Program : World Class Research
Nama Ketua Tim : Dr. techn. Endry Nugroho Prasetyo, S.Si.,MT.
Judul : Pengembangan Sistem Regenerasi Antioksidan Berbasis Enzimatik sebagai Agen Penyembuh Luka Kronis

1.Artikel Jurnal

No	Judul Artikel	Nama Jurnal	Status Kemajuan*)
1.	**In vivo analysis of cellobiose dehydrogenase based wound dressing hydrogel for acute wound healing**	**Biotechnology Journal (BTJ)**	**Persiapan**

*) Status kemajuan: Persiapan, *submitted*, *under review*, *accepted*, *published*

2. Artikel Konferensi

No	Judul Artikel	Nama Konferensi (Nama Penyelenggara, Tempat, Tanggal)	Status Kemajuan*)
1.	**Effect of polymer material on antimicrobial activity of wound dressing hydrogel**	**5th International Biology Conference (IBOC) 2020, 17 Oktober 2020**	**Persiapan**

*) Status kemajuan: Persiapan, *submitted*, *under review*, *accepted*, *presented*

3. Paten

No	Judul Usulan Paten	Status Kemajuan

*) Status kemajuan: Persiapan, *submitted*, *under review*

4. Buku

No	Judul Buku	(Rencana) Penerbit	Status Kemajuan*)

*) Status kemajuan: Persiapan, *under review*, *published*

5. Hasil Lain

No	Nama Output	Detail Output	Status Kemajuan*)

*) Status kemajuan: cantumkan status kemajuan sesuai kondisi saat ini

6. Disertasi/Tesis/Tugas Akhir/PKM yang dihasilkan

No	Nama Mahasiswa	NRP	Judul	Status*)

*) Status kemajuan: cantumkan lulus dan tahun kelulusan atau *in progress*

LAMPIRAN 2 DRAFT PAPER

In vivo analysis of cellobiose dehydrogenase based wound dressing hydrogel for acute wound healing

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Abstract. Wounds cause interference on the anatomical functions and structures of body. Acute wounds heal faster than chronic wounds. Acute wounds are infected by the *Staphylococcus aureus* bacteria could become a chronic status due to the mechanism of bacterial infection. An enzymatic based wound dressing hydrogel of cellobiose dehydrogenase (CDH) has been proposed as an antioxidant regeneration system that counteracts reactive oxygen species in the wounds and generates hydrogen peroxide against microbial infections. The CDH enzyme in this study was produced by *Trametes versicolor* mold. Meanwhile, the enzymatic based wound dressing hydrogels were supported by κ-carrageenan matrices by preparing into 3 layers containing CDH, lactose and gallic acid respectively. The application of enzyme based wound dressing hydrogels of CDH in the acute non-infectious wounds and acute wounds infected with *S. aureus* did not have a significant effect on the wound closure compared to control of gallic acid hydrogels and plain hydrogels. However, the negative control had significantly faster wound closure. Assessment of epidermal and dermal healing on day 12 of each treatment showed no

significant difference, but enzymatic-based wound dressing hydrogel CDH had a better tendency of dermal and epidermal healing than the control treatments.

Keywords: acute wound, cellobiose dehydrogenase, *Staphylococcus aureus*, wound dressing hydrogel.

1. Introduction

Wound causes disruption in the function and anatomical structure of the body[1]. Based on the duration of healing process, the wound can be classified into acute and chronic wound. Acute wound is a tissue injury that can be recovered within 8-12 weeks period [2]. While chronic wounds are slower recovery process up to 12 weeks and sometimes can cause disability [3]. Physiological processes of wound healing pass through four overlapping stages includes hemostasis, inflammation, proliferation, and remodeling [4]. In the inflammatory phase, phagocytic cells such as neutrophils and macrophages work predominantly. Neutrophils release ROS and proteases which prevent bacterial contamination and clean the wound from cellular debris. Blood monocytes arrive at the wound site and differentiate into tissue macrophages. Macrophages not only eliminate bacteria and non-viable tissue by phagocytosis but also release various growth factors and cytokines that recruit fibroblasts, endothelial cells, and keratinocytes to repair damaged blood vessels [5].

Wound infected by *Staphylococcus aureus* could become chronic if the infection stay too long until it forms biofilms [6,7]. Toxin α can be directly lyse the host cells, including macrophages in the perivascular space (spaces between cells and blood vessels) of the skin, which leads to failure or delay in the recruitment of polymorphonuclear cells [8]. The failure of polymorphonuclear cells towards the perivascular space causes inhibition of the production of hydrogen peroxide to counteract infections that enter the wound [9]. While biofilms will prevent bacteria from attacking immune cells or from antibiotic exposure [7].

Failure in the inflammatory phase will cause a wound in a prolonged inflammatory phase that forms chronicity of the wound. Chronic wounds also are able to form reactive oxygen species (ROS) which lyse extracellular matrix proteins thereby cell damage [10]. ROS is a toxic oxygen biradical molecule especially when converted to superoxide radicals ($\cdot O_2^-$) that considered to be the main ROS in biological systems. These free radicals are a byproduct of normal aerobic metabolism, but their generation is relatively increased during infection [11].

Chronic wound healing can be successful if the physiological balance become closer to acute wound which has a normal healing phase condition. The antioxidant regeneration system has been carried out by incorporating continuous scavenging/quenching system of ROS and the continuous income of H_2O_2 from enzymatic cellobiose dehydrogenase (CDH) oxidation [12]. Plant phenolic antioxidant compounds can be regenerated again after scavenging ROS from the wound and can be returned to their original form continuously with the help of lactose as a donor electron [13]. While continuous income H_2O_2 can function as antibacterial agent through Fenton reactions and biofilm formation by bacteria [14]. It needs to be proven whether this antioxidant regeneration system is effectively used *in vivo*. The usage of *Mus musculus* test animals which have been acutely injured and infected with *S. aureus* is an important step to review the effectiveness of the use of this antioxidant regeneration system.

2. Material and methods

Wound dressing hydrogel preparation

All of concentration must considered to total volume (Table 1 (3)). Unautoclavable composition was separately prepared, including enzyme and gallic acid. CDH enzyme was produced by *Trametes versicolor* [14] . Gallic acid stock solution was sterilized by filtering using a 0.2 µm sterile filter. $CaCl_2$ stock solution [15] and hydrogels compositions [12] that prepared before autoclaved (Table 1 (4) and (5)) were prepared separately and sterilized at autoclave 121 °C 15 minutes. The powder poured aseptically to the flasks and mixed at hot plate. The mixture kept warm

at 45 °C at waterbath. The mixture added by solution that added after autoclaved (Table 1 (6)) and mixed completely. Enzymatic-based CDH Hydrogel (ECH), immediately poured in an ordinary sequence of layer on Petri dish (d: 15 cm). Gallic acid hydrogel (GAH) and plain hydrogel (PH) also poured immediately on the dish. The hydrogel kept at room temperature until solidified then cut into round shape (d: 1 cm). The cut hydrogel kept at 2-8 °C before used.

Table 1. Wound dressing hydrogels compositions. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. The concentration must considered to total volume of hydrogel.

Wound Dressing Hydrogel	Layer	Volume	Prepared before autoclaved		Added after autoclaved
			Flasks	Powder	
(1)	(2)	(3)	(4)	(5)	(6)
ECH	1	30 ml	dH_2O	2% κ-carrageenan	2.4 U/ml CDH + 0.4 mM $CaCl_2$
	2	20 ml	400 µM lactose	2% κ-carrageenan	0.4 mM $CaCl_2$
	3	30 ml	dH_2O	2% κ-carrageenan	100 µM gallic acid + 2.4 U/ml CDH + 0.4 mM $CaCl_2$
GAH	-	30 ml	dH_2O	2% κ-carrageenan	100 µM gallic acid + 0.4 mM $CaCl_2$
PH	-	30 ml	dH_2O	2% κ-carrageenan	0.4 mM $CaCl_2$

Bacterial preparation

A twenty-four hours-old *Staphylococcus aureus* ATCC 6538 at nutrient agar (NA) slant were diluted using 0.9% NaCl physiological saline. The last three of the dilution were plated at NA medium and incubated at 30-35 °C. The suspension kept at 2-8 °C until used as infecting agent of the wound. Growing colonies were counted using colony counter after 2 days incubation.

Animal sources and husbandry

Fifteen experimentally 8-week old male outbred Balb/c test mice (*Mus musculus*) were purchased from Indonesian Center of Veterinary Pharmacology (PUSVETMA) and were housed

one per cage in hanging clear Plexiglas cages with free access to food and water and maintained on a 12-hr light/dark cycle. All animal procedures were approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia and conducted in accordance with the National Guide for the Care and Use of Laboratory Animals. (No. 3.KE.111.07.2018).

Wound healing experiment

The experimental mice were anesthetized with Ketamine 44 mg per kg body weight of 2.5%. The dorsal part of the mice was wiped with cotton containing 70% ethanol prior surgery. Each animal was given one wound and by stamped depilating with a 6 mm skin biopsy punch and the delineated area was surgically removed with a Metzenbaum scissors to create a 6 mm full-thickness wound that extended from the epidermis to the outer fascia of the muscle below the subcutaneous layer of the dorsal skin.. The wounds were left open to heal and animals were visually monitored for infection or lethargy.

Hydrogel application

2.1.1. Wound pictures were taken with a ruler next to the wound for size comparison. Treatment of acute-infected wound with *S. aureus* (SA) were performed by adding 10 µl of *S. aureus* suspension (10^6) on the wound immediately. Whereas, treatment of acute non-infected wound (NI) did not used *S. aureus* as topical treatment. Hydrogels were applied immediately at the wound surface then covered with a bandage consisting of Hypafix plaster, sterile cotton, and Leukoplast plaster. The treatment types marked on the bandage and placed on the heating pad until fully recovered before returned to the cage. The bandage was opened at day 4 and 8 then wound pictures were taken with a ruler next to the wound for size comparison. New hydrogels were applied and bandaged again on every treatment. . Mice were sacrificed at day 12 with an overdose of chloroform. The bandage was opened at the 12th day then wound pictures were taken with a ruler next to the

wound for size comparison. The wound tissue was excised with a biopsy punch on the wound with a diameter of 10 mm to adipose tissue and put in 10% formalin solution in a sampling bottle. The excised tissue was tested by making histological slide with Haematoxylin & Eosin staining.

Wound progression

The pictures of the wound that have been taken on each day measured using ImageJ software [16] to obtain wound area. Percentage of wound area is calculated based on the size of the initial and final area of the wound. Where A_0 is the initial area of the wound and A_t is the area of the wound on the day of observation [17].

$$\text{Wound area percentage (\%)} = \left(1 - \frac{A_0 - A_t}{A_0}\right) \times 100\%$$

Histological analysis

Histological slides were observed by compound microscope. The epidermal healing value and dermal healing value determined at 0-4 scale according to Yates et al [17].

Data analysis

Each treatment was performed 4 repetitions. All values are expressed as mean ± SEM. Parametric data, which are wound area percentage, were performed ANOVA analysis with Tukey's post-hoc ($p < 0.05$). For nonparametric data, which are EV and DV, were performed Kruskal-Wallis test with Dunn's posttest [18].

3. Results and Discussion

3.1. Wound Closure at Non-infected Acute Wound

Non-infected acute (NI) wound haven't added by bacteria to the wound surface. However, the acute wound could be infected from the surrounding environment including skin, hair, air, dust, as well as endogenous sources in the body [19]. Negative control (NC) of NI wounds on the day 4 had an area percentage of 38% ± 8% which was significantly different ($p < 0.01$) to PH of NI wounds

with an area percentage of 116% ± 17% (Figure 1). NC of NI wounds were also significantly different ($p < 0.05$) to ECH of NI wounds with an area percentage of 83% ± 4%. ECH of NI wounds were not significantly different to GAH of NI wounds (68% ± 10%) and PH of NI wounds (116% ± 17%).

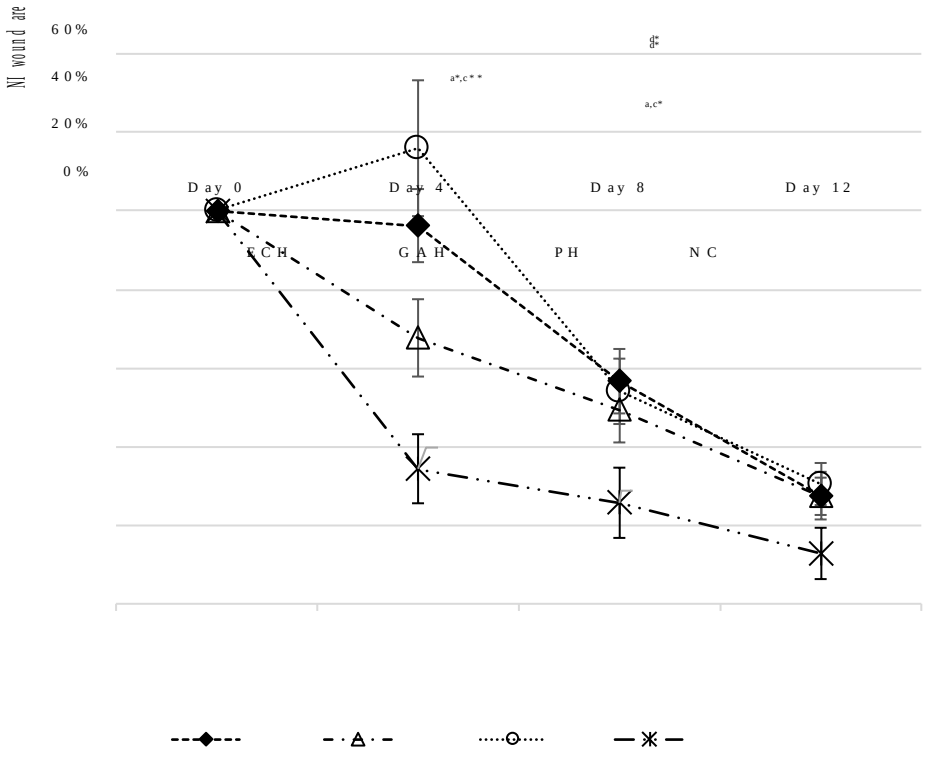

Figure 1. Non-infected acute (NI) wound area percentage of tested animals observed on days 0, 4, 8 and 12. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. Data served as mean ± SEM with significance values $p<0.001$ = ***, $p<0.01$ = ** and $p<0.05$ = *; the letter symbol shows the comparison of significance to: c = PH, and d = NC.

On the day 8, NC of NI wound has performed reduction of the wound area percentage to 25% ± 3% ($p < 0.05$) that significantly different PH of NI wound (54% ± 8%) and ECH of NI wound (46% ± 5%), but not significantly different to GAH of NI wound (49% ± 8%).

On the day 12, all treatment of NI wound didn't have a significant difference of wound area percentage. ECH of NI wound has wound area percentage of 28% ± 6%, GAH of NI wound

has wound area percentage of 27% ± 5%, PH of NI wound has wound area percentage of 32% ± 4%, and NC of NI wound has wound area percentage of 14% ± 5%.

Wounds enter the inflammatory phase, proliferation, and migration of fibroblasts on day 4 [20]. The role of ECH is very important in the inflammatory phase. In this phase, immune cells will be recruited by hydrogen peroxide signaling which released by neutrophils [5]. Hydrogen peroxide produced by ECH of NI wounds does not have much influence on the wound area closure. While the low ROS content of NI wounds [10] does not have an impact on ROS scavenging by GAH. PH shows an increase of the wound area that was possible because the wound condition was still at the inflammatory phase .

NC of NI wounds has a different wound environment to NI wounds with wound dressing hydrogels treatment. Wound dressing hydrogel could hold water to keep the moist of the wound [20]. NC does not have a barrier to maintain moisture in the wound which makes the wound dry. Dry wound could perform the formation of scab which was a natural wound dressing. Scab is a crust formed by dry serum with erythrocytes trapped in it. Scab can provide functions such as the wound dressing which includes protection against foreign objects, reduce pain, hold the wound edges, facilitate wound contraction, and minimize fluid and protein loss [21]. NC of NI wounds experienced rapid wound closure and were significantly different compared to the three wound dressing hydrogel treatments. This was caused by wound contractions facilitated by scab which accelerated wound closure [22]. Although scab has an advantage in wound dressing, it is not an ideal deal. Scabs could slow epithelialization and hold bacteria on the wound surface[23]. In addition, scab formation can cause scars [24].

On days 8 and 12, wound dressing hydrogels treatment have not made a significant difference from each other. In this proliferative phase, the wound did not released exudates that could enter the ECH antioxidant regeneration system or ROS scavenging by GAH. However, the support of carrageenan hydrogel would be able to provide tissue compatibility, provide moist

condition, and avoids irritation that guarantees cells to properly proliferate [25]. Whereas, the NC treatment has a smaller wound area and is significantly different from wound dressing hydrogels treatments. The rapid closure caused by the formation of scabs which accelerated the wound healing process.

3.1.1. Epidermal and Dermal Healing of Non-infected (NI) Wound

Histological observation at epidermal tissue of NI wound on the day 12 showed that all treatments did not show any differences. The epidermal tissue has undergone complete migration with complete keratinization with an epidermal healing value of 3.00 (Figures 2 and 3). Wound dressing hydrogel treatment accelerated the epithelialization process shown at Figure 2 ECH, GAH, and PH. Wound dressing hydrogel provided good compatibility to the proliferation of epidermal tissue . Whereas NC caused hyperplasia on epidermal tissue which is a sign of scar formation shown at Figure 2 NC [26].

Dermal tissue observation of NI wound on the day 12 showed that ECH had a dermal healing value of 2.75 ± 0.25, GAH and PH had a dermal healing value of 2.67 ± 0.33, and NC had a dermal healing value of 2.25 ± 0.25. Dermal healing value on all treatments of NI wounds did not have a significant difference in the Kruskal Wallis non-parametric statistical test, but ECH tended to have better dermal healing than other treatments in the presence of hair follicle growth (Figure 2 ECH). Hair follicle growth is a sign of maturing wound tissue that would improve wound healing and reduce scar formation [27]. NC had the lowest dermal assessment compared to the treatment with wound dressing hydrogel. This shows that although the wound closure of NC treatment was good, it is not better than the wound dressing hydrogel treatments in dermal healing. Scab formation of NC caused increased of granulation tissue and delayed remodeling [28].

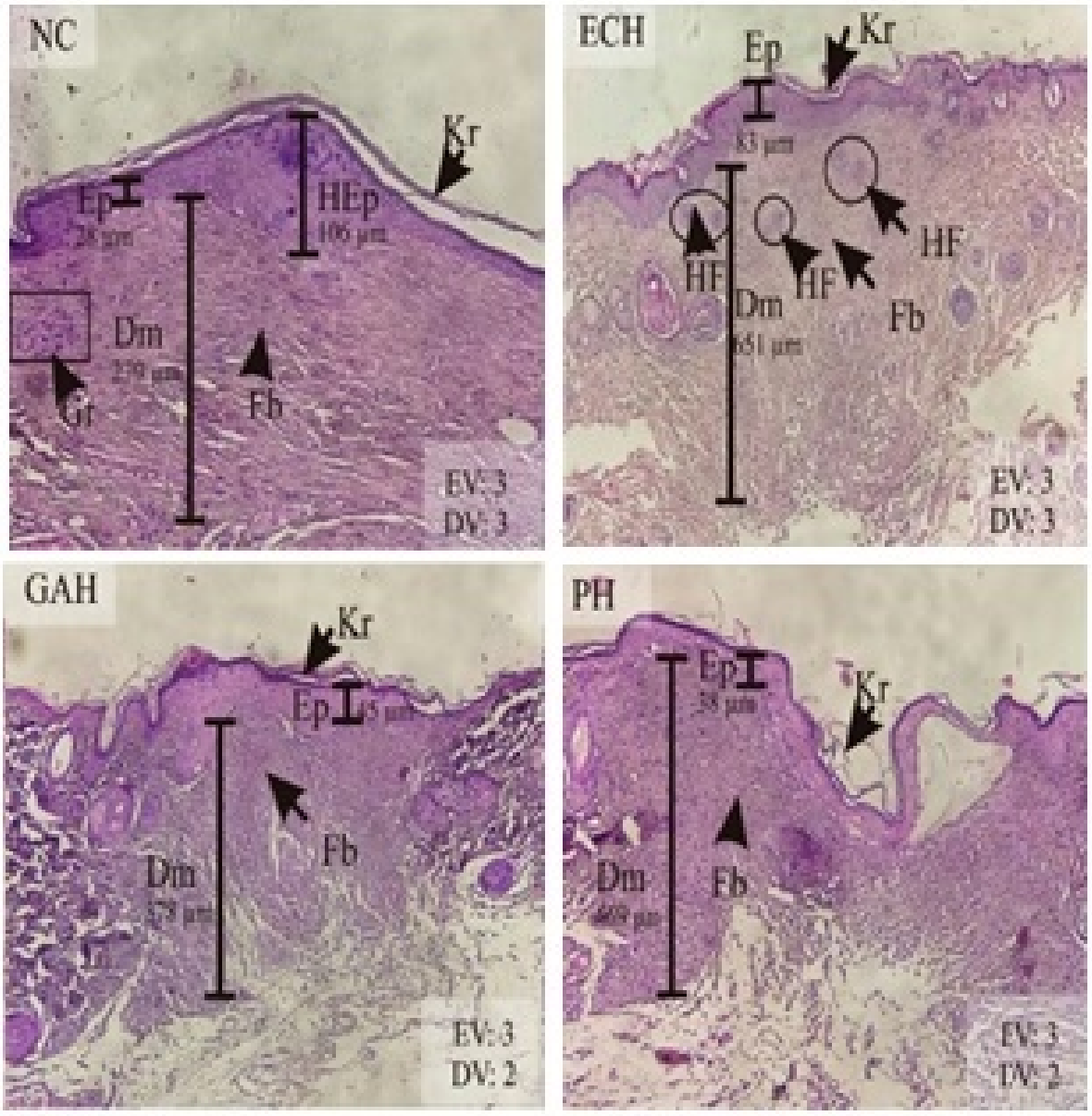

Figure 2. Histological tissue observation of non-infected acute (NI) wound on the day 12. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. Dm: dermis, Ep: epidermis, Fb: fibroblast, Gr: granulation tissue, HEp: hyperplasia of epidermis, HF: hair follicle, Kr: keratin. The tissue was stained with Haematoxylin & Eosin staining and observed with a 100x magnification microscope.

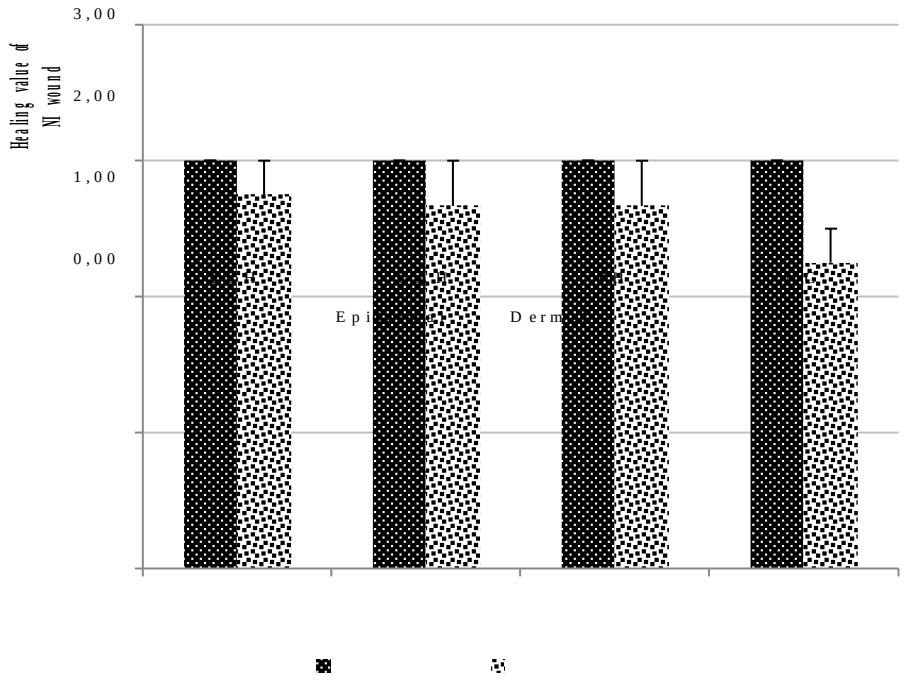

Figure 3. Epidermal and dermal healing value of acute non-infected acute (NI) wound on the 12th day. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. Data are presented in mean ± SEM with data not showing significance between treatments with the Kruskal Wallis non-parametric statistical test.

3.2. Wound Closure at *Staphylococcus aureus* Infected Acute Wound

The number of *S. aureus* topically used in SA wound was 1.6×10^4 cfu/ml. The wound area percentage of *Staphylococcus aureus* infected acute (SA) wound on the 4th day in all treatment did not have a significant difference ($p<0.05$) (Figure 4). ECH of SA wound has a wound area percentage of 68% ± 13%, GAH of SA wound has a wound area percentage of 97% ± 12%, PH of SA wound has a wound area percentage of 42% ± 8%, and NC of SA wound has a wound area percentage of 60% ± 10%.

The wound area percentage of NC of SA wound on day 8 (9% ± 1%) has a significant difference ($p<0.01$) to GAH of SA wound which has a wound area percentage of 62% ± 8%, and

significant difference ($p<0.05$) to ECH of SA wound which has a wound area percentage of 55% ± 11% and PH which has a wound area percentage of 42% ± 8%.

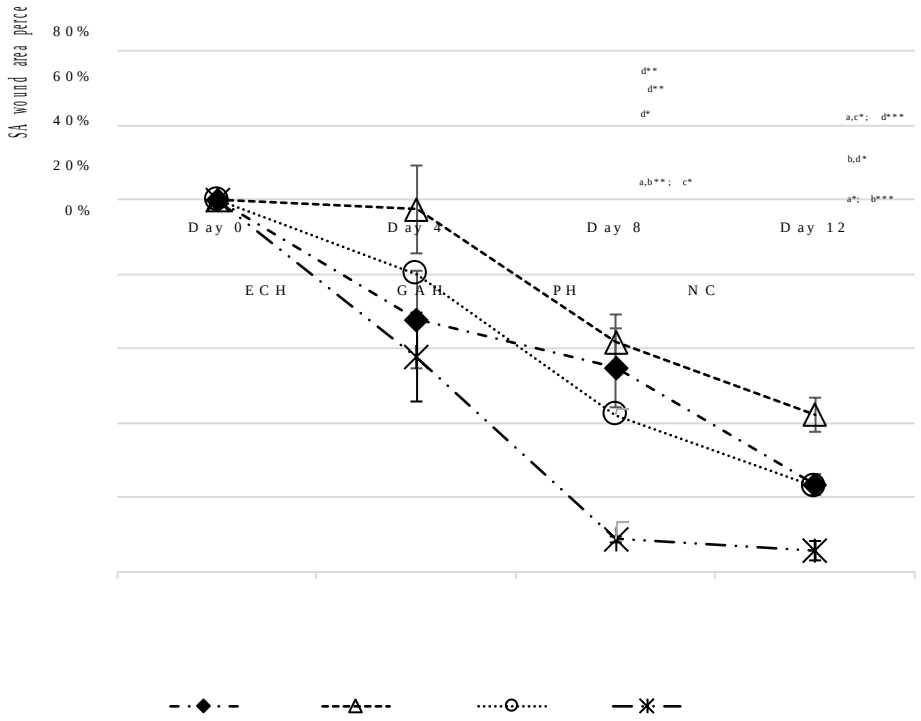

Figure 4 Percentage of *Staphylococcus aureus* infected acute (SA) Wound in test animals observed on days 0, 4, 8 and 12. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. Data are presented in mean ± SEM with significance values $p<0.001$ = ***, $p<0.01$ = ** and $p<0.05$ = *; the letter symbol shows the comparison of significance to: a = ECH, b = GAH, c = PH, and d = NC.

On day 12, NC of SA wound had a significant difference of wound area percentage of 6% ± 3% ($p<0.05$) with ECH of SA wound which had a wound area percentage of 23% ± 3% and significant difference ($p<0.01$) with GAH of SA wound which has a wound area percentage of 42% ± 5%. Whereas PH of SA wound had a wound area percentage of 23% ± 6% were not significant different to NC of SA wound. ECH and PH of SA wound were significantly different from GAH of SA wound.

S. aureus identified as one of the bacteria that composes the aggregate of chronic wounds [29]. Wound caused by *S. aureus* could become chronic if the infection left too long to forms

biofilms [6,7] and excretes virulence factors in the form of α toxin (haemolysin α) – pore-forming toxins to lyse the host cells [30]. Both of the factor would trigger the wound to become more chronic [31]. Wounds infected with bacteria could increases ROS levels due to the inflammation condition of the tissue [10]. ECH could scavenge ROS from SA wound so that it does not worsen the wound condition due to oxidation of biomolecules [11]. In addition, ECH also supplies peroxide as an antibacterial agent and prevents biofilm forming produced by *S. aureus*. Peroxides recruits important polymorphonuclear cells in the inflammatory process [9].

The high ROS content of SA wound causes GAH to become an radical antioxidant [12]. GAH did not have an antioxidant regeneration system like in ECH. These antioxidant radicals could cause wound difficult to heal as indicated by poor healing of GAH of SA wound. It showed extensive wound area on the day 12. This extent caused by phenolic antioxidant radicals that could increase oxidative stress and biomolecular oxidation of the wound that inhibiting wound healing process [32]. The extent is significantly different from the ECH, PH, and NC of SA wound.

PH provides good compatibility of wound tissue and has no negative effect. Hydrogels known to be able to encourage wound healing by maintain permeability and water content in the wound, become a barrier of bacteria from the environment, and avoid irritation of the wound [27]. NC of SA wound shows rapid wound closure because of the dry wound causes scab formation which help wound contraction [25]. Scab holds bacteria on the wound surface [24], but SA wound does not have a significant impact on wound healing. Bacteria in the number of 10^4 unable to properly infect the wound. The number of bacteria that could cause inhibition of wound healing is more than 10^5 organisms/gram tissue. This number does not depend on the status of the host immune system and the number and species of infecting bacteria [20].

3.3. Epidermal and Dermal Healing of *Staphylococcus aureus* infected acute (SA) wound

Histological observation at epidermal tissue of SA wound on the day 12 showed that ECH and GAH of SA wound had an epidermal healing value of 3.00, PH of SA wound had an epidermal healing

value of 2.75 ± 0.25, and NC of SA wound had an healing value of 2.67 ± 0.33 (Figure 5). Epidermal healing value of SA wound did not have a significant difference between treatments in the Kruskal Wallis non-parametric statistical test but in the ECH and GAH treatment performed complete epidermal migration and complete keratinization (Figure 5 ECH and GAH). This shows the relationship between the ROS and infection handling to keratinization of the epidermal tissue. Whereas, complete migration and partial keratinization in PH and NC of SA wound shown in Figure 5 (PH and NC). Keratinocyte cells, which are the main constituents in epidermal cells, become apoptosis if the ROS content increases. The use of antioxidants could overcome the process of apoptosis that occur in keratinocyte cells [33].

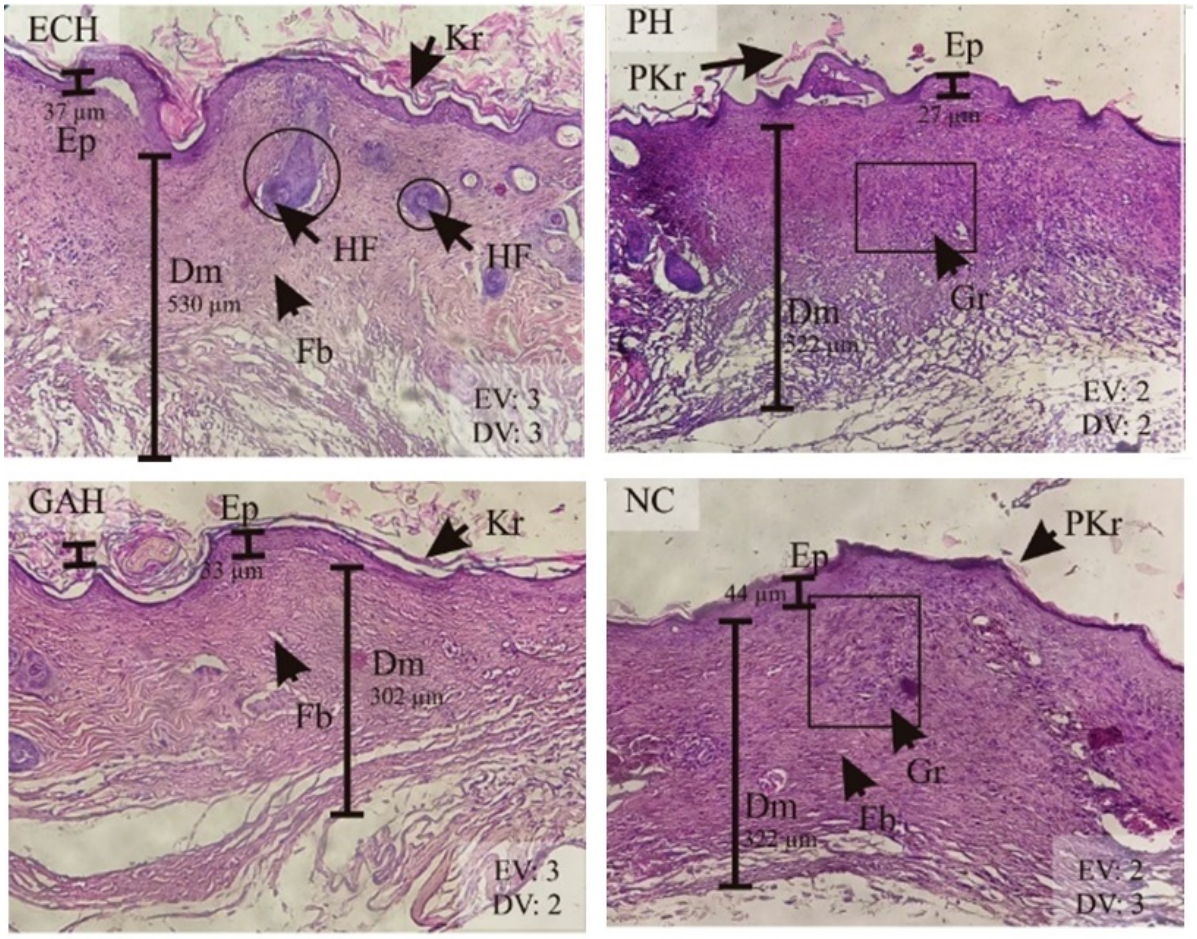

Figure 5. Histological tissue observation of *Staphylococcus aureus* infected acute (SA) wound on day 12. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. Dm: dermis, Ep: epidermis, Fb: fibroblast, Gr: granulation tissue, HF: hair follicle, Kr: keratin, PKr:

partial keratinization. The tissue was stained with Haematoxylin & Eosin staining and observed with a 100x magnification microscope.

Dermal tissue observation of SA wound showed that the ECH of SA wound had a dermal healing value of 2.75 ± 0.25, GAH of SA wound had a dermal healing value of 2.50 ± 0.29, PH of SA wound had a dermal healing value of 2.00 and NC had a dermal healing value of 3.00. Dermal healing value of SA wound did not have a significant difference between treatments in the Kruskal Wallis non-parametric statistical test.

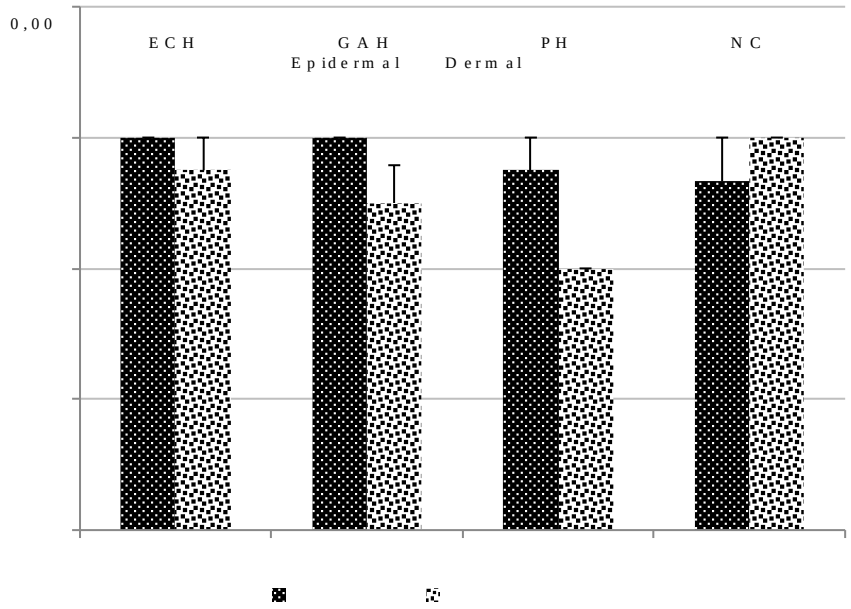

Figure 6. Epidermal and dermal healing value of *Staphylococcus aureus* infected acute (SA) Wound on the day 12. ECH: enzymatic-based CDH hydrogel, GAH: gallic acid hydrogel, PH: plain hydrogel. Data are presented in mean ± SEM with data not showing significance between treatments with the Kruskal Wallis non-parametric statistical test.

The dermal healing value on ECH shows a lower value but is seen maturing in the wound dermis tissue in the presence of hair follicles (Figure 5 ECH). The growth of hair follicles could reduce scar formation [29]. GAH of SA wound, there is no growth of hair follicles (Figure 5 GAH).

The lower healing value is PH of SA wound which did not show collagen deposition in granulation tissue (Figure 5 PH). NC treatment has the highest dermal healing value compared to all treatments in the presence of collagen deposition but still shows the presence of granulation tissue (Figure 5 NC). The longer of granulation tissue presence in the tissue shows a slow dermal healing [30].

4. Conclusion

Wound healing in the enzymatic CDH-based wound dressing hydrogel had no significant effect on wound closure compared to gallic acid hydrogel control and plain hydrogel. The negative control has a significantly faster wound closure to the wound dressing hydrogel treatment. Assessment of epidermal and dermal healing on the day 12 on all treatment did not performed a significant difference but had a tendency that CDH enzymatic-based wound dressing hydrogel had better healing than control treatment.

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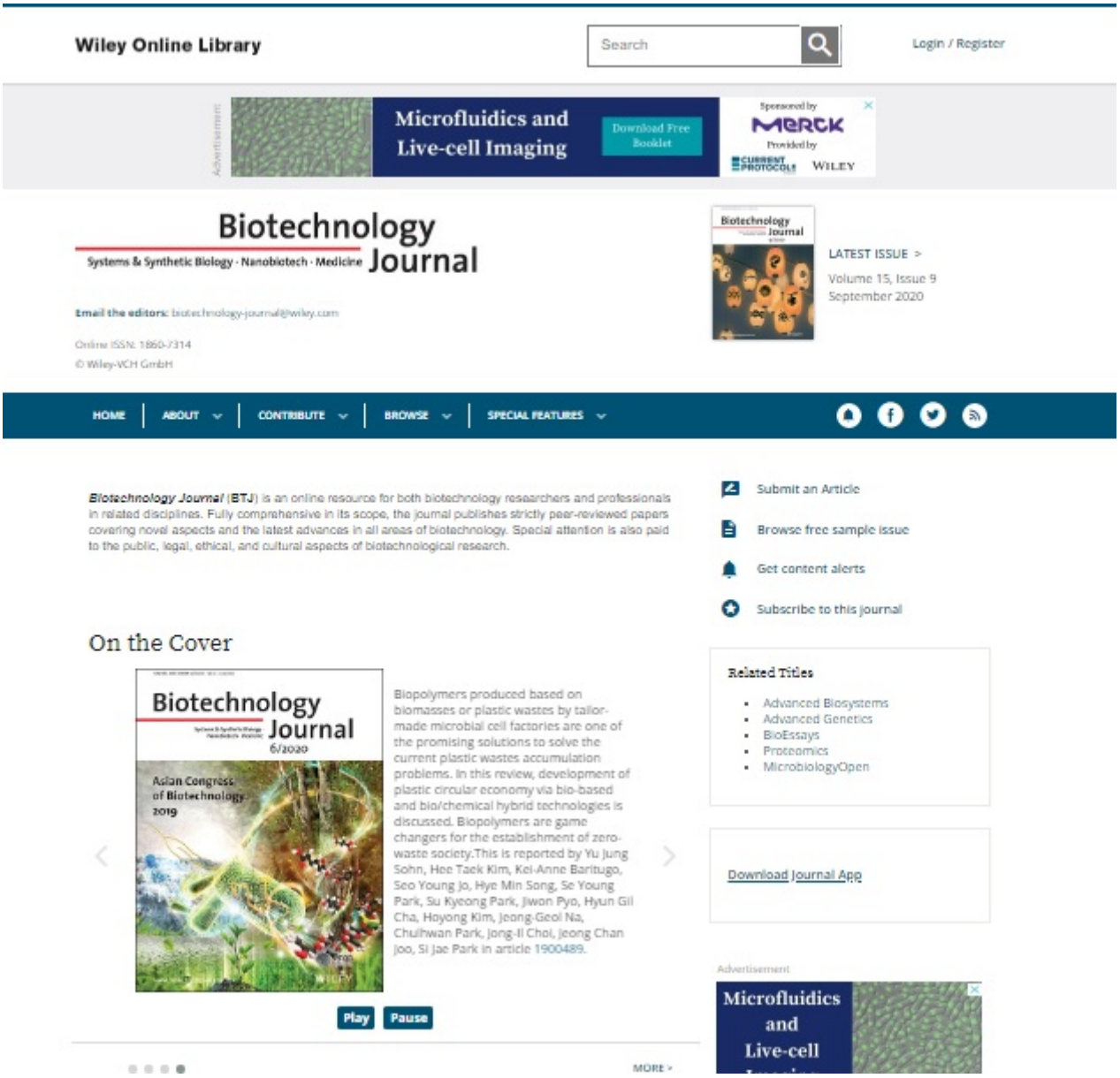

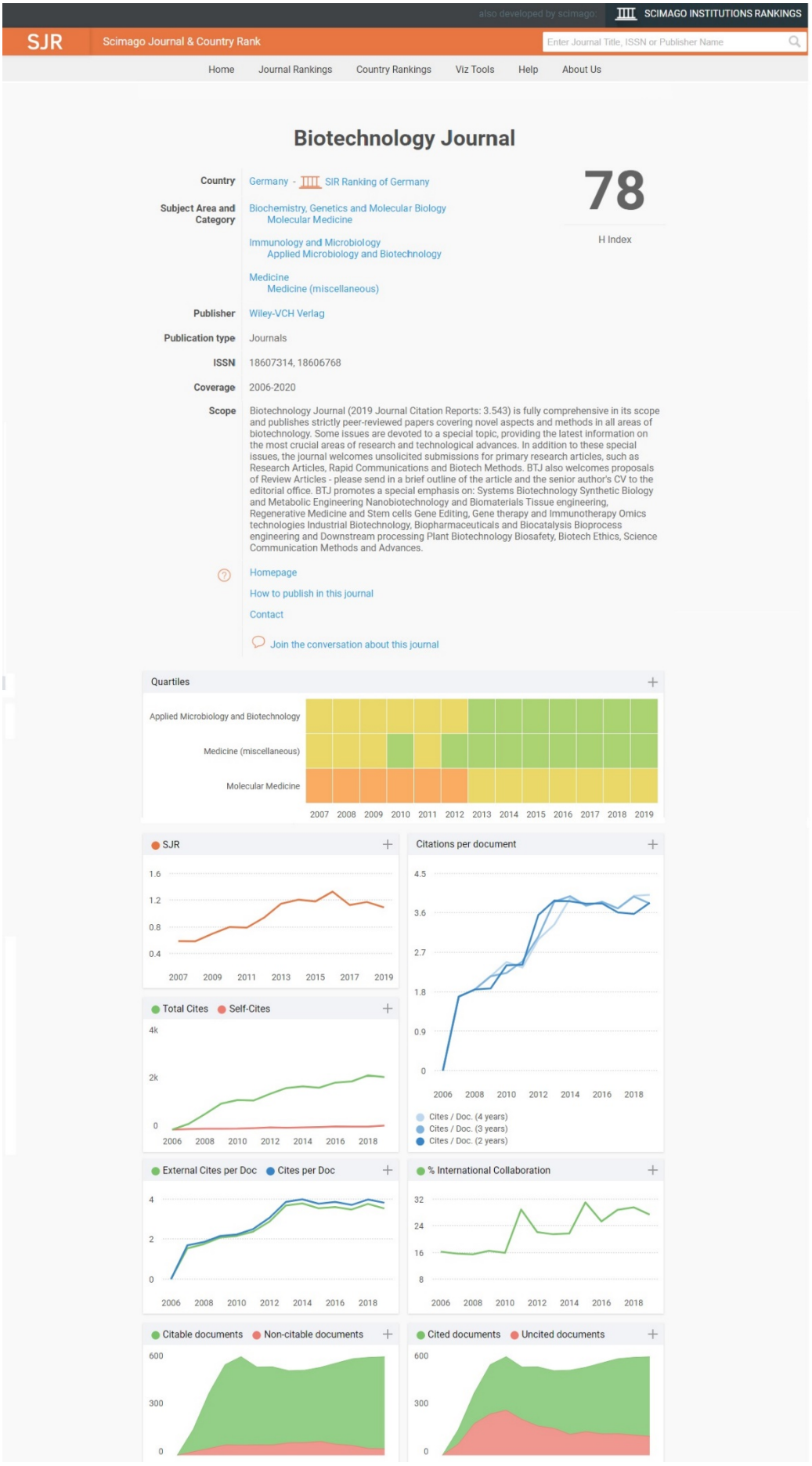
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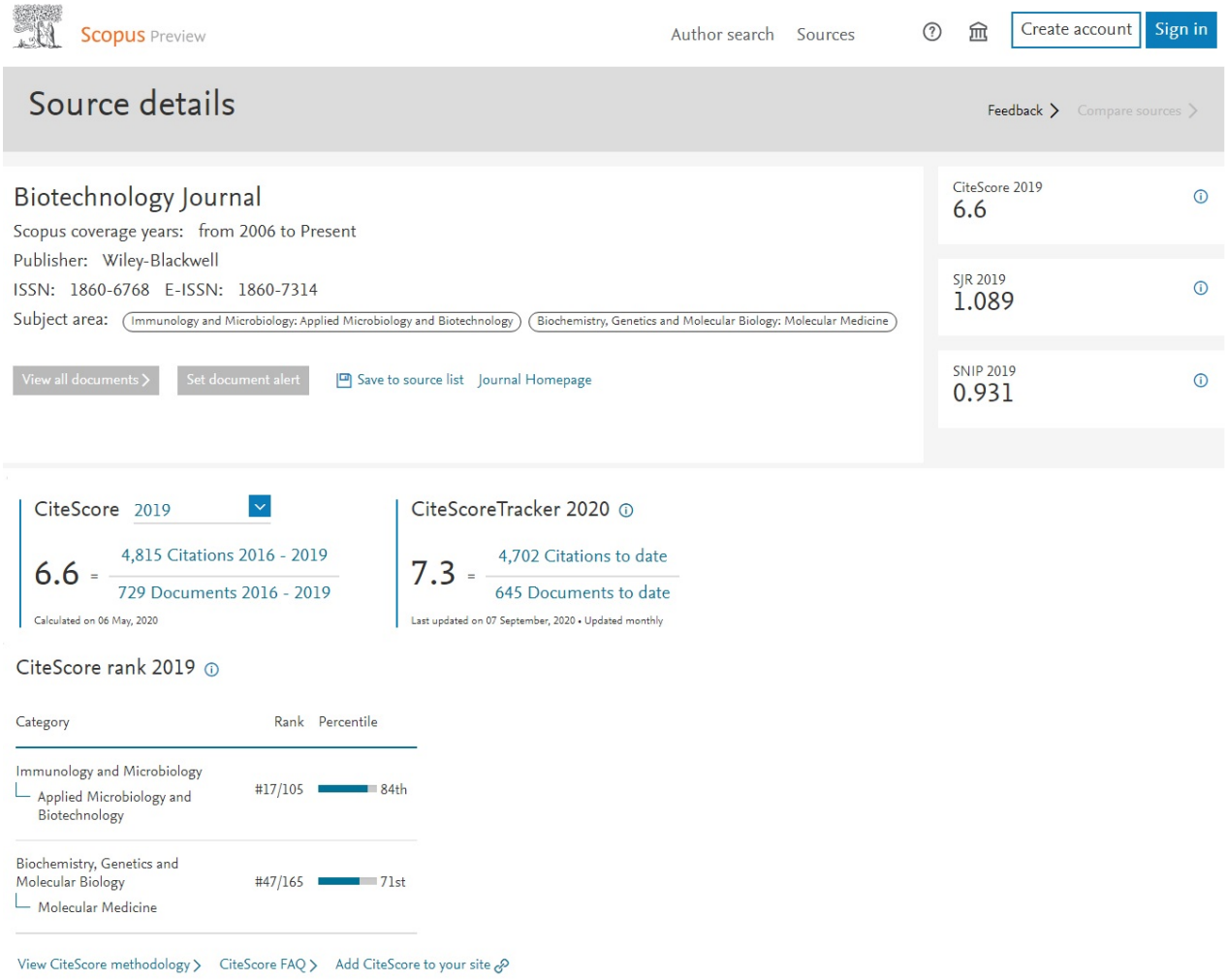
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