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ADELMO CAVALCANTI ARAGÃO NETO

AVALIAÇÃO DA CICATRIZAÇÃO DE FERIDAS CUTÂNEAS TRATADAS COM HIDROGEL DE POLICAJU E QUITOSANA ASSOCIADO A LASER TERAPÊUTICO

Recife/PE
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Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco

Orientadora: Prof[a]. Dr[a]. Maria das Graças Carneiro da Cunha.

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Adelmo Cavalcanti Aragão Neto

"Avaliação da cicatrização de feridas cutâneas tratadas com hidrogel de policaju e quitosana associado a laser terapêutico"

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Aprovado por:

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Profa. Dra. Maria das Graças Carneiro da Cunha

____

Profa. Dra. Maria Tereza dos Santos Correia

____

Prof. Dr. Luiz Bezerra de Carvalho Júnior

____

Profa. Dra. Elizabeth Arruda Carneiro Ponzi

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Aos meus pais, à minha noiva, à minha orientadora, aos amigos e colegas do laboratório de biotecnologia, aos alunos e colegas professores de odontologia. Um forte abraço.

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"*Certo dia, Chao-chou caiu na neve e gritou: - Ajudem-me a levantar, ajudem-me a levantar! Apareceu um monge, que se deitou a seu lado. Chao-chou levantou-se e foi-se embora*".

(Koan Zen)

AVALIAÇÃO DA CICATRIZAÇÃO DE FERIDAS CUTÂNEAS TRATADAS COM HIDROGEL DE POLICAJU E QUITOSANA ASSOCIADO A LASER TERAPÊUTICO

RESUMO

O desenvolvimento de protocolos para o tratamento de ferimentos cutâneos é uma área em constante evolução, onde se fazem necessárias várias estratégias visando uma eficiente cicatrização. Dentre os tipos de tratamentos que podem ser empregados para este fim, destacam-se os hidrogéis a base de polissacarídeos, pela sua biocompatibilidade e capacidade de manutenção da umidade da região lesionada, e a laserterapia de baixa intensidade ("Low level laser therapy" - LLLT), em função do potencial de certos comprimentos de onda em estimular tecidos vivos, aumentando o metabolismo local, produção de ATP, assim como de estimular a ação de fibroblastos. Desta forma, o objetivo do presente trabalho foi a avaliação do processo de cicatrização de feridas cutâneas induzidas em ratos Wistar, tratadas com um hidrogel a base dos polissacarídeos, policaju, extraído da goma do cajueiro (*Anacardium occidentale* L) e quitosana, sendo o mesmo denominado POLI-CHI, associado ou não a LLLT no espetro do vermelho (660 nm). Foram utilizados 45 animais, machos, com idade entre 90 e 120 dias, os quais foram submetidos a procedimento cirúrgico para a confecção de ferida circular (Ø = 0,8 cm) na região dorsal torácica. Os mesmos foram divididos em 3 grupos de acordo com o tratamento empregado: Controle (C), tratado com NaCl 0,1M; Tratado com Hidrogel (H); e tratados com Hidrogel associado a LLLT (HL). As avaliações macroscópicas, evolução do processo de reparo do ponto de vista clínico e mensuração da área da ferida, acompanhada por paquímetro digital, foram realizadas durante todo o período experimental e as microscópicas, através de escores histológicos. A análise estatística foi realizada através do método de analise de variância (ANOVA) e do teste de Bonferroni para comparações múltiplas ($p < 0,05$). Com relação a avaliação clínica, os grupos H e HL apresentaram cicatrizes mais estéticas, com coloração mais próxima do tecido maduro e uma maior regressão da área da ferida, com significância estatística aos 7 e 14 dias, ambos em relação ao Controle. Com relação a avaliação microscópica, foram identificados os seguintes achados: presença de crosta fibrino-leucocitária mais intensa no grupo HL; maior presença de colágeno nos grupos H e HL; menor presença de necrose focal aos 7 e 14 dias no grupo H; menor presença de exudato neutrofílico nos grupos H e HL; menor presença de edema nos grupos H e HL; regressão da neoformação vascular aos 7 dias no grupo H e modulação da mesma no grupo HL. Os resultados obtidos demonstram que a utilização do hidrogel POLI-CHI contribuiu para uma cicatrização mais eficiente dos ferimentos induzidos e modulação do processo inflamatório, além disso, o uso combinado com a LLLT atuou de forma sinérgica neste processo.

Palavras-Chave: Hidrogel, Quitosana, Policaju, Laser, Cicatrização.

EVALUATION OF THE SKIN WOUND HEALING USING A HYDROGEL OF POLICAJU AND CHITOSAN ASSOCIATED WITH THERAPEUTIC LASER

ABSTRACT

The development of protocols for the skin wound treatment is a constant evolving area, where a variety of strategies are necessary aiming for an effective healing. Among the available treatments that can be used for this, must be highlighted the hydrogels based on polysaccharides, because of its biocompatibility and the capacity to maintenance the humidity within the lesion area, and the low level laser therapy (LLLT), that is based on the potential of specific wave lengths to stimulate live tissues, increasing its local metabolism, ATP production, and fibroblast stimulation. In this context, the aim of this research work was to evaluate the wound healing process of skin induced lesions in Wistar rats, treated with a hydrogel based on the polysaccharides, policaju, extracted from the *Annacardium occidentale* L. gum, and chitosan, being the same referred as POLI-CHI, combined or not with LLLT in the red spectrum (660 nm). Were used, 45 animals, males, age ranging from 90 to 120 days, which were subjected to surgical procedures to create circular full thickness wounds (Ø = 0,8cm) in the dorsal thoracic region. They were divided in 3 groups, according to the applied treatment, being: Control (C), treated using 0.1M NaCl; Treated using the hydrogel (H); and treated using the hydrogel and LLLT (HL). Regarding to macroscopic evaluation, the evolution of the wound healing process, by the clinical point of view and wound area measurement, using a digital caliper, were performed during all experimental period and the microscopic ones, using histological criteria patterns. The statistical analysis was applied using the method of analysis of variance (ANOVA) and the Bonferroni's multiple comparison test ($p < 0.05$). Concerning the clinical evaluation, groups H and HL presented more esthetical scar tissue, with more similar to mature tissue coloration and a more notable wound area regression, being statistical significant at 7 and 14 days, both in comparison to Control. Regarding to the microscopic evaluation, were identified the following finds: more intense presence of fibrin-leucocite crust in HL group; larger collagen presence in groups H and HL; minor presence of focal necrosis at 7 and 14 days in H group; minor neutrohilic exudate in groups H and HL; regression of the vascular neoformation at 7 days in group H and modulation of the same in group HL. This obtained data showed that the use of POLI-CHI contributes to a more efficient healing process of the induced wounds and modulation of the inflammatory process, furthermore, the combined use with LLLT potentiates this process.

Keywords: Hydrogel, Chitosan, Policaju, Laser, Wound Healing.

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1. Introdução

Tratar uma ferida não é um desafio novo. Desde a antiguidade há relatos sobre este cuidado. Atualmente, muitos protocolos vêm sendo estabelecidos visando acelerar o processo *cicatricial,* fenômeno pelo qual o organismo tende a reparar uma porção lesada, de modo a guiar ou gerir este processo, para que sejam obtidos resultados mais eficientes em relação ao tempo para a completude do processo e à qualidade da cicatriz.

A cicatrização de feridas é um processo muito complexo e afetado por uma série de fatores, incluindo a coagulação do sangue, inflamação, fibroplasia, deposição de colágeno e contração da ferida (BUSILACCHI et al., 2013).

Os agentes curativos são ferramentas terapêuticas que auxiliam na resolução de lesões e ferimentos cutâneos, onde suas principais funções são: proteção de contaminações secundárias; prevenção da maceração dos tecidos circunvizinhos pela absorção do excesso de exsudato e, o favorecimento da re-epitelização e migração celular, uma vez que estas ocorrem mais rapidamente em locais cuja umidade é mantida (WANG et al., 2012).

Dentre os diversos tipos de agentes curativos que ajudam a manter a umidade no local lesionado, pode-se destacar os hidrogéis. Os quais representam uma classe de sistema de liberação controlada de drogas que tem se destacado na entrega inteligente das mesmas. São definidos como uma rede polimérica reticulada capaz de absorver grande quantidade de água ou fluído biológico, sem se dissolverem e têm sido utilizados em aplicações médicas e biológicas devido as suas características físico-químicas, podendo assim serem utilizados para conservar células, nutrientes, drogas ou proteínas (HOARE e KOHANE, 2008; ANUMOLU et al., 2011).

Dos polímeros hidrofílicos mais utilizados na formulação de hidrogéis estão os sintéticos como o álcool polivinílico (PVA), o *polietilenoglicol* (PEG), a policaprilactona (PCL)*,* o *poli*ácido *glicólico (*PGA)*,* o *poli(*ácido *láctico-co-ácido glicólico) (*PLGA) (MELO et al., 2012) e, entre os naturais estão os polissacarídeos como a quitosana (BHATTARAI et al., 2010) e alginato (THU e ZULFAKAR, 2012), porém existem hidrogéis constituidos por misturas desses diferentes polímeros (OPRENYESZK et al., 2013).

A busca por novos hidrogéis com potencial aplicação na área biomédica e farmacêutica se encontra em expansão, como pode-se citar os hidrogéis a base de policaju (polissacarídeo da gama do cajueiro)/quitosana (SOARES et al., 2012), de alginato/quitosana (OPRENYESZK et al., 2013), de PEG/PLC contendo curcumina imobilizada em micelas poliméricas (GONG et al., 2013), de PEG/quitosana com ou sem micelas poliméricas (ITO et al., 2013), de CM-quitosana/gelatina (HUANG et al., 2013), de PEG/PCL/PEG (NI et al., 2014) e de quitosana/PVA (LIU et al., 2014) .

Há evidências de que várias estratégias terapêuticas são capazes de modular eventos em todas as fases do processo de cicatrização de feridas cutâneas, entre elas está a laserterapia de baixa intensidade (LLLT). Aplicações da LLLT incluem o tratamento de feridas resultantes de traumas ou lesões vasculares, restauração da função neural normal após a lesão, a atenuação da dor e modulação do sistema imune. Combinações de terapias são muitas vezes necessárias para melhorar o efeito terapêutico sinérgico e para reduzir a dose ou frequência de cada uma das drogas para o tratamento de lesões, e, por conseguinte, reduzir o risco de efeitos adversos (KIM et al., 2013).

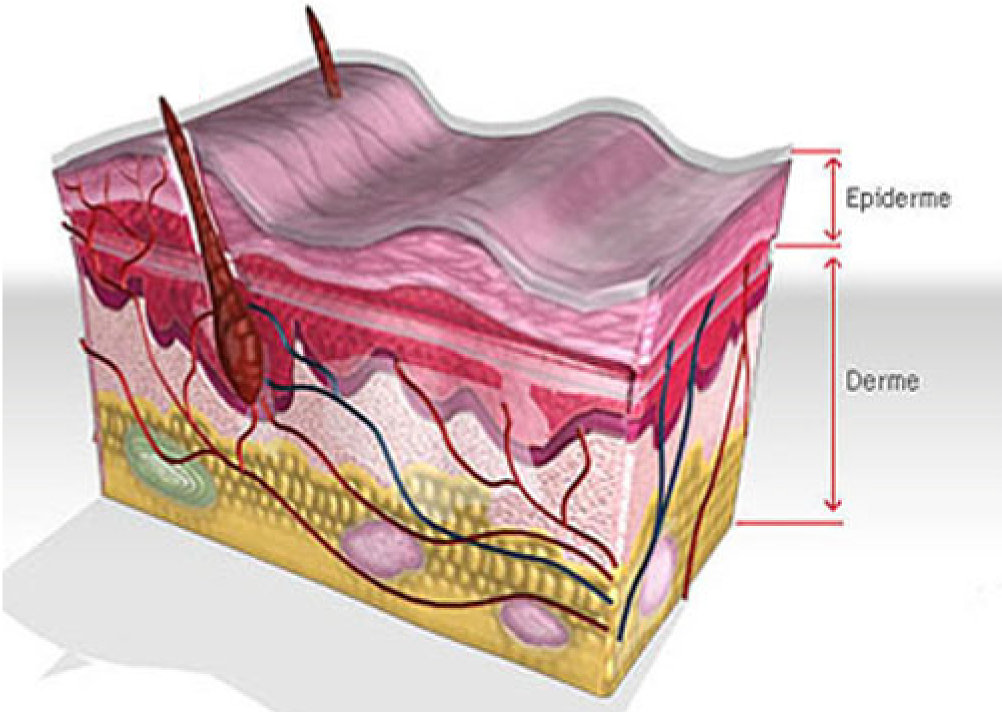
Recentemente foi demonstrado que a combinação de curativos a base de polissacarídeos e LLLT acelera os eventos biológicos envolvidos no processo de cicatrização (DANTAS et al., 2011). Portanto, o objetivo deste estudo foi avaliar o processo de cicatrização de feridas cutâneas induzidas em ratos Wistar, tratadas com o hidrogel de policaju/quitosana associado ou não à Laser terapia de baixa intensidade.

2. Revisão Bibliográfica

2.1 Anatomofisiologia da Pele

A pele é o maior dos órgãos do corpo e um dos mais ativos, tendo cerca de 1,7 m² que corresponde aproximadamente a 16% do peso corporal total. Sua espessura varia de 0,05 a 6 mm conforme a área revestida, e recebe aproximadamente um terço do volume sanguíneo circulante (BENBOW, 2005). A mesma é formada por duas camadas distintas, epiderme e derme (Figura 1), porém, conta também com uma estrutura de suporte, conhecida como hipoderme ou tecido subcutâneo (JUNQUEIRA e CARNEIRO, 2008).

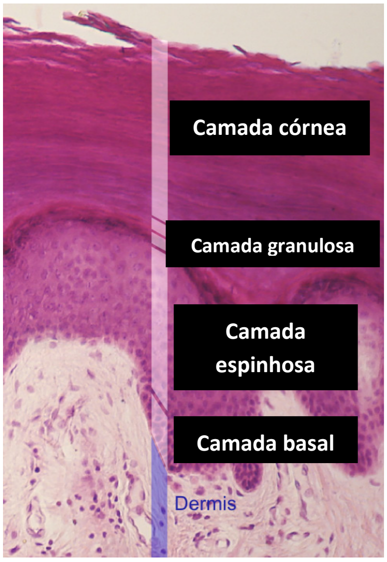
Figura 1. Estrutura da Pele: Epiderme e Derme.



Fonte: http://4.bp.blogspot.com/_jY7xNVPELlA/TUND2-WbqSI/AAAAAAAAAbw/hz_b0FE1YMY/s1600/pele.jpg

A epiderme, camada mais externa, deriva do folheto embrionário ectoderma e é definida como um epitélio estratificado, queratinizado pavimentoso, composto por quatro camadas celulares (Figura 2): **a) Camada germinativa ou basal**, composta por células basais e melanócitos, onde as células basais caracterizam-se por sua intensa atividade mitótica, que dão origem às demais células epidérmicas e os melanócitos, juntamente com os queratinócitos, são responsáveis pela produção de melanina, a qual atua na proteção da pele contra a radiação ultravioleta; **b) Camada espinhosa ou malpighiana**, composta por células de conformação poliédrica que vão se achatando até a epiderme, onde suas características conferem resistência ao atrito; **c) Camada granulosa,** composta por células escuras, achatadas, com núcleo de difícil visualização e sua principal característica é a significativa presença de grânulos de querato-hialina, envolvidos na queratinização da epiderme; **d) Camada córnea,** composta por células mortas, anucleadas, constituídas por queratina e proteína fibrosa resistente, onde esse conjunto de células previne a perda de fluido corporal (JUNQUEIRA e CARNEIRO, 2008; PORTO, 2009; KAMEL et al., 2013)

Figura 2. Camadas celulares da epiderme: A) Basal; B) Espinhosa; C) Granulosa e D) Córnea.



Fonte: http://upload.commons/e/e4/Epidermal_layers.png

A derme, originária do folheto mesodérmico, confere sustentação à epiderme e envolve anexos cutâneos, vasos sanguíneos e linfáticos, terminações nervosas sensoriais e músculos. Sua espessura é variável e atinge um máximo de 3 mm na planta dos pés (JUNQUEIRA e CARNEIRO, 2008). Ao contrário da epiderme, a derme não possui uma organização regular e é constituída por um material transparente amorfo, com características de um gel semilíquido rico em glicosaminoglicanas, que confere resistência mecânica à pele, e fibras elásticas, colágenas e reticulares (BURKITT et al., 1994; KAMEL et al., 2013).

Fibroblastos, células essenciais ao processo cicatricial, estão também presentes na derme, e os mesmos sintetizam e secretam colágeno e elastina, fundamentais ao processo cicatricial, além disso, desempenham papel crucial na contração e retração da ferida (PORTO, 2009).

A hipoderme, formada por tecido conjuntivo frouxo, possui espessura variável e é constituída exclusivamente por tecido adiposo. Atua como isolante térmico, protetor mecânico contra traumas e pressão e, reservatório nutritivo (SILVA et al., 2007; KAMEL et al., 2013).

Em conjunto, epiderme, derme e hipoderme constituem o revestimento externo do corpo e atuam como primeiro mecanismo de defesa do organismo. Entretanto, por sua complexidade, composta por tecidos de natureza distinta, além de proteção, a pele está adaptada para exercer diferentes funções, tais como termorregulação, percepção, absorção, secreção e formação de vitamina D (JUNQUEIRA e CARNEIRO, 2008).

2.2 – Cicatrização e Tratamentos

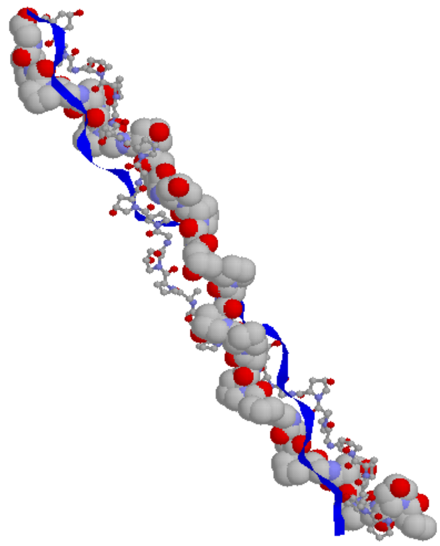
O conhecimento relativo tanto ao processo de cicatrização de ferimentos quanto à utilização de técnicas curativas tem expandido e mudado dramaticamente nas últimas três décadas. Antes disso, o processo de cicatrização era considerado de cunho temporal, ficando o clínico passivo diante do mesmo (BARANOSKI e AYELLO, 2012).

Com relação à fisiopatologia do reparo tecidual, os ferimentos cutâneos cicatrizam em quatro fases ou períodos: hemostasia, inflamatória, proliferativa e remodelamento. Nos casos de ferimentos crônicos, esta progressão natural é afetada, ocorrendo a ausência ou a lentificação deste processo de reparo (WILLIAMSON e HARDING, 2004; PARK et al., 2011).

Durante as fases iniciais do processo de cicatrização ocorre o aumento da concentração local dos fatores de crescimento, onde as citocinas se elevam, os processos de regeneração vascular e fibroplasia se intensificam através da angiogênese, migração e proliferação fibroblástica, formando um tecido rico em elementos vasculares, celulares e, a produção do tecido de granulação, que aos poucos vai se alastrando e preenchendo o vazio resultante dos tecidos eliminados. Na fase precoce do processo cicatricial existe deposição de fibronectina e ácido hialurônico que propiciam uma atmosfera favorável para a movimentação celular. O avanço do processo modifica os substratos sintetizados localmente, os quais passam a ser compostos por proteoglicanos que fixam as células, beneficiando a troca de fenótipo celular, sem contar que à medida que se formam novas camadas de tecido de granulação, as mais antigas, profundamente situadas, vão perdendo sua riqueza em vasos, e os fibroblastos e feixes de colágeno passam a predominar (BALBINO et al., 2005).

A produção de colágeno (colagenização) na área da ferida representa um dos fatores mais significativos para a recuperação dérmica após uma agressão (MOURA et al., 2014). O colágeno constitui um grupo de proteínas compostas por três cadeias polipeptídicas dispostas em tripla hélice (Figura 3), e é o principal componente da matriz extracelular (MEC) perfazendo aproximadamente 25% da massa protéica total do organismo (GHADIALLY, 1997). De modo geral, as fibras colágenas se dispõem num padrão ondulado e exibem extensão bastante variada, e têm papel fundamental na arquitetura tecidual, na resistência dos tecidos e em uma ampla variedade de interações célula-célula e célula-matriz (RICH e WHITTAKER, 2005; CHENG et al., 2013).

Figura 3. Estrutura do colágeno.



Fonte: http://www.cryst.bbk.ac.uk/PPS2/projects/pauly/proline/collagen2.gif

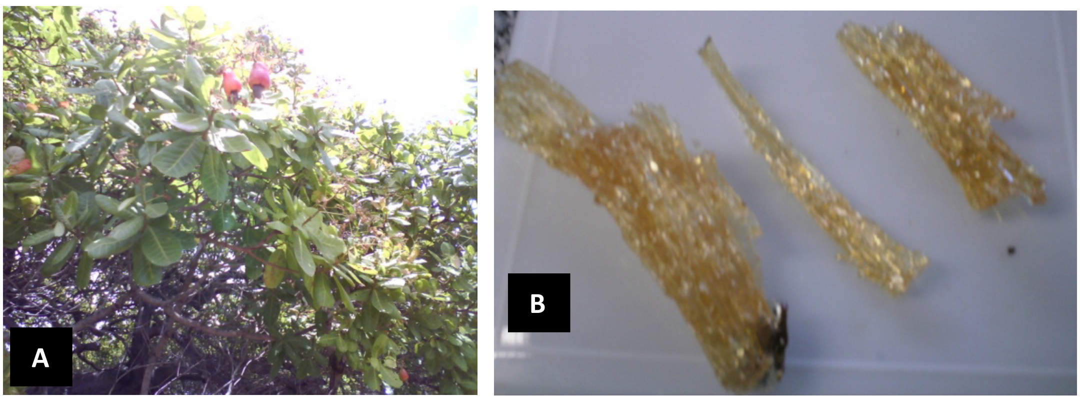
Certas condições favoráveis ao processo de cicatrização podem ser estabelecidas pela utilização e manutenção de curativos (SINGH et al., 2013). Após a limpeza criteriosa da região lesionada, esta deve ser submetida ao curativo cuja função é a proteção de contaminações secundárias; prevenção da maceração dos tecidos circunvizinhos pela absorção do excesso de exsudato e favorecimento da re-epitelização e migração celular, uma vez que estas ocorrem mais rapidamente em locais cuja umidade é mantida (WANG et al., 2012).

Dentre os diversos tipos de agentes curativos, cada um atuando de forma mais efetiva em certos tipos de lesões, pode-se citar: soluções de polissacarídeos como o policaju (SCHIRATO et al., 2006) e alginato de cálcio (THU e ZULFAKAR, 2012), soluções de lectina de *Cratylia mollis*, Cramoll (MELO et al., 2011), filmes a base de quitosana (LI et al., 2012) e de alginato/quitosana associado a laser terapia (DANTAS et al., 2011), gazes impregnadas com antibióticos (MUELLER e KREBSBACH, 2008) e com solução de policaju com tripsina imobilizada (MONTEIRO et al., 2007), curativos não-aderentes a base de silicone (AHMADI e WILLIAMS, 2009), hidrocoloide do tipo Karayahesive® (FUJIMOTO et al., 2008), absorventes de exsudado contendo nanoparticulas de prata (FERNANDEZ et al., 2009), gel de silicone (JIA et al., 2011), sprays hemostáticos a base de cyanoacrilato (WALIA et al., 2013) e hidrogéis a base de quitosana (MADHUMATHI et al., 2009), de carbopol contendo Cramoll imobilizada (PEREIRA et al., 2012) e, de PEG/PCL/PEG com micelas imobilizadas contendo curcumina (GONG et al., 2013).

2.3 – Polissacarídeos

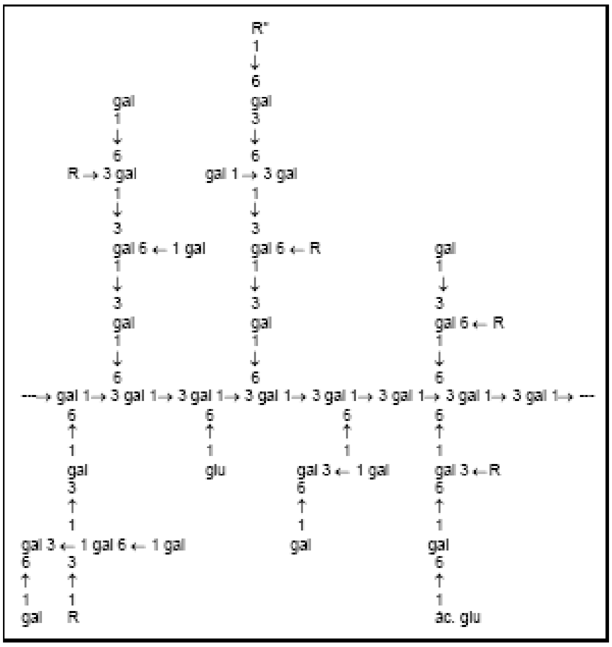
Os polissacarídeos, polímeros hidrofílicos naturais de cadeia longa linear ou ramificada, constituídos de monossacarídeos, são atóxicos, biocompatíveis, biodegradáveis e de fácil solubilização, podendo formar hidrogéis ou cristais líquidos em solução (CHANDRA e RUSTGI., 1998). O polissacarídeo policaju extraído da goma do cajueiro *Anacardium occidentale* L. (Figura 4), encontrado em países tropicais, tem apresentado resultados eficientes no processo de cicatrização de lesões cutâneas (SCHIRATO et al., 2006; MONTEIRO et al., 2007).

Figura 4. A) Cajueiro (*Anacardium occidentale*) e B) Goma do cajueiro.



O policaju (figura 5) é um polissacarídeo ácido complexo (arabinogalactana ácida) com massa molecular de 1,6 x 10^5 Da, composto por uma cadeia principal formada por unidades de D-Gal*p* unidas por ligações glicosídicas β-(1→3) substituídos em O-6, tendo como resíduos terminais a arabinose, raminose, ácido glucurônico, ácido 4-O-metilglucurônico, xilose, glicose e manose (DE PAULA e RODRIGUES, 1995). Este polissacarídeo tem sido relatado como potencial constituinte de filmes e espessantes (MENESTRINA et al., 1998, CARNEIRO-DA-CUNHA et al., 2009, SOUZA et al., 2010), além disso, outros estudos confirmaram a atividade anti-tumoral, anti-parasitária e efeitos cicatrizantes (MENESTRINA et al., 1996; SCHIRATO et al., 2006). O fácil acesso a este material de baixo custo, não tóxico, hidrofílico, biocompatível e biodegradável, o qual ainda apresenta interessante atividade biológica e boas propriedades reológicas são fatores que fazem com que seja viável o seu uso como matriz para imobilização e distribuição de drogas (MONTEIRO et al., 2007).

Figura 5. Esquema de um fragmento da estrutura do Policaju. R representa D–manose, D–xilose, L–raminose, L–arabinose ou cadeias de arabinose com ligação 1,2; R'' representa D-glicose ou ácido D-glucurônico.

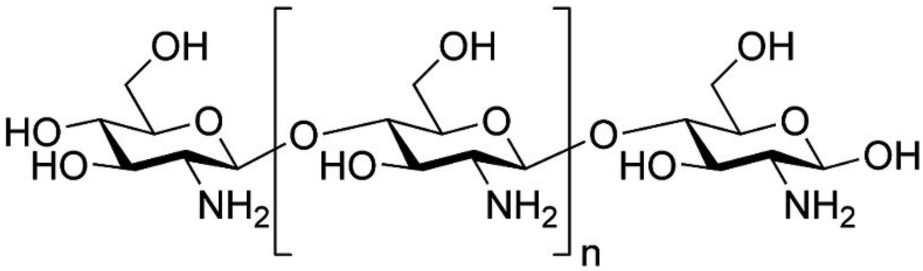


Fonte: (ANDERSON e BELL, 1975).

A quitosana (Figura 6), polissacarídeo derivado da quitina, obtida por desacetilação da mesma, e também encontrada naturalmente em alguns fungos (VINSOVA e VAVRIKOVA, 2008; MUZZARELLI et al., 2012; 2013), também tem sido investigada pela comunidade científica em aplicações biomédicas e terapêuticas, por possuir propriedades curativas e hemostáticas, bem como atividade antimicrobiana (MADHUMATHI et al., 2009; ARAIN et al., 2013).

Com relação às atividades biológicas, a quitosana provoca inibição do crescimento de micro-organismos, uma vez que em contato com os fluidos fisiológicos, seus grupos amínicos são protonados e ligam-se aos micro-organismos, resultando na aglutinação das células microbianas e inibição do seu crescimento (KOIDE, 1998; SIMONCIC e TOMSIC, 2010). Este mecanismo da atividade antimicrobiana da quitosana está intimamente relacionado às suas propriedades físico-químicas e às características da membrana do micro-organismo, (SILVA et al., 2006).

Figura 6. Estrutura da quitosana. Evidenciando unidades de D-glicosamina.



Fonte: http://www.mn.uio.no/kjemi/english/people/aca/bony/research/chitosan.html

2.4 – Hidrogéis

Os hidrogéis são definidos como uma rede polimérica tridimensional capaz de absorver grande quantidade de água ou fluído biológico. Quimicamente, os hidrogéis são baseados em polímeros hidrofílicos, que são intercruzados para prevenir a sua dissolução em água, podendo assim ser utilizados para conservar células, nutrientes, drogas ou proteínas. Em um ambiente aquoso, os grupos hidrofílicos da rede polimérica são hidratados causando inchaço gerando a estrutura em "rede" e a forma do hidrogel. Esse termo implica no intercruzamento químico ou físico entre os grupamentos ativos dos polímeros em composição. Além disso, os hidrogéis podem ser formulados em uma variedade de formas físicas, incluindo filmes e revestimentos comestíveis, sendo micro ou nanoparticulados (HOARE e KOHANE, 2008).

Reológicamente, as soluções aquosas de polímeros hidrofílicos em concentrações baixas ou moderadas normalmente apresentam um comportamento newtoniano. Por outro lado, uma vez que ligações cruzadas entre as diferentes cadeias de polímeros são introduzidas, as "redes" assim obtidas mostram um comportamento visco-elástico e, por vezes, um comportamento puramente elástico (CUGGINO et al., 2008).

Em geral, os hidrogéis são biocompatíveis, sendo a biocompatibilidade promovida pelo seu alto teor de água e as semelhanças físico-químicas que possuem com a matriz extracelular nativa de tecidos orgânicos, tanto em composição, quanto mecanicamente (GEEVER et al., 2008).

A biodegradabilidade da matriz polimérica pode ser projetada através de vias enzimáticas, além de vias hidrolítica ou ambiental como por exemplo, pH, temperatura ou campo elétrico, no entanto, a degradação nem sempre é desejável, dependendo do tempo de liberação e local de entrega da biomolécula (TOMME e HENNINK, 2007).

Devido à sua capacidade de absorção de água, os hidrogéis possuem ampla aplicação em diferentes áreas biotecnológicas, como por exemplo, são utilizados como materiais para lentes de contato; separação de biomoléculas ou células; matrizes para a imobilização de células; como dispositivos para a liberação controlada de compostos bioativos (MIRONI-HARPAZ et al., 2012); em práticas clínicas da medicina experimental para a engenharia e regeneração de tecidos (ZHU e MARCHANT, 2011), além de servirem de materiais de barreira para regular aderências biológicas (WANG et al., 2010).

A natureza elástica dos hidrogéis hidratados inchados permite minimizar a irritação dos tecidos circundantes após implantação. A baixa tensão interfacial entre a superfície do hidrogel e do fluido corporal minimiza a adsorção de proteína e adesão celular, o que reduz as chances de uma reação imunológica negativa. Além disso, os hidrogéis possuem várias características que os tornam excelentes veículos de entrega de drogas (BHATTARAI et al., 2010).

Com relação a capacidade de deformação, os hidrogéis são relativamente deformáveis e podem se adaptar à forma da superfície a qual são aplicados. Neste último contexto, as propriedades de muco ou bioadesividade de alguns hidrogéis podem ser vantajosas para imobilizá-los no local da aplicação, mesmo que a superfície tópica não seja horizontal (HOARE e KOHANE, 2008).

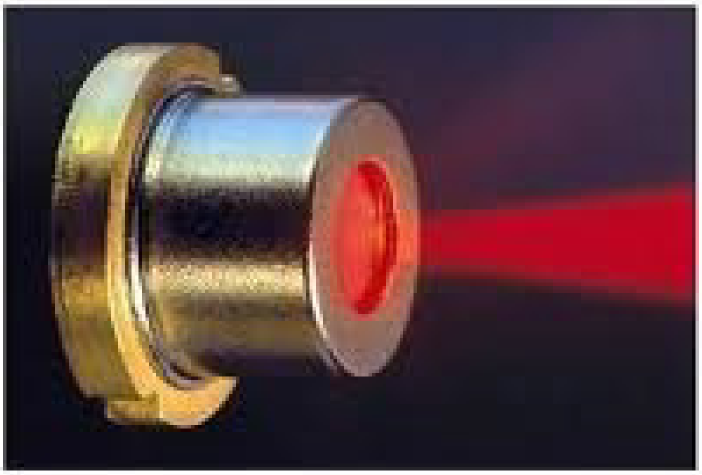
2.5 – Laserterapia

Uma recente modalidade terapêutica utilizada em processos de cicatrização é baseada na própria luz. A idéia de que esta possa ser utilizada curativamente não é nova, foi reconhecia como uma potencial forma de terapia ao longo da história. Os antigos egípcios e gregos, por exemplo, acreditavam que o sol fortificava e curava o corpo (BASFORD, 1995; COULTER, 2003). Na idade média, o banho de sol foi considerado um aliado na luta contra doenças virulentas como a praga (BASFORD, 1995). No entanto, a radiação LASER, sigla correspondente a "Light Amplification by the Stimulated Emission of Radiation", apenas foi divulgada no mundo científico na década de 60, sendo uma técnica para a geração de radiação muito monocromática na região infra-vermelha do espectro óptico através da utilização de vapor alcalino como meio ativo (MAIMAN, 1960).

A propriedade terapêutica dos lasers foi identificada a baixa intensidade, sendo então reconhecida a irradiação atérmica, sendo esta denominada laserterapia. A aplicação dos lasers em medicina foi explorada pelo Dr. Mester, um professor de cirurgia em Budapeste e suas descobertas serviram de base para muitos outros estudos (MESTER et al., 1971; 1985).

Os primeiros lasers utilizados terapeuticamente foram baseados no uso de gases inertes, como Hélio-Neônio e Argônio. Os lasers com diodos semicondutores foram posteriormente introduzidos, incluindo Gálio-Arsênio, e Gálio-Alumínio-Arsênio (Figura 7), os quais vêm sendo amplamente utilizados com comprimento de onda variando de 632 até 980 nm, e dosagem compreendendo de 1 a 4 J/cm^2. Estes parâmetros foram definidos por investigações iniciais e permanecem como as exposições mais utilizadas para o tratamento de ferimentos (WHINFIELD e AITKENHEAD, 2009).

Figura 7. Laser de diodo (GaAlAs).



Fonte: http://www.novaeletronica.net/tutoriais/laser/laserpin1t.jpg

Dentre as propriedades dos lasers nos tecidos vivos, podemos citar os efeitos de foto-sensibilização e foto-resposta celular. Estes irão se manifestar, da seguinte forma: primeiramente vão agir diretamente na célula, produzindo um efeito inicial ou imediato, aumentando o metabolismo celular através do aumento da síntese de endorfinas e diminuição da liberação de transmissores nosciceptivos como a bradicinina e serotonina, levando a uma ação estimulativa e analgésica; secundariamente ou indiretamente, a aplicação aumentará o fluxo sanguíneo e estimulará a drenagem linfática, agindo assim na inflamação; e por fim, haverá a instalação de efeitos terapêuticos gerais como a estimulação do sistema imunológico (KRESLAVSK et al., 2012).

Com relação a alguns efeitos terapêuticos dos lasers de baixa intensidade, pode-se mencionar: proliferativo, tendo em vista que os mesmos estimulam a angiogênese, síntese de fibroblastos, colágeno e ATP; fibrinolítico; anti-edematogênico; analgésico; e bactericida, aumentando a quantidade de interferon presente (HAWKINS et al., 2007; SANATI et al., 2011).

Recentemente, Dantas et al. (2011) reportaram a eficiência da laser terapia na cicatrização de queimaduras induzidas em ratos, os quais foram divididos em 6 grupos: sem tratamento; filme de celulose; filmes à base de alginato de sódio/quitosana; laser terapia; filme de celulose associado a laser terapia e filmes de alginato de sódio/quitosana associado a laserterapia. Os resultados revelaram que: a reação inflamatória foi significativamente mais intensa no grupo sem tratamento do que nos grupos irradiados com laser; a laser terapia estimulou a diferenciação miofibroblástica, com ou sem os filmes e que a laserterapia combinada com os filmes resultou numa melhor epitelização, formação de vasos sanguíneos e colagenização, promoveu uma substituição rápida do colágeno tipo III por tipo I e favoreceu um melhor arranjo das fibras de colágeno recém-formados.

As contraindicações da laserterapia são mínimas, excetuando-se a irradiação direta na retina que pode desencadear dano permanente como qualquer radiação luminosa com alto poder de penetrância, e se referem basicamente aos meios que seriam estimulados negativamente por sua ação de acelerar o metabolismo, como a existência de lesões malignas na área irradiada, e focos bacterianos agudos, uma vez que a terapia poderia intensificar o processo patológico (PROCKT et al., 2008; KHAN et al., 2013).

3. Objetivos

3.1 – Objetivo Geral

Avaliação do processo de cicatrização de feridas cutâneas induzidas em ratos Wistar, tratadas com hidrogel de policaju/quitosana associado ou não à Laserterapia de baixa intensidade.

3.2 – Objetivos Específicos

- Obter o hidrogel de policaju e quitosana (POLI-CHI);
- Realizar o tratamento tópico de lesões cutâneas experimentais em ratos Wistar utilizando POLI-CHI ou POLI-CHI associado à laserterapia de baixa intensidade;
- Acompanhar a evolução do processo de reparo do ponto de vista clínico (avaliação clínica das feridas e mensuração de sua área) durante 14 dias;
- Acompanhar a cicatrização do ponto de vista histopatológico, através de biópsias no 3º, 7º e 14º dias do pós-operatório (Crosta fibrino-leucocitária, Colágeno, Necrose focal, Depósitos de fibrina, Exsudato neutrofílico, Edema, Exudato eosinofílico, Infiltrado mononuclear, Infiltração macrofágica, Granulomas, Neovascularização, Proliferação fibroblástica, e Fibrose).

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5. Artigo

5.1 ARTIGO A SER SUBMETIDO AO PERIÓDICO JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART A.

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Combined therapy using Low Level Laser and Chitosan-Policaju Hydrogel for Wound Healing

Combined therapy using Low Level Laser and Chitosan-Policaju Hydrogel for Wound Healing

Adelmo C. Aragão-Neto[a], Paulo A.G. Sorares[a], Maria T.S. Correia[a], Maria H.M. Lima-Ribeiro[b], Elaine J.A. Carvalho[c], Maria G. Carneiro-da-Cunha[a*]

[a]Departamento de Bioquímica, Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego, s/n, Cidade Universitária - CEP: 50670-420 - Recife, PE - Brazil.

[b]Biotério do Laboratório de ImunopatologiaKeizoAsami, UFPE, Av. Prof. Moraes Rego, s/n, Cidade Universitária - CEP: 50670-901 - Recife, PE - Brazil.

[c]Departamento de Odontologia Preventiva, UFPE, , Av. Prof. Moraes Rego, 1235, Cidade Universitária - CEP: 50670-901 - Recife, PE - Brazil.

(*) Correspondingauthor: Phone: +55.81.21268547; Fax: +55.81.21268576

E-mail address: mgcc@ufpe.br (M.G. Carneiro-da-Cunha)

Abstract

This paper aimed to evaluate the wound healing of skin lesions in Wistar rats, using a hydrogel based on polysaccharides, policaju (from *Anacardiumoccidentale* L. gum), and chitosan, being this hydrogel termed POLI-CHI, combined or not with Low level laser therapy (LLT). Lesions were made on the dorsal region of 45 male, assigned into three groups: 0.9% (w/v) NaCl Control (C); POLI-CHI hydrogel (H) and POLI-CHI and LLLT (HL). Macroscopic evaluations were carried out using clinical observations and area measurements and microscopic by histological criteria. H and HL presented more esthetical scar tissue, and larger wound area regression, statistically significant at 7 and 14 days in comparison to C. Histopathological analyzes showed: more intense fibrin-leucocite crust in HL; larger collagen presence in H and HL; minor presence of focal necrosis at 7 and 14 days in H; minor neutrophilic exudate in H and HL; regression of the vascular neoformation at 7 days in H, and modulation of the same in HL. These results demonstrated that POLI-CHI contributed to more efficient healing process and modulation of the inflammation, larger collagen presence, minor focal necrosis, and regression of vascular neoformation, furthermore, the combined use with LLLT subtle potentiated this process.

Key-words: Hydrogel, Chitosan, Policaju, Laser, Wound Healing.

1. Introduction

Concerning to the pathophysiology of the tissue repair, skin wounds heals basically in four phases or periods: hemostasis, inflammatory, proliferative and remodelative, and in chronic wounds, the natural progression is affected, causing the absence or slowing of this repair process[1]. There is available for clinical use, a variety of wound treatments, each, acting more effectively in specific lesions, being: polysaccharide[2], chitosan films[3], antibiotic impregnated gauzes[4], silicon based non adherent bandages[5], Karaya gum hydrocolloid[6], calcium alginate solutions[7], silver nanoparticles exudate absorbents[8], silicone gel[9], cyanoacrylate haemostatic spray[10], and chitosan hydrogels[2].

Regarding wound treatments based on polysaccharides, may be highlighted the use of one obtained from the gum of *Anacardiumoccidentale* L. tree (policaju), it has been studied and has shown satisfactory results in wound healing, anti parasitic and anti tumors effects[11], as well as chitosan, a polysaccharide derived from the chitin by deacetylation, that also has being investigated by the scientific community for biomedical and therapeutic applications, showing excellent biocompatibility, biodegradability, low toxicity, hemostatic, healing properties, and antimicrobial activity[12,13]. Another property that can generally be found in polysaccharides is its ability to form hydrogels or crystals in solutions[14].

Rheologically, aqueous solutions of hydrophilic polymers in low to moderate concentrations typically exhibit a Newtonian behavior, although, once the cross-links between different polymer chainsare introduced, the “nets” start to show a visco-elastic and sometimes even a purely elastic behavior[15,16], which is the basic principle of the hydrogels. They are defined as three-dimensional polymer nets capable of absorbing large amounts of water or biological fluid, being used to preserve cells, nutrients, drugs or proteins, and also represents a drug delivery system class that has excellent intelligent drug delivery[17].Due to its physicochemical similarities with the organic matrix, such as high water content, and in the case of the ones based on carbohydrates, its composition and mechanical aspects, the hydrogels are generally biocompatible[18]. In addition, hydrogels are relatively deformable and can adapt to the shape of the surface to which they are applied. In this context, the properties of mucus or bioadhesiveness of some hydrogels can be advantageous to immobilize them at the application site, even if the topical surface is not horizontal[15].

Another therapeutic modality used in wound healing is based on the light itself. The low level laser therapy (LLLT) is a treatment that applies a monochromatic, intense, coherent and collimated light, whose emission of radiation is done by stimulating the external field. This therapy acts causing several biological effects, such as: in behavior of lymphocytes, increasing their proliferation and activation; on macrophages, increasing phagocytosis; and on fibroblasts, increasing the secretion of growth factors and enhancing the uptake of both fibrin as collagen. In addition, it contributes to increase the motility of epithelial cells, the amount of granulation tissue and may reduce the synthesis of inflammatory mediators[19].

Concerning combined therapies, they are often used to cause a synergistic effect and in reducing the frequency of the drug/ treatment applied, thus, minimizing adverse effects[20]. As an example, may be mentioned a study carried out by Dantas et al.[21] which used the combination of laser therapy and sodium alginate/chitosan-based dressing for induced burn wounds in rats, obtaining a synergic effect, apparently by modulating the epithelisation, blood vessels formation and collagenization processes.

Based on what was previously exposed, the aim of this study was to evaluate the healing process of skin wounds induced in Wistar rats treated primarily with a hydrogel based on two polysaccharides, policaju and chitosan, termed (POLI-CHI), previously studied and characterized by this research group[22], which presented good perspectives for biomedical use, also, was accessed the combined use with LLLT.

2. Matherials and Methods

2.1 Matherials

Polysaccharide from *Anacardiumoccidentale* L. tree gum (collected in South coast of Pernambuco, Brazil) was obtained according to Souza et al.[23] and termed policaju. The chitosan (deacetylation> 75%) was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2 Hydrogel preparation

The preparation of the hydrogel composed of chitosan and policaju in a ratio of 1:4, respectively, was performed according to Soares et al.[22]. Briefly, stock solutions of 10% (w/v) of policaju and 1% (w/v) of chitosan dissolved in 1% lactic acid (v/v), were prepared in advance. In a separated beaker was added a known volume of chitosan solution plus 200 µL of 0.1 M $CaCl_2$ and kept under stirring in a Ultra-Turrax (IKA, USA) at 7000 rpm for 20 min to form a chitosan pre-gel, and then immediately after using a 27 G syringe and a flow of 1 ml/min was added a known volume of policaju solution. The mixture was left under stirring (7000 rpm) for 20 min. The pH was adjusted to 5.0 with 1 M NaOH solution and called pre-gel. The filmogenic solution was distributed in petri dishes and kept in an oven at 40 ^{o}C for 16 h for drying and polymerization, and then it was hydrated with distilled water and termed POLI-CHI hydrogel, and stored under refrigeration at 4 ^{o}C.

2.3 Animals and treatment groups

Forty-five male rats of the Wistar strain (*Rattusnorvegicus*) [90-120 day-old, weighing 250- 300 g] were submitted to experimental surgical procedures, being anesthetized intraperitoneally with 2 % (w/v) of xylazine hydrochloride and 10 % (w/v) ketamine hydrochloride at 1:1 ratio. The antisepsis of dorsal thoracic region was made using 1 % (w/v) povidone-iodine and 0.9 % (w/v) NaCl sterile solutions. Full thickness circular surgical wounds (Ø = 0.8 cm) were made in the skin using a biopsy punch and a scalpel blade number 15. The tissue divulsion was performed using Metzenbaum and Iris scissors. After the surgery the animals were randomly divided into three groups (n = 15) according to the treatment: (C) Control, 0.1 ml of 0.9% (w/v) NaCl; (H) POLI-CHI hydrogel and (HL) POLI-CHI hydrogel and LLLT irradiation. After the surgical procedures they were placed in isolated cages. The light-dark cycle was of 12 h, beginning the brightly one at 6 h am. The environment temperature was set at 23 ± 1 ^{o}C and the water and food (ration) was *ad libitum*. All animal procedures were in accordance with the ColégioBrasileiro de Experimentaçãoem Animal (COBEA) and the Animal Ethical Committee of the Universidade Federal de Pernambuco approved the experimental protocol n^{o} 23076.050933/2012-10. The groups H and HL received daily application of 0.1 ml of hydrogel for dressing, after the laser irradiation if it applies, and the group C received saline solution of 0.1M NaCl. In the matter of the laser irradiation, the animals that were subjected to LLLT (group HL) were irradiated in a punctually way starting from the center of the wound with Therapy XT (DMC medical, USA). The irradiation was carried out after surgery and at a 48 h interval until the euthanasia time. The irradiation parameters used were: λ = 660 nm, A = 1 cm^2; ED = 4 J/cm^2, P = 100 mW, F = 50 Hz. The exposure mode was continuous through optical fiber, and the application was performed at 2 mm from the skin.

2.4 Macroscopic evaluation

After the surgical procedures, the animals were clinical evaluated daily according to the presence of the following criteria: edema, hyperemia, presence of exsudate, crust, detachment and reepithelialization. The wound area calculus was made using for measure a decimal digital caliper, Neiko 01407A (USA), and the equation: A = (πab), where (A) the area, (a) the minor ray and (b) the large ray. The regression wound (mm^2) was defined by the difference between the initial and the final area.

2.5 Euthanasia and histological processing

Five animals from each group were sacrificed after 3, 7 and 14 days after the surgical procedure. They were subjected to lethal doses of sodium tiopental (200 $mg.Kg^{-1}$), and skin fragments are collected with a wide margin (± 1 cm) from the original lesion and storaged in 10 % (v/v) formalin, according to Michalany[24]. The histological specimens were included in paraffin and after microtome cut, the sections were stained using hematoxylin-eosine (HE), for cellular observation, and picrosirius (PS) for collagen fibers.

2.6 Microscopic evaluation

Regarding light microscopic evaluation, the microscopy slides were analyzed using histological criteria in order to evaluate the presence and intensity, such as: Fibrin-leukocyte crust, Collagen, Focal necrosis, Fibrin deposits, Neutrophilic exudates, Edema, Eosinophilic exudates, Mononuclear infiltrate, Macrophage infiltrate, Granuloma, Neovascularization, Fibroblast proliferation and Fibrosis.

2.7 Statistical analysis

Concerning statistical analysis, were applied the method of analysis of variance (ANOVA) and Bonferroni's multiple comparison test. The statistical significance was set at 5% ($p<0.05$) and the software used for data entry and processing was the Graphpad Prism for Windows, version 5.0 from Graphpad Software, Inc.

3. Results and Discussion

3.1 Macroscopic evaluation

Regarding clinical observation, from the first to the third day after surgery, all studied groups presented edema, hyperemia and crust, although, was found that the HL group showed a thicker and dry crust in comparison to other groups with absence of exudate. From the 4^{th} to the 6^{th} day, all groups still presented Crust, being Edema and Hyperemia absente in all groups. At the 6^{th} day, the HL group started to lose its crust, characterizing the detachment. From 7^{th} to the 9^{th} day, all groups presented a similar pattern with crust, detachment and reepitelization. From the 10^{th} to 12^{th} day, detachment and reepitelization, and from the 13^{th} to 14^{th}, just reepitelization (Fig. 1).

- Insert Figure 1 -

According to Soon & Acton[25] animals subjected to stress and pain had a poor wound healing process, so, must be highlighted that in our study, one day after the surgical procedures, the animals from HL group started to feed and drink, while in the other groups it was found only in the second day, which may indicate that this animals were feeling, at least, a minor amount of post-operatory pain than those of other groups. This may be due to the analgesic properties of LLLT, being corroborant of the findings of Pozza et al.[26], once they identified pain relief patterns comparing Laser irradiated animals with control.

As may be observed in Figure 1, the scar tissue formed after the surgical procedure in experimental groups was much reduced in comparison to control and its coloration was paler and more similar to mature surrounding tissue, evidencing H and HL groups at 14 days post operatively. This findings are in accordance with other studies, such as those that evaluated the reduction of hypertrophic scars in human patients carried out by Moravvaej et al.[27] and Avci et al.[28] using LLLT, although, in our research, the group treated with just POLI-CHI hydrogel presented the same pattern than the combined modality treatment (HL), which may suggest a modulation in the scar tissue aided by the hydrogel. This finding remit to a study carried out by Zhanget al.[29], which evaluated the action of a sulfated anionic polysaccharide in reduction of scar tissue and inflammation that obtained clinical improvements in rosacea skin disease treatment, this may suggest a positive influence of one or both polysaccharides of the hydrogel.

Concerning the evaluation of the regression of the wounds using a digital caliper, at the third day post surgery, as can be seen in Figure 2, the H group presented the high arithmetical mean (44.1475 mm^2 ± 1.954 SEM – Standard error of mean), followed by HL (38.3765 ± 5.231) and C (31.4699 mm^2 ± 3.532) . Despite the predominance of the H group, there was no statistical significance.

- Insert Figure 2 -

Observing the evaluation at the seventh day the HL group surpasses the H group by a very little difference (66.3406 ± 2.006 and 66.1515 mm^2 ± 1.596, respectively), being both statistically significant in comparison to C group (31.2894 mm^2 ± 6.613). In the evaluation carried out at 14 days after surgery, maintained statistical significance in all experimental groups in comparison to Control, being the best result the HL group followed by H and C groups (75.5008 ± 0.6630, 73.9520 ± 0.5315, and 66.0442 mm^2 ± 1.7520, respectively). As may be observed in Figure 2, the experimental groups presented a similar trend for repair and wound regression among them. This findings corroborate with others studies that suggests that use of polysaccharide, such as chitosan[3,30] and policaju[2,11] that presented themselves as efficient healing agents, and the use of LLLT in the improvement of the wound regression and healing[28,31]. Thus, may be emphasized that the use of POLI-CHI significantly stimulated the wound regression, being the Laser irradiation optative, once the perceived improvements in wound regression were discrete.

3.2 Microscopic evaluation

Among the evaluated histological criteria, the presence and intensity of several items were observed. The main objectives of this analysis were to delineate a histological overview of the subjects and to compare the different findings in a temporal and effectiveness fashion.

The fibrin-leukocite crust is one of the most important criteria, once is responsible for keeping the wound environment humid and protected from external aggression. Evaluating the groups at the 3rd day post-operatively, was perceived a larger amount of crust in HL group in comparison to H and C groups (Figure 3A), but at the 7th day happened a inversion, presenting the HL group with a weak presence.

This may suggest, as occurred in clinical evaluation, an early detachment of the crust by the borders, being so a hint of acceleration of the wound healing process. At the 14th day, there was not fibrin-leukocyte crust in any evaluated specimen. Similar results were found by Pinheiro et al.[31], including the early detachment, beginning by the extremities of the lesion until the total substitution by scar tissue in groups treated with LLLT.

A very important criteria that follows is collagen presence, initially, it presented similar results in all groups at the 3th day, presenting itself scarcely in all groups, evolving to moderately present at 7 days in groups H and HL (Figure 3B), diverging from C group that keep unshaken, and in the 14th day evaluation all groups presented similar results. The data suggesting early collagen formation in both experimental groups (H and HL), which may imply a modulation of the repair process. Another aspect that emerged was the thickening of collagen arrange in experimental groups in comparison to C group. This may be due to an increase in collagen fiber maturation aided by the healing agents. This data goes in accordance with other studies that evaluated different healing agents[32,33].

- Insert Figure 3 –

Evaluating focal necrosis, at the 3th day after surgery, the HL group presented a minor amount (scarcely) in comparison to groups H and C, which presented it in a moderate form. At the 7th day the H group do not presented any sign of focal necrsosis differing from HL and C groups that presented it in a scarcely form. This regression pattern in H group may suggest an improvement in the healing process and phagocytosis of the necrotic loci modulated by the POLI-CHI hydrogel, and the possibility of a negative stimulus in this period caused by the LLLT irradiation, once the focal necrosis persisted in the new formed matrix, which goes against the work of Neves et al.[34] that had positive results regarding reduction of necrosis areas in rat's skin flaps using LLLT.

The macrophage presence is fundamental to the repair process, being the main effector cell, degrading and removing components of damaged connective tissue, as collagen, elastin, and proteoglycans, and beyond this role in phagocytosis of cell fragments, they also secrete chemotactic factors which attract other inflammatory cells to the site of wound and produce prostaglandins, which act as potent vasodilators, affecting the permeability of microvessels[35]. At the 3rd day evaluation, all groups presented macrophage infiltrate in a scarcely way, being the H group lightly superior. At the 7th day, H and HL groups presented in an almost absente way, and in C group no presence was found. This may indicate the necessity to phagocytosis involving the hydrogel remaining.

The presence of Edema is a flogistic sign that must be thorough evaluated. In HL group it was found strongly present, and moderated in H and C groups at the 3rd day, and at the 7th day it showed almost absente in H and HL and scarcely at C groups. At the 14th day it was completely absent in H and HL and still scarcely present in C groups. This may indicate an elongating of the wound repairing process in C group, which may suggest a modulation and controlling of the inflammatory process, as indicated by Sezer et al.[36], concerning a chitosan hydrogel, and Lima et al.[37], regarding LLLT.

Evaluating the Neovascularization criteria, at the 3rd day, was found minimal variation between groups, all of them appearing scarcely present. At the 7th day, occurred a major variation in group C that showed it strongly present, while in H group the presence was moderated, and in group HL was found in a scarcely form. It may suggest that the vasculature regression after the inflammatory stage was stimulated in treated groups, which may be the contributive factor for the tissue maturation, emphasizing the H group, once it presented improvement in the neovascular formation in the early stage and regression at the proliferative stage, suggesting a modulatory effect.

Regarding the light microscopic evaluation, when observing the 3rd Day specimens, may be found a more prevalent collagen distribution in groups H and HL in comparison to C group, once the collagen fibers presented a more homogeneous distribution while in control it was found basically in the peripheral region, and a major formation of granulation tissue within the defect (Figure 3A). Evaluating at the 7thday was found a pattern of thicker collagen fibers in H and HL groups, which may indicate an early maturation and healing modulation. At the 14th day, all groups presented closed wounds, but must be highlighted the contraction present in some specimens of H group (Figure 3C), being an important factor for the validation of the healing properties of the POLI-CHI hydrogel.

4.0 Conclusions

In conclusion, the collected data showed that the POLI-CHI hydrogel contributed for a most effective wound healing and modulation of the inflammatory process, statistical significance regarding wound regression, larger collagen presence, minor focal necrosis, early epithelizathion, and additionally, the combined use of POLI-CHI hydrogel with LLLT was able to subtle improve this process.

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Figure Legends

Figure 1.Macroscopic aspects of the induced lesions by the time of evaluation, using for treatment: (C) Control, 0.1 M NaCl, (H) POLI-CHI, (HL) POLI-CHI + LLLT.

Figure 2.Evaluation of regression in wound area by time. Comparison of the Arithmetical Mean (AM) and Standard Error of Mean (SEM) between experimental groups and control using the method of analysis of variance (ANOVA) and Bonferroni's multiple comparisons test. There was found statistical significance (*) between groups H and HL in comparison to C at 7 and 14 days ($p < 0.05$).

Figure 3. Light microscopy of stained specimens (Picrosirius – PS and Hematoxilin-Eosin – HE). A) 3rd day, PS, 4x, from HL group; B) 7th, PS, 4x, from H group; C) 14th , HE, 4x, from H group; where: ANE – Dermal Annexes; BOR – Mature Border; COL – Collagen Fibers; CRU – Fibrin Leukocyte Crust; GRA – Granulation tissue.

Figures

Figure 1.

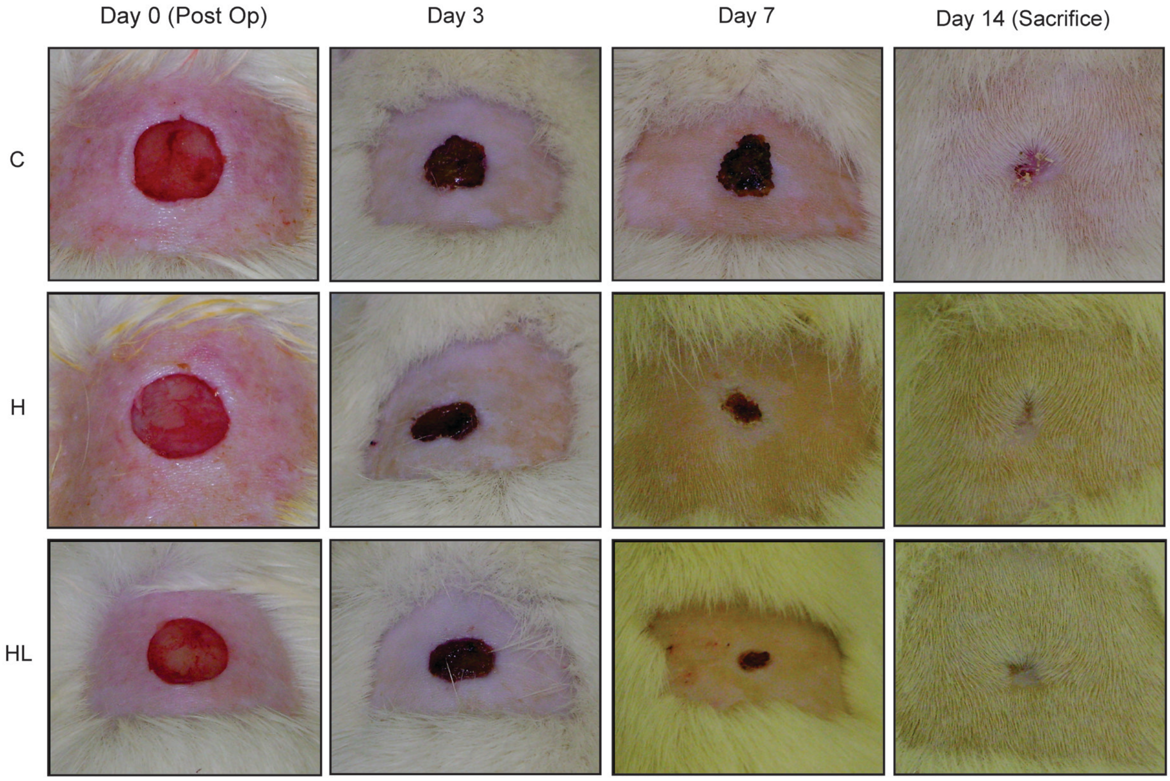
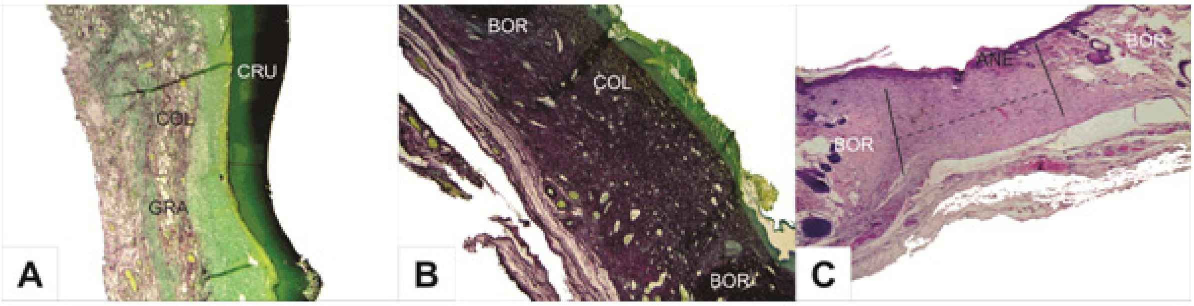


Figure 2.

Groups:	AM:	SEM:
C3	31.4699	±3.532
H3	44.1475	±1.954
HL3	38.3765	±5.231
C7	31.2894	±6.613
H7	66.1515*	±1.596
HL7	66.3406*	±2.006
C14	66.0442	±1.7520
H14	73.9520*	±0.5315
HL14	75.5008*	±0.6630

Figure 3.



6. Conclusões

Os resultados obtidos permitem concluir, que:

- O tratamento utilizando o hidrogel a base dos polissacarídeos POLICAJU e Quitosana (POLI-CHI) contribuiu para a cicatrização de ferimentos cutâneos induzidos em ratos Wistar, bem como na modulação do processo inflamatório. Os animais apresentaram cicatrizes mais estéticas, maior regressão da área da lesão, maior formação de collageno, menor presença de necrose focal, menor presença de exudato neutrofílico, menor presença de edema, e regressão da neoformação vascular.

- O tratamento utilizando o POLI-CHI associado à Laserterapia de baixa intensidade (Low level laser therapy – LLLT) atuou potencializando o reparo desencadeado pelo hydrogel, bem como induzindo um maior conforto pós-operatório com sinalização de atividade analgésica e aceleração dos estágios cicatriciais.

- Tendo em vista o que foi exposto, o POLI-CHI acrescido ou não da LLLT atuou de forma a estimular a cicatrização, e havendo a possibilidade do tratamento combinado, este deve ser indicado por atuar de forma sinérgica.

Anexo A

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2. Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990. 1223 p.

3. Gilmor ML, Rouse ST, Heilman CJ, Nash NR, Levey AI. Receptor fusion proteins and analysis. In: Ariano MA, editor. Receptor localization. New York: Wiley-Liss; 1998. p 75-90. Please note that journal title abbreviations should conform to the practices of Chemical Abstracts.

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Anexo B

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Av. Prof. Nelson Chaves, s/n
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Recife, 02 de abril de 2013.

Ofício nº 552/13

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Prof. Maria das Graças Carneiro da Cunha**
Universidade Federal de Pernambuco
Departamento de Bioquímica
Processo nº 23076.050933/2012-10

Os membros da Comissão de Ética no Uso de Animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEUA-UFPE) avaliaram seu projeto de pesquisa intitulado, **"Avaliação da Cicatrização de Feridas Cutâneas com a utilização de Hidrogel de Quitosana e Policaju associado a Laser Terapêutico".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEUA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 11.794 de 08 de outubro de 2008, que trata da questão do uso de animais para fins científicos e didáticos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais a serem realizados.

Origem dos animais: Biotério de criação do Departamento de Nutrição/UFPE. Animais: ratos Wistar (Rattusnovergicus); Peso: 250-300g; sexo: machos; idade: 90 a 120 dias; nº total de animais: 60 ratos.

Atenciosamente,