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Efeito inibitório de micropartículas de vidro bioativo dopado com prata sobre formas promastigotas de *Leishmania amazonensis* e *Leishmania braziliensis*

Emanuene Galdino Pires

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do título de Mestre em Odontologia – Área de Concentração em Ciências Odontológicas.

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EMANUENE GALDINO PIRES

EFEITO INIBITÓRIO DE MICROPARTÍCULAS DE VIDRO BIOATIVO DOPADO COM PRATA SOBRE FORMAS PROMASTIGOTAS DE *LEISHMANIA ARMAZONENSIS* E *LEISHMANIA BRASILIENSIS*

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DEDICATÓRIA

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“Talvez não tenhamos conseguido fazer o melhor, mas lutamos para que o melhor fosse feito. Não somos o que deveríamos ser, não somos o que iremos ser. Mas graças a Deus, não somos o que éramos!”

Martin Luther king

NOTAS PRELIMINARES

A presente Dissertação foi redigida conforme o Manual para Normatização da Defesa do Trabalho Final proposto pelo Programa de Pós-Graduação em Odontologia da Universidade Federal da Paraíba, adotando o formato alternativo (Anexo A). Um artigo científico compõe este trabalho de Dissertação, o qual foi redigido de acordo com as exigências da revista a ser enviado (Plos One*).*

RESUMO

A leishmaniose é uma doença parasitária infecciosa causada por várias espécies de protozoários do gênero *Leishmania* que acomete o homem e diferentes espécies de animais, induzindo lesões mucocutâneas. O objetivo deste estudo foi avaliar o efeito inibitório de micropartículas de vidro bioativo dopado com prata sobre formas promastigotas de *Leishmania amazonenses* (*L.Amazonensis*) e *Leishmania braziliensis* (*L. braziliensis*). Micropartículas de vidro bioativo puro (*Bioactive Glass*-BG) e dopado com prata (BGAg1 e BGAg2) pertencentes ao sistema $58SiO_2 \cdot (36-x)CaO \cdot 6P_2O_5 \cdot xAg_2O$ com x = 0, 1 e 2 mol%, respectivamente, foram sintetizadas através do método sol-gel e caracterizadas por Microscopia Eletrônica de Varredura (SEM), Análise Termogravimétrica (TGA), Difratometria de Raios-X (XRD) e Espectroscopia de Adsorção no Infra-Vermelho com Transformada de Fourier (FTIR). A citotoxicidade do BG e BGAg em células humanas foi avaliada a partir da resposta de células de adenocarcinoma pulmonar da linhagem A549. As culturas de formas promastigotas de *L. Amazonensis* e *L. braziliensis* foram expostas às amostras de BG, BGAg1 e BGAg2 por 24h. Em seguida, 0,5 mM de resazurina foi adicionada à cultura e leituras de absorbância foram realizadas após 48, 72 e 96h de incubação. O percentual de redução da resazurina por grupo foi obtido a partir de uma calculadora (ABD Serotec®) e as formas promastigotas viáveis foram contadas em câmara de Neubauer. As imagens da SEM mostraram partículas micrométricas com superfície irregular e porosa. A TGA demonstrou, para as amostras com prata, um pico endotérmico em torno de 497ºC associado à decomposição do óxido de prata. Os padrões de XRD exibiram características essencialmente amorfas correspondentes ao BG. As curvas de FTIR revelaram os principais modos vibracionais, incluindo: vibração assimétrica de alongamento do Si-O-Si (1000-1200 cm^{-1}), vibração simétrica de alongamento do Si-O-Si (750-800 cm^{-1}), modo de flexão do Si-O-Si (450-480 cm^{-1}) e flexão assimétrica do PO_4^{-3}(570-600 cm^{-1}). No que se refere à citotoxicidade, o BGAg mostrou comportamento não tóxico. O BGAg1- 300µg/mL inibiu *L. amazonensis* e o BGAg2- 300µg/mL inibiu *L. braziliensis* com 97,6% e 92% de eficácia, respectivamente. A contagem em câmara de Neubauer confirmou a eficácia do BGAg2 nas concentrações de 150 e 300µg/mL revelando a ausência de células viáveis. Em conclusão, o BGAg2- 150 e 300 µg/mL inibiu o crescimento e proliferação de *L.amazonensis* e *L. braziliensis* na forma promastigota e poderia ser utilizado em outros estudos, como investigações *in vivo,* que são necessários para verificar a atividade do BGAg nas formas amastigotas de *Leishmania*.

Palavras- chave: Leishmaniose, Prata, Vidro Bioativo, Síntese Sol-gel.

ABSTRACT

Leishmaniasis is a parasitic infectious disease caused by several species of protozoa of the genus *Leishmania* affecting humans and different animal species, inducing mucocutaneous lesions. The purpose of this study was to evaluate the inhibitory effect of silver doped bioactive glass microparticles over promastigote forms of *Leishmania amazonensis* (*L.Amazonensis*) and *Leishmania braziliensis* (*L. braziliensis*)*.* Microparticles of bioactive glass (BG) and doped with silver (BGAg1 and BGAg2) belonging to the system $58SiO_2{\cdot}(36\text{-}x)CaO{\cdot}6P_2O_5{\cdot}xAg_2O$ with x = 0, 1 and 2 mol%, respectively were synthesized via sol–gel method and characterized by Scanning Electron Microscopy (SEM), Thermal Gravimetric Analysis (TGA), X-Ray Diffraction (XRD) and Fourier Transform Infrared Espectroscopy (FTIR). The cytotoxity of the BG and BGAg in human cells was assessed from the response of A549 lung adenocarcinoma ephitelial cells line. *L.Amazonensis* and *L. braziliensis* promastigote cultures were exposed to BG, BGAg1 and BGAg2 for 24h. Then, 0.5 mM resazurin was added to culture and absorbance readings were assessed after 48, 72 and 96 hours of incubation. The percentage of resazurin reduction per group was obtained from a calculator (AbDSerotec®) and viable promastigote forms were counted on Neubauer chamber. SEM images showed micrometric particles with irregular and porous surface. TGA analysis demonstrated, for samples doped with silver, an endotermic peak around 497ºC associated to silver oxide decomposition. The XRD patterns exhibit mainly amorphous characteristics corresponding to BG. FTIR curves revealed main vibrational modes including Si-O-Si asymmetric stretching vibration (1000-1200 cm^{-1}), Si-O-Si symmetric stretching vibration (750-800 cm^{-1}), Si-O-Si bending mode (450-480 cm^{-1}) and PO_4^{-3} antisymmetric bending (570-600 cm^{-1}). Reffering to cytotoxicity, BGAg showed non-toxic behavior. BGAg1-300µg/mL inihibited *L. amazonensis* and BGAg2 300 µg/mL inihibited *L. braziliensis* with 97,6 % and 92% of efficacy, respectively. The count on Neubauer chamber confirmed the efficacy of BGAg2 in 150 e 300 µg/mL concentrations revealing absence of viable cells. In conclusion, the BGAg2- 150 and 300 µg/mL inhibited the growth and proliferation of *L.amazonensis* and *L. braziliensis* on promastigote form and could be used in other studies, like *in vivo* investigations that are necessary to verify the BGAg ativity on *Leishmania* amastigotes forms.

Keywords: Leishmaniasis, Silver, Bioactive glass, Sol-gel synthesis.

LISTA DE ABREVIATURAS E SIGLAS

BG-Bioactive Glass

BGAg- Silver doped bioactive glass

CaO– Calcium oxide

DNA – Desoxyribonucleic acid

XRD- X-Ray Diffraction

FBS- Fetal bovine serum

FTIR - Fourier Transform Infrared Espectroscopy

L.-*Leishmania*

LTA- Leishmaniose Tegumentar Americana

Min- Minutes

mL– Mililiter

Na_2O - Sodium oxide

pH - Hydrogenionic potential

P_2O_5 - Phosphorus pentoxide

RNA-Ribonucleic acid

SiO_2–Silicon dioxide

TCP - Tricalcium phosphate

TEOS- Tetraethylorthosilicate

TEP - Triethyl phosphate

TGA - Thermal Gravimetric Analysis

SEM - Scanning Electron Microscopy

µg- Microgram

ºC – Celsius

µm-Micrometer

mM-Milimolar

SUMÁRIO

1. INTRODUÇÃO

A leishmaniose é uma doença parasitária causada por protozoários do gênero *Leishmania*, que acomete o homem e diversas espécies animais silvestres e domésticos. Pode manifestar-se em diferentes formas clínicas, como a Leishmaniose tegumentar americana (LTA) que representa um problema de saúde pública (CARRION *et al*, 2008) e se apresenta clinicamente como lesões na forma de úlceras, nódulos, pápulas ou placas nas partes expostas do corpo ou membranas mucosas (MEARS *et al*, 2015).

A LTA é endêmica em 88 países e afeta cerca de 12 milhões de pessoas (WHO/TDR, 2013). No Brasil, no período de 1985 a 2005, verificou-se uma média anual de 28.568 casos autóctones registrados e coeficiente de detecção médio de 18,5 casos/100.000 habitantes (BRASIL, 2010). As principais espécies de *Leishmania* responsáveis pela transmissão da LTA no Brasil são *L. braziliensis* e *L. amazonensis*. A *L. amazonensis* está associada à leishmaniose cutânea difusa, enquanto a *L. braziliensis* causa lesões cutâneas e está relacionada à leishmaniose mucosa, sendo esta condição de difícil tratamento e prognóstico regular quanto à possibilidade de cura. A transmissão da doença para o homem se dá através da picada das fêmeas de flebotomíneos infectadas com *Leishmania* (WHO/TDR, 2013).

O trato respiratório, nariz e cavidade oral são os sítios iniciais mais frequentes da leishmaniose mucosa. Na cavidade oral, esta ocasiona danos como perdas dentárias e obstrução respiratória. As lesões são destrutivas e podem acometer membranas mucosas do nariz, mucosa jugal, lábio, palato (duro e mole), orofaringe e laringe (STRAZZULLA *et al*, 2013).

A *Leishmania* se apresenta durante o seu ciclo de vida nas formas promastigota e a amastigota. A forma promastigota é encontrada no tubo digestivo do vetor, sendo esta, a principal responsável pela transmissão da doença, enquanto a forma amastigota é encontrada no hospedeiro vertebrado. Os macrófagos constituem o reservatório da LTA e são as primeiras células a serem infectadas após a inoculação do parasita na da pele (NEVES *et al*, 2011).

O antimonial pentavalente é a base terapêutica no tratamento da leishmaniose e começou a ser utilizado em 1912, sendo que a formulação corrente

tem sido usada desde 1945. São utilizados dois antimoniais pentavalentes: o Estibogluconato de Sódio (Pentostan do Wellcome Foundation, London, UK) e o Antimoniato de N-metilglucamina (Glucantime do Rhone Poulenc, Paris, France) (VRIES, 2015).

No Brasil, o fármaco de primeira escolha é o Glucantime. Esse medicamento, no entanto, apresenta dificuldades de administração e alto custo. Os antimoniais pentavalentes, no geral, apresentam importantes efeitos colaterais que incluem mialgia, artralgia, aumento sérico das enzimas hepáticas, pancreatite, disfunção gastrintestinal, dores musculares difusas, enrijecimento das articulações, arritmias, pancitopenia, insuficiência renal reversível e cardiotoxicidade (SUNDAR; OLLIARO, 2007).

A miltefosina (hexadecilfosfocolina), assim como a Anfotericina B, têm sido usadas como drogas de segunda escolha nos casos de resistência aos antimoniais (CONTI, PINTO JUNIOR, 2015; SILVA-LÓPEZ, 2010). Esses medicamentos, no entanto, também apresentam toxicidade elevada e são ineficazes em muitos casos (TEIXEIRA *et al*, 2013).

Assim, apesar da existência de tratamento para a leishmaniose, muitas desvantagens limitam o uso das drogas de escolha (MORAIS-TEIXEIRA *et al*, 2013). Apesar do contínuo desenvolvimento de pesquisas relacionadas às doenças negligenciadas, os resultados obtidos nem sempre se revertem em avanços terapêuticos. Uma das razões para esse quadro é o baixo interesse da indústria farmacêutica nesse tema, justificado pelo reduzido potencial de retorno lucrativo para a indústria, uma vez que a população atingida é, em sua maioria, de baixa renda (MINISTÉRIO DA SAÚDE, 2010).

Diante disso, o desenvolvimento de novas terapias e drogas para o tratamento da leishmaniose ainda são uma necessidade urgente (FREITAS-JUNIOR *et al*, 2012).

Na ciência médica atual os biomateriais desempenham um papel importante, substituindo órgãos, tecidos e modificando as suas funções. Entre eles, os vidros bioativos estão sendo usados nas áreas de odontologia e ortopedia (BALAMURUGAN *et al*, 2008).

O vidro bioativo (*Bioactive Glass* -BG), descrito inicialmente por Hench, é composto principalmente por dióxido de silício (SiO_2), óxido de cálcio(CaO), pentóxido

de fósforo(P_2O_5) e óxido de sódio (Na_2O)(HENCH *et al*, 1971). A sua estrutura consiste em uma rede de SiO_2, tendo o P_2O_5 como coadjuvante e o CaO e o Na_2O como modificadores (SHIRTLIFF; HENCH, 2003). O BG tem como principal método de produção o processamento Sol-Gel que consiste na hidrólise do tetraetilortosilicato $Si(OC_2H_5)$ em meio ácido, produzindo SiO_2 na forma semelhante ao vidro (HENCH; WEST, 1990). A bioatividade desse material concedeu sua aplicação no campo da regeneração e engenharia tecidual (JONES *et al*, 2006; SHIRTLIFF; HENCH, 2003).

Dentre as aplicações do BG, pode-se citar o uso como: enxertos para preenchimento de espaços corpóreos (GOH *et al*, 2007), revestimento para implantes ósseos (AL-NOAMAN *et al*, 2013a; AL-NOAMAN *et al*, 2013b) arcabouço para reparo tecidual com arranjo poroso semelhante ao trabeculado ósseo (JONES *et al*, 2006). Materiais bioativos, como o BG, podem desenvolver no osso ligações químicas capazes de permitir a integração do biomaterial à área receptora (AL-NOAMAN *et al*, 2013b).

O emprego do BG vai além dos tecidos duros. Pesquisas apontam para notáveis propriedades em tecidos moles. Ostomel *et al* (2006) observaram uma diminuição do tempo de coagulação sanguínea induzida pela presença de microesferas porosas de BG. Esse material também pode ter atividade na angiogênese, o que é importante para a cicatrização de feridas em tecidos moles (HATCH *et al*, 2014; HANDEL*et al*, 2013). A indução do reparo tecidual pelo BG 58S (SGBG-58S), BG em escala nanométrica (NBG-58S) e do derivado do BG 45S5 pode ser constatada no estudo de Lin *et al* (2012), onde feridas em ratos tratadas com esse material tiveram o tempo de cicatrização reduzido quando comparadas ao controle. Esse resultado sugere a possibilidade de uso do BG para facilitar o processo de cicatrização de ferimentos em pele, como é o caso das lesões ocasionadas pela leishmaniose.

O BG pode, ainda, ser conjugado à Prata (Ag), conferindo-lhe propriedades antimicrobianas. Day *et al* (2005b), utilizando esferas de sílica porosas produzidas em passo único com brometo de cetiltrimetilamônio e conjugadas ao $Ca(NO_3)_2$ e ao $AgNO_3$, observaram efeito antimicrobiano sobre *E. coli* e *S. aureus*. Os ensaios de Zhu *et al* (2014) constataram que o BG poroso 58S (SM58S), preparado a partir da modificação superficial do BG poroso 58S (M58S) pelosilano KH-550 e carregado com

íons de Ag, também tiveram significante atividade antibacteriana contra *E. coli* e *S. aureus.* No entanto, o mesmo efeito não foi observado pelo BG com ausência de prata.

Alguns trabalhos (ALLAHVERDIYEV, 2011; BAIOCCO *et al*, 2010; MOHEBALI *et al*, 2009) também relataram a eficácia da Ag como agente leishmanicida. O mecanismo pelo qual o ion prata exerce a sua toxicidade contra espécies de *Leishmania* não é bem esclarecido, mas sabe-se que em bactérias, sua atividade antimicrobiana pode estar relacionada com sua ligação ao DNA, à interação com componentes celulares e à interferência com o transporte de elétrons, pois íons Ag reagem com carboxilatos de proteína, hidroxilos e tióis (BALAMURUGAN *et al*, 2008; BAIOCO *et al*, 2010). A ação de ions Ag+ deriva da sua ligação com proteínas microbianas carregadas negativamente impedindo a sua replicação. Além disso, podendo se fixar a grupos sulfidrila, inibindo assim sua proliferação (HOLT; BARD, 2005).

Navarro *et al* (2006) demonstraram que os complexos de prata polipiridilpaládio são biologicamente ativos contra *Leishmania mexicana*, onde interagem com o DNA. Proteínas que contêm enxofre e elementos que contêm fósforo, como o DNA, são os sítios prefenciais de ligação com a prata, uma vez que íons Ag^+ apresentam tendência em complexar com substâncias contendo enxofre ou fósforo. Sugere-se que o efeito inibitório dos ions Ag^+ está associado à desativação de enzimas celulares com comprometimento da permeabilidade da membrana e, por último, morte e eventual lise celular (PERCIVAl *et al*, 2005; SAMBHY *et al*, 2006; PAUL*et al*, 2007).

Apesar da existência de estudos sobre o efeito antibacteriano do vidro bioativo dopado com prata e das suas propriedades no processo de reparo tecidual de lesões em pele, não foram encontrados na literatura trabalhos que verifiquem a atuação desse biomaterial em culturas de *Leishmania*. Diante disso, o objetivo deste trabalho foi avaliar o efeito inibitório de micropartículas de BG dopado com prata sobre formas promastigotas de *L. amazonensis* e *L. braziliensis.*

2. CAPÍTULO 1

Effect of silver doped bioactive glass microparticles over promastigote forms of *Leishmania amazonensis* and *Leishmania braziliensis*

ABSTRACT

The purpose of this study was to evaluate the inhibitory effect of silver doped bioactive glass microparticles over promastigote forms of *Leishmania amazonensis* and *Leishmania braziliensis.* Microparticles of bioactive glass (BG) and doped with silver (BGAg1 and BGAg2) belonging to the system $58SiO_2 \cdot (36\text{-}x)CaO \cdot 6P_2O_5 \cdot xAg_2O$ with x = 0, 1 and 2 mol%, respectively were synthesized via sol–gel method and characterized by Scanning Electron Microscopy (SEM), Thermal Gravimetric Analysis (TGA), X-Ray Diffraction (XRD) and Fourier Transform Infrared Espectroscopy (FTIR). The cytotoxity of the BG and BGAg in human cells was assessed from the response of A549 lung adenocarcinoma ephitelial cells line. *Leishmania amazonensis* (*L.Amazonensis*) and *Leismania braziliensis* (*L. braziliensis*) promastigote cultures were exposed to BG, BGAg1 and BGAg2 for 24h. Then, 0.5 mM resazurin was added to culture and absorbance readings were assessed after 48, 72 and 96 hours of incubation. The percentage of resazurin reduction per group was obtained from a calculator (AbDSerotec®) and viable promastigote forms were counted on Neubauer chamber. SEM images showed micrometric particles with irregular and porous surface. TGA analysis demonstrated, for samples doped with silver, an endotermic peak around 497ºC associated to silver oxide decomposition. The XRD patterns exhibit mainly amorphous characteristics corresponding to BG. FTIR curves revealed main vibrational modes including Si-O-Si asymmetric stretching vibration (1000-1200 cm^{-1}), Si-O-Si symmetric stretching vibration (750-800 cm^{-1}), Si-O-Si bending mode (450-480 cm^{-1}) and PO_4^{-3} antisymmetric bending (570-600 cm^{-1}). Reffering to cytotoxicity, BGAg showed non-toxic behavior. BGAg1- 300µg/mL inihibited *L. amazonensis* and BGAg2 300 µg/mL inihibited *L. braziliensis* with 97,6 % and 92% of efficacy, respectively. The count on Neubauer chamber confirmed the efficacy of BGAg2 in 150 e 300 µg/mL concentrations revealing absence of viable cells. In conclusion, the BGAg2- 150 and 300 µg/mL inhibited the growth and proliferation of *L.amazonensis* and *L. braziliensis* on promastigote.

Keywords: Leishmaniasis, Silver, Bioactive glass, Sol-gel synthesis.

1. INTRODUCTION

Leishmaniasis is a parasitic infectious disease caused by several species of protozoa of the genus *Leishmania* that affects humans and different domestic or wild animal species. It can be manifested in different clinical forms, such as the American Cutaneous Leishmaniasis, which in general can present as single, multiple or disseminated ulcerated lesions in the skin or mucous membranes [1, 2]. This disease is endemic in 88 countries and affects about 12 million people worldwide [3]. Between the main species of *Leishmania* responsible for the transmission of American Cutaneous Leishmaniasis in Brazil are the *Leishmania braziliensis* (*L.braziliensis*) and *L. amazonensis* (*L.amazonensis*). The morphological forms shown by *Leishmania* during its life cycle are the promastigote and amastigote. The promastigotes are found in the vector gut, being the main responsible for disease transmission while the amastigotes are found in vertebrate host [4].

Pentavalent antimonals remain the first-choice treatment for leishmaniasis in most countries, mainly Sodium stibogluconate (Pentostan®,Wellcome Foundation, London, UK), and N-methylglucamine antimoniate (Glucantime®, Rhone Poulenc, Paris, France) [5]. In cases of resistance to antimoniate, Amphotericin B and Miltefosine (hexadecylphosphocholine) have been used as second-line drugs. These drugs, however, have high toxicity, are ineffective in many cases, present high cost and significant side effects which include myalgia, arthralgia, increased liver serum enzymes, pancreatitis, gastrointestinal dysfunction, diffuse muscular pains, stiffness of the joints, arrhythmias, pancytopenia, reversible renal impairment and cardiotoxicity [6,7, 8].

Thus, although the existence of treatment for leishmaniasis, these disadvantages limit the use of these drugs [9]. Hence, the study and development of new therapies and drugs for the treatment of leishmaniasis is still an urgent need [10].

The bioactive glass (BG) was first described by Hench, composed mainly of silicon dioxide (SiO_2), calcium oxide(CaO), phosphorus pentoxide (P_2O_5) and sodium oxide (Na_2O). The structure consists of a network of SiO_2, having P_2O_5as an adjuvant and CaO and Na_2O as modifiers [11, 12]. Sol-gel processing is one of the main techniques used for BG production [13]. The bioactivity of this material allows its application in the field of regeneration and tissue engineering [14].

BG can be used in a wide range of applications, such as bioactive fillers in the bone regeneration field [15], coating for implants [16, 17] and as scaffold for tissue repair, with porous arrangement similar to trabecular bone [14]. Bioactive materials, such as BG, may develop in the bone chemical bonds, which are stronger than the ceramic material alone [17]. BG is commonly used as hard tissue replacement material, although some studies show remarkable properties in soft tissues. Ostomel et al. (2006) [18] observed a decrease of the blood coagulation time induced by the presence of porous BG microspheres. Lin et al. (2012) [19] showed the induction of tissue repair by BG 58S (SGBG-58S), BG at the nanoscale (NBG-58S) and a derivative of the BG 45S5 , where mice with wounds treated with this material had reduced healing time as compared to the control. These results suggest the possibility of using BG to facilitate the healing of skin wounds, such as leishmaniasis lesions.

The BG may also be conjugated to antimicrobial ions like silver (Ag). Dayet et al. (2005) [20], using porous silica spheres, produced in one step with cetyltrimethylammonium bromide and conjugated to $Ca(NO_3)_2$ and $AgNO_3$, it was observed an antimicrobial effect against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Zhu et al. (2014) [21] found that the porous BG 58S (SM58S), prepared from the surface modification of the porous BG 58S (M58S) by KH-550 silane and loaded with Ag ions had a significant antibacterial activity against *E. coli* and *S. aureus*. The same effect was not observed in the absence of silver doped

BG. Ag ions damage bacterial RNA and DNA, inhibiting replication. Ag containing products are also applied in wound repair. Ag treatment can reduce the inflammatory and granulation phases of healing and induce epidermal repair [22].

Despite the existence of studies on the antibacterial effect of the BG doped with Ag (BGAg) and your properties in the skin lesions repair, no studies verified the effect of this biomaterial in *Leishmania* cultures. The aim of this work was to evaluate the inhibitory effect of BGAg microparticles on promastigote forms of *L. amazonensis* and *L. braziliensis.*

2. EXPERIMENTAL

2.1Sol-gel synthesis of BGAg microparticles

Samples belonging to the system $58SiO_2 \cdot (36\text{-}x)CaO \cdot 6P_2O_5 \cdot xAg_2O$ with x = 0, 1 and 2 mol% (Neat BG, BGAg1 and BGAg2) were prepared by sol–gel method. Hydrolysis and condensation of tetraethyl orthosilicate (TEOS), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), triethyl phosphate (TEP) from Sigma Aldrich and silver nitrate ($AgNO_3$) from PlatLab were used to obtain the gels. The molar ratio of EtOH: TEOS was of 1:1. The other precursors were dissolved in distillated water. The pH of the solutions was adjusted around 2 by using HNO_3. The obtained gels were dried for 3 days at ambient temperature and 2 days in a drying oven, at 120° C. The dried gels were heat treated up to 700 °C for 2 h, at a constant rate of 3ºC min^{-1}[23].

2.2 Characterization of BGAg microparticles

The morphology of the BG was observed using a scanning electron microscope (SEM) model Zeiss Quanta 450 (FEI ™, FEI Company). Samples were fixed on aluminum stubs and their surfaces covered with gold using a K 550X Emitech sputter coater. Particles size was evaluated

by an image analyzer software (Image J, National Institutes of Health, USA). For each sample, the average size and its dispersion were determined from the analysis of 60 random particles. Differential thermal analysis (DTA) and thermal gravimetric analysis (TGA) measurements were performed on Shimadzu analyzer DTG-60H, in nitrogen atmosphere, from room temperature to 800 °C, using alumina crucibles, with heating rate of 10 °C/min. The X-Ray Diffraction (XRD) spectra was recorded with a Shimadzu XRD-6000 diffractometer, using Cu K_α radiation (λ = 1.5418 Å), with Ni-filter. Specimens were aligned perpendicular to the incident beam and the analysis parameters were step size 0.01º and a diffraction range of 10–90º. The diffractograms were recorded after the samples being annealed at 700 °C. The infrared spectra of the prepared glasses were obtained using a Fourier transformer infrared spectrophotometer (IRPrestige-21, Shimadzu®) in a wave number range of 400-4000 cm^{-1} and 20 scans with a resolution of 4 cm^{-1}.

2.3 Cytotoxicity of BGAg microparticles

A549 human lung adenocarcinoma epithelial cells were cultured at 37º in a humidified atmosphere of 95% air and 5% CO_2, in DMEM (Dulbecco's modified Eagle's medium, Gibco, Germany) with 1 vol.% penicillin/streptomycin (Sigma-Aldrich, Germany), 10 vol.% fetal bovine serum (FBS, Sigma-Aldrich, Germany) and 0.25 mg ml^{-1}amphotericin B (Sigma-Aldrich, Germany). Cells were grown for 48 h to confluence in 75 cm^2 culture flasks. Cells were counted by a automatic counter cell (VI-CELL XR® BECKMAN COULTER) and diluted to a final concentration of 8 $x10^4$ cells ml^{-1}. Thus, cells were cultured in media containing the soluble products of the glass. In the preparation of the dissolution product, each BG sample in 5 concentrations (450, 300, 150, 75 and 35 µg/ml) was shaked in DMEM culture medium at 37º C and 150 RPM for 24, 36 and 48h. After these times of immersion, culture medium was filtered to remove the glass particles with a 0,22 µm filter. The cytotoxity was evaluated from

the response of A549 cells to the dissolution products of the glass. The cell line was seeded with this supernantant in a 24-well culture plate and stored at 37ºC in a humidified atmosphere containing 5%CO_2, for 24 hours. The test was done in triplicate. As a positive control, culture medium without the soluble glass product was used. A latex fragment was used as a negative control. The reading was performed using viability automatic cell counter(VI-CELL XR® BECKMAN COULTER) [24, 25]. The percentage values of viability was considered when at least 90 % of cells were live comparing with the percentual of positive growth [26].

2.4 Promastigote assay

The 199 strain of *Leishmania amazonensis* and 2903 strain of *Leishmania braziliensis*were routinely maintained as promastigotes in RPMI 1640 medium (Gibco) at 26 °C supplemented with FBS (Sera Laboratories International, Horsted Keynes, UK) and 100 U/mL penicillin + 100 μg/mL streptomycin (BioWhittaker, Verviers, Belgium) in 75 cm^2 culture flasks[27].

The experiment with promastigotes were made in flat-bottomed 96-well cell culture microtiter plates with lid (Falcon II, BD, Bedford, MA, USA). Cultures were carried out at 26 °C in aerated culture chamber or incubated in a 95% air/5% CO_2 humidified atmosphere. Culture media (RPMI 1640) with additional 20 mM Hepes was used. To perform these assay, 5×10^5 per well of mid-log phase promastigotes of *L.amazonensis* and *L. braziliensis* were added per well, with a culture media, in a final volume of 190 μL/well in 96-microtiter plates. Ten microliters of 10mM Amphotericin B (AmpB) (Sigma-Aldrich, St Louis, US) and different concentrations of neat BG, BGAg 1 and BGAg2 (300, 150 and 75 μg/ml) were added. Sterile control was obtained after 4 cycles of 480s on ultrasound appliance. Cultures were carried out at 26°C in plates with lid under a 5% CO_2 atmosphere. After 24 h incubation, 20 μL of 0.5 mM Resazurin (Sigma Aldrich, St Louis, US) was added and the plates were kept for 48, 72 and 96h. The readings of

absorbance were obtained using a dual filter of 570 and 600 nm [28]. After 168h of initial inoculation, a count was made using Neubauer chamber to estimate the number of viable cells per well. The percentage reduction of resazurin per group was obtained from a calculator (ABDSerotec®).

3 RESULTS AND DISCUSSION

3.1Characterization

3.1.1 SEM

The SEM showed irregular and porous surface for all samples (**Figure 1**). Ethanol evaporation as well as nitrate decomposition during the stabilization process that was carried out to convert the dry gel into glass, can explain the porous nature of BG. Those textural properties promote and accelerate the *in vitro* hydroxyapatite layer formation [29].

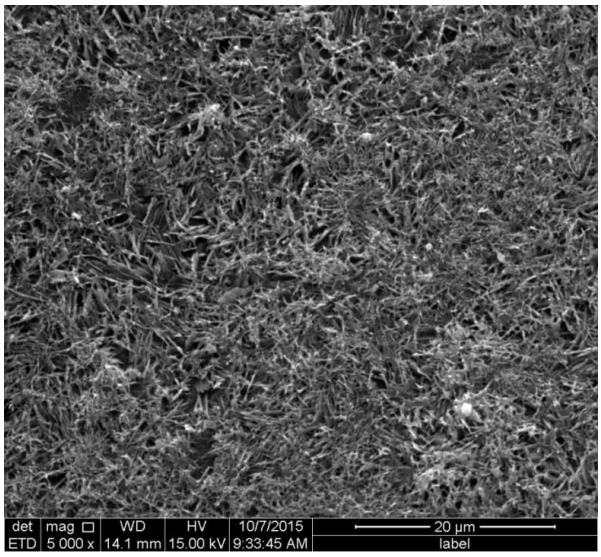

Figure 1. SEM photomicrograph showing the porous and irregular surface of a neat BG particle.

The average particle size is shown in **Table 1**.The particle size can be related to the type of catalysts that were used to adjust the pH of the solution. In this study, microparticulate samples

were obtained, which can be related to the use of conventional sol-gel technique, using one-step acid catalysis [30].

Table1. Average size and standard desviation of the BG particles.

Sample	Size (μm) (±sd)
BG	286,093 ± 165,197
BGAg1	140,115 ± 90,217
BGAg2	64,388 ± 38,675

The average size of the BG particles decreased with the increasing of the silver concentration. This result differs from those reported by Vulpoi et al (2012) [23] and can be related with differences in synthesis methodology. The decrease of particle size, in the present work, may not be necessarily related to the increase of silver, but to the grinding process of BG in the synthesis method.

3.1.2 Thermal analysis

DTA and TGA curves for samples neat BG, BG/Ag1 and BG/Ag2 are shown in **Figure 2.**

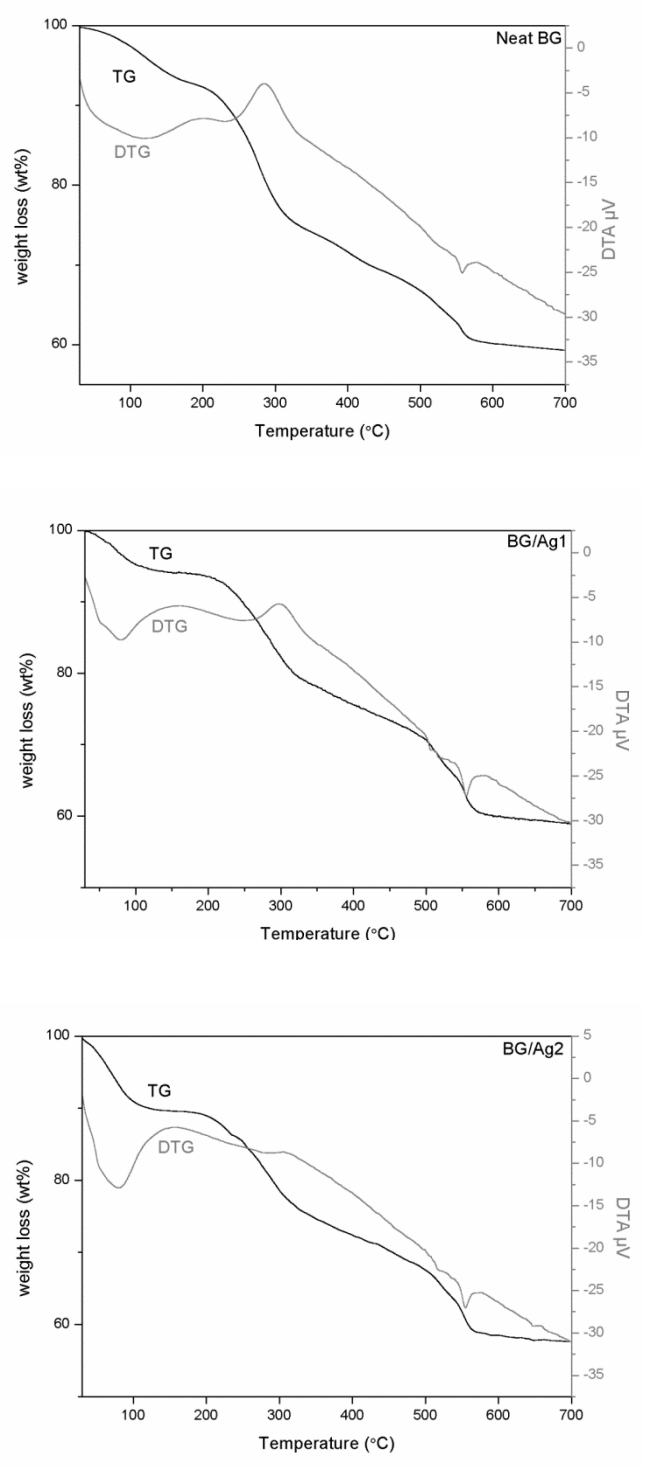

Figure 2. DTA and TGA curves for samples neat BG(A), BGAg1(B) and BGAg2(C).

For all samples, the first weight loss appeared at the temperature interval between 65ºC - 95ºC. It is an endothermic peak associated with the elimination of residual alcohol and physisorbed water from the polycondensation reaction that were not removed during drying. The second

peak is exothermic, with an onset aroud 237ºC, for all samples, and could be associated with the desorption of chemically adsorbed water or to the removal of organic residues. For the silver doped samples, an endotermic peak appears around 497ºC and could be attributed to the silver oxide decomposition and possibly metalic silver nanocrystals formation. For all samples, a weight loss located around 534ºC could be assigned to the decomposition of the unreacted $Ca(NO_3)_2$ [23,30].Taking into account these results, the temperature of 700ºC was chosen for the stabilization of all the glasses powders, since all residuals could be removed before 700ºC.

3.1.3 XRD

The XRD results of the investigated samples, shown in **Figure 3,** exhibit mainly amorphous characteristics corresponding to glass. All samples showed incipient crystallization of a tricalcium phosphate (TCP) phase identified as $Ca_3(PO_4)_2 \cdot 2H_2O$ centered at $2\theta=32^o$. This result suggests that the incorporation of silver into the investigated BG samplesdid not compromise its bioactivity [23].

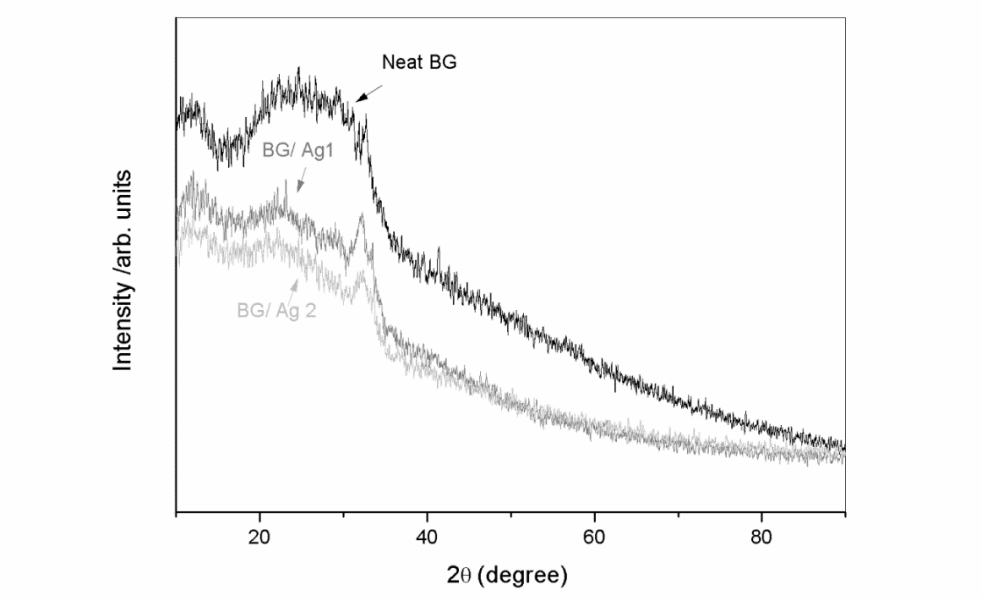

Figure 3. XRD patterns of the $58Si0_2 \cdot (36\text{-}x)CaO \cdot 4P_2O_5 \cdot xAg_2O$ samples.

3.1.4 FTIR

FTIR was used to characterize the presence of specific chemical groups in the BG samples. IR spectra of neat BG, BGAg1 and BGAg2 are summarized in **figure 4**.

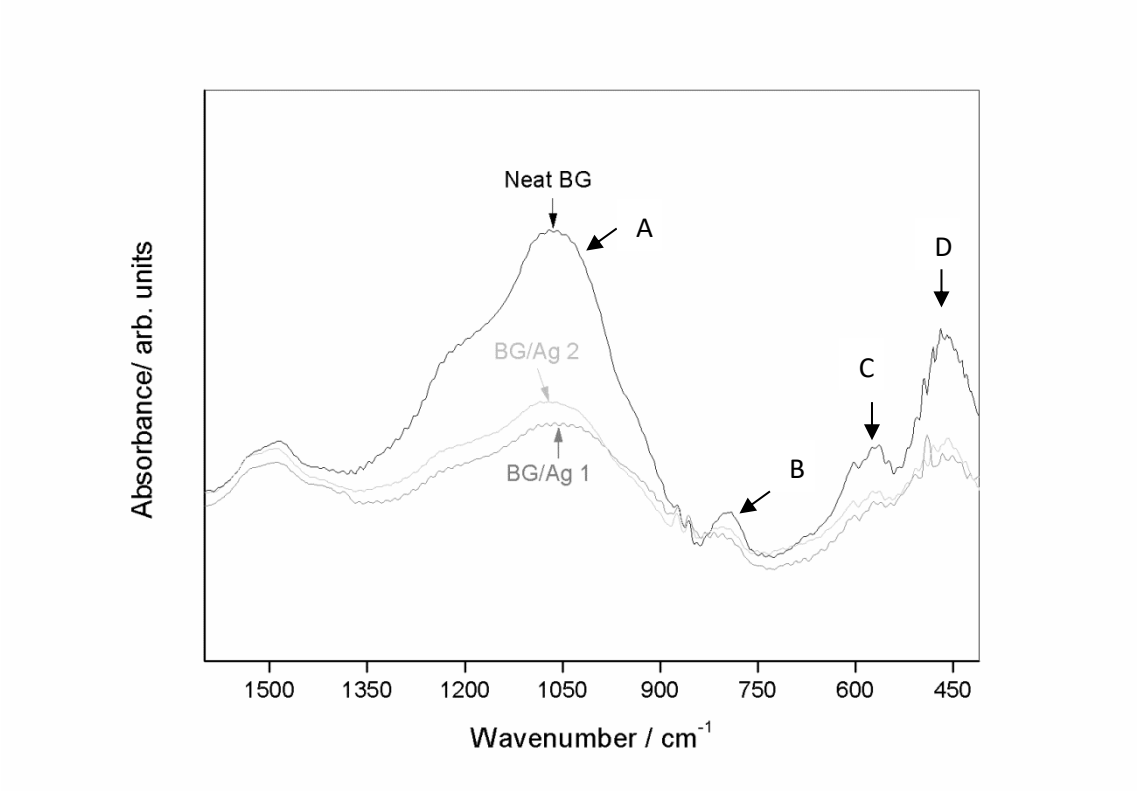

Figure 4. FTIR spectra of BG, BGAg1and BGAg2 samples.

In FTIR curves were identified the main vibrational modes: Si-O-Si asymmetric stretching vibration correspond to the band located in the range of 1000-1200 cm^{-1}(A), Si-O-Si symmetric stretching vibration near 750-800 cm^{-1}(B), Si-O-Si bending mode at 450-480 cm^{-1} (D) and PO_4^{-3} (C) antisymmetric bending at 570-600 cm^{-1}, associated with phosphorous in a crystal-like environment [30, 23, 31]. The presence of Si-O-Si groups in all samples demonstrates the preservation of the glass structure, which has an atomic arrangement characterized by an extended three-dimensional network, which has no periodicity and symmetry and whose basic element is silica [32].

3.2 Cytotoxicity

The cytotoxicity is the first *in vitro* assay performed to evaluate the biocompatibility of the material. When the material has mild or non-toxicity, biocompatibility tests should proceed with *in vivo* tests using laboratory animals [33].

Table 2 presents the mean of percentage of viable cells in contact with BG, BGAg1and BGAg2 samplescompared to growth control, after 24 hours of culture according to time interval of dissolution. Based on the Kaur et al (2014) methodology,the percentage of viable cells of test groups was obtained comparing their results with the growth control, in which the cell viabilityis considered to be 100%. Survival of 90 to 100% cells after the contact with a certain concentration of test material, compared to control cells (without any test material), show that the material is basically not affecting the cellular proliferation as well as cell viability and suggests that it is non-toxic[26].

Table 2. Mean of percentage (%) of viable cells in contact with BG, BGAg1and BGAg2 samples compared to growth control, after 24 hours of culture according to time interval of dissolution.

Dissolution (μg/mL)	BG	BG Ag1 35	BG Ag1 75	BG Ag1 150	BG Ag1 300	BG Ag1 450	BG Ag2 35	BG Ag2 75	BG Ag2 150	BG Ag2 300	BG Ag2 450	BG 35	BG 75	BG 150	BG 300	BG 450
24h	90.6	97.9	94.4	99.9	94.5	100	100	100	100	100	94.2	86.2	85.6	95.9	92
36h	99.9	92.5	94.2	87.6	94.4	95.9	97.8	95.8	96.9	98.2	95.9	100	95.5	96.7	95.7
48h	100	97	99.8	93.6	100	100	94.7	94	95.8	81.6	94.6	85.5	88.3	100	95.9

Except for the samples BGAg1 – 300 μg/mL with 36h of dissolution, with value close to 90% and BGAg2- 450 μg/mL with 48h of dissolution, all the BGAg show non-toxic behavior to A549 Cells and could be used for biological applications. The neat BG (75μg/mL and 150μg/mL) with 24 and 48h of dissolution, showed a percentage of viable cells slightly below

90%. These results do not invalidate the use of these BG concentrations in biological applications. However, other biocompatibility studies with neat BG are still necessary.

3.3 Promastigote assay

The tests with *L. amazonensis* and *L. brasiliensis* were conducted using resazurin colorimetric method. Resazurin is a blue dye that is reduced to resofurin in the presence of viable cells. Non-viable cells lose their metabolic capacity to reduce resazurin in the mitochondria [34]. This method has some advantages including: rapidity, simplicity of use, reliability, low cost, and can be used with long incubation periods [27]. Percentage of reduction of 0.5mM Resazurin according to BG groups during time intervals with *L. amazonensis* and *L. brasiliensis* inoculum are shown in **Tables 3 and 4**.

Table 3. Percentage reduction of 0.5mM Resazurin according to BG groups during time intervals with *L. amazonensis* inoculum.

Reading	BG 300 µg/mL	BG 150 µg/mL	BG 75 µg/mL	BGAg1 300 µg/mL	BG Ag 1 150 µg/mL	BG Ag 1 75 µg/mL	BG Ag 2 300 µg/mL	BG Ag 2 150 µg/mL	BGAg2 75 µg/mL
48 h	69.9	66.6	70.7	2.6	4.4	6.3	14.0	7.2	6.1
72 h	60.9	56.6	63.7	2.2	3.0	7.7	10.5	5.6	4.0
96 h	65.3	61.5	67.1	2.4	3.7	7.0	12.2	6.4	5.0

Table 4. Percentage reduction of 0.5mM Resazurin according to BG groups during time intervals with *L. braziliensis* inoculum.

Reading	BG 300 µg/mL	BG 150 µg/mL	BG 75 µg/mL	BGAg1 300 µg/mL	BG Ag 1 150 µg/mL	BG Ag 1 75 µg/mL	BG Ag 2 300 µg/mL	BG Ag 2 150 µg/mL	BGAg2 75 µg/mL
48 h	47.3	49.4	68.5	17.9	12.1	33.6	7.5	8.5	14.1
72 h	49.6	50.8	64.6	23.7	23.2	39.9	8.5	9.3	19.0
96 h	14.0	15.7	23.4	20.4	22.5	32.5	8.2	9.1	19.8

It was observed that in the wells containing BGAg there was a lower percentage of resazurin reduction when compared to neat BG, which indicates the small number of viable microorganisms in wells containing BGAg. For *L. brasiliensis*, in the wells containing Amphotericin B, the percentage of reduction of 0.5mM Resazurin, for 48, 72 and 96h was 3.1, 3.3, and 5, respectively. A similar study developed in parallel with this work by our group showed that in cultures of 2.5 $x10^5$ cells / well of *L. amazonensis*, after 136h of the initial inoculation, in the wells containing Amphotericin B, there was a reduction of 3.8 % (Data not shown).

The cell counting (per well) after 168h on Neubauer chamber according to groups and species is shown in **table 5**.

Table 5. Cell counting (per well) after 168h on Neubauer chamber according to groups and species.

	Leishmaniaamazonensis	***Leishmaniabraziliensis***
Sterile Control	0	0
Growth Control	$2.14x10^7$	$2.37X10^7$
BG pure (300 µg /ml)	$4.78x10^7$	$8.33x10^7$
BG/Ag 1 (300 µg /ml)	0	$4.76x10^7$
BG/Ag 1 (150 µg /ml)	0	$4.47x10^7$
BG/Ag 1 (75 µg /ml)	$4.4x10^5$	$5.23x10^7$
BG/Ag 2 (300 µg /ml)	0	0
BG/Ag 2 (150 µg /ml)	0	0
BG/Ag 2 (75 µg /ml)	0	$5.8x10^6$

In the counting in the Neubauer chamber, viable microorganisms were not found in the groups BGAg2 and BGAg1-300µg/mL and BGAg1-150µg/mL, for *L. amazonensis*. For *L. brasiliensis* were not found viable microorganisms in the groups BGAg2-300 µg/mL and BGAg2-150 µg/mL. The neat BG had no effect on the viability of *Leishmania* species. In agreement with the results presented here, Bellantone et al. (2002)[35]and Balamurugan et al. (2008)[22] reported that neat BG had no antimicrobial effect. The antileishmania effect observed for

AgBG may be attributed to the leaching of ionic silver from the glass matrix.

Some works [36, 37, 38] also report the effectiveness of Ag as antileishmanial agent. The mecanism by which the silver ion exerts its toxicity towards *leishmania* species is unclear, but it is known that in bacteria, Ag ion antimicrobial activity can be associated with: binding to DNA, interaction with cell components and the interference with electron transport, since it reacts with protein carboxylates, hydroxyls, and thiols [22, 39]. The Ag ion action derives from the binding with the negatively charged microbial proteins preventing their replication, and via attachment to sulfhydryl groups, resulting in inhibiting their proliferation [40].

Navarro et al (2006) [41] showed that silver polypyridyl complexes are biologically active against *Leishmania mexicana*, where they interact with DNA. Proteins that contain sulfur and components that contain phosphorous such as DNA are preferential binding sites for silver, since Ag + ions have a tendency to form complex with substances containing sulfur or phosphorous. It is suggested that the inhibitory effect of Ag^{+} ions is associated with deactivation of cellular enzymes with impaired membrane permeability and finally, death and eventual cell lysis [42, 43, 44].

BG may also be effective in promoting vascularization, which is important to healing of soft tissue wounds. Studies indicated that, genes related to wound healing, such as the CD44 antigen hematopoietic form precursor, fibronectin receptor beta subunit, fibroblast growth factor receptor1 precursor (N-sam), vascular endothelial growth factor (VEGF) precursor and vascular cell adhesion protein 1 precursor (V-CAM 1) can be activated by BG [45,46,47].

Our results shown the effectiveness of BGAg microparticles to inhibit the growth and proliferation of *Leishmania* species studied and suggest the future use of BG in combination or alternatively to current antileishmanial drugs.

4. CONCLUSIONS

The silver doped BG, in the present study, were sucessfully produced by sol-gel method. SEM images showed micrometric particles with irregular and porous surface. The XRD patterns of the investigated samples showed incipient crystallization of tricalcium phosphate (TCP). These results suggests that the incorporation of silver into the investigated samples did not compromise their bioactivity. In FTIR curves, it was observed the presence of Si-O-Si groups in all samples, which demonstrates the conservation of the structure of the glass even with the addition of silver. BGAg showed non-toxic behaviorand can be used for biological applications. The BGAg2- 150 and 300 µg/mL inhibited the growth and proliferation of *L.amazonensis* and *L. braziliensis* on promastigote form and could be used in other studies, like *in vivo* investigations that are necessary to verify the BGAg activity on *Leishmania* amastigotes forms.

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References

1. Carrion J, Folgueira C, Alonso C. Transitory or long-lasting immunity to Leishmania major infection: the result of immunogenicity and multicomponent properties of histone DNA vaccines. Vaccin. 2008; 26: 1155-1165.
2. ReveizL, Maia-Elkhoury ANS, Nicholls RS, Romero GAS, Yadon ZE. Interventions for American cutaneous and mucocutaneousleishmaniasis: a systematic review update.Plos ONE.2013; 8: 1-14.
3. WHO/TDR World Health Organization. Tropical Disease Research – Leishmaniasis, 2013.
4. Neves DP, Melo AL, Linardi PM, Vitor RWA. Parasitologia humana. 11 ed. São Paulo: Atheneu; 2005.
5. Vries HJC, Reedijk SH, SchalligHenk DFH. Cutaneous leishmaniasis: recent developments in diagnosis and management. Am J Clin Dermatol. 2015; 16: 99-109.
6. Sundar S, Olliaro P L. Miltefosine in the treatment of leishmaniasis: clinical evidence for informed clinical risk management. Ther Clin Risk Manag. 2007; 3: 733.
7. Silva-López RE. Leishmania proteases: new targets for rational drug development. Quim Nova. 2010; 33: 1541-1548.
8. Conti RV, Pinto Junior VL. Therapeutic approach of visceral leishmaniasis in Brazil - a review for the clinicians. Rev Med Saúde Brasília. 2015; 4: 240-249.
9. Morais-Teixeira E, Gallupo MK, Rodrigues LF, Romanha AJ, Rabello A. In vitro interaction between paromomycinsulphate and four drugs whit leshmanicidal activity against three new world leishmania species. J Antimicrob Chemother. 2013; 69: 150-154.

10. Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL. Visceral leishmaniasis treatment: what do we have, what do we need and how to deliver it? Int JParasitol. 2012; 2: 11–19.
11. Shirtliff VJ, Hench LL. Bioactive materials for tissue engineering, regeneration and repair. J Mater Sci. 2003; 38: 4697-4707.
12. Hench LL, Splinter RJ, Allen WC, GreenleeJr TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res. 1971; 2: 117-141.
13. Hench LL, West JK. The sol-gel process. Chem Rev**.** 1990; 90: 33-72.
14. Jones JR, Ehrenfried LM, Hench LL. Optimising bioactive glass scaffolds for bone tissue engineering. Biomaterials. 2006; 27: 964-973.
15. Chouzouri G, Xanthos M. In vitro bioactivity and degradation of polycaprolactone composites containing silicate fillers. Acta Biomater. 2007; 3: 745-756.
16. Al-Noaman A, Rawlinson SCF, Hill RG. Bioactive glass-stoichimetricwollastonite glass alloys to reduce tec of bioactive glass coatings for dental implants. Mater Lett. 2013; 94: 69-71.
17. Al-Noaman A, Karpukhina N, Rawlinson SCF, Hill RG. Effect of fa addition on bioactivity of bioactive glass coating for titanium dental implant:part ii - composite coating. J Non-Cryst Solids. 2013; 364: 99-106.
18. Ostomel TA, Shi Q,Tsung CK, Liang H, Stucky GD. Spherical bioactive glass with enhanced rates of hydroxyapatite deposition and hemostatic activity. Small. 2006; 2: 1261-1265.
19. Lin C, Mao C, Zhang J, Li Y, Chen X. Healing effect of bioactive glass ointment on full-thickness skin wounds. Biomed Mater. 2012; 7: 1-13.

20. Day RM, Maquet V, Boccaccini AR, Jerome R, Forbes A. in vitro and in vivo analysis of macroporous biodegradable poly(d,l-lactide-co-glycolide) scaffolds containing bioactive glass. J Biomed Mater Res A. 2005; 75a: 778-787.
21. Zhu H, Hu C, Zhang F, Feng X, Li J, Liu T, et al. Preparation and antibacterial property of silver-containing mesoporous 58s bioactive glass. Mater SciEng, C. 2014; 42: 22-30.
22. Balamurugan A, Balossier G, Laurent-Maquin D, Pina S, Rebelo AHS, Faure J, et al. An in vitro biological and anti-bacterial study on a sol–gel derived silver-incorporated bioglass system. Dent Mater. 2008; 24: 1343-51.
23. Vulpoi A, Baia L, Simon S, Simon V. Silver effect on the structure of SiO 2-CaO-P 2 O 5 ternary system. Mater Sci Eng*: C*. 2012, *32*: 178-183.
24. Luo SH, Xiao W, Wei X J, Jia WT, Zhang CQ, Huang WH, et al. In vitro evaluation of cytotoxicity of silver-containing borate bioactive glass. J BiomedMater Res B ApplBiomater. 2010; 95: 441-448.
25. Hoppe A, Brandl A, Bleiziffer O, Arkudas A, Horch RE, Jokic B, et al. In vitro cell response to co-containing 1393 bioactive glass. Mater Sci.2015; 57:157-163.
26. Kaur G, Pickrell G, Kimsawatde G, Homa D, Allbee HA,Sriranganathan N. Synthesis, cytotoxicity, andhydroxyapatiteformation in 27-tris-sbf for sol-gel based cao-p2o5-sio2-b2o3-zno bioactiveglasses. Scientific Reports. 2014; 4: 1-14.
27. Castellano LR, Filho DC, Argiro L, Dessein H, Prata A, Dessein A, Rodrigues V. th1/th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-gamma production. Hum Immunol. 2009; 70:383-90.

28. Corral MJ, Gonzalez E, Cuquerella M, Alunda JM. Improvement of 96-well microplate assay for estimation of cell growth and inhibition of leishmania with alamar blue. J Microbiol Methods. 2013; 94: 111–116.
29. El-KadyAM, Farag MM. Bioactive glass nanoparticles as a new delivery system for sustained 5-fluorouracil release: characterization and evaluation of drug release mechanism. Journal of Nanomaterials. 2015; 2015: 1-11.
30. El-KadyAM, Ali AF, Rizk RA, Ahmed MM. Synthesis, characterization and microbiological response of silver doped bioactive glass nanoparticles. Ceram Int. 2012; 38: 177-188.
31. Delben JRJ, Pimentel OM, Coelho MB, Candelorio PD, Furini LN, dos Santos FA, et al. Synthesis and thermal properties of nanoparticles of bioactive glasses containing silver. J Therm Anal Calorim. 2009; 97: 433-436.
32. Li R, Clark AE, Hench LL. An investigation of bioactive glass powders by sol-gel processing. J ApplBiomater. 1991; 2: 231-239.
33. Mundstock KB, de Oliveira APN, Hotza D, Rogero SO. Evaluation of the biocompatibility of glass and glass ceramics of the SNCP (SiO_2-Na_2O-CaO-P_2O_5) system. Quim Nova. 2012; 35: 665-670.
34. Marinho FA, Gonçalves KCS, Oliveira SCO, Gonçalves DS, Matteoli FP, Seabra SH, et al. The calpain inhibitor mdl28170 induces the expression of apoptotic markers in leishmaniaamazonensis promastigotes. Plos ONE. 2014; 9:e87659. doi:10.1371/journal.pone.0087659.
35. Bellantone M, Williams HD, Hench LL. Broad-spectrum bactericidal activity of Ag2O-doped bioactive glass. Antimicrob Agents Chemother. 2002; 46: 1940-1945.
36. Mohebali M, Rezayat MM, Gilani K, Sarkar S, Akhoundi B, Esmaeili J, et al. Nanosilver in the treatment of localized cutaneous leishmaniasis caused by

leishmaniamajor (mrho/ir/75/er): an in vitro and in vivo study. J Pharm Sci. 2009; 17: 285-289.

37. Baiocco P, Ilari A, Ceci P, Orsini S, Gramiccia M, Di Muccio T, et al. Inhibitory effect of silver nanoparticles on trypanothione reductase activity and leishmaniainfantum proliferation. ACS Med Chem Let. 2010; 2: 230-233.
38. Allahverdiyev AM, Abamor ES, Bagirova M, Ustundag CB, Kaya C, Kaya F, et al. Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light.Int J Nanomed. 2011; 6: 2705.
39. Baiocco P, Ilari A, Ceci P, Orsini S, Gramiccia M, Di Muccio T, et al. Inhibitory effect of silver nanoparticles on trypanothione reductase activity and leishmaniainfantum proliferation. ACS Med Chem Let. 2010; 2: 230-233.
40. Holt KB, Bard AJ. Interaction of silver (I) ions with the respiratory chain of Escherichia coli: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag+. Biochem. 2005; 44:13214–23
41. Navarro M, Cisneros-Fajardo EJ, Marchan E. New silver polypyridyl complexes: synthesis, characterization and biological activity on Leishmania mexicana. Arzneimittelforschung. 2006; 56: 600–604.
42. Sambhy V, MacBrid MM, Peterson BR, Sem A. Silver bromide nanoparticle/polymer composite: dual action tunable antimicrobial material. J Am Chem C. 2006; 128: 9798-9808.
43. Paul S, Tak Y, Song JM. Does the antibacterial activity of silver nanoparticles depend on the Shape of the nanoparticles? A study of the gram negative bacterium Escherichia coli. Appl Environ Microbiol. 2007; 73: 1712-1720.
44. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. J Hosp Infect. 2005; 60: 1-7.

45. Kent Leach J, Kaigler D, Wang Z, Krebsbach PH, Mooney DJ. Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration. Biomater. 2006; 2: 73249–55
46. Xynos ID, Edgar AJ, Buttery LDK, Hench LL , Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass R45S5 dissolutionJ. Biomed Mater Res. 2001; 5: 5151–7.
47. Handel M, Hammer TR, Nooeaid P, Boccaccini AR, Hoefer D . 45S5-Bioglass® -Based 3D-Scaffolds seeded with human adipose tissue-derived stem cells induce in vivo vascularization in the CAM angiogenesis assay. Tissue Eng A. 2013; 19: 2703-2712.

3. CONSIDERAÇÕES GERAIS

A LTA pode apresentar lesões na forma de úlceras, nódulos, pápulas ou placas nas partes expostas do corpo ou membranas mucosas. Há aproximadamente 1 milhão de novos casos de LTA a cada ano no mundo, no entanto, é difícil estimar a sua verdadeira incidência, devido à falta de sistemas de vigilância em áreas remotas e populações marginalizadas. Estima-se que existam 20 espécies de *Leishmania* que causam a LTA, mas no Brasil assumem importante papel na parasitologia dessa doença a *L. amazonensis* e a *L. braziliensis*, escolhidas para a realização do presente trabalho (MEARS *et al*, 2015).É uma doença de importância também para a Odontologia, uma vez que pode apresentar manifestações na cavidade oral com lesões destrutivas, podendo acometer membranas mucosas do nariz, mucosa jugal, lábio, palato (duro e mole), orofaringe e laringe (STRAZZULLA *et al*, 2015).

Embora os fámacos de escolha para o tratamentodesta doença tenham uma eficácia aceitável, apresentam alto custo, baixa aderência, devido ao uso prolongado de injecções intravenosas ou intramusculares, toxicidade sistêmica, e, em alguns casos, a resistência. Estas desvantagens reforçam a necessidade de se estudar outras opções de tratamento para a LTA(CARDONA-ARIAS et al, 2014).

As propriedades antimicrobiamas BGAg e a sua atividade no reparo tecidual de ferimentos já têm sido estudadas. Porém, não foi possível encontrar na literatura trabalhos que verifiquem a atuação desse biomaterial em culturas de *Leishmania*. Diante disso, o objetivo desse trabalho foi avaliar o efeito inibitório de micropartículas de BGAg sobre formas promastigotas de *L. amazonensis* e *L. braziliensis.* Os resultados mostraram que nas amostras de BGAg, houve um menor percentual de redução da resazurina quando comparado ao BG puro, o que indica a efetividade das amostras com prata na inibição da proliferação de *L. amazonensis* e *L. braziliensis*. Por outro lado, no que se refere a citotoxicidade em células humanas, o BGAg mostrou comportamento não tóxico.

Esses resultados sugerem que o uso do BGAg pode representar uma futura alternativa ou ser utilizado em conjunto com as atuais drogas antileishmaniose. Poderia ser administrado de forma tópica sobre as lesões ocasionadas pela doença,

porém outros estudos ainda se fazem necessários incluindo outros testes de biocompatibilidade e ensaios *in vivo*. As próximas etapas deste trabalho incluem a realização ensaios de invasão celular e atividade das amostras de vidro bioativo sobre as formas amastigotas de *L. amazonensis* e *L. braziliensis*.

4. CONCLUSÃO

Os BGAgs, no presente estudo, foram produzidos com sucesso pelo método sol-gel. As imagens do SEM mostraram partículas micrométricas com superfície irregular e porosa. Os padrões de DRXdas amostras investigadas mostraram cristalização incipiente do fosfato tricálcico (TCP), sugerindo que a incorporação de prata nas amostras investigadas não comprometeu a sua bioatividade. Nas curvas de FTIR, foi observada a presença de grupos Si-O-Si em todas as amostras, o que demonstra a conservação da estrutura do vidro, mesmo com a adição de prata. No que se refere à citotoxicidade, o BGAg mostrou comportamento não tóxico, o que sugere que pode vir a ser utilizado para aplicações biológicas. O BGAg2, nas maiores concentrações, inibiu ambas as cepas de *L. braziliensis* e *L. amazonensis* e poderia ser utilizado em outros estudos, como ensaios que verifiquem a atuação do BGAg sobre formas amastigotas das espécies de *Leishmania*.

REFERÊNCIAS*

1. Allahverdiyev AM, Abamor ES, Bagirova M, Ustundag CB, Kaya C, Kaya F, et al. Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light. Int J Nanomed. 2011; 6: 2705.
2. Al-Noaman A, Rawlinson SCF, Hill RG. Bioactive glass-stoichimetric wollastonite glass alloys to reduce tec of bioactive glass coatings for dental implants. Mater Lett. 2013a; 94: 69-71.
3. Al-Noaman A, Karpukhina N, Rawlinson SCF, Hill RG. Effect of fa addition on bioactivity of bioactive glass coating for titanium dental implant: part ii - composite coating. J Non-Cryst Solids. 2013b; 364: 99-106.
4. Baiocco P, Ilari A, Ceci P, Orsini S, Gramiccia M, Di Muccio T, et al. Inhibitory effect of silver nanoparticles on trypanothione reductase activity and leishmaniainfantum proliferation. ACS Med Chem Let. 2010; 2: 230-233.
5. Balamurugan A, Balossier G, Laurent-Maquin D, Pina S, Rebelo AHS, Faure J, *et al*. An in vitro biological and anti-bacterial study on a sol–gel derived silver-incorporated bioglass system. Dent Mater. 2008; 24: 1343-51.
6. BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Manual de Vigilância da Leishmaniose Tegumentar Americana– 2. ed. atual. – Brasília : Editora do Ministério da Saúde, 2010.

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* De acordo com as normas do PPGO/UFPB, baseadas na norma do International Committee of Medical Journal Editors - Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

7. Cardona-Arias JA, Vélez ID, López-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: a meta-analysis of controlled clinical trials. PloS one. 2014; 10: e0122569-e0122569.
8. Carrion J, Folgueira C, Alonso C. Transitory or long-lasting immunity to Leishmania major infection: the result of immunogenicity and multicomponent properties of histone DNA vaccines. Vaccin. 2008; 26: 1155-1165.
9. Conti RV, Pinto Junior VL. Therapeutic approach of Visceral Leishmaniasis in Brazil - a review for the clinicians. Rev Med Saúde Brasília. 2015; 4: 240-249.
10. Day RM, Maquet V, Boccaccini AR, Jerome R, Forbes A. In vitro and in vivo analysis of macroporous biodegradable poly(d,l-lactide-co-glycolide) scaffolds containing bioactive glass. J Biomed Mater Res A. 2005b; 75a: 778-787.
11. Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL. Visceral leishmaniasis treatment: what do we have, what do we need and how to deliver it? Int J Parasitol. 2012; 2: 11–19.
12. Goh YF, Alshemary AZ, Akram M, Kadir A, Rafiq M, Hussain R. Bioactive glass: an in-vitro comparative study of doping with nanoscale copper and silver particles. Int J Appl *Glass Sci*. 2014; 5: 255-266.
13. Handel M, Hammer TR, Nooeaid P, Boccaccini AR, Hoefer D. 45S5-Bioglass®-Based 3D-Scaffolds Seeded with Human Adipose Tissue-Derived Stem Cells Induce In Vivo Vascularization in the CAM Angiogenesis Assay. Tissue Eng A. 2013; 19: 2703-2712.
14. Hench LL, Splinter RJ, AllenWC,GreenleeJrTK. Bonding Mechanisms at the Interface of Ceramic Prosthetic Materials. J Biomed Materials Research.1971; 2: 117-141.
15. Hench LL, West JK. The sol-gel process. Chem Rev**.** 1990; 90: 33-72.

16. Holt KB, Bard AJ. Interaction of silver (I) ions with the respiratory chain of Escherichia coli: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag+. Biochem. 2005; 44:13214–23
17. Jones JR, Ehrenfried LM, Hench LL. Optimising bioactive glass scaffolds for bone tissue engineering. Biomaterials. 2006; 27: 964-973.
18. Lin C, Mao C, Zhang J, Li Y, Chen X. Healing effect of bioactive glass ointment on full-thickness skin wounds. Biomed Mater. 2012; 7: 1-13.
19. MearsER, Modabber F, Don R, Johnson GE. A Review: The Current In Vivo Models for the Discovery and Utility of New Anti-leishmanial Drugs Targeting Cutaneous Leishmaniasis. PLoS Negl Trop Dis.2015; *9*: e0003889.
20. Ministério da Saúde. Doenças negligenciadas: estratégias do Ministério da Saúde. Rev Saúde Pública. 2010; 44: 200-2.
21. Mohebali M, Rezayat MM, Gilani K, Sarkar S, Akhoundi B, Esmaeili J, et al. Nanosilver in the treatment of localized cutaneous leishmaniasis caused by leishmaniamajor (mrho/ir/75/er): an in vitro and in vivo study. Journal of Pharm Sci. 2009; 17: 285-289.
22. Morais-Teixeira E, Gallupo MK, Rodrigues LF, Romanha AJ, Rabello A. In vitro interaction between paromomycin sulphate and four drugs whit leshmanicidal activity against three new world leishmania species. J antimicrob chemother. 2013; 69: 150-154.
23. Navarro M, Cisneros-Fajardo EJ, Marchan E. New silver polypyridyl complexes: synthesis, characterization and biological activity onLeishmania mexicana. Arzneimittelforschung. 2006; 56: 600–604.

24. Neves DP, Melo AL, Linardi PM, Vitor RWA. Parasitologia humana. 11 ed. São Paulo: Atheneu; 2011.
25. Ostomel TA, Shi Q, Tsung CK, Liang H, Stucky GD. Spherical bioactive glass with enhanced rates of hydroxyapatite deposition and hemostatic activity. Small. 2006; 2: 1261-1265.
26. Paul S, Tak Y, Song JM. Does the antibacterial activity of silver nanoparticles depend on the Shape of the nanoparticles? A study of the gram negative bacterium Escherichia coli. Appl. Environ. Microbiol. 2007; 73: 1712-1720.
27. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. J Hosp Infect. 2005; 60: 1-7.
28. Rath SN, Brandl A, Hiller D, Hoppe A, Gbureck U, Horch RE, *et al*. Bioactive copper-doped glass scaffolds can stimulate endothelial cells in co-culture in combination with mesenchymal stem cells. PloS one. 2014; 9: e113319.
29. Sambhy V, MacBrid MM, Peterson BR, Sem A. Silver bromide nanoparticle/polymer composite: dual action tunable antimicrobial material. J Am Chem C. 2006; 128: 9798-9808.
30. Shirtliff VJ, Hench LL. Bioactive materials for tissue engineering, regeneration and repair. J Mater Sci. 2003; 38: 4697-4707.
31. Silva-López RE. Leishmania proteases: new targets for rational drug development. Quim Nova. 2010; 33: 1541-1548.
32. Strazzulla A, Cocuzza S, Pinzone MR, Postorino MC, Cosentino S, Serra, *et al.* Mucosal leishmaniasis: an underestimated presentation of a neglected disease. BioMed research international.2013; *2013*: 1-7.

33. Sundar S, Olliaro P L. Miltefosine in the treatment of leishmaniasis: clinical evidence for informed clinical risk management. Ther Clin Risk Manag. 2007; 3: 733.
34. Vries HJC, Reedijk SH, Schallig Henk DFH. Cutaneous leishmaniasis: recent developments in diagnosis and management. Am. j. clin. dermatol. 2015; 16: 99-109.
35. WHO/TDR World Health Organization. Tropical Disease Research – Leishmaniasis, 2013.
36. Zhu H, Hu C, Zhang F, Feng X, Li J, Liu T, *et al*. Preparation and antibacterial property of silver-containing mesoporous 58s bioactive glass. Mater Sci Eng, C. 2014; 42: 22-30.

ANEXOS

ANEXO A – Normas para a Defesa da Dissertação do Programa de Pós-Graduação em Odontologia da UFPB

Formato alternativo.

- Capa
- Folha de rosto (primeira folha interna)
- Ficha catalográfica (verso da folha de rosto)
- Folha de aprovação
- Dedicatória (Opcional)
- Agradecimentos (Opcional)
- Epígrafe (Opcional)
- Resumo (com no máximo quinhentas palavras)
- Abstract (com no máximo quinhentas palavras)
- Lista de Abreviaturas e Siglas (Opcional)
- Sumário

1. Introdução (trata-se, como no formato tradicional, da parte inicial do texto, da formulação clara e simples do tema investigado, constando a delimitação do assunto tratado, sua justificativa e objetivos da pesquisa)
2. Capítulos (devem ser inseridas as cópias de artigos de autoria ou co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem. Cada capítulo deve conter sua indicação, seguido do número (em arábico) correspondente. Ex.: Capítulo1, Capítulo 2 e assim sucessivamente)
3. Discussão ou Considerações Gerais (de caráter opcional, esta parte poderá conter argumentos para estabelecer relações entre os artigos apresentados nos capítulos)
4. Conclusão

- Referências
- Anexo (Opcional)
- Apêndices (Opcional)