

of immune response. In brief, mice inoculated with chimeric protein were able to control parasitemias and exhibited an immune response against *T. cruzi* in comparison with controls.

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#### DEVELOPMENT OF COVID-19 MONOCLONAL ANTIBODIES AND RECOMBINANT PROTEINS AS REAGENTS FOR BIOMEDICAL RESEARCH AND DIAGNOSTIC TESTS

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Since SARS-COV-2 virus spread worldwide and COVID-19 turned rapidly into a pandemic illness, the necessity for vaccines and diagnostic tests became crucial. The viral surface is decorated with Spike, the major antigenic determinant and main target for vaccine development. Within Spike, the receptor binding domain (RBD), constitutes the main target of highly neutralizing antibodies found in COVID-19 convalescent plasma. Besides vaccination, another important aspect of Spike (and RBD) is their use as immunogen for the development of poli- and monoclonal antibodies (mAbs) for therapeutic and diagnostic purposes. Here we report the development and preliminary biochemical characterization of a set of monoclonal antibodies against the Spike RBD domain along with the recombinant expression of two mayor COVID-19 protein reagents: the viral Spike RBD domain and the extracellular domain of the human receptor ACE2. RBD and the extracellular domain of ACE2 (aa 1-740) were obtained through transient gene transfection (TGE) in two different mammalian cell culture systems: HEK293T adherent monolayers and Expi293F™ suspension cultures. Due to its low cost and ease scale-up, all transfections were carried with polyethyleneimine (PEI). Expressed proteins were purified from culture supernatants by immobilized metal affinity chromatography. Anti-RBD mAbs were developed from two different immunization schemes: one aimed to elicit antibodies with viral neutralizing potential, and the other with the ability to recognize denatured RBD for routine lab immunoassays. To achieve this, the first group of mice was immunized with RBD in aluminum salts (RBD/Al) and the other with RBD emulsified in Freund's adjuvant (RBD/FA). Polyclonal and monoclonal antibody reactivities against native or denatured RBD forms were then assessed by ELISA. Complete RBD denaturation was followed by intrinsic fluorescence spectral changes upon different physicochemical stress treatments. As expected, RBD/Al immunized mice developed an antibody response shifted to native RBD while those immunized with RBD/FA showed a high response against both forms of the protein. In accordance with the observed polyclonal response, RBD/FA derived mAbs recognize both, native and denatured RBD. On the contrary, hybridomas generated from the RBD/Al protocol mostly recognize RBD in its native state. Further ELISA binding assays revealed that all RBD/FA derived mAbs can form a trimeric complex with ACE2 and RBD, denoting they would not have viral neutralizing activity. ELISA competition assays with the RBD/ACE2 complex aimed to determine the neutralization potential of the RBD/Al derived mAbs are under way. Overall, the anti-Spike RBD mAbs and the recombinant RBD and ACE2 proteins presented here constitute valuable tools for diverse COVID-19 academic research projects and local immunity surveillance testing.

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#### GROWTH OF ELECTRO-ACTIVE BACTERIA WITH BIOCHAR AS CHEMICAL ELECTRON ACCEPTOR AND ELECTRODE MATERIAL

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Recently, conductive materials started being applied as filling material of treatment wetlands, giving rise to the new technology of Bioelectrochemical Wetlands or METland filters. The conductive material enhances bacterial activity on the system boosting treatment efficiency. It allows the occurrence of a process known as direct interspecies electron transfer (DIET) in which electro-active microorganisms exchange electrons either by direct contact or through conductive materials, without relying on chemical intermediates. Major drawbacks for the application of bioelectrochemical wetlands are the cost and the availability of the conductive materials. Biochar is a conductive and biocompatible material obtained through the thermal decomposition (pyrolysis) of biomass residues and vegetable wastes and appears as a valid candidate for its use as filling material on bioelectrochemical wetlands. Its electrical conductivity, a parameter of major importance for the process of DIET, increases with pyrolysis temperature, but also does its cost. At low pyrolysis temperature chemicals such as quinones and other aromatic compounds that can be used as electron acceptors and electron donors for the growth of bacteria are produced and remain biochar. Thus, low biochar obtained at low temperatures may also enhance bacterial activity. In this work, we show the results of our first experiments aimed at finding the pyrolysis conditions that result in an enhance of the bacterial activity and wastewater treatment without compromising the cost. Biochar were obtained through pyrolysis of pruning residues at different operational temperatures ranging from 400 to 1200 °C. The composition of the materials was analyzed through infrared spectroscopy (FT-IR) and Raman spectroscopy assays, to determine the relative amount of possible bacterial electron donors or acceptors. To analyze the growth of electro-active bacteria with biochar as chemical electron acceptor, *Geobacter sulfurreducens*, a model electro-active bacteria was grown in batch with this material as the sole electron acceptor and its growth was followed by counting in a Neubauer chamber. Also, the electrical conductivity of the materials was measured